Linking microbially mediated soil organic matter turnover and nitrogen mineralisation to phosphorus and pH management in grassland soils

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Abstract

Nitrogen (N) is an essential nutrient for plant growth that is often limiting in agricultural production systems. While soil organic matter (SOM) represents a large terrestrial store of N, N stored in SOM is not directly available for plant uptake. The conversion of organic N in SOM to inorganic (plant-available) N is dependent on microbial activity, which in turn is influenced by plant-derived organic inputs to soil (rhizodeposition). Predicting when and in what form N becomes available from SOM can enhance farm nutrient management practices by linking nutrient cycling with plant growth. This has benefits agronomically and economically, by reducing reliance on expensive N fertilisers; and environmentally, by reducing nutrient losses to waterways and as greenhouse gas emissions, as N use becomes more soil, field and crop specific.

It is currently poorly understood how phosphorus addition, in both inorganic and organic forms, and soil pH management, through lime addition, impacts the processes involved in SOM N turnover. The main aim of this thesis was to address this knowledge deficit by investigating how these soil management practices impact the plant-soil-microbial processes (i.e., SOM decomposition and priming) that are involved in N mineralisation, and the associated changes in the microbial community. This was achieved through several hypotheses-driven experiments using analytical methods, novel in their combination of use, including a seven-day anaerobic assay (for the measurement of potential N mineralisation), stable ^{13}C isotope labelling through both $^{13}CO_2$ and ^{13}C glucose solution additions (for the partitioning and quantification carbon (C) fluxes in the soil system), and high throughput Illumina sequencing of 16S and ITS genes.

The impact of P management on N mineralisation potential was inconsistent, and long-term P addition had the capacity to either increase or decrease N mineralisation. However, when P was applied in organic form N mineralisation consistently increased. Higher P input caused a greater magnitude of priming (both positive and negative) compared to treatments which received less P. High P addition in the organic form reduced SOM decomposition rates, compared to when P was applied in an inorganic form, and resulted in a negative priming response. Therefore, there was a decrease in the release of C from SOM in this treatment, which will have implications for both increasing soil health and the sustainability of organic nutrient addition. P addition, both in the organic and inorganic form, had an impact on the bacterial and fungal microbial community structure.

Overall, N mineralisation potential increased with pH and this was associated with a shift in microbial community structure. This was particularly evident with the prokaryotic (16S) community, where there was an increase in diversity with increasing pH. For both the 16S and fungal communities, the largest variations in abundance were seen between treatments where the differences in pH were

greatest. Unexpectedly, the lime-derived CO_2 emissions could be detected more than 12 months after field application of lime. This has potentially important implications when considering the C budget of agricultural soils in grassland systems, where lime is typically a common agronomic management strategy and requires further research.

This thesis highlights the effects of soil management on C and N cycling in grassland soils and the findings will contribute towards sustainable management practices in grassland ecosystems to promote soil health and reduce nutrient losses to the environment.

Declaration of Authorship

I, Sorcha Kelly, declare that this thesis and the work presented in it are my own and have been performed by myself as a result of my own original research, and it has not been submitted in a previous application for a degree. Where I have consulted and quoted the published work of others the reference and name of the author is always given. I have acknowledged all main sources of help.

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1.1 Introduction

Nitrogen (N) is vital for all living organisms and is one of the key nutrients required for plant growth. Even though atmospheric N_2 makes up 79% of air (the biggest pool of N in the biosphere), N is a limiting nutrient in most terrestrial ecosystems, particularly in systems used for agricultural production (Rütting *et al.*, 2018). N_2 is bound by triple bonds, which are difficult and energy intensive to break down, and these bonds can only be broken by a few natural processes (Robertson and Vitousek, 2009). One such natural process is mediated by N-fixing microbes, for example rhizobia, that are capable of producing enzymes, such as nitrogenase, that convert N_2 to more plant available forms such as NH_3 and NH_4; this is the most prevalent way of fixing N in soils (Robertson and Vitousek, 2009). A well as the natural processes that operate in soils to overcome N limitation in agricultural systems, it is common agronomic practice to apply synthetic N fertiliser (Erisman *et al.*, 2008; Bouwman *et al.*, 2013; Bodirsky *et al.*, 2014; Schröder *et al.*, 2016). Even 100 years ago the agricultural and economic importance of N in agricultural systems was well known (Rütting *et al.*, 2018). However, current policy ambitions, particularly as part of the EU Green Deal, have targeted a decrease in the use of both pesticides and fertilisers on farms due to their detrimental impacts on the environment. The EU green deal has targeted a reduction in nutrient losses by up to 50% by 2030; it is thought that this would require at least a 20% reduction in fertiliser use in agricultural systems (Montanarella and Panagos, 2021). This could increase the importance of alternative sources of nutrients in agricultural systems.

Soil organic matter (SOM) is the largest store of N in soils (96-98%, 0.095×10^{18}g of total N globally; (Booth *et al.*, 2005)). However, in SOM, N is in an organic form and generally not available directly to plants. Therefore, to be accessible for plants, it needs to be transformed to inorganic forms of N prior to uptake. The transformation of organic N to inorganic N from SOM is a microbially mediated process known as N mineralisation. Even in intensive agricultural systems where N fertilisers are applied regularly, some studies have found that almost half of crop N uptake comes from SOM-derived N (Engels and Kuhlmann, 1993; McDonald *et al.*, 2014). N supply through net mineralisation over a growing season in Irish grasslands ranges from 56-220kg N ha^{-1} yr^{-1} (McDonald *et al.*, 2014). However, current fertiliser application strategies are a 'one size fits all' and do not consider the specific N mineralisation rates on a field-by-field basis. The impacts of soil nutrient management strategies, such as the application of fertilisers and how this impacts soil N mineralisation potential, are still relatively unknown and were examined as part of this thesis.

The negative environmental impacts of N fertiliser use are numerous and wide ranging. Over-application of N in agricultural systems can cause leaching of N to waterways which is detrimental for the health of aquatic systems (Savci, 2012). Emissions of N_2O are highest in agriculture compared to all other sectors globally(Duffy *et al.*, 2014). Larger N_2O are associated with higher N application rates in Irish grasslands (Rafique *et al.*, 2011). As well as this, there are large GHG emissions associated with the manufacturing of N through the Haber-Bosh processes , which is responsible for 1.2% of global CO_2 emissions (Smith *et al.*, 2020). N fertilisers and larger CO_2 emissions are associated with the transport of fertilisers globally (Poore and Nemecek, 2018). In the context of global change and achieving the targets set by the EU Green Deal, a reduction in N fertiliser use in agricultural systems is of international importance. Achieving soil- (or potentially field-) specific fertiliser plans that take into account N supply from SOM mineralisation have potentially significant environmental benefits, including a reduction of N losses to the environment via N leaching to waterways and reduced associated GHG. Furthermore, the economic benefits that accrue to the farmer through reduced use of fertiliser translating into reduced expenditure have become especially relevant in recent years as fertiliser prices have risen considerably.

1.2 The nitrogen cycle

N is the fourth most abundant element in cellular biomass (Stein and Klotz, 2016), being one of the primary components in nucleotides and proteins (Booth *et al.*, 2005; Robertson and Vitousek, 2009). In soils, the nitrogen cycle has several microbially mediated processes that are associated with N supply and loss. These include N fixation, N mineralisation, nitrification, and denitrification (Hayatsu *et al.*, 2008).

1.2.1 N_2 and nitrogen fixation

N_2 is not directly available to most organisms. For example, plants are not capable of N_2 fixation except in symbiosis with microbial N-fixers, acquiring N in the forms of NO_3^- and NH_4^+ from soil (Bardgett *et al.*, 2014). N_2 is made available through N fixation, the change from dinitrogen gas (N_2) in the atmosphere to 'reactive N', which is carried out by microbial and geochemical processes (Stein and Klotz, 2016; Coskun *et al.*, 2017). There are a limited number of bacteria and fungi that are capable of reducing N_2 to NH_4^+, which can then be converted into amino acids or other available organic N compounds, and this process is referred to as biological N fixation (Hoffman *et al.*, 2014; Krapp, 2015). N_2 fixation can be carried out by several prokaryotes that use nitrogenase, along with other enzymes and cofactors, to break down the tightly bound triple bond in N_2 (Soumare *et al.*, 2020). Bacteria that carry out N fixation can be either free-living in soil or symbiotic bacteria that are associated with

certain plant groups; in grasslands systems this can be carried out by red and white clover (Soumare *et al.*, 2020). Free-living bacteria involved in N fixation include *Azotobacter, Azospirillum, Bacillus*, and *Clostridium* (Ravikumar *et al.*, 2007; Soumare *et al.*, 2020) and symbiotic bacteria commonly associated with plants include *Rhizobium* with legumes, *Frankia* with actinorhizal plants, and *Cyanobacteria* with cycads (Ininbergs *et al.*, 2011; Soumare *et al.*, 2020). Methanogens are an archaeal group that are also associated with N fixation (Welte, 2018). By far the most studied of these biological N fixation microbial groups is the symbiotic relationship between rhizobia and legumes. The root nodules formed as part of this symbiotic relationship can fix between 20-300 Kg ha^{-1} yr^{-1} (Soumare *et al.*, 2020). For example, where red clover (*Trifolium pratense L.*), which is capable of generating up to 80% of its available N needs from symbiotic N_2 fixation (Hammelehle *et al.*, 2018), is typically grown in a grass mixture, it facilitates a comparatively moderate application of mineral N fertiliser. There is thus potential for biologically fixed N to reduce the need for N addition which in turn will lead to a reduction in N losses (Iannetta *et al.*, 2016) from soils.

1.2.2 Nitrification and denitrification

During nitrification, NH_4^+ can be oxidised by soil microbes to form hydroxylamine (NH_2OH), nitrite (NO_2^-), and nitrate (NO_3^-) (Coskun *et al.*, 2017). Nitrification is performed under aerobic conditions (Signor and Cerri, 2013). Historically it was thought that there were two main stages in nitrification: firstly, the oxidation of NH_4^+ to NO_2^-, which is carried out by ammonia oxidising prokaryotes (AOA and AOB); and secondly, the oxidation of NO_2^- to NO_3^-, this part is carried out by ammonia oxidising bacteria (AOB) (Hu *et al.*, 2021). However, it has been found that *Nitrospira* are able to perform both steps of nitrification (complete nitrification) in one cell (commammox) (Daims *et al.*, 2015; Van Kessel *et al.*, 2015; Daims *et al.*, 2016; Hu *et al.*, 2021). *Nitrospira* have been found in a range of agricultural soils and are positively correlated with soil nitrification potential (Pjevac *et al.*, 2017; Xia *et al.*, 2018; Lin *et al.*, 2020; Hu *et al.*, 2021). As NH_4^+ is oxidised as part of nitrification, the concentration of NO_2^- increases. However, NO_2^- concentration decreases as NO_2^- is subsequently oxidised to NO_3^- (Signor and Cerri, 2013).

Nitrate (NO_3^-) is the most oxidised form of N (Stüeken *et al.*, 2016). Denitrification is the reduction of NO_3^- to N gases such as N_2, N_2O, and NO (Stüeken *et al.*, 2016) and occurs in anaerobic soil conditions (Hayatsu *et al.*, 2008; Robertson and Groffman, 2015; Zhu-Barker and Steenwerth, 2018; Dubeux and Sollenberger, 2020). Denitrifying microorganisms use NO_2^- or NO_3^- instead of O_2 as an electron acceptor (as a means of energy generation) during respiration (Robertson and Groffman, 2015; Dubeux and Sollenberger, 2020). There are both bacterial and archaea groups that are capable of carrying out

denitrification, including *Pseudomonas, Alcalligenes, Bacillus, Agribacterium, Flavibacterium, Thiobacillus,* and *Propiobacterium* (Firestone, 1982; Robertson and Groffman, 2015). Microorganisms are capable of carrying out the process of denitrification through enzymes such as nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase (Hochstein and Tomlinson, 1988). However, it is not only bacteria that are able to perform denitrification, fungi can also mediate the process which is also referred to as aerobic denitrification, as fungi are able to denitrify and use O_2 for respiration at the same time (Robertson and Kuenen, 1990; Zhou *et al.*, 2001; Zhu-Barker and Steenwerth, 2018). Fungi, including *Ascomycota*, *Cylindrocapon tonkinerse*, *Gibberella fujikuroii*, and *Trichosporon cutaneum*, contribute to denitrification processes (Hayatsu *et al.*, 2008).

1.2.3 Soil nitrogen mineralisation

N is different from other elements as it does not have a mineral-bound weatherable pool in soils. Other elements, such a P and Ca have a potentially available source in soil bedrocks; however N has to be sourced from outside the soil system (Robertson and Vitousek, 2009). SOM contains a wide range of N compounds which are obtained from different processes such as animal excreta, N_2 fixation, atmospheric deposition, the incorporation of dead and decaying plant material, and microbial residues (Farrell *et al.*, 2014). Mineralisation of SOM occurs from the activity of microorganisms that are growing on decomposed organic material (Chen *et al.*, 2014). N mineralisation in soil is the transformation of complex organic N polymers to mineral N compounds (Murphy, 2015). The amount of organic N that can be mineralised is referred to as the potentially mineralised N pool (Ros *et al.*, 2011). Organic N is usually in the form of complex polymers such as humic and fulvic acids, which are the stabilised products of the decomposition process. However, these molecules do not have well-defined structures which make them resistant to microbial mineralisation; in some cases, the production of enzymes can overcome this barrier. Humic acids can have a residency time of up to 500 years in soil. In contrast, the mean residency time for organic and amino acids, as well as simple sugars, is usually less than one hour, as these forms are readily utilised by microbial communities (Piccolo, 2002).

Mineralisation of complex organic N comprises of a series of reactions, involving a combination of enzymes, organic substrates and microorganisms. Complex organic N is broken down to amides, amino sugars, and amino acids (the process of aminization) by N-acetyl glucosaminidase and arylamidase (Muruganandam *et al.*, 2009). Chitin, a relatively recalcitrant N-containing organic molecule, specifically derived from fungal cell walls, is a common organic N compound in most soils and is broken down by the N-acetyl-B-glucosaminidase enzyme (Muruganandam *et al.*, 2009).

Chitinase and leucine aminopeptides are other common enzymes that are used in decomposing organic N compounds (Chen *et al.*, 2014). The rate of supply of available N in agroecosystems is determined by N mineralisation activities i.e., the enzymes produced by microorganisms (Muruganandam *et al.*, 2009).

Soil microbial N mineralisation is impacted by several factors, such as SOM recalcitrance and concentration (Booth *et al.*, 2005), the C to N ratio within the SOM, the composition and abundance of the microbial community, and physical and chemical soil factors such as structure, pH, and water content (Kemmitt *et al.*, 2008). An increase in the soil C:N ratio is associated with a decrease in mineralisation (Ros *et al.*, 2011). However, SOM is not a viable C-substrate for microbes, as it takes more energy to decompose SOM-C than is returned by decomposition. However, if there is sufficient available C for biomass and energy, microbial growth can become limited by nutrients, such as N, and therefore mobilisation of nutrients from SOM can become a required and viable strategy to maintain microbial growth. Kemmitt *et al.* (2008) showed that when labile C is present, a diversity of microorganisms is important as not all microbes are capable of using labile C to break down SOM. Murphy *et al.* (2015) also showed that given sufficient C, microbes can preferentially mineralise N-rich components of SOM.

Soil aggregates vary in size and provide a variety of microhabitats for microorganisms; this influences microbial community structure and activity, which in turn influences enzymatic activity in soil (Sollins *et al.*, 1996; Muruganandam *et al.*, 2009). Physical protection of SOM occurs within and between soil aggregates and through adsorption on minerals (Six *et al.*, 2002). Biological availability of organic N is affected by clay particles, and the formation of protected aggregates in SOM (Ros *et al.*, 2011). Clay soils generally have lower rates of N mineralisation, and this may be due to binding of substrates (organic compounds) and products (NH_4^+) to clay surfaces (Ros *et al.*, 2011). Intermediate aggregate size fractions have been known to encourage fungal growth and activity (Muruganandam *et al.*, 2009). Muruganandam *et al.* (2009) also found that all enzymatic activities were significantly reduced in tilled systems in comparison to no-till systems and that no-till systems supported higher rates of potential N mineralisation. Furthermore, under no-till systems there is a larger proportion of fungi in microbial communities, which increases the potential N mineralisation, as fungi are generally considered better adapted to utilisation of complex organic forms, such as SOM (Muruganandam *et al.*, 2009).

SOM N mineralisation is an important source of N in agricultural systems (McDonald *et al.*, 2014), but accurately predicating SOM N mineralisation is difficult, as there are many interacting factors such as soil characteristics, management practices, and climate (Montgomery, 2007; Ashman and Puri, 2013).

Ratjen and Kage (2018) showed that climate and soil fertility both impact N mineralisation. Information on the potential amount of N mineralisation occurring in a soil is essential to determine the amount of N fertiliser needed. This is important for both crop yields and to prevent unnecessary losses to the environment by nitrate leaching to waterways or GHGs.

1.3 Soil organic matter

As noted in section 1.1 above, soil organic matter (SOM) is mostly made of dead organic material in soils such as decaying plant material, proteins, and polymers such as humic and fulvic acids. However, there is no universally agreed definition of SOM. While SOM mostly refers to dead organic matter, some authors refer to SOM as including living aspects of soil such as plant roots, microbes and other soil fauna (Kuzyakov *et al.*, 2000). The organic compounds found in SOM comprise different ages, molecular structures, stability, nutrient contents, and biological availability (Ros *et al.*, 2011). The total SOM stock is made up of different pools with varying mean residence times (MRTs) and recalcitrance (von Lützow *et al.*, 2007). The function of SOM varies with each different component, the youngest compounds are the most active, and contribute most to the physical soil status (Ros *et al.*, 2011). When considering SOM pools this is normally a theoretical classification, and when referring to fractions this usually relates to the parts of SOM that are measurable (Ros *et al.*, 2011). There are three main factors that are known to alter SOM pool MRT: recalcitrance of SOM; physical protection of SOM in soil aggregates; and relationships between soil minerals and SOM (Dungait *et al.*, 2012; Cotrufo *et al.*, 2013). Soils that have more clay, having more reactive sites, allow for SOM and nutrients to be adsorbed through ligand exchange and polyvalent cation bridging, which in turn reduces SOM mineralisation (Sarker *et al.*, 2018). Regardless of the level of physical protection of SOM, it has long been established that SOM is still mineralisable by soil microbes, when these microbes have access to an available energy source (Löhnis, 1926; Broadbent, 1948), and therefore soil microbes are considered the primary mediators of SOM turnover. SOM contains organic forms of C and all major plant nutrients such as N, P, S. (Kirkby *et al.*, 2011; Murphy, 2015). The quantity of SOM in a soil is dependent on the balance of C, N, and P entering the soil through plants or fertiliser addition, and leaving the soil through microbial processes such as mineralisation, leaching and gaseous emissions (Rumpel *et al.*, 2015).

SOM is central to ecosystem functions, including: supply and store of nutrients; C storage; maintenance of increased soil biodiversity; and improved water retention capacity (Rees *et al.*, 2001; Janzen, 2005; Janzen, 2006; Garcia-Pausas and Paterson, 2011). SOM contains two-to-five times as much C as above-ground biomass, and two-to-four times as much as the atmosphere (Ciais *et al.*,

2013). Some studies have shown that the capacity of soils and SOM to store C can be even higher (Glaser and Birk, 2012; Minasny *et al.*, 2017). However, stores of soil organic C have decreased and are continuing to decrease in agricultural soils with increasing agricultural intensification (Veerman *et al.*, 2020). The impacts of N enrichment on the SOM C pool is uncertain (Riggs *et al.*, 2015); increased understanding in this area is important not only for nutrient supply but also for C sequestration. N has been found to increase (Frey *et al.*, 2014), decrease (Waldrop *et al.*, 2004) and have no effect (Zeglin *et al.*, 2007) on the total C pool in SOM. However, it is thought that N has an impact on below-ground C cycling due to the close interactions between the C and N cycles (Janssens *et al.*, 2010b; Chen *et al.*, 2014).

1.4 Plant-soil-microbe interactions

Microbes associated with plants are critical for plant health and growth, and plant-microbes interactions are also important for wider function in the rhizosphere (Taylor *et al.*, 2009; Becher *et al.*, 2013).The rhizosphere is considered to be the area of soil influenced by plant roots. Plants can influence the microbial population density, structure and activity by their root exudates within the rhizosphere (Badri *et al.*, 2009; Berendsen *et al.*, 2012). The main function of plant roots is to anchor the plant, take up water and mineral nutrients. However, roots also release labile organic C compounds, which provide an energy source for microbial communities in the rhizosphere (Lambers *et al.*, 2009). Plants and microbes also often compete for resources such as nutrients, including N in the form of low weight peptides and amino acids in soils (Kuzyakov and Xu, 2013).

Plants can influence the rhizosphere and soil microbial communities through exudation of C molecules (Hu *et al.*, 2018; Griffiths *et al.*, 2021). These molecules are usually made up of sugars, amino acids, or carboxylic acids (Hartmann *et al.*, 2009; Cesco *et al.*, 2010; Hu *et al.*, 2018); they have a set function, and all plants release all of the compounds on a regular basis (Lambers *et al.*, 2009). Up to 20% of the C taken up through photosynthesis is used as part of root exudates (Haichar *et al.*, 2008). Rhizodeposition may be more important than litter inputs in promoting SOM accumulation, as the dominant form of SOM is microbial necromass and residues, rather than recalcitrant plant-derived compounds (Cotrufo *et al.*, 2013; Hoffland *et al.*, 2020). The quality and quantity of root exudates differ with plant species and environmental factors, such as pH and P concentration in soil (Lambers *et al.*, 2009; Griffiths *et al.*, 2021). Previous studies have highlighted the positive effect of root exudation on soil C (Henneron *et al.*, 2020a; Henneron *et al.*, 2020b).

Roots influence SOM decomposition through decreasing nutrient availability for microbes as plant growth competes for soil nutrients, as well as this plant roots can enhance organic substrate quantities in soil through root exudation, which increases microbial SOM turnover (Rumpel *et al.*, 2015). Root exudates released by the plant to the rhizosphere is at a cost for the plant, as this is a potential C source that will not be used for plant biomass (Lambers *et al.*, 2009). Plants can trigger SOM N transformations which are catalysed by microorganisms in the rhizosphere by providing these microbes with an energy source through C root exudates (Cassman *et al.*, 2002; Schlesinger, 2009). Such C inputs increase the activity of microbes, potentially resulting in SOM mineralisation (Kuzyakov *et al.*, 2000), a process referred to as the priming effect.

1.5 Priming

Priming is the change in the rate of SOM decomposition resulting from the addition of an easily available organic matter input. This can be in the form of root exudate, plant litter material, animal manure or excreta (Kuzyakov *et al.*, 2000; Paterson, 2003; Zhu and Cheng, 2011). SOM decomposition and priming is measured using CO_2 effluxes from soil (Kuzyakov, 2010). Experiments that measure priming in soil systems typically use isotope labelling techniques (Kuzyakov *et al.*, 2000; Paterson *et al.*, 2009), and are established as one treatment that receives a isotope labelled C based input (through plant root exudates or from a laboratory added C cocktail) and a reference treatment that does not receive these inputs. With isotope labelling, it is therefore possible to distinguish between mineralisation of SOM and of the added substrate, and primed SOM decomposition is calculated by subtracting the SOM-derived respiration in the isotope labelled treatment from the SOM-derived respiration in the treatment not receiving inputs (Paterson *et al.*, 2007).

It is common in studies examining the priming effect that ^{13}C labelled glucose is used as the added energy source, as glucose is one of the primary sugars in rhizodeposits and a product of macromolecule (e.g. cellulose) decomposition (Derrien *et al.*, 2004). Furthermore, the use of living plants in experiments investigating priming is important, as planted systems are more likely to represent rhizosphere soil and the presence of a plant allows for a more detailed study on the links between SOM decomposition and plant growth (e.g., root and shoot biomass). Plant roots impact the rhizosphere soil which in turn could potentially impact the rate of priming in soils (Hinsinger, 2001). Plant roots, via the microbial activity stimulated by root exudates, can decrease SOM decomposition by up to 50% or increase SOM decomposition by up to 380% (Cheng *et al.*, 2014). There are several processes involved in priming that can lead to this increase or decrease in SOM decomposition, which are described in section 1.5.1 below.

1.5.1 Processes involved in priming

The direction of priming can be either positive or negative. Positive priming occurs when there is an increase in SOM decomposition with addition of C-substrate, whereas negative priming is when there is a decrease in SOM decomposition. Some of the hypotheses and theories involved in both positive and negative priming are outlined below.

1.5.1.1 Positive priming

Positive priming can occur when labile or recalcitrant substrates are added to soil and this has been linked with co-metabolism (Zhang *et al.*, 2017; Zhu *et al.*, 2018). Positive priming occurs when inputs of more recalcitrant organic substrates (such as plant material, straw, or grass) stimulates SOM decomposition via enzymes that can decompose both added substrates and SOM (Murphy, 2015). The positive 'priming-effect' is related to the amount of C substrate added (Paterson and Sim, 2013), and may also be influenced by the form of addition. For example, in systems where glucose is added compared to plant--based systems, this can potentially have an impact on the magnitude of priming as glucose is a more readily available substrate than other root exudate compounds(Kuzyakov *et al.*, 2000; Paterson, 2003).

Positive priming effects can also be associated with both nutrient-rich and nutrient-poor soil systems. Nutrient availability has been shown to affect decomposition rates (Kuzyakov, 2010; Dijkstra *et al.*, 2013; Chen *et al.*, 2014; Murphy *et al.*, 2017). There are opposing theories that potentially explain this outcome. Firstly, the *activation theory* highlights the impact that labile substrate addition has on both microbial activity and biomass (Hessen *et al.*, 2004). This theory postulates that the stimulation of the microbial community following the addition of a labile substrate will increase microbially mediated processes, including SOM decomposition. Paterson *et al.* (2007) showed that after labile C addition, this C is taken up quickly by soil microbes and is also associated with SOM mineralisation. Secondly, the microbial *nitrogen mining hypothesis* considers that when N is limiting, microbes use labile C sources, such as plant root exudates, to provide energy required to decompose organic N sources in SOM (Moorhead and Sinsabaugh, 2006; Craine *et al.*, 2007). This hypothesis considers that in nutrient-poor but C-rich environments such as the rhizosphere, priming is a response of the microbial community to break down SOM for essential nutrients such as N (Craine *et al.*, 2007; Murphy *et al.*, 2017; Guyonnet *et al.*, 2018). However, microbial mining does not only occur in nutrient-limited systems. This process can occur in systems where there is a nutrient imbalance and therefore microbes mine for nutrients to return the nutrient balance (Craine *et al.*, 2007). This also links with a *stoichiometric decomposition theory* which suggests that microbial activity and decomposition are

highest when C and N inputs match microbial demand, i.e., when inputs of C and N correspond to the C to N ratios (Hessen *et al.*, 2004).

1.5.1.2 Negative priming

The process involved in negative priming is considered to be when easily available additions of C sources such as root exudates are decomposed preferentially instead of SOM (Blagodatskaya *et al.*, 2007). Negative priming usually occurs in systems that are both nutrient- and C-rich (Kuzyakov and Bol, 2006). The scenario where these readily available sources of C and N are used (at the expense of SOM decomposition) is known as the *preferential substrate hypothesis* (Blagodatskaya and Kuzyakov, 2008).

1.5.2 Soil microbes and priming

Priming can almost be considered as an indirect symbiosis, as the microbial community and the plants are not physically linked (Cheng *et al.*, 2014). While several factors have been identified that impact priming activities – including nutrient status, plant species, and the soil horizon (Garcia-Pausas and Paterson, 2011) – microorganisms play several key roles in the priming process (Fontaine *et al.*, 2003), which often depend on their life strategies. Both r and K strategists can be involved in substrate and SOM decomposition (Fontaine *et al.*, 2003; Chen *et al.*, 2014; de Graaff *et al.*, 2014; Lonardo *et al.*, 2017). Fast growing r-strategists use easily-available substrates, while slower growing K-strategists use more recalcitrant organic material (Chen *et al.*, 2014). R-strategists increase the release of enzymes which break down fresh organic substrates, and potentially SOM (Pianka, 1970; Fontaine *et al.*, 2003; Lambers *et al.*, 2009). Metabolites that are released by r-strategists as part of the breakdown of labile substrates or SOM stimulate K-strategists, and this results in decomposition of SOM by the K-strategists, as these organisms (including fungi) are considered better adapted to utilisation of complex organic compounds (Lambers *et al.*, 2009). A study by Chen *et al.* (2014) showed that under differing N conditions, different microbial strategists were involved in priming. In a soil with low N availability, K-strategists were more involved in priming and SOM decomposition; whereas, in soils with high N, r-strategists were the drivers of priming (Chen *et al.*, 2014).

1.6 Grassland nutrient management

In the EU, grasslands are one of the most important agricultural land use types, with significant grassland coverage in Ireland (67%), UK (40%) and Germany (23%) (Buchen *et al.*, 2017). Grasslands arguably represent one of the primary agricultural systems offering potential to maximise C sequestration (Crème *et al.*, 2020). Scurlock *et al.* (2002) showed that grasslands can store 0.5 Pg soil

organic C per year. Grassland soils also contain large N stores in SOM. N supply over a growing season in Irish grasslands is 56-220kg N ha^{-1} yr^{-1} (McDonald *et al.*, 2014). N stocks in agricultural grasslands can be impacted by several factors such as site conditions, sward age, soil properties, and farm management practices (Buchen *et al.*, 2017). In terms of sustainable grassland management and productivity, it is important to be able to understand and predict organic N pools and their turnover rates (Prendergast-Miller *et al.*, 2015). This would potentially translate into specific on-farm fertiliser plans, thus reducing N losses to the environment, as well as economic costs to farmers. Encouraging specific management practices that promote SOM accumulation can also have important co-benefits for soil health and ecosystem services (Audette *et al.*, 2021).

Grassland soils generally have a high OM content, which can improve soil quality and in turn increase microbial activity and diversity, nutrient and water availability (Rumpel *et al.*, 2015). Highly productive grassland systems, that receive large amounts of nutrients and maintain a high plant biomass, can have a positive impact on soil organic C storage, through increased organic matter inputs from plant material (Lal, 2004; Curtin *et al.*, 2014; Richardson *et al.*, 2014; Crème *et al.*, 2020). However, intensive agricultural systems can also decrease SOM stocks (Lal, 2004). Differences in SOM storage can result from differences in the grazing and cutting system, stocking rate, fertilisation, nutrient status, and plant and microbial species composition, as these factors affect SOM turnover through OM input and rhizosphere activity (Rumpel *et al.*, 2015).

There are several processes in grassland systems that contribute to stabilisation and turnover of soil C and N; these include root turnover, rhizodeposition, root respiration, and microbial responses (Rumpel *et al.*, 2015). Grazing and cutting encourages plant root exudation, which enhances soil microbial activity and nutrient turnover (Rumpel *et al.*, 2015). However, in grazing systems when the livestock stocking rate is too high and plants are overgrazed, both shoots and root biomass is reduced (Naeth *et al.*, 1991; Zhou *et al.*, 2017). This can have knock-on impacts on root exudation of C and hence decrease SOM build up (Zhou *et al.*, 2017). However, up to a particular livestock density, grazing increases SOM build-up through excretal returns from grazing livestock when compared with the biomass loss associated with cutting (Rumpel *et al.*, 2015). However, cutting-based grassland systems induce regular root turnover and this is a source of C-input to soils (Hammelehle *et al.*, 2018). The effects of grazing vs cutting on N mineralisation are less well understood. It has been shown that regular defoliation through grazing can increase N mineralisation; however, it is not known if defoliation through a cutting-based system has the same effect on N mineralisation potential (Hamilton *et al.*, 2008).

1.6.1 Nitrogen addition

The addition of mineral N changes the composition and function of soil microbial communities which can affect SOM decomposition and other ecosystem processes (Robertson and Vitousek, 2009). With increasing soil N availability there is a higher N content in grassland litter, and N additions can change the abundance of plant species in the grass sward (Henry *et al.*, 2005). However, N additions can also decrease relative allocation of assimilate to below-ground plant biomass, as there is less investment in roots needed for nutrient acquisition (FENG *et al.*, 2010; Janssens *et al.*, 2010a); this can impact on N availability in soils and N stores in SOM (Li *et al.*, 2015), and also affect the relative resource availabilities of C and P, not only to plants but also to the microbial biomass. N additions can decrease microbial respiration of SOM by 29% (Riggs *et al.*, 2015) and this highlights a shift in microbial community activity and can also change microbial community structure (Dai *et al.*, 2018). Results from Murphy *et al.* (2017) showed that nutrient addition reduced SOM decomposition in a planted system.

1.6.2 Phosphorus addition

P is also commonly a growth-limiting nutrient for both plant and microbes in agricultural systems (Sharma *et al.*, 2013). P is an important nutrient for both plants and microbes as it is required in the formation of cell membranes, nucleic acids, energy molecules such as ATP, and seed development (Cordell and White, 2014). P application, similar to N application, is essential to maintain production in agricultural systems. In Ireland, with increasing agricultural intensification, P has over time been removed from soil stocks in animal products, leading to P limitation in some grassland soils (McDonald *et al.*, 2019). Nutrient limitation in agricultural systems for P is often overcome by the addition of fertilisers (Bouwman *et al.*, 2013; Bodirsky *et al.*, 2014; Schröder *et al.*, 2016). Similar to N-based fertilisers, the addition of P fertilisers can alter the soil microbial community activity and structure in grazed grasslands (Randall *et al.*, 2019). Nottingham *et al.* (2018) found that, when P was added in combination with other nutrients, bacteria out-competed fungi for N. Hence, the addition of nutrients in agricultural systems is likely to cause changes to microbial community composition, activity and microbial mediated nutrient cycling (Fang *et al.*, 2020). This has knock-on effects on a range of soil microbial functions and mechanisms, including those involved in nutrient cycling processes such as SOM mineralisation and priming (Sullivan and Hart, 2013; Fang *et al.*, 2020). The effects of P on SOM decomposition and priming are relatively understudied in comparison to N addition.

1.6.3 Soil pH and lime management

Soil pH is often referred to in the literature as the “master soil variable”, as it influences soil physical, chemical, and biological processes (Essington, 2015; Neina, 2019; Žurovec *et al.*, 2021). The optimum

soil pH for productivity in Irish grasslands is 6.3 (Wall and Plunkett, 2021). In the context of grassland nutrient management soil, pH is an important environmental variable as it directly impacts nutrient availability. In Ireland, acidic agricultural soils are common due to high rainfall and also high fertiliser additions (Tunney *et al.*, 2010). The application of lime is a commonly used agricultural practice to combat the detrimental effects of soil acidification by increasing soil pH (Aye *et al.*, 2016; Holland *et al.*, 2018). Liming generally has a positive influence on soil nutrient availability, soil structure and soil microbial activity (Holland *et al.*, 2018; Žurovec *et al.*, 2021). Liming can affect nutrient availability, and microbial communities structure and activity through its impact on the quality and stability of soil organic C (Audette *et al.*, 2021). Liming soil improves the availability of N, P, K, and other nutrients (Johnson *et al.*, 2005). In studies of long term-liming experiments it has been shown that the formation of organo-mineral fractions, with calcium ions acting as cation bridges between SOM and clay particles, leads to improved aggregate formation and structure as well as lowering the C:N ratio of the bulk soil (Fornara *et al.*, 2011; Briedis *et al.*, 2012a). This can have impacts on SOM decomposition through altering the solubility of soil organic C, but also through changes to microbial activity (Andersson *et al.*, 2000; Briedis *et al.*, 2012b; Wang and Tang, 2017). Soil microbial community activity and structure are influenced by soil pH (Bertrand *et al.*, 2007; Herold *et al.*, 2018). Microbial and enzymatic activity are higher in soils ranging in pH from 5-8 than in soils that have lower pH (Blagodatskaya and Anderson, 1998). Wang *et al.* (2016) found that with liming and increasing soil pH there was a shift from fungal-dominated to bacterial-dominated communities. It was hypothesised that this would contribute to the regulation of SOM decomposition by increasing the longer-lasting recalcitrant soil-organic C (Wang *et al.*, 2016). This has far-reaching impacts on soil biogeochemical processes, particularly those involved in nutrient cycling and C storage (Neina, 2019). Previous studies have found that N mineralisation can be affected by soil pH (Bolan *et al.*, 2003; Bolan and Hedley, 2003). The optimum soil pH for N mineralisation was reported as approximately pH 6 or higher (Blagodatskaya and Anderson, 1998).

1.7 Aims and Objectives

N dynamics in agricultural soils and the challenge to optimise N fertiliser advice are linked to the farm management system (Rütting *et al.*, 2018), including the addition of other nutrients such as P or of lime. The main challenge of N management is to supply enough N so that plants can maximise growth, but also to prevent excess N ending up in the environment where it can be harmful to waterways and the atmosphere (Robertson and Vitousek, 2009). It is important to understand the effect that agricultural practices have on the supply of nutrients via SOM mineralisation. As SOM contains a large

store of organic N, this information can be used to increase the productivity, profitability, and environmental sustainability of farming systems (Sarker *et al.*, 2018).

The main aim of this thesis was to further understanding of the processes and mechanisms involved in the release of N from SOM, as well as the nutrient management practices that effect these processes. The specific focus was on assessing the extent of priming and N mineralisation as a function of grassland nutrient management strategies. The effect of grassland management on microbial activity and community structure was also investigated.

1.7.1 Objectives

It is known that N mineralisation potential is highly variable with soil type. However, it is less well known what effects agricultural nutrient management has on N mineralisation and also the mechanisms involved in N mineralisation. Additionally, there is limited knowledge on the effects of agricultural management nutrient management practices on the mediators of this process, i.e. enzymes and soil microbial communities. The first experimental chapter of this thesis (Chapter 2) was designed to examine the differences in potential N mineralisation, enzyme activity and microbial community structure across nutrient management strategies in grassland systems across a range of soil types. Nutrient management included variations in the type of P addition (inorganic vs organic) and also soil pH amendment with lime addition. N mineralisation potential was measured using the seven-day anaerobic incubation assay and enzyme activity was measured using fluorimetric microplate assays. The hypotheses were:

1) The application of lime and organic P would increase N availability, N mineralisation of SOM, and enzymatic activity.

2) Chemical P application would decrease the activity of enzymes, and N mineralisation of SOM.

3) The microbial community structure would be influenced by nutrient management and soil conditions.

The impacts of N addition on priming have been investigated more often in the literature (Conde *et al.*, 2005; Murphy *et al.*, 2017; Li *et al.*, 2018; Mason-Jones *et al.*, 2018; Fan *et al.*, 2020; Thilakarathna and Hernandez-Ramirez, 2021) in comparison to the effects of P addition, particularly P addition on its own. Experimental chapters 3 and 4 investigated the impacts of P addition in the form of inorganic or organic fertiliser addition on priming and N mineralisation. Chapter 3 focused on how the impacts of P fertiliser management (in a planted system) on SOM decomposition and priming effects, which

were measured using a $^{13}CO_2$ labelling chamber. In contrast, Chapter 4 assessed the effects of P fertiliser management strategy using a soil-based system with ^{13}C glucose addition to stimulate priming. Chapter 4 expanded upon the work of Chapter 3 as it also examined the difference(s) between a grazed and cut grassland system. Furthermore, Chapter 4 examined the differences in microbial community structure with P nutrient management under cut and grazed grassland systems. The hypotheses tested were:

Chapter 3:

1) The addition of P would increase plant productivity, N mineralisation and the rate of priming.

2) That P added in an organic form would increase soil nutrient availability, N mineralisation and the rate of priming, due to increased microbial and enzymatic activity.

Chapter 4:

1) The addition of P would increase N mineralisation and the rate of priming.

2) That P added in an organic form would increase N mineralisation and the rate of priming.

3) In the cut and remove for silage system there would be increased priming and potential N mineralisation.

4) Soil microbial community structure would be affected by P addition and also grassland management.

It is well known that pH impacts nutrient availability and microbial activity in grassland agricultural systems. However, it is still important to consider the effects of pH on N mineralisation potential and SOM decomposition, as this may have important implications for soil specific fertiliser application. Chapter 5 examined the impact of soil pH and liming on grassland soils on priming and potential N mineralisation. This experiment also used a $^{13}CO_2$ labelling chamber technique to measure priming in a planted system. It is rare in the literature the effect of pH on priming has been measured without lime being added as part of the experiment and also using a planted grass system. The hypotheses were:

1) That the priming affect will have the highest magnitude in the soils with a pH of 6-7.

2) N mineralisation would increase with increasing soil pH and liming, and hence the highest potential mineralisation rate would be in the soil with the highest pH.

3) Microbial community diversity and abundance would be affected by soil pH and that the highest soil pH would have the most diverse microbial community.

1.8 Methodologies

Throughout this thesis there were a number of experimental procedures used consistently. These include the seven-day anaerobic incubation for the measurement of potential N mineralisation, stable isotope labelling techniques to measure SOM decomposition and priming, and finally Illumina sequencing to examine bacterial, archaeal, and fungal community structure.

There are a number of techniques to measure N mineralisation in soils. To measure gross N mineralisation the15N dilution technique is widely used (Goerges and Dittert, 1998; Murphy et al., 1999; Murphy et al., 2017; Braun et al., 2018; Braun et al., 2019; Fiorentino et al., 2019) Fan et al., 2022). The 15N dilution method uses the addition of a 15N labelled NH4+ tracer, this is usually in the form of a labelled N fertiliser which is then applied to the experiment. In these experiments any N released from SOM will be seen as 14N of the soil NH4+ pool. Therefore, it is possible to determine if N was used from the fertiliser or mineralisation from the organic N pool using the applied labelling approach (Goerges and Dittert, 1998). The 15N dilution method would accounts for differentiate N applied as part of the fertiliser, N mineralised from SOM, and N immobilisation into soil microbial biomass, and thus enables which gives a gross N mineralisation measurement. However, it was decided not to use this technique as adding an N fertiliser could have knock on effects on both the microbial mediated processed that would be measure (N mineralisation and priming), as well as on microbial community structure.

N mineralisation can also be measured using incubations, these can be either aerobic or anaerobic. Incubation experiments aim to capture biological activity involved in N mineralisation. As this thesis aimed to examine the effects of microbial mediated processes, a biological measurement of potential N mineralisation was selected. The aerobic method for measuring N mineralisation was designed by Stanford and Smith (1972). This method is carried out over 30 weeks, soils are incubated at 35°C with aeration for at least 5 minutes per day (Ros et al., 2011; McDonald et al., 2014). Both NO3-N and NH4-N are measured, and the rate of N mineralisation is calculated using a kinetic model (McDonald et al., 2014). However, this method is not always practical due to the time length of the assay. To avoid the issues associated with long term incubations this thesis chose the 7-day anaerobic incubation method. This method was first established by Waring and Bremner (1964) and has previously been used on a variety of Irish grassland soils by McDonald et al. (2014). Soils are kept in anaerobic conditions through

waterlogging in a sealed glass test tube and incubated at 40°C for 7 days. These conditions allow for aminization and ammonification to occur, while nitrification is inhibited, and therefore this incubation is NH4-N based (Waring and Bremner, 1964; McDonald et al., 2014). A potential downfall of the selected 7-day anaerobic incubation method is that it provides a net measured of N mineralisation. Therefore, it was not possible to quantify the N that was immobilised by the microbial biomass.

In order to quantify SOM-C decomposition and priming, the C in the plant and soil system needs to be traced. One of the main methods to follow C through these complex systems is to use stable C isotope labelling. This technique has been increasing in use (Kuzyakov, 2010). There are two main labelling techniques used in stable isotope labelling known as pulse and continuous (Paterson et al., 2009). Pulse labelling approaches apply a 13CO2 label at discrete time points, for example this could be over a number of hours on a certain day over an experimental period. This type of labelling technique is effective for measuring plant C inputs into soil systems (Meharg, 1994; Nguyen, 2003) . However, pulse labelling does not distribute the label evenly throughout the plant and soil system. This makes it difficult to correctly quantify whole fluxes in the plant-soil system, particularly as the C pool is dynamic and C is constantly being exchanged between both plant and soil pools (Paterson et al., 2005). As the pulse label is thought to be limited to more recently developed plant C pools and in active growth areas it is unlikely that older C pools in SOM have been labelled, and therefore it is not possible to quantity the C flow from recalcitrant pools (Thornton et al., 2004; Paterson et al., 2006). Continuous labelling offers a potential solution to the issues that pulse labelling has. Kuzyakov (2010) stated that the correct way to measure SOM decomposition and priming was to use isotope labelling as it is the only way to truly separate C flows from their different sources. Continuous labelling can be achieved through the use of a naturally depleted 13CO2 system (used in Chapter 5) and also through continuously enriched 13CO2 labelling systems (used in Chapter 3), over the whole growth period of the plant. The continuous labelling allows for uniform labelling of the plant and therefore full quantification of plant inputs to soil through rhizodeposits and root respiration (Paterson et al., 2009). This allows for isotope partitioning of the plant and soil contribution to C in the system and therefore priming and SOM decomposition can be calculated as outlined in Chapters 3 and 5.

However, not all experimental set ups related to C tracing, SOM decomposition, and priming use planted systems. Alternatively, fallow soil systems receiving a labelled C input such as glucose or cellulose may be used (Kuzyakov et al., 2000; Paterson, 2003; Kuzyakov, 2010). The most common form of C added to stimulate a primed response is glucose, as glucose is often released by the plant in root exudates (Derrien et al., 2004). There are advantages to using a system without a plant. For

example, there is more control of the soil system compared to a planted system which includes additional influences of plants such as through changes to water fluxes, impacts on soil structure, the chemistry of the rhizosphere (e.g., pH), changes to microbial community composition, and nutrient availability. Chapter 4 in this thesis used a glucose labelling system in order to further examine the effect of P treatment on priming, SOM decomposition, and microbial communities.

This thesis also aimed to assess the structure of bacterial, archaeal, and fungal communities and how these structures were impacted by P and pH management, as well as SOM-C decomposition and priming. In the past, microbial community analysis was limited to bacteria that could be cultured or microbes were grouped by morphology and physiological features (Staley and Konopka, 1985; Frostegård et al., 2011; Alteio et al., 2021). These methods have also led to more quantitative approaches of measuring the microbial community such as measuring microbial biomass using the chloroform fumigation method (Brookes et al., 1982; Brookes et al., 1985), and PLFA - phospholipid fatty acid profiling (Frostegård et al., 1991). However, chloroform fumigation extraction only measures the size of the microbial biomass and does not give an indication of which microbes are present (Bailey et al., 2002). Whereas PLFA use the lipids in cell membranes to identify some of the microorganisms in the community (Hinojosa et al., 2010). The PLFA analysis has been used as an indicator for the changes in microbial communities with different management techniques (Alteio et al., 2021) .However, in more recent times Illumina sequencing particularly of the 16s bacteria and archaea community has become increasingly popular (Niu et al., 2016; Choi et al., 2017; Schöler et al., 2017; Nkongolo and Narendrula-Kotha, 2020). Other micorbial markers such as ITS for analysing fungal communities can also be used (Alteio et al., 2021). Amplicon sequencing using Illumina technologies is being more accessible to the wider scientific community as the accessibility of DNA extraction and PCR assays techniques have increased, costs have reduced, and the availability of free analysis software packages has improved and become more user friendly (Caporaso et al., 2012). Some have suggested that sequencing has now become the most cost and time effective approach to analyse microbial communities (Alteio et al., 2021). However, data from amplicon sequencing using PCR based gene markers such as 16S are compositional and the results cannot infer function of the microbial community (Gloor et al., 2017). Other techniques such as metagenomics or metatranscriptomics are required if more information about the functioning of the community is desired (Regalado et al., 2020). However, Illumina sequencing still is an important technique when considering future studies that may have the potential for more focused targets using metatranscriptomics for example (Regalado et al., 2020). However, coupling sequencing data with high quality meta data such as

microbial biomass data from chloroform fumigation extraction, as was used in this study, can also contribute important information about soil microbial communities.

1.9 References

Alteio, L.V., Séneca, J., Canarini, A., Angel, R., Jansa, J., Guseva, K., Kaiser, C., Richter, A., Schmidt, H., 2021. A critical perspective on interpreting amplicon sequencing data in soil ecological research. Soil Biology and Biochemistry 160, 108357.

Andersson, S., Nilsson, S.I., Saetre, P., 2000. Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biology and Biochemistry* 32, 1-10.

Ashman, M., Puri, G., 2013. *Essential soil science: a clear and concise introduction to soil science*. John Wiley & Sons.

Audette, Y., Congreves, K.A., Schneider, K., Zaro, G.C., Nunes, A.L.P., Zhang, H., Voroney, R.P., 2021. The effect of agroecosystem management on the distribution of C functional groups in soil organic matter: A review. *Biology and Fertility of Soils* 57, 881-894.

Aye, N.S., Sale, P.W.G., Tang, C., 2016. The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils. *Biology and Fertility of Soils* 52, 697-709.

Badri, D.V., Weir, T.L., van der Lelie, D., Vivanco, J.M., 2009. Rhizosphere chemical dialogues: plant-microbe interactions. *Curr. Opin. Biotechnol*. 20, 642-650.

Bailey, V.L., Peacock, A.D., Smith, J.L., Bolton, H., 2002. Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biology and Biochemistry 34, 1385-1389.

Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground: Root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution* 29, 692-699.

Becher, D., Bernhardt, J., Fuchs, S., Riedel, K., 2013. Metaproteomics to unravel major microbial players in leaf litter and soil environments: challenges and perspectives. *Proteomics* 13, 2895-2909.

Berendsen, R.L., Pieterse, C.M.J., Bakker, P., 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science* 17, 478-486.

Bertrand, I., Delfosse, O., Mary, B., 2007. Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: Apparent and actual effects. *Soil Biology and Biochemistry* 39, 276-288.

Blagodatskaya, E.V., Anderson, T.-H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO_2 of microbial communities in forest soils. *Soil Biology and Biochemistry* 30, 1269-1274.

Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. Applied Soil Ecology 37, 95-105.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45, 115-131.

Bodirsky, B.L., Popp, A., Lotze-Campen, H., Dietrich, J.P., Rolinski, S., Weindl, I., Schmitz, C., Müller, C., Bonsch, M., Humpenöder, F., Biewald, A., Stevanovic, M., 2014. Reactive nitrogen requirements

to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nature Communications* 5, 3858.
Bolan, N.S., Adriano, D.C., Curtin, D., 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Advances in Agronomy* 78, 5-272.
Bolan, N.S., Hedley, M.J., 2003. *Role of carbon, nitrogen, and sulfur cycles in soil acidification*. CRC Press.
Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.* 75, 139-157.
Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H.W., Van Vuuren, D.P., Willems, J., Rufino, M.C., Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences* 110, 20882-20887.
Braun, J., Mooshammer, M., Wanek, W., Prommer, J., Walker, T.W.N., Rütting, T., Richter, A., 2018. Full 15N tracer accounting to revisit major assumptions of 15N isotope pool dilution approaches for gross nitrogen mineralization. Soil Biology and Biochemistry 117, 16-26.
Braun, S., Warrinnier, R., Börjesson, G., Ulén, B., Smolders, E., Gustafsson, J.P., 2019. Assessing the ability of soil tests to estimate labile phosphorus in agricultural soils: Evidence from isotopic exchange. Geoderma 337, 350-358.
Briedis, C., de Moraes Sá, J.C., Caires, E.F., de Fátima Navarro, J., Inagaki, T.M., Boer, A., de Oliveira Ferreira, A., Neto, C.Q., Canalli, L.B., dos Santos, J.B., 2012a. Changes in Organic Matter Pools and Increases in Carbon Sequestration in Response to Surface Liming in an Oxisol under Long-Term No-Till. *Soil Science Society of America Journal* 76, 151-160.
Briedis, C., Sá, J.C.d.M., Caires, E.F., Navarro, J.d.F., Inagaki, T.M., Boer, A., Neto, C.Q., Ferreira, A.d.O., Canalli, L.B., Santos, J.B.d., 2012b. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. *Geoderma* 170, 80-88.
Broadbent, F.E., 1948. Nitrogen Release and Carbon Loss from Soil Organic Matter during Decomposition of Added Plant Residues. *Soil Science Society of America Journal* 12, 246-249.
Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319-329.
Buchen, C., Well, R., Helfrich, M., Fuß, R., Kayser, M., Gensior, A., Benke, M., Flessa, H., 2017. Soil mineral N dynamics and N2O emissions following grassland renewal. *Agriculture, Ecosystems & Environment* 246, 325-342.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME journal 6, 1621-1624.
Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. *Ambio* 31, 132-140.
Cesco, S., Neumann, G., Tomasi, N., Pinton, R., Weisskopf, L., 2010. Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant and Soil* 329, 1-25.
Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Global Change Biology 20, 2356-2367.

Cheng, W., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., McNickle, G.G., Brzostek, E., Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. New Phytologist 201, 31-44.
Choi, S., Song, H., Tripathi, B.M., Kerfahi, D., Kim, H., Adams, J.M., 2017. Effect of experimental soil disturbance and recovery on structure and function of soil community: a metagenomic and metagenetic approach. Scientific reports 7, 1-15.
Ciais, P., C. , Sabine, G., Bala, L., Bopp, V., Brovkin, J., Canadell, A., Chhabra, R., DeFries, J., Galloway, M., Heimann, C., Jones, C., Le Quéré, R.B., Myneni, S., Piao, Thornton, a.P., 2013. Carbon and Other Biogeochemical Cycles. In: Intergovernmental Panel on Climate, C. (Ed.), Carbon and other biogeochemical cycles. Climate change 2013: the physical science basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp. 465-570.
Conde, E., Cardenas, M., Ponce-Mendoza, A., Luna-Guido, M., Cruz-Mondragón, C., Dendooven, L., 2005. The impacts of inorganic nitrogen application on mineralization of 14C-labelled maize and glucose, and on priming effect in saline alkaline soil. *Soil Biology and Biochemistry* 37, 681-691.
Cordell, D., White, S., 2014. Life's bottleneck: sustaining the world's phosphorus for a food secure future. Annual Review of Environment and Resources 39, 161-188.
Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017. How Plant Root Exudates Shape the Nitrogen Cycle. Trends in Plant Science 22, 661-673.
Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988-995.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. Ecology 88, 2105-2113.
Crème, A., Rumpel, C., Malone, S.L., Saby, N.P.A., Vaudour, E., Decau, M.-L., Chabbi, A., 2020. Monitoring Grassland Management Effects on Soil Organic Carbon—A Matter of Scale. Agronomy 10, 2016.
Curtin, D., Beare, M.H., Scott, C.L., Hernandez-Ramirez, G., Meenken, E.D., 2014. Mineralization of Soil Carbon and Nitrogen Following Physical Disturbance: A Laboratory Assessment. *Soil Science Society of America Journal* 78, 925-935.
Dai, Z., Su, W., Chen, H., Barberán, A., Zhao, H., Yu, M., Yu, L., Brookes, P.C., Schadt, C.W., Chang, S.X., Xu, J., 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. Glob Chang Biol 24, 3452-3461.
Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., 2015. Complete nitrification by Nitrospira bacteria. Nature 528, 504-509.
Daims, H., Lücker, S., Wagner, M., 2016. A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria. Trends in Microbiology 24, 699-712.
de Graaff, M.-A., Jastrow, J.D., Gillette, S., Johns, A., Wullschleger, S.D., 2014. Differential priming of soil carbon driven by soil depth and root impacts on carbon availability. *Soil Biology and Biochemistry* 69, 147-156.

Derrien, D., Marol, C., Balesdent, J., 2004. The dynamics of neutral sugars in the rhizosphere of wheat. An approach by13C pulse-labelling and GC/C/IRMS. *Plant and Soil* 267, 243-253.
Dijkstra, F., Carrillo, Y., Pendall, E., Morgan, J., 2013. Rhizosphere priming: a nutrient perspective. Frontiers in Microbiology 4.
Dubeux, J.C.B., Sollenberger, L.E., 2020. Chapter 4 - Nutrient cycling in grazed pastures. In: Rouquette, M., Aiken, G.E. (Eds.), Management Strategies for Sustainable Cattle Production in Southern Pastures. Academic Press, pp. 59-75.
Duffy, P., Hanley, E., Hyde, B., O'Brien, P., Ponzi, J., Cotter, E., Black, K., 2014. Ireland National Inventory Report 2014, Greenhouse Gas Emissions 1990-2012. Reported to the United Nations Framework Convention on Climate Change. Environmental Protection Agency, Johnstown Castle Estate, Co. Wexford, Ireland.
Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18, 1781-1796.
Engels, T., Kuhlmann, H., 1993. Effect of the rate of N fertilizer on apparent net mineralization of N during and after cultivation of cereal and sugar beet crops. Zeitschrift für Pflanzenernaehrung und Bodenkunde 156, 149-154.
Erisman, J.W., A. Sutton, M., Galloway, J., Klimont, Z., Winiwarter, W., 2008. How a century of ammonia synthesis changed the world.
Essington, M.E., 2015. Soil and water chemistry: an integrative approach. CRC press.
Fan, Y., Zhong, X., Lin, T.-C., Lyu, M., Wang, M., Hu, W., Yang, Z., Chen, G., Guo, J., Yang, Y., 2020. Effects of nitrogen addition on DOM-induced soil priming effects in a subtropical plantation forest and a natural forest. *Biology and Fertility of Soils* 56, 205-216.
Fang, Y., Singh, B.P., Farrell, M., Van Zwieten, L., Armstrong, R., Chen, C., Bahadori, M., Tavakkoli, E., 2020. Balanced nutrient stoichiometry of organic amendments enhances carbon priming in a poorly structured sodic subsoil. *Soil Biology and Biochemistry* 145, 107800.
Farrell, M., Prendergast-Miller, M., Jones, D.L., Hill, P.W., Condron, L.M., 2014. Soil microbial organic nitrogen uptake is regulated by carbon availability. *Soil Biology and Biochemistry* 77, 261-267.
Feng, X., Simpson, A.J., Schlesinger, W.H., Simpson, M.J., 2010. Altered microbial community structure and organic matter composition under elevated CO_2 and N fertilization in the duke forest. Global Change Biology 16, 2104-2116.
Fiorentino, N., Sánchez-Monedero, M.A., Lehmann, J., Enders, A., Fagnano, M., Cayuela, M.L., 2019. Interactive priming of soil N transformations from combining biochar and urea inputs: A 15N isotope tracer study. Soil Biology and Biochemistry 131, 166-175.
Firestone, M., 1982. Biological denitrification. Nitrogen in agricultural soils 22, 289-326.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. Biochem. 35, 837-843.
Fornara, D.A., Steinbeiss, S., McNamara, N.P., Gleixner, G., Oakley, S., POULTON, P.R., MacDonald, A.J., Bardgett, R.D., 2011. Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland. Global Change Biology 17, 1925-1934.
Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B.A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P.H., Wickings, K., 2014. Chronic nitrogen additions

suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121, 305-316.
Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621-1625.
Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. *Soil Biology and Biochemistry* 43, 1705-1713.
Glaser, B., Birk, J.J., 2012. State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (terra preta de Índio). Geochimica et Cosmochimica acta 82, 39-51.
Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. Frontiers in Microbiology 8.
Goerges, T., Dittert, K., 1998. Improved diffusion technique for 15N:14N analysis of ammonium and nitrate from aqueous samples by stable isotope spectrometry. Communications in Soil Science and Plant Analysis 29, 361-368.
Griffiths, M., Delory, B.M., Jawahir, V., Wong, K.M., Cody Bagnall, G., Dowd, T.G., Nusinow, D.A., Miller, A.J., Topp, C.N., 2021. Optimization of root traits to provide enhanced ecosystem services in agricultural systems: a focus on cover crops. Plant, Cell & Environment n/a.
Guyonnet, J.P., Cantarel, A.A.M., Simon, L., Haichar, F.e.Z., 2018. Root exudation rate as functional trait involved in plant nutrient-use strategy classification. Ecology and evolution 8, 8573-8581.
Haichar, F., Marol, C., Berge, O., Rangel-Castro, J., Prosser, J., Balesdent, J., Heulin, T., Achouak, W., 2008. Plant host habitat and root exudates shape soil bacterial community structure. The ISME Journal 2: 1221-1230. https. doi. org/10.1038/ismej.
Hamilton, E.W., Frank, D.A., Hinchey, P.M., Murray, T.R., 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology and Biochemistry* 40, 2865-2873.
Hammelehle, A., Oberson, A., Lüscher, A., Mäder, P., Mayer, J., 2018. Above- and belowground nitrogen distribution of a red clover-perennial ryegrass sward along a soil nutrient availability gradient established by organic and conventional cropping systems. *Plant and Soil* 425, 507-525.
Hartmann, A., Schmid, M., Van Tuinen, D., Berg, G., 2009. Plant-driven selection of microbes. *Plant and Soil* 321, 235-257.
Hayatsu, M., Tago, K., Saito, M., 2008. Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition* 54, 33-45.
Henneron, L., Cros, C., Picon-Cochard, C., Rahimian, V., Fontaine, S., 2020a. Plant economic strategies of grassland species control soil carbon dynamics through rhizodeposition. *Journal of Ecology* 108, 528-545.
Henneron, L., Kardol, P., Wardle, D.A., Cros, C., Fontaine, S., 2020b. Rhizosphere control of soil nitrogen cycling: a key component of plant economic strategies. *New Phytologist* 228, 1269-1282.
Henry, H.A., Cleland, E.E., Field, C.B., Vitousek, P.M., 2005. Interactive effects of elevated CO_2, N deposition and climate change on plant litter quality in a California annual grassland. *Oecologia* 142, 465-473.

Herold, M.B., Giles, M.E., Alexander, C.J., Baggs, E.M., Daniell, T.J., 2018. Variable response of nirK and nirS containing denitrifier communities to long-term pH manipulation and cultivation. *FEMS Microbiology Letters* 365.
Hessen, D.O., Agren, G.I., Anderson, T.R., Elser, J.J., De Ruiter, P.C., 2004. Carbon, sequestration in ecosystems: The role of stoichiometry. *Ecology* 85, 1179-1192.
Hinsinger, P., 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* 237, 173-195.
Hinojosa, M.B., García-Ruiz, R., Carreira, J.A., 2010. Utilizing microbial community structure and function to evaluate the health of heavy metal polluted soils. Soil heavy metals. Springer, pp. 185-224.
Hochstein, L.I., Tomlinson, G.A., 1988. The enzymes associated with denitrification. *Annual Reviews in Microbiology* 42, 231-261.
Hoffland, E., Kuyper, T.W., Comans, R.N.J., Creamer, R.E., 2020. Eco-functionality of organic matter in soils. *Plant and Soil* 455, 1-22.
Hoffman, B.M., Lukoyanov, D., Yang, Z.-Y., Dean, D.R., Seefeldt, L.C., 2014. Mechanism of Nitrogen Fixation by Nitrogenase: The Next Stage. *Chemical Reviews* 114, 4041-4062.
Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of The Total Environment* 610-611, 316-332.
Hu, J., Zhao, Y., Yao, X., Wang, J., Zheng, P., Xi, C., Hu, B., 2021. Dominance of comammox Nitrospira in soil nitrification. *Science of The Total Environment* 780, 146558.
Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., Schlaeppi, K., Erb, M., 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* 9, 2738.
Iannetta, P.P.M., Young, M., Bachinger, J., Bergkvist, G., Doltra, J., Lopez-Bellido, R.J., Monti, M., Pappa, V.A., Reckling, M., Topp, C.F.E., Walker, R.L., Rees, R.M., Watson, C.A., James, E.K., Squire, G.R., Begg, G.S., 2016. A Comparative Nitrogen Balance and Productivity Analysis of Legume and Non-legume Supported Cropping Systems: The Potential Role of Biological Nitrogen Fixation. *Frontiers in Plant Science* 7.
Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D.A., Nilsson, M.C., 2011. Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. *New Phytologist* 192, 507-517.
Janssens, I., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., 2010a. Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci*. 3, 315-322.
Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Piao, S.L., Schulze, E.D., Tang, J., Law, B.E., 2010b. Reduction of forest soil respiration in response to nitrogen deposition. *Nat. Geosci*. 3, 315-322.
Janzen, H., 2005. Soil carbon: A measure of ecosystem response in a changing world? *Canadian Journal of Soil Science* 85, 467-480.
Janzen, H.H., 2006. The soil carbon dilemma: Shall we hoard it or use it? *Soil Biology and Biochemistry* 38, 419-424.

Johnson, D., Leake, J.R., Read, D.J., 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. *Plant and Soil* 271, 157-164.
Kemmitt, S.J., Lanyon, C.V., Waite, I.S., Wen, Q., Addiscott, T.M., Bird, N.R.A., O'Donnell, A.G., Brookes, P.C., 2008. Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass—a new perspective. *Soil Biology and Biochemistry* 40, 61-73.
Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C., Batten, G., 2011. Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. *Geoderma* 163, 197-208.
Krapp, A., 2015. Plant nitrogen assimilation and its regulation: a complex puzzle with missing pieces. *Current Opinion in Plant Biology* 25, 115-122.
Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363-1371.
Kuzyakov, Y., Bol, R., 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biology and Biochemistry* 38, 747-758.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* 32, 1485-1498.
Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist* 198, 656-669.
Lal, R., 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. Science 304, 1623-1627.
Lambers, H., Mougel, C., Jaillard, B., Hinsinger, P., 2009. Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. *Plant and Soil* 321, 83-115.
Li, L.-J., Zhu-Barker, X., Ye, R., Doane, T.A., Horwath, W.R., 2018. Soil microbial biomass size and soil carbon influence the priming effect from carbon inputs depending on nitrogen availability. *Soil Biology and Biochemistry* 119, 41-49.
Li, W., Jin, C., Guan, D., Wang, Q., Wang, A., Yuan, F., Wu, J., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. *Soil Biology and Biochemistry* 82, 112-118.
Lin, Y., Ye, G., Ding, W., Hu, H.-W., Zheng, Y., Fan, J., Wan, S., Duan, C., He, J.-Z., 2020. Niche differentiation of comammox Nitrospira and canonical ammonia oxidizers in soil aggregate fractions following 27-year fertilizations. *Agriculture, Ecosystems & Environment* 304, 107147.
Löhnis, F., 1926. Nitrogen availability of green manures. *Soil Science* 22, 253-290.
Lonardo, D.P.D., Boer, W.d., Gunnewiek, P.J.A.K., Hannula, S.E., Wal, A.v.d., 2017. Priming of soil organic matter: Chemical structure of added compounds is more important than the energy content. *Soil Biol. Biochem*. 108, 41-54.
Mason-Jones, K., Schmücker, N., Kuzyakov, Y., 2018. Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming. *Soil Biology and Biochemistry* 124, 38-46.
McDonald, N.T., Wall, D.P., Mellander, P.E., Buckley, C., Shore, M., Shortle, G., Leach, S., Burgess, E., O'Connell, T., Jordan, P., 2019. Field scale phosphorus balances and legacy soil pressures in mixed-land use catchments. *Agriculture, Ecosystems & Environment* 274, 14-23.

McDonald, N.T., Watson, C.J., Laughlin, R.J., Lalor, S.T.J., Grant, J., Wall, D.P., 2014. Soil tests for predicting nitrogen supply for grassland under controlled environmental conditions. *The Journal of Agricultural Science* 152, 82-95.
Meharg, A.A., 1994. A critical review of labelling techniques used to quantify rhizosphere carbon-flow. Plant and Soil 166, 55-62.
Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., 2017. Soil carbon 4 per mille. *Geoderma* 292, 59-86.
Montanarella, L., Panagos, P., 2021. The relevance of sustainable soil management within the European Green Deal. *Land Use Policy* 100, 104950.
Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. *Proceedings of the National Academy of Sciences* 104, 13268.
Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. *Ecol. Monogr.* 76, 151-174.
Murphy, B.W., 2015. Impact of soil organic matter on soil properties—a review with emphasis on Australian soils. *Soil Research* 53, 605-635.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2015. Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry* 81, 236-243.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. *Plant and Soil* 417, 499-510.
Muruganandam, S., Israel, D.W., Robarge, W.P., 2009. Activities of Nitrogen-Mineralization Enzymes Associated with Soil Aggregate Size Fractions of Three Tillage Systems. *Soil Science Society of America Journal* 73, 751-759.
Murphy, D.V., Bhogal, A., Shepherd, M., Goulding, K.W.T., Jarvis, S.C., Barraclough, D., Gaunt, J.L., 1999. Comparison of 15N labelling methods to measure gross nitrogen mineralisation. Soil Biology and Biochemistry 31, 2015-2024.
Naeth, M., Chanasyk, D., Rothwell, R., Bailey, A., 1991. Grazing impacts on soil water in mixed prairie and fescue grassland ecosystems of Alberta. Canadian Journal of Soil Science 71, 313-325.
Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. Applied and Environmental Soil Science 2019, 5794869.
Nguyen, C., 2003. Rhizodeposition of organic C by plants: mechanisms and controls. Agronomie 23, 375-396.
Niu, J., Rang, Z., Zhang, C., Chen, W., Tian, F., Yin, H., Dai, L., 2016. The succession pattern of soil microbial communities and its relationship with tobacco bacterial wilt. BMC microbiology 16, 1-10.
Nkongolo, K.K., Narendrula-Kotha, R., 2020. Advances in monitoring soil microbial community dynamic and function. Journal of Applied Genetics 61, 249-263.
Nottingham, A.T., Hicks, L.C., Ccahuana, A.J.Q., Salinas, N., Bååth, E., Meir, P., 2018. Nutrient limitations to bacterial and fungal growth during cellulose decomposition in tropical forest soils. *Biology and Fertility of Soils* 54, 219-228.
Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. European Journal of Soil Science 54, 741-750.
Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytologist 173, 600-610.

Paterson, E., Midwood, A.J., Millard, P., 2009. Through the eye of the needle: A review of isotope approaches to quantify microbial processes mediating soil carbon balance. *New Phytologist* 184, 19-33.
Paterson, E., Sim, A., 2013. Soil-specific response functions of organic matter mineralization to the availability of labile carbon. *Global Change Biology* 19, 1562-1571.
Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. European Journal of Soil Science 54, 741-750.
Pianka, E.R., 1970. On r- and K-Selection. The American Naturalist 104, 592-597.
Piccolo, A., 2002. The supramolecular structure of humic substances: A novel understanding of humus chemistry and implications in soil science. *Advances in Agronomy*. Academic Press, pp. 57-134.
Pjevac, P., Schauberger, C., Poghosyan, L., Herbold, C.W., van Kessel, M.A., Daebeler, A., Steinberger, M., Jetten, M.S., Lücker, S., Wagner, M., 2017. AmoA-targeted polymerase chain reaction primers for the specific detection and quantification of comammox Nitrospira in the environment. *Frontiers in Microbiology* 8, 1508.
Poore, J., Nemecek, T., 2018. Reducing food's environmental impacts through producers and consumers. *Science* 360, 987-992.
Prendergast-Miller, M.T., de Menezes, A.B., Farrell, M., Macdonald, L.M., Richardson, A.E., Bissett, A., Toscas, P., Baker, G., Wark, T., Thrall, P.H., 2015. Soil nitrogen pools and turnover in native woodland and managed pasture soils. *Soil Biology and Biochemistry* 85, 63-71.
Rafique, R., Hennessy, D., Kiely, G., 2011. Nitrous oxide emission from grazed grassland under different management systems. Ecosystems 14, 563-582.
Randall, K., Brennan, F., Clipson, N., Creamer, R., Griffiths, B., Storey, S., Doyle, E., 2019. Soil bacterial community structure and functional responses across a long-term mineral phosphorus (Pi) fertilisation gradient differ in grazed and cut grasslands. *Applied Soil Ecology* 138, 134-143.
Ratjen, A.M., Kage, H., 2018. Predicting the site specific soil N supply under winter wheat in Germany. *Nutrient Cycling in Agroecosystems* 110, 71-81.
Ravikumar, S., Kathiresan, K., Alikhan, S.L., Williams, G.P., Gracelin, N.A.A., 2007. Growth of Avicennia marina and Ceriops decandra seedlings inoculated with halophilic azotobacters. *Journal of Environmental Biology* 28, 601.
Rees, R., Ball, B., Campbell, C., Watson, C., 2001. *Organic Matter the Sustenance of Soil. Sustainable Management of Soil Organic Matter*. CABI Publ., Wallingford, UK.
Regalado, J., Lundberg, D.S., Deusch, O., Kersten, S., Karasov, T., Poersch, K., Shirsekar, G., Weigel, D., 2020. Combining whole-genome shotgun sequencing and rRNA gene amplicon analyses to improve detection of microbe–microbe interaction networks in plant leaves. The ISME journal 14, 2116-2130.
Richardson, A.E., Kirkby, C.A., Banerjee, S., Kirkegaard, J.A., 2014. The inorganic nutrient cost of building soil carbon. *Carbon Management* 5, 265-268.
Riggs, C.E., Hobbie, S.E., Bach, E.M., Hofmockel, K.S., Kazanski, C.E., 2015. Nitrogen addition changes grassland soil organic matter decomposition. *Biogeochemistry* 125, 203-219.
Robertson, G.P., Groffman, P.M., 2015. Chapter 14 - Nitrogen Transformations. In: Paul, E.A. (Ed.), Soil Microbiology, Ecology and Biochemistry (Fourth Edition). Academic Press, Boston, pp. 421-446.

Robertson, G.P., Vitousek, P.M., 2009. Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. *Annual Review of Environment and Resources* 34, 97-125.
Robertson, L.A., Kuenen, J.G., 1990. Combined heterotrophic nitrification and aerobic denitrification in Thiosphaera pantotropha and other bacteria. *Antonie van Leeuwenhoek* 57, 139-152.
Ros, G.H., Hanegraaf, M.C., Hoffland, E., van Riemsdijk, W.H., 2011. Predicting soil N mineralization: Relevance of organic matter fractions and soil properties. *Soil Biology and Biochemistry* 43, 1714-1722.
Rumpel, C., Crème, A., Ngo, P.T., Velásquez, G., Mora, M.L., Chabbi, A., 2015. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. *Journal of Soil Science and Plant Nutrition* 15.
Rütting, T., Aronsson, H., Delin, S., 2018. Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems* 110, 1-5.
Sarker, J.R., Singh, B.P., Dougherty, W.J., Fang, Y., Badgery, W., Hoyle, F.C., Dalal, R.C., Cowie, A.L., 2018. Impact of agricultural management practices on the nutrient supply potential of soil organic matter under long-term farming systems. *Soil and Tillage Research* 175, 71-81.
Savci, S., 2012. Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia* 1, 287-292.
Schlesinger, W.H., 2009. On the fate of anthropogenic nitrogen. *Proceedings of the National Academy of Sciences of the United States of America* 106, 203-208.
Schröder, J.J., Schulte, R.P.O., Creamer, R.E., Delgado, A., van Leeuwen, J., Lehtinen, T., Rutgers, M., Spiegel, H., Staes, J., Tóth, G., Wall, D.P., 2016. The elusive role of soil quality in nutrient cycling: a review. *Soil Use and Management* 32, 476-486.
Schöler, A., Jacquiod, S., Vestergaard, G., Schulz, S., Schloter, M., 2017. Analysis of soil microbial communities based on amplicon sequencing of marker genes. Springer, pp. 485-489.
Scurlock, J.M., Johnson, K., Olson, R.J., 2002. Estimating net primary productivity from grassland biomass dynamics measurements. *Global Change Biology* 8, 736-753.
Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2, 1-14.
Signor, D., Cerri, C.E.P., 2013. Nitrous oxide emissions in agricultural soils: a review. *Pesquisa Agropecuária Tropical* 43, 322-338.
Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil* 241, 155-176.
Smith, C., Hill, A.K., Torrente-Murciano, L., 2020. Current and future role of Haber–Bosch ammonia in a carbon-free energy landscape. *Energy & Environmental Science* 13, 331-344.
Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma* 74, 65-105.
Soumare, A., Diedhiou, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., Kouisni, L., 2020. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. *Plants* 9, 1011.
Staley, J.T., Konopka, A., 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annual review of microbiology 39, 321-346.
Stanford, G., Smith, S.J., 1972. Nitrogen Mineralization Potentials of Soils. Soil Science Society of America Journal 36, 465-472.

Stein, L.Y., Klotz, M.G., 2016. The nitrogen cycle. *Current Biology* 26, R94-R98.
Stüeken, E.E., Kipp, M.A., Koehler, M.C., Buick, R., 2016. The evolution of Earth's biogeochemical nitrogen cycle. *Earth-Science Reviews* 160, 220-239.
Sullivan, B., Hart, S., 2013. Evaluation of mechanisms controlling the priming of soil carbon along a substrate age gradient. *Soil Biology and Biochemistry* 58, 293–301.
Taylor, L.L., Leake, J.R., Quirk, J., Hardy, K., Banwart, S.A., Beerling, D.J., 2009. Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology* 7, 171-191.
Thilakarathna, S.K., Hernandez-Ramirez, G., 2021. How does management legacy, nitrogen addition, and nitrification inhibition affect soil organic matter priming and nitrous oxide production? *Journal of Environmental Quality* 50, 78-93.
Thornton, B., Paterson, E., Midwood, A.J., Sim, A., Pratt, S.M., 2004. Contribution of current carbon assimilation in supplying root exudates of Lolium perenne measured using steady-state 13C labelling. Physiologia Plantarum 120, 434-441.
Tunney, H., Sikora, F.J., Kissel, D., Wolf, A., Sonon, L., Goulding, K., 2010. A comparison of lime requirements by five methods on grassland mineral soils in Ireland. *Soil Use and Management* 26, 126-132.
Van Kessel, M.A., Speth, D.R., Albertsen, M., Nielsen, P.H., den Camp, H.J.O., Kartal, B., Jetten, M.S., Lücker, S., 2015. Complete nitrification by a single microorganism. *Nature* 528, 555-559.
Veerman, C., Pinto Correia, T., Bastioli, C., Biro, B., Bouma, J., Cienciala, E., Emmett, B., Frison, E., Grand, A., Hristov, L., Kriaučiūnienė, Z., Pogrzeba, M., Soussana, J., Vela, C., Wittkowski, R., 2020. *Caring for soil is caring for life : ensure 75% of soils are healthy by 2030 for healthy food, people, nature and climate: interim report of the mission board for soil health and food*. Publications Office of the EU.
von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. *Soil Biology and Biochemistry* 39, 2183-2207.
Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Marcy, G., Chris, L., 2004. Nitrogen Deposition Modifies Soil Carbon Storage through Changes in Microbial Enzymatic Activity. *Ecological Applications* 14, 1172-1177.
Wall, D., Plunkett, M., 2021. *Major and micro nutrient advice for productive agricultural crops*. Teagasc, Johnstown Castle, Environmental Research Centre, Wexford.
Wang, X., Tang, C., 2017. The role of rhizosphere pH in regulating the rhizosphere priming effect and implications for the availability of soil-derived nitrogen to plants. Annals of Botany 121, 143-151.
Wang, X., Tang, C., Baldock, J., Butterly, C., Gazey, C., 2016. Long-term effect of lime application on the chemical composition of soil organic carbon in acid soils varying in texture and liming history. *Biology and Fertility of Soils* 52, 295-306.
Waring, S.A., Bremner, J.M., 1964. Ammonium Production in Soil under Waterlogged Conditions as an Index of Nitrogen Availability. Nature 201, 951-952.
Welte, C.U., 2018. Revival of archaeal methane microbiology. Msystems 3, e00181-00117.
Xia, F., Wang, J.-G., Zhu, T., Zou, B., Rhee, S.-K., Quan, Z.-X., 2018. Ubiquity and diversity of complete ammonia oxidizers (comammox). *Appl. Environ. Microbiol*. 84, e01390-01318.

Zeglin, L.H., Stursova, M., Sinsabaugh, R.L., Collins, S.L., 2007. Microbial responses to nitrogen addition in three contrasting grassland ecosystems. *Oecologia* 154, 349-359.
Zhang, X., Han, X., Yu, W., Wang, P., Cheng, W., 2017. Priming effects on labile and stable soil organic carbon decomposition: Pulse dynamics over two years. *PLOS ONE* 12, e0184978-e0184978.
Zhou, G., Zhou, X., He, Y., Shao, J., Hu, Z., Liu, R., Zhou, H., Hosseinibai, S., 2017. Grazing intensity significantly affects belowground carbon and nitrogen cycling in grassland ecosystems: A meta-analysis. *Global Change Biology* 23, 1167-1179.
Zhou, Z., Takaya, N., Sakairi, M.A.C., Shoun, H., 2001. Oxygen requirement for denitrification by the fungus Fusarium oxysporum. *Archives of Microbiology* 175, 19-25.
Zhu-Barker, X., Steenwerth, K.L., 2018. Chapter Six - Nitrous Oxide Production From Soils in the Future: Processes, Controls, and Responses to Climate Change. In: Horwath, W.R., Kuzyakov, Y. (Eds.), *Developments in Soil Science*. Elsevier, pp. 131-183.
Zhu, B., Cheng, W., 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. *Global Change Biology* 17, 2172-2183.
Zhu, Z., tida, G., Luo, Y., Liu, S., Xu, X., Tong, C.-l., Shibistova, O., Guggenberger, G., 2018. Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil. *Soil Biology and Biochemistry*, 121, 67-76.
Žurovec, O., Wall, D.P., Brennan, F.P., Krol, D.J., Forrestal, P.J., Richards, K.G., 2021. Increasing soil pH reduces fertiliser derived N2O emissions in intensively managed temperate grassland. *Agriculture, Ecosystems & Environment* 311, 107319.

Chapter 2

The impact of lime and phosphorus amendment on potential nitrogen mineralisation and enzymatic activity in grassland soils

2.1 Introduction

Nitrogen (N) is a limiting nutrient in most terrestrial ecosystems, particularly in systems used for agricultural production, and is one of the key nutrients required for plant growth (Rütting *et al.*, 2018). Agricultural systems often overcome N limitation by applying synthetic N fertiliser (Bouwman *et al.*, 2013; Bodirsky *et al.*, 2014; Schröder *et al.*, 2016). However, the over application and reliance on chemical fertiliser is considered environmentally detrimental – as N is lost to waterways and as greenhouse gas nitrous oxide to the atmosphere – and economically unsustainable, particularly for the farmer as the cost of N fertiliser continues to rise (Guignard *et al.*, 2017; Rütting *et al.*, 2018). Soil organic matter (SOM) is the main store of N in soils – 96-98%, $0.095x10^{18}$g of total N globally (Booth *et al.*, 2005) – but this store of organic N is not available directly to plants and needs to be transformed. The conversion of organic N in SOM to inorganic (plant-available) N, known as N mineralisation, is dependent largely on soil microorganisms through their associated enzyme production. N supply through net mineralisation over a growing season in Irish grasslands ranges from 56-220kg N ha^{-1} yr^{-1} (McDonald *et al.*, 2014b). Nutrient management strategies such as the application of fertilisers to manage phosphorus (P) and lime to manage soil pH have the potential to change soil N availability, and also impact microbial mediated N cycling processes including N mineralisation in managed grassland agricultural systems. However, to what extent soil nutrient management practices change soil N availability is difficult to measure and predict. A variety of biological and chemical indices to determine the effect of these nutrient management practices on a soil's capability to supply its own plant available N through microbial mediated SOM turnover would be useful in guiding soil N supply predictions and nuancing fertilisation advice.

Microbial activities are sensitive to changes in nutrient availability in soils (Fujita et al., 2019). Zhang et al. (2019) found that soils with increasing nutrient content – particularly with manure addition but also with mineral fertiliser addition – increased enzymatic activity. Sinsabaugh and Follstad Shah (2012) discuss that the relative production of C, N and P mobilising enzymes is related to which element is the limiting resource in the niche that the microbes are occupying. Depending on nutrient availability, microorganisms change their allocation of C, N, and P acquiring enzymes (Sinsabaugh, 1994; Sinsabaugh and Follstad Shah, 2012; Zhang et al., 2019). Enzymes in soil play an important role in SOM decomposition and enzymatic activity can be an indicator of soil health (Sinsabaugh et al., 2008; Schloter et al., 2018; Zhang et al., 2019). The relationship between soil nutrient management practices, soil microbial community structure and soil enzymatic activity is poorly understood.

However, it has been shown that use of chemical fertilisers causes changes in nutrient stoichiometry in soils that can in turn lead to both increases and decreases in soil enzymatic activity (Cleveland and Liptzin, 2007). Changes to enzymatic activity with fertiliser addition could suggest efforts to increase production of limited nutrient(s) to increase their availability while utilising nutrient(s) that are in excess. Therefore, enzyme activities may be useful indicators of the impacts of diverse management practices on microbial functions mediating nutrient mobilisation from SOM.

Application of lime is a commonly used agricultural practice to manage and increase the productivity of acidic soils (Goulding, 2016). Liming soil improves the availability of N, P, K, and many other nutrients (Johnson *et al.*, 2005); it can also contribute to reduced N_2O emissions from agricultural soils (Žurovec *et al.*, 2021). Soil microbial activity, microbial community composition, microbial SOM turnover (mineralisation activities), and microbial biomass size are affected by soil pH (Johnson *et al.*, 2005; Bertrand *et al.*, 2007; Paradelo *et al.*, 2015; Herold *et al.*, 2018). Lime application can also cause a physical response in soils through altering the physio-chemical reactions between soil particles affecting flocculation, formation, and stabilisation of macro and microaggregates (Six *et al.*, 2004; Bronick and Lal, 2005; Briedis *et al.*, 2012b). These changes to soil aggregates could potentially expose previously protected SOM particles to mineralisation (Caires *et al.*, 2006; Briedis *et al.*, 2012a). However, the impacts of liming (and altered soil pH) on the mechanisms of microbially mediated N mobilisation from SOM remains unclear (Bertrand et al., 2007, Briedis et al., 2012).

Information about nutrient balance and availability in soil is important to inform the application of the correct amounts of nutrient fertiliser and lime (Mooshammer *et al.*, 2012). P is an essential nutrient for plant productivity and is therefore also one of the main constituents of chemical fertilisers (Guignard et al., 2017). P deficiency not only affects plants but can also limit microbial growth and affect microbial activity (Zhu *et al.*, 2018). As such, it is important to understand the effect of P nutrient addition to predict microbial nutrient cycling in soils. P addition can also be made through organic fertilisers such as slurry or farmyard manure. The application of organic fertilisers is a common agricultural practice especially in livestock and mixed farm systems (Svanbäck *et al.*, 2019). As is the nature of organic fertilisers, they add significant amounts of organic compounds to the soil which have direct and indirect effects on plant, microbial and enzymatic activity (Liu *et al.*, 2017), and can improve SOM quality. Zhang *et al.* (2018) found that adding cattle manure increased both SOM and total N contents, when compared to control plots that did not receive any organic fertiliser. Sun *et al.* (2015) highlighted that manure applications contribute to soil microbial community stability and diversity. Furthermore, enzyme activity in soil changes rapidly in response to C addition from organic fertilisers (Zhang et al., 2019). Chemical fertiliser application can also cause rapid changes in soil enzymatic

activity. Organic nutrient forms require microbial enzyme production for mineralisation into available forms (influencing measured enzyme activities 'directly'), whereas mineral forms do not, but do influence nutrient stoichiometry – which can result in differential enzyme production. However, the impact and differences in the effect of the addition of chemical P vs organic P fertiliser on nutrient balances and how this affects microbial community structure, enzymatic activity, and potential N mineralisation in soils is also poorly understood.

Grassland nutrient management practices affect soil nutrient balances, nutrient availability, and soil-microbe interactions. However, the extent to which nutrient management practices affect a soil's ability to supply N through microbially mediated SOM N mineralisation is uncertain. This study focused on assessing the effect of liming and P application (via chemical or organic fertiliser) on N mineralisation, and microbial community structure, with enzymatic activity as an indicator for microbial nutrient cycling activity. It was hypothesised that: 1) the application of lime and P application would increase N mineralisation of SOM, and enzymatic activity; 2) chemical P application would decrease the activity of enzymes, and N mineralisation of SOM; 3) organic P application would increase the activity of enzymes, and N mineralisation of SOM; 4) the microbial community structure would be influenced by nutrient management conditions.

2.2 Methods

2.2.1 Experimental details and design

Soils from four grassland sites were selected with varying levels of N mineralisation potential and organic matter (OM) content: two sites in Moorepark, Co. Cork, one in Grange, Co. Meath, and one in Johnstown, Co. Wexford (Table 2.1) (McDonald et al. 2014). Pots were established in a soil microcosm facility (area enclosed by netting of 29 x 11 x 2 meters) at the experimental research station at Teagasc, Johnstown Castle, Co. Wexford, Ireland (52° 17' N; 6° 30' W) in 2015. Pots were black 16 litres (30cm diameter x 28cm height). They were packed with bulk soil to a bulk density of ~1g cm^3. The pots were then randomly distributed in a block design. The microcosm was set up as a grassland P and lime management trial with a monoculture ryegrass sward (*Lolium perenne*). There were four treatments per soil type: a control (no lime or no P addition), a lime addition (5t ha^{-1}), lime with a chemical P addition triple superphosphate (16%P) (40kg P ha^{-1}), and lime with an organic P addition (dairy cattle slurry) (20kg P ha^{-1}). Nitrogen was applied at 100kg ha^{-1} $year^{-1}$.

2.2.2 Sampling

The sampling order of experimental units was selected using a random number generator and samples were taken by block using an 8mm pencil soil corer to a depth of 10cm. The timing of sampling was designed to match the time required for analyses, such that the storage of samples was equivalent. Therefore, random samples were taken from blocks over a 4-week period. There were two sampling days per week. Cores were taken in a "W" shape across the pot and approximately 200 g of soil was collected per pot. To reduce the potential of cross-contamination of samples, the corer and sampling utensils were disinfected with 70% ethanol between samples. Samples were stored in labelled sterile plastic bags and transferred to the lab within an hour of sampling and 5 g subsamples were taken from each pot for molecular analysis and frozen at -80°C. Microbial biomass and enzyme assays requiring fresh soil were performed on the day of sampling. A subsample of soil, approximately 100g, was sieved to 2mm dried for chemical analysis.

Table 2.1: Details of field sites where soil was collected for this study. Results for pH, SOM, and N mineralisation here are representative of the field samples before the pots were established. Adapted from McDonald et al. (2014).

Site	**Soil Classification**	**pH**	**SOM** ($g\ kg^{-1}$)	**N mineralisation** (7-day anaerobic incubation test, $NH_4\ g^{-1}$	**Location**
Moorepark 1	Typic Hapludalf	6.0	83	161.4	52°09′43.197′N, 8°13′59.209′W
Moorepark 4	Typic Hapludalf	5.5	81	151.1	52°10'15.187'N 8°14'21.408'W
Johnstown Castle	Typic Dystrudept	5.7	92	260.6	52°17′46.107'N, 6° 30′22.526'W
Grange	Typic Endoaquept	6.0	104	335.8	53°31'22.649'N, 6°40'07.597'W

2.2.3 Seven-day anaerobic incubation test for biological nitrogen mineralisation

Soil was sieved to 2mm and dried in an oven at 40°C for three days. For each collected soil sample, a 5g subsample of soil was weighed into each of two paired tubes, tube 1 (day 0 samples) and tube 2 (day 7 samples). Samples representing day zero in tube 1 were extracted with 25ml 2 M KCl and 12.5ml dH_2O. For samples representing day seven in tube 2, 12.5ml dH_2O was added to each tube to waterlog the samples. Tube 2 samples were sealed by using a screw cap. All tube 2 samples were placed in an incubator at 40°C for 7 days. Thereafter, soil was extracted using 25ml 2 M KCl, and the tube 2 day 7

samples and were analysed for inorganic N (NO_2–N, NO_3–N, and NH_4–N) using an Aquakem 600A (Aquakem 600A, 01621, Vantaa, Finland). Mineralised N was calculated as the NH_4–N on day 7 minus the NH_4–N on day zero (McDonald *et al.*, 2014a), i.e., tube 2 (day 7) - tube 1 (day 0) equals potential mineralised N.

2.2.4 Microbial biomass carbon, nitrogen, and phosphorus

Microbial biomass C, N, and P were determined using chloroform fumigation-extraction on fresh soil samples (Brookes *et al.*, 1985; Vance *et al.*, 1987). For microbial biomass C and N, 10g fresh soil was weighed in two sets, fumigated (F) and non-fumigated (NF). The F samples were placed into a desiccator with 50ml chloroform for 24 hours under vacuum. Both F and NF samples were extracted using 40ml 0.5 M K_2SO_4. Total organic C and total N were measured from the extracts on a Shimadzu, TOC-UCPH analyser with ASIV auto sampler. Microbial biomass C and N were calculated by subtracting values obtained from the NF sample from the corresponding F sample. The result was then divided by set conversion factors of 0.45 for microbial biomass C and 0.54 for microbial biomass N (Brookes et al., 1985; Vance et al., 1987).

2.2.5 Soil nutrient analyses

Total C and N were analysed using 0.2kg of a ball-milled soil dried at 40^{o}C for 48 hours and were then analysed on an elemental analyser (Leco Truespec CN elemental analyser, US). Available P was measured using the Morgan's method (McCormack, 2002; Massey, 2012), it was also done spectrophotometrically at 800mm using the phosphomolydbated method (Murphy & Riley, 1962). Soil pH was analysed using 1:2 soil: deionised water (w/v) ratio suspension on a mettler Toledo glass calomel electrode (McCormack, 2002). Soil moisture content was calculated by drying a subsample at 105^{o}C for 24 hours and subtracting the water weight. The OM content was calculated using the loss on ignition method at 500^{o}C for 16 hours.

2.2.6 Potential Enzyme Activity Assays

Enzyme activity of all samples was determined using fluorimetric microplate assays of 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) based substrates (Marx *et al.*, 2001). The enzymes assayed in this study were chosen as they are involved in C, N and P cycles. The following enzymes were selected: β -1,4-glucosidase (BGLU, C cycle), α -1, 4-glucosidase (AGLU, C cycle), β -N-acetyl-glucosaminidase (NAG, C and N cycle), phosphatase (P.1, P cycle). Each of these used MUF based substrates. Leucine aminopeptidase (AMI, N cycle) was also examined, and this is an AMC based substrate. Fresh sieved (2mm) soil was weighed (5g fresh weight) into a 50ml centrifuge tube with 50ml sterile dH_2O. The solution was placed on a Gyratory Shaker (New Brunswick Scientific)

at 150 rpm for 10 mins and centrifuged for 10 mins at 4°C at 800 rpm (Fox *et al.*, 2017). Plates were prepared to a total volume of 250µl in each well. Wells contained 200µl sample soil solution, 10µl MOPS buffer (pH 7.4 for MUF substrates and pH 7.8 for AMC substrates), and 40µl enzyme substrate. Suitable substrate concentrations for each enzyme were optimised in preliminary experiments. Control and blank wells had 50µl of buffer solution and 200µl of soil solution or sterile dH_2O, respectively. Plates were incubated at 30°C for two hours prior to taking measurements. All plates were run in a microplate reader (SYNERGY/HTX, multi-mode reader, BioTek, with Gen5 version 3.03 software) for fluorescence measurements, wavelength was set at 360nm excitation and 460nm emission. Plates were run for 90 minutes at 30°C, and there were 5-minute intervals between measurements. For standard curve calculation MUF and AMC salts were added at four different concentrations to the soil water solution (Hendriksen *et al.*, 2016; Fox *et al.*, 2017). There were two standard curves on each plate one for MUF substrates and one for AMC substrates. The activity of each enzyme was calculated as in Hendriksen *et al.* (2016).

2.2.7 DNA extraction and sequencing

DNA was extracted from 0.25g frozen soil using the Qiagen Power Soil kit (Qiagen, USA) according to the manufacturer's instructions. Extracted DNA was eluted in 25µl C6 buffer before freezing samples at -80°C until further analysis. DNA concentrations were measured using a Qubit Fluorimeter (Thermo Fisher, Ireland) with Qubit™ dsDNA BR Assay Kit (Thermo Fisher, Ireland). DNA quality was checked using a nanodrop spectrophotometer (Thermofisher, Ireland) and electrophoresis gels. Sequencing of the 16S rRNA (bacteria, archaea and eukaryote) and ITS regions was carried out for all samples. Briefly, the library preparation was a 2-step PCR process with PCR1 consisting of a 25µl reaction containing the raw DNA template (5ng μl^{-1}), 2X Kapa hifi hot start ready Taq mix (Roche, Ireland) and 0.2 µM final concentration of primers of each of the forward and reverse primers. Primers had overhang adapters attached. The primers used for 16S rRNA were 515F (Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGYCAGCMGCCGCGGTAA]) and 926R (Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [CCGYCAATTYMTTTRAGTTT]). The primers for ITS were 86F (Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGAATCATCGAATCTTTGAA]) and 4R (Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [TCCTCCGCTTATTGATATGC]) (Quince *et al.,* 2011, Op de Beeck *et al.,* 2014) The PCR1 product was cleaned using AMPure XP beads to ensure that the PCR product was free of primer dimer. The clean PCR1 product was run on a 1% agarose gel and visualised under UV light to check both the strength of bands and the absence of primer dimer. PCR2 is an index PCR with dual indices and Illumina Sequencing adapters attached to the samples using the Nextera XT Index Kit (Illumina, Ireland). PCR2 consists of 50µl reactions containing 5µl PCR1 template, each index

primers at a final concentration of 0.2 µM, and 2X Kapa hifi hot start ready mix (Roche, Ireland). PCR2 product was cleaned using the Ampure XP beads as described for PCR1. The beads were eluted in 15µl TE buffer. The concentration of PCR2 was quantified using a Qubit fluorometer. The generated 16S rRNA and ITS libraries were then pooled at equimolar concentration. Pools were quality checked on a Bioanalyser using the DNA 1000 chip and sent to the sequencing lab in Teagasc Research Centre, Moorepark, Co. Cork, Ireland for sequencing on the Illumina MiSeq platform.

2.2.8 Data Handling and Statistics

All data analyses were performed in *Rstudio* software 4.2.0 (R Core Team, 2021). All graphing was carried out in RStudio. Data was checked for normality using visual assessment and the Schapiro-Wilks test. Where data was not normally distributed, data was transformed using standard transformation techniques (logging the data, inverse, or the square root). Data was also checked for homogeneity using the Levenes Test. ANOVAs and Tukey HSD tests were used to determine significant differences between treatments and soil types, when all assumptions for the tests were met (normally distributed and homogenous). In the case where data was still not normally distributed, a Kruskal-Wallis test was used and a dunn-test for the pairwise comparison, with p-values adjusted using the Benjamin-Hocberg method. Statistical significance was determined at $p<0.05$. Principal component analysis was used to visually assess relationships between enzyme activity and soil type and treatment.

2.2.9 Sequencing data handling

FASTQ files were generated through the Illumina BaseSpace platform. Data was cleaned (negative controls and singletons removed) and analysed using the DADA2 pipeline in RStudio. ASVs tables were also analysed in R studio. Data was analysed using the *phyloseq* (McMurdie and Holmes, 2013) and *vegan* (Oksanen et al., 2020) packages in R Studio. After forming a phyloseq object containing taxa, ASVs table, tree tables and metadata, alpha diversity indexes were calculated for each treatment and significant differences determined between treatments using Kruskal-Wallis tests. Relative abundances of the dominant 20 phylum in each treatment were visualised. However, further analyses of all sequence data were log transformed, using the centred log ratio as outlined in Gloor *et al.*, (2017). This transformation was performed using the *microbiome* (Lahti *et al.,* 2017). After data transformation, a new phyloseq object was made and vectors formed from the new phyloseq object for use in the *vegan* package. Principal component analysis was used to visually represent the distribution of the 16S and ITS communities. PERMANOVA analyses were performed using the *pairwiseAdonis* package (Arbizu 2017). To determine significant effect(s) of treatment on the microbial community structure based on 16S and ITS data. Differences between treatments were analysed using the pairwise Adonis function. Redundancy analysis models were conducted to assess the significant

effect of the measured environmental variables on the microbial community structure. In order to assess specific differences in the enrichment (differential abundance) of ASVs between both soil types and treatments, down to genus level the *Deseq2* package (Love *et al.,* 2014) was used.

2.3 Results

2.3.1 Potential nitrogen mineralisation treatment and soil type

Potential N mineralisation was significantly affected by soil type ($p < 0.0001$); however, there was no significant interaction between soil type and treatment ($p=0.167$). The highest absolute potential N mineralisation was found in the GR1 soil (mean = 145.7 NH_4 g^{-1} dry soil), and the lowest absolute N mineralisation potential was in MP4 (mean = 85.9 NH_4 g^{-1} dry soil) (Figure2.1). There was a significant difference in N mineralisation potential between GR1 and MP4 ($p<0.002$), and between MP1 and MP4 ($p<0.05$) and a marginal difference between GR1 and JC2 ($p<0.05$).

There was also a significant effect of treatment on potential N mineralisation ($p< 0.002$). The highest N mineralisation potential was in the organic treatment (mean = 134.8 NH_4 g^{-1} dry soil), and the lowest in the control treatment (mean = 85.5 NH_4 g^{-1} dry soil) (Figure2.1). There was a significant difference between the organic and the control treatments ($p<0.01$). However, when examining treatment within each soil type, there was no significant effect of treatment on potential N mineralisation in GR1, MP4, and JC2. There was a significant effect of treatment in MP1 ($p<0.03$). In MP1 organic treatment had significant higher N mineralisation potential compared to the control treatment (Tukey HSD, $p<0.002$).

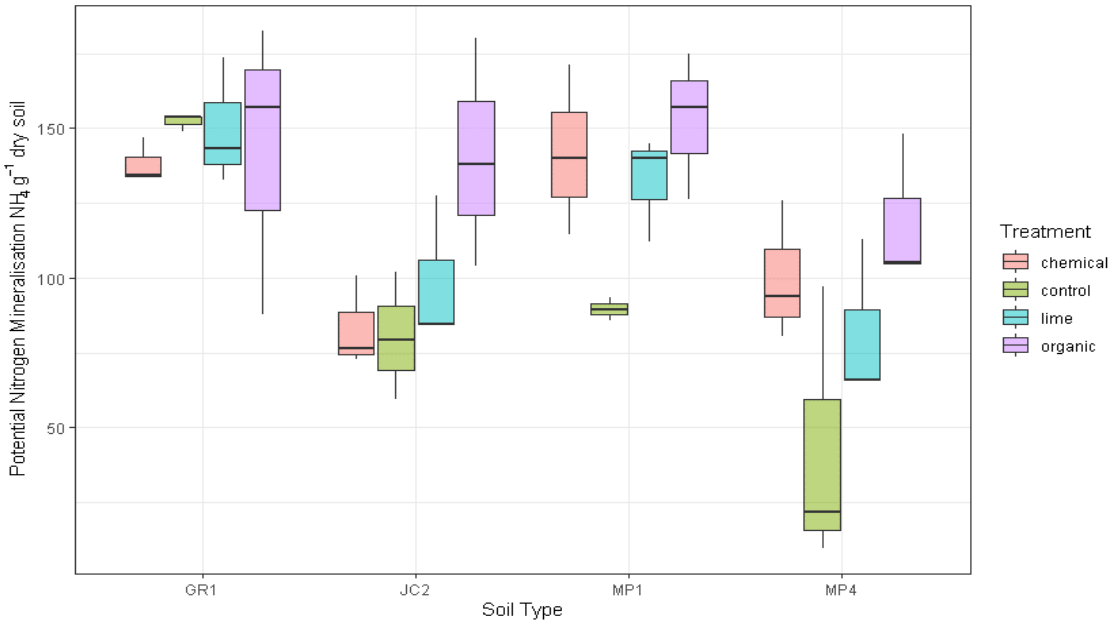


Figure2.1: Potential nitrogen mineralisation, measured using the seven-day anaerobic incubation method (NH_4 g^{-1} dry soil). Error bars represent standard error, n = 3. Soil types are represented along the x-axis and treatments are highlighted by colours described in the table legend.

2.3.2 Relationships between potential nitrogen mineralisation and soil environmental factors

There was a significant effect of OM content on potential N mineralisation (p=0.006). The GR1 soil had the highest average organic matter (OM) content measured as part of this study (Table 2.2) and was significantly higher than all the other soil types (p<0.0001). OM content was also significantly different between MP4 and JC2 (p=0.0001). Total C (p=0.017), total N (p=0.008), and total P (p=0.015) significantly affected potential N mineralisation. Available P, measured as P Morgan's (PM), also significantly affected potential N mineralisation (p=0.027) (Figure 2.2). Increased PM in the MP1 soil significantly increased potential N mineralisation (p=0.007). However, note that the MP1 soil had a higher starting PM level than the other three soils (Figure 2.2).

Table 2.2: Soil nutrients measured as part of this experiment for each soil type and treatment (n=3). Standard error is recorded in the parentheses.

	pH	OM %	C %[1]	N %	P mg Kg^{-1}	K mg Kg^{-1}	PM mg Kg^{-1}	KM mg Kg^{-1}	MBC ug g^{-1}	MBN ug g^{-1}	Mineral-isation NH_4 g^{-1} dry soil
MP 1 (organic)	6.27 (±0.08)	7.37 (±0.07)	3.42 (±0.07)	0.33 (±0.02)	1233.88 (±62.03)	2457.77 (±430.30)	21.33 (±2.03)	55.37 (±16.37)	173.66(±22.46)	28.70 (±4.01)	152.70 (±14.14)
MP 1 (control)	5.61 (±0.13)	7.37 (±0.15)	3.32 (±0.08)	0.32 (±0.01)	1118.33 (±152.59)	3046.79 (±325.18)	10.64 (±3.19)	46.53 (±5.19)	43.14 (±11.30)	17.57 (±2.07)	89.54 (±2.17)
MP 1 (chemical)	6.09 (±0.08)	7.00 (±0.15)	3.30 (±0.13)	0.33 (±0.01)	1311.15 (±26.70)	2513.26 (±838.87)	22.83 (±1.22)	37.87 (±4.32)	100.94 (±25.96)	27.32 (±4.05)	141.91 (±16.47)
MP 1 (lime)	6.11 (±0.03)	7.27 (±0.09)	3.23 (±0.06)	0.31 (±0.01)	1250.10 (±37.58)	2545.04 (±230.09)	20.43 (±1.05)	42.30 (±3.31)	99.64 (±36.74)	27.57 (±3.07)	132.47 (±10.22)
GR 1 (organic)	5.63 (±0.09)	9.90 (±0.10)	4.37 (±0.04)	0.43 (±0.01)	1117.46 (±19.83)	3808.42 (±315.77)	6.01 (±0.29)	52.67 (±9.02)	292.77 (±27.83)	54.11 (±4.17)	142.44 (±28.28)
GR 1 (control)	5.17 (±0.07)	9.83 (±0.09)	4.42 (±0.02)	0.45 (±0.00)	1198.55 (±9.02)	3601.46 (±407.17)	5.72 (±0.19)	49.33 (±2.67)	264.16 (±96.22)	36.13 (±3.71)	152.24 (±1.72)
GR 1 (chemical)	5.62 (±0.12)	9.60 (±0.10)	4.30 (±0.13)	0.43 (±0.00)	1142.92 (±40.68)	3406.59 (±456.23)	6.09 (±0.21)	44.73 (±4.77)	328.64 (±41.93)	55.48 (±7.86)	138.23 (±4.30)
GR 1 (lime)	5.58 (±0.04)	9.73 (±0.07)	4.26 (±0.07)	0.43 (±0.02)	1117.24 (±9.04)	3913.36 (±532.25)	5.28 (±0.35)	54.03 (±9.63)	255.12 (±29.97)	41.78 (±1.00)	149.96 (±12.28)
MP 4 (organic)	6.16 (±0.08)	7.93 (±0.03)	3.63 (±0.09)	0.34 (±0.01)	800.12 (±7.20)	3282.79 (±798.96)	5.52 (±0.84)	117.43 (±19.10)	181.87 (±22.38)	31.51 (±3.44)	119.18 (±14.47)
MP 4 (control)	5.13 (±0.07)	7.70 (±0.06)	3.49 (±0.03)	0.31 (±0.01)	780.27 (±29.67)	3681.88 (±664.30)	3.05 (±0.16)	53.10 (±2.70)	128.56 (±15.96)	26.18 (±1.44)	42.81 (±27.40)
MP 4 (chemical)	5.62 (±0.04)	7.37 (±0.15)	3.47 (±0.19)	0.31 (±0.01)	946.91 (±140.22)	3684.67 (±888.96)	7.72 (±3.00)	63.57 (±9.32)	144.50 (±32.78)	34.66 (±9.01)	100.03 (±13.48)
MP 4 (lime)	5.77 (±0.08)	7.33 (±0.12)	3.61 (±0.05)	0.33 (±0.00)	767.39 (±24.80)	3281.86 (±461.87)	3.69 (±0.61)	87.93 (±16.17)	151.03 (±36.62)	29.71 (±5.21)	81.64 (±15.67)
JC 2 (organic)	6.07 (±0.05)	7.30 (±0.21)	3.31 (±0.06)	0.30 (±0.01)	761.80 (±12.94)	1790.54 (±88.78)	5.10 (±0.16)	108.83 (±12.69)	174.32 (±22.53)	30.11 (±4.13)	140.68 (±21.98)

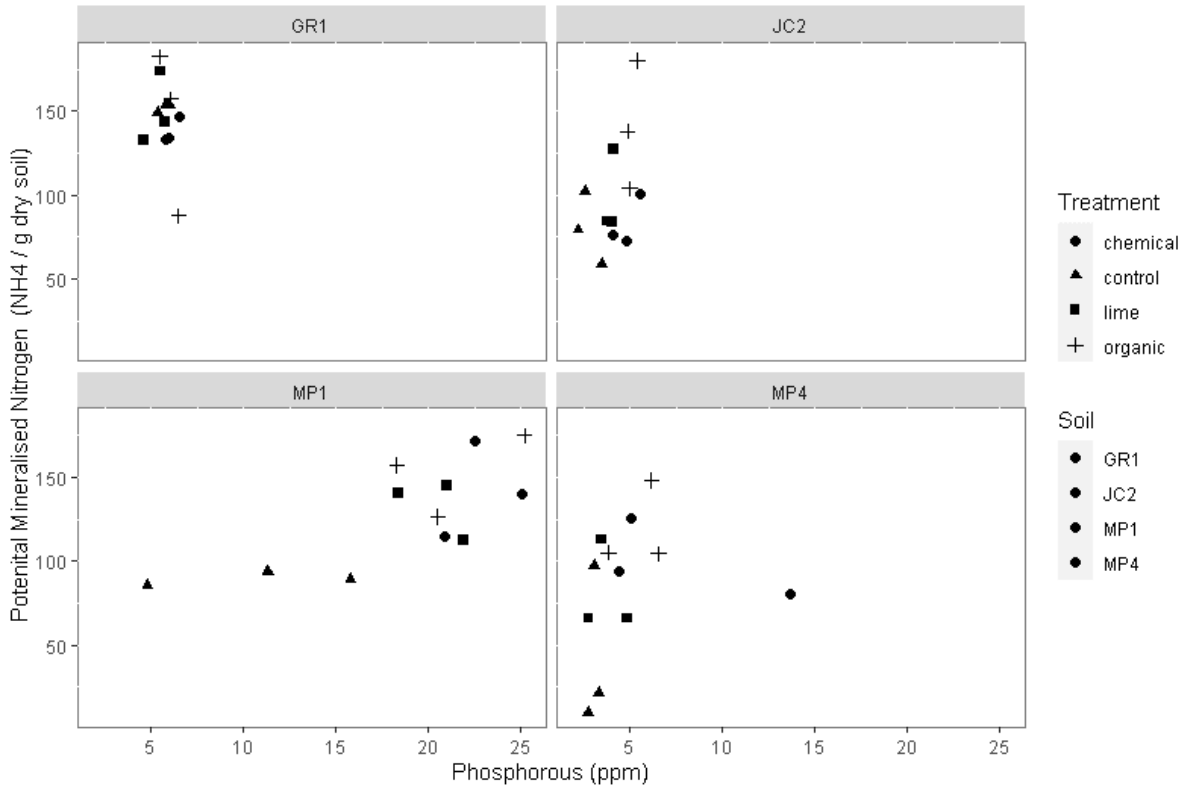


Figure 2.2: Potential nitrogen mineralisation by soil type and treatment with available phosphorus measured using Morgan's test (n=3). Soil types are separated into grids, GR1 and JC2 are on the top and MP1 and MP4 are on the bottom. Treatments are represented by shapes, as explained in the figure legend.

There was also a significant effect of pH on potential N mineralisation ($p<0.003$). Assessing the soil types individually, there was a significant effect of pH on N mineralisation potential in MP1 ($p=0.002$), MP4 ($p=0.007$), and JC2 ($p=0.031$). As pH increased across three of the soils (MP1, MP4, JC2) N mineralisation potential also increased, with pH values above 6.1 associated with a greater potential N mineralisation in these soils (Figure 2.3). The control treatment had the lowest pH across all soil types (mean pH = 5.3). The organic treatment had the highest pH (mean pH= 6.05).

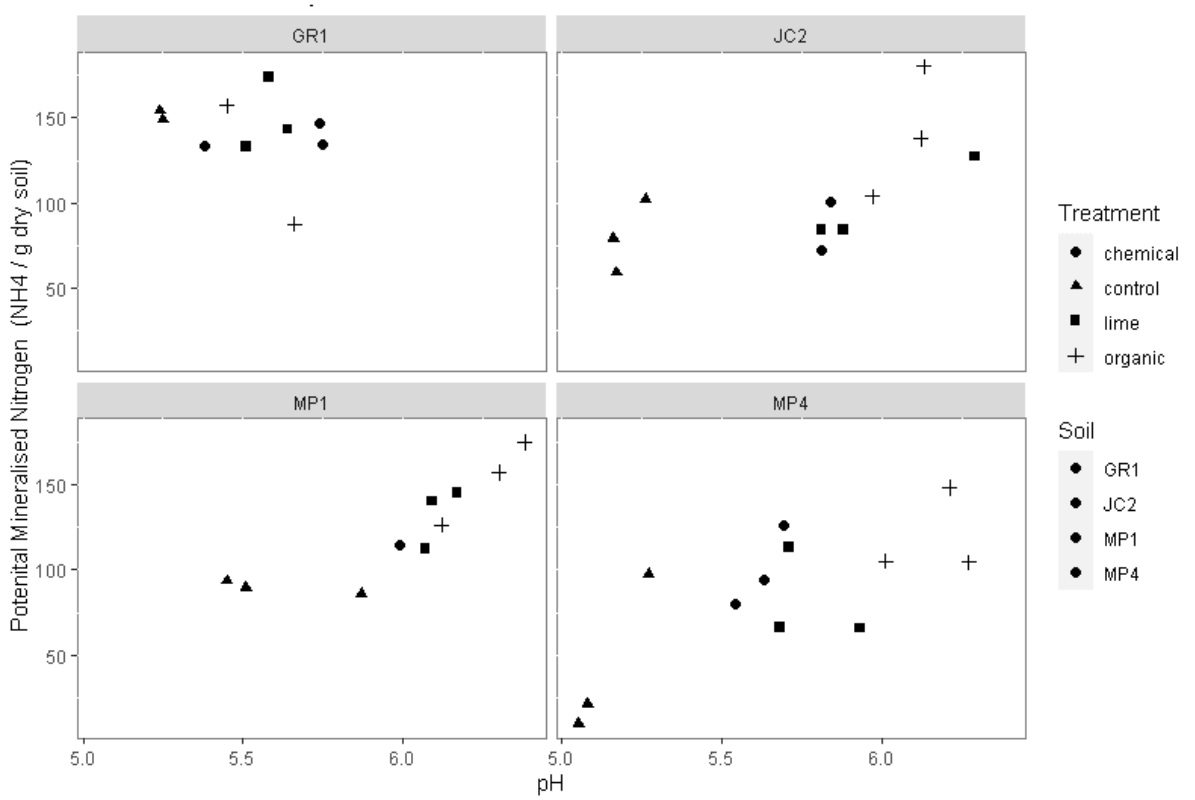


Figure 2.3: Potential nitrogen mineralisation and pH by soil type and treatment. Soil types are separated into grids, GR1 and JC2 are on the top and MP1 and MP4 are on the bottom. Treatments are represented by shapes, as explained in the figure legend.

2.3.3 Microbial Biomass carbon and nitrogen

The GR1 soil had the largest microbial biomass C and N (MBC, MBN) compared with the other soil types (Table 2.2), there was significantly larger MBC and MBN in the GR1 compared to the other three soil types ($p<0.001$). There were no significant differences between the other three soils (Figure 2.4). There was no significant effect of treatment on MBC in the GR1, JC2, and MP4 soils. In the MP1 soil there was a significant difference in MBC between the organic and the control treatment. The organic treatment had significantly higher MBC ($p<0.05$).

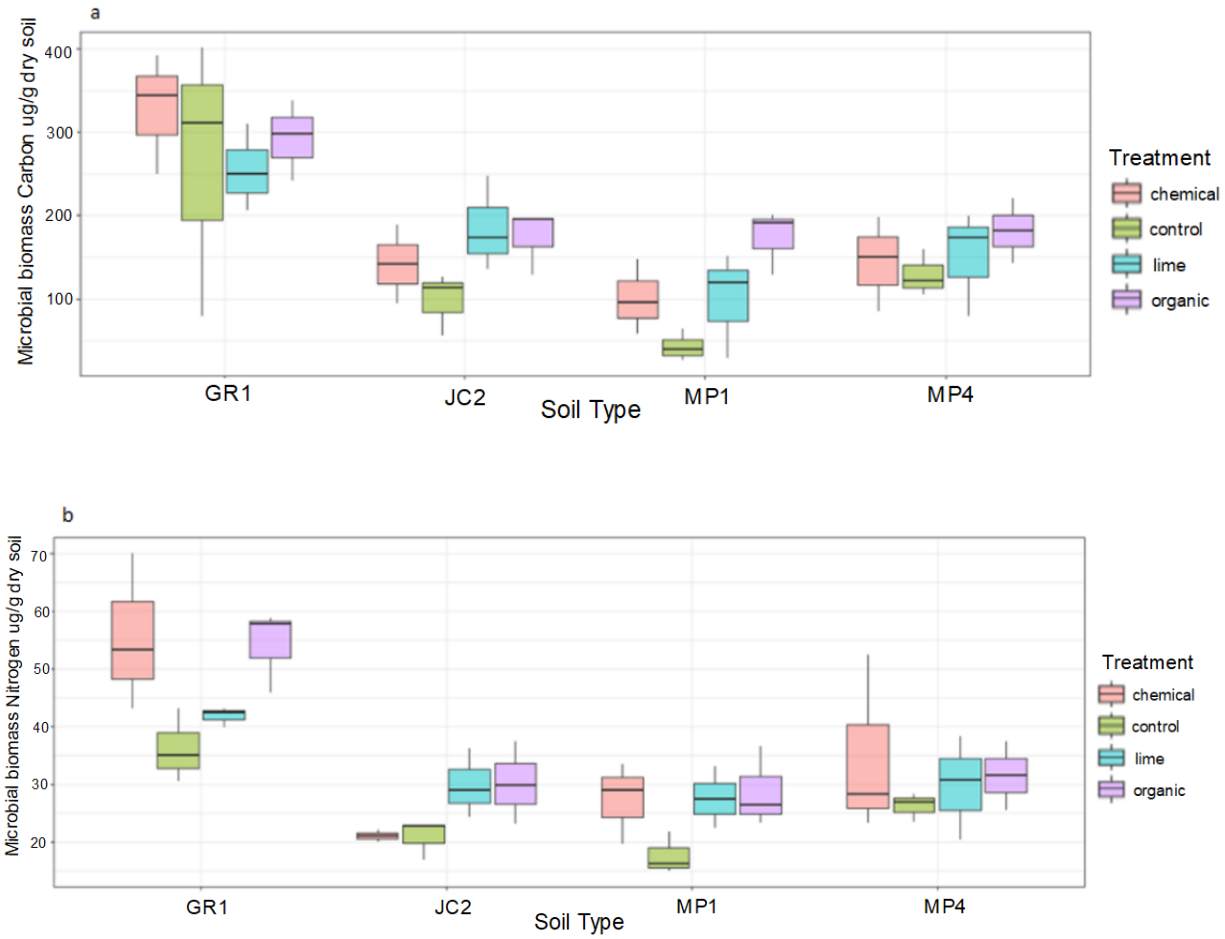


Figure 2.4: Microbial biomass C and N with soil type and treatment. Graph a shows Microbial Biomass Carbon with four different soil types (GR1, JC2, MP1 and MP4) and treatments (chemical, control, lime and organic). Graph b shows Microbial Biomass Nitrogen with four different soil types (GR1, JC2, MP1 and MP4) and treatments (chemical, control, lime and organic).

2.3.4 Enzymes

There was no significant effect of soil type (p=0.765) or treatment (p=0.084) on enzyme activity. Principal component analysis (PCA) (Figure 2.5) showed that there was minimal clustering of samples based on soil type or treatment. Environmental factors that affected enzyme activity included OM which significantly affected AGLU (p=0.013) and soil pH which significantly affected NAG (p=0.048) and P.1 (p=0.048). Significant interactions between enzyme activities were found across soils and treatments. Enzymes significantly interacted with each other. The interactions between AGLU and BGLU were significant (p <0.0001). The interactions between AGLU and AMI were also significant (p=0.016). BGLU significantly interacted with NAG (p=0.006). NAG significantly interacted with P.1 (p=0.001). The interaction between AMI and AGLU was significant (p=0.016).

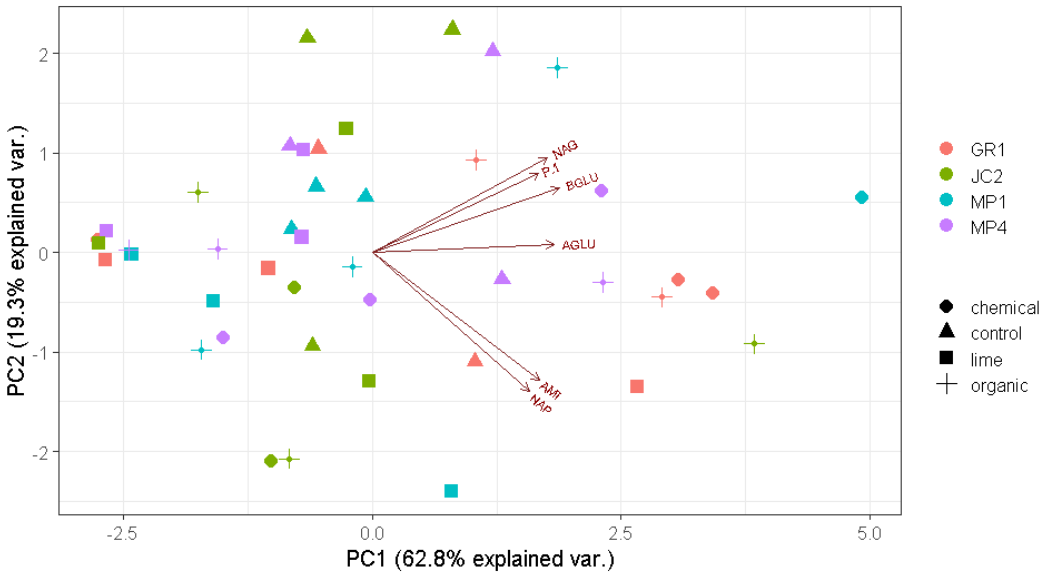


Figure 2.5: Principal component analysis of enzymatic activity for each enzyme within each soil type (n=12) and for each treatment (n=3)

2.3.5 16S Bacterial community composition

The dominant 20 most relatively abundant bacterial genera across the soil types and treatments are shown in Figure 6. Within this the five most relatively abundant genera were *Candidatus udaeobacter*, *Gaiella*, *Pseudolabrys*, *MND1*, *Mycobacterium* (Figure 2.6). The alpha diversity between soil types varied from an average of 1221 to 2304 (Chao1), and 6.41 to 6.89 (Shannon), (Table 2.3). There were significant differences in the alpha diversity indexes tested between the soil types. However, within each soil type there were no significant differences in alpha diversity with treatment. The Shannon index was significantly higher in JC2 and MP1 than in GR1 and MP4 ($p<0.01$). MP4 was significantly lower than GR1, JC2, and MP1 ($p<0.001$) for observed species and Chao1. Results from the PCA analysis showed separate clustering of the 16S communities with soil type (Figure 2.7a). Along PC1 all soil types were separate; however, along PC2 only the MP4 and JC2 had separate clusters. There was less distinction between clusters when considering treatments (Figure 2.7b), only the control treatment showed marginal separation along PC1. Permutational analysis of variance (PERMANOVA) results showed a significant effect of both soil type ($p<0.001$) and treatment ($p<0.001$), and an interaction between soil type and treatment ($p<0.02$) on the bacterial community composition. An analysis of similarities (ANOSIM) further confirmed a significant effect of soil type ($R =0.46$, $p <0.001$) and treatment ($R=0.14$, $p<0.001$). Pairwise Adonis analysis showed that all four soils were significantly different from each other ($p<0.001$). However, pairwise Adonis analysis of the treatment effect showed that there were only significant differences when considering the organic vs the control ($p<0.001$), the lime vs the control ($p<0.001$), and the chemical vs the control ($p<0.001$), there were no other significant differences between treatments. For specific genus that varied between the soil types

and the treatments, refer to the deseq2 analysis tables in the supplementary materials. RDA analysis showed that the 16S community was significantly affected by soil pH and the activity of the BGLU enzyme (Figure 2.8). The MP1 and JC2 soils were particularly influenced by soil pH (Figure 2.8). However, the effect of pH on treatments was much more variable.

Table 2.3: Alpha diversity indexes for the 16S community for each soil type and treatment. There were no significant differences in treatments, so letters here represent the differences between soil types.

	Chao	Shannon	Simpson
GR 1	a	b	b
Control	1375	6.393	197.0
Organic	1998.2	6.718	278.0
Lime	1410.6	6.274	164.9
Chemical	1442	6.419	184.0
MP1	a	a	a
Control	1465	6.675	345.8
Organic	2293	7.015	463.4
Lime	1629	6.725	299.6
Chemical	3828	7.145	499.7
MP4	a	b	b
Control	1188	6.414	247.9
Organic	1259	6.436	219.04
Lime	1180.5	6.278	195.58
Chemical	1257	6.532	270.8
JC2	a	a	a
Control	1970	6.787	317.5
Organic	1520	6.608	281.5
Lime	1945	6.932	407.4
Chemical	1987	6.885	374.6

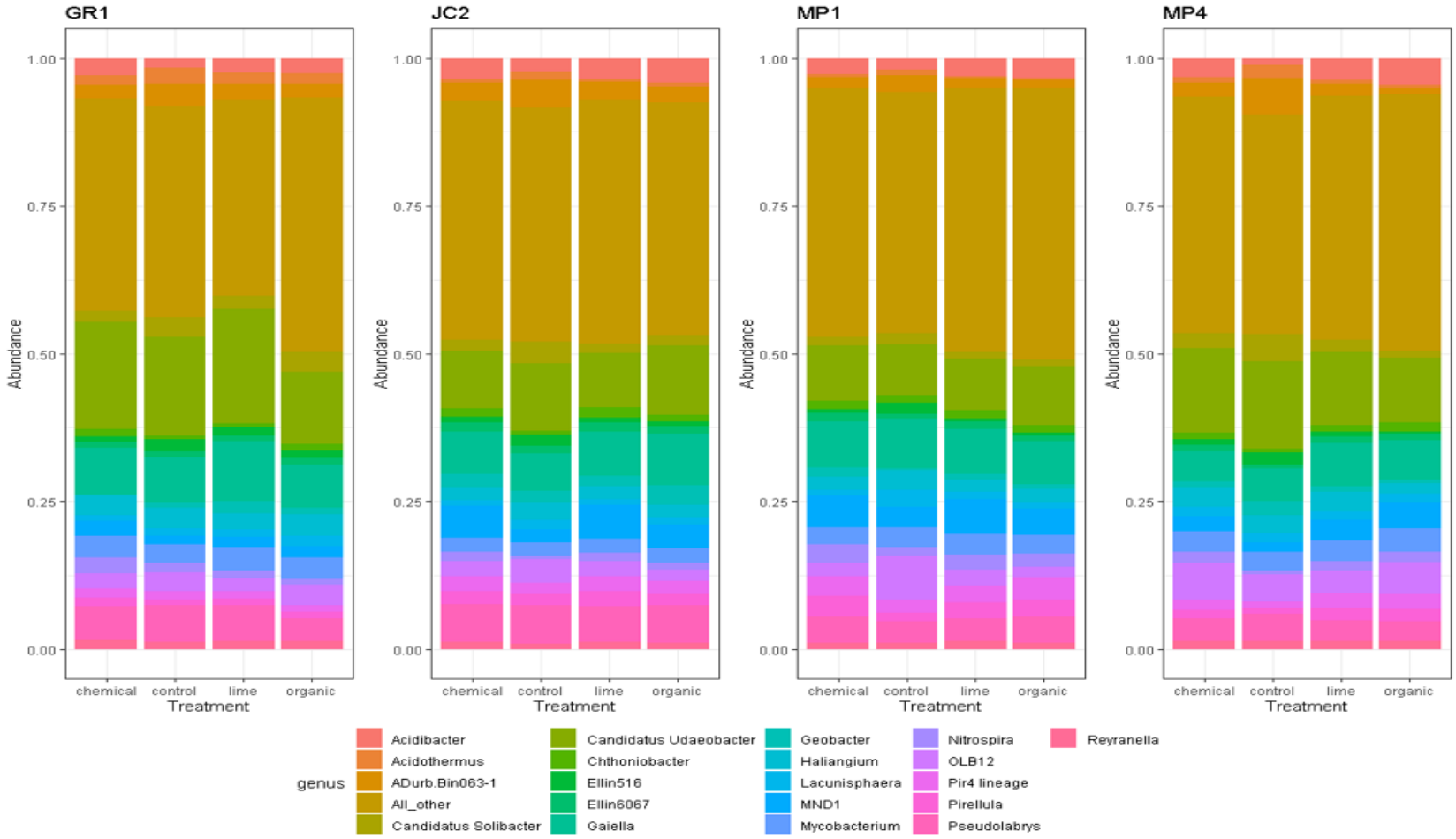


Figure 2.6: Relative abundance of the dominant 20 bacterial genera based on 16S rDNA sequencing across all soil types and treatments. The genus "All_other" as quoted in the legend represents the rest of the genus that were not present in the dominant 20 genera. Each treatment contains 3 replicate samples.

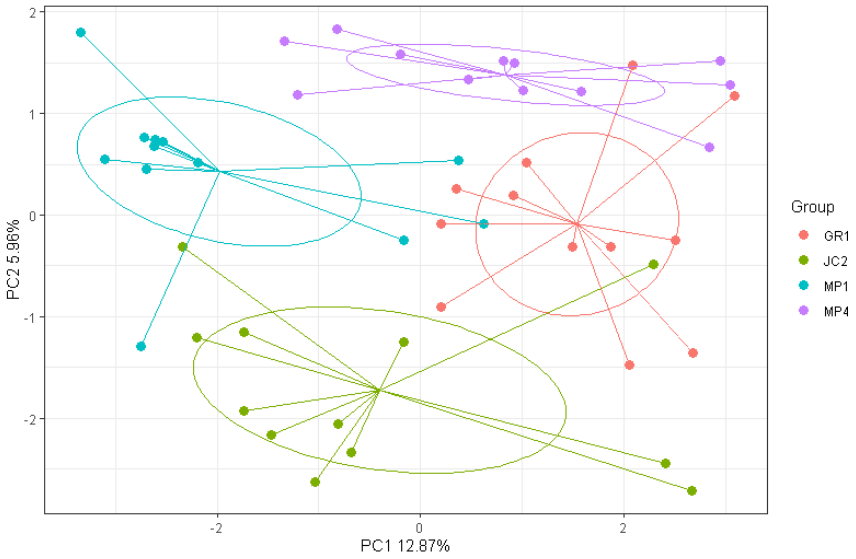


Figure 2.7a: Principal component analysis showing clustering of 16S microbial community with soil type (n=12). Colours are representing the different soil types

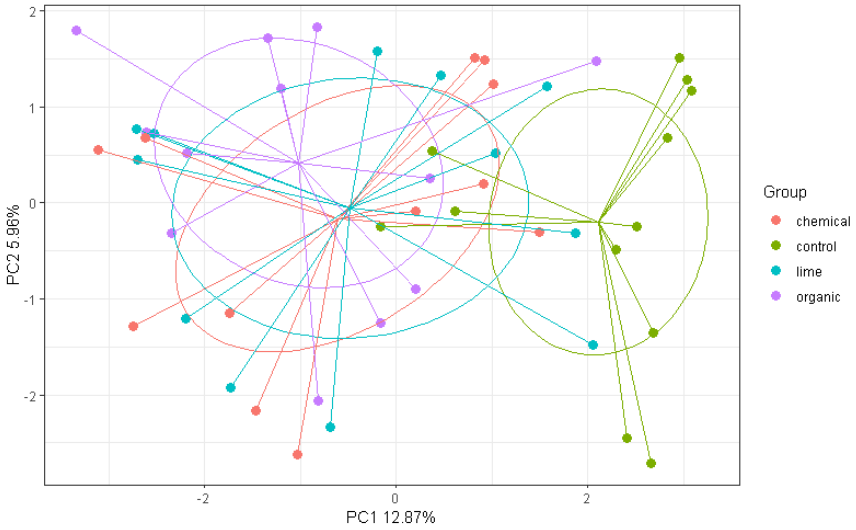


Figure 2.7b: Principal component analysis showing clustering of 16S microbial community with treatment (n=12). Colours represent the different treatment

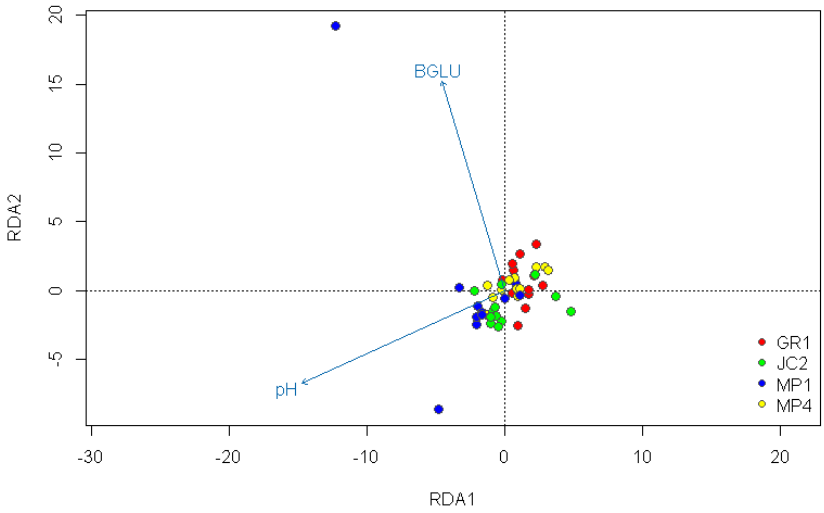


Figure 2.8: Redundancy analysis by soil type (n=12). This graph shows which soil nutrient properties are significantly influencing the 16S community structure. In this graph both soil pH and BGLU enzyme activity are significantly influencing the 16S community structure.

2.3.6 Fungal community composition

The dominant 20 most relatively abundant fungal genera across the soil types and treatments are shown in Figure 9. The five most abundant genera in the fungal community based on ITS sequencing were *Mortierella, Lachnum, unidentified_3513, Claroideoglomus,* and *unidentified_77* (Figure 2.9). The alpha diversity was significantly different between soil types; however, there were no significant differences between the treatments (Table 2.4). When using the Shannon index, the diversity in MP1 was significantly different from all other soil types ($p<0.05$); when using the Simpsons index, MP1 was significantly different from MP4 and GR1 ($p<0.01$); when using the Chao index, JC2 was significantly different from GR1 and MP1 ($p<0.01$) (Table 2.4). Results from the PCA analysis showed separate clustering of the ITS communities with soil type (Figure 2.10a). Along PC1, GR1 was separate from the other soil types; however, along PC2 only JC2 had a separate cluster compared to the other soils. There was no distinction between clusters when considering treatments (Figure 2.10b). PERMANOVA showed a significant effect of soil type ($p=0.001$) and treatment ($p=0.004$) on the fungal community, but no significant interaction between soil type and treatment. ANOSIM also showed a significant effect of soil type ($R=0.62$, $p <0.001$). Pairwise Adonis analysis showed that all soil types were significantly different from each other ($p<0.001$). However, only the organic vs the control ($p< 0.001$), the lime vs the control ($p< 0.027$), and the chemical vs the control ($p< 0.021$), were significantly different among the treatments. For specific genus that varied between the soil types and the treatments refer to the deseq2 analysis tables in the supplementary materials. RDA analysis showed that the ITS community was significantly affected by soil pH and total C, total N, available K, and OM content (Figure 2.11). The GR1 soil fungal community was significantly influenced by OM. The MP1 were significantly influenced by soil pH (Figure 2.11). However, the effect of the environmental soil factors on treatments was much more variable.

Table 2.4: Alpha diversity indexes for the ITS community for each soil type and treatment. There were no significant differences in treatments, so the letters here represent the differences between soil types.

	Chao1	Shannon	Simpson
GR 1	b	a	a
Control	175.3	3.997	25.65
Organic	198.3	4.338	33.93
Lime	184.0	3.946	24.31
Chemical	254.7	4.420	31.81
MP1	b	b	b
Control	156.3	3.575	18.41
Organic	202.7	3.683	14.72
Lime	174.0	3.276	8.806
Chemical	159.7	3.504	11.843
MP4	ab	a	a
Control	192.3	4.253	36.28
Organic	317.0	4.469	35.32
Lime	176.0	4.095	27.24
Chemical	268	3.850	22.932
JC2	a	a	ab
Control	263.7	4.098	20.63
Organic	197.3	4.043	17.16
Lime	296.7	4.128	20.57
Chemical	362.7	4.295	21.04

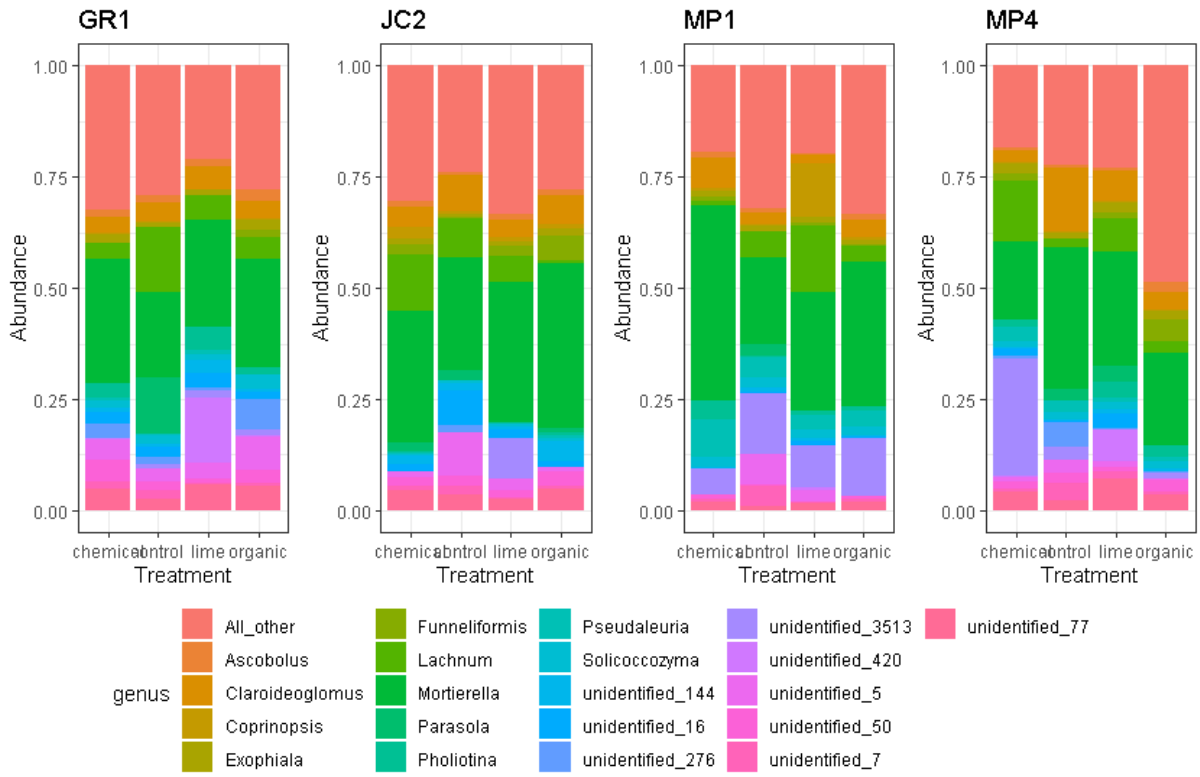


Figure 2.9: Relative abundance of the dominant 20 bacterial genera based on ITS sequencing across all soil types and treatments. The genus "All_other" as quoted in the legend represents the rest of the genus that were not present in the dominant 20 genera. Each treatment contains 3 replicate samples.

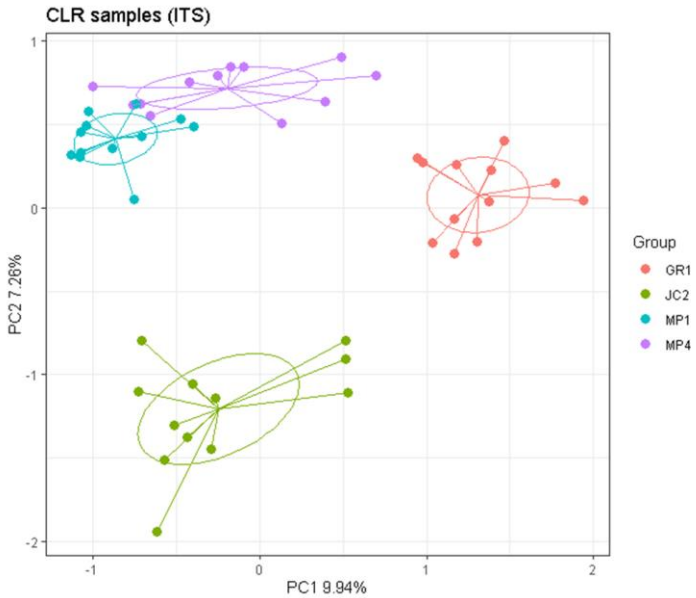


Figure 2.10a: Principal component analysis showing clustering of ITS microbial community with soil type (n=12). Colours represent the different soil types.

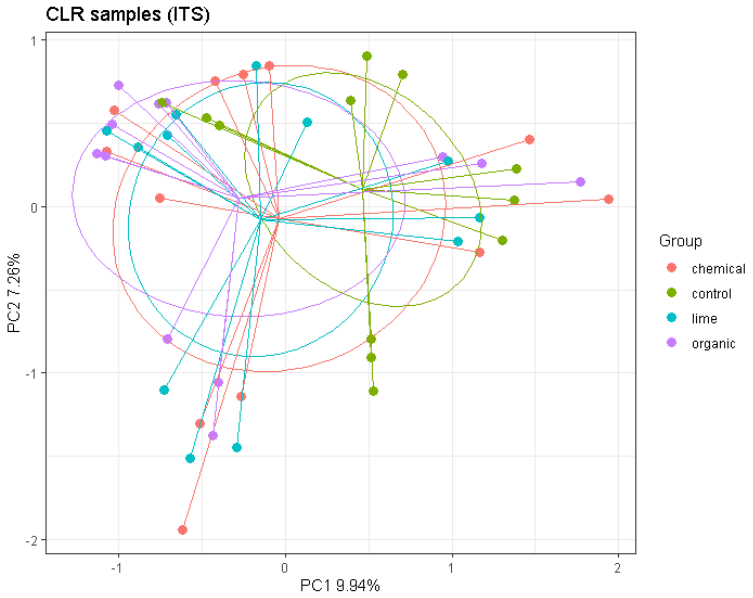


Figure 2.10b: Principal component analysis showing clustering of ITS microbial community with treatment (n=12). Colours represent the different treatments.

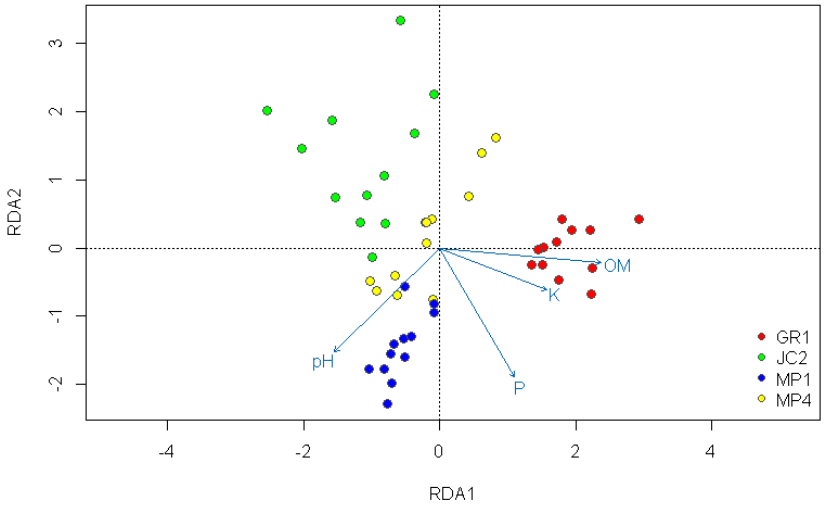


Figure 2.11: Redundancy analysis by soil type (n=12). This graph shows which soil nutrient properties are significantly influencing the ITS community structure. In this graph both soil pH and BGLU enzyme activity are significantly influencing the 16S community structure.

2. 4 Discussion

This experiment aimed to determine whether there was an effect of soil P, P fertiliser type (chemical and organic), and lime addition on N mineralisation potential, enzyme activity and microbial community structure. Nutrient management strategies such as the provision of P and the amendment of soil pH through lime addition are important for ensuring nutrient availability for plant growth. It is important to examine how these nutrient management practices affect N mineralisation, as a better understanding of the nutrient management dynamics and their effects on natural processes will assist in tailoring more specific nutrient management advice.

This study found that N mineralisation potential varied with soil type. This has been seen in other studies of Irish grassland soils (McDonald et al., 2014; Murphy *et al.*, 2017). The GR1 soil had the highest N mineralisation potential and was significantly higher than JC2 and MP4 soils. The GR1 soil also had a significantly higher OM content when compared with all other soil types. Organic matter contents are linked with mineralisation rates. Higher levels of potential N mineralisation with increasing OM was also found by van Vliet *et al.*, (2007) in Dutch grasslands. The Grange soil, GR1, was the only soil type where the control treatment did not have the lowest N mineralisation potential. There was also minimal variance in P level in this soil and the pH range was also lower than other soil types.

Except for the GR1 soil, there was an increase in potential N mineralisation with the P addition treatments (chemical and organic) in MP1 and MP4, and an increase with organic P addition in JC2, when compared with the control treatment. Therefore, the hypothesis that with P addition N mineralisation would increase was accepted. The effect of P on potential N mineralisation was most evident in the MP1 soil, with available P (P Morgan's) greatest in this soil type. In the MP1 soil the control had a significantly lower N mineralisation potential than the other treatments. The high available P in MP1 showed that there is great potential for increasing N mineralisation with P additions. An increase in potential N mineralisation with P availability has also been seen by (Bicharanloo *et al.*, 2022) in their study on soils growing wheat. The absence of P in the control pots resulted in the lowest N mineralisation potential in the control treatments in most soil types. It has been hypothesised that in soils low in P, the microbial community mineralise organic forms of P (Ikoyi *et al.*, 2018). Ikoyi et al. (2018) also found in the treatment not receiving P, bacterial and fungal feeding nematodes were more abundant, AMF colonisation increased, and bacteria containing the *phoD* gene such as *Actinobacteria* were also more abundant. Low P soils have been shown to have a selective pressure that may lead to increased abundance of bacteria involved in P mineralisation (Mander *et al.*, 2012). In this study the control treatment had a significantly higher relative abundance in eight

genera of *Actinobacteria* (*Bryobacter, Candidatus solibacter, Catenulispora, Edaphobacter, Granulicella, Occallatibacter, Streptacidiphilus,* and *Terracidiphilus*). Therefore, the hypothesis that nutrient management would influence microbial communities was accepted, as significant differences were seen between treatments. This could suggest that the control treatment in this study is also under a selective pressure to stimulate the microbial community responsible for P mineralisation rather than N mineralisation in a P limited environment. The coupling of C, N, and P in terms of mineralisation is variable in terms of their links. For C and N, mining for N also mobilises C (i.e., N is contained within organic macromolecules of SOM, meaning mobilising N also releases C that is metabolised by microbes). For P, the coupling with C and N mineralisation is less strong as P can be mobilised from SOM macromolecules via phosphatases that 'snip' phosphate from the ends of these compounds, without breaking down the whole molecule.

The addition of an organic form of P, in this study in the form of cattle slurry, increased N mineralisation potential across all four soil types. Therefore, the hypothesis that organic P addition would increase N mineralisation potential was cautiously accepted as this increase was not significant. The comparison decrease in N mineralisation with the chemical treatment hypothesis was rejected as there was no significant decrease. The organic treatment also could be creating conditions which encourage N mineralisation. Slurry contains a diverse mixture of organic nutrient compounds, including sources of labile C, organic N and P. The increased availability of labile C sources in the organic P treatment is a possible reason for increase in N mineralisation potential seen in this study. As microbes are often C limited (Schimel and Bennett, 2004) the C sources in organic fertilisers such as slurry could potentially stimulate microbial activity, mineralisation, and increase nutrient cycling in soil (Lazcano *et al.*, 2013; Ling *et al.*, 2016). However, it is possible that a high rate of N mineralisation is due to a residual effect of repeated additions of organic forms of N over time (Cavalli *et al.*, 2016). In this study *Proteobacteria* were significantly higher in the organic treatment than any of the other treatments. A study by Francioli *et al.* (2016) also found that *Proteobacteria* abundance increased under organic fertiliser treatment.

Potential N mineralisation was significantly higher in the limed treatment vs the control in three soil types. Therefore the hypothesis that lime increases N mineralisation potential was accepted. It can be seen in all soil types, even those with narrower pH ranges, that there is an increase in N mineralisation potential with increasing soil pH. Increased N mineralisation potential with increasing pH and liming activities has also been found (Mkhonza *et al.*, 2020). Soil pH can impact on SOM mineralisation through an increase in the dissolved organic compounds that are present in soil as pH increases (Andersson *et al.*, 2000; Neina, 2019), these organic compounds are less recalcitrant and easier to

break down. It has also been shown that soil pH and liming can affect microbial community structure (Holland *et al.*, 2018). This has knock-on effects in terms of the production of enzymes and functioning of the microbial community that could be involved in increasing SOM mineralisation (Aye *et al.*, 2018).The addition of lime also had an effect on microbial community structure in this study and therefore the hypothesis that the microbial community would be affected by nutrient management practice was accepted. However, it is important to note that the pH range in this study is narrow, and the chemical and organic P treatments also received lime, so therefore the main comparisons drawn here were between the control and lime treatments. In terms of 16S community, *Acetobacteraceae* was present in the control treatment but not in the lime treatment. Interestingly, these bacteria are part of an N-fixing bacteria family, and have been found to grow in acidic environments with pHs close to 3.0–3.5, but 5.0–6.5 is there preferred pH (Reis and Teixeira, 2015). In terms of the ITS fungal community, many genera that were present in the lime treatment were not present in the control, including *Didymellaceae, Tubeufiaceae, Hyaloscyphaceae, Leotiaceae, Psathyrellaceae, Tetraplosphaeriaceae, Pleosporaceae,* and *Xylariaceae.*

Enzymatic activity varied greatly both between soil types and also between treatments. There were few trends that held true for all soils and treatments. However, a large proportion of the variability was explained as part of PCA analysis (82.1%). Although there were few trends with soil type and treatments, enzymatic activity was linked to other enzymatic activity. C cycling enzymes BGLU and AGLU were coupled together as were NAG and P. It was hypothesised that the chemical treatment would reduce enzymatic activity when compared to other treatments. This does not appear to be the case for most soil types and enzymes, and this hypothesis was rejected. Only JC2 had lower enzymatic activity in the chemical treatment. Enzyme activity was also general not influenced by other soil properties such as C, N or P content in this study. It is worth noting that there were only three replicates taken as part of this study and that if there were a larger number of samples taken perhaps this variability could have been somewhat reduced. However, enzyme activity is often used as an indicator of microbial community activity and C, N, and P cycling (Meena and Rao, 2021), the range of activity in the enzyme examined in this study could be an indicator of both health soils and a continuum of biological activity and nutrient cycling.

2.5 Conclusion

This study found that N mineralisation and microbial community structure were significantly affected by both P and lime application. Soils that received P in comparison to controls had a higher N mineralisation potential. The type of P addition (chemical vs organic) was not found to be significant in this study; however, the organic treatment did have the highest absolute N mineralisation potential.

This suggests that further study will be required to investigate in more depth the effect of chemical vs organic P addition and their effects on N mineralisation potential and SOM turnover. The lime application also had a significant effect on potential N mineralisation in this study. N mineralisation was found to increase with increasing soil pH, despite the narrow range found here. This indicates that pH is an important factor in determining potential N mineralisation rate if differences can be found across a narrow range. For future fertiliser and N management plans, the N supply from SOM N mineralisation in the context of the management of P and lime would require more study. However, these results highlight that it is important to consider other management practices when considering N supply from SOM.

2. 6 References

Andersson, S., Nilsson, S.I., Saetre, P., 2000. Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biology and Biochemistry* 32, 1-10.

Aye, N.S., Butterly, C.R., Sale, P.W.G., Tang, C., 2018. Interactive effects of initial pH and nitrogen status on soil organic carbon priming by glucose and lignocellulose. *Biology and Biochemistry* 123, 33-44.

Bertrand, I., Delfosse, O., Mary, B., 2007. Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: Apparent and actual effects. *Soil Biology and Biochemistry* 39, 276-288.

Bicharanloo, B., Bagheri Shirvan, M., Keitel, C., Dijkstra, F.A., 2022. Nitrogen and phosphorus availability have stronger effects on gross and net nitrogen mineralisation than wheat rhizodeposition. *Geoderma* 405, 115440.

Bodirsky, B.L., Popp, A., Lotze-Campen, H., Dietrich, J.P., Rolinski, S., Weindl, I., Schmitz, C., Müller, C., Bonsch, M., Humpenöder, F., Biewald, A., Stevanovic, M., 2014. Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nature Communications* 5, 3858.

Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.* 75, 139-157.

Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H.W., Van Vuuren, D.P., Willems, J., Rufino, M.C., Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences* 110, 20882-20887.

Briedis, C., de Moraes Sá, J.C., Caires, E.F., de Fátima Navarro, J., Inagaki, T.M., Boer, A., de Oliveira Ferreira, A., Neto, C.Q., Canalli, L.B., dos Santos, J.B., 2012a. Changes in Organic Matter Pools and Increases in Carbon Sequestration in Response to Surface Liming in an Oxisol under Long-Term No-Till. *Soil Science Society of America Journal* 76, 151-160.

Briedis, C., Sá, J.C.d.M., Caires, E.F., Navarro, J.d.F., Inagaki, T.M., Boer, A., Neto, C.Q., Ferreira, A.d.O., Canalli, L.B., Santos, J.B.d., 2012b. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. *Geoderma* 170, 80-88.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. *Geoderma* 124, 3-22.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* 17, 837-842.
Caires, E.F., Barth, G., Garbuio, F.J., 2006. Lime application in the establishment of a no-till system for grain crop production in Southern Brazil. *Soil and Tillage Research* 89, 3-12.
Cavalli, D., Marino Gallina, P., Sacco, D., Bechini, L., 2016. Soil mineral nitrogen dynamics following repeated application of dairy slurry. *European Journal of Soil Science* 67, 804-815.

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry* 85, 235-252.
Fox, A., Ikoyi, I., Creamer, R., Lanigan, G., Schmalenberger, A., 2017. Microbial community structure and function respond more strongly to temporal progression than to the application of slurry in an Irish grassland. *Applied Soil Ecology* 120, 97-104.
Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., Reitz, T., 2016. Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies. *Frontiers in Microbiology* 7.
Fujita, K., Miyabara, Y., Kunito, T., 2019. Microbial biomass and ecoenzymatic stoichiometries vary in response to nutrient availability in an arable soil. *European Journal of Soil Biology* 91, 1-8.
Gloor, G.B., Macklaim, J.M., Pawlowsky-Glahn, V., Egozcue, J.J., 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology* 8.
Goulding, K.W.T., 2016. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use and Management* 32, 390-399.
Guignard, M.S., Leitch, A.R., Acquisti, C., Eizaguirre, C., Elser, J.J., Hessen, D.O., Jeyasingh, P.D., Neiman, M., Richardson, A.E., Soltis, P.S., Soltis, D.E., Stevens, C.J., Trimmer, M., Weider, L.J., Woodward, G., Leitch, I.J., 2017. Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture. *Frontiers in Ecology and Evolution* 5.
Hendriksen, N.B., Creamer, R.E., Stone, D., Winding, A., 2016. Soil exo-enzyme activities across Europe—The influence of climate, land-use and soil properties. *Applied Soil Ecology* 97, 44-48.
Herold, M.B., Giles, M.E., Alexander, C.J., Baggs, E.M., Daniell, T.J., 2018. Variable response of nirK and nirS containing denitrifier communities to long-term pH manipulation and cultivation. *FEMS Microbiology Letters* 365.
Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of The Total Environment* 610-611, 316-332.
Ikoyi, I., Fowler, A., Schmalenberger, A., 2018. One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. *Science of The Total Environment* 630, 849-858.
Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, Eduard Szoecs and Helene Wagner (2020). vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan
Johnson, D., Leake, J.R., Read, D.J., 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. *Plant and Soil* 271, 157-164.

Leo Lahti, Sudarshan Shetty et al. (2017). *Tools for microbiome analysis in R*. Version URL: http://microbiome.github.com/microbiome.
Liu, Q., Wang, J., Bai, Z., Ma, L., Oenema, O., 2017. Global animal production and nitrogen and phosphorus flows. *Soil Research* 55, 451-462.
Love MI, Huber W, Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." *Genome Biology*, 15, 550. doi: 10.1186/s13059-014-0550-8.
Mander, C., Wakelin, S., Young, S., Condron, L., O'Callaghan, M., 2012. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. *Soil Biology and Biochemistry* 44, 93-101.
Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry* 33, 1633-1640.
McDonald, N.T., Watson, C.J., Lalor, S.T.J., Laughlin, R.J., Wall, D.P., 2014a. Evaluation of Soil Tests for Predicting Nitrogen Mineralization in Temperate Grassland Soils. *Soil Science Society of America Journal* 78, 1051-1052.
McDonald, N.T., Watson, C.J., Laughlin, R.J., Lalor, S.T.J., Grant, J., Wall, D.P., 2014b. Soil tests for predicting nitrogen supply for grassland under controlled environmental conditions. *The Journal of Agricultural Science* 152, 82-95.
McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE* 8, e61217.
Meena, A., Rao, K.S., 2021. Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region, India. Ecological Processes 10, 16.
Mkhonza, N.P., Buthelezi-Dube, N.N., Muchaonyerwa, P., 2020. Effects of lime application on nitrogen and phosphorus availability in humic soils. *Scientific Reports* 10, 8634.
Mooshammer, Wolfgang, W., Jörg, S., Birgit, W., Sonja, L., Florian, H., Andreas, B., Ieda, H., H., F.A., Lucia, F., M., K.K., Sophie, Z.-B., Andreas, R., 2012. Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. *Ecology* 93, 770-782.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. Plant and Soil 417, 499-510.
Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. *Applied and Environmental Soil Science* 2019, 5794869.
Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., Colpaert, J.V., 2014. Comparison and Validation of Some ITS Primer Pairs Useful for Fungal Metabarcoding Studies. *PLOS ONE* 9, e97629.
Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. *Agriculture, Ecosystems & Environment* 202, 98-107.
Pedro Martinez Arbizu (2017). pairwiseAdonis: Pairwise Multilevel Comparison using Adonis. R package version 0.4.
Quince, C., Lanzen, A., Davenport, R.J., and Turnbaugh, P.J. (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* 12: 38
R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
Reis, V.M., Teixeira, K.R.d.S., 2015. Nitrogen fixing bacteria in the family Acetobacteraceae and their role in agriculture. *J Basic Microbiol* 55, 931-949.

Rütting, T., Aronsson, H., Delin, S., 2018. Efficient use of nitrogen in agriculture. *Nutrient Cycling in* Agroecosystems 110, 1-5.
Schloter, M., Nannipieri, P., Sørensen, S.J., van Elsas, J.D., 2018. Microbial indicators for soil quality. *Biology and Fertility of Soils* 54, 1-10.
Schröder, J.J., Schulte, R.P.O., Creamer, R.E., Delgado, A., van Leeuwen, J., Lehtinen, T., Rutgers, M., Spiegel, H., Staes, J., Tóth, G., Wall, D.P., 2016. The elusive role of soil quality in nutrient cycling: a review. *Soil Use and Management* 32, 476-486.
Sinsabaugh, R.L., Follstad Shah, J.J., 2012. Ecoenzymatic Stoichiometry and Ecological Theory. Annual Review of Ecology, Evolution, and Systematics 43, 313-343.
Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.
Sinsabaugh, R.S., 1994. Enzymic analysis of microbial pattern and process. Biology and Fertility of Soils 17, 69-74.
Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79, 7-31.
Sun, R., Zhang, X.-X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biology and Biochemistry 88, 9-18.
Svanbäck, A., McCrackin, M.L., Swaney, D.P., Linefur, H., Gustafsson, B.G., Howarth, R.W., Humborg, C., 2019. Reducing agricultural nutrient surpluses in a large catchment – Links to livestock density. Science of The Total Environment 648, 1549-1559.
van Vliet, P.C.J., van der Stelt, B., Rietberg, P.I., de Goede, R.G.M., 2007. Effects of organic matter content on earthworms and nitrogen mineralization in grassland soils. European Journal of Soil Biology 43, S222-S229.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19, 703-707.
Zhang, F., Huo, Y., Cobb, A.B., Luo, G., Zhou, J., Yang, G., Wilson, G.W.T., Zhang, Y., 2018. Trichoderma Biofertilizer Links to Altered Soil Chemistry, Altered Microbial Communities, and Improved Grassland Biomass. *Frontiers in Microbiology* 9.
Zhang, Y., Sun, C., Chen, Z., Zhang, G., Chen, L., Wu, Z., 2019. Stoichiometric analyses of soil nutrients and enzymes in a Cambisol soil treated with inorganic fertilizers or manures for 26 years. *Geoderma* 353, 382-390.
Zhu, Z., tida, G., Luo, Y., Liu, S., Xu, X., Tong, C.-l., Shibistova, O., Guggenberger, G., 2018. Microbial stoichiometric flexibility regulates rice straw mineralization and its priming effect in paddy soil. *Soil Biology and Biochemistry* 121
Žurovec, O., Wall, D.P., Brennan, F.P., Krol, D.J., Forrestal, P.J., Richards, K.G., 2021. Increasing soil pH reduces fertiliser derived N2O emissions in intensively managed temperate grassland. *Agriculture, Ecosystems & Environment* 311, 107319.

Chapter 3

The effect of long-term phosphorus fertiliser additions on soil organic matter priming and nitrogen mineralisation in a grassland agricultural system.

3.1 Introduction

Nutrient limitation in agricultural systems, especially of nitrogen (N) and phosphorus (P), is often overcome by the addition of inorganic fertilisers (Bouwman *et al.*, 2013; Bodirsky *et al.*, 2014; Schröder *et al.*, 2016). The use of inorganic fertilisers can affect soil organic matter (SOM) quality and quantity, soil pH, and also impacts the environment by release of greenhouse gas emissions and nutrient losses to waterways (Dinesh *et al.*, 2010; Ning *et al.*, 2017). When fertilisers are applied to soil this influences the soil nutrient balance and also influences other nutrients, which may not have been added or added to the same extent and can be limiting to both plant and microbial growth (Blair *et al.*, 2006; Gong *et al.*, 2009; Zheng *et al.*, 2019). SOM represents the largest stock of plant macronutrients in soils, but in organic forms that are not directly available for plants. Transformation to mineral nutrient forms via microbial activity (known as mineralisation) is one of the most important biogeochemical processes that fertilisers can affect (Murphy *et al.*, 2017). Microbial use of nutrients held in SOM may be expected to decline when readily available forms of these nutrients are applied to soil via fertilisers. As N and P make up the majority of fertiliser addition in agricultural systems it is important to examine their effects on soil nutrient cycling, in particular their interaction with each other. There is a need to understand the impacts of fertiliser application (in a variety of forms) on carbon (C), N, and P mineralisation, and interactions between the relative availabilities of these elements in soils. A deeper understanding may help in better defining the fertiliser rates required to support plant growth without providing excess nutrients, which has economic and environmental consequences. This study investigated how the addition of inorganic P affects N mineralisation.

An alternative to inorganic fertilisers is the use of organic by products materials with high nutrient value, such as livestock slurry and farmyard manure (FYM), both of which are commonly used as part of farm nutrient management practice (Scotti et al., 2015, Liu et al., 2017). The application of organic fertilisers enables recycling of valuable nutrients that enhance crop production (Butler et al., 2013, Cervantes et al., 2013), and reduces reliance on mineral fertilisers that have strong environmental impacts, both through their energy-intensive manufacturing, and following their application to soil (Van Grinsven *et al.*, 2012; Gaidajis and Kakanis, 2021). Organic fertilisers contain organic materials which can increase SOM and nutrient cycling in soil (Edmeades, 2003, Monaco et al., 2008, Chuan-Chuan et al., 2017, Zhang et al.,

2019). This increase in soil nutrient cycling is a consequence of increased microbial activity – brought about by the use of added organic materials as substrate with associated enzyme production to mobilise organic nutrient forms (Crecchio et al., 2001). These impacts on microbial activity and enzyme production may also influence utilization of native SOM, for example as a consequence of microbial mining of nutrients in response to nutrient imbalance of organic fertilisers, or through co-metabolism where enzymes produced in response to organic additions also act on components of SOM. Hence, the addition of organic fertilisers affects biological processes in soils, particularly as they contain a diversity of labile and more recalcitrant organic compounds (Dickson et al., 1981, Liu et al., 2017). Priming is one such biological process, whereby a change in available carbon (C), often considered in the context of plant root exudates, causes changes in microbial-mediated SOM mineralisation rates (Kuzyakov et al., 2000). However, the addition of an organic fertiliser also causes changes to available C in soils, and hence could potentially influence the rate of SOM priming.

Increased nutrient availability can both increase microbial activity through enzyme production and decrease microbial activity in soil as they may not be required as mineralisation nutrients (Cleveland et al., 2002, Ilstedt & Singh, 2005, Mehnaz et al., 2019a). This has knock-on effects on a range of soil microbial functions and mechanisms, including those involved in nutrient cycling processes such as potential SOM mineralisation and priming (Sullivan & Hart, 2013, Chowdhury et al., 2014, Stewart et al., 2015, Fang et al., 2020). Previous studies have shown that the magnitude of priming is linked to N availability (Craine et al., 2007); however, the effects of P on priming are in comparison unstudied.

There are several theories and hypotheses in the literature which describe the effect of nutrient availability on SOM priming. The *activation hypothesis* suggests that priming will be maximal when the amount of C, N, and P in available forms meets the demand for microbial growth and activity (Hessen et al., 2004, Craine et al., 2007, Mehnaz et al., 2019a). However, when nutrient availability is limiting to microbial growth and activity, but there is a C-source available, the *N mining hypothesis* is an alternative proposed theory. The *N mining* theory *hypothesis* considers that in nutrient-poor but C-rich environments such as the rhizosphere, priming is a response of the microbial community to break down SOM for essential nutrients such as N (Craine et al., 2007, Murphy et al., 2012, Murphy et al., 2017). When both C and N are readily available to the microbial community, usually through root exudates in combination with the addition of N-fertiliser, negative priming effects are possible (Blagdatskaya & Kuzaykov, 2008). The scenario where these readily available sources of C and N are used (at the expense of SOM decomposition) is known as the *preferential substrate hypothesis* (Blagdatskaya & Kuzaykov, 2008). The

effects of N fertiliser management on priming have been researched extensively; however, in terms of P, there is still no defined link with priming (Mehnaz et al., 2019a). Some studies have argued that N limitation is more important than P limitation for the rate of priming, as N in its organic form is linked with C as part of the structure of the macromolecules and therefore C is released concurrently with N, whereas P in its organic form does not require the breakdown of a molecular C backbone (Bradley and Fyles, 1995, Dijkstra et al., 2013, Boilard et al., 2019).

How organic fertilisers influence the rate of priming and the mechanisms involved remain largely unknown (Kuzyakov et al., 2000, Fontaine et al., 2003, Luo et al., 2016, Fang et al., 2020). Some studies have shown that the direction and rate of priming is influenced by the quality and quantity of the C added as part of the organic fertiliser (Chowdhry et al., 2014, Wang et al., 2015, Fang et al., 2017,2018a, Liu et al., 2017, Fang et al., 2020). The addition of an organic fertiliser such as slurry would be expected to increase soil respiration rates through increased microbial activity due to increased decomposition of the added organic material (Cui and Holden, 2017). The addition of inorganic fertilisers alters nutrient availability and can indirectly affect the soil C balance (through altered rates of SOM formation and decomposition); however, the addition of organic fertilisers will directly affect the C balance in soil (Zhang et al., 2019). Any changes to the C balance in soil will also impact nutrient availability (Zhang et al., 2019), through changes in microbial activity and hence nutrient cycling. Differences in organic and inorganic inputs will affect the C:N:P ratio of soil, along with the availability of N and P, and the stoichiometric differences in these fertilisation strategies may cause shifts in microbial activity and processes (Xu et al., 2013, Cleveland and Liptzin 2007, Spohn, 2016).

One of the main aims of soil research directed toward resource-efficient and sustainable agricultural practices is to gain greater knowledge of how biological and human factors, such as nutrient management practices, interact to control the release of nutrients from SOM (Boilard *et al.*, 2019). This study aimed to build understanding in this research area by examining the effect of P management on plant growth, SOM priming, and N mineralisation in a grassland system. It was hypothesised that the addition of P would increase plant productivity, N mineralisation and the rate of priming. It was further hypothesised that P added in an organic form would increase soil nutrient availability, N mineralisation and the rate of priming. These hypotheses were tested using soils from a long-term P field experiment, where mineral fertilisation and organic slurry treatments were applied in the field, that were then incubated in a ^{13}C labelling chamber under *Lolium perenne*, a common grass species used in Irish pastures

3.2 Methods

3.2.1 Site and Soil sampling

The field site used for soil sampling is located at Teagasc Research site, Johnstown Castle, Co. Wexford, Ireland, 52° 17' N, 06° 30' W (Daly and Casey, 2006). The site was established in February 1995 on a loam-textured soil, sown as a *Lolium perenne* monoculture. The sward is still currently a *Lolium perenne* monoculture, and has been previously described (Massey, 2012; Sheil *et al.*, 2016; Randall *et al.*, 2019). This site was never grazed, and the grass was cut 8 times a year to a sward height of 5-6cm using a plot harvester, to simulate grazing (Massey *et al.*, 2016; Randall *et al.*, 2019). The experiment was originally set up as a long-term P trial with each plot receiving 16% superphosphate annually at fertilisation rates of 0 (P0), 15 (P15), 30 (P30) and 45 (P45) kg P ha^{-1} yr^{-1} with 16 plots in total (Sheil *et al.,* 2016; Randall *et al.,* 2019). Since 2016, all the plots have been split, with a cattle slurry treatment applied annually at a mean rate of 37, 432 kg ha^{-1} yr^{-1} (Massey et al., 2016). This added another four treatments with fertilisation (via slurry) rates of 0 (P0 + slurry), 15 (P15 + slurry), 30 (P30 + slurry) and 45 (P45 + slurry) kg P in cattle slurry ha^{-1} yr^{-1}. This generated 32 plots (5m x 1m) containing 8 treatments, and 4 replicates were available for each treatment. For the purpose of this study, only the P0 and P45 treatments were selected from both the inorganic and organic P sources (P0, P0 + slurry, P45, P45 + slurry). These treatments were selected in order to examine the effect of high and low P, as well as slurry addition. Soils were sampled (March 2019) using a soil corer to a depth of 10 cm; six intact cores were taken in the shape of a W across each replicated plot. Cores from each replicated treatment plot were placed into a plastic bag, homogenised into composite samples for each plot and stored at 4°C prior to experimental work.

3.2.2 Pot and $^{13}CO_2$ labelling tank set up, and measurements

Soils were sieved to 2mm and water holding capacity (WHC) was determined prior to the packing of pots. WHC was then maintained at 65% throughout the experiment. Soils were packed in pots (1500g wet weight of the soil) at a dry bulk density of 0.9g cm^3. The pots used in this experiment were 2L in size, measuring 110mm x 110mm x 200mm (l x b x h). The bottom of each pot was covered with a PVC mesh (1mm) to prevent soil loss. In the center of each pot a gas sampling chamber (210ml headspace with a gas inlet and outlet port, controlled by a tap) was inserted to a depth of 2cm into the soil. The gas sampling chamber was stabilised using four glass rods inserted into the soil, secured with an elastic band wrapped around the chamber above soil level (Murphy et al., 2017).

Pots were placed in a randomised design within a Perspex labelling chamber within a controlled growth room (Conviron, Winnipeg, Canada) (Paterson *et al.,* 2005, 2006, Murphy *et al.,* 2017). The pots were kept in the labelling chamber for two weeks before the first sampling to allow time for seedling establishment and to adjust to the environmental conditions. During this time, WHC was maintained with additions of deionised water. The conditions in the growth room and labelling chamber were as follows: the room was set to have a 16-hour day (light hours at a light density of 420umol m-2 s-1 photon flux density) and 8-hour night (dark hours). This was applied so that the hours of darkness occurred during the working day in order to be able to carry out sampling and to water the pots, with no disturbance to the $\delta^{13}C$ values of the labelling chamber during the photoperiod. The growth room temperature during light hours was set at 5°C in order to have a temperature of 21°C in the labelling chamber; during dark hours the temperature was set at 18°C so that the temperature in the tank was 18°C.

From the day of sowing to harvest at the end of the experiment, the labelling chamber was flushed with CO_2-free air at 10L min^{-1}, CO_2 with a $\delta^{13}C$ value of -29.2 at 4.2ml min^{-1} and $^{13}CO_2$ (99At%) at 0.086ml min^{-1}. This ensured a concentration of approximately 400umol mol^{-1} of CO_2 and a $\delta^{13}C$ value of 1376‰, giving an At% of 2.6 ^{13}C enrichment. Flows were controlled using Mass Flow Controllers (Flotech Solutions, Stockport, UK). The delta value and CO_2 level were checked regularly throughout the experiment using an isotopic gas bench (details below) and EGM-4 infrared gas analyser (EGM-4, PP-Systems, Amesbury, USA).

The four treatments (P0, P0 + slurry, P45, P45 + slurry) were also divided into two groups (planted and unplanted). For planted pots, grass seeds were sown around the headspace chamber in each pot to ensure that only soil and root respiration were measured. The seeds used were *Lolium perenne* C.V. Kent, planted to a density of 31.6 g m^{-2}. Gas sampling started two weeks after the grass seeds were sown. Pots were watered at 0800, two and a half hours before sampling. For gas sampling, chambers were first flushed with CO_2-free air for 5 minutes until a value of less than 5ppm CO_2 was recorded. The pots were then placed back into the labelling tank for 1 hour. Thereafter, a 20ml gas sample was taken from the gas sampling chamber. Approximately 8ml of this sample was injected into the EGM-4 and CO_2 concentration recorded. The remainder of the sample was injected into an N_2 flush-filled 12 mL gas vial (Labco) and analysed for $^{13}C–CO_2$ (details below). Sampling took place once a week for five weeks.

Gas samples were analysed using an isotopic gas bench system. The ^{12}C:^{13}C ratio of CO_2 in the sample were analysed using a gas bench II connected to a Delta plus Advantage isotope ratio mass spectrometer (both, Thermo Finnigan, Bremen, Germany). Before analysis of a sample, reference materials NBS19 and USGS44

with the addition of 2M HCl were used as a reference measure of CO_2. This allowed for normalising to the VPDB scale. Repeated measurements of these quality control standards measured the precision of the gas bench for analysing $\delta^{13}C$ of CO_2 at ± 0.24 ‰ (± SD of the mean).

At the end of the gas sampling period pots were harvested. Soil was collected from the pots and used for soil nutrient analysis such as total C, N, and OM etc. The shoots and roots were also harvested, dried and weighed. Before harvesting the roots were washed. Roots and shoots were also analysed for their nutrient contents.

3.2.3 Calculation of root-derived, soil organic matter-derived respiration and Priming

3.2.3.1 Root-derived respiration

Root respiration was calculated by isotopic mass balance as in equation 1 below. Briefly, the delta value (δ) describes the ratio of ^{12}C to ^{13}C in a given sample. $\delta^{13}C$ sample represents the planted pot and the $\delta^{13}C$ control represents the unplanted pots. The $\delta^{13}C$ root represents the δ values found in the milled root sample.

Equation 1

Root respiration = (δ13C sample – δ13C control)/ (δ13C root – δ13C control)

3.2.3.2 Soil organic matter-derived respiration

SOM-derived respiration was calculated (equation 2) by subtracting the root-derived respiration from the total CO_2 respiration.

Equation 2

SOM respiration = Total respiration – root respiration

3.2.3.3 Priming

The rhizosphere priming effect is calculated as the rate of SOM-derived respiration from planted pots, minus the SOM mineralisation in unplanted pots (equation 3).

Equation 3

Priming = SOM-derived respiration of the planted pot – SOM-derived respiration of the corresponding fallow pot

Priming is represented as ug C g^{-1} dry soil day^{-1}.

It is important to note that the C source from the slurry addition had the same ^{13}C signature as the native SOM, and the SOM and slurry respiration sources could not be distinguished from each other and only the combination of both from the root-derived respiration flux were measured.

3.2.4 Seven-day anaerobic incubation – Potential nitrogen mineralisation measurement

Soil was dried in an oven at 40°C for three days. Five g of soil was weighed into separate screw-cap glass test tubes, labelled 'tube 1', and 'tube 2'. The 'tube 1' samples were extracted with 25ml 2M KCl and 12.5ml dH_2O on day 0, these are referred to as day zero samples. To 'tube 2', only 12.5ml of dH_2O were added. The 'tube 2' samples were then placed in an incubator at 40°C for 7 days. After 7 days, the soil was extracted using 25ml 2M KCl, these are referred to as the day 7 samples. Samples were analysed for inorganic N (NO2–N, NO3–N, and NH4–N) using an Aquakem 600A (Aquakem 600A, 01621, Vantaa, Finland). The N that was mineralised was calculated as the NH4–N on day 7 minus the NH4–N on day zero (Mc Donald et al., 2014, Keeney and Bremmer 1966, Schonberg et al., 2009).

3.2.5 Microbial biomass extraction carbon, nitrogen, and phosphorus

Microbial biomass C, N, and P were determined using chloroform fumigation-extraction on fresh soil samples (Brookes *et al.*, 1982; Brookes *et al.*, 1985; Vance *et al.*, 1987). For microbial biomass C and N, 10 g fresh soil was weighed in two sets, fumigated (F) and non-fumigated (NF). The F samples were placed into a desiccator with 50 ml chloroform for 24 hours under vacuum. Both F and NF samples were extracted using 40 ml 0.5 M K_2SO_4. Total organic C and total N were measured from the extracts on a Shimadzu, TOC-UCPH analyser with ASIV auto sampler. Microbial biomass C and N were calculated by subtracting values obtained from the NF sample from the corresponding F sample. The result was then divided by set conversion factors of 0.45 for microbial biomass C and 0.54 for microbial biomass N (Brookes *et al.,* 1985; Vance *et al.,* 1987). For microbial biomass P, 2 g of soil was weighed in three sets: F, NF, and a correction sample (CS). Fumigation method for the P sample was the same as C and N. The F and NF samples were extracted with 39 ml of 0.5 M $NAHCO_3$ (pH 8.5) and 1 ml of dH_2O. The CS samples were extracted with 39 ml of 0.5 M $NAHCO_3$ (pH 8.5) and 1 ml of 250 ug P ml^{-1} of a P stock solution KH_2PO_4. After extraction, the pH of all samples was adjusted to approximately 6.1 using 3 ml of 10% HCL solution (Massey, 2012). Microbial biomass P was calculated by subtracting values obtained for the NF sample from the corresponding F sample. This value was also adjusted using a conversion factor, 0.4 (Brookes *et al.,* 1982; Massey 2012).

3.2.6 Mineral soil nitrogen

20 g fresh weight of soil was weighed into a plastic tube and 50ml of 2M KCl was added. The tube was placed on a shaker for one hour at 150rpm. After shaking, the tubes were left to settle on the lab bench before being filtered through Whatmann no.2 filter paper. Samples were analysed for inorganic N (NO_2–N, NO_3–N, and NH_4–N) using an Aquakem 600A (Aquakem 600A, 01621, Vantaa, Finland).

3.2.7 Soil nutrient analyses

Soil nutrient analysis for total C and total N, available P and K measured as Morgan's test, organic matter content and soil pH were measured as in Chapter 2 section 2.2.5.

3.2.8 Statistical analyses

Data analysis was performed in R Studio 4.2.0 (R Core Team, 2021). Statistical significance was determined at $p<0.05$. Prior to analyses the Shapiro-Wilk's and Levene's tests were used to assess normality and homogeneity of variance. When data was found to be normally distributed and homogenous, one-way ANOVA was used to determine significant differences and Tukey's HSD post-hoc test was used for analysing pairwise differences ($p<0.05$). Data that was found not to be normally distributed was transformed via log or square root. In the case where data was still not normally distributed, a Kruskal-Wallis test was used and a dunn-test for the pairwise comparison, with p-values adjusted using the Benjamin-Hocberg method.

3.3 Results

3.3.1 Plant biomass, root-shoot partitioning and tissue nutrient content

The results expressed here represent plant material harvested on the final day of the experiment. At the end of the experiment there was a higher root biomass in the higher P treatments (i.e., P45 and P45 + slurry). The P45 + slurry treatment had the highest average root biomass (Table 3.1). There were significant differences in root biomass between treatments. P45 + slurry was significantly higher than all other treatments, P45 was significantly higher than the two lower P treatments (P0 and P0 +slurry), (p values <0.01). There was no statistically significant difference between P0 and P0 + slurry. This trend was also seen in the shoots, the shoot biomass was higher in the higher P treatments than the lower P treatments. P45 had the highest shoot biomass on average (Table 3.2). The shoot biomass in the P45 and P45 + slurry treatments were significantly higher than the two lower treatments ($p<0.0001$). There was

no statistically significant difference between P45 and P45 +slurry. P0 + slurry was also significantly higher than the P0 treatment ($p<0.0001$).

There was a lower shoot-to-root ratio in the P45 + slurry treatment than in the P45 treatment (Table 3.1 & 3.2). This trend was also seen in the P0 and P0 + slurry treatments. The P45 slurry and P0 slurry treatments had a lower shoot-to-root ratio than treatments receiving chemical application or no application of P.

There was a higher N concentration in the roots of the plants given lower P treatments (P0 and P0 + slurry) and a higher P content in the roots of the plants that received higher P treatments (Table 3.1). In shoots, there was also a higher N content with the lower P treatments (Table 3.2). On the day of harvest, the plants given the P45 and P45 slurry treatments had symptoms of N deficiency (i.e., yellowing of the leaves).

Table 3.1: Root nutrient data, taken on the final day 42 of the experiment. Figures shown here are the average (n=4). Standard error measures are inside the parentheses. Data is missing from the P0 treatment as there was not enough root material to carry out all analyses.

Treatment	*Biomass (g)*	*C %*	*N %*	*P%*	*K%*	*S%*
P0	0.45 (±0.03)	36.35 (±4.01)	1.81 (±0.15)	-	-	-
P45	2.64 (±0.65)	35.75 (±1.85)	0.88 (±0.03)	0.21 (±0.01)	0.75 (±0.03)	0.11 (±0.00)
P0 slurry	0.99 (±0.42)	36.80 (±1.25)	1.52 (±0.04)	0.13 (±0.00)	1.20 (±0.02)	0.14 (±0.00)
P45 slurry	3.44 (±0.46)	30.35 (±2.04)	0.84 (±0.03)	0.18 (±0.01)	0.68 (±0.02)	0.11 (±0.00)

Table 3.2: Shoot nutrient data, taken on the final day 42 of the experiment. Figures shown here are the average (n=4). Standard error measures are inside the parentheses.

Treatment	*Biomass (g)*	*C %*	*N %*	*P%*	*K%*	*S%*
P0	0.86 (±0.05)	41.55 (±0.15)	3.65 (±0.10)	0.10 (±0.00)	2.81 (±0.10)	0.29 (±0.01)
P45	3.62 (±0.66)	42.33 (±0.13)	0.94 (±0.01)	0.22 (±0.00)	0.50 (±0.01)	0.14 (±0.00)
P0 slurry	1.55 (±0.02)	42.28 (±0.23)	2.84 (±0.05)	0.11 (±0.00)	2.39 (±0.06)	0.23 (±0.00)
P45 slurry	3.57 (±0.53)	41.98 (±0.20)	1.08 (±0.02)	0.23 (±0.00)	0.44 (±0.01)	0.17 (±0.00)

3.3.2 Soil properties in planted and unplanted treatments

There was a trend in the absolute values for soil organic matter content to be higher in the lower P treatments than in the higher P treatments in the planted pots (Table 3.3); however, these differences in organic matter content between the treatments and pot types (planted vs unplanted) were not significant. Interestingly, the trend in the absolute values in soil C content tended to be higher in the higher P treatments than in lower P treatments in the planted pots, although this difference was not statistically significant. There was no statistically significant difference in total N (TN) concentrations in the planted and unplanted pots (Tables 3.3 and 3.4). Available P (PM) was highest in the P45 treatment, and was lower as per the set treatments in the P0 and P0 + slurry treatments. There were significant differences in PM between all treatment combinations ($p<0.001$), except between the available P in the P0 and the P0 + slurry treatment (Table 3.3).

Table 3.3: Planted pots total soil nutrient data, as measured on the final day of experiment when the pots were harvested. Figures shown here are averages (n= 4) with ± standard errors.

Planted pot Treatment	*Organic matter %*	*Total C ug g^{-1}*	*Total N ug g^{-1}*	*Available N as NO3-N (mg kg^{-1})*	*7-day Mineralised N (NH_4 g^{-1} dry soil)*	*Available P mg Kg^{-1}*	*Available K mg Kg^{-1}*	*pH*
P0	9.03 (±0.05)	327.5 (±11.06)	29.0 (±1.08)	32.05 (±6.82)	141.69 (±8.49)	2.15 (±0.06)	88.13 (±7.66)	6.06 (±0.03)
P45	8.85 (±0.17)	352.3 (±15.13)	30.5 (±1.50)	1.66 (±0.13)	134.70 (±2.03)	8.96 (±0.08)	31.53 (±0.43)	6.39 (±0.02)
P0 slurry	9.13 (±0.25)	328.5 (±14.67)	28.8 (±1.49)	2.63 (±0.15)	159.96 (±4.42)	2.44 (±0.04)	53.33 (±2.65)	6.41 (±0.02)
P45 slurry	8.73 (±0.05)	335.5 (±8.09)	28.8 (±0.75)	1.27 (±0.12)	152.00 (±5.40)	7.95 (±0.12)	38.18 (±4.57)	6.49 (±0.02)

In the unplanted pots, the absolute value of organic matter content was highest in the P0 + slurry unplanted pot (Table 3.4). Organic matter content between the rest of the unplanted treatments was not statistically different. The absolute value of total C content was highest in the P45 + slurry unplanted pot. Total N contents were not statistically different between all treatments in the unplanted pots. The absolute average potential N mineralised was highest in the P0 unplanted pot, this absolute value was higher than that in all planted pots.

Table 3.4: Unplanted pots total soil nutrient contents, as measured on the final day of the experiments when the pots were harvested. Figures shown here are averages (n= 4) with ± standard errors.

Unplanted pot Treatment	*Organic matter %*	*Total C ug g^{-1}*	*Total N ug g^{-1}*	*Available N as NO3-N mg Kg^{-1}*	*Mineralised N (NH_4 g^{-1} dry soil)*	*Available P mg Kg^{-1}*	*Available K mg Kg^{-1}*	*pH*
P0	8.85 (±0.13)	347.75 (±21.82)	31.00 (±2.08)	67.37 (±0.05)	168.22 (±5.75)	2.30 (±0.03)	94.88 (±1.41)	5.98 (±0.03)
P45	8.83 (±0.05)	341.25 (±21.31)	30.00 (±2.27)	64.56 (±4.56)	138.85 (±2.40)	10.04 (±0.10)	44.05 (±3.35)	6.11 (±0.01)
P0 slurry	9.30 (±0.23)	332.25 (±11.47)	30.00 (±1.08)	75.17 (±3.70)	155.74 (±13.61)	2.51 (±0.03)	78.00 (±4.63)	6.07 (±0.02)
P45 slurry	8.60 (±0.23)	351.50 (±16.08)	31.25 (±1.55)	75.53 (±17.71)	145.36 (±3.26)	8.99 (±0.21)	44.53 (±3.17)	6.22 (±0.01)

3.3.3 Soil nitrogen availability and potential nitrogen mineralisation

The available N concentration was higher in the unplanted pots than in the planted pots (Table 3.4). The slurry treatments had a higher available N concentration than the P45 and P0 treatments in the unplanted pots. In the unplanted pots, P45 + slurry and P0 + slurry had a significantly higher NO_3 concentrations than the P45 and P0 unplanted treatment ($p < 0.001$). However, in the planted pots P0 had a significantly higher available nitrate concentration than any of the other treatments ($p<0.007$). There was very little available N left in the other three treatments at the end of the experiment. Plants in these three treatments were larger than the plants in the P0 treatment, and hence may have used the available N. P0 + slurry in the planted pots also had a significantly higher NO_3 concentration than the P45 and the P45 + slurry planted treatment ($p<0.04$).

Potential N mineralisation varied with treatment and pot type (planted vs unplanted) (Figure 3.1). Measured using the seven-day anaerobic incubation method, N mineralisation potential was significantly affected by treatment ($p =0.0237$). A significant result ($p =0.0115$) was also found with mineralisation and the availability of P in the soil ($p =0.0115$), measured using the P Morgan's method. The P0 unplanted treatment had the highest absolute potential N mineralisation (Table 3.4). In the planted pots there was a significant effect of slurry addition on potential N mineralisation ($p =0.0063$). The P0 + slurry treatment had the highest mean potential N mineralisation in the planted pots (Table 3.3). There was a significantly higher N mineralisation potential in the P0 + slurry planted treatment than the P45 planted treatment ($p=0.03$).

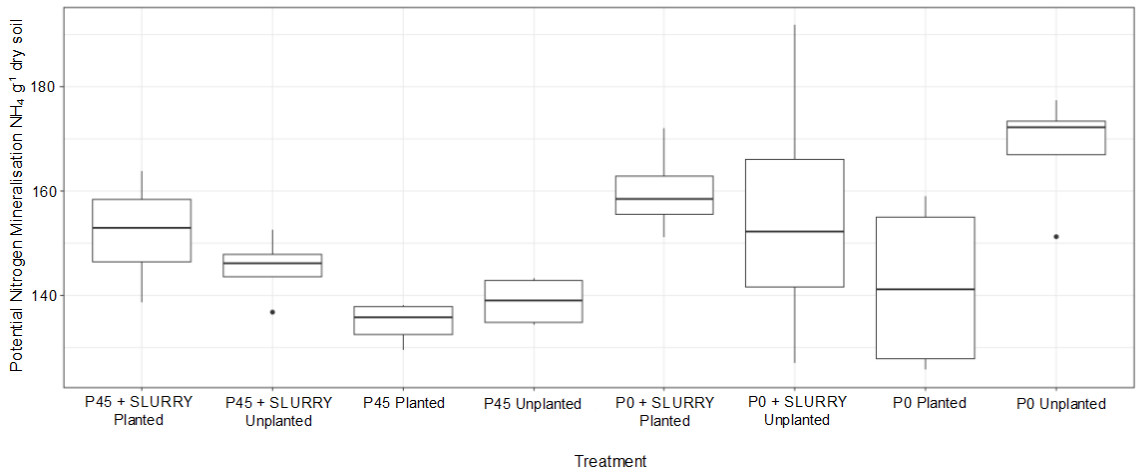


Figure 3.1: Nitrogen mineralisation potential measured between eight different treatments (NH_4^- g^{-1} dry soil), n=4. Both planted and unplanted pots are represented in this graph.

3.3.4 Soil microbial biomass carbon, nitrogen, and phosphorus

There were no significant differences in microbial biomass C (MBC) across all treatments, or between the planted and unplanted pots. However, there was a significant difference ($p < 0.05$) between treatments found for microbial biomass N (MBN) and P (MBP). Results from ANOVA analysis showed there was no significant effect of microbial biomass C, N and P on potential N mineralisation. Microbial biomass P (MBP) was significantly lower in the P45 planted treatment than all other treatments ($p<0.01$). In the unplanted pots, MBP in the P0 treatment was significantly higher than in P45 and the P45 + slurry treatments ($p<0.01$). Microbial biomass N was lowest in the P45 unplanted treatment, and highest in the P45 + slurry treatment in the planted pots. Microbial biomass N was higher in the planted pots than in the unplanted pots for all treatments, except the P0+ slurry where MBN was not significantly different between the planted and unplanted pots. P45 + slurry in the planted pots had a significantly higher MBN ($p<0.01$) than the P0 + slurry planted pots. The P0 treatment had the highest microbial biomass N in the unplanted pots; however, there were no significant differences in MBN between treatments across the unplanted pots (Figure 3.2).

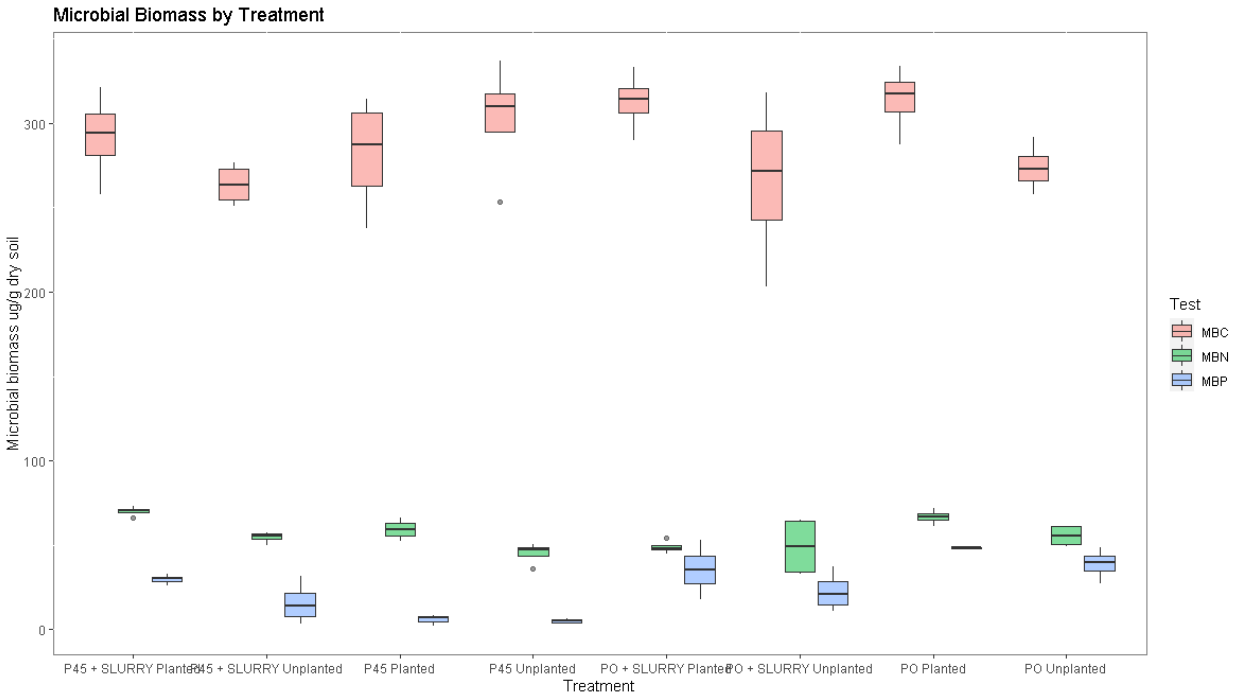


Figure 3.2: Microbial biomass (ug g^{-1} dry soil) carbon (MBC), nitrogen (MBN), and phosphorus (MBP) between the treatments in both planted and unplanted pots. Colours distinguish the different microbial biomass groups.

3.3.5 Soil organic matter-derived respiration

There was a significantly higher rate of total CO_2 respiration in the planted then unplanted treatments ($p<0.001$). Over the course of the experiment CO_2 respiration increased by an average of 67% in the planted pots; and increased by 62% in the P45 treatment, 63% in the P45 + slurry treatment, 59% in the P0, and 67% in the P0 + slurry treatment (Figure 3.3). In the planted pots, there were significant differences in CO_2 between the high and low P treatments ($p<0.001$). On day 35, only the P0 treatment was significantly different from the other treatments. On the final day of sampling (day 42), only the P0-P45, and P0- P0 + slurry treatments were significantly different.

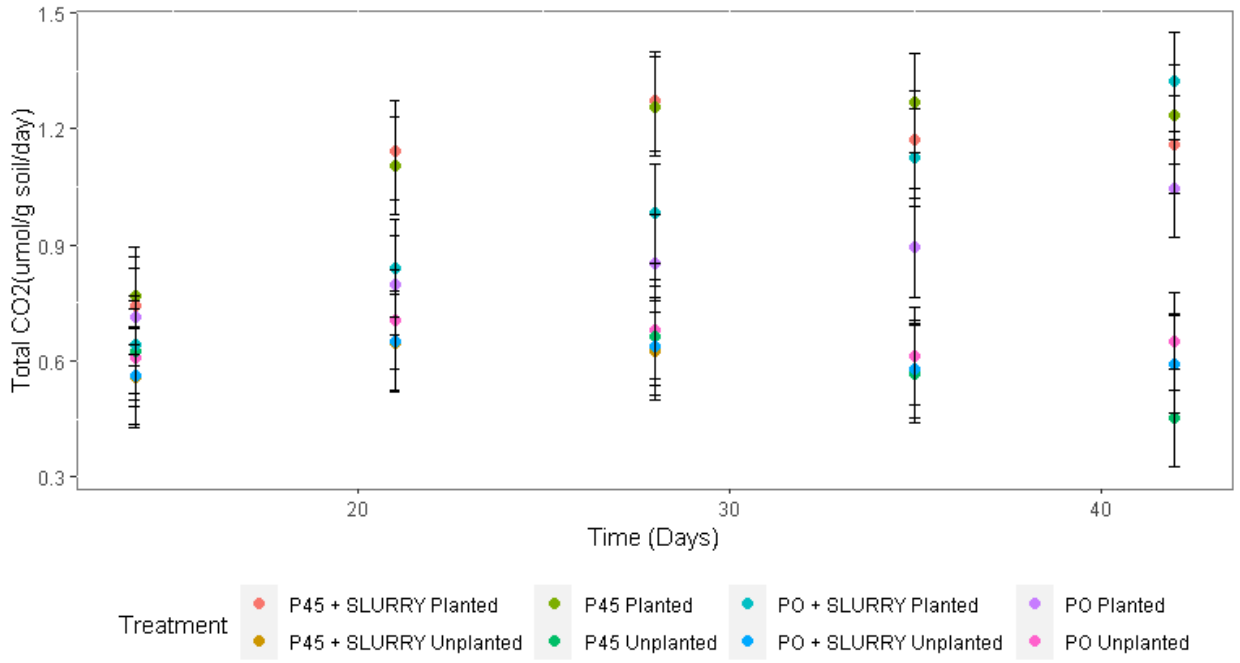


Figure 3.3: Total CO_2 efflux (ppm g^{-1} dry soil $hour^{-1}$) for the duration of the experiment. Colours represent planted or unplanted pots.

SOM-derived CO_2-C is a measure of the decomposition rate of non-plant sources throughout the experiment. Overall, there was a significant effect of treatment ($p<0.04$) and sampling time point ($p<0.01$) on the SOM decomposition rate. The greatest amount of SOM-derived CO_2-C for almost all treatments in the unplanted pots occurred in the middle of the experiment, day 28. This was also true for the P0 and P0 + slurry planted pots which were also at their peak on day 28 (Figure 3.4). However, the planted pots for the P45 and P45 + slurry treatments reached their peak one week earlier on day 21 (Figure 3.4). Sampling time points had significantly different ($p<0.01$) values from each other; these varied from increases in SOM-derived C to decreases in SOM-derived C over time. In the unplanted pots the rate of SOM-derived C remained similar throughout the experiment in both of the slurry treatments. However, the unplanted pots in the P45 and P0 treatment were variable, with a significant decrease in SOM-derived C in the P45 unplanted pot on day 42 ($p <0.01$). In the P45 planted pots, SOM-derived C decreased over time (Figure3. 4). A similar trend was seen in the P45 + slurry treatment, with a significant ($p<0.01$) decrease in SOM-derived CO_2 efflux at the end of the experiment, day 42.

In all treatments there were significant differences in SOM decomposition between planted and unplanted pots, which varied in magnitude over the course of the experiment ($p<0.0001$). There was

significantly less SOM-derived CO_2 efflux in the P45 +slurry planted pot, relative to the P45 + slurry unplanted pot ($p<0.0001$). There was also significantly less SOM-derived CO_2 efflux from the P45 + slurry planted pots than from the P0 planted pots ($p<0.0001$). In the P45 treatment there was significantly less SOM-derived CO_2 efflux in the unplanted pots than in the planted pots ($p<0.0001$). There was a significant difference in the SOM-derived C between P0 planted and P45 planted over time ($p<0.0001$).

Overall, when the data from SOM-derived CO_2 for both planted and unplanted systems were analysed separately, there was a significant effect of slurry addition in both the planted ($p<0.03$) and unplanted pots ($p<0.04$). Only on sampling days 21 and 28 was there no effect of either P or slurry addition. On day 14 there was a significant effect of slurry addition in the lower P treatments ($p<0.01$). On day 35 there was a significant difference between the higher and low P treatments ($p<0.02$). On the final day (day 42) of sampling both slurry addition and P were significant ($p<0.01$).

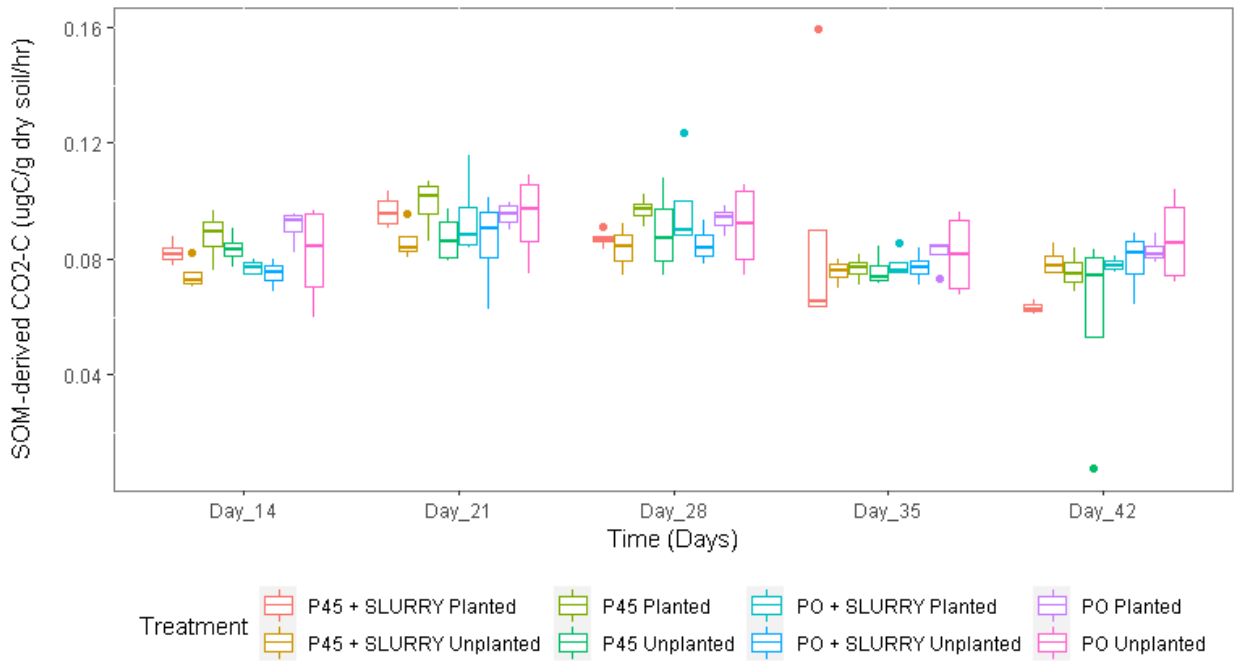


Figure 3.4: Total soil organic matter-derived CO_2-C (ug C g^{-1} dry soil $hour^{-1}$) for the duration of the experiment. Treatments are highlighted with colour, and both planted and unplanted pots are represented in this graph (n=4).

The specific activity of the microbial biomass was measured to assess how active the community was in decomposing SOM (i.e., SOM mineralisation per unit microbial biomass C). There was no significant difference in MBC between the treatments; however, there were significant differences in SOM-derived C ($p<0.01$). Pot type (planted vs unplanted pots) has a significant effect of on the specific activity of the

MBC ($p<0.05$). The unplanted pots had higher absolute SOM-mineralisation activity than the planted pots in all treatments except P45 (Figure 3.5). In the planted pots, P45 had the highest absolute MBC-specific activity, but in the unplanted pots it had the lowest.

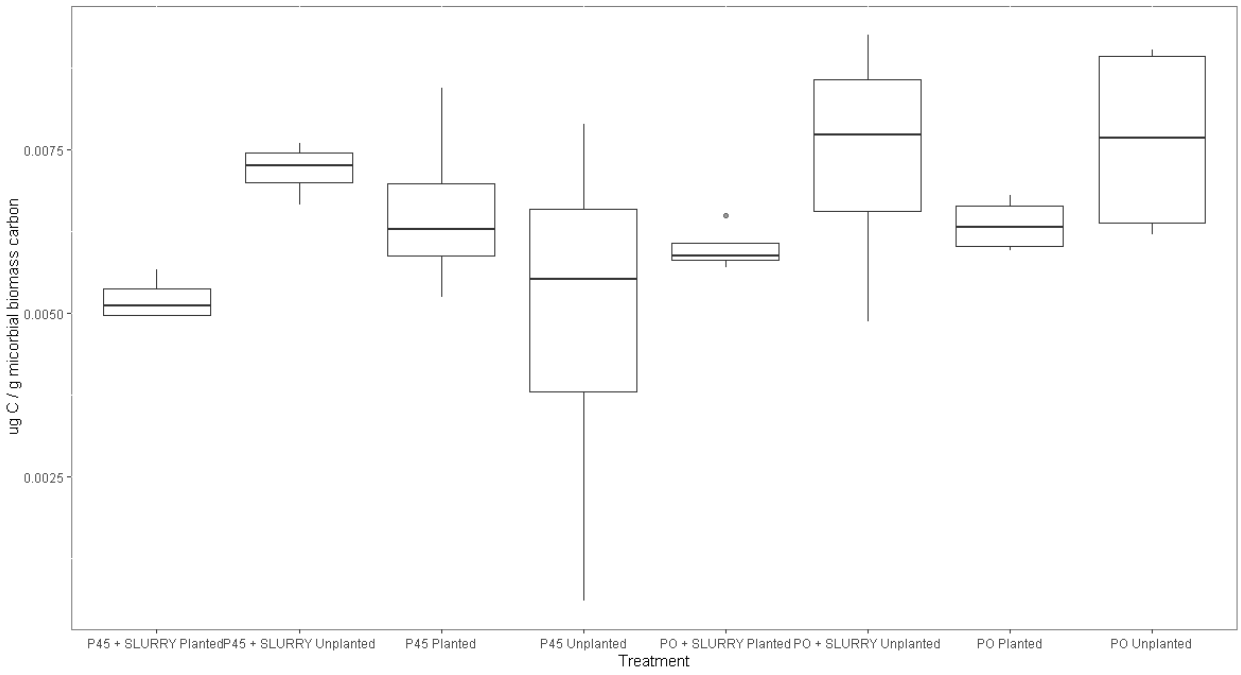


Figure 3.5: Specific activity of microbial biomass carbon. Measured as the SOM-derived carbon divided by the microbial biomass carbon for each treatment. The measurements represented here are from day 42 the final sampling point of the experiment.

3.3.6 Priming

P treatment significantly affected the magnitude (and direction) of SOM priming ($p = 0.03$), and both positive and negative priming responses were evident. On day 14 of the experiment, the P treatments were not significantly different from one another. Midway in this experiment, days 21-35, differences between treatments started were evident (Figure 3.6). On day 35, SOM mineralisation was significantly less ($p<0.05$) for the P45 + slurry treatment compared to all other treatments, including unplanted controls (i.e., exhibiting negative priming). On the final day of sampling, day 42, SOM mineralisation from the P45 + slurry treatment was still significantly lower than all other treatments ($p<0.05$). Also, on the final day of sampling, P45 was significantly higher than all other treatments ($p<0.05$). When looking at the effect of slurry addition (i.e., treatments that received slurry vs treatments that did not), there was a

significant effect of slurry addition on the rate of priming (p=0.03). However, there was no significant effect of priming on potential N mineralisation measured at the end to the experiment (p =0.06).

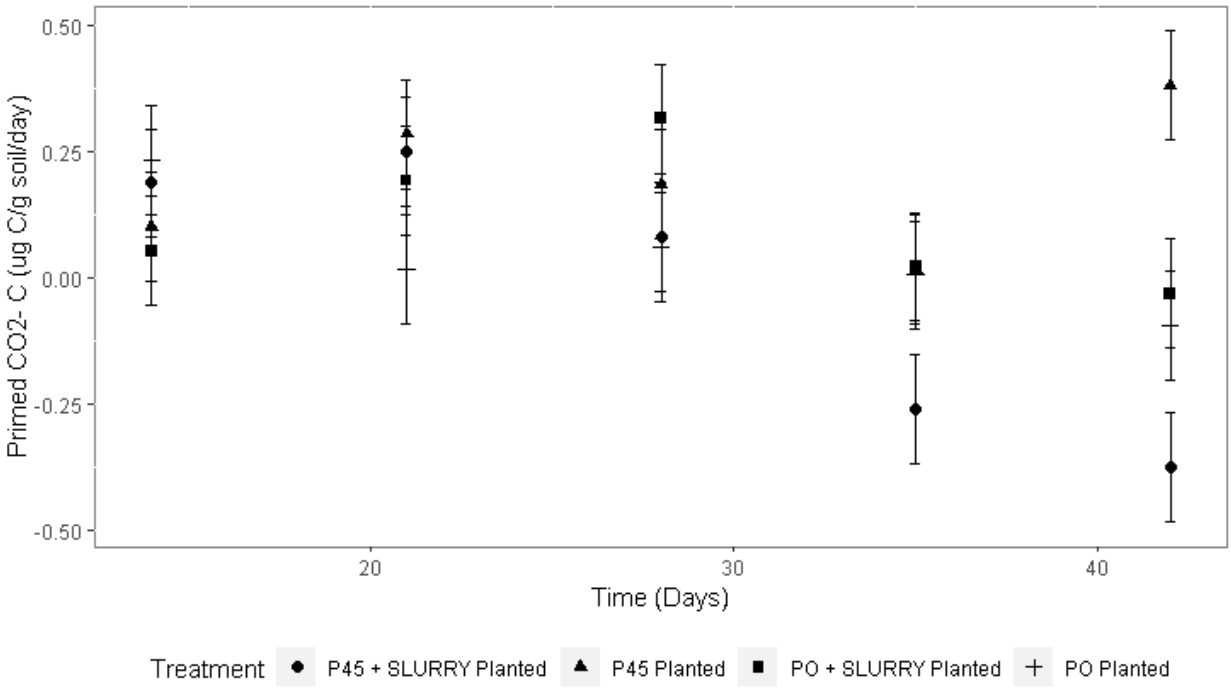


Figure 3.6: Total primed soil organic matter carbon CO_2-C (ug C g^{-1} dry soil $hour^{-1}$) for the duration of the experiment. Shapes represent treatment. Error bars represent standard error (n=4).

3.4 Discussion

This experiment aimed to determine whether there was an effect of soil phosphorus, achieved by varying the levels of added P fertiliser (chemical and organic), from a long-term P trial, on N mineralisation potential and the rate of priming. Both N and P are essential nutrients for plant and microbial activity, and hence when one is in excess, or limiting, it is important to examine the effect of this on microbially-mediated processes of SOM mineralisation and priming, as well as on plant growth. Four phosphorus treatments were selected for this study two high level P treatments, one with a chemical P addition and one with slurry; and two low P treatments, one with no P addition, and the other with a small slurry P top up. Root and SOM -derived respiration measurements were taken over a five-week period in order to non-destructively quantify the priming effect over the course of the experiment. Bulk soil samples were taken

at the end of the experiment to determine if there were any effects of treatments on microbial biomass, soil nutrient status and potential N mineralisation.

3.4.1 Plant growth was affected by soil phosphorus addition

The response of the plant-to-soil P treatment was evident in both the root and shoot biomass. As expected, plant biomass responded to the nutrient status of the soil and the treatments with a higher available P had significantly more biomass on day 42 compared with the treatments with lower available P. The effect of the P treatments was also seen in the root-to-shoot ratios. The soils with less P had a higher root-to-shoot ratio compared to the treatments receiving more P.

The effect on plant growth over the course of the experiment can also be seen in the nutrient analyses of the plant material. The plants with the highest P concentration comprised the higher P treatments, as expected. However, the plants in the higher P45 and P45 + slurry treatments had lower N concentrations in their shoot and root material than the lower P treatments (Table 3.1 and 3.2). This suggests that, although these plants had higher nutrient availability over the course of the experiment, by the end of the experiment they have consumed more N to produce a large plant biomass and available N was beginning to run out. Towards the end of the experiment the P45 and P45 slurry plants had yellowing of the older leaves which is indicative of plant N deficiency.

3.4.2 Nitrogen mineralisation potential is impacted by phosphorus addition

The first hypothesis that N mineralisation potential would be higher when nutrient availability of P was higher was rejected as there was a significant negative effect of increasing soil P on N mineralisation potential. The treatments which received less P, and therefore had lower soil P, had a higher N mineralisation potential. This was particularly evident in the unplanted system where the treatments receiving less P significantly increased N mineralisation potential, but it was also evident in the planted system where P0 slurry had significantly higher N mineralisation potential than P45. The results of this study indicate that a soil's N mineralisation potential was affected by soil P status. Similarly, Akbari *et al.* (2020) found that a buildup of P in a calcareous soil with high P fertiliser addition every year reduced N mineralisation from crop residue. A similar trend was seen in this study, where repeated P application, and hence a build-up of soil P (measured as part of this experiment, see Table 3.3 and 3.4), reduced N mineralisation potential. However, the relationship between the N and P cycles is complex. The reduction in N mineralisation potential with increased P addition could be caused by a shift in the microbial community structure and functioning with increased P availability. Other studies have noted a change in

microbial community and activity with P additions (Cleveland *et al.*, 2002; Raiesi and Ghollarata, 2006). Although microbial community structure was not measured as part of this experiment, a change in microbial community composition, for example a shift in the abundance of N cycling genera and/or microbial activity via increased enzymatic activity, is one possible explanation for the variation seen here in N mineralisation potential. A previous study carried out on this site by Randall *et al.* (2019) found that the fungal community changed with increased P addition. If this was also affecting potential N mineralisation at this site further analysis of the soil microbial community would be required.

Although overall (i.e., all treatments, including unplanted controls) there was no significant effect of slurry addition on potential N mineralisation, there was a significant effect of slurry addition in planted systems. Particularly in the planted treatments that received a higher P addition, slurry addition increased N mineralisation potential measured at the end of the experiment. However, the planted P0 + slurry treatment also had higher N mineralisation potential than the P45 treatment, suggesting that in the planted system a slurry addition could be an important determining factor in N mineralisation potential. Slurry contains a diverse mixture of organic nutrient compounds, including sources of labile C. The increased availability of labile C sources in the slurry-treated, planted pots is one possible explanation for the increase in N mineralisation potential seen in this study. As microbes are often C limited (Schimel and Bennett, 2004) the C sources in organic fertilisers, such as the slurry used in this experiment, could potentially stimulate microbial activity, mineralisation, and increase nutrient cycling in soil (Lazcano *et al.*, 2013; Ling *et al.*, 2016).

3.4.3 Soil organic matter-derived carbon in soil CO_2 efflux is not only altered by the presence of a plant, but also by organic phosphorus addition

The unplanted systems in this experiment showed no effect of differing P treatments, or application type, on the rate of SOM decomposition. In the unplanted pots, although the rate varied between sampling time points within treatments there was no significant difference between the treatments. However, when examining specific microbial biomass activity (i.e., the rate of SOM decomposition per unit microbial biomass), the P45 slurry treatment increased the specific activity on day 42. This suggests that although there was not a significant difference in the decomposition rate overall, there was potentially an effect on the allocation of resources in the unplanted system (slurry treatment may be increasing soil biomass P) produced during the decomposition process. The unplanted systems had a higher specific microbial SOM-mineralising activity compared to the planted system. This could be due to the increased need in the unplanted pots to mine the SOM for C or other nutrients. This might also explain why N mineralisation

potential was higher in the unplanted pots. However, measuring SOM-derived CO_2 efflux this does not include microbial processing of root exudates (i.e., with a plant-derived ^{13}C signature). As microbial biomass size was not significantly affected by the presence of a plant, less SOM would be needed to maintain the microbial biomass size when there was co-utilisation of root exudates. This result suggests that part of the microbial activity during the course of the experiment was supported by the plant through root exudation use, but this was not associated with increased activity in mineralisation of SOM (positive priming), rather root exudates were used in preference to SOM (preferential substrate use, negative priming).

Previous studies have shown that the addition of an organic fertiliser such as slurry increases soil respiration (Cui and Holden, 2017, Sorensen, 1998). In this experiment the total respiration rate was not significantly different between the P45 and P45 slurry treatment. However, slurry was applied in the field and not directly to the pots themselves, and slurry mineralisation prior to sampling, or during soil preparation, was not captured in the experiment. Another factor was that, when soil samples were taken in March 2019, Ireland was experiencing dry weather conditions and as such the slurry applied may not have had time to incorporate fully at that time. However, it has been reported that regular application of slurry, such as occurred in soils used in this experiment, results in 34-38% of fresh slurry C remaining in soil for 12 weeks or more after decomposition (Sorensen, 1998). This is important to note for this experiment as when measuring SOM derived C there was no isotopic label to distinguish between SOM-C and slurry-C. Therefore, the measure of SOM-C reported here would also include the decomposition of slurry-derived C sources.

The addition of slurry had a significant effect on SOM mineralisation in planted systems. Particularly towards the end of the experiment, the P45 + slurry treatment had a lower decomposition rate than all other treatments. The P45 + slurry treatment, which received the most slurry addition of any of the treatments had a significantly lower SOM decomposition rate over the course of the experiment than the P45 treatment. This highlights that the type of fertiliser application is important when considering the amount of C in SOM that is being broken down. Despite both P45 and P45 +slurry treatments being nutrient rich, there is a clear distinction in SOM decomposition rate; on each sampling day except day 35 the SOM decomposition rate was higher in the P45 + slurry treatment. This may suggest that the P45 treatment is mining SOM for other nutrients that both plant and microbial community may be limited in, as the plant continued to grow. In particular N may be limiting, as available soil N in the P45 treatment on day 42 was depleted (see Table 3.3 for soil nutrient results in the planted pots). Nutrient availability has

been shown to affect decomposition rates in other studies (Kuzakov 2010; Dijkstra *et al.*, 2013; Murphy *et al.*, 2017). If all freely-available nutrients have been used previously by the plant or immobilised into microbial biomass, this may promote mineralisation of SOM stocks to mobilise limiting nutrients. This phenomenon has been observed and is termed as the microbial *N mining hypothesis* (Craine et al., 2007, Moorhead & Sinsabaugh, 2006). The microbial *N mining hypothesis* considers that when N is limiting, microbes use labile C sources, such as plant root exudates, to provide energy required to decompose organic N sources in SOM (Craine et al., 2007, Moorhead & Sinsabaugh, 2006). Although the P45 treatment is nutrient rich, there is no source of slow released nutrients outside of the SOM pool. Even though the plants in the P45 treatment had more nutrient supply throughout, it seems these nutrients had been exhausted by the end of the experiment, as is seen in the low concentration of N in the plant material at final measurement (Table 3.1 and 3.2). In contrast, slurry treatments at higher P application seem to be satisfying the needs of the plants and microbial community more effectively than the mineral fertiliser P application, and hence SOM decomposition rates are lower.

3.4.4 Phosphorus treatment affects the rate and direction of SOM priming

Continuous ^{13}C-labelling was used to quantify and partition CO_2 efflux from plant and soil derived sources, and hence calculate the rate of priming as described in Paterson *et al.* (2007) and more recently by Murphy *et al.* (2017). The rhizosphere priming effect was impacted by both P treatment applied to the soil (i.e., P0 or P45), and the type of application (i.e., inorganic or organic fertiliser). The primed response was larger in the P treatments with more P (P45 and P45 slurry) compared with the treatments with less P (P0 and P0 slurry). This could be due in part to the size of the root system, as in this study the P0 treatments had a smaller root biomass than the P45 treatments. As the plants themselves were smaller in the P0 treatments, perhaps their demand for nutrients was also lower meaning they were less likely to mine SOM for their N requirement. On the other hand, it could also be that the P0 treatment is highly P limited and so there is less demand for N, but when P is abundant then N becomes limiting. There is a higher stoichiometric demand on the soils with a higher amount of P and therefore there is a larger response of the soil microbial community to balance out the C: N: P ratios (Griffiths *et al.*, 2012). It has also been suggested that in P-limited systems such as the P0 treatments found in this study, it may be difficult to observe the potential primed response of the system. This is because organic P is broken down by hydrolysis and hence is not oxidised releasing CO_2 (Dijkstra *et al.*, 2013) and McGill and Cole 1981.

The second element which is affecting the rate of priming is the type of P addition. The addition of a chemical P fertiliser resulted in a significant positive, plant-mediated priming effect. This is where root

exudates are being used to break down SOM. On the other hand, when P is added in an organic slurry form, the end result was negative priming where there was reduced breakdown of the SOM. For negative priming, soil microbes are considered to be using a more readily available C source in preference to SOM. In this experiment this readily available C source could be coming from either the plant, or alternatively from the C sources found within slurry itself. However, if this was the case the use of slurry C would still be measured as priming as the slurry and SOM-derived C have the same isotopic signature, so this is unlikely to be the case. Negative priming is normally found in systems were nutrients such as N, and P, as well as C are abundant (Kuzyakov and Bol, 2006; Murphy *et al.*, 2017). The addition of slurry could be creating the conditions that would satisfy the *preferential substrate hypothesis*. This is because the addition of a rich nutrient source to soils when nutrient availability is high causes a negative priming response as microbes switch from decomposing recalcitrant SOM C sources to using the more freely available labile C source (root exudates) as their primary energy source (Cheng, 1999; Blagodatskaya *et al.*, 2007; Guenet *et al.*, 2010). If the slurry addition had a higher N concentration compared to SOM, then less of the slurry would need to be mineralised compared to the SOM, to mobilise the same amount of N, resulting in less C mineralised. As well as this a lower C-N ratio (and a more labile source of nutrient) of slurry would be expected to increase microbial C use efficiency resulting in less respiration per unit C consumed. It is possible that there was a switch from positive to negative priming in this system as the slurry C was beginning to run out and that the microorganisms needed a fresh C supply to further break down the nutrients in the slurry. Future studies could apply a labelled slurry source to examine which C source is being used in this system.

Both P45 and P45 + slurry treatments resulted in soils with high P availability. However, the P45 inorganic treatment showed a positive priming effect and the P45 + slurry organic treatment showed a negative priming effect. These treatments have been under this management system in the field for several years and hence their microbial communities should be well adapted to the type of nutrient supply they received. Although not measured as part of this study, it could be hypothesised that different microbial community structures are driving the different response of these systems. It has been noted previously that changes in microbial communities can influence rhizosphere priming effects (Fontaine *et al.*, 2003). The addition of a slurry treatment may have promoted faster growing microbes (r-strategists) whereas high and freely available nutrients in the P45 treatment could have promoted slower growing microbes (K-strategists). Along with this, the demand to mine nutrients by the microbial community could be higher in the P45 treatment, as inorganic fertilisers are not held for long in soil systems before being lost to the

environment in greenhouse gases such as nitrous oxide or nitrate and phosphate leaching to waterways (Hati and Bandyoopadhay, 2011; Bodirsky *et al.*, 2014). Despite no difference in the quantity of nutrients applied to the higher P treatments and no significant difference in SOM contents, the P45 treatment had a higher SOM decomposition rate than the P45 slurry treatment. The increased release of CO_2 as part of positive priming when applying inorganic fertiliser compared to the negative priming and decreased CO_2 release of the treatment that received some organic fertiliser is an important policy consideration in the context of C storage in soils and also greenhouse gas emission under different soil nutrient management practices.

3.5 Conclusion

Nitrogen mineralisation potential was higher in the treatments with lower P. N mineralisation was also significantly affected by the type of P addition, N mineralisation was higher in organic treatments. The rate of priming was affected by the type of P addition in this study. Treatments with lower P primed less then treatments with higher P. The P treatments however, also effected the size of the plant biomass both above and below ground. The treatments with high plant biomass showed a stronger priming effect. The addition of an organic form of P reduced the amount of SOM breakdown, and also changed the direction of priming from positive to negative in the higher P treatment. Therefore, there was less C broke down and released in the P45 +slurry treatment. This could have implications for C storage in grassland based agricultural system. Perhaps the addition of organic nutrient source to grasslands could reduce both C loss and inorganic fertiliser use, both of which have positive impacts on reducing GHG emissions and increasing agricultural sustainability.

3.6 References

Akbari, F., Fallah, S., Dahmardeh, M., Pessarakli, M., 2020. Interaction effects of nitrogen and phosphorus fertilizer on nitrogen mineralization of wheat residues in a calcareous soil. *Journal of Plant Nutrition* 43, 1-12.

Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology* 37, 95-105.

Blair, N., Faulkner, R.D., Till, A.R., Poulton, P., 2006. Long-term management impacts on soil C, N and physical fertility: Part I: Broadbalk experiment. *Soil and Tillage Research* 91, 30-38.

Bodirsky, B.L., Popp, A., Lotze-Campen, H., Dietrich, J.P., Rolinski, S., Weindl, I., Schmitz, C., Müller, C., Bonsch, M., Humpenöder, F., Biewald, A., Stevanovic, M., 2014. Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nature Communications* 5, 3858.

Boilard, G., Bradley, R.L., Paterson, E., Sim, A., Brown, L.K., George, T.S., Bainard, L., Carubba, A., 2019. Interaction between root hairs and soil phosphorus on rhizosphere priming of soil organic matter. *Soil Biology and Biochemistry* 135, 264-266.
Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H.W., Van Vuuren, D.P., Willems, J., Rufino, M.C., Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences* 110, 20882-20887.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry* 17, 837-842.
Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* 14, 319-329.
Cheng, W., 1999. Rhizosphere feedbacks in elevated CO_2. *Tree Physiology* 19, 313-320.
Cleveland, C.C., Townsend, A.R., Schmidt, S.K., 2002. Phosphorus Limitation of Microbial Processes in Moist Tropical Forests: Evidence from Short-term Laboratory Incubations and Field Studies. *Ecosystems* 5, 0680-0691.
Dijkstra, F., Carrillo, Y., Pendall, E., Morgan, J., 2013. Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology* 4.
Dinesh, R., Srinivasan, V., Hamza, S., Manjusha, A., 2010. Short-term incorporation of organic manures and biofertilizers influences biochemical and microbial characteristics of soils under an annual crop [Turmeric (Curcuma longa L.)]. *Bioresource Technology* 101, 4697-4702.
Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biol. *Biochem*. 35, 837-843.
Gaidajis, G., Kakanis, I., 2021. Life Cycle Assessment of Nitrate and Compound Fertilizers Production—A Case Study. *Sustainability* 13, 148.
Gong, W., Yan, X., Wang, J., Hu, T., Gong, Y., 2009. Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat–maize cropping system in northern China. *Geoderma* 149, 318-324.
Guenet, B., Neill, C., Bardoux, G., Abbadie, L., 2010. Is there a linear relationship between priming effect intensity and the amount of organic matter input? *Applied Soil Ecology* 46, 436-442.
Hati, K., Bandyoopadhay, K., 2011. Fertilizers (Mineral, Organic), Effect On Soil Physical Properties. In: Gliński, J., Horabik, J., Lipiec, J. (Eds.), *Encyclopedia of Agrophysics*. Springer Netherlands, Dordrecht, pp. 296-299.
Griffiths, B.S., Spilles, A., Bonkowski, M., 2012. C:N:P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. *Ecological Processes* 1, 6.
Kuzyakov, Y., Bol, R., 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biology and Biochemistry* 38, 747-758.
Lazcano, C., Gómez-Brandón, M., Revilla, P., Domínguez, J., 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. *Biology and Fertility of Soils* 49, 723-733.
Ling, N., Zhu, C., Xue, C., Chen, H., Duan, Y., Peng, C., Guo, S., Shen, Q., 2016. Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. *Soil Biology and Biochemistry* 99, 137-149.
Massey, P.A., 2012. *Interactions between phosphorus fertilisation and soil biota in managed grasslands systems*. Doctor of Philosophy, Cranfield.

Massey, P.A., Creamer, R.E., Whelan, M.J., Ritz, K., 2016. Insensitivity of soil biological communities to phosphorus fertilization in intensively managed grassland systems. *Grass and Forage Science* 71, 139-152.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. *Plant and Soil* 417, 499-510.
Ning, C.-c., Gao, P.-d., Wang, B.-q., Lin, W.-p., Jiang, N.-h., Cai, K.-z., 2017. Impacts of chemical fertilizer reduction and organic amendments supplementation on soil nutrient, enzyme activity and heavy metal content. *Journal of Integrative Agriculture* 16, 1819-1831.
Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytologist* 173, 600-610.
R Core Team (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing*, Vienna, Austria. URL https://www.R-project.org/.
Raiesi, F., Ghollarata, M., 2006. Interactions between phosphorus availability and an AM fungus (Glomus intraradices) and their effects on soil microbial respiration, biomass and enzyme activities in a calcareous soil. *Pedobiologia* 50, 413-425.
Randall, K., Brennan, F., Clipson, N., Creamer, R., Griffiths, B., Storey, S., Doyle, E., 2019. Soil bacterial community structure and functional responses across a long-term mineral phosphorus (Pi) fertilisation gradient differ in grazed and cut grasslands. *Applied Soil Ecology* 138, 134-143.
Schimel, J.P., Bennett, J., 2004. NITROGEN MINERALIZATION: CHALLENGES OF A CHANGING PARADIGM. *Ecology* 85, 591-602.
Schröder, J.J., Schulte, R.P.O., Creamer, R.E., Delgado, A., van Leeuwen, J., Lehtinen, T., Rutgers, M., Spiegel, H., Staes, J., Tóth, G., Wall, D.P., 2016. The elusive role of soil quality in nutrient cycling: a review. *Soil Use and Management* 32, 476-486.
Sheil, T.S., Wall, D.P., Culleton, N., Murphy, J., Grant, J., Lalor, S.T.J., 2016. Long-term effects of phosphorus fertilizer on soil test phosphorus, phosphorus uptake and yield of perennial ryegrass. *The Journal of Agricultural Science* 154, 1068-1081.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry* 19, 703-707.
Van Grinsven, H., Ten Berge, H., Dalgaard, T., Fraters, B., Durand, P., Hart, A., Hofman, G., Jacobsen, B.H., Lalor, S.T., Lesschen, J.P., 2012. Management, regulation and environmental impacts of nitrogen fertilization in northwestern Europe under the Nitrates Directive; a benchmark study. *Biogeosciences* 9, 5143-5160.
Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Dietrich, M., Herbold, C.W., Eichorst, S.A., Woebken, D., Richter, A., 2019. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil Biology and Biochemistry* 136, 107521.

Chapter 4

The effect of grazing and organic amendment on soil organic matter nitrogen mineralisation potential

4.1 Introduction

Nitrogen (N) is a limiting nutrient in many terrestrial ecosystems (Vitousek and Howarth, 1991). Soil organic matter (SOM) is the largest soil store of N; however, organic forms of N in SOM are not directly available for plant uptake (Leinweber *et al.*, 2013; Sun *et al.*, 2017). Transformation of these organic N forms to inorganic N is a microbially-mediated process, known as mineralisation. Although plant growth is often limited by N, it is seldom limited by carbon (C) as, given sufficient light and water, photosynthesis supplies the plant C requirement (Farrell *et al.*, 2014). However, microbes in soils are often C limited (Schimel and Bennett, 2004), as a large proportion of SOM is physically protected (associated with mineral surfaces, or within aggregates) and/or chemically recalcitrant. It is essential for plant growth for plants to be able to access nutrients in SOM, through soil microbial processes such as N mineralisation (Phillips *et al.*, 2011), and understanding these processes would allow better matching of fertilisation rates with plant demand, reducing environmental impact and economic costs. Plants promote microbial processes in the rhizosphere through root exudation of labile C compounds that are easily accessible to soil microorganisms (i.e. priming) and hence can stimulate microbial activity, such as enzyme production, which increases mobilisation of nutrients from organic compounds to mineral forms available to plants (Kuzyakov, 2010; Phillips *et al.*, 2013; Sun *et al.*, 2017). If soil fungal biomass contains more C than bacterial biomass (i.e. the C: nutrient ratio is higher in fungi), this would imply that fungi are more likely to be C limited due to their higher C demand, although bacteria are limited more by other nutrients such as N and P (Keiblinger *et al.*, 2010). A consequence of this is that fungi can maintain growth with a lower nutrient availability, relative to bacteria (Keiblinger *et al.*, 2010, Žifčáková *et al.*, 2016; Frąc *et al.*, 2018). Soil microorganisms are an essential part of grassland ecosystems particularly because of their involvement in nutrient cycling, supporting plant productivity; it is important to understand how management practices impact microbial community structure and functioning (Dengler *et al.*, 2014).

In grassland agricultural systems, plant nutrient limitations are often overcome by the addition of nutrients in the form of inorganic mineral fertilisers (Schröder *et al.*, 2016) or organic fertilisers (slurry or farmyard manure) (Liu *et al.*, 2017). However, the addition of nutrients to soil will not only encourage plant growth but also affect soil microbial nutrient cycling, growth, and activity (Griffiths *et al.*, 2012; Liu *et al.*, 2017). In managed agricultural systems, the stoichiometry of the soil microbial biomass can be changed by the addition of nutrients (Griffiths *et al.*, 2012). As the balance of nutrient limitation on the soil microbial community changes, this will shift microbial resource allocation as they

scavenge for the limiting nutrient (Cleveland *et al.*, 2002; Cleveland and Liptzin, 2007). A study carried out by Yin *et al.* (2014) found that even minor C additions from root exudation can have notable effects on N cycling, particularly in ecosystems were there are limited soil nutrients. Nottingham *et al.* (2018) found that, when P was added in combination with other nutrients, bacteria out-competed fungi for N. Hence, the addition of nutrients in large quantities, as may occur in agricultural systems, is likely to cause changes to microbial community composition, activity and microbially-mediated nutrient cycling (Fang *et al.*, 2020). The addition of organic fertilisers affects biological processes in soils, particularly as they contain a diversity of labile and more recalcitrant organic compounds (Dickson et al., 1981, Liu et al., 2017). Changes in nutrient availability has knock-on effects on a range of soil microbial functions and mechanisms, including those involved in nutrient cycling processes such as SOM mineralisation and priming (Sullivan & Hart, 2013, Chowdhury et al., 2014, Stewart et al., 2015, Fang et al., 2020).

Both N and phosphorus (P) cycling in grazed grasslands are influenced by root-microbe interactions (Sun *et al.*, 2017). In grazed grasslands, livestock influence soil nutrient availability (Bardgett *et al.*, 2005; Hamilton *et al.*, 2008; Sun *et al.*, 2017). This can occur in two ways: firstly, the addition of organic nutrients through livestock excreta (Olsen *et al.*, 2011); and, secondly, as livestock graze the grass sward this promotes root exudation which encourages soil C turnover. Both of these pathways promote positive feedback between aboveground (plant and livestock nutrition) and belowground (plant-microbe interactions) (Bardgett and Wardle, 2003; Hamilton *et al.*, 2008). Sun *et al.* (2017) found that after grazing the increased release of labile C from plant root exudates influenced enzyme activity, increased soil N mineralisation, and plant nutrient intake. The increase in plant nutrient uptake found by Sun *et al.* (2017) suggests that after grazing, as a result of increased root exudation and subsequent microbial activity, there was a higher N and P mineralisation. This benefits both the plant and the grazing livestock, as their respective nutritional needs are met, resulting in a positive root-microbe feedback within the ecosystem (Sun *et al.*, 2017). However, it is important to note that stocking density plays a key role in these feedback systems. If there is heavy or intensive grazing, plant growth, particularly of roots, is severely limited, and hence rates of root exudation per plant are reduced (Parsons *et al.*, 2011; Parsons *et al.*, 2013). However, grassland systems are not always grazed; in some management systems grass is cut and removed for winter feed as silage or hay. Therefore, even though grazing removes grassland vegetation it does so at a lower and spatially more variable rate than a homogeneous cut and remove silage-based system; this, coupled with the excretal returns from the livestock, allows grazing-based systems to have a more significant nutrient reintroduction pathway belowground (Rumpel *et al.*, 2015; Randall *et al.*, 2019). In a silage-based system the nutrient-rich plant material is completely removed and not returned to soil. The effect of cutting and

removing aboveground vegetation, as opposed to animal grazing, in grassland systems is an important distinction as clipping is often used as an experimental treatment to simulate grazing (Hamilton III and Frank, 2001; Murphy *et al.*, 2017). These studies found increases in rhizosphere processes such as SOM-C decomposition and N availability with clipping (Hamilton III and Frank, 2001; Murphy *et al.*, 2017). However, the direct comparison between a cut and a grazed grassland and management effects on rhizosphere process and microbially-mediated nutrient cycling is relatively understudied. Therefore this study has three interlinked research strands, namely to: a) investigate how the addition of C, phosphorus (P) management, and grassland management strategies effects microbial SOM decomposition and N mineralisation in a soil environment of high and low nutrient availability; b) examine how long-term P addition compared with long-term P limitation affected microbial community structure and microbial nutrient cycling activity in terms of SOM decomposition and potential N mineralisation; and c) to explore the effect of a cut and remove silage-based system with a grazed grassland in terms of the belowground effects on SOM-C decomposition, potential N mineralisation, and microbial community structure

Grassland management strategies through the addition of nutrients, inorganic and organic, along with cutting for silage or grazing for livestock affect both plant and microbial communities (Grayston *et al.*, 2004), as well as nutrient cycling and organic matter quality and quantity (Poeplau, 2021). It was therefore hypothesised that the addition of P compared to treatments that had not received P over several years would have a higher potential N mineralisation, a higher rate of SOM-C decomposition and priming, and that there would be impacts on both bacterial and fungal community structure. It was further hypothesised that: the type of P addition would also affect these factors; the addition of an organic source of P in the form of slurry would lead to an increase potential N mineralisation, a higher rate of SOM-C decomposition and priming; and that the form (mineral or organic) of fertilisation would impact both bacterial and fungal community structure. It was also hypothesised that the type of grassland management would affect microbially-mediated nutrient cycling – specifically, that in a cut and remove for silage system there would be increased SOM-C decomposition and potential N mineralisation. These hypotheses were tested using grassland soils from an experimental platform under long term P management treatments.

4. 2 Methods

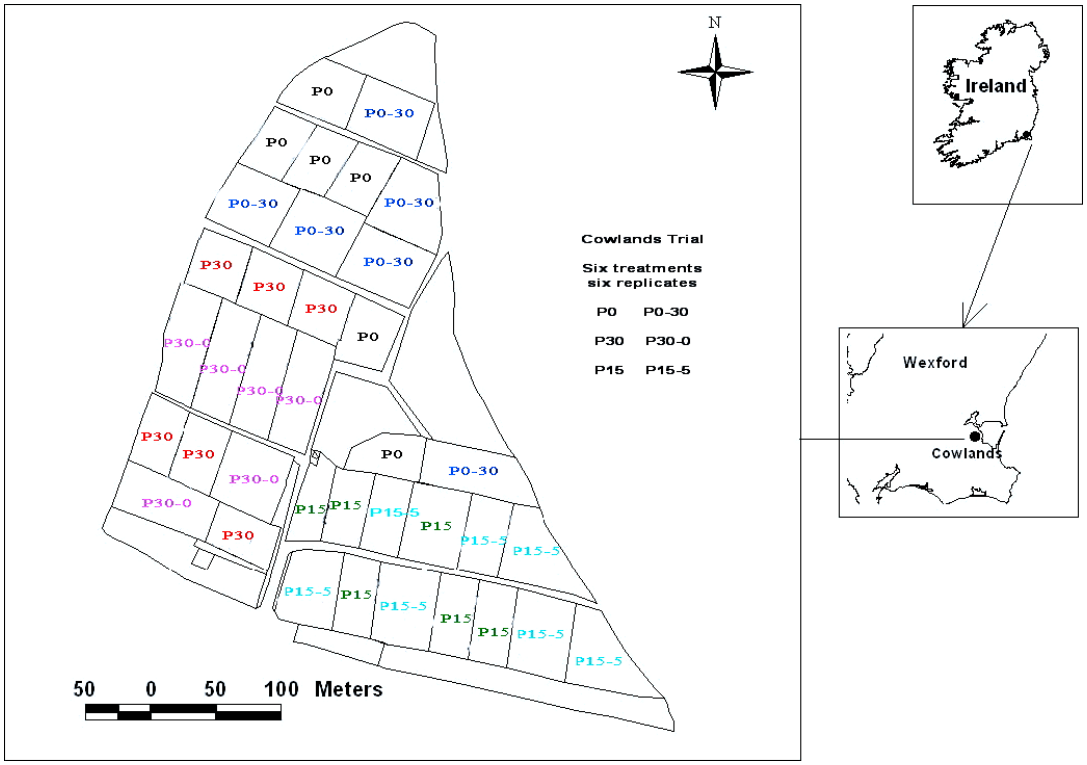
4.2.1 Site description and sample collection

There were two sampling sites (cut and grazed) used in this study, both were under a long-term P management trial and both sites are located at the Teagasc Johnstown Castle Research Centre. The cut site is located on the Johnstown Castle, Co. Wexford, Ireland, dairy farm [52^{o} 17'N, 06^{o} 30 W] (Daly

and Casey, 2005; Randall *et al.*, 2019). This site operates a cut and remove grassland system (equivalent to silage). The site was first set up in 1995 on a loam texture soil under *Lolium perenne* monoculture, in a fully randomised and replicated (n=4) block design (Massey, 2012; Randall *et al.*, 2019). The plant biomass is harvested eight times per year. After each harvest, all plots receive 40kg N ha^{-1} as calcium ammonium nitrate (CAN), and potassium (as potash) at a rate of 125kg K ha^{-1} $year^{-1}$ (Massey, 2012). For the P treatments, there was an application of 16% superphosphate at fertilisation rates of 0 (P0), 15 (P15), 30 (P30) and 45 (P45) kg P ha^{-1} yr^{-1} with 16 plots in total (Sheil *et al.*, 2016; Randall *et al.*, 2019). For this experiment, only P0 and P30 were sampled (Figure 4.1 and Table 4.1). In 2016, the plots were split to include a slurry treatment. Cattle slurry was applied at a rate of 37432kg ha^{-1} (Massey *et al.*, 2016). This added another four replicate treatments with fertilisation (via slurry) rates of 0 (P0 + slurry), 15 (P15 + slurry), 30 (P30 + slurry) and 45 (P45 + slurry). For the purpose of this study the P0 + slurry and the P30 + slurry treatments were selected (Figure 4.1 and Table 4.1).

The grazed site is located at Johnstown Castle, Co. Wexford, Ireland, dairy farm 52° 16 N, 06° 30 W (Tunney et al, 2010). There have been many studies that have described this site to date (Culleton et al, 2002; King-Salter, 2008(Griffiths *et al.*, 2012; Chen *et al.*, 2014; Randall *et al.*, 2019). Briefly, this site was first set up in 1968, sown with *Lolium perenne* (same species as is at the cut site). The soil is a Humic Gleysol (IUSSWRB, 2015). There were three P treatments: P0, P15 and P30. There were 4 plots per P application, and each plot also received 250kg ha^{-1} $year^{-1}$ N as ammonium nitrate and K 20kg ha^{-1} as KCl. From 1999 onwards the grazing stocking rate was (3300kg stock ha^{-1}). In this study only the P0 and P30 plots were sampled, in order to match the P treatments of the cut site.

Samples were collected from each of the six selected treatments (Table 4.1). At both the grazed and cut sites, each of the four replicate paddocks was sampled using bulk soil sampling to a depth of 10cm in a “W” across each paddock. Samples from each of the four replicates were homogenised to form one representative sample for each of either the grazed or cut treatments. Samples were brought back to the lab, sieved to 5mm and stored at 4°C.



108- P15 incl Slurry	107- P15	106 - P0* + slurry	105 - P0	104 - P45 incl slurry	103- P45	102 - P30 incl slurry	101 - P30	Block 1
208 P45 incl slurry	207 - P45	206 - P30 incl slurry	205 - P30	204 - P15 incl slurry	203 - P 15	202 - P0* + slurry	201 - P0	Block 2
308 - P15 incl slurry	307 - P15	306 - P0* + slurry	305 - P0	304 - P30 incl slurry	303 - P30	302 - P45 incl slurry	301 - P45	Block 3
408 - P30 incl slurry	407 - P30	406 - P45 incl slurry	405 - P45	404 - P0* + slurry	403 - P0	402- P15 incl slurry	401- P15	Block 4

Figure 4.1: Layout of both cut and grazed experimental sites at Johnstown Castle, Co. Wexford, Ireland, research centre. The top graph represents the grazed site. The grid represents the cut site.

Table 4.1: Summary of treatments used in this study.

Treatment	Site	P level	Slurry Addition	Grazing
P0 cut	Cut site	No P	No	No
P30 cut	Cut site	High P	No	No
P0 Slurry	Cut site	No P	Yes	No
P30 slurry	Cut site	High P	Yes	No
P0 grazed	Grazed	No P	No	Yes
P30 grazed	Grazed	High P	No	Yes

4.2.2 Soil nutrient analyses

Soil nutrient analyses were carried out as previously described in Chapter 3 section 3.2.6.

4.2.3 Soil microbial biomass

Soil microbial biomass C, N, and P were measured as part of this study. Analysis was carried out as described in Chapter 3 section 3.2.4

4.2.4 Seven-day anaerobic incubation

A seven-day anaerobic incubation assay was used to measure N mineralisation potential in this study. Methods for this assays are previously described in Chapter 3 section 3.2.3.

4.2.5 Microcosm set up and 13C Glucose addition

The design used in this experiment has been previously described by Paterson et al (2007) and Murphy et al (2015). The experimental units (troughs) used were made of PVC tubing (100mm length and 50mm diameter), cut longitudinally (Figure S4.1 supplementary material). The troughs were covered during the experiment with a PVC cover plate drilled with 3mm holes; this allowed air flow and reduced moisture loss during the experiment. All soils were packed into the troughs at a bulk density of 0.9g cm^3 and moisture content was adjusted so that all treatments had a 65% water holding capacity (WHC). There were four replicate troughs for each treatment. The troughs were placed into an incubator at 20^oC for the duration of the experiment. Troughs were watered daily to a predetermined mass to maintain soil water content. The troughs were incubated for one week prior to the experiment as a stabilisation period. To measure if the soils had stabilised respiration, measurements were taken daily (method below).

Following stabilisation, the glucose treatment was applied. There were two sets of troughs (each treatment, four replicates) one set received ^{13}C glucose and the other control did not receive glucose. The ^{13}C glucose was added as a solution, 0.5mg g^{-1} dry soil, as previously used by Murphy et al, 2015 and Paterson & Sim, 1999. This rate was selected here and in previous studies as it mimics known root exudation of grasslands and is within the range that would cause a primed response (Paterson & Sim, 2013). The glucose solution used for this experiment had a 3% atom enrichment. Glucose was added every second day (six times) over two weeks, with added water required to maintain 65% WHC.

4.2.6 Respiration Measurements

Soil respiration measurements were taken after each ^{13}C glucose addition. Respiration measurements were taken from all treatment replicates. Microcosms were placed into a 1L Kilner glass jar, the jar was sealed airtight with a fitted lid with two 3-way valves. The jars were flushed with CO_2 free air for

5 minutes at a flow rate of 800ml min^{-1}. The gas flow was measured on a gas analyser (EGM^{-4}, CO_2 monitor, PP systems) until the CO_2 concentration in the headspace was less than 10µL L^{-1}. The jar was then sealed and incubated for 1 hour at 20°C. Thereafter, a sample (24ml) was taken from the jar using a gas syringe. Fourteen ml of the sample was injected into the gas analyser (EGM^{-4}), PP – systems, Amesbury, USA) to measure the CO_2 efflux from soil (reference gas used for calibration, BOC 450 µL L^{-1} carbon dioxide certified gas). Then 10ml was injected into a 12ml gas vial (Labco) and sent for ^{13}C-CO_2 isotopic gas analysis. For $^{13}C/^{12}C$ isotope analysis, the gas samples were measured on an isotope gas bench ($Delta^{plus}$ advantage, Thermo Scientific Bremen Germany). Reference gases (IAEA reference material NBS 19 TS-limestone) were used to calibrate the instrument, prior to each run. Within each sample run, a quality control standard ($^{13}CO_2$ 0.24‰ ± SD of the mean) was included. Each sample was sampled eight times with four of the values taken to give a single end value.

Partitioning of the CO_2 fluxes was required in order to be able to calculate fluxes from SOM decomposition and priming. The partitioning equation used was described in previous work by (Garcia-Pausas and Paterson, 2011). The total CO_2 efflux from each sample was partitioned into glucose and soil-derived components using the following mass balance equation:

CO_2glucose = (δ13C sample − δ13C control)/ (δ13C glucose − δ13C control)

Where:

CO_2glucose = the total CO_2 derived from glucose

δ13C sample = $\delta^{13}C$-CO_2 signature of the measured gas sample at each time point

δ13C control = the average $\delta^{13}C$-CO_2 signature from the control treatments at each sampling time point

δ13C glucose = $\delta^{13}C$ signature of the glucose added to the troughs

4.2.7 DNA extraction

At the end of the sampling period, the troughs were harvested and all soil was collected for nutrient analysis. A subsample was frozen at -80 °C for molecular work. DNA was extracted using a modification of the Griffiths *et al.* (2000) method. A sample of 0.5g of soil was extracted from each trough (n=48). After soil was weighed it was placed into a Lysing Matrix E tube (MP Biomedicals, USA), with 0.5ml of CTAB buffer (equal volumes of 10% (w/v)) hexadecyltrimethylammonium bromide (Merck Ireland) in 0·7 mol l^{-1} NaCl (Promega, Southampton, UK) and 240 mmol l^{-1} potassium phosphate, pH 8·0 (Sigma)).

0.5ml of liquid Phenol:Chloroform:Isoamyl alcohol (24:1)(Sigma-Aldrich) was also added. Tubes were then placed in the Prep-24 (MP Biomedicals, USA) for 15 seconds at 5m/s and subsequently centrifuged using a refrigerated micro-centrifuge (Eppendorf) at 13,000rpm for 20 minutes at 4^{o}C. The aqueous layer was then removed and placed into a sterile 1.5ml microcentrifudge tube. To remove any remaining phenol, 0.5ml of Chloroform:Isoamyl (24:1, Merck Ireland) was added and the tubes were inverted several times. The tubes were then centrifuged for 5 minutes at 13rpm at 4^{o}C. Again, the top aqueous layer was removed and placed into a sterile 1.5ml microcentrifudge tube. Two volumes of 30% PEG solution (1ml total) were added to each tube and mixed well to precipitate the DNA. The tubes were then left on ice for 2 hours. After this time, tubes were centrifuged at 13rpm for 30 minutes at 4^{o}C. The PEG solution was carefully removed leaving behind the pellet. 1ml of ice cold 70% ethanol was added to the pellet and centrifuged for 30 minutes at 13rpm at 4^{o}C. the ethanol wash was repeated twice to make sure all the PEG was removed. The ethanol was removed, and the pellets were resuspended in 50µl of T.E buffer. The DNA was visualised on a 0.8%(w/v) agarose gel containing 0.01% (v/v) cyberSafe(Sigma) (Roche Diagnostics, Ireland). DNA was quantified on a Qubit Fluorimeter (Thermo Fisher, Ireland) with Qubit™ dsDNA BR Assay Kit (Thermo Fisher, Ireland).

4.2.8 Amplicon sequencing of bacterial and fungal communities

Sequencing of the 16S rRNA (bacteria, archaea and eukaryote) and ITS (fungi) regions was carried out for all samples. Briefly, the library preparation was a 2-step PCR process with PCR 1 consisting of a 25µl reaction containing the raw DNA template (5ngµl^{-1}), 2X Kapa Hifi hot start ready mix Taq (Roche, Ireland) and 0.2 µM final concentration of both forward and reverse primers. Primers had overhang adapters attached. The primers used for 16S rRNA were 515F (Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGYCAGCMGCCGCGGTAA]) and 926R (Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [CCGYCAATTYMTTTRAGTTT]). The primers for ITS were 86F (Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGAATCATCGAATCTTTGAA]) and 4R (Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [TCCTCCGCTTATTGATATGC]) (Quince *et al.*, 2011; Op De Beeck *et al.*, 2014). The PCR1 product was cleaned using AMPure XP beads to ensure that the PCR product was free of primer dimer. The clean PCR1 product was run on a 1% agarose gel and visualised under UV light to check both the strength of bands and the absence of primer dimer. PCR2 is an index PCR with dual indices and Illumina Sequencing adapters attached to the samples using the Nextera XT Index Kit (Illumina, Ireland). PCR2 consists of 50µl reactions containing 5µl PCR1 template, each index primers at a final concentration of 0.2µM, and 2X Kapa Hifi hot start ready mix (Roche, Ireland). PCR2 product was cleaned using the Ampure XP beads as described for PCR1. The beads were eluted in 15 µl TE buffer. The concentration of PCR2 was quantified using a Qubit fluorometer. The generated 16S

rRNA and ITS libraries were then pooled at equimolar concentration. Pools were quality checked on a Bioanalyser (Agilent, Ireland) using the DNA 1000 chip (Agilent, Ireland) and sent to the sequencing lab in Teagasc Research Centre, Moorepark, Co. Cork, Ireland for sequencing on an Illumina MiSeq platform.

4.2.9 Data handling for sequencing

FASTQ files were generated through the Illumina BaseSpace platform and this data was run through the same sequencing analysis pipeline as was described in Chapter 2 section 2.3.8.

4.2.10 Statistical analyses

Data analysis was performed in R Studio 4.2.0 (R Core Team, 2021). Statistical significance was determined at $p<0.05$. Statistical analysis was carried out as previously described in Chapter 2 section 2.3.7 and Chapter 3 section 3.2.7.

4.3 Results

4.3.1 Soil properties in the control and glucose treatments

A summary of soil nutrient properties can be seen in Table 4.2. The P0 slurry treatment in both the control and the glucose troughs had the highest absolute OM content (Table 4.2). Within the slurry treatments, the OM content in the P30 slurry 13C soils was significantly lower than the P0 slurry 13C ($p<0.05$). The P0 slurry control trough soils had a significantly higher OM content than P30 cut, and P30 slurry control troughs ($p<0.05$). There were no significant differences in OM in the grazed treatments. Available P measured as P Morgan's (PM) was also significantly different between some treatments ($p<0.05$). The P30 grazed treatments (control and glucose) had significantly higher PM than all the other treatments ($p<0.05$). In the cut, grazed, and slurry treatments the P0 treatment was significantly lower in PM than the equivalent P30 treatments ($p<0.05$). There were also some significant differences in total C content. In the control troughs the P0 cut treatment had significantly lower total C compared to the P0 grazed ($p<0.03$). The P0 grazed 13C treatment had significantly lower total C then the P0 grazed control treatment ($p<0.05$). There was no significant difference in total C in the grazed treatments. There were some differences found in total N between the treatments. There was a significant difference in total N between the P0 grazed control and the P0 grazed 13C treatment, total N was significantly higher in the control ($p<0.05$). There were significant differences in pH between treatments. The grazed treatments had a significantly lower pH than all of the other treatments ($p<0.05$). The lowest absolute pH treatment in this study was in the P0 grazed control troughs.

Table 4.2: Summary of soil nutrient analysis (n=4, error=standard error). Letters refer to statistical differences between treatments. PM and KM are available P and K measured by P Morgan's method. OM stands for organic matter content. TC and TN represent total carbon and nitrogen. N-min shows potential nitrogen mineralisation. MBC, MBN, and MBP are microbial biomass carbon, nitrogen and phosphorus. Statistical significances have been left out of N-min, MBC, MBN, and MBP as these will be described later in the text.

Treatment	PM mg Kg^{-1}	OM %	TC %	TN %	KM mg Kg^{-1}	pH	N- Min NH_4 g^{-1} dry soil	MBC ug g^{-1}	MBN ug g^{-1}	MBP ug g^{-1}
P0 cut 13C	4.63 (±0.02) a	9.46 (±0.02) ab	4.22 (±0.02) ab	0.39 (±0.01) a	97.25 (±0.71) d	5.41 (±0.00) e	50.88 (±17.19)	498.56(± 35.62)	180.69(± 15.98)	31.70 (±0.75)
P0 cut control	4.59 (±0.07) a	9.09 (±0.03) a	4.20 (±0.08) a	0.38 (±0.01) a	100.25(± 1.11) d	5.29 (±0.01) d	81.95 (±7.29)	340.70(± 42.67)	198.86(± 2.59)	32.43 (±1.79)
P30 cut 13C	7.97 (±0.07) e	9.23 (±0.16) a	4.40 (±0.08) ab	0.39 (±0.01) a	41.22 (±0.40) b	5.41 (±0.01) e	86.25 (±8.33)	673.33 (±58.08)	221.86(± 12.88)	36.36 (±2.39)
P30 cut control	7.84 (±0.03) e	9.28 (±0.12) a	4.19 (±0.05) a	0.38 (±0.00) a	39.40 (±0.35) b	5.31 (±0.01) d	(92.86 (±5.94)	465.9 (±69.93)	206.26(± 17.36)	32.75 (±2.50)
P0 slurry 13C	5.50 (±0.03) bc	9.91 (±0.20) b	4.63 (±0.05) ab	0.41 (±0.01) ab	56.75 (±0.62) c	5.51 (±0.00) f	92.88 (±3.30)	568.29 (±45.65)	220.39 (±9.60)	34.11 (±2.31)
P0 slurry control	5.38 (±0.08) b	9.91 (±0.14) b	4.62 (±0.04) ab	0.42 (±0.01) ab	55.90 (±1.09) c	5.39 (±0.01) e	88.98 (±4.81)	501.95 (±64.26)	186.23 (±11.04)	34.53 (±1.99)
P30 slurry 13C	7.41 (±0.08) d	9.24 (±0.10) a	4.28 (±0.03) ab	0.38 (±0.00) a	32.33 (±0.58) a	5.71 (±0.01) h	82.32 (±1.04)	649.90(± 68.55)	181.44 (±10.24)	32.80 (±1.52)
P30 slurry control	7.39 (±0.13) d	9.29 (±0.06) a	4.20 (±0.06) a	0.38 (±0.00) a	32.68 (±0.71) a	5.56 (±0.01) g	69.71 (±8.35)	478.51(± 73.20)	175.79(± 24.43)	30.69 (±3.34)
P0 grazed 13C	5.51 (±0.06) bc	9.54 (±0.14) ab	4.18 (±0.34) a	0.39 (±0.03) a	145.50(± 1.66) e	4.75 (±0.00) b	64.74 (±10.92)	746.63 (±155.72)	173.12(± 17.21)	34.33 (±0.38)
P0 grazed control	5.88 (±0.11) c	9.56 (±0.03) ab	4.82 (±0.16) b	0.46 (±0.03) b	148.25(± 2.02) e	4.66 (±0.02) a	35.13 (±6.18)	477.12 (±43.27)	163.33 (±35.35)	41.08 (±1.18)
P30 grazed 13C	13.70 (±0.11) f	9.32 (±0.12) a	4.44 (±0.04) ab	0.41 (±0.00) ab	59.86 (±1.40) c	4.89 (±0.00) c	54.24 (±16.94)	760.17(± 144.68)	242.53(± 31.07)	41.60 (±2.19)
P30 grazed control	14.08 (±0.13) f	9.37 (±0.03) ab	4.47 (±0.07) ab	0.42 (±0.01) ab	61.00 (±1.34) c	4.74 (±0.01) b	56.14 (±14.85)	432.82 (±112.35)	188.84 (±21.52)	31.35 (±2.32)

4.3.2 Nitrogen mineralisation potential with control and glucose treatment

Potential N mineralisation varied with treatment and the addition of glucose (Figure 2). Potential N mineralisation was significantly affected by treatment ($p<0.01$). However, the troughs that had glucose applied had no significant differences in potential N mineralisation between the treatments. In the control troughs there were significant differences ($p<0.01$) in potential N mineralisation. The P0 grazed control treatment had a significantly lower N mineralisation potential compared to the P0 cut,

P0 slurry, and P30 cut control treatments ($p<0.05$). There was a significant effect of both slurry addition ($p=0.035$), and grazing ($p<0.001$) on potential N mineralisation (Figure 4.2).

When examining the effect of soil nutrients on potential N mineralisation using regression analysis, there was a significant effect of total N ($p=0.04$). There was also a significant effect of soil pH ($p<0.02$) and available potassium (K) ($p<0.02$). In the control troughs, total C ($p<0.05$, $r^2=0.17$), total N ($p<0.02$, $r^2=0.22$), pH ($p<0.001$, $r^2=0.42$), and K ($p<0.01$, $r^2=0.29$) had a significant effect on potential N mineralisation. There were no significant effects of the measured soil nutrients in the glucose troughs. In the slurry treatment, potential N mineralisation was significantly affected by PM ($p<0.01$, $r^2=0.35$), total C ($P<0.001$, $r^2=0.42$), total N ($p<0.02$, $r^2=0.32$), and K ($p<0.01$, $r^2=0.34$). Soil nutrients concentrations did not have a significant effect on potential N mineralisation in the grazed treatment.

4.3.3 Microbial biomass carbon, nitrogen, phosphorus

The only significant difference found for microbial biomass C (MBC) was between the P30 grazed 13C and P0 cut control treatments ($p<0.05$). There was no significant effect of the soil nutrients measured as part of this study on MBC. As well as this, there was no significant effect of treatments or soil nutrients on MBN (Figure 4.3a).

There was a significant effect of treatment on MBP ($p<0.01$) (Figure 4.3c). There were significant differences between P30 slurry control and P0 grazed control, P30 grazed control and P30 grazed 13C, P30 slurry control and P30 grazed 13C ($p<0.05$). In the glucose-treated soils there were significant differences between the P30 grazed 13C and P0 cut 13C, and P30 slurry 13C and P30 grazed 13C ($p<0.05$). There were no significant differences in the slurry treatment. There was a significant effect of total C, and N, as well as soil pH on MBP in this study ($p<0.01$).

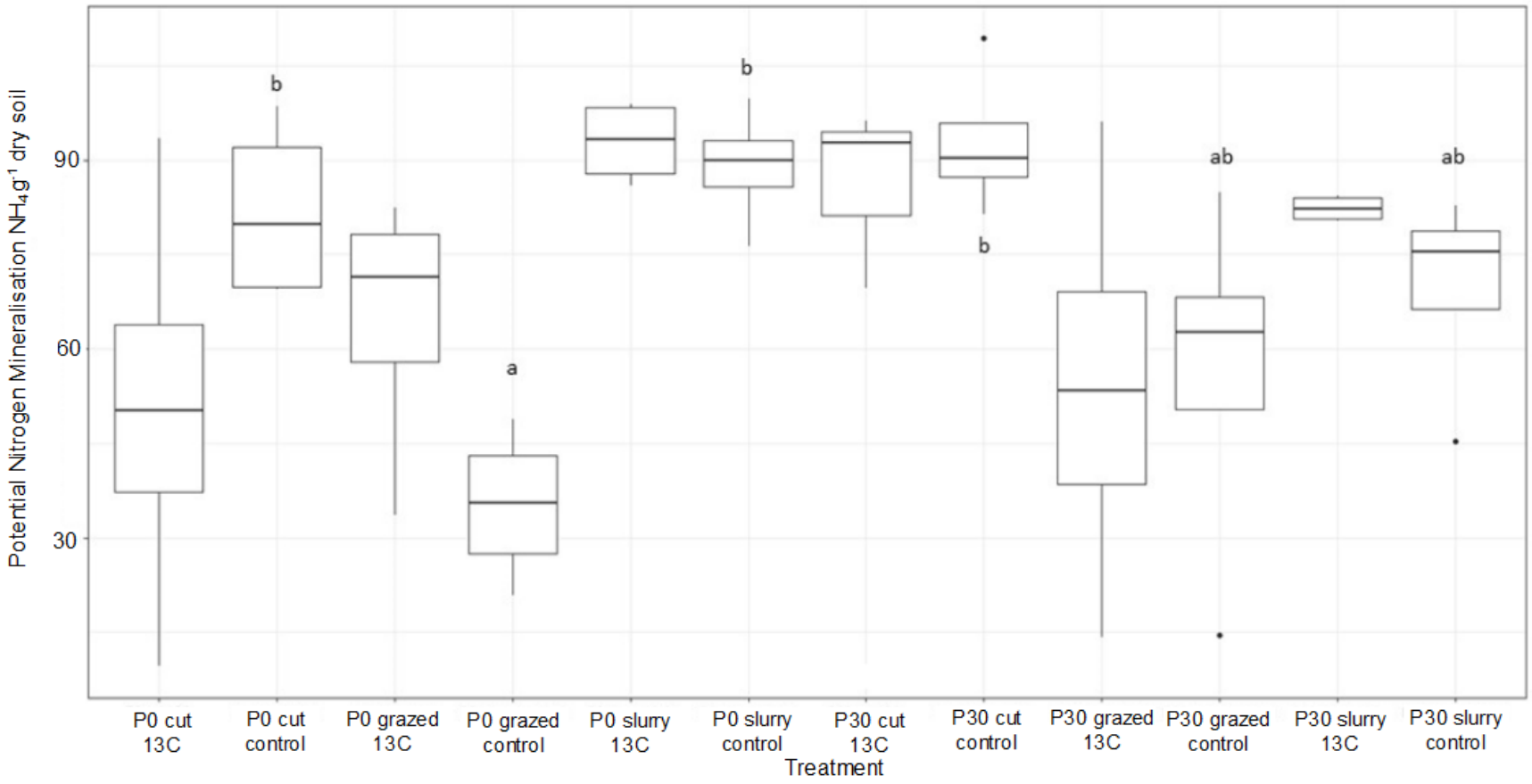


Figure 4.2: Potential nitrogen mineralisation between 6 different treatments (NH4- g-1 dry soil) (n=4). There were two sets of troughs for each treatment, a trough that received glucose, these treatments are labelled with 13C, and also a control, which is labelled as control. The letter above the control treatments indicate significant difference in N mineralisation potential. There were no significant difference in the glucose troughs.

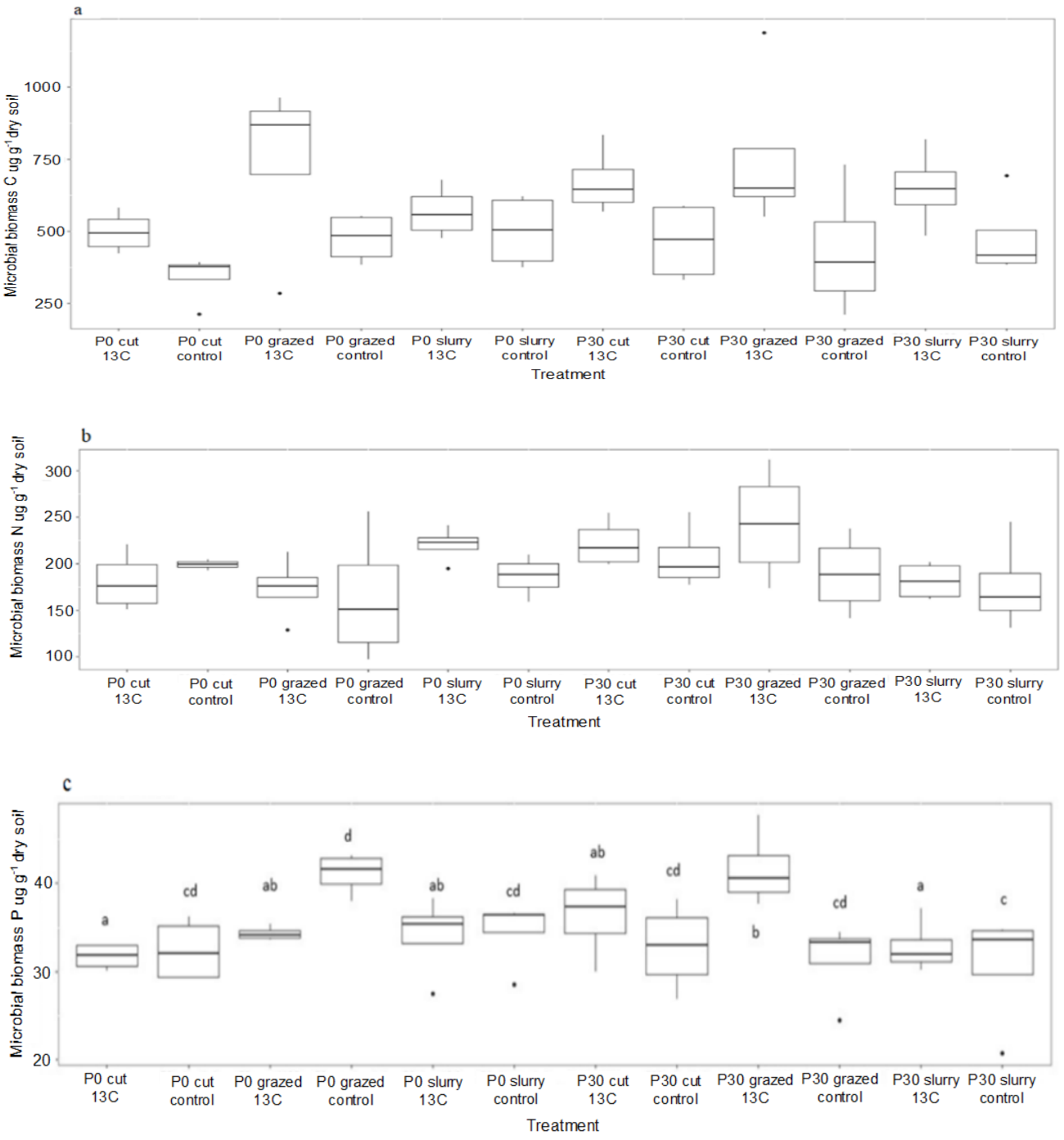


Figure 4.3 (a, b, c): a showing microbial biomass carbon in both the glucose and control soils across the different treatments (n=4). b is showing microbial biomass nitrogen in both the glucose and control soils across the treatments (n=4).c is showing microbial biomass phosphorus in both the glucose and control soils across the treatments (n=4). Letters on figure c show the statistically significant differences between the treatments in microbial biomass P.

4.3.4 Soil organic matter derived respiration

There was a significant effect of treatment and sampling time point on the total respiration from soils during the experiment ($p<0.01$). There was a significantly higher total respiration in the soils that received ^{13}C glucose when compared with the control ($p<0.01$) (Figure 4.4a). Overall, there was no significant effect of slurry or grazing on respiration rate. The P30 grazed 13C treatment had the largest increase in respiration over the course of the experiment. In the glucose soils there were significant differences between the treatments on days 0, 4, 7, 9, and 11 (Figure4.4b). In the control soils (not receiving glucose additions) there were significant differences between experimental treatments on days 4, 7, 9, and 11. However, these differences were only between the P30 slurry treatment and P30 cut ($p<0.05$) and P30 slurry and P0 grazed ($p<0.05$). On day 4 P30 slurry was significantly different to all treatments except P0 cut, where at day 11 P30 slurry was only significantly different to P0 grazed (Figure 4.4a).

SOM derived CO_2-C is a measure of the decomposition rate of non-glucose sources throughout the experiment. There was no significant effect of sampling time point on SOM decomposition rate. There was a significant effect of treatment on SOM decomposition rate in the glucose-addition troughs ($p<0.01$). In the glucose treatment, the P0 grazed treatment had the lowest rate of SOM decomposition throughout the experiment (Figure 4.5b). There was a significant effect of the grazed treatment on OM decomposition rate ($p<0.01$). The P0 grazed glucose treatment had a significantly lower SOM decomposition rate than both the P0 slurry glucose and P30 cut glucose treatments on each sampling day ($p<0.05$) (Figure4.5b) but not at day 2. On day 0 and day 11, the P30 grazed treatment had a significantly higher SOM decomposition rate compared to the P0 grazed treatment in the glucose amended troughs ($p<0.05$)(Figure 4.5b). There was also a significant effect of slurry addition on SOM decomposition rate ($p<0.01$). SOM mineralisation from the P0 slurry treatment was significantly higher than the P30 slurry treatment ($p<0.05$) on days 0 and 2. There was no significant effect of P level, as this also depended on other aspects of the treatment. Overall, P level significantly influenced SOM mineralisation in the slurry and grazed treatments: in the slurry treatment, P0 slurry was significantly higher than P30 slurry, and in the grazed treatments P0 grazed was significantly lower than P30 grazed overall ($p<0.05$). There was no significant effect of treatment on SOM decomposition rate in the control troughs with treatment. However, variability within the treatments did increase with time over the experiment (Figure 4.5a).

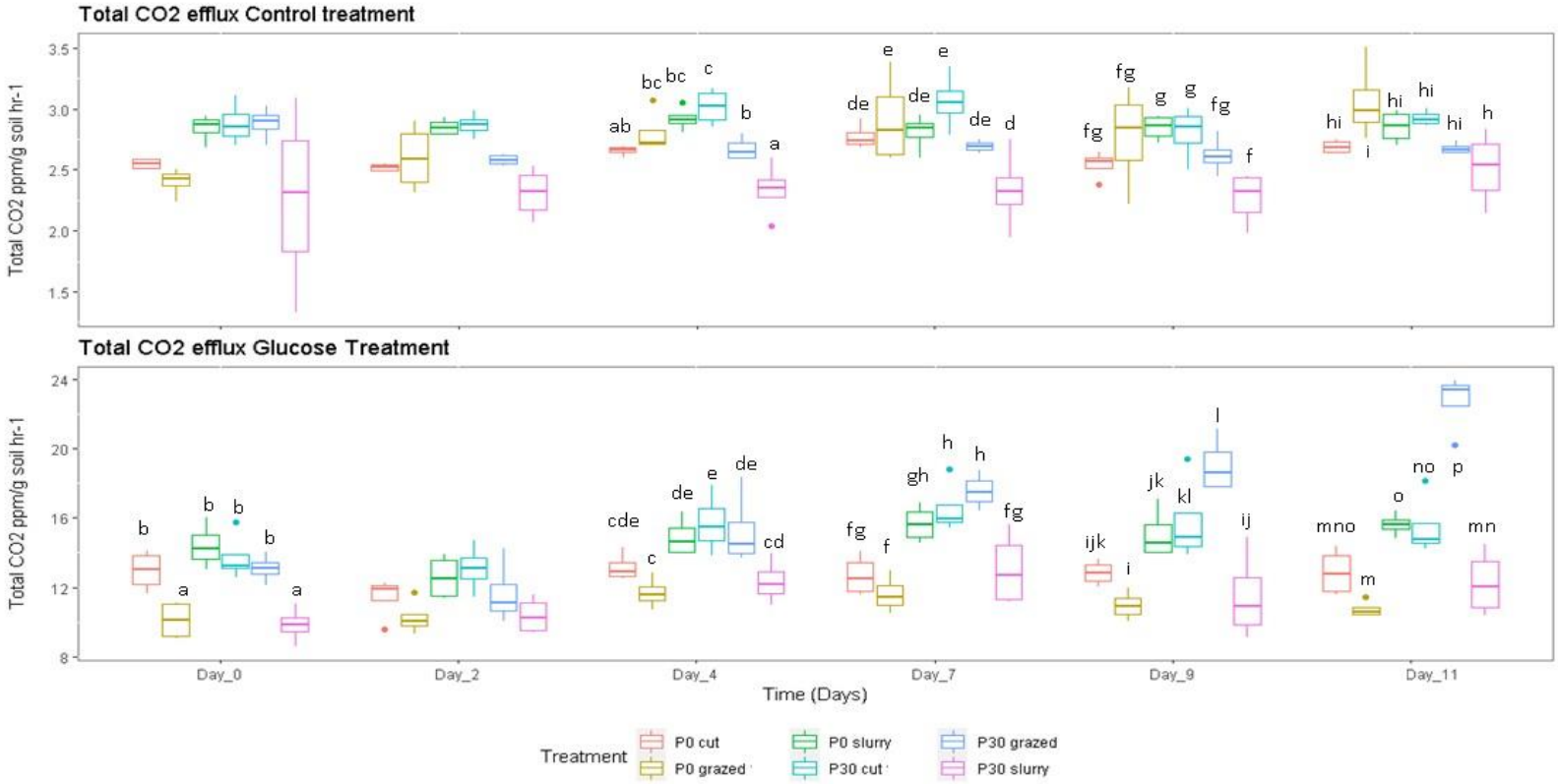


Figure 4.4: Total CO_2 efflux (ppm g^{-1} dry soil $hour^{-1}$) for the duration of the experiment. The top graph represents respiration in the control trough (n=4), and letters donate significant differences between treatments at each sampling point ($p<0.05$). The bottom graph represents respiration in the control trough (n=4), and letters denote significant differences between treatments at each sampling point ($p<0.05$).

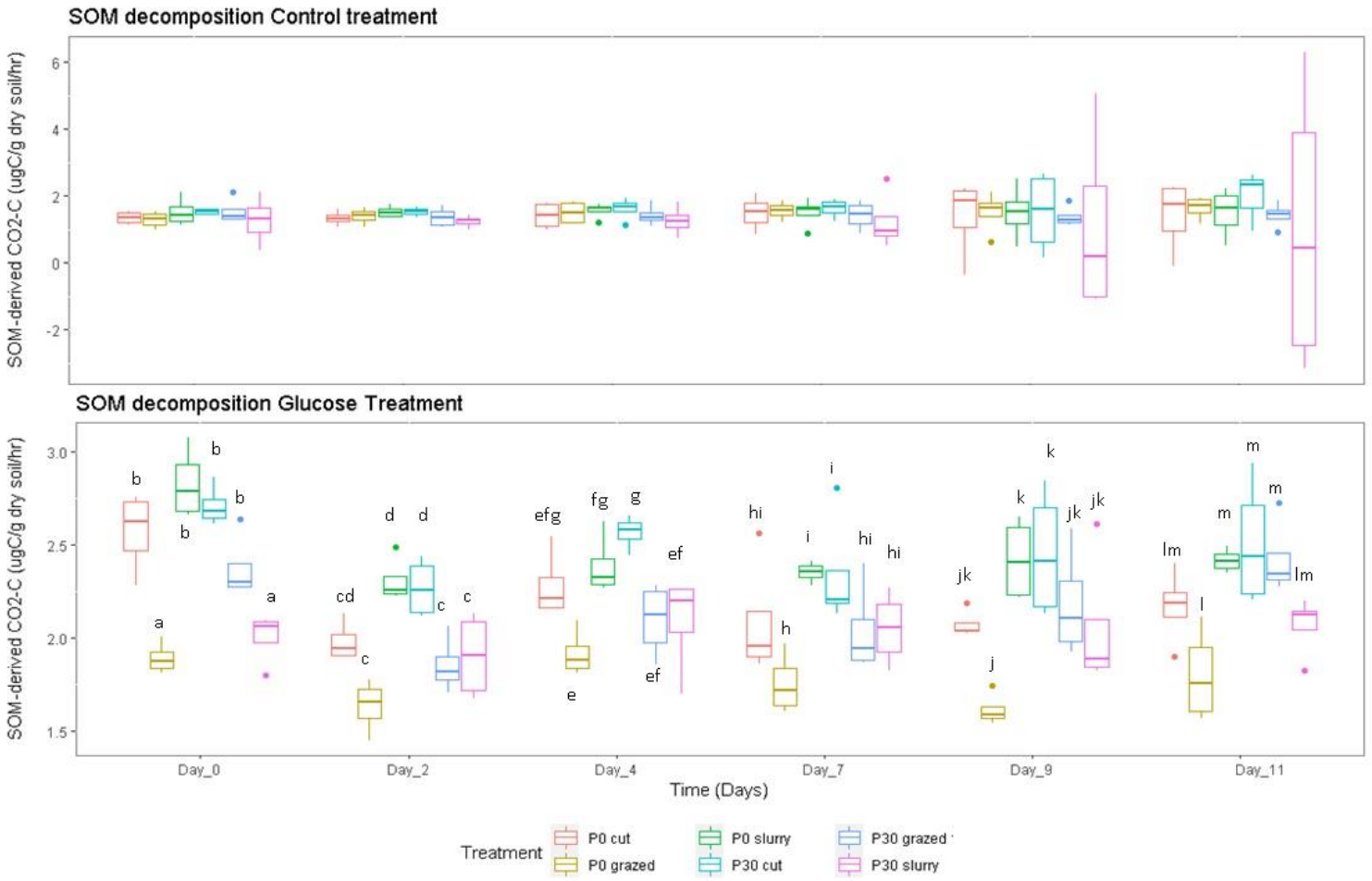


Figure 4.5: Total soil organic matter-derived CO_2-C (ug C/g dry soil hour^{-1}) for the duration of the experiment. The top graph shows the 6 treatments over time in the control troughs (n=4). The bottom graph shows the 6 treatments over time in the glucose troughs (n=4). Letters on the bottom graph represent significant difference between the treatments at each measured time point.

4.3.5 Priming

There was a significant effect of treatment on the rate of priming ($p<0.05$) with both grazing and slurry treatments affecting the rate of priming ($p<0.01$). Sampling time had an effect on priming ($p<0.01$). At each sampling point the P0 grazed treatment was significantly lower in its primed response compared to all other treatments ($p<0.05$) (Figure 4.6). The highest rate of priming was seen on day 0 in the P0 slurry treatment (Figure 4.6). Only the P30 slurry treatment had a lower rate of priming on day 0 of the experiment compared to the final day 11 (Figure 4.6). The significant differences between the treatments on each sampling day can be seen in Table 4.3. There was no significant effect of P level on the rate of priming in this study.

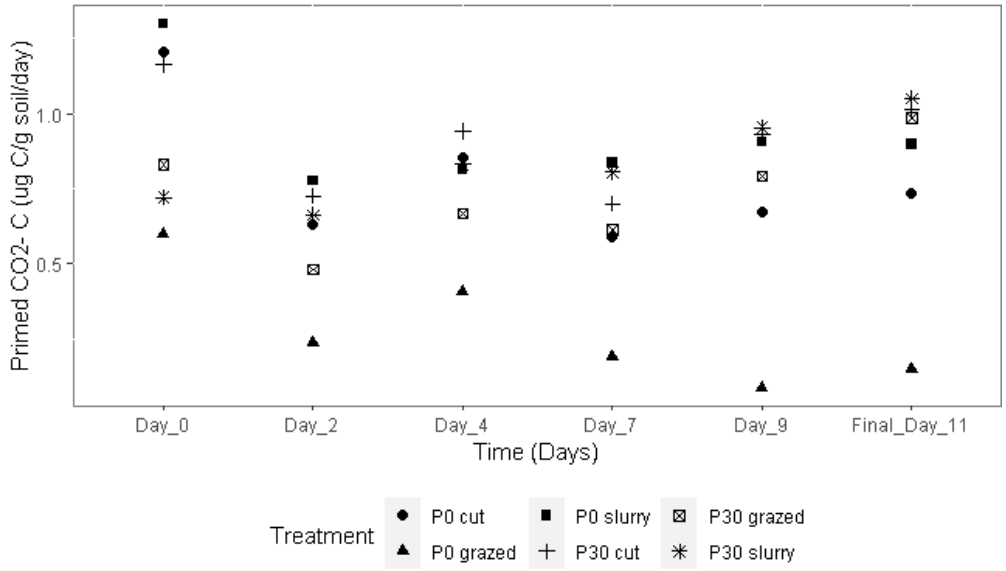


Figure 4.6: Total primed soil organic matter carbon CO_2-C (ug C g^{-1} dry soil $hour^{-1}$) for the duration of the experiment. Shapes represent the treatments (n=4). The standard errors for the above graph can be seen in table 4.3.

Table 4.3: Total primed soil organic matter carbon between the six treatments (n=4, error= standard error). Results in the table below shows means and standard errors at each sampling point. The letters denote statistically significant differences between treatments at each time point.

Treatment	Day 0	Day 2	Day 4	Day 7	Day 9	Day 11
P0 cut	1.21 (0.10) bc	0.62 (0.05) abc	0.86 (0.09) ab	0.59 (0.16) ab	0.67 (0.04) ab	0.73 (0.10) b
P30 cut	1.16 (0.05) bc	0.73 (0.08) bc	0.94 (0.04) b	0.70 (0.16) ab	0.93 (0.18) b	1.02 (0.17) b
P0 slurry	1.34 (0.11) c	0.98 (0.19) c	1.03 (0.19) b	1.02 (0.17) b	1.00 (0.19) b	1.07 (0.15) b
P30 slurry	0.72 (0.07) a	0.66 (0.12) abc	0.83 (0.13) ab	0.81 (0.10) b	0.95 (0.19) b	1.05 (0.08) b
P0 grazed	0.59 (0.04) a	0.24 (0.07) a	0.41 (0.06) a	0.19 (0.08) a	0.09 (0.04) a	0.15 (0.13) a
P30 grazed	0.83 (0.09) ab	0.48 (0.07) ab	0.67 (0.10) ab	0.61 (0.12) ab	0.79 (0.15) b	0.97 (0.10) b

4.3.6 Bacterial community structure

It was hypothesised that P addition, type of P addition, and grassland management system (cut vs grazed), would all have an impact on the microbial community structure. This was seen in both the glucose and control treatments as part of the deseq2 analysis and significant differences are reported in the supplementary tables. The top 20 dominant genera are displayed in the supplementary materials. In both the glucose and control *Candidatus Udaeobacter* and *Gaiella* were the most relative abundant genera (Supplementary Material). As well as this, significant differences in alpha diversity were also recorded between the treatments in the glucose and control soils. In the glucose soils when using the Simpsons index, the P30 slurry 13C treatment had the highest diversity and was significantly higher than the P30 grazed treatment ($p<0.05$) (Table 4.4). In the control troughs, alpha diversity was found to be significant when measured using both the Shannon and the Simpsons diversity indexes (Table 4.5). The P30 slurry control had the highest diversity according to the Simpsons index and was significantly higher than all other treatments ($p<0.05$). Measured using the Shannon index, the P30 Slurry treatment also had a significantly higher diversity compared with the P30 grazed and P0 grazed controls ($p<0.05$) (Table 4.5).

Across both the grazed and cut sites, at different P levels and slurry addition, differences in bacterial community structure were found (Supplementary Tables). PCA analysis performed on the data set showed a distinction between the grazed and cut sites in both the glucose and the control troughs (Figures 4.7 & 4.9). PCA analysis was also performed on the cut site to see differences between chemical fertiliser and slurry addition treatments. In the glucose troughs the P30 cut and the P30 slurry treatments were different from each other on both PC1 and PC2 (Figure 4.8). In the control troughs the P0 slurry and P30 slurry treatments were different, along PC1, as well as being different from the treatments that had not receive slurry (Figure 4.10). Further analysis with a PERMANOVA showed there was a significant effect of treatment on 16S bacterial community structure in both the glucose and the control troughs ($p<0.001$). Pairwise Adonis analysis showed significant difference between each of the treatments for both the glucose (Table 4. 6) and the control (Table 4.7) troughs. In the glucose trough there was no significant difference between the P0 slurry and P30 slurry bacterial community structure (Table 4.6). There was also no significant difference in the P0 cut and P0 slurry treatments (Table 4.7). All treatment combinations were significantly different in the control troughs (Table 4.7). Specific differences in bacterial genus groups were analysed using the deseq2 pipeline (Love et al., 2014), these differences can be seen in the supplementary material. In the glucose troughs, results from the RDA analyses showed that the bacterial community was significantly influenced by OM content, available P, available K, and pH ($p<0.05$) (Figure 4.11). The bacterial

community in the control troughs were also significantly influenced by available P, as well as potential N mineralisation and total C content ($p<0.05$) (Figure 4.12).

Table 4.4: Alpha diversity indexes (n=4) for the glucose 16S communities. Values shown here represent mean richness in each treatment. Letters represent significant differences between treatment ($p<0.05$).

Glucose Treatment	Chao1	Shannon	InvSimpson
P0 cut 13C 16S	1589	6.470	170.9 abc
P30 cut 13C 16S	2109	6.702	193.5 ab
P0 Slurry 13C 16S	1675	6.473	154.0 bc
P30 Slurry 13C	1911	6.650	210.1 a
P0 grazed 13C 16S	1874	6.528	169.5 abc
P30 Grazed 13C 16S	1708	6.382	130.8 c

Table 4.5: Alpha diversity indexes (n=4) for the control 16S communities. Values shown here represent mean richness in each treatment. Letter represent significant differences between treatment ($p<0.05$).

Control Treatment	Chao1	Shannon	Simpson
P0 cut control 16S	2116	6.721 ab	202.4 b
P30 cut control	2291	6.710 ab	175.4 bc
P0 slurry control	1751	6.497 ab	147.4 cd
P30 slurry control	2332	6.835 a	248.1 a
P0 grazed control	1475	6.356 b	145.9 cd
P30 Grazed control	2017	6.468 b	117.8 d

4.3.7 Fungal community structure

Fungal communities were also impacted by P addition, type of P addition (inorganic vs organic), and grassland management system (cut vs grazed). There were differences in communities identified down to genus level using deseq2, and these differences are highlighted in the supplementary materials. The top 20 dominant fungal genera are shown in figures in the supplementary materials.The most dominant genera in the control treatments were *Mortierella* and *Ascobolus*. In the glucose soils the most dominant genera were *Mortierella* and *Cladosporium* (Supplementary Materials).There were also significant differences in alpha diversity in both the glucose and control soils (Table 4.8 & 4.9). Interestingly, these were the opposite to what was seen in the 16S communities. In the glucose soils there was significantly higher diversity in the P0 grazed 13C treatment for both the Shannon and Simpsons indexes when compared with the P30 cut 13C and P30 slurry 13C ($p<0.05$) (Table 4.8). In the control treatments, the alpha diversity was significantly higher in both P0 grazed and P30 grazed when compared with P0 slurry, P30 slurry, and P30 cut ($p<0.05$)(Table 4.9).

Across the grazed and cut sites, at different P levels (P0 and P30), differences in bacterial community structure were found (supplementary tables). PCA analysis preformed on the data set showed a distinction between the grazed and cut sites in both the glucose and the control troughs (Figure 4.13 & 4.15). PCA analysis was also performed on the cut site in order to see differences between chemical fertiliser and slurry addition treatments. In both the glucose and the control soils there were distinct differences between all 4 treatments at the cut sight (Figure 4.14 & 4.16). Further analysis with a PERMANOVA showed there was a significant effect of treatment on the ITS fungal community structure in both the glucose and the control troughs ($p<0.001$). Pairwise Adonis analysis showed significant differences between each of the treatments for both the glucose (Table 4.6) and the control (Table 4.7) troughs. All treatments were significantly different from each other in the glucose troughs (Table 4.6). There was no significant difference in the P0 cut and P0 grazed treatments in the control troughs (Table 4.7). All other treatment combinations were significantly different in the control troughs (Table 4.7). Specific differences in fungal genus groups were analysed using the deseq2 pipeline (Love et al., 2014), these differences can be seen in the supplementary material. In the glucose troughs, results from the RDA analyses showed that the fungal community was significantly influenced by available K, and pH ($p<0.05$) Figure 4.17). The fungal community in the control troughs were also significantly influenced by pH ($p<0.05$) (Figure 4.18).

Table 4.6: Results from the PERMAONVA pairwise analysis of the 16S bacterial community and ITS fungal community assessing the difference between treatments in the glucose troughs (n=4)

Treatment Comparison Glucose	16S p-value	ITS p-value
P0 cut – P30 cut	0.03	0.04
P0 slurry – P30 slurry	0.09	0.03
P0 grazed – P30 grazed	0.03	0.03
P0 cut – P0 slurry	0.07	0.03
P0 cut – P0 grazed	0.03	0.03
P0 slurry –P0 grazed	0.03	0.03
P30 cut – P30 slurry	0.03	0.03
P30 cut – P30 grazed	0.04	0.03
P30 slurry – P30 grazed	0.03	0.03

Table 4.7: Results from the PERMANOVA pairwise analysis of the 16S bacterial community and ITS fungal community assessing the difference between treatments in the control troughs (n=4).

Treatment Comparison Control	16S p-value	ITS p-value
P0 cut – P30 cut	0.05	0.03
P0 slurry – P30 slurry	0.03	0.03
P0 grazed – P30 grazed	0.03	0.03
P0 cut – P0 slurry	0.04	0.03
P0 cut – P0 grazed	0.03	0.10
P0 slurry –P0 grazed	0.02	0.04
P30 cut – P30 slurry	0.03	0.03
P30 cut – P30 grazed	0.04	0.03
P30 slurry – P30 grazed	0.02	0.03

Table 4.8: Alpha diversity indexes (n=4) for the glucose ITS communities. Values shown here represent mean richness in each treatment. Letter represent significant differences between treatment (p<0.05).

Glucose Treatment	Chao1	Shannon	Simpson
P0 cut 13C ITS	246.00 a	3.96bc	20.73 ab
P30 cut 13C ITS	239.75 a	3.91 bc	17.48 b
P0 Slurry 13C ITS	312.75 a	3.986 bc	17.99 b
P30 Slurry 13C ITS	222.75 a	3.690c	13.54 b
P0 grazed 13C ITS	357.75 a	4.400 a	31.67 a
P30 Grazed 13C ITS	282.00 a	4.22ab	29.25 a

Table 4.9: Alpha diversity indexes (n=4) for the control ITS communities. Values shown here represent mean richness in each treatment. Letter represent significant differences between treatment (p<0.05).

Control Treatments	Chao1	Shannon	Simpson
P0 Cut control	294.00 a	3.97 ab	18.58 ab
P30 Cut control	295.00 a	3.70 b	11.73 b
P0 Slurry control	205.00 a	3.74 b	14.86 b
P30 Slurry control	222.75 a	3.71 b	12.44b
P0 Grazed control	276.66 a	4.15 a	24.62 a
P30 Grazed control	340.00 a	4.16 a	26.27 a

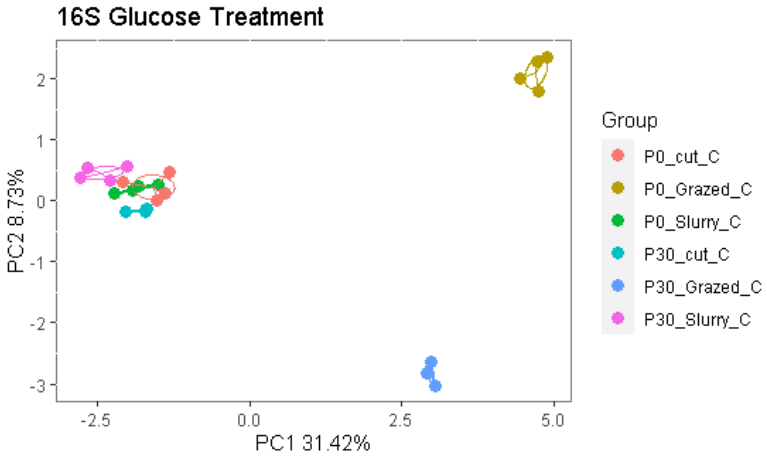


Figure 4.7: Principal component analysis showing differences in bacterial community structure with treatment in the glucose troughs (n=4).

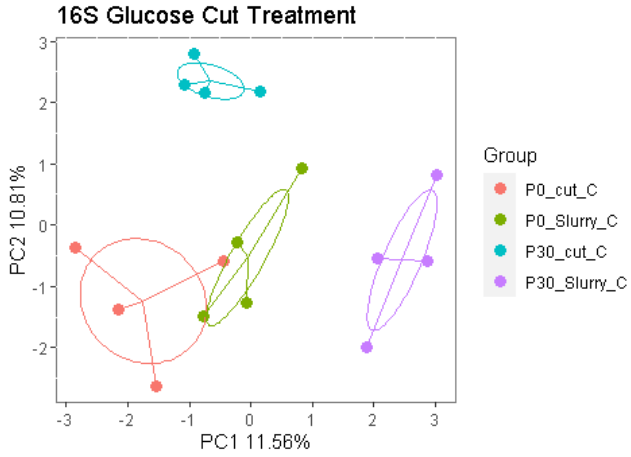


Figure 4.8: Principal component analysis showing the differences in bacterial community structure with treatment at the cut site in the glucose troughs (n=4).

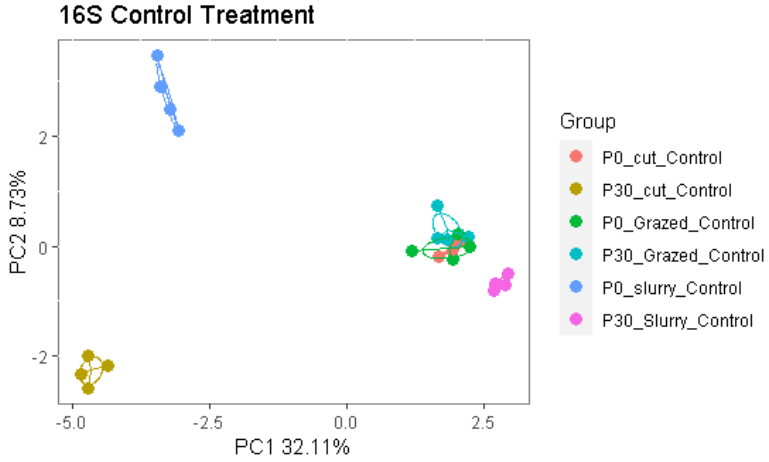


Figure 4.9: Principal component analysis of bacterial community structure in the control troughs with treatment (n=4).

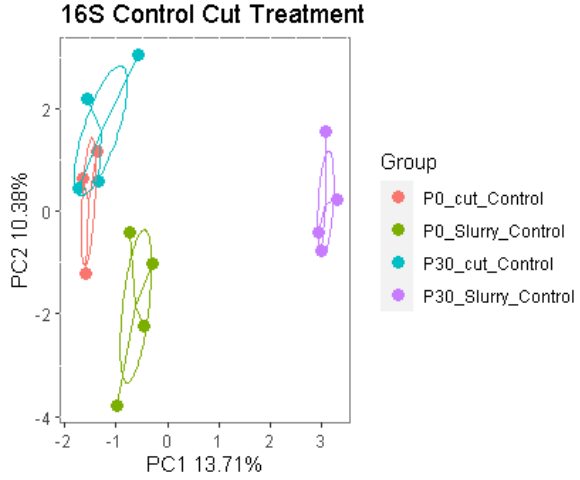


Figure 4.10: Principal component analysis of bacterial community structure from the cut site in the control troughs with treatment (n=4).

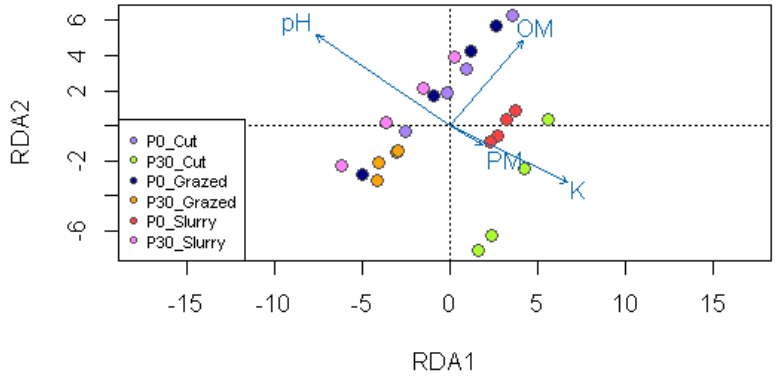


Figure 4.11: Redundancy analysis of the glucose troughs by treatment (n=4). This graph highlights which of the soil nutrient properties are significantly influencing the bacterial community structure in these troughs. OM refers to organic matter content, PM refers to P Morgan's measure of available P, K refers to K Morgan's available K, and pH represents the soil pH. Each of these nutrient measurements significantly affected the bacterial community structure ($p<0.05$).

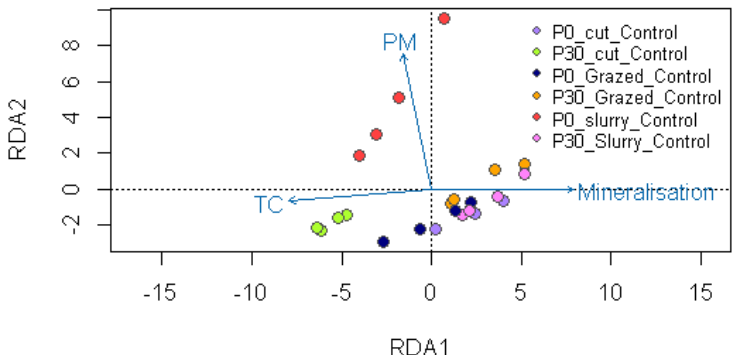


Figure 4.12: Redundancy analysis of control troughs by treatment (n=4). This graph shows which soil nutrient properties are significantly influencing the bacterial community structure in the control troughs. In this graph, PM refer to available P as measured by P Morgan's test, TC refers to total carbon content, and mineralisation shows the results from the 7-day anaerobic incubation measure of potential N mineralisation.

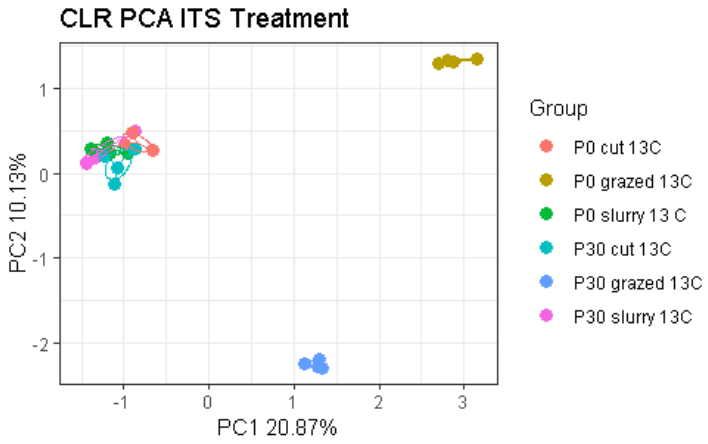


Figure 4.13: Principal component analysis showing differences in fungal community structure with treatment in the glucose troughs (n=4).

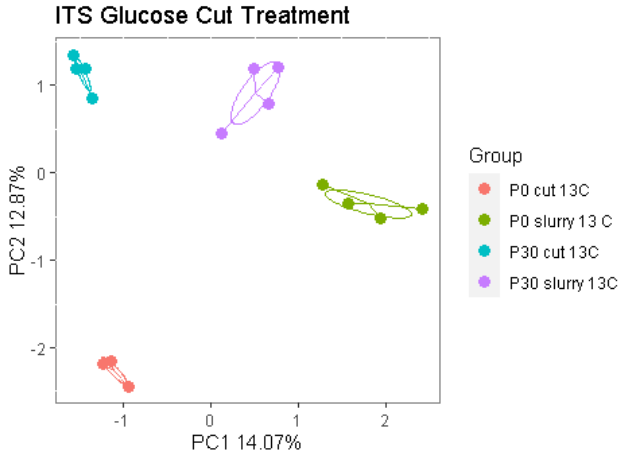


Figure 4.14: Principal component analysis showing the fungal in bacterial community structure with treatment at the cut site in the glucose troughs (n=4).

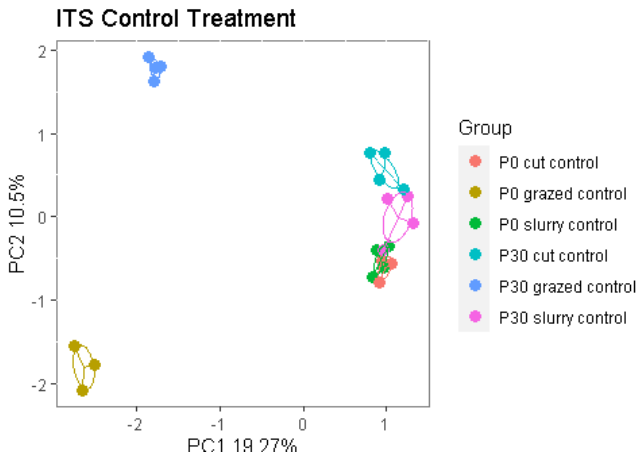


Figure 4.15: Principal component analysis of fungal community structure in the control troughs with treatment (n=4).

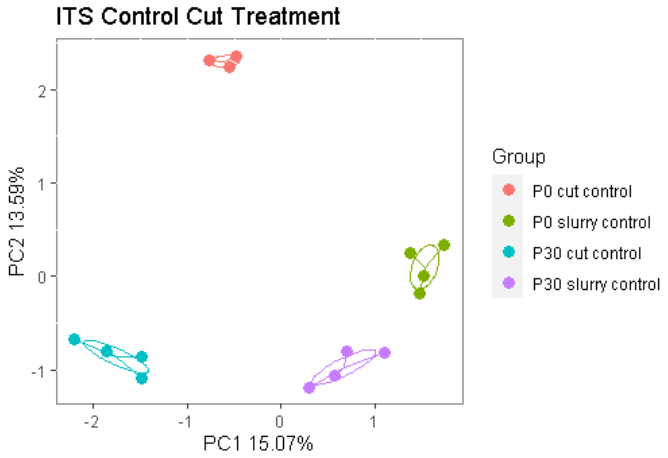


Figure 4.16: Principal component analysis of fungal community structure from the cute site in the control troughs with treatment (n=4).

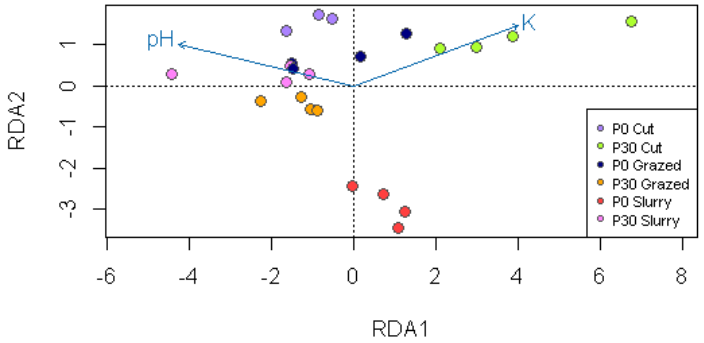


Figure 4.17: Redundancy analysis of the glucose troughs by treatment (n=4). This graphs highlights which of the soil nutrient properties are significantly influencing the fungal community structure in these troughs. K refers to K Morgan's available K, and pH represents the soil pH. Each of these nutrient measurements significantly affected the fungal community structure ($p<0.05$).

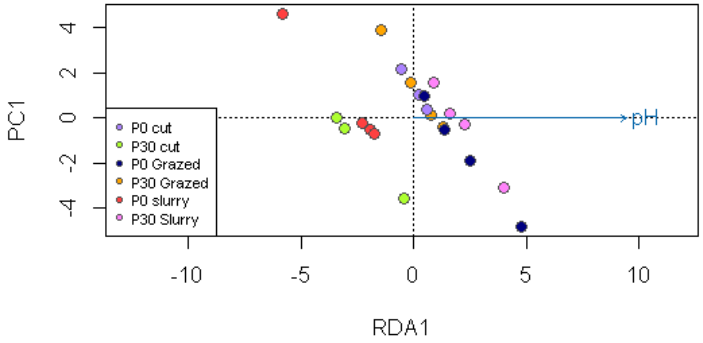


Figure 4.18: Redundancy analysis of control troughs by treatment (n=4). This graph shows which soil nutrient properties are significantly influencing the fungal community structure in the control troughs. In this graph, pH refers to the soil pH values. pH was significantly impacting microbial community structure in the control soils.

4.6 Discussion

This study aimed to examine the addition of C, P, and grass management strategies on SOM decomposition, potential N mineralisation and microbial community structure. This was achieved by using soils of varying levels of added P fertiliser and fertiliser type (chemical and organic), from long-term P trials. Alongside the varying P management, grassland management systems (cut silage system vs grazed by livestock system) and how this effects N mineralisation potential, the rate of SOM decomposition and priming, and microbial community structure were also examined. As was seen in other studies at both the cut (Massey *et al.*, 2016; Sheil *et al.*, 2016; Randall *et al.*, 2019) and grazed (Griffiths *et al.*, 2012; Chen *et al.*, 2014) grassland sites, there was a significant effect of P addition treatments on the amount of available P (measured as P Morgan's P). The treatments which did not receive any P had a lower amount of P compared to the P30 treatments that were receiving annual P additions (table 4.2) (Griffiths *et al.*, 2012; Chen *et al.*, 2014; Massey *et al.*, 2016; Sheil *et al.*, 2016; Randall *et al.*, 2019).

4.6.1 Potential nitrogen mineralisation is affected by grassland management and glucose addition

There were differences in N mineralisation potential in the soils collected from both cut vs grazed grassland management systems; however, differences between P treatments were less defined here than in Chapter 3 where *L. perenne* was grown in the soils. In Chapter 3 the high P treatment in the cut system had the lowest N mineralisation potential and was significantly lower than the P0 slurry treatment. However, here the high P cut treatment is almost mineralising at the same rate as the P0 slurry treatment. Overall in the planted system in Chapter 3, the rate of potential N mineralisation was higher than seen here in Chapter 4. In the control troughs there was a difference in N mineralisation potential between the grazed and cut system. The grazed treatments with both high and low P additions had a lower N mineralisation potential then the cut treatments. Therefore, the hypothesis that potential N mineralisation would be higher in the grazed system was rejected. It was expected that potential N mineralisation would be higher in the grazed treatments compared with the cut treatments due to the addition of livestock excreta. The excreta addition in the field would provide both the C energy source for mineralising activity and also nutrients to be mineralised. However, the potential N mineralisation assay used in this study is a net measure, so therefore N that becomes immobilised in soil is not measured as part of the NH_4 pool. From the glucose soils, it is possible to hypothesise that immobilisation of available N occurred due to the increase in both microbial biomass C (which would require N to build up) and also the microbial biomass N size increase compared to the control P0 and P30 grazed treatments (Table 2). The repeated addition of glucose throughout the experiment could have promoted the use and immobilisation of more readily available organic N sources such as peptides and amino acids, and as the potential N mineralisation assay was only

preformed after the final day of addition the change in mineralisation and immobilisation of N over the course of the experiment cannot be quantified.

There was no significant effect of glucose addition on potential N mineralisation and no significant differences between treatments in the glucose troughs. It was hypothesised that the addition of glucose to the troughs would increase potential N mineralisation. Despite the absolute values being higher, the difference between the glucose troughs and the control troughs was not statistically significant. However, there was an increase in microbial biomass C and N across all treatments with glucose addition (Table 4.2). There were also differences in microbial community structure between glucose and control soils (supplementary tables). This would suggest that immobilisation of N sources was occurring to increase the microbial biomass size. Again, the N that may have been immobilised from the SOM pool may not have been picked up by the net amount measured with the potential N mineralisation assay used in this study. During SOM-C decomposition it is expected that N is also released (Murphy *et al.*, 2015). The rates of SOM-C decomposition measured as part of this study showed significant differences between treatments (Figure 4.5), this would suggest that N is also being released at different rates depending on the treatment. In order to measure the release of N as a gross measurement from SOM rather than the net amount measured in this study, the ^{15}N poll dilution technique should be applied (Goerges and Dittert, 1998). Briefly, this methods uses the addition of a ^{15}N labelled source, and therefore any N released from SOM will have a ^{14}N labelling. Using the two labels it is possible to determine the gross N mineralised in the soil, as any N release from SOM would have the native isotopic signature (Goerges and Dittert, 1998).

In the P0 cut treatment, potential N mineralisation was higher in the control treatment than in the glucose treatment. The P0 cut control was also the only treatment where microbial biomass N was higher in the control compared to the glucose soil. However, as the microbial biomass C was higher in the glucose soil it is possible that N was mobilised to increase the microbial biomass C and, hence, this N was not measured in the potential N mineralisation assay. This is likely to be the case particularly as there is an increase in the rate of priming in the P0 cut treatment over the second week of the experiment (Figure 4.6). Also, as potential N mineralisation was only measured on the final day of the experiment, N that had been mineralised previously and used up was not recorded. As the P0 cut treatment has a history of being C limited, the increased rate of priming towards the end of the experiment and the lower N mineralisation in the glucose treatment but higher microbial biomass C could potentially indicate that N had been used by the microbial community to build soil C stocks while the labile C glucose source was available.

4.6.2 Soil organic matter decomposition was altered by organic nutrient addition and cutting

As has been reported in previous studies, the addition of glucose increased total CO_2 respiration (Garcia-Pausas and Paterson, 2011; Murphy *et al.*, 2015). All of the P treatments in this study had an increase in total CO_2 with glucose addition. However, the magnitude of this increase differed with the P and grassland management treatment. Although towards the end of the experiment variability in the control troughs increased in terms of the SOM-C decomposition rate, there were no significant differences between the treatments but also between each of the sampling time points. This highlights that the soils were well stabilised in the system prior to experimental analysis. It was expected that SOM-C decomposition rate would be larger in the higher P treatment, due to the increased microbial activity with increasing nutrient availability. In the cut and grazed management system, this hypothesis was confirmed. The P limited treatments had lower SOM-C decomposition rates compared with the higher P treatments. Due to P limitation, the microbial community in the P0 grazed treatment was less active than all other treatments, even with glucose addition. P limitation has been seen to reduce microbial activity in grassland soils in other studies (Randall *et al.*, 2019).

However, in the slurry treatments the hypothesis that treatments with higher P would have a higher rate of SOM-C decomposition was rejected as the SOM-C decomposition rate in the P0 slurry treatment was higher than the P30 slurry treatment in terms of absolute values at all time points and was significantly higher on days 2 and 4. This trend in the slurry treatments was also seen in the planted system described in Chapter 3. There was a significant effect of the slurry treatment on soil--derived C mineralisation in this study. In this context, it is important to remember, as was outlined in Chapter 2, that the slurry and SOM decomposition sources to soil CO_2 efflux cannot be distinguished, as there was no isotope label used in the slurry and hence it had the same isotope signature as SOM. It is possible that the decomposition rate in the P0 slurry treatment is higher as this system is more reliant on nutrients provided by the slurry addition and hence, as slurry cannot be distinguished from SOM in this study, it is possible that it is slurry nutrients being decomposed instead of SOM. Previous studies have shown that the addition of an organic fertiliser, such as slurry, increases soil microbial activity and hence soil respiration (Sørensen, 1998; Cui, 2017), which may be the case in the P0 slurry treatment. However, in the P30 slurry treatment SOM-C decomposition rate was the second lowest of all treatments. As the slurry added to this soil in the field was a mixture of C, N and P compounds, this could be in agreement with Blagodatskaya and Kuzyakov (2008) who hypothesised that the addition of a combination of C and N would reduce SOM decomposition as microbial communities use more freely available sources of C and N. However, in this study if the slurry was being mineralised it would have been seen in the CO_2 flux.

It was hypothesised that SOM-C decomposition would be higher in the cut grassland system when compared to the grazed grassland system. This was supported as the rate of SOM-C decomposition was higher at both the P0 cut and P30 cut site compared with the P0 grazed and P30 grazed sites, respectively. There are several of potential explanations for this. Firstly, in the cut system there is a uniform removal of plant material, and hence nutrients and C in plant biomass are not returned to soil (Parsons *et al.*, 2013); this can lead to limitation of the microbial biomass and therefore to an increased need to mineralise both C and N to maintain growth and activity. In comparison, in a grazed system, although nutrients are removed through grazing, these nutrients are returned to the soil via livestock excreta. The majority of N and P (65Kg yr^{-1} N and 13Kg yr^{-1} P) are returned in animal excreta and up to 25-40% of C is also returned to the soil (Soussana *et al.*, 2010; Parsons *et al.*, 2013). Hence, the need to mineralise nutrients from SOM in a grazed site may be lower, as the addition of more labile forms of nutrients are available. Secondly, the starvation of the microbial biomass, particularly of C in the cut system, may encourage the decomposition of SOM to meet their C demand. The average microbial biomass C content was lower in the cut sites compared to that of the grazed sites (Table 4.2). The demand for nutrients can also be seen in potential N mineralisation, which is also higher in the cut sites (Figure 4.2). However, as the starvation state of the microbial biomass is potentially higher in the cut system this may have made this treatment more responsive to glucose addition. Similarly, when the grass is cut and removed there is a flush of C from the roots into the soil system. The higher decomposition of SOM-C in the cut sites may be a contributing factor in studies which has seen lower rates of C sequestration in cut silage-based grassland systems compared to in long term grazed systems (Fitter *et al.*, 1997; Senapati *et al.*, 2014; Ottaviani *et al.*, 2021).

4.6.3 The rate of priming was affected by phosphorus limitation and slurry addition

It was expected that the rate of priming would increase with higher soil P. The increase demand of nutrients, and larger microbial biomass size, with higher P addition over time would increase the rate of priming as has been seen in Chapter 2. This was the case across each of the management systems: in the cut system – the rate of priming was higher in the P30 cut compared to the P0 cut, in the grazed system P30 grazed had a higher rate of priming than P0 grazed. However, on days 9 and 11 the P30 slurry treatment had a higher rate of priming compared to the P0 slurry. At all other sampling times the P0 slurry treatment had a higher rate of priming. The P0 slurry treatment had the highest rate of priming on days 0, 2, and 7. However, the decomposition of SOM and slurry cannot be distinguished, and therefore it is possible that the nutrients applied in slurry are being primed here. Although, as this nutrient limited system is supplied with a labile C and N energy source, the P0 slurry treatment may be better equipped or alternatively have a microbial community that is better adapted to mining for nutrients compared to both the P0 cut and P0 grazed systems. Previous studies have shown that when

C and N are available, even in lower productivity systems, the rate of priming can increase (Murphy *et al.*, 2015). The results of this study highlight that organic compounds in slurry additions may be used in preference to stabilised, native SOM, promoting SOM retention and sequestration.

4.6.4 Bacterial community structures were altered by both phosphorus treatment and grassland management strategy

Results from previous studies at these sites showed differences in microbial community structure, particularly at the grazed grassland site with soil P treatments (Chen *et al.*, 2014; Randall *et al.*, 2019). It is important in the context of this study to note that the differences between the cut and grazed sites cannot be fully explained by the management strategy, as there are slight differences in the grazed site soil type as the clay content, for example, was marginally higher. As well as this the soil pH in the cut sites was higher than at the grazed site and this was affecting the microbial community structure (Table 4.1). Results from the PCA analysis showed that there were significant differences in bacterial community with P fertilisation level (i.e. high vs low P) in this experiment. It has been previously shown that differences in P have an impact on soil microbial community structure (Tan *et al.*, 2013; Chen *et al.*, 2014; Ling *et al.*, 2017; Ikoyi *et al.*, 2018; Randall *et al.*, 2019). In addition, the RDA analysis in this study showed that available P (PM) was significantly affecting the microbial community structure in both the glucose and control soils.

The effect of P level occurred in all of the management strategies, cut, slurry, and grazed. However, more significantly, different genera were found between P0 grazed and P30 grazed (Supplementary Tables). *Acidobacteria* are one of the most abundant bacterial phyla in soils (Ge *et al.*, 2008; Lee *et al.*, 2008) and in this study there were 132 significantly different genera found across the treatment combinations. In the cut site, of the significant differences, *Acidobacteria* genera were significantly more abundant in the P30 cut compared to the P0 cut treatment in the glucose soils. The relative abundance of *Acidobacteria* was also found to increase with higher P at this grazed site (Randall *et al.* (2019). In the current study, significant differences in the enrichment of *Acidobacteria* genera were found in the control soils where abundances were higher with the P30 grazed treatment (Supplementary Tables). This same trend also followed for the slurry treatment, where abundances were higher in the P30 slurry treatment. *Acidobacteria* are commonly reported in studies of agricultural soils; however, their ecological functions are not well understood (Nunes da Rocha *et al.*, 2013). Studies by Chen *et al.* (2014) and Wakelin *et al.* (2012) found that the abundance of *Actinobacteria* were linked with soil P status. The *Streptomyces* a member of *Actinobacteria* was found to be significantly higher in the P0 grazed treatment when compared with the P30 grazed, P0 cut, and P0 slurry treatments. Interestingly, Nelson *et al.* (2016) reported that *Streptomyces* are involved in N cycling activities, particularly the denitrification pathway. However, the P0 grazed treatment was not

associated with high N mineralisation. However, the abundance of *Streptomyces* may also be influenced by other factors as part of this experiment such as the rate of plant growth prior to soil sampling. Wakelin *et al.* (2012) reported that *Actinobacteria* abundances respond negatively to P fertilisation and this was also found to be the case in the P0 grazed control soils in this study when compared with the P30 grazed control soils. However, in the slurry treatment significant differences in the enrichment of *Actinobacteria* were found in the P30 slurry treatment when compared with the P0 slurry treatment.

The addition of slurry at the cut site caused significant changes in microbial community structure. However, this was not seen at this site by Randall *et al.* (2019), this could be due to time of sampling (i.e. there has been more slurry addition in between the two studies) but also the difference in data analysis techniques used. In the P0 cut treatment, *Chthoniobacter* was significantly more abundant compared with the P0 slurry treatment. It is interesting to note that from the RDA analysis the bacterial communities from the glucose soils were influenced by organic matter content. It is thought that *Chthoniobacter* are involved in the breakdown of soil organic C (Sangwan *et al.*, 2004). However, it is possible that the addition of glucose was stimulating this genus as a more labile form of C had been added to the soil. There were more significant differences between the control soils than the glucose soils for P0 cut vs P0 slurry. In the control P0 slurry treatment, *Candidatus Nitrososphaera* and *Sideroxydans* were significantly more abundant, both of which are involved in N cycling (Nelson *et al.*, 2016). However, it is not possible to say if this contributed to increased N mineralisation potential in the P0 slurry compared to the P0 cut treatment in the control soils. There were more differences in the enrichment of ASVs between the higher P treatments P30 cut vs P30 slurry (Supplementary tables).

4.6.5 Fungal community structures were altered by both phosphorus treatment and grassland management strategy

The dominant fungal group in agricultural grasslands is *Ascomycetes* (Deacon *et al.*, 2006). This same trend was seen in this study where *Ascomycota* phylum had the most significant differences in terms of ASVs abundance between the different treatment combinations (fungi Supplementary Tables). Soil fungal communities are influenced by long-term fertiliser application in agricultural systems (Parfitt *et al.*, 2010; Chen *et al.*, 2014; Leff *et al.*, 2015; Cassman *et al.*, 2016). Significant differences in fungal community composition were seen in all treatment combinations except the P0 cut and P0 grazed treatment control soils (Table 4.5). This is in contrast to the bacterial community composition, where significant differences were found between the P0 cut and P0 grazed treatments (Table 4.5). Previous studies have found that in grazed grassland systems the application of P can alter the fungal: bacterial ratio in soil, and hence lead to changes to microbial community structure (Parfitt *et al.*, 2010). Results

from the PCA analyses showed that there were distinct community groups between the grazed and the cut site, as well as distinct community groups within the cut site (Figures 4.13 and 4.14). It is important to note that soil pH was driving community structure in the fungal data set (Figures 4.17 and 4.18). This is important as the pH at the cut site was higher than at the grazed site and may be the driver of the differences seen in this study.

There were only 3 significant differences in fungal ASVs within the slurry treatment in the glucose soils. In fact, all of these differences were associated with the P30 slurry 13C soil. This could suggest that these genera (Clavaria, Absidia, Pseudogymnoascus) in the slurry and glucose soil were influenced by higher soil P. In the P0 slurry soils *Glomeromycota (Archaeospora*) was enriched. *Glomeromycota* are arbuscular mycorrhizal fungi (AMF) which form symbiotic relationships with plant roots, scavenge for nutrients in the rhizosphere, and have been known to assist in the uptake of immobile nutrients such as phosphate (Sanders and Tinker, 1971; Hodge *et al.*, 2001). AMF symbiosis can also contribute to the decomposition of grass leaves and increase N capture (Hodge *et al.*, 2001). In the context of this study, as *Glomeromycota* and in particular the genus *Funneliformis* was more often found in the lower P treatments (i.e. the P0s), it is possible that these groups were involved in P uptake in P limited systems. In the P0 grazed vs the P30 grazed control soil, the genus *Paraglomerales* was found to be enriched in the P0 grazed treatment. This genus is also part of the AMF group and previous studies have found that this genus is associated with soils lower in P as it assists plant roots in P uptake (Bolan, 1991; Cassman *et al.*, 2016).

There were significantly different genera enriched between the cut (inorganic fertiliser addition) and slurry (organic fertiliser addition) treatments in both the glucose and control soils. Ikoyi *et al.* (2020) also found significant differences in the ITS community with inorganic and organic P fertiliser addition. However, only 3 genera (Supplementary Materials) in the P0 slurry control were enriched when compared with the P0 cut control. This would suggest that, although the treatments were significantly different, there was not a strong shift in the community structure in the P0 slurry control with the slurry addition. However, with the addition of glucose there were 11 genera (Supplementary Materials) that were enriched in the P0 slurry treatment compared with the P0 cut glucose soil. The addition of glucose appeared to stimulate the growth of some genera in the P0 slurry treatment (Supplementary Tables).

4.7 Conclusion

There were three main research strands as part of this study. First, the effect of the addition of P. It was hypothesised that P addition would increase N mineralisation, SOM decomposition, priming, and impact microbial community structure. This hypothesis was not supported in terms of N mineralisation

as treatments that received less P had a higher mineralisation potential. However, in terms of SOM decomposition and priming this hypothesis was supported as both increased with P addition. The effect on the microbial community structure was more difficult to support as although no differences were seen in terms of alpha diversity there was an effects of P addition on the relative abundance of ASVs.

Secondly, the effect of type of P addition, was hypothesised that organic addition would increase N mineralisation, SOM decomposition, priming, and also impact on microbial community structure. In terms of N mineralisation potential, although higher with organic addition in the P0 slurry treatment, this hypothesis was not supported as differences in N mineralisation potential were not significant when considering the type of P addition. The hypothesis that SOM decomposition rate would increase was also not supported as in the P30 slurry treatment there was less SOM decomposition compared with the P30 cut treatment. However, the hypothesis that organic addition would impact on the microbial community structure was supported as there were differences in relative abundance as well as alpha diversity with organic addition.

Finally, grassland management system was considered, with a comparison between cut and grazed grassland systems examined. It was hypothesised that in a cut grassland system, N mineralisation and SOM decomposition would be increased. Both hypotheses were supported, as both were found to be higher in the cut grassland system. Grassland management was also hypothesised to have an impact on microbial community and this was supported as there were significant difference in the relative abundance of both 16S and ITS ASVs between the grassland systems, as well as significant differences in alpha diversity.

4. 8 References

Bardgett, R.D., Bowman, W.D., Kaufmann, R., Schmidt, S.K., 2005. A temporal approach to linking aboveground and belowground ecology. *Trends in Ecology & Evolution* 20, 634-641.
Bardgett, R.D., Wardle, D.A., 2003. Herbivore-mediated linkages between aboveground and belowground communities. *Ecology* 84, 2258-2268.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45, 115-131.
Bolan, N., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil* 134, 189-207.
Cassman, N.A., Leite, M.F., Pan, Y., de Hollander, M., van Veen, J.A., Kuramae, E.E., 2016. Plant and soil fungal but not soil bacterial communities are linked in long-term fertilized grassland. *Scientific Reports* 6, 1-11.
Chen, X., Daniell, T.J., Neilson, R., O'Flaherty, V., Griffiths, B.S., 2014. Microbial and microfaunal communities in phosphorus limited, grazed grassland change composition but maintain homeostatic nutrient stoichiometry. *Soil Biology and Biochemistry* 75, 94-101.

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85, 235-252.
Cleveland, C.C., Townsend, A.R., Schmidt, S.K., 2002. Phosphorus Limitation of Microbial Processes in Moist Tropical Forests: Evidence from Short-term Laboratory Incubations and Field Studies. *Ecosystems* 5, 0680-0691.
Cui, J., 2017. The Effect of Grassland Management History on Soil Carbon Response to Slurry and Urea. *Open Access Library Journal* 4, 1.
Daly, K., Casey, A., 2005. Environmental aspects of soil phosphorus testing. *Irish Journal of Agricultural and Food Research*, 261-279.
Deacon, L.J., Pryce-Miller, E.J., Frankland, J.C., Bainbridge, B.W., Moore, P.D., Robinson, C.H., 2006. Diversity and function of decomposer fungi from a grassland soil. *Soil Biology and Biochemistry* 38, 7-20.
Dengler, J., Janišová, M., Török, P., Wellstein, C., 2014. Biodiversity of Palaearctic grasslands: a synthesis. *Agriculture, Ecosystems & Environment* 182, 1-14.
Fang, Y., Singh, B.P., Farrell, M., Van Zwieten, L., Armstrong, R., Chen, C., Bahadori, M., Tavakkoli, E., 2020. Balanced nutrient stoichiometry of organic amendments enhances carbon priming in a poorly structured sodic subsoil. *Soil Biology and Biochemistry* 145, 107800.
Farrell, M., Prendergast-Miller, M., Jones, D.L., Hill, P.W., Condron, L.M., 2014. Soil microbial organic nitrogen uptake is regulated by carbon availability. *Soil Biology and Biochemistry* 77, 261-267.
Fitter, A., Graves, J., Wolfenden, J., Self, G., Brown, T., Bogie, D., Mansfield, T., 1997. Root production and turnover and carbon budgets of two contrasting grasslands under ambient and elevated atmospheric carbon dioxide concentrations. *The New Phytologist* 137, 247-255.
Frąc, M., Hannula, S.E., Bełka, M., Jędryczka, M., 2018. Fungal Biodiversity and Their Role in Soil Health. *Frontiers in Microbiology* 9.
Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. *Soil Biology and Biochemistry* 43, 1705-1713.
Ge, Y., Zhang, J.-b., Zhang, L.-m., Yang, M., He, J.-z., 2008. Long-term fertilization regimes affect bacterial community structure and diversity of an agricultural soil in northern China. *Journal of Soils and Sediments* 8, 43-50.
Goerges, T., Dittert, K., 1998. Improved diffusion technique for 15N:14N analysis of ammonium and nitrate from aqueous samples by stable isotope spectrometry. *Communications in Soil Science and Plant Analysis* 29, 361-368.
Grayston, S., Campbell, C., Bardgett, R., Mawdsley, J., Clegg, C., Ritz, K., Griffiths, B., Rodwell, J., Edwards, S., Davies, W., 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. *Applied Soil Ecology* 25, 63-84.
Griffiths, B.S., Spilles, A., Bonkowski, M., 2012. C:N:P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. *Ecological Processes* 1, 6.
Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA-and rRNA-based microbial community composition. *Appl. Environ. Microbiol.* 66, 5488-5491.
Hamilton, E.W., Frank, D.A., Hinchey, P.M., Murray, T.R., 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. *Soil Biology and Biochemistry* 40, 2865-2873.
Hamilton III, E.W., Frank, D.A., 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* 82, 2397-2402.
Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413, 297-299.

Ikoyi, I., Egeter, B., Chaves, C., Ahmed, M., Fowler, A., Schmalenberger, A., 2020. Responses of soil microbiota and nematodes to application of organic and inorganic fertilizers in grassland columns. *Biology and Fertility of Soils* 56, 647-662.
Ikoyi, I., Fowler, A., Schmalenberger, A., 2018. One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. *Science of The Total Environment* 630, 849-858.
Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K., Richter, A., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. *FEMS Microbiology Ecology* 73, 430-440.
Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363-1371.
Lee, S.-H., Ka, J.-O., Cho, J.-C., 2008. Members of the phylum Acidobacteria are dominant and metabolically active in rhizosphere soil. *FEMS Microbiology Letters* 285, 263-269.
Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J., Fierer, N., 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences* 112, 10967.
Leinweber, P., Kruse, J., Baum, C., Arcand, M., Knight, J.D., Farrell, R., Eckhardt, K.-U., Kiersch, K., Jandl, G., 2013. Chapter Two - Advances in Understanding Organic Nitrogen Chemistry in Soils Using State-of-the-art Analytical Techniques. In: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 83-151.
Ling, N., Chen, D., Guo, H., Wei, J., Bai, Y., Shen, Q., Hu, S., 2017. Differential responses of soil bacterial communities to long-term N and P inputs in a semi-arid steppe. *Geoderma* 292, 25-33.
Liu, Z., Rong, Q., Zhou, W., Liang, G., 2017. Effects of inorganic and organic amendment on soil chemical properties, enzyme activities, microbial community and soil quality in yellow clayey soil. *PLOS ONE* 12, e0172767.
Massey, P.A., 2012. *Interactions between phosphorus fertilisation and soil biota in managed grasslands systems*. Doctor of Philosophy, Cranfield
Massey, P.A., Creamer, R.E., Whelan, M.J., Ritz, K., 2016. Insensitivity of soil biological communities to phosphorus fertilization in intensively managed grassland systems. *Grass and Forage Science* 71, 139-152.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2015. Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry* 81, 236-243.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. *Plant and Soil* 417, 499-510.
Nelson, M.B., Martiny, A.C., Martiny, J.B.H., 2016. Global biogeography of microbial nitrogen-cycling traits in soil. *Proceedings of the National Academy of Sciences* 113, 8033.
Nottingham, A.T., Hicks, L.C., Ccahuana, A.J.Q., Salinas, N., Bååth, E., Meir, P., 2018. Nutrient limitations to bacterial and fungal growth during cellulose decomposition in tropical forest soils. *Biology and Fertility of Soils* 54, 219-228.
Nunes da Rocha, U., Plugge, C.M., George, I., van Elsas, J.D., van Overbeek, L.S., 2013. The Rhizosphere Selects for Particular Groups of Acidobacteria and Verrucomicrobia. *PLOS ONE* 8, e82443.
Olsen, Y.S., Dausse, A., Garbutt, A., Ford, H., Thomas, D.N., Jones, D.L., 2011. Cattle grazing drives nitrogen and carbon cycling in a temperate salt marsh. *Soil Biology and Biochemistry* 43, 531-541.
Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., Colpaert, J.V., 2014. Comparison and Validation of Some ITS Primer Pairs Useful for Fungal Metabarcoding Studies. *PLOS ONE* 9, e97629.

Ottaviani, G., Lubbe, F.C., Lepš, J., Lisner, A., Martínková, J., Mudrák, O., Klimešová, J., 2021. Strong impact of management regimes on rhizome biomass across Central European temperate grasslands. *Ecological Applications* 31, e02317.
Parfitt, R.L., Yeates, G.W., Ross, D.J., Schon, N.L., Mackay, A.D., Wardle, D.A., 2010. Effect of fertilizer, herbicide and grazing management of pastures on plant and soil communities. *Applied Soil Ecology* 45, 175-186.
Parsons, A., Edwards, G., Newton, P., Chapman, D., Caradus, J., Rasmussen, S., Rowarth, J., 2011. Past lessons and future prospects: plant breeding for yield and persistence in cool-temperate pastures. *Grass and Forage Science* 66, 153-172.
Parsons, A.J., Thornley, J.H.M., Newton, P.C.D., Rasmussen, S., Rowarth, J.S., 2013. Soil carbon dynamics: The effects of nitrogen input, intake demand and off-take by animals. *Science of The Total Environment* 465, 205-215.
Phillips, R.P., Brzostek, E., Midgley, M.G., 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist* 199, 41-51.
Phillips, R.P., Finzi, A.C., Bernhardt, E.S., 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecology Letters* 14, 187-194.
Poeplau, C., 2021. Grassland soil organic carbon stocks along management intensity and warming gradients. *Grass and Forage Science* 76, 186-195.
Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing Noise From Pyrosequenced Amplicons. *BMC Bioinformatics* 12, 38.
Randall, K., Brennan, F., Clipson, N., Creamer, R., Griffiths, B., Storey, S., Doyle, E., 2019. Soil bacterial community structure and functional responses across a long-term mineral phosphorus (Pi) fertilisation gradient differ in grazed and cut grasslands. *Applied Soil Ecology* 138, 134-143.
Rumpel, C., Crème, A., Ngo, P., Velásquez, G., Mora, M., Chabbi, A., 2015. The impact of grassland management on biogeochemical cycles involving carbon, nitrogen and phosphorus. *Journal of Soil Science and Plant Nutrition* 15, 353-371.
Sanders, F.E., Tinker, P.B., 1971. Mechanism of Absorption of Phosphate from Soil by Endogone Mycorrhizas. *Nature* 233, 278-279.
Sangwan, P., Chen, X., Hugenholtz, P., Janssen, P.H., 2004. Chthoniobacter flavus gen. nov., sp. nov., the first pure-culture representative of subdivision two, Spartobacteria classis nov., of the phylum Verrucomicrobia. *Appl. Environ. Microbiol.* 70, 5875-5881.
Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85, 591-602.
Schröder, J.J., Schulte, R.P.O., Creamer, R.E., Delgado, A., van Leeuwen, J., Lehtinen, T., Rutgers, M., Spiegel, H., Staes, J., Tóth, G., Wall, D.P., 2016. The elusive role of soil quality in nutrient cycling: a review. *Soil Use and Management* 32, 476-486.
Senapati, N., Chabbi, A., Gastal, F., Smith, P., Mascher, N., Loubet, B., Cellier, P., Naisse, C., 2014. Net carbon storage measured in a mowed and grazed temperate sown grassland shows potential for carbon sequestration under grazed system. *Carbon Management* 5, 131-144.
Sheil, T.S., Wall, D.P., Culleton, N., Murphy, J., Grant, J., Lalor, S.T.J., 2016. Long-term effects of phosphorus fertilizer on soil test phosphorus, phosphorus uptake and yield of perennial ryegrass. *The Journal of Agricultural Science* 154, 1068-1081.
Sørensen, P., 1998. Effects of storage time and straw content of cattle slurry on the mineralization of nitrogen and carbon in soil. *Biology and Fertility of Soils* 27, 85-91.
Soussana, J.-F., Tallec, T., Blanfort, V., 2010. Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. *animal* 4, 334-350.
Sun, G., Zhu-Barker, X., Chen, D., Liu, L., Zhang, N., Shi, C., He, L., Lei, Y., 2017. Responses of root exudation and nutrient cycling to grazing intensities and recovery practices in an alpine meadow: An implication for pasture management. *Plant and Soil* 416, 515-525.

Tan, H., Barret, M., Mooij, M.J., Rice, O., Morrissey, J.P., Dobson, A., Griffiths, B., O'Gara, F., 2013. Long-term phosphorus fertilisation increased the diversity of the total bacterial community and the phoD phosphorus mineraliser group in pasture soils. *Biology and Fertility of Soils* 49, 661-672.
Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13, 87-115.
Wakelin, S., Mander, C., Gerard, E., Jansa, J., Erb, A., Young, S., Condron, L., O'Callaghan, M., 2012. Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. *Applied Soil Ecology* 61, 40-48.
Yin, H., Wheeler, E., Phillips, R.P., 2014. Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biology and Biochemistry* 78, 213-221.
Žifčáková, L., Větrovský, T., Howe, A., Baldrian, P., 2016. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. *Environmental Microbiology* 18, 288-301.

Chapter 5

The effect of soil pH on soil organic matter turnover and nitrogen mineralisation in grassland soils

5.1 Introduction

N is a limiting nutrient in most terrestrial ecosystems, particularly in systems used for agricultural production, and is one of the key nutrients required for plant growth (Vitousek and Howarth, 1991; Rütting *et al.*, 2018). N limitation in some agricultural systems is overcome by the addition of inorganic fertilisers (Bouwman *et al.*, 2013; Bodirsky *et al.*, 2014; Schröder *et al.*, 2016) that support plant growth but may lead to losses of N via leaching to waterways or losses to the atmosphere via greenhouse gases, such as N_2O (Guignard *et al.*, 2017; Rütting *et al.*, 2018). Soil organic matter (SOM) is the largest store of soil N, however, this store of N is not generally available for direct plant uptake (Leinweber *et al.*, 2013). Microbially mediated SOM mineralisation is required to transform the N stored in SOM to plant available forms (Murphy *et al.*, 2017). Plants can promote microbial processes through root exudation of labile C compounds that are easily accessible to soil microorganisms and hence can stimulate microbial activity such as enzyme production, that increases N release from SOM (Kuzyakov, 2010; Phillips *et al.*, 2013; Sun *et al.*, 2017). Understanding the microbially mediated processes involved in SOM mineralisation, and also other plant-microbial interactions (e.g. microbial community development in the rhizosphere) would allow better matching of N fertilisation rates with plant demand, reducing environmental losses and economic costs of N. However, there are a number of environmental factors that can also influence microbially mediated processes in soils such as water status, temperature and pH (Booth *et al.*, 2005; Wang *et al.*, 2015). Soil pH is often referred to in the literature as the "master soil variable", as it influences soil physical, chemical, and biological processes (Brady *et al.*, 2008; Neina, 2019; Žurovec *et al.*, 2021). The effects of soil pH on microbial communities, structure, activity and abundance have been widely reported in the literature (Kemmitt *et al.*, 2006; Page *et al.*, 2009; Rousk *et al.*, 2009; Rousk *et al.*, 2010a; Rousk *et al.*, 2010b; Zhalnina *et al.*, 2015; Trivedi *et al.*, 2016; Zheng *et al.*, 2019; Abalos *et al.*, 2020). It has been found that fungal communities are generally less sensitive to pH than bacterial communities (Lauber *et al.*, 2008; Liu *et al.*, 2018), and at low pH, fungal communities can have a higher abundance than bacterial communities (Fierer and Jackson, 2006; Rousk *et al.*, 2009). However, the effect of soil pH on microbially mediated processes, such as those involved in SOM turnover, priming, and N mineralisation are less well understood. It can be considered that the microbial mediated processes could also be affected y by soil pH, as the effects on microbial community are so great (Cheng *et al.*, 2013).

N transformation, and N transformation rates, particularly of nitrification and denitrification, have been shown to be influenced by soil pH in a variety of agricultural systems (Zhang *et al.*, 2018; Zhu *et al.*, 2019). Soil pH can also impact on SOM decomposition, and thus N release from the SOM pool, through altering the solubility of soil organic carbon (SOC) or through changes to microbial biomass and activity (Andersson *et al.*, 2000; Bertrand *et al.*, 2007; Briedis *et al.*, 2012; Wang and Tang, 2017; Herold *et al.*, 2018). Microbial and enzymatic activity are higher in soils ranging in pH from 5-8 than in soils that have lower pHs (Blagodatskaya and Anderson, 1998). A study by Perelo and Munch (2005) showed that the magnitude of the priming effect was higher in soils with a pH of 6.1 compared with soil of a pH of 5.9 or less and the optimum pH for soil priming is considered to be in the range pH 6-8 (Blagodatskaya and Kuzyakov, 2008; Aye *et al.*, 2017; Aye *et al.*, 2018). Previous studies have also found that N mineralisation can be effected by soil pH (Bolan *et al.*, 2003; Bolan and Hedley, 2003), with the optimum soil pH for N mineralisation reported to be approximately pH 6 (Blagodatskaya and Anderson, 1998). However, there are few studies that have examined the effect of soil pH on SOM decomposition, priming and N mineralisation in a planted system (Ahmad *et al.*, 2013), as most studies have examined these effects through the addition of glucose or lignin based stable isotope labels. This study provides a unique opportunity to examine the effect of long-term soil pH treatments on SOM decomposition in a planted grassland soil.

In areas where the amount of rainfall is often higher than the rate of evapotranspiration, such as Ireland, this can lead to the acidification of soils (Corbett *et al.*, 2021). Lime is a commonly used agricultural practice to combat the detrimental effects of soil acidification (Paradelo *et al.*, 2015; Aye *et al.*, 2016; Holland *et al.*, 2018; Corbett *et al.*, 2021). The main purpose of lime addition is to neutralise hydrogen ions (H^+) that are in excess in the soil solution, and hence increase soil pH (Bolan *et al.*, 2003). Liming generally has a positive influence on soil, as it can improve soil structure, increase nutrient availability, and soil microbial activity (Holland *et al.*, 2018; Žurovec *et al.*, 2021). Liming of soils can improve soil structure through increasing soil water holding capacity, improving aggregate stability by increasing the pore space between aggregates, which also improves soil hydraulic conductivity (Haynes and Naidu, 1998; Bolan *et al.*, 1999; Tran *et al.*, 2014; Blomquist *et al.*, 2018). Changes to aggregate stability (particularly between clay and organic particles) can also cause changes to SOC stability (and accessibility to microbial communities), and hence SOM mineralisation (Fuentes *et al.*, 2006). Liming of soils also plays an important role in nutrient availability through increasing the cation exchange capacity and soil surface charge (Li *et al.*, 2019). Lime neutralises acidic cations such as H^+, which increases the availability of nutrients such as P, K, and S (Bolan *et al.*, 2003), as well as supplying Ca^{2+} and Mg^{2+} (depending on the type of lime used) which are also important nutrients for crop growth. Also, liming has also been reported to increase root density (Tang *et al.*, 2003), which

has potential to increase root respiration and exudation (Kunhikrishnan *et al.*, 2016), with potential impacts on the rate of priming and SOM decomposition. Plant- soil-microbe interactions impact on nutrient availability in agricultural systems, including through SOM decomposition and N mineralisation. However, how the impact of liming soils effects the mechanisms involved in nutrient cycling and in particular the mechanisms involved N cycling dynamics is still poorly understood.

In Chapter 2 of this thesis provided evidence that as soil pH increased N mineralisation also increased, indicating a potentially important role for pH in the rate transformation of N from the SOM pool. Further there was evidence in both Chapter 2 and Chapter 4 that pH had a significant influence on microbial community structure. The combination of N mineralisation potential, SOM decomposition, and microbial community dynamic is not widely reported in the literature. Therefore, this study will examine the impacts of soil pH and liming on priming, SOM decomposition, N mineralisation and the associated microbial community structure, to investigate in more detail the findings of Chapter 2 and 3. In this study it was hypothesised that N mineralisation would increase with increasing soil pH and liming, and that the highest potential mineralisation rate would be in soil with the highest pH, which received the most lime. It was further hypothesised that SOM decomposition rate would be highest in soils with higher pH, and would increase with increasing pH. Finally, it was hypothesised that microbial community structure would be altered by soil pH, with the greatest differences in microbial communities observed where differences in pH were greatest. These hypotheses were tested using soils from a long-term pH field experiment, where lime treatments were applied in the field over eight years that were then incubated in a ^{13}C labelling chamber under *Lolium perenne*, a common grass species used in Irish pastures

5.2 Methods

To address the stated hypotheses soil from a long-term grassland pH trial was used as a basis of an incubation experiment that sought to measure potential N mineralisation, SOM decomposition rate, and the associated microbial community structure. This field site last received lime more than 12 months before this study was undertaken. However, SOM decomposition rate was labelled as Soil-C decomposition rate in this chapter as there was an enrichment from lime dissolution (part of the CO_2 flux that was measured as SOM was CO_2 flux from $CaCO_3$ lime dissolution) as part of the VH treatment. However, the VL, L, and H treatments did not show any lime dissolution.

5.2.1 Site description and soil sampling

The selected trial site was located at Johnstown Castle Research Centre Farm, Co. Wexford, Ireland (52°17'47''N 6°30'25''W). This site was first established in 2011 as a liming and phosphorus trial under a perennial ryegrass (*Lolium perenne*) monoculture, to investigate lime, N, P, and K dynamics in a

grassland system (Sheil *et al.*, 2016; Žurovec *et al.*, 2021). The soil at this site is a moderately drained brown earth (Stagnic Cambisol, ISSU-WRB and group (2015)). It has a loamy texture with sand 49%, silt 29%, and clay 22% content (Žurovec *et al.*, 2021). This trial was set up as a randomised block design, with each plot measuring 1.5 X 6m and four replicate plots for each treatment. There were four lime and four phosphorus treatments, as well as two K treatments (Figure 5.1). Lime application was as ground limestone, $CaCO_3$, P as triple super phosphate, 16% P, and K as Potash (Figure 5.1). N was applied equally across all treatments as Sulfa CAN (calcium ammonium nitrate with sulphur, 26% – N; 5% – S). Application rates of all nutrient management additions can be seen in Figure 1. Plots were harvested as a cut and remove silage-based system 8-9 times a year depending on winter growth rate (Figure1).

Lime and pH treatments in this field trial were as follows:

VL – no lime application
L – 5 t ha^{-1} lime applied in 2011
H- 5 t ha^{-1} lime applied in 2014 and 1.5 t ha^{-1} applied in January 2019
VH- 5 t ha^{-1} lime applied in 2011, 2014 and 5 t ha^{-1} applied in January 2019

While all pH treatment levels were sampled, only the P60 treatment receiving 250 kg ha^{-1} K (Figure 5.1) was selected for this study. This was to ensure that there was no nutrient limitation within the treatments. Soil samples were taken in December 2019 and were taken using bulk sampling to a depth of 10cm following a “W” pattern from each replicated plot and homogenised to form one composite sample per treatment. Samples were stored at 4^{o}C prior to the establishment of the stable isotope labelling experiment.

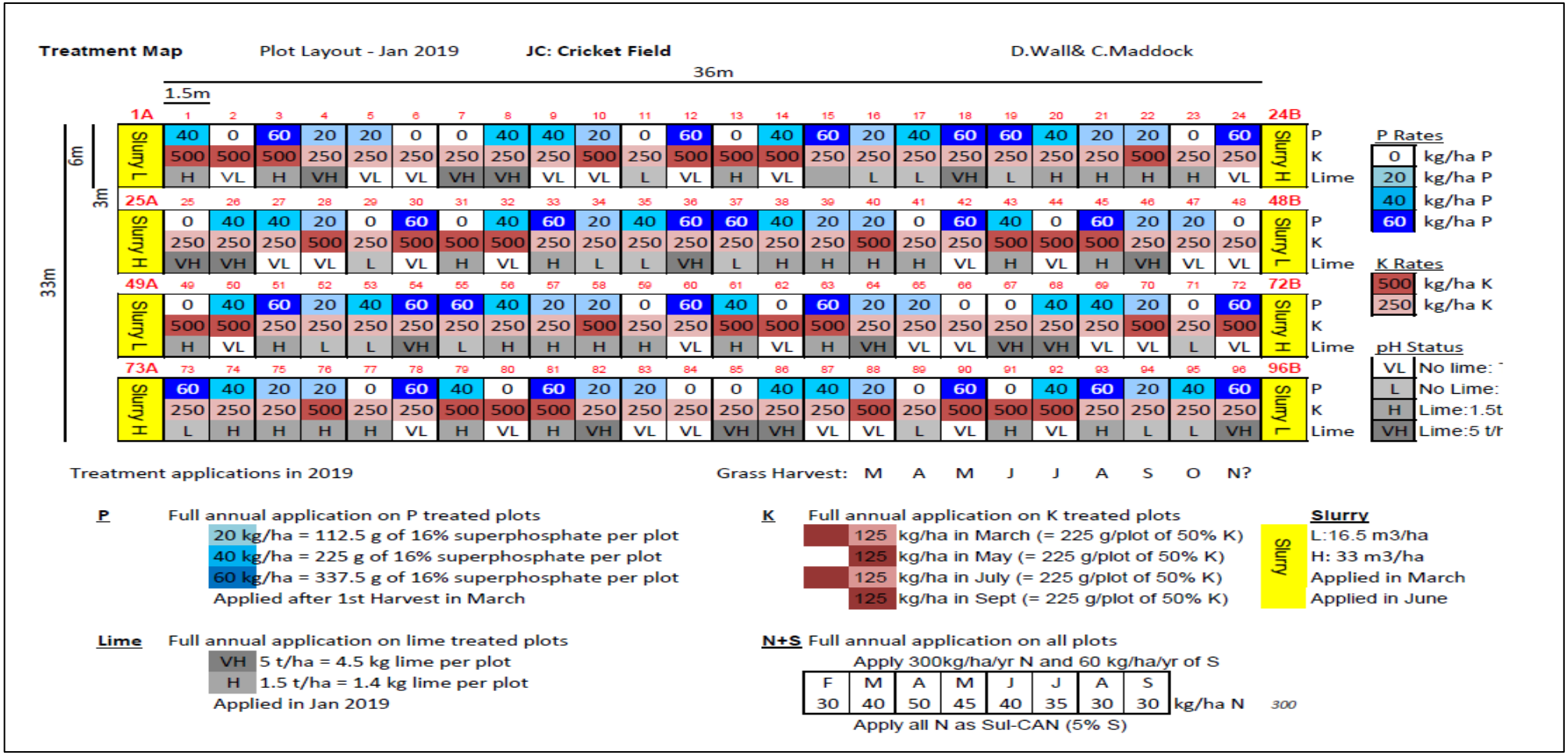


Figure 5.1: Plot trial layout and nutrient addition information at the Johnstown Castle Research Centre Farm. For the purpose of this study, all pH treatments (VL, L, H, VH) at P60 and K250 were sampled.

5.2.2 Pot and $^{13}CO_2$ depleted labelling tank set up, and measurements

Methods here were similar to those used in Chapter 3 (3.2.2). The pots used and were packed as described in Chapter 3 (3.2.2). The same plant species (*Lolium perenne*) was also used, and sowing, and maintenance of the pots was the same as in Chapter 3 (3.2.2). The environmental setting for the labelling chamber such as daylight hours and temperature were also previous described in Chapter 3 (3.2.2). The style of isotope labelling used in this chapter differed slightly from Chapter 3. From the day of sowing to harvest at the end of the experiment, the labelling chamber was flushed with CO_2-free air at 10L min^{-1}, and with ^{13}C depleted CO_2 (-38‰) supplied at 25L min^{-1} with a CO_2 concentration of 400µl L^{-1} (Paterson *et al.*, 2007). Flows were controlled using Mass Flow Controllers (Flotech Solutions, Stockport, UK). The delta value and CO_2 level were checked regularly throughout the experiment using an isotopic gas bench (details in Chapter 3 (3.2.2) and EGM-4 infrared gas analyser (EGM-4, PP-Systems, Amesbury, USA).

Gas sampling was carried out as previously described in Chapter 3 (3.2.2). However, the duration of this experiment was shorter, as there were only four sampling periods. Gas samples were analysed on the isotopic gas bench as described in Chapter 3 (3.2.2).

5.2.3 Soil and crop sampling on the final day (Day 32) of the experiment

At the end of the gas sampling period (Day 32) pots were harvested. Soil was collected from the pots and analysed for total pH, total C, total N, OM, available P, and available K. Shoots and roots were also harvested, dried, and weighed as described in section 3.2.2. Roots and shoots were also analysed for their nutrient content C, N, P, K, and sulphur (S). A subsample of soil was also placed directly into the -80°C freezer for DNA-based microbial studies. Calculations were also carried out as described in Chapter 3 (section 3.2.2, Equation 1-2)

5.2.4 Seven-day anaerobic incubation – Potential nitrogen mineralisation assay

This procedure was carried out as was described in Chapter 3 section (3.2.3). There were no modifications to the method for this chapter.

5.2.5 Microbial biomass extraction carbon, nitrogen, and phosphorus

Microbial biomass C, N, and P were determined using chloroform fumigation-extraction on fresh soil samples, methods were previously described in Chapter 3 section 3.2.4

5.2.6 Soil nutrient analyses

Nutrient analyses of total C, total N, OM, and available P and K were carried out as previously described in section Chapter 3 section 3.2.6.

5.2.7 DNA extraction and sequencing

Methods for DNA extraction and sequencing were identical to Chapter 4 section 4.2.7 and section 4.2.8 as samples were extracted and sent for sequencing at the same time under the same laboratory conditions.

5.2.8 Statistical analyses

As with Chapter 2 section 2.3.7 and Chapter 3 section 3.2.7.data analysis was performed in R Studio 4.2.0 (R Core Team, 2021). Statistical significance was determined at $p<0.05$.

5.2.9 Data handling and analyses of microbial data

Analysis of the data produce from the sequencing of the 16S and ITS communities was carried out as described in Chapter 2 section 2.3.8.

5.3 Results

5.3.1 Plant biomass and root and shoot tissue nutrient concentration

The results expressed here represent material harvested on the final day of the experiment (Day 32). At the end of the experiment the H treatment had the highest absolute mean shoot biomass, and the L treatment had the highest absolute mean root biomass (Table 5.1). There were no significant differences in biomass of either shoot or root material between the treatments.

In the shoot material the VH treatment had a significantly higher K (mg g^{-1}) concentration than the VL treatment ($p<0.01$) (Table 5.1). The N concentration of the shoot material was significantly higher in the VL treatment when compared with the L and H treatments ($p<0.01$) (Table 5.1). The shoot sulphur concentration was also significantly higher in the VL treatment compared to the VH treatment ($p<0.01$). There were no significant differences in plant shoot P and C. In the root material, total N content was significantly higher in the VL treatment ($p<0.01$) when compared with the L treatment (Table 5.1). There were no significant differences in P, K, sulphur, C in the root material between the different treatments.

Table 5.1: Plant biomass, root and shoot tissue data (n=4) data in parentheses = standard error. Letters represent significant differences between treatments (p<0.05).

Treatment	Biomass (g)	C (%)	N (%)	P (%)	K (%)	S (%)
Shoot						
VL	1.52 (±0.04)	39.90 (±0.76) a	2.13 (±0.14) b	0.24 (±0.00) a	0.81 (±0.03) a	0.19 (±0.00) a
L	1.52 (±0.01)	38.18 (±0.96) a	1.50 (±0.04) a	0.23 (±0.00) a	0.93 (±0.03) ab	0.15 (±0.00) ab
H	1.76 (±0.26)	37.73 (±1.65) a	1.49 (±0.09) a	0.26 (±0.01) a	0.99 (±0.05) b	0.14 (±0.01) a
VH	1.58 (±0.09)	38.13 (±0.85) a	1.87 (±0.10) ab	0.24 (±0.01) a	1.09 (±0.05) b	0.17 (±0.01) bc
Root						
VL	2.91 (±0.09)	23.22 (±1.03) a	1.03 (±0.01) b	0.17 (±0.00) a	0.61 (±0.03) a	0.10 (±0.00) a
L	3.93 (±0.60)	17.28 (±1.38) a	0.76 (±0.05) a	0.16 (±0.01) a	0.56 (±0.05) a	0.08 (±0.01) a
H	2.78 (±0.21)	22.25 (±1.35) a	0.86 (±0.06) ab	0.17 (±0.01) a	0.68 (±0.04) a	0.09 (±0.01) a
VH	2.79 (±0.48)	20.45 (±1.98) a	0.94 (±0.08) ab	0.17 (±0.01) a	0.65 (±0.05) a	0.10 (±0.01) a

5.3.2 Soil properties in the planted and fallow treatments

Soil results here are also representative of soil samples taken on the final day (Day 32) of the experiment. As was expected, there was a significant difference in soil pH between pH treatments ($p<0.01$) (Table 5.2). The lowest pH on average was in the VL fallow treatment and the highest pH on average was in the VH fallow treatment (Table 5.2). In the planted pots, the H treatment had a significantly lower total N concentration then the L planted treatment ($p<0.01$). There was no significant difference in total N in the fallow pots. The OM content in the H treatment in both the planted and fallow pots were significantly lower than all other treatments ($p<0.01$) (Table 5.2). P, measured as available P Morgan's, was significantly higher in the VH planted and fallow pots when compared with all other treatments ($p<0.01$). The H treatment was also significantly higher in available P when compared with the L and VL planted pots ($p<0.01$) (Table 5.2). The fallow pots had significantly higher available K compared to the planted pots ($p<0.01$). In the planted pots the VL treatment had significantly higher K compared with the L and VH treatments ($p<0.01$) (Table 5.2).

Table 5.2: Soil nutrient data from plant and fallow pots (n=4) data in parentheses = standard error. Letters represent significant differences between treatments (p<0.01).

	Potential N Min	Soil pH	TN	OM	TC	PM	KM	MBC	MBN	MBP
Planted	NH_4 g^{-1} dry soil		%	%	%	mg Kg^{-1}	mg Kg^{-1}	ug g^{-1}	ug g^{-1}	ug g^{-1}
VL	23.99 (±7.56) ab	4.67 (±0.01) b	0.30 (±0.00) ab	7.35 (±0.06) bc	3.10 (±0.02) ab	6.57 (±0.10) a	34.65 (±0.56) b	230.81 (±6.37) a	17.30 (±3.06) a	23.15 (±7.45) a
L	45.65 (±4.29) abc	5.11 (±0.01) d	0.33 (±0.03) b	7.31 (±0.06) abc	3.61 (±0.38) b	7.80 (±0.06) ab	30.10 (±0.57) a	209.01 (±24.03) a	42.78 (±2.19) abc	25.48 (±6.16) a
H	56.48 (±3.44) bc	5.75 (±0.02) f	0.27 (±0.00) a	6.93 (±0.07) ab	2.93 (±0.07) a	10.34 (±0.31) d	30.65 (±0.93) ab	243.37 (±7.57) a	51.35 (±0.89) bc	24.29 (±1.34) a
VH	68.93 (±10.16) c	6.41 (±0.06) g	0.29 (±0.00) ab	7.65 (±0.18) c	3.12 (±0.04) ab	16.05 (±0.41) e	28.35 (±0.39) a	200.19 (±45.93) a	56.37 (±12.31) c	23.50 (±2.22) a
Fallow										
VL	17.37 (±8.17) a	4.45 (±0.01) a	0.32 (±0.00) ab	7.64 (±0.09) c	3.15 (±0.04) ab	8.17 (±0.12) bc	41.35 (±0.95) c	228.20 (±9.20) a	24.74 (±12.33) ab	20.25 (±3.50) a
L	53.21 (±3.14) bc	4.90 (±0.01) c	0.31 (±0.00) ab	7.57 (±0.22) c	3.24 (±0.05) ab	8.50 (±0.03) bc	52.28 (±0.44) d	216.79 (±5.38) a	58.32 (±1.55) c	18.19 (±3.62) a
H	50.64 (±10.84) abc	5.61 (±0.02) e	0.28 (±0.00) ab	6.69 (±0.18) a	2.94 (±0.02) a	9.70 (±0.18) cd	49.38 (±0.85) d	202.06 (±9.59) a	35.08 (±2.93) bc	18.68 (±4.36) a
VH	77.88 (±5.74) c	6.54 (±0.04) g	0.31 (±0.01) ab	7.48 (±0.14) bc	3.25 (±0.07) ab	18.86 (±0.74) e	44.90 (±1.72) c	182.06 (±13.90) a	57.93 (±1.21) c	18.60 (±1.44) a

5.3.3 Potential nitrogen mineralisation

Potential N mineralisation varied with treatment (Figure 5.2), and was significantly affected by pH treatment ($p<0.0001$). However, there was no significant difference in N mineralisation potential between planted vs fallow pots. Results from the Tukey analysis showed that the VH treatment had a significantly higher N mineralisation potential compared to the VL treatment ($p<0.01$) in both the planted and the fallow pots. There was a significant effect of soil pH on potential N mineralisation ($p<0.001$, $r^2=0.6$). There was also a significant effect of available soil P (measured as P Morgan's) on potential N mineralisation ($p<0.001$, $r^2=0.5$).

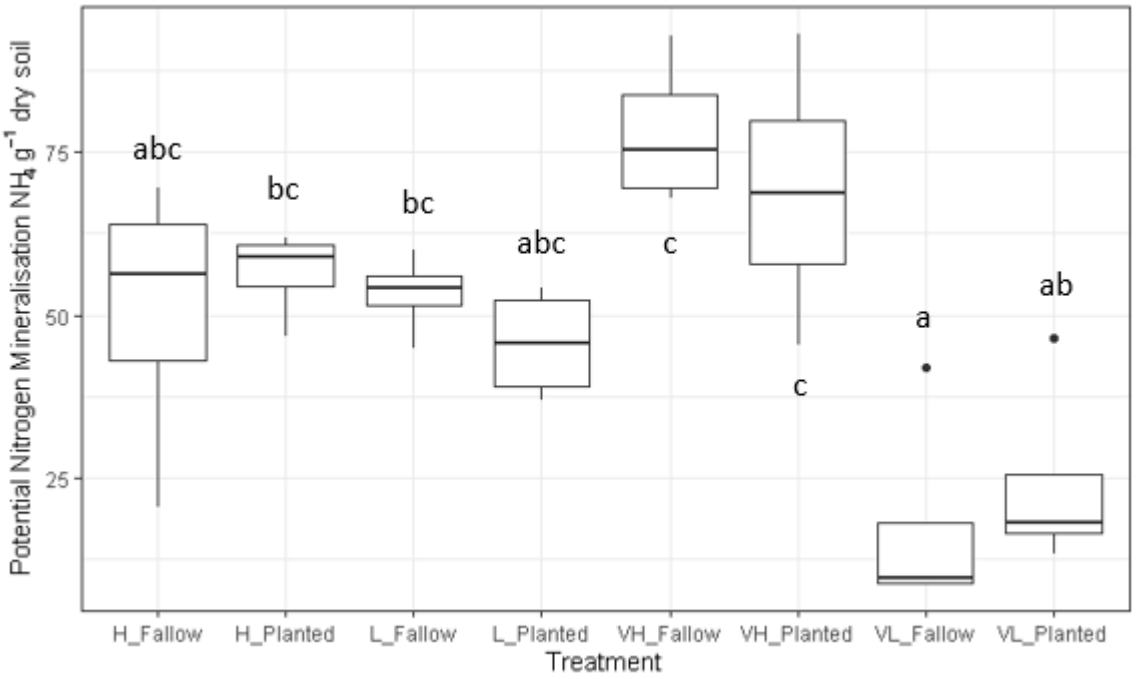
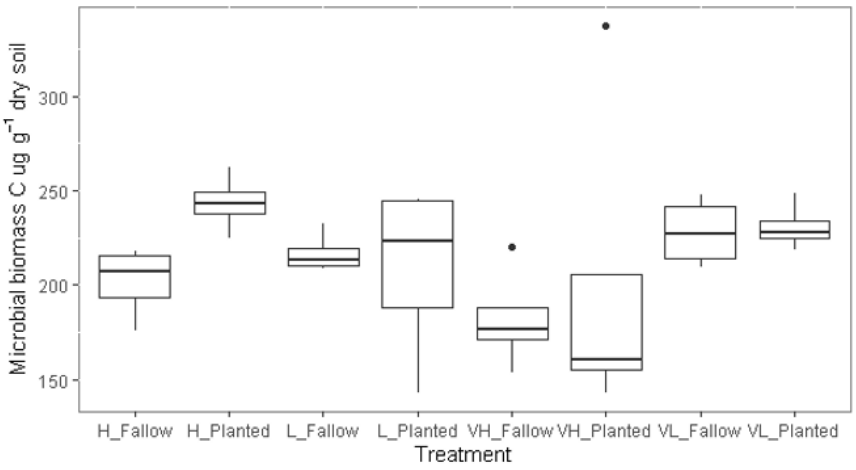


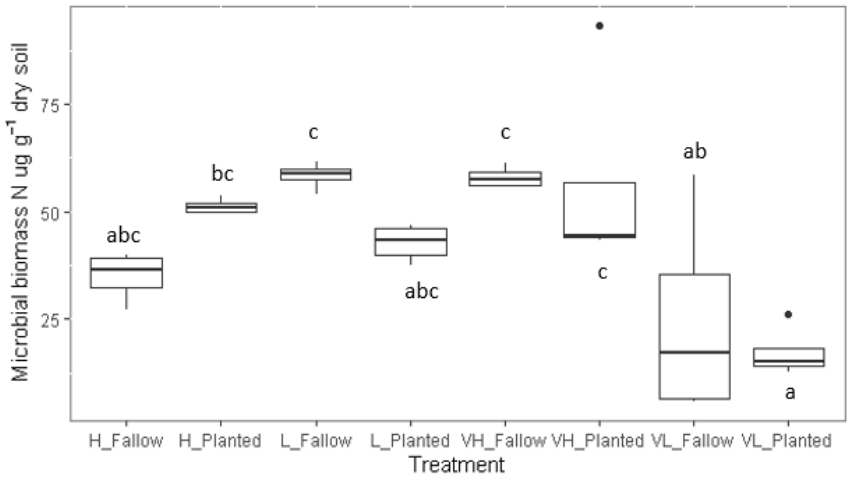
Figure 5.2: Potential nitrogen mineralisation measure using a 7-day anaerobic incubation test (n=4). This graph shows all treatments in both the planted and the fallow pots. Letters denote statistically significant differences between the treatments measure using ANOVA and Tukey test (p<0.05).

5.3.4 Soil microbial biomass carbon, nitrogen, phosphorus

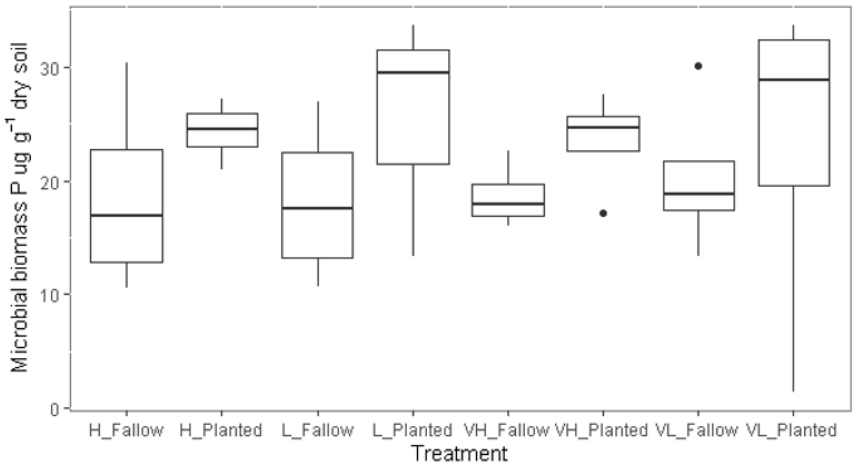
There were no significant differences in microbial biomass C and P between the treatments and planted vs fallow pots (Figure 5.3). The H planted treatment had the highest mean microbial biomass C (Table 5.3). Microbial biomass N was significantly difference between treatments (Figure 5.3). The VL planted treatment had a significantly lower microbial biomass nitrogen compared with the H planted, L fallow, VH fallow and VH planted treatments ($p<0.05$). Regression analysis showed that microbial biomass C and P were not significantly influenced by soil pH. However, microbial biomass N was significantly affected by soil pH ($p=0.001$, $r^2=0.3$). Potential N mineralisation was significantly influenced by microbial biomass C ($p=0.015$, $r^2=0.2$), and microbial biomass N ($p=0.002$, $r^2=0.03$). There was no significant effect of microbial biomass P on N mineralisation potential.



5.3a



5.3b



5.3c

Figure 5.3 (a-c): Microbial biomass carbon (a), nitrogen (b), and phosphorus (c) (n=4). Significant differences between the treatments in microbial biomass N are marked with letters (p<0.05).

5.3.5 Total CO_2 respiration and soil organic matter carbon decomposition

There was a significant effect between planted and fallow pots on total CO_2 efflux ($p<0.05$), this trend varied between treatments (Figure 5.4). However, the general trend was that CO_2 respiration was higher in the planted pot compared to the fallow pot (Figure 5.4). There were significant differences in total respiration between treatments ($p<0.05$), at each sampling point (Figure 5.4). At days 14 and 21 only the VH treatments were different to the other treatments (Figure 5.4). At day 27, the 2 H treatments were different to each other; VH fallow was different to all except VH planted and VL fallow; VH planted was different to VL fallow (Figure 5.4). At day 32, H-fallow and VL fallow were different to all other treatments except L fallow, L planted, it should be noted that VL fallow was different to L planted (Figure 5.4). Respiration rate increased over time in the VL, L, and H planted treatments (Figure 5.4).

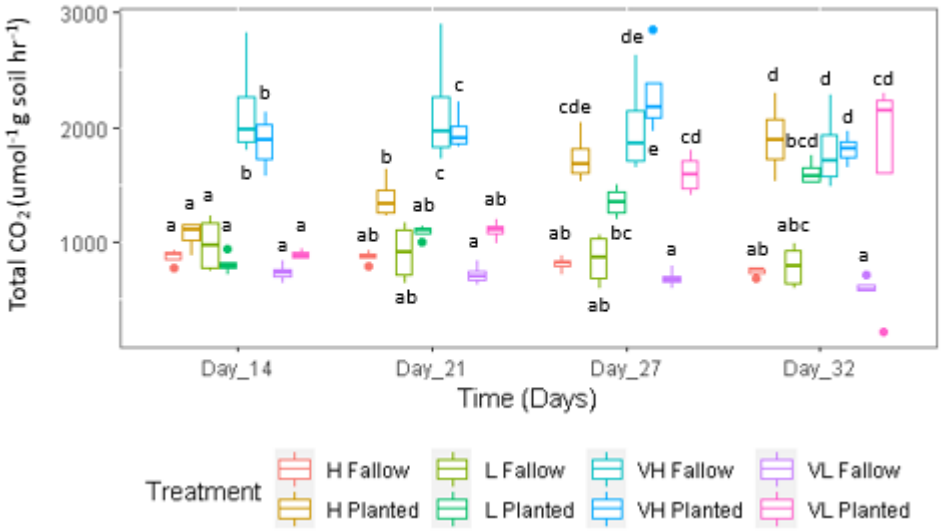


Figure 5.4: Total CO_2 flux as measure during the experimental period for both the planted and fallow pots (n=4). Different letters indicate significant differences between treatments at each time point ($p<0.05$).

Soil derived CO_2-C is a measure of the decomposition rate of the non-plant sources throughout the experiment. As previously stated, in the VH treatment part of the CO_2 from lime dissolution was recorded with the SOM derived and so therefore this flux is being referred to as the Soil-C decomposition. However, there was no lime dissolution found in the VL, L, and H treatments. There was an overall significant effect of treatment on Soil-C decomposition rate ($p<0.05$). However, there was no significant difference in Soil-C decomposition between sampling time points. There was also no significant difference in Soil-C decomposition rate between the planted and fallow pots. The highest rate of average of Soil-C decomposition occurred in the VH fallow treatment on days 21, 27, and 32 (Figure 5.5). The VH planted and fallow pots were significantly higher than all other treatments on days 14 and 21 ($p<0.05$) (Figure 5.5).

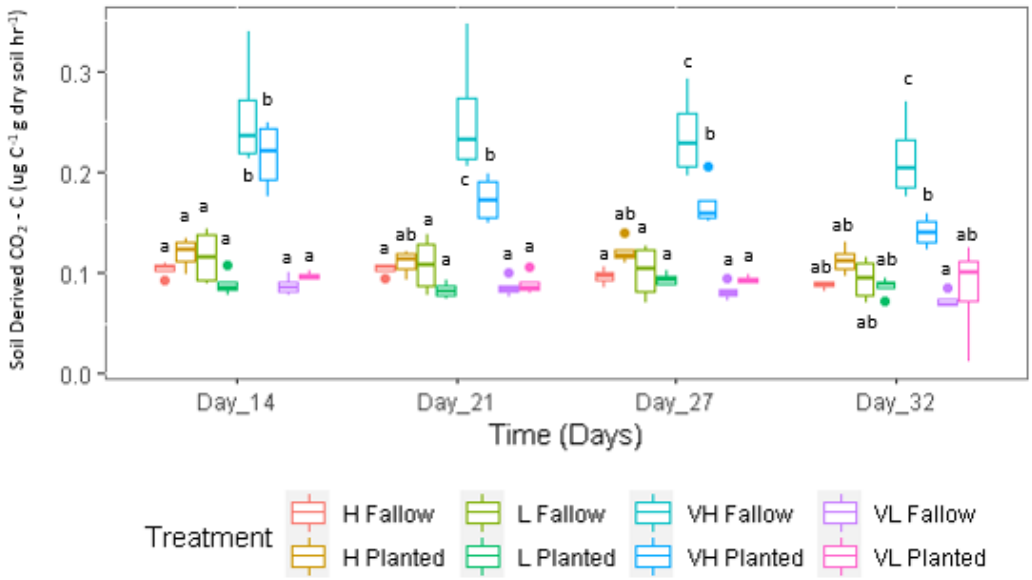


Figure 5.5: Soil carbon decomposition measured over time (n=4). Treatments are represented by colours. Letters represent significant differences between treatments at each sampling time point (p<0.05).

Specific microbial biomass C activity was measured on the final day of the experiment (Day 32) With the exception of L planted and VH planted, VH fallow pots were significantly higher in specific microbial activity compared with the other treatments ($p<0.05$) (Figure 5.6). For the liming treatments, there were no significant differences between the planted and the fallow pots. There was a significant effect of soil pH status on the specific microbial activity ($p<0.01$, $r^2= 0.4$). The specific microbial activity was also found to significantly affect N mineralisation potential ($p<0.001$, $r^2= 0.3$).

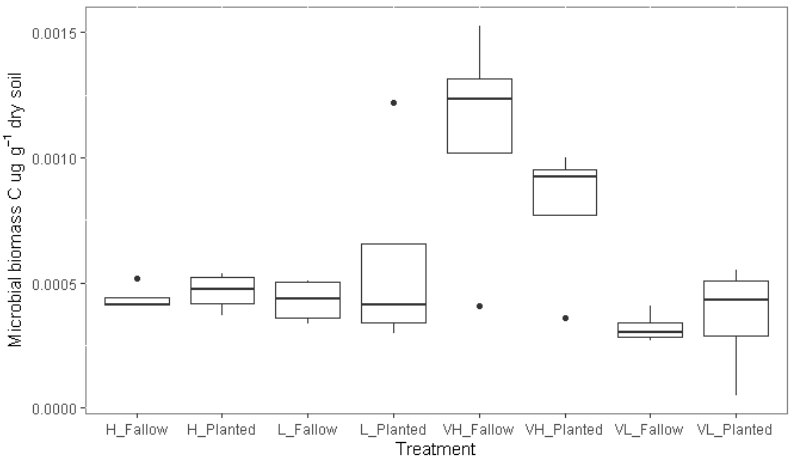


Figure 5.6: Specific activity of microbial biomass carbon (n=4). Measured as the soil organic matter derived carbon divided by the microbial biomass carbon for each treatment. The measurements represented here are from day 32 the final sampling point of the sampling. Letters represent significant differences between the treatments (p<0.05).

5.3.7 16S bacterial and archaeal community structure

The dominant 20 most abundant microbial genera across each of the treatments and pot types are shown in Figure 5.8. *Candidatus Udaeobacter* and *Gaiella* were dominant in all treatments (Figure 5.8). As well as this *Candidatus Nitrosotalea* was dominant in the VL treatments (Figure 5.8). Significant differences in alpha diversity were found for the Shannon index between VH fallow vs VL planted and VH fallow vs VL fallow, for the Simpsons index VH fallow vs VL planted, VL fallow, H planted, H fallow, and L fallow, and for the Chao index VH planted vs VH fallow vs VL planted and VL fallow ($p<0.05$) (Table 5.3). Results from the PCA analysis show that PC1 separates VL and L treatments from each other and all other treatments; H and VH are also separated (Figure 5.7). PC2 separates VL and L from H and VH but VL and L on PC2 are not separated from each other, nor is H and VH (Figure 5.7). There were significant differences in microbial 16S community structure between the different pH treatments (PERMANOVA, $p<0.01$). Pairwise Adonis analysis showed that there were significant differences between the pH treatments in both the planted and the fallow pots (Table 5.4). When comparing the planted pots with the fallow pots in the same pH treatment, there was only a significant difference in 16S community structure between the planted and fallow pots in the L treatment ($p<0.05$) (Table 5.4). Specific differences in microbial community structure at genus level using ASV data were assessed using the Deseq2 pipeline (Love et al., 2014) and resulting tables can be seen in supplementary material, overall, the most differences were found between the VH and VL treatments. An RDA analysis showed that the 16S community structure was significantly influenced by pH and OM content ($p<0.01$) (Figure 5.9). pH is particularly driving the community in the L planted and fallow treatments (Figure 5.9).

Table 5.3: Alpha diversity indexes (n=4) for the 16S communities. Values shown here represent mean richness in each treatment. Letters represent significant differences between treatment ($p<0.05$).

Treatment	Chao1	Shannon	In v. Simpson
VH Planted	3638 b	7.263 ab	387 ab
VH Fallow	4002 b	7.327 b	420.5 b
H Planted	2240.6 ab	6.643 ab	198 a
H Fallow	2270 ab	6.725 ab	203.2 a
L Planted	3066 ab	6.9 ab	220.3 ab
L Fallow	2057 ab	6.573 ab	173.8 a
VL Planted	1839.6 a	6.259 a	156.3 a
VL Fallow	1759 a	6.208 a	130.3 a

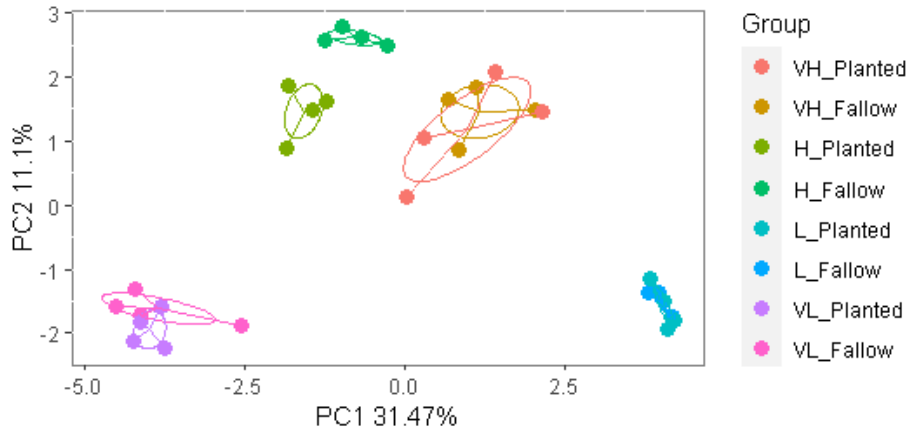


Figure 5.7 Principal component analysis showing clustering of microbial 16S community structure (n=4). Treatments are highlighted by colour.

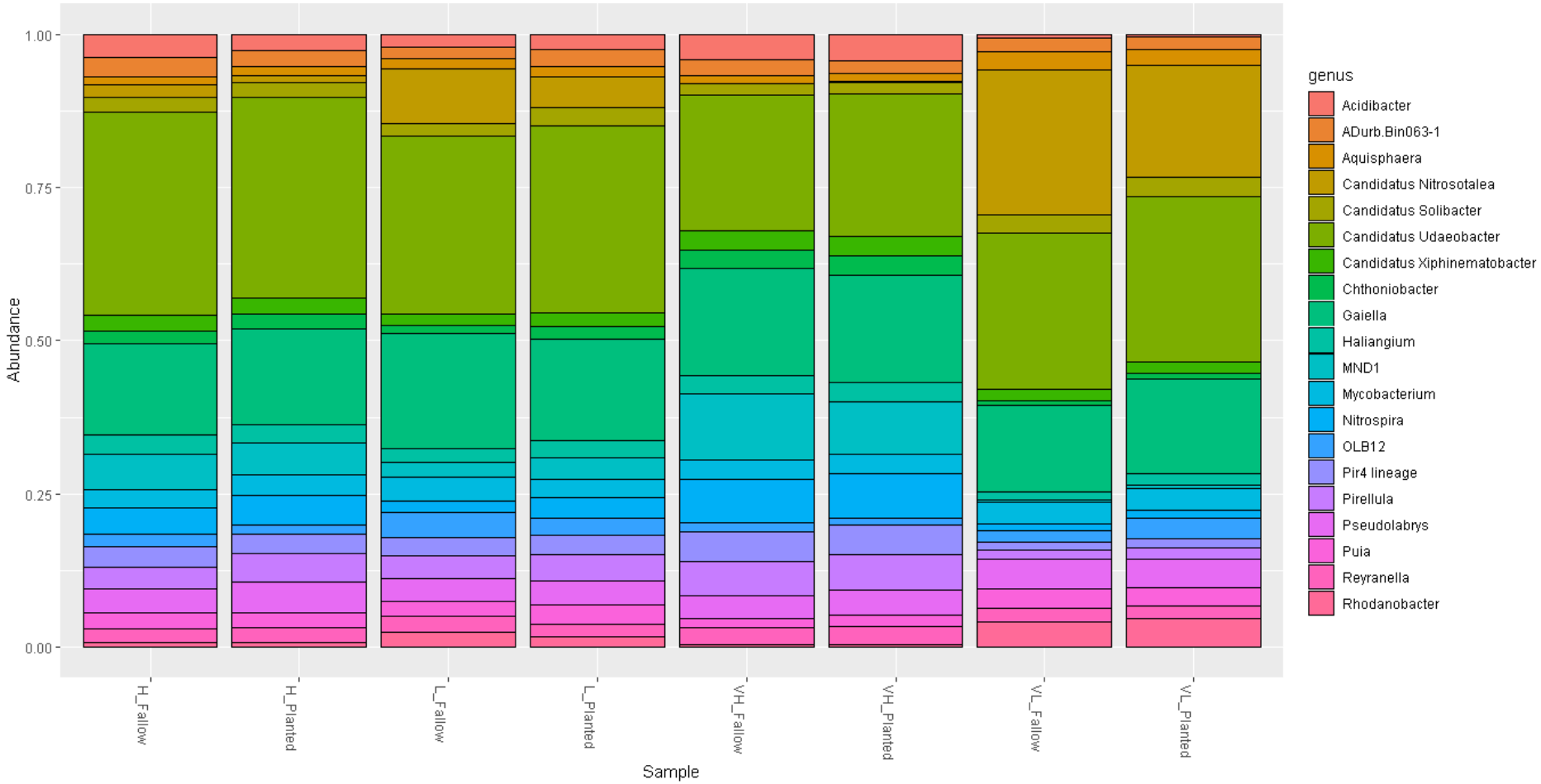


Figure 5.8: The twenty dominant microbial genera in terms of relative abundance of 16S sequencing in each of the pH treatments within the planted and the fallow pots (n=4)

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Table 5.4: Results from the PERMANOVA pairwise analysis of the 16S bacterial community assessing the difference between treatments in the planted and fallow pots (n=4). The second set of treatments assessing the significant difference in community with pH treatment between planted and fallow pots.

Treatment	Planted p-value	Fallow p-value	Treatment	p-value
VH vs H	0.028	0.028	VH planted vs VH Fallow	0.102
VH vs L	0.024	0.023	H planted vs H fallow	0.601
VH vs VL	0.028	0.037	L planted vs L fallow	0.048
H vs L	0.024	0.030	VL planted vs VL fallow	0.361
H vs VL	0.037	0.039	-	
L vs VL	0.029	0.043	-	

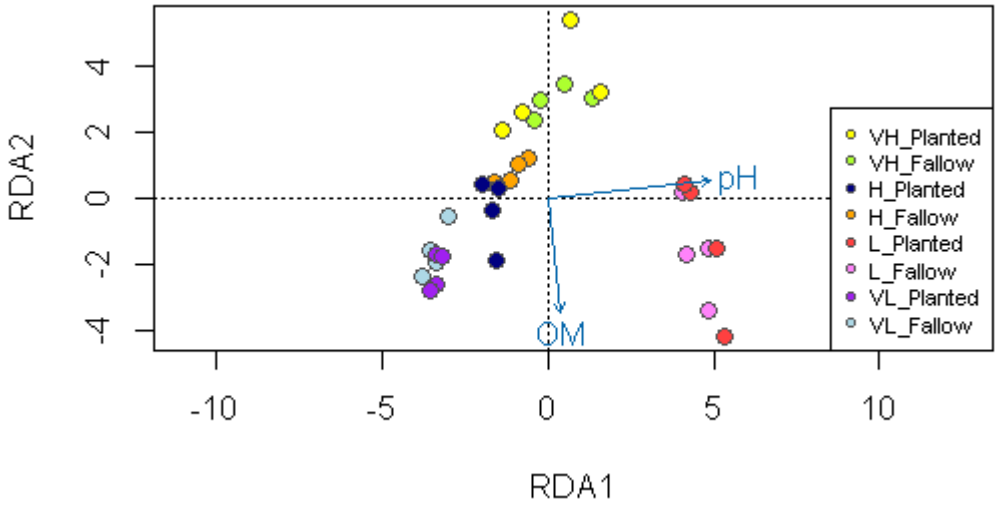

Figure 5.9: Redundancy analysis by pH treatment in the planted and fallow pots (n=4). This graph shows which soil nutrient properties are significantly influencing the 16S community structure. In this graph both soil pH and organic matter content are significantly influencing the 16S community structure.

5.3.8 ITS fungal community structure

The dominant 20 most relatively abundant fungal genera across each of the treatments and pot types are shown in Figure 5.10. The VL planted treatment was removed due to errors while sequencing. The dominant genus in all treatments was *Mortierella* (Figure5.10). Overall, alpha diversity was significantly different with pH treatment when measured using the Shannon and Simpson indexes ($p<0.01$), however, there was no significant difference under the Chao index. Significant differences in alpha diversity with the Shannon index included VL fallow vs L fallow and VH plant vs VH fallow ($p<0.05$) (Table 5.5), with the Simpsons index VL fallow vs VH fallow were significantly different ($p<0.05$) (Table 5.5). Results from a PCA analysis showed along PC1 the VL fallow treatment was

clustered separately to all other treatments (Figure 5.11). On PC2 both the H planted and fallow pots clustered separately from the other treatments. Overall, there were significant differences in ITS community structure between the different pH treatments (PERMANOVA, $p<0.01$), further to this the Pairwise Adonis analysis showed that there were significant differences between the pH treatments in both the planted and the fallow pots (Table 5.6). However, within treatment there were no significant differences between planted and fallow pots. When comparing the planted pots with the fallow pots in the same pH treatment there were no significant difference (Table 5.6). Specific differences in fungal community structure, at genus level using ASV data were assessed using the Deseq2 pipeline (Love et al., 2014) and resulting tables can be seen in supplementary material, overall, there were less differences in the fungal communities compared with the bacterial communities. An RDA analysis showed that the fungal ITS community structure was similarly significantly influenced by pH and OM content ($p<0.01$) (Figure 5.12). pH is correlated with the L planted and L fallow communities and OM is driving the H planted treatment (Figure 5.12).

Table 5.5: Alpha diversity indexes for the ITS communities (n=4). Values shown here represent mean richness in each treatment. Letters represent significant differences between treatment (p<0.05).

Treatment	Chao1	Shannon	Simpson
VH Planted	358.5	4.177 ab	18.51 c
VH Fallow	349.5	3.939 b	17.768 c
H Planted	443.2	4.575 ab	35.8 abc
H Fallow	390.8	4.429 a	29.43 ab
L Planted	287	4.269 ab	29.14 abc
L Fallow	428.2	4.317 ab	23.36 bc
VL Fallow	430.8	4.53 a	40.01 a

Table 5.6: Results from the PERMANOVA pairwise analysis of the ITS fungal community assessing the difference between treatments in the planted and fallow pots (n=4). The second set of treatments assessing the significant difference in community with pH treatment between planted and fallow pots.

Treatment	Planted (p-value)	Fallow (p-value)	Treatment	p-value
VH vs H	0.032	0.029	VH planted vs VH Fallow	0.544
VH vs L	0.046	0.366	H planted vs H fallow	0.452
VH vs VL	NA	0.026	L planted vs L fallow	0.184
H vs L	0.025	0.029	VL planted vs VL fallow	NA
H vs VL	NA	0.024	-	
L vs VL	NA	0.025	-	

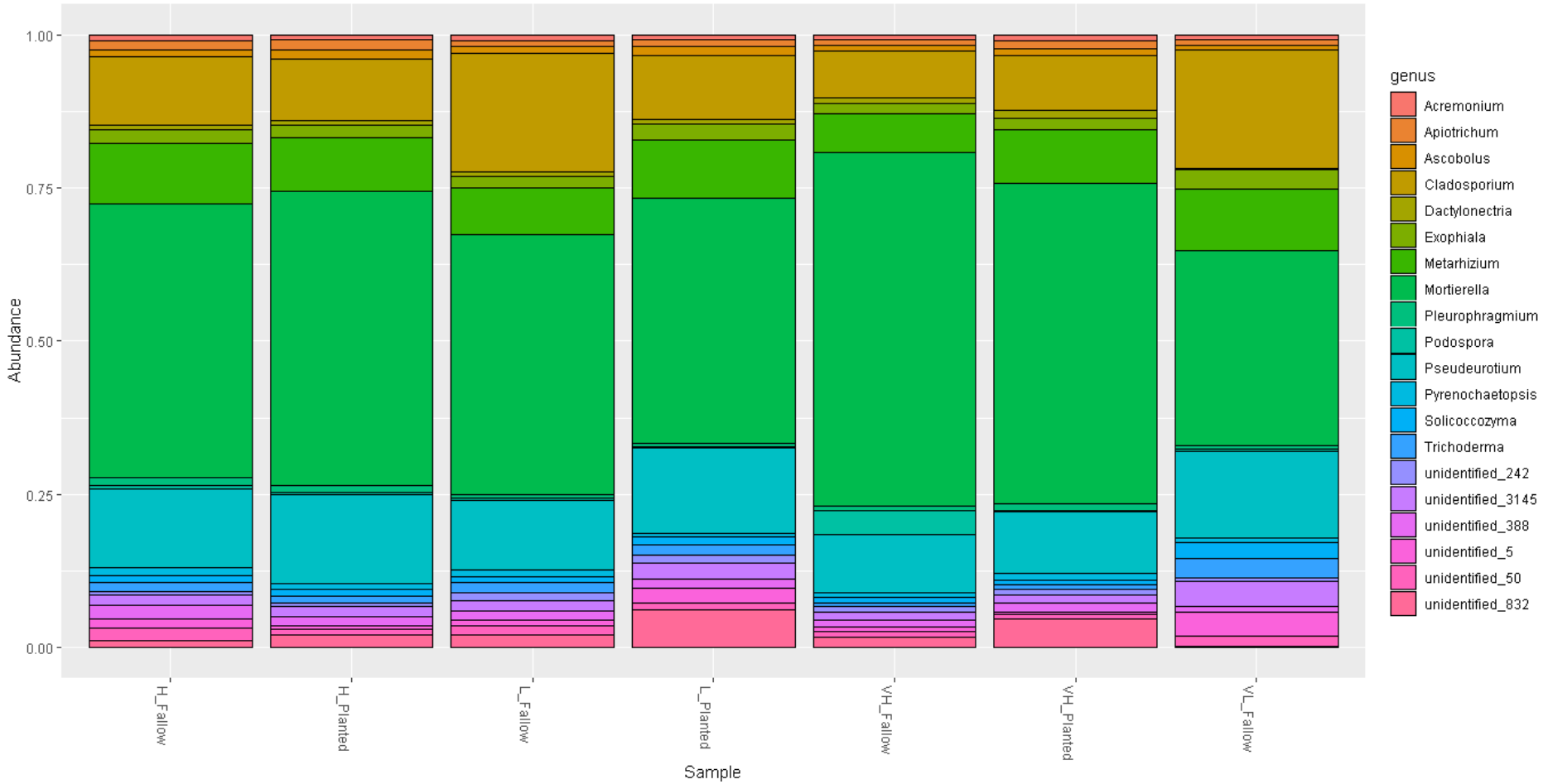

Figure 5.10: The twenty dominant fungal genera in terms of abundance of the ITS sequencing in each of the pH treatments and pot types (n=4).

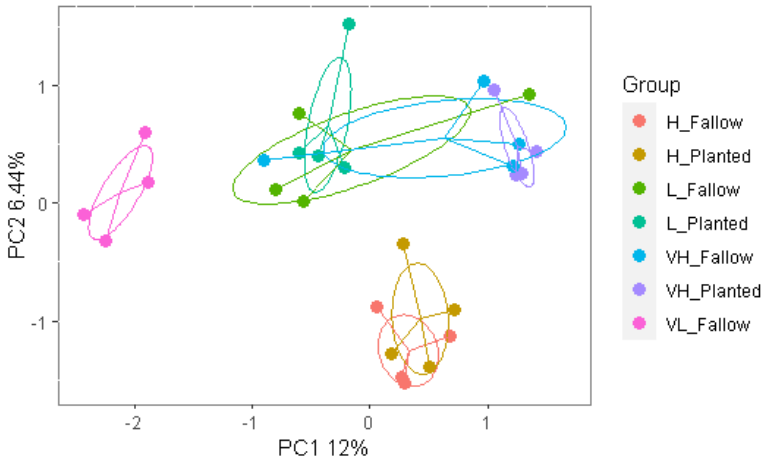

Figure 5.11: Principal component analysis of the ITS fungal communities showing clustering of communities with pH treatment (n=4).

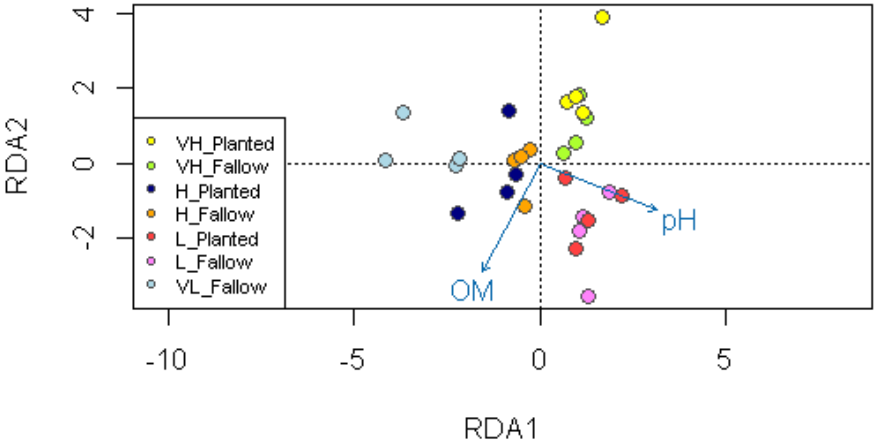

Figure 5.12: Redundancy analysis by pH treatment in the planted and fallow pots (n=4). This graph shows which soil nutrient properties are significantly influencing the ITS fungal community structure. In this graph both soil pH and organic matter content are significantly influencing the ITS fungal community structure.

5.4 Discussion

This study aimed to determine whether there was an effect of soil pH on potential N mineralisation, soil-C decomposition and the associated microbial community structure. Utilising soils from a long-term pH trial with a pH gradient enabled us to assess the longer-term effects of pH management.

Soil pH is an important factor in determining soil nutrient availability. Soil pH can both increase and decrease soil nutrient availability through the balance of cations and anions which can cause particular nutrients to become locked up (Rengel, 2002; Essington, 2015) and also through the influence of soil pH on microbial community structure and processes such as SOM decomposition (Wang and Tang, 2017). It is therefore important to examine the effect of soil pH on microbially mediated N mineralisation, as well as on plant growth, to gain a deeper understanding of N availability with soil pH, and also to build towards soil specific N advice.

5.4.1 Plant tissue nutrient content was affected by soil pH

Shoot and root biomass were not significantly different between the pH treatments. A field study carried out at the same site as this study also found no significant effect of lime addition on overall grass yield (Žurovec *et al.*, 2021).Variation in grass yields with lime also occurred in a study by Sheils et al. (2014), who found that with low soil pH grass yield increased at higher soil pH yield decreased. Responses of crops yields to lime addition can be dependent on a number of reasons such as crop species, timing of lime application, the method of application, soil type and texture (Li *et al.*, 2019). A potential explanation for the lack of biomass variation found in this study is that the fertilisation treatments received were the same for each pH treatment and perhaps these were sufficient to maintain available nutrients for plant growth.

Although there was no effect on plant biomass this does not mean that pH did not affect grass growth in terms of nutrient composition. In this study, the effect of pH treatment on plant growth over the course of the experiment could be seen in the nutrient analysis of the plant material. Impacts of lime addition and soil pH and their effect on grass nutrient analysis is not well understood and reports in the literature are highly variable (Adams and Martin, 1984; Bailey, 1995). A previous study at Johnstown Castle found that with lime application there was a decrease in grass P concentration (Sheils et al., 2014). Other studies have reported an increase in crop P concentration with lime application (Bailey, 1995). However, neither an increase nor decrease was seen in this study. In this study the lowest pH treatment, VL, had the highest absolute N concentration on average in both root and shoot material, and the lowest absolute K concentration on average in the shoot material (Table 5.1). Uptake of K from the soil to the plant may have been affected by the low soil pH. In more acidic soil environments, the cation exchange capacity is reduced, therefore the ability of the soil to hold on

to positively charged cations such as K^+ is reduced, and hence there is increased availability of K^+ in the soil solution (Blue and Ferrer, 1986). In terms of the higher N concentration in the VL shoot and root material, this may have been affected by reduced plant-microbial biomass competition in these pots, as the microbial biomass N concentration was significantly lower in the VL treatment compared with the VH treatment. Also, the S concentration in the highest pH treatment, VH, was significantly higher in the shoot material. Previous studies have shown that increasing soil pH can also increase mineralisation of sulphur from both SOM and Fe and Al sulphates (Bolan and Hedley, 2003; Kunhikrishnan et al., 2016). Such increased mineralisation of sulphur forms leads to more freely available S for both plant and microbial uptake (Kunhikrishnan et al., 2016). Perhaps the results seen in this study and the variability in the literature on the effects of lime and pH on grass nutrient concentrations suggest that impacts of pH in a grassland are site-specific (e.g. strongly dependent on soil type) and may be reflected by impacts on grass tissue quality (i.e. nutrient composition), rather than by biomass production only and this area requires more research.

5.4.2 Potential nitrogen mineralisation increased with soil pH

The hypothesis that potential N mineralisation would increase with increasing soil pH was supported. There was a significant effect of both pH treatment and soil pH on potential N mineralisation. The treatments with lower soil pH had a lower N mineralisation potential. The results reported in this study are in line with others that have examined the effect of soil pH on N mineralisation potential (Curtin *et al.*, 1998; Khalil *et al.*, 2005; Senwo and Tabatabai, 2005; Neina, 2019). Adams and Martin (1984) and Aciego Pietri and Brookes (2008) found that N mineralisation decreases below pH 6. Aciego Pietri and Brookes (2008) hypothesised that the optimum pH for N mineralisation is between pH 6-8. Possible explanations for this could be both chemical and biological. Chemically, the amount of dissolved organic compounds increases with increasing soil pH, as the bounds between organic compounds and clay particles weakens (Andersson *et al.*, 2000; Neina, 2019). Hence, increasing the availability of organic compounds for mineralising. However, it is more likely that this effect is biologically driven as soil pH has a direct effect on the soil microbial community and hence microbial community functioning such as the production of enzymes (Aye *et al.*, 2018; Neina, 2019).

5.4.3 Soil derived CO_2 efflux was highest with the highest pH and increased over time in the planted pots

Many previous studies examining the effect of soil pH and lime on SOM decomposition and priming, have limed the soils (i.e. sample soils in the field and adjusted the pH in the lab with lime) to create pH treatments, and therefore contributions of lime as part of the experiment would be expected (De Nobili *et al.*, 2001; Conde *et al.*, 2005; Hamer and Marschner, 2005; Blagodatskaya *et al.*, 2007).

However, in this experiment soils were selected from a long-term pH field trial with the rationale that both the soil pH would not need to be adjusted, and also that the microbial communities would be more truly representative of the soils (from the same soil type) at different pHs. However, although it was thought that taking samples from this field site more than 12 months after lime application that lime dissolution would not affect CO_2 fluxes from soil. However, this was not the case, and in the VH treatment, in a unique scenario due to the stable isotope partitioning set up of this study, it was possible to identify that lime dissolution had occurred and contributed to soil CO_2 efflux in this treatment. Therefore, there is a contribution of lime to CO_2 efflux despite it not being added as part of the experiment, and this needs to be considered in terms of data analysis and discussion. Due to the contribution of lime, particularly in the VH treatment, fluxes were partitioned into plant- and soil-derived components, the latter being from both SOM decomposition and lime dissolution. The focus is on soil-derived CO_2 efflux in this chapter, and not priming per se, as results from the priming calculation would be influenced by the CO_2 flux from lime and could, potentially be confounded by lime dissolution, not reflecting microbial mineralisation of SOM sources.

It has been shown that plants grown in soils that have had lime added can affect both lime dissolution and SOM decomposition (Cheng *et al.*, 2014; Kunhikrishnan *et al.*, 2016). This could be occurring in the VH soil particularly as this soil had the highest soil pH which not only encourages increased root exudation, and hence the priming effect, but also increases lime dissolution (Ahmad *et al.*, 2013). In the fallow pots the VH treatment had a significantly higher Soil-derived CO_2 efflux rate compared to the other treatments at each sampling time point (Figure 5.4), however as previously stated the dissolution of lime could be a contributing factor to this significant difference. In subsequent experiments, the contribution of lime to soil CO_2 efflux could be calculated by adding a treatment where plants were grown under identical conditions, but without isotopic labelling. In this treatment, the isotopically distinct sources to soil CO_2 efflux would be plant- and SOM-derived C (combined) and from lime. Therefore, the enriched natural abundance ^{13}C signature of lime could be used to calculate its contribution to soil CO_2 efflux, via the two-source mass balance approach used elsewhere in the thesis.

Although not calculated as part of this study, previous studies have shown that initial soil pH has a strong influence on the priming effect (Luo *et al.*, 2011; Aye *et al.*, 2016). Blagodatskaya and Kuzyakov (2008) showed that across 12 studies the magnitude of priming increased with increasing soil pH. However, those studies were not of a planted system, instead a substrate such as glucose which can miss some of the important plant-soil interactions (e.g. plant uptake of nutrients, affecting nutrient availability to microbial communities). For negative priming to occur, soil microbes use more readily

available C sources instead of breaking down SOM-C, and therefore negative priming is also associated with systems that are rich in soil nutrients (Kuzyakov and Bol, 2006; Murphy *et al.*, 2017). One of the benefits of applying lime and increasing soil pH in agricultural systems is the associated increase in nutrient availability (Haynes and Naidu, 1998; Bolan *et al.*, 1999). An increase in nutrient availability associated with lime addition has been seen in this study. Despite all treatments receiving the same P addition there was significantly higher available P (measured as P Morgan's) in the VH treatment. The increase in soil pH, contributing to increased nutrient availability, could potentially reduce microbial demand for these nutrients from SOM mineralisation in this treatment. Liming can increase root exudation as it improves plant growth and root activity (FORNARA *et al.*, 2011). The increase in root activity and potentially root exudation, along with increased nutrient availability with increasing soil pH, could be causing the negative priming response seen in this study. Although further investigation would be required, this could have important knock-on effects in terms of decreased SOM decomposition and increased longer-term C storage in SOM. Increased C storage with lime was seen by Fornara et al. (2011). It is important to remember that the effects of soil pH and liming on SOM-C decomposition and priming are likely to be affected by the changes in microbial community structure and function due to varying soil pH status (Rousk *et al.*, 2010b), which will now be discussed in section 5.4.4.

5.4.4 Microbial community structure was influenced by soil pH

Microbial communities in limed soils can have increased diversity and are potentially better at mineralising a wide range of organic compounds and also use them more efficiently (Fuentes *et al.*, 2006; Page *et al.*, 2009; Paradelo *et al.*, 2015; Curtin *et al.*, 2016). In this study, as reported elsewhere, there were pronounced differences in soil microbial communities with pH treatment as measured by the diversity and abundances of 16S (bacteria and archaea) and ITS (fungal) genera via sequencing (Rousk *et al.*, 2010a; Rousk *et al.*, 2010b; Zhalnina *et al.*, 2015; Trivedi *et al.*, 2016; Zheng *et al.*, 2019; Abalos *et al.*, 2020), and therefore the hypothesis that pH influences microbial communities was supported. However, some studies have seen that the effects on the fungal communities were less strongly associated with soil pH than bacterial communities (Lauber *et al.*, 2008; Liu *et al.*, 2018). It is important to note that this discussion is based on general assessment of the microbial community structure in response to the pH treatments applied in this study, and enrichment of ASVs in particular treatments does not mean that direct conclusions can be drawn about the exact processes they are involved in.

5.4.4.1 16S microbial community structure

The Alpha diversity index Chao 1 showed significant differences between the VH and VL treatments in both the planted and the fallow pots. However, the Shannon index only showed significant differences between the VH and VL fallow pots, alpha diversity was higher in the VH treatment (Table 5.3). Previous studies have also found that alpha diversity is influenced by soil pH and also higher in soils with higher pH (Cho *et al.*, 2016; Xia *et al.*, 2020). An RDA analyses in this study showed that there was a significant effect of soil pH and OM on the microbial community structure. This influenced of pH treatment was further confirmed by the results of the PERMANOVA which showed significant differences between the pH treatments (Table 5.4). The specific genera differences can be seen from the results of the Deseq2 analysis present in the supplementary materials. However, *Acidobacteria* are one of the most common bacterial groups in soil environments and have been known to be negatively correlated with soil pH (Trivedi *et al.*, 2016; Zheng *et al.*, 2019). In this study, *Acidobacteria* were one of the most abundant bacterial phyla, and their relative abundance was higher in soils with a higher pH. *Verrucomicrobiota* also commonly found in soil environments (Trivedi *et al.*, 2016) were in this study found to be frequently enriched in the VL planted and fallow pots (Supplementary materials) when compared to the other pH treatments (e.g., H planted). *Verrucomicrobiota* are oligotophs (K–strategists) (Fierer et al., 2007, Trivedi et al., 2013) which are associated with growing on recalcitrant C sources. However, in this study there was no strong evidence that the VL treatment was using recalcitrant C sources more than the L, H and VH treatments as the SOM decomposition rate was low and only a slight positive priming effect was evident. The ecology and functioning of *Verrucomicrobiota* however, are not well understood particularly as they are difficult to culture in the lab (Bergmann et al., 2011, Fierer et al., 2013), however, advances in genomic analysis using metagenomics has provide increased recover of genetic material and potential for increasing the understanding of the *Verrucomicrobiota* phylum (Kielak *et al.*, 2010). *Firmicutes* was enriched in soils with higher pH, in particularly VH compared to VL and L treatments but also with H compared to VL and L compared to VL treatments, with differences among the planted and the fallow pots (Supplementary materials). *Firmicutes* are important chitin degrading bacteria in soil (Gooday, 1990; Beier and Bertilsson, 2013). Potential N mineralisation and SOM –C decomposition, linked with chitin degradation were higher in treatments that were enriched with *Firmicutes* in this study. This concurs with previous studies that have reported increases in *Firmicutes* with increasing soil pH (Anderson *et al.*, 2018).

5.4.4.2 ITS microbial community structure

This study as has also been shown in previous studies showed that soil pH has a significant effect on fungal community structure (Zhang *et al.*, 2016; Liu *et al.*, 2018; Ning *et al.*, 2020; Li *et al.*, 2021). However, in terms of alpha diversity there was no significant difference when measured using the Shannon index between the treatments in this study. However, when looking at the Simpsons index there were significant differences in alpha diversity between the VL fallow pots and the VH fallow and planted pots (Table 5.5). From the deseq 2 analyses there were fewer differences between the fungal communities in the pots compared with the differences between the bacterial communities (Supplementary materials). This is consistent with previous studies that reported bacterial communities (16S) were more strongly affected by soil pH then fungal communities (Liu *et al.*, 2016; Liu *et al.*, 2018). Using a multivariate analytical approach, an RDA analysis highlighted a significant effect of soil pH on fungal community structure and PERMANOVA also indicated significant differences in fungal communities between the pH treatments (Table 5.6). However, there were no significant differences between the treatments in the fallow pots (PERMANOVA; Table 5.6).

The results of the deseq2 analyses showed that there was a significant enrichment of certain *Ascomycota* ASVs in the VH treatment (Supplementary materials). *Ascomycota* fungi are commonly found in soils (Ning *et al.*, 2020) and previous studies have also found that *Ascomycota* abundance increased with increasing soil pH (Lauber *et al.*, 2008). *Ascomycota* are also associated with having a positive effect on soil aggregation (Lehmann *et al.*, 2020), which is also with a consequence of lime addition. Interestingly, *Chytridiomycota* were significantly enriched when comparing the higher soil pH treatments with lower soil pH treatments. This is noteworthy as *Chytridiomycota* are not often found in soil ecosystems, however, they are more commonly found at higher altitudes (Freeman *et al.*, 2009). *Chytridiomycota* can often be found in fallow soil systems although in this study they were found in both the planted and fallow pots. A study by Liu *et al.* (2018) found a significant correlation between soil pH and the abundance of *Chytridiomycota*. In this study *Chytridiomycota* was enriched in treatments with lower soil pH, with lower soil pH potentially being an indicator for the increased enrichment of this phyla.

5.5 Conclusion

The hypothesis that N mineralisation potential would increase with soil pH, and would be highest with the highest pH was supported by the results of this study. The hypothesis that soil microbial communities was influence by soil pH was also supported by the results of this study. Soil microbial communities for both 16S and ITS were significantly influenced by soil pH. However, the effects of soil

pH were more clearly seen in the 16S community compared to the ITS community. 16S diversity was found to be higher in the higher pH treatments. The largest differences in abundances of both 16S and ITS genera were seen when comparing the VH and VL treatments. The hypotheses related to SOM-C decomposition was also neither accepted nor rejected as due to the impacts of lime it was not summarised as in previous chapters. However, the result that lime dissolution can be seen more than 12 months after lime application is one that requires further study. Lime may promote soil C storage, but the lime itself is a potential contributor to soil CO_2 emissions while is dissolving in soils but also the emissions associated with the mining and extraction of lime at the primary source. This could have potential important implications for grassland greenhouse gases budgeting.

5.6 References

Abalos, D., Liang, Z., Dörsch, P., Elsgaard, L., 2020. Trade-offs in greenhouse gas emissions across a liming-induced gradient of soil pH: Role of microbial structure and functioning. *Soil Biology and Biochemistry* 150, 108006.

Aciego Pietri, J.C., Brookes, P.C., 2008. Nitrogen mineralisation along a pH gradient of a silty loam UK soil. *Soil Biology and Biochemistry* 40, 797-802.

Adams, F., Martin, J.B., 1984. Liming effects on nitrogen use and efficiency. *Nitrogen In Crop Production*, 417-426.

Ahmad, W., Singh, B., Dijkstra, F.A., Dalal, R.C., 2013. Inorganic and organic carbon dynamics in a limed acid soil are mediated by plants. *Soil Biology and Biochemistry* 57, 549-555.

Anderson, C.R., Peterson, M.E., Frampton, R.A., Bulman, S.R., Keenan, S., Curtin, D., 2018. Rapid increases in soil pH solubilise organic matter, dramatically increase denitrification potential and strongly stimulate microorganisms from the Firmicutes phylum. *PeerJ* 6, e6090-e6090.

Andersson, S., Nilsson, S.I., Saetre, P., 2000. Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biology and Biochemistry* 32, 1-10.

Aye, N.S., Butterly, C.R., Sale, P.W., Tang, C., 2017. Residue addition and liming history interactively enhance mineralization of native organic carbon in acid soils. *Biology and Fertility of Soils* 53, 61-75.

Aye, N.S., Butterly, C.R., Sale, P.W.G., Tang, C., 2018. Interactive effects of initial pH and nitrogen status on soil organic carbon priming by glucose and lignocellulose. *Soil Biology and Biochemistry* 123, 33-44.

Aye, N.S., Sale, P.W.G., Tang, C., 2016. The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils. *Biology and Fertility of Soils* 52, 697-709.

Bailey, J., 1995. Liming and nitrogen efficiency: some effects of increased calcium supply and increased soil pH on nitrogen recovery by perennial ryegrass. *Communications In Soil Science And Plant Analysis* 26, 1233-1246.

Beier, S., Bertilsson, S., 2013. Bacterial chitin degradation—mechanisms and ecophysiological strategies. *Frontiers in Microbiology* 4, 149.

Bertrand, I., Delfosse, O., Mary, B., 2007. Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: Apparent and actual effects. *Soil Biology and Biochemistry* 39, 276-288.

Blagodatskaya, E., Blagodatsky, S.A., Anderson, T.-H., Kuzyakov, Y., 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. applied soil ecology 37, 95-105.

Blagodatskaya, E.V., Anderson, T.-H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO_2 of microbial communities in forest soils. *Soil Biology and Biochemistry* 30, 1269-1274.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45, 115-131.
Blomquist, J., Simonsson, M., Etana, A., Berglund, K., 2018. Structure liming enhances aggregate stability and gives varying crop responses on clayey soils. Acta Agriculturae Scandinavica, Section B — *Soil & Plant Science* 68, 311-322.
Blue, W., Ferrer, M., 1986. Potassium retention in a sandy Florida spodosol with variable organic matter concentrations and liming histories. *Proceedings-Soil and Crop Science Society of Florida* (USA).
Bodirsky, B.L., Popp, A., Lotze-Campen, H., Dietrich, J.P., Rolinski, S., Weindl, I., Schmitz, C., Müller, C., Bonsch, M., Humpenöder, F., Biewald, A., Stevanovic, M., 2014. Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. Nature Communications 5, 3858.
Bolan, N.S., Adriano, D.C., Curtin, D., 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Advances in Agronomy* 78, 5-272.
Bolan, N.S., Hedley, M.J., 2003. *Role of carbon, nitrogen, and sulfur cycles in soil acidification*. CRC Press.
Bolan, N.S., Naidu, R., Syers, J., Tillman, R., 1999. Surface charge and solute interactions in soils. *Advances in Agronomy* 67, 87-140.
Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol. Monogr.* 75, 139-157.
Bouwman, L., Goldewijk, K.K., Van Der Hoek, K.W., Beusen, A.H.W., Van Vuuren, D.P., Willems, J., Rufino, M.C., Stehfest, E., 2013. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences* 110, 20882-20887.
Brady, N.C., Weil, R.R., Weil, R.R., 2008. *The nature and properties of soils*. Prentice Hall Upper Saddle River, NJ.
Briedis, C., Sá, J.C.d.M., Caires, E.F., Navarro, J.d.F., Inagaki, T.M., Boer, A., Neto, C.Q., Ferreira, A.d.O., Canalli, L.B., Santos, J.B.d., 2012. Soil organic matter pools and carbon-protection mechanisms in aggregate classes influenced by surface liming in a no-till system. *Geoderma* 170, 80-88.
Cheng, W., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., McNickle, G.G., Brzostek, E., Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. *New Phytologist* 201, 31-44.
Cheng, Y., Wang, J., Mary, B., Zhang, J.-b., Cai, Z.-c., Chang, S.X., 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. *Soil Biology and Biochemistry* 57, 848-857.
Cho, S.-J., Kim, M.-H., Lee, Y.-O., 2016. Effect of pH on soil bacterial diversity. *Journal of Ecology and Environment* 40, 10.
Conde, E., Cardenas, M., Ponce-Mendoza, A., Luna-Guido, M., Cruz-Mondragón, C., Dendooven, L., 2005. The impacts of inorganic nitrogen application on mineralization of 14C-labelled maize and glucose, and on priming effect in saline alkaline soil. *Soil Biology and Biochemistry* 37, 681-691.
Corbett, D., Wall, D.P., Lynch, M.B., Tuohy, P., 2021. The influence of lime application on the chemical and physical characteristics of acidic grassland soils with impeded drainage. *The Journal of Agricultural Science* 159, 206-215.
Curtin, D., Campbell, C.A., Jalil, A., 1998. Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. *Soil Biology and Biochemistry* 30, 57-64.
Curtin, D., Peterson, M.E., Anderson, C.R., 2016. pH-dependence of organic matter solubility: Base type effects on dissolved organic C, N, P, and S in soils with contrasting mineralogy. *Geoderma* 271, 161-172.

De Nobili, M., Contin, M., Mondini, C., Brookes, P., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. *Soil Biology and Biochemistry* 33, 1163-1170.
Essington, M.E., 2015. Soil and water chemistry: an integrative approach. CRC press.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences* 103, 626-631.
Fornara, D.A., Steinbeiss, S., Mcnamara, N.P., Gleixner, G., Oakley, S., Poulton, P.R., Macdonald, A.J., Bardgett, R.D., 2011. Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland. *Global Change Biology* 17, 1925-1934.
Freeman, K.R., Martin, A.P., Karki, D., Lynch, R.C., Mitter, M.S., Meyer, A.F., Longcore, J.E., Simmons, D.R., Schmidt, S.K., 2009. Evidence that chytrids dominate fungal communities in high-elevation soils. *Proceedings of the National Academy of Sciences* 106, 18315.
Fuentes, J.P., Bezdicek, D.F., Flury, M., Albrecht, S., Smith, J.L., 2006. Microbial activity affected by lime in a long-term no-till soil. *Soil and Tillage Research* 88, 123-131.
Gooday, G., 1990. Physiology of microbial degradation of chitin and chitosan. 611 *Biodegradration*.
Guignard, M.S., Leitch, A.R., Acquisti, C., Eizaguirre, C., Elser, J.J., Hessen, D.O., Jeyasingh, P.D., Neiman, M., Richardson, A.E., Soltis, P.S., Soltis, D.E., Stevens, C.J., Trimmer, M., Weider, L.J., Woodward, G., Leitch, I.J., 2017. Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture. *Frontiers in Ecology and Evolution* 5.
Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. *Soil Biology and Biochemistry* 37, 445-454.
Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling In Agroecosystems* 51, 123-137.
Herold, M.B., Giles, M.E., Alexander, C.J., Baggs, E.M., Daniell, T.J., 2018. Variable response of nirK and nirS containing denitrifier communities to long-term pH manipulation and cultivation. *FEMS Microbiology Letters* 365.
Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Science of The Total Environment* 610-611, 316-332.
ISSU-WRB, group, W., 2015. World Reference Base for Soil Resources 2014, update 2015.
Kemmitt, S.J., Wright, D., Goulding, K.W., Jones, D.L., 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. *Soil Biology and Biochemistry* 38, 898-911.
Khalil, M., Hossain, M., Schmidhalter, U., 2005. Carbon and nitrogen mineralization in different upland soils of the subtropics treated with organic materials. *Soil Biology and Biochemistry* 37, 1507-1518.
Kielak, A., Rodrigues, J.L., Kuramae, E.E., Chain, P.S., van Veen, J.A., Kowalchuk, G.A., 2010. Phylogenetic and metagenomic analysis of Verrucomicrobia in former agricultural grassland soil. *FEMS Microbiol Ecol* 71, 23-33.
Kunhikrishnan, A., Thangarajan, R., Bolan, N.S., Xu, Y., Mandal, S., Gleeson, D.B., Seshadri, B., Zaman, M., Barton, L., Tang, C., Luo, J., Dalal, R., Ding, W., Kirkham, M.B., Naidu, R., 2016. Chapter One - Functional Relationships of Soil Acidification, Liming, and Greenhouse Gas Flux. In: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 1-71.
Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry* 42, 1363-1371.
Kuzyakov, Y., Bol, R., 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biology and Biochemistry* 38, 747-758.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biology and Biochemistry* 40, 2407-2415.
Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., Maaß, S., Rillig, M.C., 2020. Fungal Traits Important for Soil Aggregation. *Frontiers in Microbiology* 10.
Leinweber, P., Kruse, J., Baum, C., Arcand, M., Knight, J.D., Farrell, R., Eckhardt, K.-U., Kiersch, K., Jandl, G., 2013. Chapter Two - Advances in Understanding Organic Nitrogen Chemistry in Soils Using State-

of-the-art Analytical Techniques. In: Sparks, D.L. (Ed.), *Advances in Agronomy*. Academic Press, pp. 83-151.
Li, S., Ji, X., Chao, C., Liu, Z., Zhu, J., Peng, H., 2021. Effects of increasing lime application rates on microbial diversity and community structure in paddy soils. *Applied Soil Ecology* 161, 103837.
Li, Y., Cui, S., Chang, S.X., Zhang, Q., 2019. Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. Journal of Soils and Sediments 19, 1393-1406.
Liu, D., Liu, G., Chen, L., Wang, J., Zhang, L., 2018. Soil pH determines fungal diversity along an elevation gradient in Southwestern China. *Science China Life Sciences* 61, 718-726.
Liu, D., Wu, X., Shi, S., Liu, H., Liu, G., 2016. A hollow bacterial diversity pattern with elevation in Wolong Nature Reserve, Western Sichuan Plateau. *Journal of Soils and Sediments* 16, 2365-2374.
Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry* 43, 2304-2314.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. Plant and Soil 417, 499-510.
Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. *Applied and Environmental Soil Science* 2019, 5794869.
Ning, Q., Chen, L., Jia, Z., Zhang, C., Ma, D., Li, F., Zhang, J., Li, D., Han, X., Cai, Z., Huang, S., Liu, W., Zhu, B., Li, Y., 2020. Multiple long-term observations reveal a strategy for soil pH-dependent fertilization and fungal communities in support of agricultural production. *Agriculture, Ecosystems & Environment* 293, 106837.
Page, K.L., Allen, D.E., Dalal, R.C., Slattery, W., 2009. Processes and magnitude of CO_2, CH4, and N2O fluxes from liming of Australian acidic soils: a review. *Soil Research* 47, 747-762.
Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agriculture, Ecosystems & Environment 202, 98-107.
Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytologist* 173, 600-610.
Perelo, L.W., Munch, J.C., 2005. Microbial immobilisation and turnover of 13C labelled substrates in two arable soils under field and laboratory conditions. *Soil Biology and Biochemistry* 37, 2263-2272.
Phillips, R.P., Brzostek, E., Midgley, M.G., 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytologist* 199, 41-51.
Rengel, Z., 2002. Handbook of Plant Growth pH as the Master Variable. CRC Press.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010a. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* 4, 1340-1351.
Rousk, J., Brookes, P.C., Baath, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* 75, 1589-1596.
Rousk, J., Brookes, P.C., Bååth, E., 2010b. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry* 42, 926-934.
Rütting, T., Aronsson, H., Delin, S., 2018. Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems* 110, 1-5.
Schröder, J.J., Schulte, R.P.O., Creamer, R.E., Delgado, A., van Leeuwen, J., Lehtinen, T., Rutgers, M., Spiegel, H., Staes, J., Tóth, G., Wall, D.P., 2016. The elusive role of soil quality in nutrient cycling: a review. *Soil Use and Management* 32, 476-486.
Senwo, Z.N., Tabatabai, M.A., 2005. Effect of Management Systems on Nitrogen Mineralization and Nitrification in Soils. *Communications in Soil Science and Plant Analysis* 36, 1313-1326.
Sheil, T.S., Wall, D.P., Culleton, N., Murphy, J., Grant, J., Lalor, S.T.J., 2016. Long-term effects of phosphorus fertilizer on soil test phosphorus, phosphorus uptake and yield of perennial ryegrass. *The Journal of Agricultural Science* 154, 1068-1081.

Sun, G., Zhu-Barker, X., Chen, D., Liu, L., Zhang, N., Shi, C., He, L., Lei, Y., 2017. Responses of root exudation and nutrient cycling to grazing intensities and recovery practices in an alpine meadow: An implication for pasture management. *Plant and Soil* 416, 515-525.
Tang, C., Rengel, Z., Diatloff, E., Gazey, C., 2003. Responses of wheat and barley to liming on a sandy soil with subsoil acidity. *Field Crops Research* 80, 235-244.
Tran, T.D., Cui, Y.-J., Tang, A.M., Audiguier, M., Cojean, R., 2014. Effects of lime treatment on the microstructure and hydraulic conductivity of Héricourt clay. *Journal of Rock Mechanics and Geotechnical Engineering* 6, 399-404.
Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C., Singh, B.K., 2016. Response of Soil Properties and Microbial Communities to Agriculture: Implications for Primary Productivity and Soil Health Indicators. *Frontiers in Plant Science* 7.
Vitousek, P.M., Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13, 87-115.
Wang, J., Zhu, B., Zhang, J., Müller, C., Cai, Z., 2015. Mechanisms of soil N dynamics following long-term application of organic fertilizers to subtropical rain-fed purple soil in China. *Soil Biology and Biochemistry* 91, 222-231.
Wang, X., Tang, C., 2017. The role of rhizosphere pH in regulating the rhizosphere priming effect and implications for the availability of soil-derived nitrogen to plants. *Annals of Botany* 121, 143-151.
Xia, Q., Rufty, T., Shi, W., 2020. Soil microbial diversity and composition: Links to soil texture and associated properties. *Soil Biology and Biochemistry* 149, 107953.
Zhalnina, K., Dias, R., de Quadros, P.D., Davis-Richardson, A., Camargo, F.A., Clark, I.M., McGrath, S.P., Hirsch, P.R., Triplett, E.W., 2015. Soil pH determines microbial diversity and composition in the park grass experiment. *Microb Ecol* 69, 395-406.
Zhang, J., Cai, Z., Müller, C., 2018. Terrestrial N cycling associated with climate and plant-specific N preferences: a review. *European Journal of Soil Science* 69, 488-501.
Zhang, T., Wang, N.-F., Liu, H.-Y., Zhang, Y.-Q., Yu, L.-Y., 2016. Soil pH is a Key Determinant of Soil Fungal Community Composition in the Ny-Ålesund Region, Svalbard (High Arctic). *Frontiers in Microbiology* 7.
Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Dietrich, M., Herbold, C.W., Eichorst, S.A., Woebken, D., Richter, A., 2019. Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil Biology and Biochemistry* 136, 107521.
Zhu, G., Song, X., Ju, X., Zhang, J., Müller, C., Sylvester-Bradley, R., Thorman, R.E., Bingham, I., Rees, R.M., 2019. Gross N transformation rates and related N2O emissions in Chinese and UK agricultural soils. *Science of The Total Environment* 666, 176-186.
Žurovec, O., Wall, D.P., Brennan, F.P., Krol, D.J., Forrestal, P.J., Richards, K.G., 2021. Increasing soil pH reduces fertiliser derived N2O emissions in intensively managed temperate grassland. *Agriculture, Ecosystems & Environment* 311, 107319.

Chapter 6

Summary and future work

6.1 Summary

One of the main aims of soil research directed toward resource-efficient and sustainable agricultural practices is to gain greater knowledge of how biological and management factors, such as nutrient management practices, interact to control the release of nutrients from SOM (Boilard *et al.*, 2019). It is important to understand the interactions between agricultural nutrient management practices and nutrient release from SOM for a number of reasons. For instance, to decrease the input of N fertilisers into agricultural systems, which could decrease associated losses of N in the form of greenhouse gas (GHG) fluxes from agricultural systems. This would also decrease GHG associated with the production of fertiliser which is an important consideration in global GHG balances. The reduction of GHG's is of global importance and soil carbon (C) sequestration processes play a key role in storing or increasing SOM, and also sustainable nutrient supply in agricultural systems. Reduced fertiliser inputs to agricultural soils would also decrease loss of nutrients to waterways. An increased understanding of nutrient release from SOM is also important for farmers; the economic cost of fertilisers has increased significantly in the last decade, in particular in 2008-'09 and 2021-'22, and this resulted in declines in farm income. With an expanding global population there are increasing demands on farmers to increase food supply while simultaneously decrease fertiliser and pesticide use on farms in line with agri-environmental policy ambitions (EU Green Deal). Sustainable practices such as increasing nutrient release from SOM could contribute to both a reduction in cost for farmers while also helping to meet sustainable management goals. The EU soil strategy has recognised the importance of SOM in ecosystem functions, including contributions to GHG mitigation, nutrient supply, as well as supporting biodiversity.

This thesis investigated the effect of soil management practice on microbially mediated soil organic matter (SOM) decomposition and in particular N mineralisation in grassland soils. The nutrient management practices focused on phosphorus (P) addition in inorganic and organic forms, and amendment of soil pH through the application of lime. To examine this, a 7-day anaerobic incubation assay was used to measure potential N mineralisation. The use of stable isotope labels, $^{13}CO_2$ and ^{13}C-glucose, were used to measure the rate of SOM decomposition and priming, and differences in microbial community structure were assessed using high throughput sequencing analysis. This combination of techniques, linking management practice with soil functioning and microbial community dynamics, adds novelty to this thesis. Chapter 2 investigated the effect of both P management and pH on N mineralisation potential, enzyme activity and microbial community

structure. Chapters 3 and 4 built on the results of Chapter 2, as the effect of P fertiliser type (inorganic vs organic) and their effects on potential N mineralisation, although different, was marginally not significantly different. Chapters 3 and 4 also examined SOM decomposition and priming, to gain further depth of understanding on the microbially mediated processes involved in N mineralisation and the effect that P addition has on these processes. Chapter 4 also examined the effect of a cut silage system vs a grazed grassland system. Chapter 3 only examined a cut silage-based system, therefore in Chapter 4 the additional influence of animal excretal returns in a grazing treatment were examined. As well as this, in the silage-based system, the influence of plant biomass removal (without excretal returns) was considered as a factor influencing grassland soil microbial community structure and soil C/N cycling processes. Chapter 5, also built on Chapter 2 and examined the effect of differential soil pH, resulting from long-term field lime applications, on potential N mineralisation, SOM decomposition and priming, as well as on microbial community structure. The effect of soil pH was examined as it was found to be an important environmental factor in previous chapters, as it influenced both N mineralisation potential and microbial community structure. Thus, despite liming being a common practice in grassland systems, there have been few studies that have examined the effect of pH on SOM decomposition and priming. This is particularly the case for experiments including plants (as opposed to use of exogenous substrate addition to mimic root exudate inputs), and therefore it was important to examine the additional interactions between soil pH, plant growth, microbial communities and C/N cycling in soils influenced by living roots.

Chapter 2 was the only study as part of this thesis that used both a range of soil types and a combination of P and pH nutrient management strategies. The soils were selected as in previous studies there was a significant difference in N mineralisation potential between these soils, and a range of organic matter (OM) contents (McDonald *et al.*, 2014; Murphy *et al.*, 2017). The soil with the highest OM content had the highest N mineralisation potential (Chapter 2, Table 1) (McDonald *et al.*, 2014). However, the main focus of this chapter was on the nutrient management treatments and their impact on N mineralisation potential. The hypotheses tested in Chapter 2 were: 1) the application of lime and organic P application would increase N mineralisation, and enzymatic activity; 2) chemical P application would decrease the activity of enzymes, and N mineralisation; and 3) the microbial community structure is altered by nutrient management conditions. Measurement of enzymatic activity is used as an indicator of biological activity in soils (Schloter *et al.*, 2018; Zhang *et al.*, 2019), and also of SOM decomposition processes (Muruganandam *et al.*, 2009; Chen *et al.*, 2014). However, the potential enzyme activities measured as part of this study were highly variable and did not correlate with either soil type or treatment. Therefore, hypotheses 1 and 2 in the context of the enzyme activity assays applied in the experiment were rejected.

Results from Chapter 2 were inconclusive on the impact of P form on N mineralisation. Therefore, the hypothesis (2) that chemical P would reduce N mineralisation potential was not accepted, and the hypothesis (1) that organic P addition would increase N mineralisation was also rejected. However, this was further investigated in Chapters 3 and 4. Increasing soil pH was correlated with increasing N mineralisation potential (Chapter 2), and therefore the hypothesis that lime addition would increase N mineralisation was supported. The effect of soil pH on N mineralisation and SOM turnover was further investigated in Chapter 5. There were significant differences in both 16S and ITS communities between soil types and treatments. Results of the alpha diversity indexes showed significant differences with soil type, although there were no significant differences between treatments (P, type of P, and lime). However, when examining abundance, there were significant differences in genera for both 16S and ITS between treatments. Therefore, the hypothesis (3) that microbial community structure would be altered by nutrient management practice was supported with recognition that more research is required to establish the functional significance of these changes in microbial community composition.

Chapter 3 used stable isotope labelling to determine the effect of P nutrient management in a planted grass system. This study aimed to further examine the effect of inorganic and organic P additions on N mineralisation. The effect of P nutrient management on SOM decomposition and priming is relatively less well understood compared to N management (Dijkstra *et al.*, 2013). However, P is an essential nutrient for plant growth and is also applied regularly in agricultural soils and, hence, it is important to understand the effect of P management on SOM decomposition and N mineralisation. Chapter 3 tested the hypotheses that: 1) the addition of P would increase plant productivity, N mineralisation and the rate of priming; and 2) that P added in an organic form would increase soil nutrient availability, N mineralisation and the rate of priming.

Similar to the results of Chapter 2, in Chapter 3 there was a significant effect of available P on potential N mineralisation. However, while in Chapter 2 the higher available P led to a higher N mineralisation potential compared to the control, in Chapter 3 the lower P treatments (P0 and P0+slurry) were associated with higher N mineralisation potential (this was seen in both the planted and unplanted treatments). The range of P and in particular P limitation was greater in Chapter 3 compared to Chapter 2. The P0 treatments in Chapter 3 had an average available P value of between 2.1-2.5mg Kg^{-1}, while in Chapter 2 the available P values were closer to 5mg kg^{-1} depending on soil type. This difference may explain the different effect of P on potential N mineralisation and the increase in N mineralisation potential with an organic P addition (Chapters 2 and 3). However, the hypothesis that P addition would increase N mineralisation potential was rejected.

Chapter 3 examined the effect of P and P addition type on the rate of SOM decomposition and priming. SOM decomposition rate in the P45 + slurry treatment was significantly lower than the P45 treatment. It was also found that organic P addition had a significant effect on SOM priming, the magnitude of priming was larger in soils that received more P. The highest magnitudes of priming were seen in the higher P treatments. Therefore, in terms of priming and SOM decomposition both hypotheses 1 and 2 in Chapter 3 were accepted. However, the direction of priming was different; the P45 inorganic treatment was associated with positive priming, and the P45 + slurry organic treatment was associated with negative priming. This may have important policy implications both in terms of nutrient management, but also practices that could be used to promote carbon (C) storage in grassland soils. The higher SOM decomposition rate and positive priming effect in P45 suggests that, despite high nutrient availability in this treatment, more SOM was being broken down to provide nutrients. In the P45 treated soils, it is possible that the supply of freely available nutrients had been exhausted by the plant or immobilised by the microbial biomass and therefore SOM stocks were mined for nutrients such as N. This result supports the microbial N mining hypothesis of plant-mediated priming, where N-limitation can promote SOM mineralisation in the rhizosphere (Craine *et al.*, 2007). Although further study would be required, this is a potentially important objective for agri-environmental policy development, as there were increased C losses from SOM with inorganic P fertiliser addition compared to treatments that also received an organic P addition. This organic source of nutrients may be important in reducing C losses by not only providing more nutrients but also providing an alternative organic substrate and energy source for the microbial community to utilise.

Chapter 4 followed on from Chapter 3 and examined the effect of P addition on potential N mineralisation, SOM decomposition and priming. However, Chapter 4 also examined the effect of a cut silage-based grassland system, compared to a grazed grassland system. In addition, building on the results of Chapter 3, differences in microbial community structure were examined through the high throughput sequencing analysis of the 16S and ITS communities. The methodology described in this chapter to measure SOM decomposition and priming also used stable isotope labelling; however, it used a ^{13}C glucose addition to stimulate root exudates (a planted system was not used in this chapter). The addition of a ^{13}C glucose label is a commonly used method for stimulating priming (Kuzyakov *et al.*, 2000; Paterson, 2003). The addition of glucose allows for more control of the soil system compared to a planted system which includes additional influences of plants such as through changes to water fluxes, impacts on soil structure, the chemistry of the rhizosphere (e.g., pH), changes to microbial community composition, and nutrient availability. It was hypothesised that: 1) long-term P addition would result in greater potential N mineralisation, a higher rate of SOM-C decomposition and priming, and that there would be impacts on both bacterial and fungal community structure; 2)

the type of P addition would also affect these factors: the addition of an organic source of P in the form of slurry would lead to an increased potential N mineralisation, a higher rate of SOM-C decomposition and priming, and that the form (mineral or organic) of fertilisation would impact both bacterial and fungal community structure; and 3) the type of grassland management would also affect microbial mediated nutrient cycling, that in a cut and remove for silage system there would be increased SOM-C decomposition and potential N mineralisation.

Overall, the rate of potential N mineralisation was higher in the planted system (Chapter 3) than in the soil only system (Chapter 4). There were no significant differences between the P treatments that received glucose. However, in the control, non-glucose-amended treatments, there was a difference in N mineralisation potential between the grazed and cut system. The grazed treatments with both high and low P additions had a lower N mineralisation potential than the cut treatments. It was expected that potential N mineralisation would be higher in the cut treatments compared with the grazed treatments due to the higher demand for SOM-derived nutrients, whereas in a grazed system some of this demand is met from livestock excreta, so therefore the hypothesis (3) in terms of N mineralisation was supported. A potential explanation was that with the C recycling through livestock excretal returns to soil, N was potentially immobilised by the microbial biomass. This could not be measured by the potential N mineralisation assay used in this study as it is a net measure, thus N that became immobilised in microbial biomass was not measured as part of the flux of NH_4 from SOM. This is also a potential explanation for the lack of differences seen in the treatments which received glucose. However, only measuring net N mineralisation is a potential limitation of this study. In order to measure the release of N from SOM as a gross N-flux, rather than the net change in NH4+ pool size, a ^{15}N pool dilution technique could be applied (Goerges and Dittert, 1998). Briefly, this method uses the addition of a ^{15}N labelled NH4+ tracer, and therefore any N released from SOM will be seen as ^{14}N dilution of the soil NH4+ pool. Using such a labelling approach, gross N mineralised can be determined in soil, as any N release from SOM would have the native isotopic signature (Goerges and Dittert, 1998). The 15N dilution method would also account for N immobilisation into soil microbial biomass that potentially was occurring as part of this study.

It was hypothesised that the treatments receiving higher P addition would have a higher rate of SOM decomposition. This hypothesis was rejected as SOM decomposition was higher in the lower P treatments. The SOM-C decomposition rate in the P0 slurry treatment was higher than the P30 slurry treatment in terms of absolute values at all time points and was significantly higher on days 2 and 4. Overall, there was a significant effect of the slurry treatment on SOM decomposition. However, SOM-C decomposition was not always higher with slurry addition and therefore the hypothesis (2) was

rejected. It was hypothesised that SOM- C decomposition would be greater in the cut grassland system when compared to the grazed grassland system. This was accepted as the rate of SOM-C decomposition was greater in soils from both the P0 cut and P30 cut site compared with the P0 grazed and P30 grazed sites, respectively. As determined by molecular sequence analyses, significant differences in both microbial (16S gene) and fungal (ITS region) communities were found in Chapter 4. The microbial communities were significantly influenced by available P. Although there were impacts on the process involved in SOM decomposition and on microbial communities, it cannot be confirmed which changes in the microbial communities were responsible for the changes in SOM C and N dynamics. There is scope here to examine this through metagenome or metatranscriptomic approaches to better understand the functioning of microbial communities in SOM decomposition.

Chapter 5 focused on the effect of soil pH and liming management on potential N mineralisation, SOM decomposition, priming and microbial community structure. Chapter 2 found pH to have a significant effect on N mineralisation and Chapter 5 aimed to investigate this further with a broader pH range, and in a system where the rate of nutrient addition was specifically controlled. The same experimental set up used in Chapter 3 was used with stable isotope labelling of a planted system. It was hypothesised that: 1) the soil decomposition would have the highest magnitude in the soils with a pH of 6-7; 2) N mineralisation would increase with increasing soil pH and liming, and hence the highest potential mineralisation rate would be in soil with the highest pH and that received the most lime; and 3) microbial community structure as determined by 16S and ITS sequencing would be altered by soil pH, and that the diversity of microbial communities would be higher between the soil with the highest and lowest pH.

The increase in N mineralisation associated with increasing soil pH observed in Chapter 2 was also seen in Chapter 5. The treatment with the highest pH (VH) had a significantly greater N mineralisation potential compared with the lower pH treatments, and so this hypothesis (2) was accepted. Perhaps an unexpected result was the CO_2 fluxes from lime in the VH treatment, as it was expected that more than 12 months after lime application that there would be no effect of lime dissolution on the CO_2 measurements. A significantly enriched $^{13}CO_2$ signature was associated with the VH treatment compared with the other treatments, indicating a direct contribution of lime-derived carbonate to the soil CO_2 efflux. All treatments were grown in a $^{13}CO_2$ depleted environment, but in the fallow soil (i.e., not receiving labelled plant-derived inputs) there were two sources of C with distinct isotopic signatures (SOM and lime) which allowed for CO_2 from lime dissolution to be measured by isotopic partitioning. As contributions of lime to soil CO_2 efflux were evident more than 12 months after in-field lime application, CO_2 fluxes from lime dissolution may be an important factor in terms of

considering greenhouse gases (GHG) budgeting in managed grassland soils. GHG emissions from N in the form of N_2O via the denitrification pathway, along with N fertiliser addition and the length of time after application these emissions can occur for, is given careful consideration in GHG emission budgets for grassland soils. Perhaps there is scope to consider the GHG emissions directly from lime dissolution also.

Overall, the addition of P increased the magnitude of SOM-C decomposition and priming when compared with treatments that are limited in P. This has important implications when considering fertiliser planning, as systems which are P limited are also not mining SOM for nutrients, and therefore could potentially also become limited in other nutrients such as N more rapidly. However, despite SOM decomposition rates being lower with lower P, N mineralisation potential with lower P was often not significantly lower than higher P treatments. As well as this, the type of P addition was also important as decomposition rates were found to be greater in treatments receiving an inorganic form of P only. The direction of priming is also variable with type of P addition, with potential for negative priming with organic nutrient additions. This has important implications for C storage in soils and perhaps inclusion of an organic input with inorganic fertilisers on all grasslands used for agriculture would both decrease the amount of inorganic fertiliser needed and contribute positively to maintenance of C stocks. C storage in grassland soils is considered an important mitigation strategy for GHG emissions from agricultural land, and this decreased SOM decomposition promotes soil health.

Overall, the addition of P increased the magnitude of SOM-C decomposition and priming when compared with treatments that were limited in P. This has important implications when considering fertiliser planning, as systems which are P limited will have reduced release of nutrients from SOM, and therefore could potentially become limited in other nutrients such as N more rapidly. In comparison to the effect of N on priming and SOM-C decomposition, there have been fewer studies on the effect of P on priming and SOM-C decomposition (Dijkstra et al., 2013; Boilard et al., 2019; Mehnaz et al., 2019; Xu et al., 2019; Lu et al., 2020). A study by Mehnaz et al. (2019) examined a system without plants and used label C solution including glucose to stimulate priming, similar to Chapter 4 in this thesis. The results from Chapter 4 are in agreement with Mehnaz et al. (2019) where it was also found that priming effects stimulated by glucose addition were also higher in soils with higher soil P. Perhaps, the main explanation for this effect is that the microbial communities in soils receiving P are not P limited and therefore have more energy to carry out SOM decomposition. Previous studies have not examined microbial communities as were examined in Chapter 4 of this thesis. Although there is much more work to be done to determine the function of the microbial

community in both N and P cycling, Chapter 4 highlighted that P addition has a significant effect on both 16S bacterial and archaeal communities as well as fungal communities. In terms of the planted systems used to examine the effects of priming both Boilard et al. (2019) examined the effect of P in a Barley system and Xu et al. (2019) examined the effect of in a wheat system Both of these studies also found that in planted systems there was an increase in the rate of priming with increased P availability. Their results are in agreement with the grass-based system examined as part of Chapter 3 in this thesis. In contrast, Lu et al. (2020), who also examined a grass based system, found that there was decreased priming effect with the addition of P addition. However, in the case of Lu et al. (2020), soils received P as part of a nutrient solution in the lab whereas the soils used in Chapter 3 were part of a long-term P trial and therefore microbial communities were not disrupted by nutrient addition during the experiment, which could have impacted the direction and magnitude of priming.

As well as this, the type of P addition was also important as decomposition rates were found to be greater in treatments receiving an inorganic form of P only. The direction of priming is also variable with type of P addition, with potential for negative priming with organic nutrient additions. This has important implications for C storage in soils and perhaps inclusion of an organic input with inorganic fertilisers on all grasslands used for agriculture would both decrease the amount of inorganic fertiliser needed and contribute positively to maintenance of C stocks. C storage in grassland soils is considered an important mitigation strategy for GHG emissions from agricultural land, and this decreased SOM decomposition may also promote soil health through build-up of SOM. However, previous studies have found that slurry C which was injected into soil was lost to the atmosphere twice as rapidly as the native soil C, as well as inducing positive priming effects (Bol et al., 2003). This study concluded that although there are savings in terms N related gases with injecting slurry into soil these may be offset by the rapid C losses from both slurry and the primed soil C (Bol et al., 2003). This is in contrast to what was found in Chapter 3 of this study. A possible explanation for the differences seen here could be due to the timings of the slurry application. In Chapter 3 slurry was applied in the field and therefore the quick loss of C from slurry may not have been captured as part of the experiment. As the slurry was more established in the system in Chapter 3 than in the study by Bol et al. (2003) it is possible that the results seen in this thesis are more in line with Blagodatskaya and Kuzyakov (2008) who hypothesised that the addition of a combination of C and N would reduce SOM decomposition as microbial communities use more freely available sources of C and N. Hence, the microbial communities in these slurry treated soils are better adapted to decompose more freely available nutrients rather than difficult to degrade degreed SOM nutrients. This is consistent with differences in microbial community structure with slurry addition as was seen in the results of Chapter 4.

However, despite SOM decomposition rates being lower with lower P, N mineralisation potential was often not significantly lower than higher P treatments in this thesis. The effect of P application on N mineralisation reported in the literature is highly variable, with some studies showing increases in N mineralisation with P application (Cadisch et al., 1994; Bicharanloo et al., 2022; Matsuoka-Uno et al., 2022), others showed decreases in N mineralisation with P addition (Cadisch et al., 1994; Akbari et al., 2020), or no effect of P addition on N mineralisation rate (Schleuss et al., 2021).Possible explanations for the variance seen across studies could be due to differences between soil types (Cadisch et al., 1994), and differences in N mineralisation potential with P application and soil type were also seen in Chapter 2. Cadisch et al. (1994) found that in sandy Entisol soils N mineralisation increased with P application but only during dry conditions, and that in clayey soils that are likely to fix P, P application also increased N mineralisation. Cadisch et al. (1994) hypothesised that when the plant was not P deficient after P addition that N cycling efficiency increased and consequently N mineralisation also increased. A similar hypothesis was also considered in Chapter 3 and 4 although it was not found to be the case as N mineralisation was not consistently higher with P addition across studies in this thesis.

Throughout the thesis, but particularly in Chapter 5, there was a significant effect of soil pH on N mineralisation potential, priming, and SOM decomposition. Chapter 5 found that there was an increase in N mineralisation potential with increasing soil pH and lime addition. This result is in agreement with numerous other studies which also found that N mineralisation increased with increasing soil pH (Blagodatskaya and Anderson, 1998; Curtin et al., 1998; Bolan et al., 2003; Bolan and Hedley, 2003; Ahmad et al., 2013; Neina, 2019). This could be due to an increase in organic compounds with increasing soil pH (Neina, 2019). However, it is more likely due to increased biological activity. More neutral soil pHs can have increased microbial diversity and activity, as well as being better at mineralising a wider range of organic compounds, and using available nutrients more efficiently (Paradelo et al., 2015; Aye et al., 2016; Curtin et al., 2016; Neina, 2019). Results from microbial community sequencing analyses in Chapter 5 are in agreement with previous studies (Wakelin et al., 2008; Rousk et al., 2010a; Rousk et al., 2010b; Trivedi et al., 2016; Grover et al., 2021), that showed a significant effect of soil pH on microbial community structure and diversity. This suggests that potential changes due to liming and hence soil pH may have a contributing effect to the increased N mineralisation potential seen in the highest pH treatment.

Results from Chapter 5 in this thesis indicated that soil derived CO2 efflux was highest with the highest pH and increased over time in the planted pots. Although it was thought that by taking samples from this field site more than 12 months after lime application that lime dissolution would not affect CO2 fluxes from soil, but this was not the case in the VH treatment. Other studies have previously reported

an increase in CO2 fluxes with lime addition (Hinsinger et al., 2003; Bertrand et al., 2007; Biasi et al., 2008; Paradelo et al., 2015; Holland et al., 2018; Lochon et al., 2018), this could potentially be attributed to an increase in soil C mineralisation or lime dissolution. Chapter 5 in this thesis presented a unique scenario where due to the stable isotope partitioning set up it was possible to identify that lime dissolution had occurred and contributed to soil CO2 efflux in this treatment. As has been mentioned previously liming and soil pH change the structure and activity of microbial communities. In this context, Keiblinger et al. (2010) hypothesised that after liming microbial communities can shift to favour microbes with lower C use efficiency and hence higher CO2 respiration rate. This is one potential explanation for the slow rate of lime dissolution seen in Chapter 5. The application of lime may promote the build up of SOC and therefore C storage (Johnson et al., 2005; Fornara et al., 2011; Eze et al., 2018). Additionally, it has been reported that lime application may also reduce both CH4 (Hilger et al., 2000; Kunhikrishnan et al., 2016) and N2O emissions (Goulding, 2016; Abdalla et al., 2022). However, it is important to consider the potential of the lime itself as a contributor to CO2 emissions while dissolving, as well as during mining and extraction. At the moment the emission factor used for CO2 emission from lime is fixed (Fornara et al., 2011, Lochon et al., 2018). Although further study would be required, the scenario presented in Chapter 5 would suggest that the CO2 emissions from lime in agricultural soils are dynamic and longer lasting than previously thought.

6.2 Future work

The experimental work carried out as part of this thesis contributes to the understanding of SOM turnover and N mineralisation in grassland agricultural systems. However, gaps remain in our knowledge and understanding, and this section aims to highlight potential future areas of research.

In Chapters 3 and 4, a field-applied slurry amendment, supplying P, was used in addition to mineral P treatments. However, the stable isotope labelling techniques used could not distinguish between SOM and the slurry sources to soil CO_2 efflux. Thus, a potential future experiment to further examine the effect of organic nutrient addition in the form of slurry on SOM decomposition rates could employ a 3-way isotope partitioning approach. One possible way of achieving this would be through labelling the plant material in a $^{14}CO_2$ environment, use the labelled plant material as feedstock for livestock and collect the resultant slurry. In theory, this would allow separate quantification of SOM-, slurry- and plant-derived sources. However, this is likely to raise some ethical issues as ^{14}C isotopes are radioactive. Also, it would be difficult to grow enough plant material to achieve the desired amount of slurry. Perhaps a more achievable way of quantifying and partitioning slurry-derived mineralisation and SOM-derived fluxes would be to use a slurry from livestock fed on a C4 plant diet (such as a maize-based feed) in a C3 soil and plant system. This would require a combinational experimental set up

similarly to the one used as part of this thesis, where one treatment receives a C4 based slurry and the other receives a C3 based slurry. The three isotope sources would then be the slurry (C3 vs C4), the plant (enriched with $^{13}CO_2$), and the SOM (natural abundances), this would allow a more specific determination of the effect of slurry treatments on SOM decomposition rate. This would require that the SOM/slurry contributions are calculated by difference; and the assumption would have to be made that the C4 and C3 slurries would have equivalent chemistries. However, it is not a given that these chemistries would in fact be the same, creating a different confounding influence.

This thesis was carried out as a series of controlled environment and laboratory-based experiments; however, it is important to also consider scaling up to field-based methods. Field based methods would include greater complexity inherent in natural ecosystems, for example environmental conditions, season, and impacts of management as applied on farm. There is potential to measure net N mineralisation in the field using a cylinder inserted into the top 10cm of soil, with a plastic bag at the top and bottom containing an acid and alkaline exchange resin. A small amount of soil and the resin bag is taken and measured using a KCl extraction to determine the N exchange (for more detailed descriptions see Risch *et al.* (2015) and Risch *et al.* (2019)). There is also some potential for labelling of $^{13}CO_2$ in field experiments, for example analogous to the use of the free air carbon dioxide enrichment (FACE) facilities (Andrews *et al.*, 1999; Ainsworth and Long, 2021). Field-based experiments may also allow for a more diverse grassland system. In this thesis a *Lolium perenne* monoculture was used, as this was based on standard Irish grasslands at the time the field trials were developed. However, now with policy drivers to deliver more sustainable management, multi-species grasslands are becoming more common, particularly grass-clover mixtures due the N-fixing capabilities of clover, which reduces the N fertiliser requirement of grasslands. There is scope to build on the results of this study using multi-species grassland swards. Field-based experiments would also allow for quantification of GHG fluxes with nutrient management strategies using chamber measurements, which were not measured as part of this thesis. Particularly in relation to N both nitrification and denitrification that were not measured as part of this study. Denitrification is a major source of potent GHG N_2O and needs to be considered in the context of GHG budgets from agricultural soils. The links between N fluxes from denitrification with nutrient management as well as SOM N turnover could contribute greater detail to life cycle assessment analysis of GHG and nutrient budgets in agricultural systems. As well as this, quantification of genes in the denitrification pathway such as *nosZ clade I* and *nosZ clade II* could contribute information on the potential activity of the microbial community with respect to N cycling in soils.

This study used high-throughput sequencing of DNA to assess the structures of microbial communities. Although this is a useful technique it does not specifically identify active components of the community, as the DNA of dead/inactive microorganisms can be sequenced, and therefore inference of function is problematic. A potential future study could also include high throughput sequencing of the ribosomal rRNA. RNA sequences can allow for the identification of the active microbes in the soil community (Blazewicz *et al.*, 2013). Combining both DNA and RNA sequences would allow for further comparison of the dormant and active members of the community (Blazewicz *et al.*, 2013; Bowsher *et al.*, 2019). This would be useful in the context of the functional effect of the microbial community in terms of SOM decomposition, as a change in active community with the addition of glucose or the presence of a plant could be measured. This would also be useful in the context of the effect of nutrient management practices on the microbial community, as changes in active members with organic slurry addition, for example, could be investigated. Future studies could use the soil samples stored for molecular work from this thesis to investigate the microbial RNA. A more detailed examination of the microbial communities through metagenomics, metaproteomics, or metatranscriptomics could contribute to a deeper understanding of the specific function of both the microbial community and enzymes involved in SOM decomposition and nutrient availability.

6.3 References

Abdalla, M., Espenberg, M., Zavattaro, L., Lellei-Kovacs, E., Mander, U., Smith, K., Thorman, R., Damatirca, C., Schils, R., ten-Berge, H., Newell-Price, P., Smith, P., 2022. Does liming grasslands increase biomass productivity without causing detrimental impacts on net greenhouse gas emissions? Environmental Pollution 300, 118999.

Ahmad, W., Singh, B., Dijkstra, F.A., Dalal, R.C., 2013. Inorganic and organic carbon dynamics in a limed acid soil are mediated by plants. Soil Biology and Biochemistry 57, 549-555.

Ainsworth, E.A., Long, S.P., 2021. 30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation? *Global Change Biology* 27, 27-49.

Akbari, F., Fallah, S., Dahmardeh, M., Pessarakli, M., 2020. Interaction effects of nitrogen and phosphorus fertilizer on nitrogen mineralization of wheat residues in a calcareous soil. Journal of Plant Nutrition 43, 1-12.

Andrews, J.A., Harrison, K.G., Matamala, R., Schlesinger, W.H., 1999. Separation of Root Respiration from Total Soil Respiration Using Carbon-13 Labeling during Free-Air Carbon Dioxide Enrichment (FACE). *Soil Science Society of America Journal* 63, 1429-1435.

Aye, N.S., Sale, P.W.G., Tang, C., 2016. The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils. Biology and Fertility of Soils 52, 697-709.

Bertrand, I., Delfosse, O., Mary, B., 2007. Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: Apparent and actual effects. Soil Biology and Biochemistry 39, 276-288.

Biasi, C., Lind, S.E., Pekkarinen, N.M., Huttunen, J.T., Shurpali, N.J., Hyvönen, N.P., Repo, M.E., Martikainen, P.J., 2008. Direct experimental evidence for the contribution of lime to CO2 release from managed peat soil. Soil Biology and Biochemistry 40, 2660-2669.

Bicharanloo, B., Bagheri Shirvan, M., Keitel, C., Dijkstra, F.A., 2022. Nitrogen and phosphorus availability have stronger effects on gross and net nitrogen mineralisation than wheat rhizodeposition. Geoderma 405, 115440.

Blagodatskaya, E.V., Anderson, T.-H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO2 of microbial communities in forest soils. Soil Biology and Biochemistry 30, 1269-1274.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115-131.
Blazewicz, S.J., Barnard, R.L., Daly, R.A., Firestone, M.K., 2013. Evaluating rRNA as an indicator of microbial activity in environmental communities: limitations and uses. *The ISME Journal* 7, 2061-2068.
Boilard, G., Bradley, R.L., Paterson, E., Sim, A., Brown, L.K., George, T.S., Bainard, L., Carubba, A., 2019. Interaction between root hairs and soil phosphorus on rhizosphere priming of soil organic matter. *Soil Biology and Biochemistry* 135, 264-266.
Bol, R., Moering, J., Kuzyakov, Y., Amelung, W., 2003. Quantification of priming and CO2 respiration sources following slurry-C incorporation into two grassland soils with different C content. Rapid Communications in Mass Spectrometry 17, 2585-2590.
Bolan, N.S., Adriano, D.C., Curtin, D., 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. Advances in agronomy 78, 5-272.
Bolan, N.S., Hedley, M.J., 2003. Role of carbon, nitrogen, and sulfur cycles in soil acidification. CRC Press.
Bowsher, A.W., Kearns, P.J., Shade, A., Deveau, A., 2019. 16S rRNA/rRNA Gene Ratios and Cell Activity Staining Reveal Consistent Patterns of Microbial Activity in Plant-Associated Soil. *mSystems* 4, e00003-00019.
Cadisch, G., Giller, K.E., Urquiaga, S., Miranda, C.H.B., Boddey, R.M., Schunke, R.M., 1994. Does phosphorus supply enhance soil-N mineralization in Brazilian pastures? European Journal of Agronomy 3, 339-345.
Chen, R., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Lin, X., Blagodatskaya, E., Kuzyakov, Y., 2014. Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. *Global Change Biology* 20, 2356-2367.
Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88, 2105-2113.
Curtin, D., Campbell, C.A., Jalil, A., 1998. Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. Soil Biology and Biochemistry 30, 57-64.
Curtin, D., Peterson, M.E., Anderson, C.R., 2016. pH-dependence of organic matter solubility: Base type effects on dissolved organic C, N, P, and S in soils with contrasting mineralogy. Geoderma 271, 161-172.
Dijkstra, F., Carrillo, Y., Pendall, E., Morgan, J., 2013. Rhizosphere priming: a nutrient perspective. *Frontiers in Microbiology* 4.
Eze, S., Palmer, S.M., Chapman, P.J., 2018. Soil organic carbon stock in grasslands: Effects of inorganic fertilizers, liming and grazing in different climate settings. Journal of environmental management 223, 74-84.
Fornara, D.A., Steinbeiss, S., McNamara, N.P., Gleixner, G., Oakley, S., POULTON, P.R., MacDonald, A.J., Bardgett, R.D., 2011. Increases in soil organic carbon sequestration can reduce the global warming potential of long-term liming to permanent grassland. Global Change Biology 17, 1925-1934.7878787878
Goerges, T., Dittert, K., 1998. Improved diffusion technique for 15N:14N analysis of ammonium and nitrate from aqueous samples by stable isotope spectrometry. *Communications in Soil Science and Plant Analysis* 29, 361-368.
Goulding, K.W.T., 2016. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use and Management 32, 390-399.
Grover, S.P., Butterly, C.R., Wang, X., Gleeson, D.B., Macdonald, L.M., Tang, C., 2021. Liming and priming: the long-term impact of pH amelioration on mineralisation may negate carbon sequestration gains. Soil Security 3, 100007.

Hilger, H.A., Wollum, A.G., Barlaz, M.A., 2000. Landfill methane oxidation response to vegetation, fertilization, and liming. Wiley Online Library.
Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant and soil 248, 43-59.
Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Science of The Total Environment 610-611, 316-332.
Johnson, D., Leake, J.R., Read, D.J., 2005. Liming and nitrogen fertilization affects phosphatase activities, microbial biomass and mycorrhizal colonisation in upland grassland. Plant and Soil 271, 157-164.
Keiblinger, K.M., Hall, E.K., Wanek, W., Szukics, U., Hämmerle, I., Ellersdorfer, G., Böck, S., Strauss, J., Sterflinger, K., Richter, A., 2010. The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. FEMS Microbiology Ecology 73, 430-440.
Kunhikrishnan, A., Thangarajan, R., Bolan, N., Xu, Y., Mandal, S., Gleeson, D., Seshadri, B., Zaman, M., Barton, L., Tang, C., 2016. Functional relationships of soil acidification, liming, and greenhouse gas flux. Advances in agronomy 139, 1-71.
Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* 32, 1485-1498.
Lochon, I., Carrère, P., Revaillot, S., Bloor, J.M.G., 2018. Interactive effects of liming and nitrogen management on carbon mineralization in grassland soils. Applied Soil Ecology 130, 143-148.
Lu, J., Yang, J., Keitel, C., Yin, L., Wang, P., Cheng, W., Dijkstra, F.A., 2020. Rhizosphere priming effects of Lolium perenne and Trifolium repens depend on phosphorus fertilization and biological nitrogen fixation. Soil Biology and Biochemistry 150, 108005.
Matsuoka-Uno, C., Uno, T., Tajima, R., Ito, T., Saito, M., 2022. Liming and Phosphate Application Influence Soil Carbon and Nitrogen Mineralization Differently in Response to Temperature Regimes in Allophanic Andosols. Agriculture 12, 142.
McDonald, N.T., Watson, C.J., Lalor, S.T.J., Laughlin, R.J., Wall, D.P., 2014. Evaluation of Soil Tests for Predicting Nitrogen Mineralization in Temperate Grassland Soils. *Soil Science Society of America Journal* 78, 1051-1052.
Mehnaz, K.R., Corneo, P.E., Keitel, C., Dijkstra, F.A., 2019. Carbon and phosphorus addition effects on microbial carbon use efficiency, soil organic matter priming, gross nitrogen mineralization and nitrous oxide emission from soil. Soil Biology and Biochemistry 134, 175-186.
Murphy, C.J., Baggs, E.M., Morley, N., Wall, D.P., Paterson, E., 2017. Nitrogen availability alters rhizosphere processes mediating soil organic matter mineralisation. *Plant and Soil* 417, 499-510.
Muruganandam, S., Israel, D.W., Robarge, W.P., 2009. Activities of Nitrogen-Mineralization Enzymes Associated with Soil Aggregate Size Fractions of Three Tillage Systems. *Soil Science Society of America Journal* 73, 751-759.
Neina, D., 2019. The Role of Soil pH in Plant Nutrition and Soil Remediation. Applied and Environmental Soil Science 2019, 5794869.
Paradelo, R., Virto, I., Chenu, C., 2015. Net effect of liming on soil organic carbon stocks: A review. Agriculture, Ecosystems & Environment 202, 98-107.
Paterson, E., 2003. Importance of rhizodeposition in the coupling of plant and microbial productivity. *European Journal of Soil Science* 54, 741-750.
Risch, A.C., Schütz, M., Vandegehuchte, M.L., van der Putten, W.H., Duyts, H., Raschein, U., Gwiazdowicz, D.J., Busse, M.D., Page-Dumroese, D.S., Zimmermann, S., 2015. Aboveground vertebrate and invertebrate herbivore impact on net N mineralization in subalpine grasslands. *Ecology* 96, 3312-3322.
Risch, A.C., Zimmermann, S., Ochoa-Hueso, R., Schütz, M., Frey, B., Firn, J.L., Fay, P.A., Hagedorn, F., Borer, E.T., Seabloom, E.W., Harpole, W.S., Knops, J.M.H., McCulley, R.L., Broadbent, A.A.D., Stevens, C.J., Silveira, M.L., Adler, P.B., Báez, S., Biederman, L.A., Blair, J.M., Brown, C.S., Caldeira, M.C., Collins, S.L., Daleo, P., di Virgilio, A., Ebeling, A., Eisenhauer, N., Esch, E., Eskelinen, A., Hagenah, N., Hautier,

Y., Kirkman, K.P., MacDougall, A.S., Moore, J.L., Power, S.A., Prober, S.M., Roscher, C., Sankaran, M., Siebert, J., Speziale, K.L., Tognetti, P.M., Virtanen, R., Yahdjian, L., Moser, B., 2019. Soil net nitrogen mineralisation across global grasslands. *Nature Communications* 10, 4981-4981.
Rousk, J., Brookes, P.C., Bååth, E., 2010b. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology and Biochemistry 42, 926-934.
Schleuss, P.M., Widdig, M., Biederman, L.A., Borer, E.T., Crawley, M.J., Kirkman, K.P., Seabloom, E.W., Wragg, P.D., Spohn, M., 2021. Microbial substrate stoichiometry governs nutrient effects on nitrogen cycling in grassland soils. Soil Biology and Biochemistry 155, 108168.
Schloter, M., Nannipieri, P., Sørensen, S.J., van Elsas, J.D., 2018. Microbial indicators for soil quality. Biology and Fertility of Soils 54, 1-10.
Trivedi, P., Delgado-Baquerizo, M., Anderson, I.C., Singh, B.K., 2016. Response of Soil Properties and Microbial Communities to Agriculture: Implications for Primary Productivity and Soil Health Indicators. Frontiers in Plant Science 7.
Wakelin, S., Macdonald, L., Rogers, S., Gregg, A.L., Bolger, T., Baldock, J., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biology and Biochemistry 40, 803-813.
Xu, Q., Wang, X., Tang, C., 2019. Rhizosphere priming of two near-isogenic wheat lines varying in citrate efflux under different levels of phosphorus supply. Annals of Botany 124, 1033-1042.
Zhang, Y., Sun, C., Chen, Z., Zhang, G., Chen, L., Wu, Z., 2019. Stoichiometric analyses of soil nutrients and enzymes in a Cambisol soil treated with inorganic fertilizers or manures for 26 years. *Geoderma* 353, 382-390.

Supplementary materials

Chapter 2 supplementary materials

2. 8 Supplementary Materials

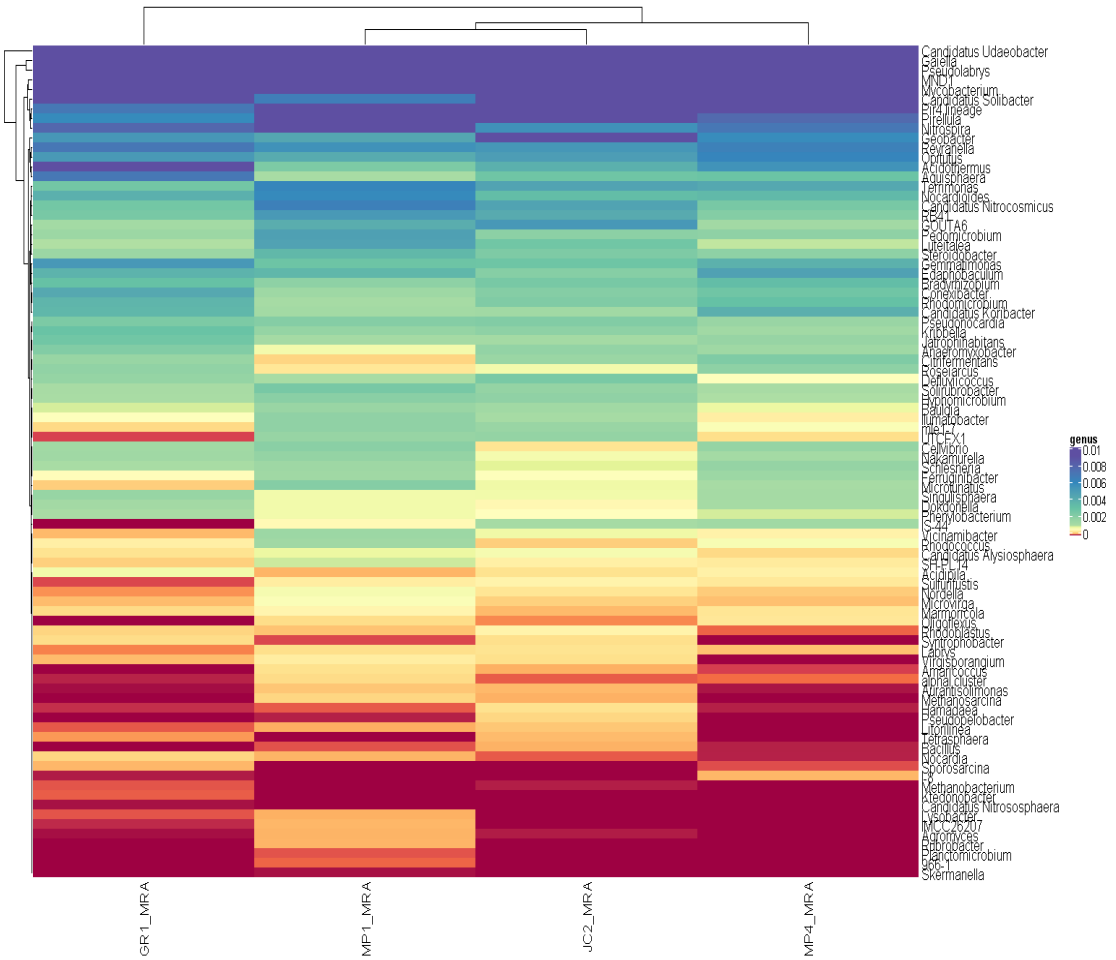

Figure S1: Heat map showing the significant differences in terms of relative abundance of 16S between soil types

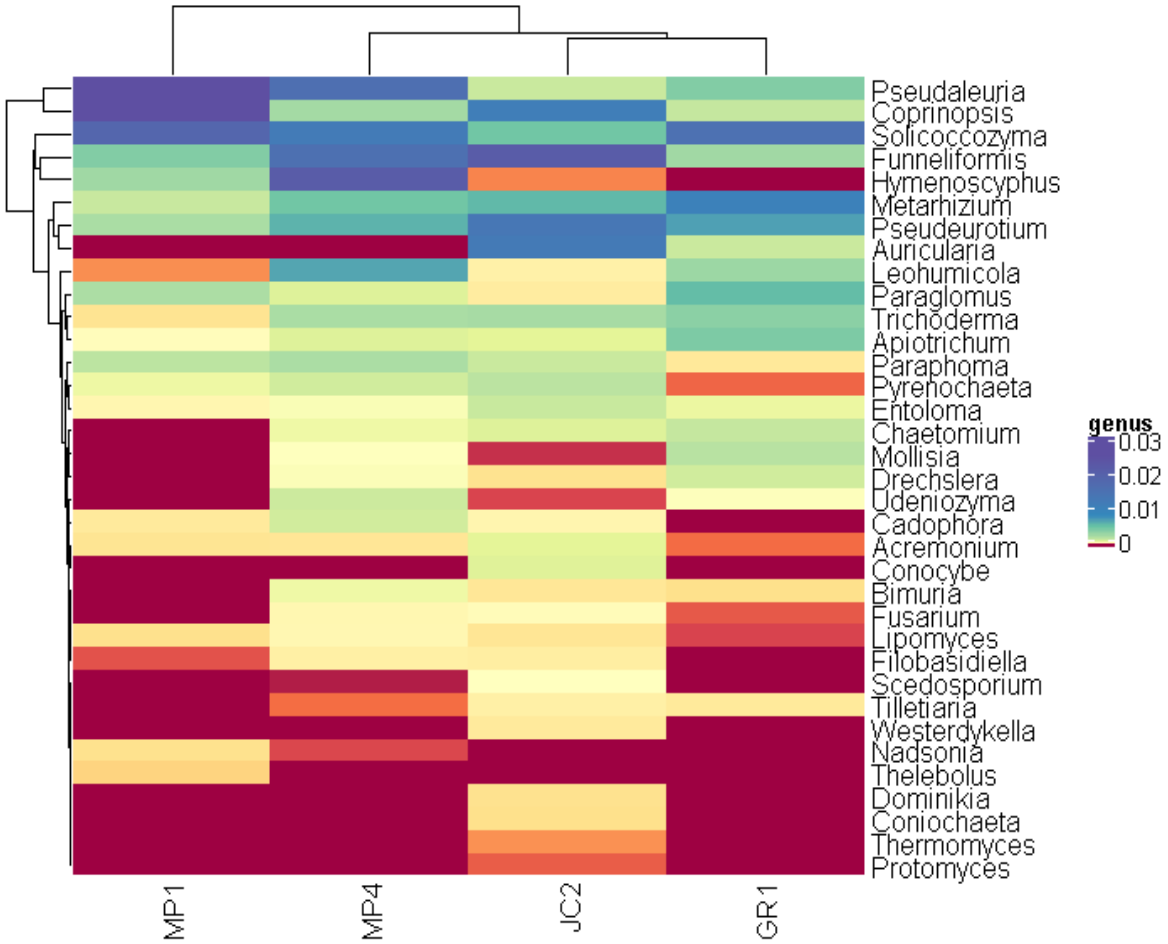

Figure S2: Heat map showing significant differences in relative abundances of ITS community with soil time

Table S1: Deseq2 analysis of 16S JC2 vs. GR1.

JC 2 vs GR1	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	IS-44	5.48	JC 2
	Proteobacteria	mle1-7	2.59	JC 2
	Actinobacteriota	Conexibacter	-0.90	GR 1
	Proteobacteria	MND1	1.28	JC 2
	Planctomycetota	Aquisphaera	-1.23	GR 1
	Actinobacteriota	Acidothermus	-1.26	GR 1
	Actinobacteriota	Mycobacterium	-0.59	GR 1
	Proteobacteria	Hyphomicrobium	0.91	JC 2
	Halobacterota	Methanosarcina	4.51	JC 2
	Planctomycetota	Pir4 lineage	0.81	JC 2
	Proteobacteria	Dokdonella	-1.18	GR 1
	Chloroflexi	UTCFX1	4.94	JC 2
	Firmicutes	Sporosarcina	-5.48	GR 1
	Proteobacteria	Ellin6067	0.59	JC 2
	Proteobacteria	Roseiarcus	-1.16	GR 1
	Gemmatimonadota	Gemmatimonas	-0.76	GR 1
	Verrucomicrobiota	Candidatus Udaeobacter	-0.61	GR 1
	Actinobacteriota	Jatrophihabitans	-1.04	GR 1
	Actinobacteriota	Microlunatus	1.89	JC 2
	Planctomycetota	Singulisphaera	-1.00	GR 1
	Planctomycetota	Pirellula	0.90	JC 2
	Proteobacteria	Dongia	1.01	JC 2

Table S2: Deseq2 analysis of 16S JC2 vs. MP4.

JC2 vs MP4	Phylum	Genus	log2FoldChange	Enriched
	Planctomycetota	Schlesneria	0.90	JC2
	Acidobacteriota	Candidatus Koribacter	1.59	JC2
	Proteobacteria	mle1-7	-1.26	MP4
	Desulfobacterota	Pseudopelobacter	-5.77	MP4
	Acidobacteriota	RB41	-0.95	MP4
	Chloroflexi	Litorilinea	-3.90	MP4
	Actinobacteriota	Mycobacterium	0.58	JC2
	Proteobacteria	Hyphomicrobium	-0.88	MP4
	Proteobacteria	Pseudolabrys	-0.71	MP4
	Proteobacteria	Dyella	7.03	JC2
	Halobacterota	Methanosarcina	-4.49	MP4
	Proteobacteria	GOUTA6	-2.04	MP4
	Actinobacteriota	Virgisporangium	-5.96	MP4
	Bacteroidota	Puia	1.08	JC2
	Proteobacteria	Defluviicoccus	-2.02	MP4
	Bacteroidota	Mucilaginibacter	1.35	JC2
	Proteobacteria	Cellvibrio	2.29	JC2
	Proteobacteria	Roseiarcus	1.35	JC2
	Bacteroidota	Edaphobaculum	1.12	JC2

Table S3: Deseq2 analysis of 16S. MP1 vs. GR1.

MP1 vs GR1	Phylum	Genus	log2FoldChange	Enriched
	Acidobacteriota	Luteitalea	2.49	MP1
	Planctomycetota	SH-PL14	2.28	MP1
	Acidobacteriota	Candidatus Koribacter	-1.31	GR1
	Proteobacteria	IS-44	4.66	MP1
	Proteobacteria	Amaricoccus	4.65	MP1
	Proteobacteria	mle1-7	2.93	MP1
	Actinobacteriota	Conexibacter	-1.42	GR1
	Verrucomicrobiota	Chthoniobacter	0.80	MP1
	Proteobacteria	Candidatus Alysiosphaera	1.49	MP1
	Acidobacteriota	RB41	1.26	MP1
	Proteobacteria	Nordella	3.13	MP1
	Proteobacteria	MND1	1.51	MP1
	Proteobacteria	Rhodomicrobium	-1.42	GR1
	Planctomycetota	Aquisphaera	-2.44	GR1
	Actinobacteriota	Acidothermus	-1.91	GR1
	Actinobacteriota	Rhodococcus	1.85	MP1
	Acidobacteriota	Vicinamibacter	3.64	MP1
	Acidobacteriota	Candidatus Solibacter	-0.83	GR1
	Crenarchaeota	Candidatus Nitrocosmicus	1.52	MP1
	Acidobacteriota	Acidipila	-2.36	GR1
	Planctomycetota	Pirellula	1.31	MP1
	Proteobacteria	Hyphomicrobium	1.19	MP1
	Halobacterota	Methanosarcina	5.82	MP1
	Proteobacteria	Steroidobacter	1.44	MP1
	Actinobacteriota	Rubrobacter	5.54	MP1

	Planctomycetota	Pir4 lineage	1.29	MP1
	Actinobacteriota	Ilumatobacter	1.86	MP1
	Proteobacteria	Bauldia	0.99	MP1
	Proteobacteria	Pedomicrobium	1.92	MP1
	Chloroflexi	UTCFX1	4.99	MP1
	Myxococcota	Anaeromyxobacter	-1.39	GR1
	Proteobacteria	Microvirga	2.27	MP1
	Proteobacteria	Roseiarcus	-2.14	GR1
	Verrucomicrobiota	Candidatus Udaeobacter	-0.72	GR1
	Actinobacteriota	Jatrophihabitans	-1.03	GR1
	Actinobacteriota	Microlunatus	3.41	MP1
	Bacteroidota	Terrimonas	1.49	MP1

Table S4: Deseq2 analysis of 16S MP1 vs. MP4.

MP1 vs MP4	Phylum	Genus	log2FoldChange	Enriched
	Acidobacteriota	Luteitalea	-2.43	MP4
	Proteobacteria	mle1-7	-1.60	MP4
	Actinobacteriota	Conexibacter	0.94	MP1
	Acidobacteriota	RB41	-1.30	MP4
	Proteobacteria	Rhodomicrobium	1.35	MP1
	Planctomycetota	Aquisphaera	1.36	MP1
	Crenarchaeota	Candidatus Nitrocosmicus	-1.41	MP4
	Planctomycetota	Pirellula	-0.77	MP4
	Proteobacteria	Hyphomicrobium	-1.11	MP4
	Halobacterota	Methanosarcina	-5.77	MP4
	Acidobacteriota	Candidatus Koribacter	1.69	MP1
	Proteobacteria	GOUTA6	-1.78	MP4

	Proteobacteria	Steroidobacter	-0.96	MP4
	Actinobacteriota	Rubrobacter	-4.77	MP4
	Planctomycetota	Pir4 lineage	-0.64	MP4
	Proteobacteria	Bauldia	-1.02	MP4
	Actinobacteriota	Virgisporangium	-6.53	MP4
	Proteobacteria	Pedomicrobium	-1.44	MP4
	Chloroflexi	UTCFX1	-2.60	MP4
	Proteobacteria	Defluviicoccus	-0.90	MP4
	Proteobacteria	Roseiarcus	2.37	MP1
	Proteobacteria	Bradyrhizobium	0.73	MP1
	Verrucomicrobiota	Candidatus Udaeobacter	0.49	MP1
	Actinobacteriota	Gaiella	-0.37	MP4
	Verrucomicrobiota	Opitutus	0.51	MP1
	Actinobacteriota	IMCC26207	-4.84	MP4

Table S5: Deseq2 analysis of 16S MP4 vs. GR1.

MP4 vs GR1	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Kribbella	-1.16	GR1
	Proteobacteria	IS-44	5.60	MP4
	Planctomycetota	Aquisphaera	-1.24	GR1

Table S6: Deseq2 analysis of 16S MP4 vs. JC2.

MP4 vs JC2	Phylum	Genus	log2FoldChange	Enriched
	Planctomycetota	Schlesneria	0.90	MP4
	Acidobacteriota	Candidatus Koribacter	1.59	MP4
	Proteobacteria	mle1-7	-1.26	JC2

	Desulfobacterota	Pseudopelobacter	-5.77	JC2
	Acidobacteriota	RB41	-0.95	JC2
	Chloroflexi	Litorilinea	-3.90	JC2
	Actinobacteriota	Mycobacterium	0.58	MP4
	Proteobacteria	Hyphomicrobium	-0.88	JC2
	Proteobacteria	Pseudolabrys	-0.71	JC2
	Proteobacteria	Dyella	7.03	MP4
	Halobacterota	Methanosarcina	-4.49	JC2
	Proteobacteria	GOUTA6	-2.04	JC2
	Actinobacteriota	Virgisporangium	-5.96	JC2
	Bacteroidota	Puia	1.08	MP4
	Proteobacteria	Defluviicoccus	-2.02	JC2
	Bacteroidota	Mucilaginibacter	1.35	MP4
	Proteobacteria	Cellvibrio	2.29	MP4
	Proteobacteria	Roseiarcus	1.35	MP4
	Bacteroidota	Edaphobaculum	1.12	MP4

Table S7: Deseq2 analysis of 16S GR1 Control vs. GR1 Chemical.

GR1 Control vs GR1 Chemical	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Ellin517	-1.76	GR1 Chemical
	Proteobacteria	Pseudorhodoplanes	-7.94	GR1 Chemical
	Bacteroidota	Chryseolinea	-8.24	GR1 Chemical
	Proteobacteria	Candidatus Accumulibacter	20.90	GR1 Control

Table S8: Deseq2 analysis of 16S GR1 Control vs. GR1 Lime

GR1 Control vs GR1 Lime	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Streptacidiphilus	-8.32	GR1 Lime

Table S9: Deseq2 analysis of 16S GR1 Organic vs. GR1 Chemical.

GR1 Organic vs GR1 Chemical	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Actinospica	20.62	GR1 Organic
	Desulfobacterota	Syntrophobacter	21.59	GR1 Organic
	Bacteroidota	WCHB1-32	21.64	GR1 Organic
	Acidobacteriota	Holophaga	19.45	GR1 Organic
	Halobacterota	Methanoregula	20.68	GR1 Organic

Table S10: Deseq2 analysis of 16S GR1 Organic vs. GR1 Control.

GR1 Organic vs GR1 Control	Phylum	Genus	log2FoldChange	Enriched
	Bacteroidota	Chryseolinea	9.35	GR1 Organic
	Proteobacteria	Candidatus Accumulibacter	-22.04	GR1 Control
	Halobacterota	Methanoregula	20.80	GR1 Organic
	Sumerlaeota	Sumerlaea	7.53	GR1 Organic

Table S11: Deseq2 analysis of 16S JC2 Control vs JC2 Chemical.

JC2 Control vs JC2 Chemical	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	MND1	-1.31	JC2 Chemical
	Acidobacteriota	Vicinamibacter	-8.46	JC2 Chemical
	Verrucomicrobiota	Chthoniobacter	-1.22	JC2 Chemical
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	6.17	JC2 Control
	Proteobacteria	Candidatus Nitrotoga	8.80	JC2 Control
	Actinobacteriota	Virgisporangium	-7.68	JC2 Chemical
	Proteobacteria	Leptothrix	7.09	JC2 Control
	Crenarchaeota	Candidatus Nitrosotalea	5.18	JC2 Control
	Bacteroidota	Ohtaekwangia	-5.18	JC2 Chemical
	Bacteroidota	Pseudoflavitalea	-22.63	JC2 Chemical
	Proteobacteria	Alkanindiges	-22.16	JC2 Chemical
	Proteobacteria	Azotobacter	-22.21	JC2 Chemical

Table S12: Deseq2 analysis of 16S JC2 Control vs JC2 Lime.

JC2 Control vs JC2 Lime	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Acidothermus	-1.51	JC2 Lime
	Proteobacteria	MND1	1.26	JC2 Control
	Verrucomicrobiota	Ellin516	-1.48	JC2 Lime
	Acidobacteriota	Vicinamibacter	8.41	JC2 Control
	Verrucomicrobiota	Chthoniobacter	1.30	JC2 Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-10.77	JC2 Lime
	Actinobacteriota	Virgisporangium	7.76	JC2 Control

	Proteobacteria	BD1-7 clade	7.45	JC2 Control
	Acidobacteriota	Granulicella	-7.12	JC2 Lime
	Proteobacteria	Leptothrix	-7.10	JC2 Lime
	Armatimonadota	Chthonomonas	-7.92	JC2 Lime

Table S13: Deseq2 analysis of 16S JC2 Control vs JC2 Chemical.

JC2 Control vs JC2 Chemical	Phylum	Genus	log2FoldChange	Enriched
	Bacteroidota	Pseudoflavitalea	-21.85	JC2 Chemical
	Proteobacteria	Alkanindiges	-21.67	JC2 Chemical
	Proteobacteria	Azotobacter	-21.42	JC2 Chemical

Table S14: Deseq2 analysis of 16S JC2 Organic vs JC2 Control.

JC2 Organic vs JC2 Control	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Actinobacteriota	Streptomyces	1.78	JC2 Organic
	Acidobacteriota	Vicinamibacter	9.40	JC2 Organic
	Acidobacteriota	Bryobacter	-1.34	JC2 Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-10.32	JC2 Control
	Bacteroidota	Ohtaekwangia	5.40	JC2 Organic
	Proteobacteria	Candidatus Nitrotoga	-8.38	JC2 Control
	Actinobacteriota	Kibdelosporangium	22.51	JC2 Organic
	Crenarchaeota	Candidatus Nitrosotalea	-8.71	JC2 Control
	Bacteroidota	Flavisolibacter	-7.53	JC2 Control
	Armatimonadota	Chthonomonas	-7.48	JC2 Control
	Myxococcota	Minicystis	-7.51	JC2 Control

Table S15: Deseq2 analysis of 16S JC2 Organic vs JC2 Control.

MP1 Chemical vs MP1 Control	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrocosmicus	-1.09	MP1 Control
	Actinobacteriota	Acidothermus	1.48	MP1 Chemical
	Bacteroidota	OLB12	1.71	MP1 Chemical
	Verrucomicrobiota	Lacunisphaera	1.68	MP1 Chemical
	Planctomycetota	Pirellula	-1.42	MP1 Control
	Verrucomicrobiota	Ellin517	-1.33	MP1 Control
	Bacteroidota	Chryseolinea	-2.67	MP1 Control
	Bacteroidota	Terrimonas	-1.49	MP1 Control
	Acidobacteriota	Occallatibacter	2.52	MP1 Chemical
	Bacteroidota	Mucilaginibacter	3.74	MP1 Chemical
	Spirochaetota	Spirochaeta 2	1.84	MP1 Chemical
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	5.86	MP1 Chemical
	Proteobacteria	Candidatus Accumulibacter	-20.29	MP1 Control
	Bacteroidota	Ohtaekwangia	-4.68	MP1 Control
	Proteobacteria	Polycyclovorans	-8.90	MP1 Control
	Bacteroidota	Cytophaga	2.85	MP1 Chemical
	Acidobacteriota	Stenotrophobacter	-6.97	MP1 Control
	Acidobacteriota	Granulicella	4.70	MP1 Chemical

	Acidobacteriota	Paludibaculum	-7.21	MP1 Control
	Proteobacteria	Luteibacter	22.97	MP1 Chemical
	Proteobacteria	Rhodopseudomonas	24.13	MP1 Chemical
	Proteobacteria	Asticcacaulis	5.32	MP1 Chemical
	Proteobacteria	Amaricoccus	-7.12	MP1 Control
	Proteobacteria	Stenotrophomonas	24.27	MP1 Chemical
	Planctomycetota	Planctopirus	-6.74	MP1 Control
	Chloroflexi	Thermosporothrix	23.21	MP1 Chemical

Table S16: Deseq2 analysis of 16S MP1 Lime vs MP1 Control.

MP1 Lime vs MP1 Control	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Acidothermus	-1.72	MP1 Control
	Bacteroidota	OLB12	-1.54	MP1 Control
	Planctomycetota	Pirellula	0.96	MP1 Lime
	Verrucomicrobiota	Ellin516	-1.86	MP1 Control
	Acidobacteriota	Bryobacter	-1.96	MP1 Control
	Acidobacteriota	Occallatibacter	-2.77	MP1 Control
	Bacteroidota	Ohtaekwangia	4.93	MP1 Lime
	Proteobacteria	Polycyclovorans	8.42	MP1 Lime

	Acidobacteriota	Paludibaculum	7.17	MP1 Lime
	Acidobacteriota	Granulicella	-8.84	MP1 Control
	Actinobacteriota	Lechevalieria	21.67	MP1 Lime
	Proteobacteria	Undibacterium	20.96	MP1 Lime
	Chloroflexi	Thermosporothrix	-21.34	MP1 Control
	Bdellovibrionota	Oligoflexus	7.23	MP1 Lime

Table S17: Deseq2 analysis of 16SMP1 Organic vs MP1 Chemical.

MP1 Organic vs MP1 Chemical	Phylum	Genus	log2FoldChange	Enriched
	Acidobacteriota	Geothrix	-20.11	MP1 Chemical

Table S18: Deseq2 analysis of 16S MP1 Organic vs MP1 Control.

MP1 Organic vs MP1 Control	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrocosmicus	1.24	MP1 Organic
	Actinobacteriota	Acidothermus	-1.83	MP1 Control
	Verrucomicrobiota	Lacunisphaera	-1.37	MP1 Control
	Bacteroidota	OLB12	-2.08	MP1 Control
	Planctomycetota	Pirellula	1.07	MP1 Organic
	Bacteroidota	Terrimonas	1.75	MP1 Organic
	Bacteroidota	Mucilaginibacter	-2.33	MP1 Control
	Verrucomicrobiota	Ellin516	-2.14	MP1 Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-8.23	MP1 Control
	Bacteroidota	Ohtaekwangia	5.49	MP1 Organic
	Bacteroidota	Chryseolinea	3.12	MP1 Organic
	Bacteroidota	Edaphobaculum	-1.33	MP1 Control
	Actinobacteriota	Catenulispora	-8.12	MP1 Control
	Acidobacteriota	Paludibaculum	7.05	MP1 Organic
	Acidobacteriota	Granulicella	-9.43	MP1 Control
	Acidobacteriota	Edaphobacter	-7.45	MP1 Control
	Proteobacteria	Luteibacter	-21.93	MP1 Control
	Planctomycetota	Phycisphaera	-4.51	MP1 Control
	Proteobacteria	Stenotrophomonas	-23.20	MP1 Control
	Planctomycetota	Planctopirus	6.94	MP1 Organic
	Chloroflexi	Thermosporothrix	-22.12	MP1 Control

Table S19: Deseq2 analysis of 16S MP1 vs JC2.

MP1 vs JC2	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	Pseudolabrys	-0.63	JC2
	Actinobacteriota	Mycobacterium	0.46	MP1
	Nitrospirota	Nitrospira	1.01	MP1
	Proteobacteria	Rhodomicrobium	-0.98	JC2
	Actinobacteriota	Nocardioides	0.87	MP1
	Proteobacteria	Pedomicrobium	1.29	MP1
	Planctomycetota	Aquisphaera	-1.29	JC2
	Acidobacteriota	Luteitalea	0.80	MP1
	Actinobacteriota	Microlunatus	1.48	MP1
	Proteobacteria	Defluviicoccus	-1.17	JC2
	Verrucomicrobiota	ADurb.Bin063-1	-0.74	JC2
	Myxococcota	Anaeromyxobacter	-1.21	JC2
	Actinobacteriota	Rhodococcus	2.63	MP1
	Actinobacteriota	Blastococcus	1.27	MP1
	Proteobacteria	Cellvibrio	2.55	MP1
	Actinobacteriota	Rubrobacter	5.51	MP1

Table S20: Deseq2 analysis of 16S MP4 Control vs MP4 Chemical.

MP4 Control vs MP4 Chemical	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Proteobacteria	Acidibacter	-1.37	MP4 Chemical
	Verrucomicrobiota	Ellin516	1.13	MP4 Control
	Proteobacteria	Pseudorhodoplanes	-7.89	MP4 Chemical
	Proteobacteria	Arenimonas	-6.98	MP4 Chemical

	Bacteroidota	Ohtaekwangia	-8.65	MP4 Chemical
	Bacteroidota	Chryseolinea	-4.94	MP4 Chemical
	Proteobacteria	Dongia	-1.62	MP4 Chemical
	Bacteroidota	Terrimonas	-1.67	MP4 Chemical
	Acidobacteriota	Luteitalea	-7.99	MP4 Chemical
	Verrucomicrobiota	ADurb.Bin063-1	1.45	MP4 Control
	Proteobacteria	Acidisoma	6.83	MP4 Control

Table S21: Deseq2 analysis of 16S MP4 Control vs MP4 Lime.

MP4 Control vs MP4 Lime	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Acidothermus	-2.07	MP4 Lime
	Proteobacteria	Acidibacter	1.44	MP4 Control
	Verrucomicrobiota	Ellin516	-1.51	MP4 Lime
	Proteobacteria	Pseudorhodoplanes	8.38	MP4 Control
	Proteobacteria	IS-44	3.83	MP4 Control
	Bacteroidota	Chryseolinea	4.89	MP4 Control
	Proteobacteria	Polycyclovorans	7.12	MP4 Control
	Bacteroidota	Terrimonas	2.41	MP4 Control
	Acidobacteriota	Luteitalea	8.17	MP4 Control
	Verrucomicrobiota	ADurb.Bin063-1	-1.71	MP4 Lime
	Actinobacteriota	Streptacidiphilus	-7.33	MP4 Lime
	Bacteroidota	Ohtaekwangia	7.34	MP4 Control
	Proteobacteria	Dyella	-7.62	MP4 Lime
	Acidobacteriota	Terracidiphilus	-6.85	MP4 Lime

	Myxococcota	Sandaracinus	7.00	MP4 Control
	Proteobacteria	Acidisoma	-6.83	MP4 Lime

Table S22: Deseq2 analysis of 16S MP4 Organic vs MP4 Chemical.

MP4 Organic vs MP4 Chemical	Phylum	Genus	log2FoldChange	Enriched
	Actinobacteriota	Acidothermus	-1.19	MP4 Chemical
	Actinobacteriota	Nocardioides	1.16	MP4 Organic
	Acidobacteriota	Candidatus Solibacter	-1.16	MP4 Chemical
	Verrucomicrobiota	Ellin516	-1.85	MP4 Chemical
	Verrucomicrobiota	ADurb.Bin063-1	-1.27	MP4 Chemical
	Bacteroidota	Puia	-2.10	MP4 Chemical
	Proteobacteria	Dokdonella	-1.88	MP4 Chemical
	Acidobacteriota	Bryobacter	-1.56	MP4 Chemical
	Bacteroidota	Mucilaginibacter	-1.94	MP4 Chemical
	Proteobacteria	Devosia	-1.43	MP4 Chemical
	Bacteroidota	Ohtaekwangia	2.04	MP4 Organic
	Proteobacteria	Microvirga	6.91	MP4 Organic
	Acidobacteriota	Paludibaculum	7.18	MP4 Organic
	Actinobacteriota	Streptacidiphilus	-7.05	MP4 Chemical
	Chloroflexi	Thermosporothrix	-7.62	MP4 Chemical
	Actinobacteriota	Demequina	7.08	MP4 Organic
	Proteobacteria	Altererythrobacter	7.29	MP4 Organic
	Acidobacteriota	Granulicella	-7.10	MP4 Chemical

	Bacteroidota	Spirosoma	-21.51	MP4 Chemical

Table S23: Deseq2 analysis of 16S MP4 Organic vs MP4 Control

MP4 Organic vs MP4 Control	Phylum	Genus	log2FoldChange	Enriched
	Planctomycetota	Pir4 lineage	1.04	MP4 Organic
	Desulfobacterota	Geobacter	-1.93	MP4 Control
	Actinobacteriota	Acidothermus	-2.27	MP4 Control
	Proteobacteria	Acidibacter	1.91	MP4 Organic
	Actinobacteriota	Nocardioides	1.65	MP4 Organic
	Proteobacteria	MND1	1.59	MP4 Organic
	Planctomycetota	Aquisphaera	-1.68	MP4 Control
	Acidobacteriota	Candidatus Solibacter	-1.97	MP4 Control
	Planctomycetota	Pirellula	1.01	MP4 Organic
	Acidobacteriota	Luteitalea	7.93	MP4 Organic
	Verrucomicrobiota	Ellin516	-2.85	MP4 Control
	Proteobacteria	Pseudorhodoplanes	9.18	MP4 Organic
	Proteobacteria	Dongia	1.77	MP4 Organic
	Bacteroidota	Puia	-1.94	MP4 Control
	Bacteroidota	Chryseolinea	6.08	MP4 Organic
	Acidobacteriota	Candidatus Koribacter	-1.89	MP4 Control
	Acidobacteriota	Vicinamibacter	8.31	MP4 Organic
	Verrucomicrobiota	Chthoniobacter	1.45	MP4 Organic
	Acidobacteriota	Bryobacter	-2.20	MP4 Control
	Acidobacteriota	Occallatibacter	-4.73	MP4 Control
	Proteobacteria	Arenimonas	6.79	MP4 Organic
	Actinobacteriota	Ilumatobacter	8.29	MP4 Organic
	Acidobacteriota	Subgroup 10	1.93	MP4 Organic
	Proteobacteria	Nordella	6.91	MP4 Organic
	Planctomycetota	SH-PL14	7.74	MP4 Organic
	Bacteroidota	Ohtaekwangia	10.83	MP4 Organic
	Actinobacteriota	Nakamurella	2.06	MP4 Organic
	Actinobacteriota	Marmoricola	7.14	MP4 Organic

	Bacteroidota	Terrimonas	1.84	MP4 Organic
	Proteobacteria	Polycyclovorans	7.92	MP4 Organic
	Proteobacteria	Microvirga	7.08	MP4 Organic
	Verrucomicrobiota	ADurb.Bin063-1	-2.60	MP4 Control
	Desulfobacterota	Desulfobulbus	-6.65	MP4 Control
	Proteobacteria	BD1-7 clade	7.38	MP4 Organic
	Acidobacteriota	Paludibaculum	7.42	MP4 Organic
	Actinobacteriota	Streptacidiphilus	-7.27	MP4 Control
	Firmicutes	Fonticella	-6.52	MP4 Control
	Acidobacteriota	Granulicella	-7.45	MP4 Control
	Acidobacteriota	Edaphobacter	-6.81	MP4 Control
	Proteobacteria	Hirschia	6.80	MP4 Organic
	Armatimonadota	Chthonomonas	-6.82	MP4 Control
	Chloroflexi	Thermosporothrix	-7.89	MP4 Control
	Proteobacteria	Dyella	-7.53	MP4 Control
	Actinobacteriota	Lechevalieria	21.31	MP4 Organic
	Actinobacteriota	Actinospica	-8.42	MP4 Control
	Bacteroidota	Crocinitomix	6.80	MP4 Organic
	Acidobacteriota	Terracidiphilus	-6.78	MP4 Control
	Proteobacteria	Acidisoma	-6.79	MP4 Control
	Proteobacteria	Micropepsis	-7.79	MP4 Control
	Actinobacteriota	Demequina	7.29	MP4 Organic
	Spirochaetota	Turneriella	6.83	MP4 Organic
	Proteobacteria	Altererythrobacter	7.48	MP4 Organic
	Chloroflexi	HSB OF53-F07	-6.97	MP4 Control

Table S24: Deseq2 analysis of ITS GR1 vs JC2.

GR1 vs JC2	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Udeniozyma	-4.98	JC2
	Basidiomycota	Coprinopsis	2.60	GR1
	Ascomycota	Pyrenochaeta	5.09	GR1
	Ascomycota	Cadophora	5.30	GR1
	Ascomycota	Drechslera	-7.33	JC2
	Ascomycota	unidentified_191	-5.85	JC2
	Basidiomycota	unidentified_420	-24.60	JC2
	Ascomycota	Mollisia	-7.31	JC2
	Glomeromycota	Paraglomus	-4.98	JC2
	Ascomycota	Clohesyomyces	21.60	GR1
	Basidiomycota	unidentified_242	6.99	GR1
	Basidiomycota	Solicoccozyma	-1.92	JC2
	Ascomycota	unidentified_9815	8.06	GR1
	Basidiomycota	Apiotrichum	-2.78	JC2
	Glomeromycota	unidentified_5039	7.07	GR1
	Chytridiomycota	unidentified_957	2.30	GR1

Table S25: Deseq2 analysis of ITS GR1 vs MP1.

GR1 vs MP1	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Udeniozyma	-5.83	MP1
	Ascomycota	Pyrenochaeta	4.38	GR1
	Ascomycota	unidentified_388	-6.30	MP1
	Ascomycota	Leohumicola	-6.22	MP1
	Ascomycota	unidentified_3145	-6.70	MP1
	Glomeromycota	unidentified_276	-9.45	MP1
	Ascomycota	Drechslera	-7.63	MP1

	Ascomycota	unidentified_191	-7.38	MP1
	Basidiomycota	Auricularia	-7.35	MP1
	Ascomycota	Pseudaleuria	3.59	GR1
	Ascomycota	Mollisia	-7.95	MP1
	Basidiomycota	unidentified_199	6.57	GR1
	Ascomycota	Hymenoscyphus	7.08	GR1
	Basidiomycota	unidentified_242	7.86	GR1
	Ascomycota	Chaetomium	-7.59	MP1
	Ascomycota	unidentified_9815	7.61	GR1
	Ascomycota	unidentified_50	-2.48	MP1
	Ascomycota	Trichoderma	-5.24	MP1
	Basidiomycota	Minimedusa	8.03	GR1
	Basidiomycota	Apiotrichum	-3.42	MP1
	Glomeromycota	unidentified_5039	8.09	GR1
	Chytridiomycota	unidentified_957	2.12	GR1

Table S26: Deseq2 analysis of ITS GR1 vs MP4.

GR1 vs MP4	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	unidentified	2.36	GR1
	Ascomycota	Pyrenochaeta	5.34	GR1
	Ascomycota	unidentified_191	-4.72	MP4
	Basidiomycota	Auricularia	-7.37	MP4
	Ascomycota	Cladorrhinum	-24.37	MP4
	Ascomycota	Hymenoscyphus	23.58	GR1
	Basidiomycota	unidentified_242	6.72	GR1
	Ascomycota	Bimuria	2.89	GR1
	Basidiomycota	Apiotrichum	-2.16	MP4
	Glomeromycota	unidentified_5039	7.62	GR1
	Ascomycota	Cadophora	7.49	GR1

Table S27: Deseq2 analysis of ITS JC2 vs MP1.

JC2 vs MP1	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Auricularia	-7.78	MP1
	Basidiomycota	Akenomyces	24.96	JC2
	Ascomycota	unidentified_388	-7.12	MP1
	Ascomycota	unidentified_3145	-5.62	MP1
	Ascomycota	Pseudaleuria	5.54	JC2
	Basidiomycota	unidentified_23	3.82	JC2
	Basidiomycota	Solicoccozyma	2.17	JC2
	Ascomycota	unidentified_50	-2.16	MP1
	Ascomycota	Chaetomium	-6.80	MP1

	Glomeromycota	Glomus	-1.75	MP1
	Ascomycota	Pseudeurotium	-2.63	MP1
	Ascomycota	Scedosporium	-5.78	MP1

Table S28: Deseq2 analysis of ITS JC2 vs MP4.

JC2 vs MP4	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Udeniozyma	6.15	JC2
	Basidiomycota	Auricularia	-8.39	MP4
	Basidiomycota	Coprinopsis	-1.68	MP4
	Ascomycota	Leohumicola	4.72	JC2
	Mortierellomycota	unidentified_983	-6.89	MP4
	Basidiomycota	unidentified_420	22.39	JC2
	Ascomycota	unidentified	1.47	JC2
	Ascomycota	Clohesyomyces	-23.11	MP4
	Basidiomycota	Solicoccozyma	1.33	JC2
	Ascomycota	unidentified_9815	-8.47	MP4
	Basidiomycota	unidentified_1560	24.73	JC2
	Ascomycota	Pseudeurotium	-1.14	MP4

Table S29: Deseq2 analysis of ITS MP1 vs MP4.

MP1 vs MP4	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Udeniozyma	7.64	MP1
	Basidiomycota	unidentified_1560	7.72	MP1
	Glomeromycota	unidentified_276	7.81	MP1
	Ascomycota	Leohumicola	8.09	MP1
	Ascomycota	unidentified_3145	5.55	MP1
	Basidiomycota	unidentified_199	-6.62	MP4
	Glomeromycota	unidentified_277	8.47	MP1
	Ascomycota	unidentified_50	2.29	MP1
	Ascomycota	Chaetomium	6.56	MP1
	Ascomycota	Bimuria	6.62	MP1
	Ascomycota	unidentified_9815	-7.60	MP4
	Ascomycota	Trichoderma	4.79	MP1
	Ascomycota	Pseudeurotium	1.93	MP1

Table S30: Deseq2 analysis of ITS GR1 chemical vs GR1 control.

GR1 chemical vs GR1 control	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	unidentified_3513	22.95	GR1 chemical

	Basidiomycota	Pholiotina	-11.38	GR1 control
	Basidiomycota	Parasola	27.44	GR1 chemical
	Basidiomycota	Tylospora	-21.38	GR1 control
	Basidiomycota	unidentified_187	21.19	GR1 chemical
	Basidiomycota	unidentified_420	-22.86	GR1 control
	Mortierellomycota	unidentified_983	-22.37	GR1 control
	Basidiomycota	Auricularia	-8.14	GR1 control
	Glomeromycota	Funneliformis	20.97	GR1 chemica

Table S31: Deseq2 analysis of ITS GR 1 control vs GR 1 lime.

GR 1 control vs GR 1 lime	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Pholiotina	12.29	GR 1 control
	Basidiomycota	unidentified_420	27.35	GR 1 control
	Basidiomycota	Serendipita	8.69	GR 1 control
	Ascomycota	unidentified_88	23.13	GR 1 control
	Ascomycota	Mollisia	-9.06	GR 1 lime
	Glomeromycota	unidentified_277	-21.82	GR 1 lime
	Basidiomycota	unidentified_151	-22.33	GR 1 lime
	Ascomycota	Neofitzroyomyces	21.89	GR 1 control
	Ascomycota	Glarea	20.29	GR 1 control
	Basidiomycota	Psathyrella	21.08	GR 1 control

Table S32: Deseq2 analysis of ITS GR 1 control vs GR 1 organic.

GR1 control vs GR1 organic	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Pholiotina	9.81	GR1 control
	Ascomycota	Gibberella	21.17	GR1 control
	Basidiomycota	unidentified_12	21.64	GR1 control
	Basidiomycota	Auricularia	19.48	GR1 control
	Glomeromycota	unidentified_277	-22.17	GR1 organic
	Basidiomycota	unidentified_151	-22.42	GR1 organic
	Basidiomycota	unidentified_420	20.58	GR1 control
	Basidiomycota	Coprinellus	20.88	GR1 control
	Glomeromycota	unidentified_408	-21.71	GR1 control

Table S33: Deseq2 analysis of ITS JC2 chemical vs JC2 control.

JC2 chemical vs JC2 control	Phylum	Genus	log2FoldChange	Enriched
	Chytridiomycota	Operculomyces	-9.14	JC2 control
	Basidiomycota	Trechispora	-21.71	JC2 control
	Glomeromycota	unidentified_277	11.03	JC2 chemical
	Basidiobolomycota	Basidiobolus	23.55	JC2 chemical
	Basidiomycota	Clavaria	-23.94	JC2 control
	Ascomycota	Cladophialophora	-19.93	JC2 control

Table S34: Deseq2 analysis of ITS JC2 control vs JC2 lime.

JC2 control vs JC2 lime	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Pholiotina	23.98	JC2 control
	Chytridiomycota	Operculomyces	10.01	JC2 control
	Basidiomycota	unidentified_3513	11.29	JC2 control
	Basidiomycota	Trechispora	22.34	JC2 control
	Ascomycota	Clohesyomyces	22.10	JC2 control
	Chytridiomycota	Paranamyces	9.82	JC2 control
	Basidiomycota	unidentified_187	-25.02	JC2 lime
	Basidiobolomycota	Basidiobolus	-23.44	JC2 lime
	Basidiomycota	Corticium	-23.50	JC2 lime

Table S35: Deseq2 analysis of ITS JC2 control vs JC2 organic.

JC2 control vs JC2 organic	Phylum	Genus	log2FoldChange	Enriched
	Chytridiomycota	Operculomyces	13.16	JC2 control
	Basidiomycota	Tylospora	-22.03	JC2 organic
	Basidiomycota	Akenomyces	22.45	JC2 control
	Glomeromycota	unidentified_276	-11.50	JC2 organic
	Ascomycota	Clohesyomyces	23.83	JC2 control
	Chytridiomycota	Paranamyces	9.84	JC2 control
	Basidiomycota	unidentified_2191	21.29	JC2 control
	Basidiomycota	Pholiotina	10.38	JC2 control
	Basidiomycota	unidentified_187	-23.88	JC2 organic
	Glomeromycota	unidentified_4174	23.27	JC2 control
	Basidiobolomycota	Basidiobolus	-22.47	JC2 organic
	Basidiomycota	Corticium	-22.74	JC2 organic

Table S36: Deseq2 analysis of ITS MP1 chemical vs MP1 control.

MP1 chemical vs MP1 control	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	unidentified_5	8.14	MP1 chemical
	Basidiomycota	Parasola	11.76	MP1 chemical
	Basidiomycota	unidentified_420	20.72	MP1 chemical
	Ascomycota	Sarocladium	28.71	MP1 chemical
	Ascomycota	unidentified_88	25.16	MP1 chemical
	Basidiomycota	Atractiella	-23.87	MP1 control
	Ascomycota	Cistella	24.40	MP1 chemical
	Glomeromycota	unidentified_6994	-23.33	MP1 control

	Ascomycota	Torula	22.38	MP1 chemical

Table S37: Deseq2 analysis of ITS MP1 control vs MP1 lime.

MP1 control vs MP1 lime	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Coprinopsis	8.27	MP1 control
	Basidiomycota	Parasola	-11.76	MP1 lime
	Ascomycota	Sarocladium	-29.25	MP1 lime
	Basidiomycota	Akenomyces	11.65	MP1 control
	Basidiomycota	unidentified_2191	25.40	MP1 control
	Basidiomycota	unidentified_1560	23.51	MP1 control
	Ascomycota	Cistella	-25.19	MP1 lime
	Basidiomycota	Kurtzmanomyces	-24.67	MP1 lime
	Ascomycota	Alatospora	22.62	MP1 control
	Ascomycota	Torula	-23.22	MP1 lime

Table S38: Deseq2 analysis of ITS MP1 control vs MP1 organic.

MP1 control vs MP1 organic	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Parasola	-11.61	MP1 organic
	Basidiomycota	unidentified_420	-21.52	MP1 organic
	Basidiomycota	Akenomyces	24.30	MP1 control
	Ascomycota	unidentified_82	9.52	MP1 control
	Ascomycota	Cladorrhinum	11.03	MP1 control
	Basidiomycota	Marasmius	24.36	MP1 control
	Ascomycota	Cistella	-25.04	MP1 organic
	Ascomycota	Pyrenophora	20.66	MP1 control
	Ascomycota	Torula	-23.07	MP1 organic
	Ascomycota	Heteroconium	21.69	MP1 control

Table S39: Deseq2 analysis of ITS MP4 chemical vs MP4 control.

MP4 chemical vs MP4 control	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Pholiotina	-11.84	MP4 control
	Ascomycota	Hymenoscyphus	-24.44	MP4 control
	Basidiomycota	Minimedusa	-22.35	MP4 control
	Glomeromycota	Funneliformis	-10.62	MP4 control
	Basidiomycota	Akenomyces	-22.04	MP4 control
	Ascomycota	Mollisia	21.58	MP4 chemical
	Basidiomycota	Clavaria	-22.41	MP4 control
	Ascomycota	Trichoglossum	-22.33	MP4 control
	Ascomycota	Chalara	-21.13	MP4 control
	Calcarisporiellomycota	Calcarisporiella	21.65	MP4 chemical
	Basidiomycota	unidentified_420	-21.97	MP4 control
	Basidiomycota	Ceratobasidium	21.50	MP4 chemical

Table S40: Deseq2 analysis of ITS MP4 control vs MP4 lime.

MP4 control vs MP4 lime	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	unidentified_3513	-25.02	MP4 lime
	Basidiomycota	Pholiotina	11.51	MP4 control
	Basidiomycota	unidentified_420	26.06	MP4 control
	Glomeromycota	Funneliformis	10.45	MP4 control
	Ascomycota	Mollisia	-21.61	MP4 lime
	Calcarisporiellomycota	Calcarisporiella	-21.69	MP4 lime
	Basidiomycota	Ceratobasidium	-21.52	MP4 lime

Table S41: Deseq2 analysis of ITS MP4 control vs MP4 organic.

MP4 control vs MP4 organic	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Pholiotina	11.17	MP4 control
	Ascomycota	Hymenoscyphus	13.21	MP4 control
	Ascomycota	unidentified_5	-3.83	MP4 organic

	Ascomycota	Slopeiomyces	8.93	MP4 control
	Basidiomycota	Minimedusa	22.40	MP4 control
	Ascomycota	Tolypocladium	25.21	MP4 control
	Glomeromycota	Funneliformis	12.23	MP4 control
	Basidiomycota	Cotylidia	-10.03	MP4 organic
	Basidiomycota	Serendipita	9.11	MP4 control
	Ascomycota	Leohumicola	-6.20	MP4 organic
	Basidiomycota	Akenomyces	19.20	MP4 control
	Glomeromycota	unidentified_276	-4.00	MP4 organic
	Ascomycota	Paraphoma	8.87	MP4 control
	Glomeromycota	unidentified_5039	7.90	MP4 control
	Ascomycota	Clonostachys	7.34	MP4 control
	Ascomycota	Fusicolla	6.95	MP4 control
	Ascomycota	Mollisia	-23.02	MP4 organic
	Glomeromycota	unidentified_277	-10.46	MP4 organic
	Ascomycota	Fusarium	6.86	MP4 control
	Basidiomycota	unidentified_3899	22.12	MP4 control
	Calcarisporiellomycota	Calcarisporiella	-23.09	MP4 organic
	Basidiomycota	Ceratobasidium	-22.87	MP4 organic

Chapter 4 Supplementary materials

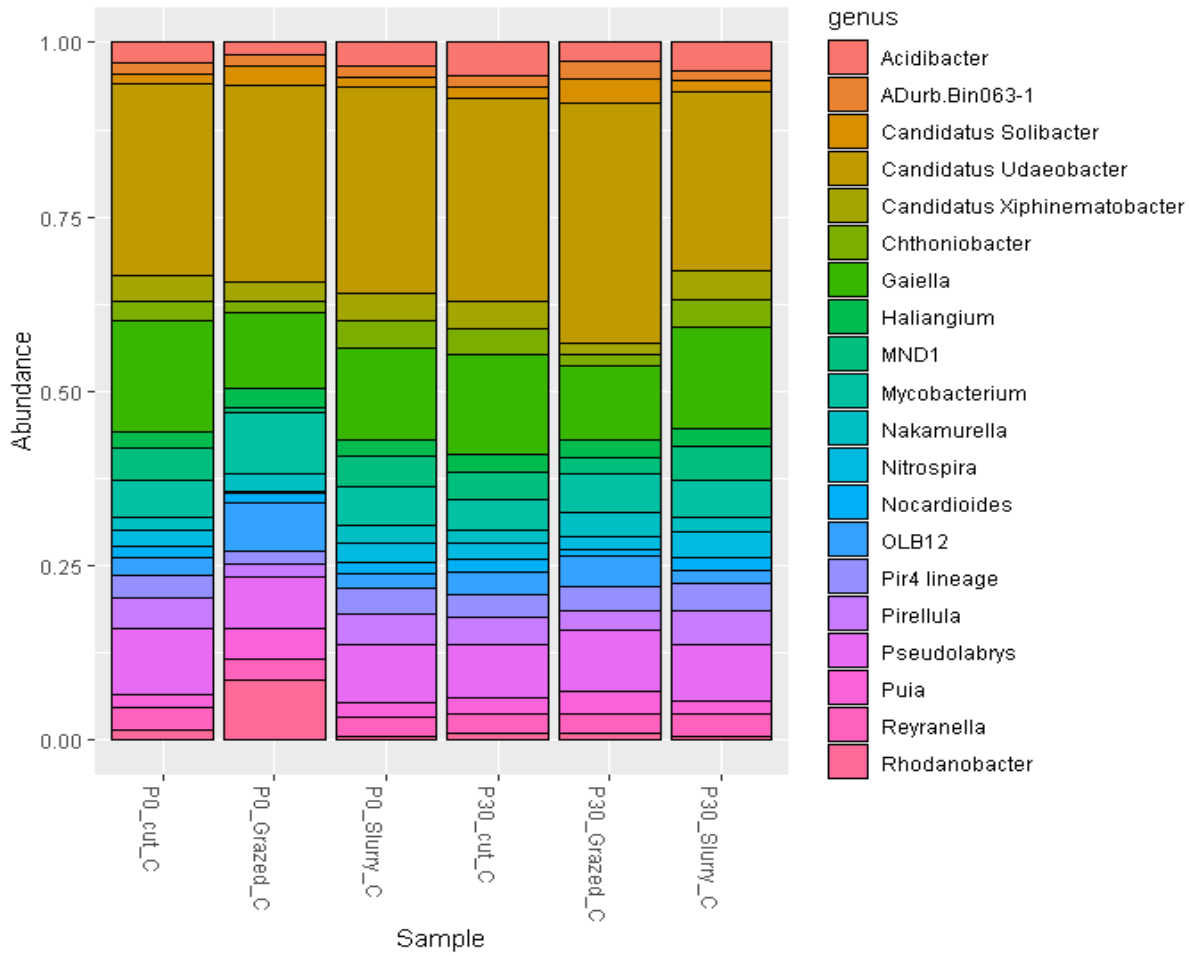

Figure S3: Realtive abundance of top 20 dominant genera of the 16S community in the glucose soils with treatment

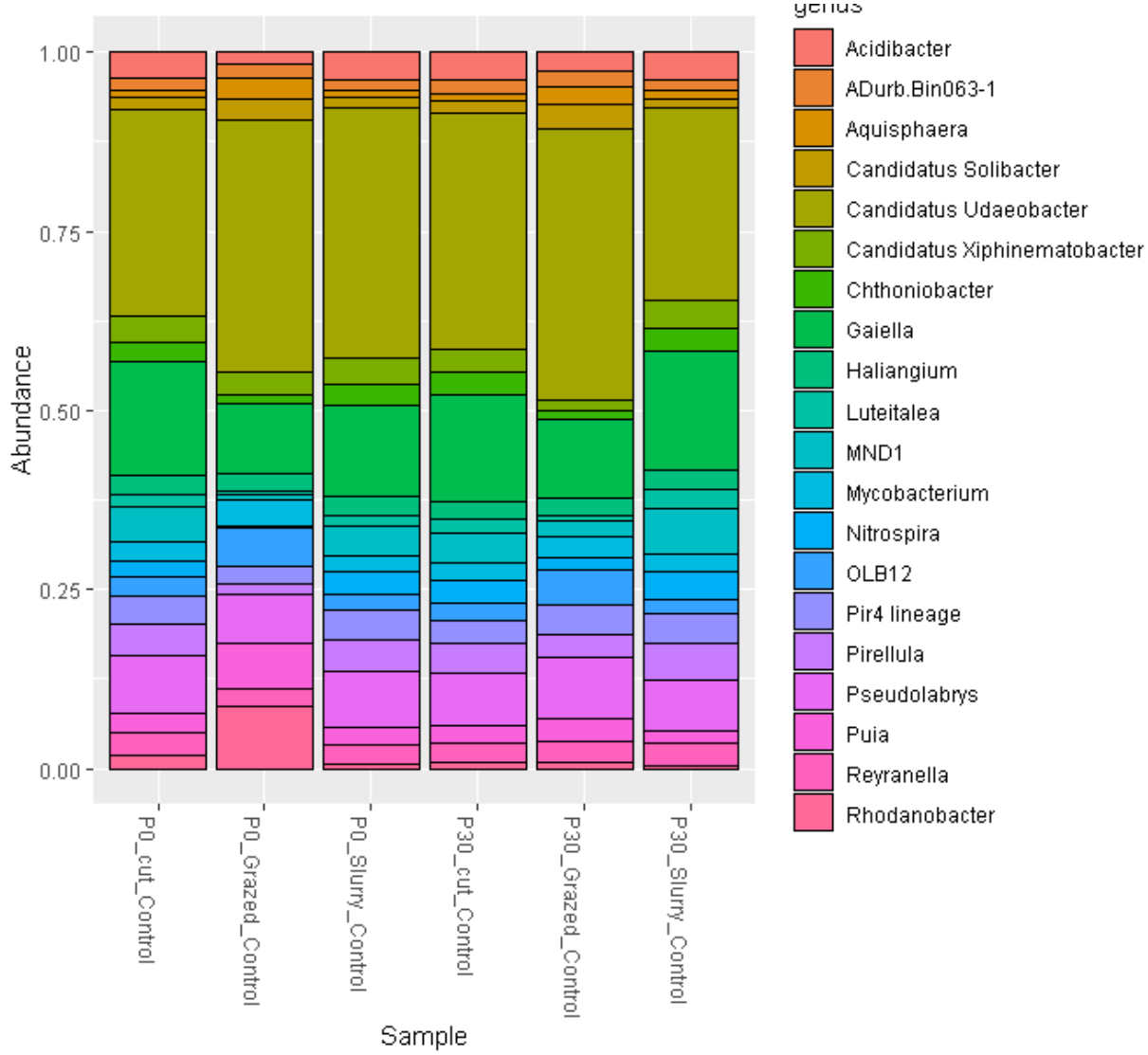

Figure S4: Realtive abundance of top 20 dominant genera of 16S community in the control soils with treatment

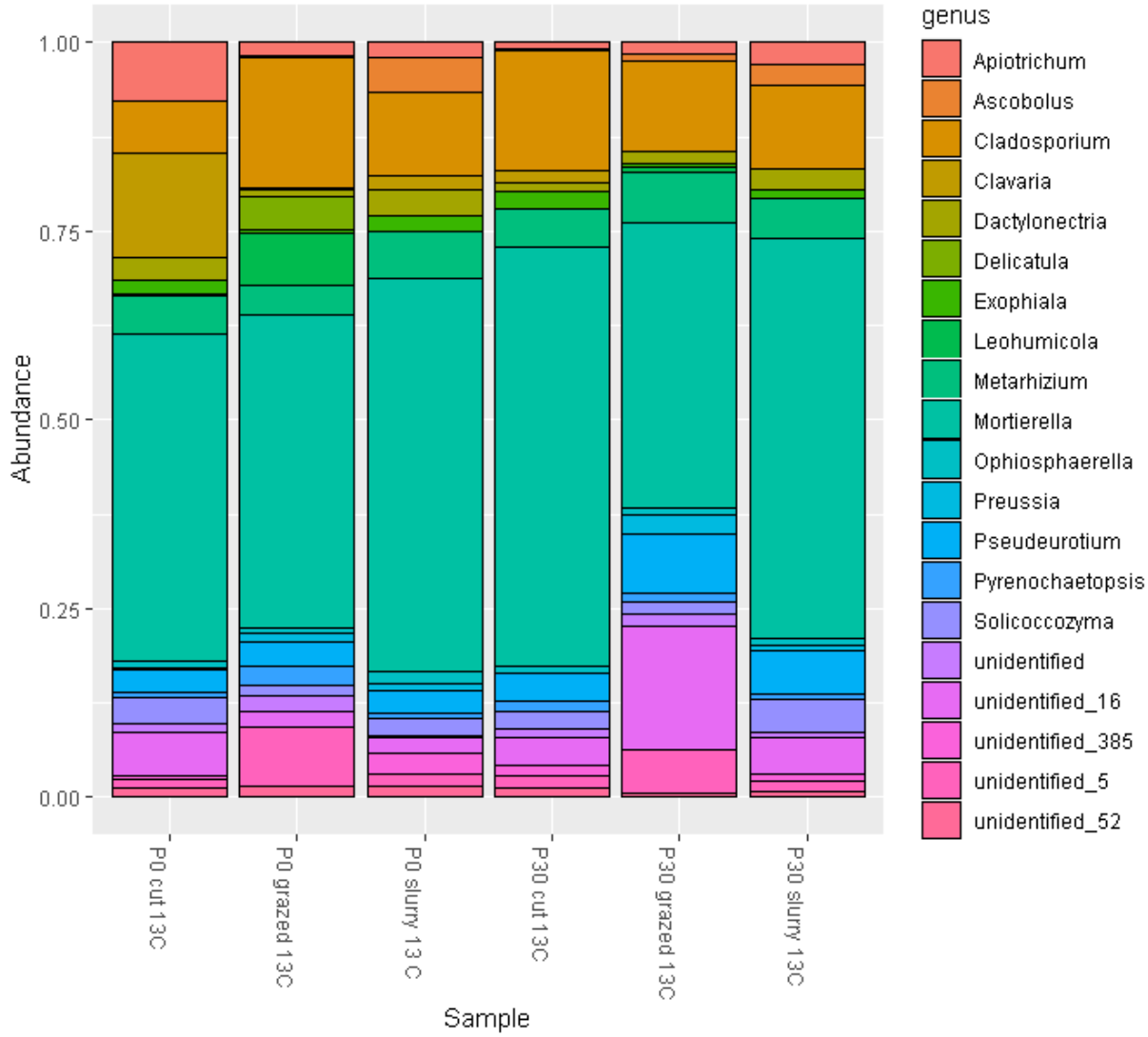

Figure S5: Realtive abundance of top 20 dominant genera of the ITS community in the glucose soils with treatment

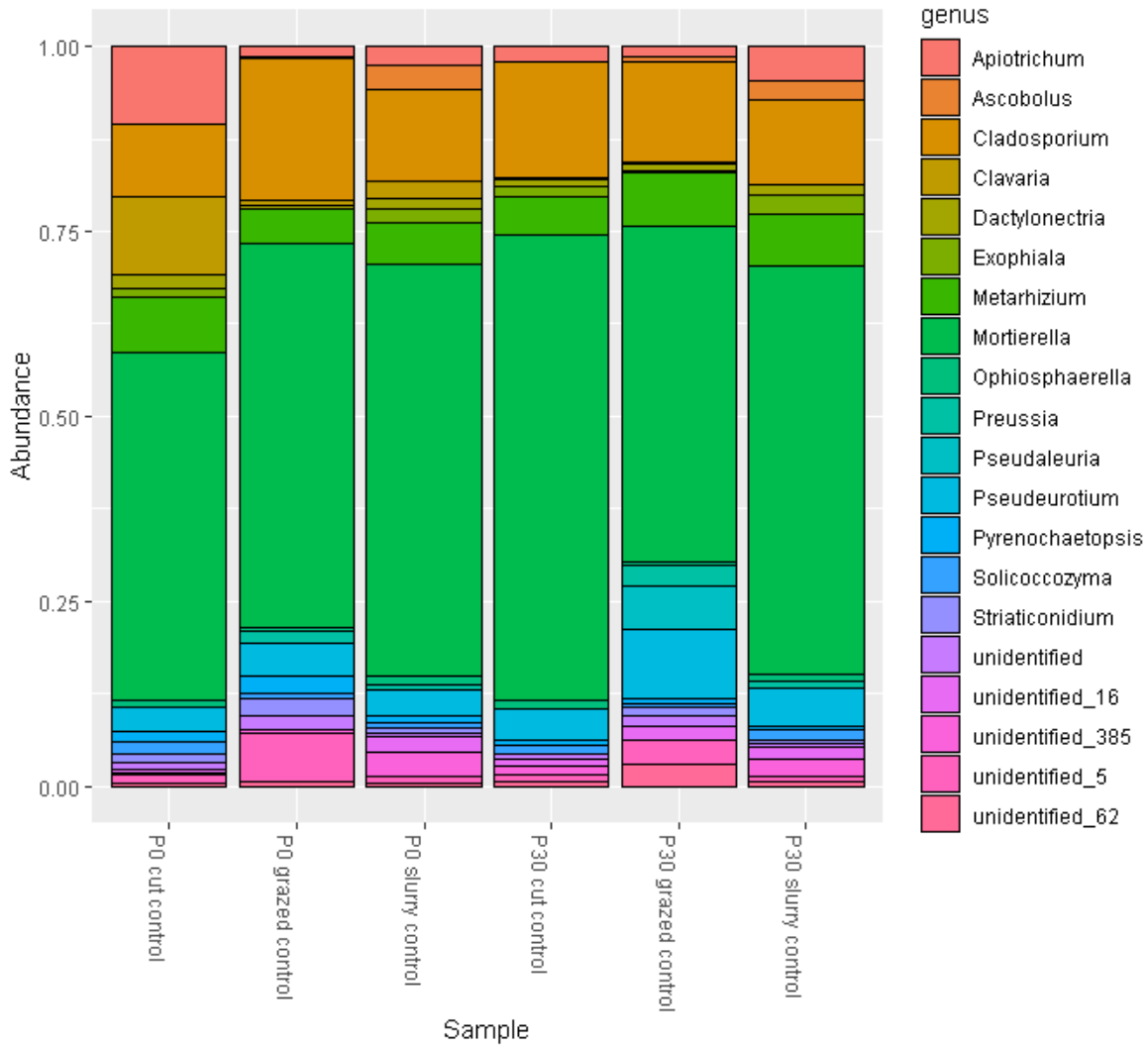

Figure 6: Realtive abundance of top 20 dominant genera of the ITS community in the control soils with treatment

Table S47: Deseq2 analysis of P0 cut 13C vs P30 cut 13C.

P0 cut 13C vs P30 cut 13C	Phylum	Genus	log2fold change	Enriched
	Proteobacteria	Rhodanobacter	-0.81	P30 cut 13C
	Proteobacteria	Acidibacter	0.69	P0 cut 13C
	Acidobacteriota	Vicinamibacter	-0.66	P30 cut 13C
	Bacteroidota	Ferruginibacter	1.32	P0 cut 13C
	Bacteroidota	Chryseolinea	-0.83	P30 cut 13C
	Acidobacteriota	Holophaga	-4.59	P30 cut 13C
	Fibrobacterota	possible genus 04	0.84	P0 cut 13C
	Bacteroidota	Terrimonas	0.5	P0 cut 13C
	Proteobacteria	Paludibacterium	-23.31	P30 cut 13C

Table S48: Deseq2 analysis of P0 cut 13C vs P0 Grazed 13C.

P0 cut 13C vs P0 Grazed 13C	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	Pseudolabrys	-0.47	P0 Grazed 13C
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.47	P0 Grazed 13C
	Planctomycetota	Pir4 lineage	-0.82	P0 Grazed 13C
	Actinobacteriota	Mycobacterium	0.56	P0 Cut 13C
	Actinobacteriota	Gaiella	-0.65	P0 Grazed 13C
	Bacteroidota	Puia	1.02	P0 Cut 13C
	Nitrospirota	Nitrospira	-3.34	P0 Grazed 13C
	Crenarchaeota	Candidatus Nitrocosmicus	-1.19	P0 Grazed 13C
	Proteobacteria	Rhodomicrobium	0.52	P0 Cut 13C
	Proteobacteria	GOUTA6	-1.47	P0 Grazed 13C
	Proteobacteria	Ellin6067	-0.73	P0 Grazed 13C
	Actinobacteriota	Nocardioides	-0.68	P0 Grazed 13C

	Planctomycetota	Aquisphaera	1.73	P0 Cut 13C
	Proteobacteria	Rhodanobacter	2.33	P0 Cut 13C
	Bacteroidota	OLB12	1.41	P0 Cut 13C
	Proteobacteria	MND1	-2.88	P0 Grazed 13C
	Proteobacteria	Roseiarcus	2.32	P0 Cut 13C
	Proteobacteria	Pedomicrobium	-1.97	P0 Grazed 13C
	Acidobacteriota	Luteitalea	-2.05	P0 Grazed 13C
	Spirochaetota	Spirochaeta 2	0.84	P0 Cut 13C
	Acidobacteriota	Occallatibacter	2.65	P0 Cut 13C
	Proteobacteria	Rhodoblastus	0.51	P0 Cut 13C
	Acidobacteriota	RB41	-1.08	P0 Grazed 13C
	Proteobacteria	Acidibacter	-0.89	P0 Grazed 13C
	Bacteroidota	Flavobacterium	-1.41	P0 Grazed 13C
	Acidobacteriota	Candidatus Solibacter	0.78	P0 Cut 13C
	Proteobacteria	Sphingomonas	1.40	P0 Cut 13C
	Proteobacteria	Dongia	-1.57	P0 Grazed 13C
	Actinobacteriota	Nakamurella	0.48	P0 Cut 13C
	Verrucomicrobiota	Chthoniobacter	-1.07	P0 Grazed 13C
	Actinobacteriota	Acidothermus	1.81	P0 Cut 13C
	Bacteroidota	Parafilimonas	0.96	P0 Cut 13C
	Proteobacteria	Rhodoplanes	-0.52	P0 Grazed 13C

	Chloroflexi	UTCFX1	-7.47	P0 Grazed 13C
	Actinobacteriota	Streptomyces	-0.89	P0 Grazed 13C
	Proteobacteria	mle1-7	-2.38	P0 Grazed 13C
	Desulfobacterota	Geobacter	-1.60	P0 Grazed 13C
	Proteobacteria	Candidatus Alysiosphaera	-2.15	P0 Grazed 13C
	Proteobacteria	Pseudorhodoplanes	-9.54	P0 Grazed 13C
	Actinobacteriota	Solirubrobacter	-1.63	P0 Grazed 13C
	Actinobacteriota	Conexibacter	1.28	P0 Cut 13C
	Actinobacteriota	Actinocorallia	-2.24	P0 Grazed 13C
	Proteobacteria	Arenimonas	-0.69	P0 Grazed 13C
	Bacteroidota	Edaphobaculum	0.72	P0 Cut 13C
	Verrucomicrobiota	Lacunisphaera	1.05	P0 Cut 13C
	Proteobacteria	Bauldia	-1.00	P0 Grazed 13C
	Crenarchaeota	Candidatus Nitrososphaera	-9.05	P0 Grazed 13C
	Actinobacteriota	Ilumatobacter	-3.00	P0 Grazed 13C
	Proteobacteria	Devosia	0.76	P0 Cut 13C
	Actinobacteriota	Rhodococcus	10.48	P0 Cut 13C
	Bacteroidota	Mucilaginibacter	2.29	P0 Cut 13C
	Actinobacteriota	Microlunatus	-2.20	P0 Grazed 13C

	Verrucomicrobiota	Ellin517	-2.14	P0 Grazed 13C
	Verrucomicrobiota	Ellin516	0.77	P0 Cut 13C
	Fibrobacterota	possible genus 04	1.93	P0 Cut 13C
	Bacteroidota	Ferruginibacter	0.64	P0 Cut 13C
	Acidobacteriota	Holophaga	3.40	P0 Cut 13C
	Acidobacteriota	Geothrix	7.27	P0 Cut 13C
	Bacteroidota	Sediminibacterium	1.65	P0 Cut 13C
	Proteobacteria	Dokdonella	1.39	P0 Cut 13C
	Planctomycetota	Pirellula	-1.47	P0 Grazed 13C
	Gemmatimonadota	Gemmatimonas	0.65	P0 Cut 13C
	Bacteroidota	Heliimonas	1.96	P0 Cut 13C
	Acidobacteriota	Acidipila	1.64	P0 Cut 13C
	Proteobacteria	Ellin6055	-3.87	P0 Grazed 13C
	Verrucomicrobiota	Terrimicrobium	0.99	P0 Cut 13C
	Crenarchaeota	Candidatus Nitrosotalea	9.32	P0 Cut 13C
	Bacteroidota	Terrimonas	-1.46	P0 Grazed 13C
	Proteobacteria	alphaI cluster	-1.28	P0 Grazed 13C
	Bacteroidota	Chryseolinea	-10.06	P0 Grazed 13C
	Planctomycetota	Schlesneria	1.42	P0 Cut 13C
	Actinobacteriota	Dactylosporangium	-1.85	P0 Grazed 13C
	Acidobacteriota	Candidatus Koribacter	3.20	P0 Cut 13C
	Acidobacteriota	Bryobacter	1.24	P0 Cut 13C

	Proteobacteria	Microvirga	-4.09	P0 Grazed 13C
	Proteobacteria	Pseudomonas	1.66	P0 Cut 13C
	Proteobacteria	Nordella	-8.18	P0 Grazed 13C
	Acidobacteriota	Granulicella	3.09	P0 Cut 13C
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	7.18	P0 Cut 13C
	Acidobacteriota	Vicinamibacter	-1.43	P0 Grazed 13C
	Verrucomicrobiota	Luteolibacter	-2.20	P0 Grazed 13C
	Proteobacteria	SWB02	-1.24	P0 Grazed 13C
	Bacteroidota	Aurantisolimonas	-1.36	P0 Grazed 13C
	Proteobacteria	966-1	-1.50	P0 Grazed 13C
	Actinobacteriota	Rhizocola	-7.69	P0 Grazed 13C
	Proteobacteria	Phenylobacterium	1.34	P0 Cut 13C
	Firmicutes	Lysinibacillus	7.93	P0 Cut 13C
	Planctomycetota	Gemmata	0.54	P0 Cut 13C
	Proteobacteria	Parablastomonas	-2.57	P0 Grazed 13C
	Proteobacteria	Duganella	2.76	P0 Cut 13C
	Proteobacteria	Asticcacaulis	2.05	P0 Cut 13C
	Actinobacteriota	Lysinimonas	2.04	P0 Cut 13C
	Planctomycetota	Singulisphaera	1.57	P0 Cut 13C
	Bdellovibrionota	Bdellovibrio	1.00	P0 Cut 13C

	Bacteroidota	Cytophaga	1.95	P0 Cut 13C
	Acidobacteriota	Terracidiphilus	8.06	P0 Cut 13C
	Verrucomicrobiota	Roseimicrobium	-1.69	P0 Grazed 13C
	Proteobacteria	Acidicaldus	2.61	P0 Cut 13C
	Proteobacteria	Nitrosospira	6.80	P0 Cut 13C
	Proteobacteria	Polaromonas	-7.34	P0 Grazed 13C
	Firmicutes	Turicibacter	6.82	P0 Cut 13C
	Proteobacteria	Uliginosibacterium	3.52	P0 Cut 13C
	Planctomycetota	Tundrisphaera	7.94	P0 Cut 13C
	Myxococcota	KD3-10	1.30	P0 Cut 13C
	Proteobacteria	Phyllobacterium	-7.25	P0 Grazed 13C
	Actinobacteriota	Virgisporangium	-6.40	P0 Grazed 13C
	Firmicutes	Sporosarcina	6.39	P0 Cut 13C
	Proteobacteria	Polycyclovorans	-6.46	P0 Grazed 13C
	Desulfobacterota	Citrifermentans	7.77	P0 Cut 13C
	Chloroflexi	Litorilinea	-4.59	P0 Grazed 13C
	Firmicutes	Romboutsia	6.28	P0 Cut 13C
	Firmicutes	Ammoniphilus	6.45	P0 Cut 13C
	Proteobacteria	Rhizomicrobium	7.61	P0 Cut 13C
	Bacteroidota	Ohtaekwangia	-6.11	P0 Grazed 13C
	Planctomycetota	SH-PL14	-1.83	P0 Grazed 13C
	Proteobacteria	Plot4-2H12	6.43	P0 Cut 13C

	Proteobacteria	Mesorhizobium	1.77	P0 Cut 13C
	Proteobacteria	Rudaea	3.55	P0 Cut 13C
	Actinobacteriota	Rugosimonospora	6.23	P0 Cut 13C
	Actinobacteriota	Kineosporia	6.72	P0 Cut 13C
	Acidobacteriota	Subgroup 10	-1.45	P0 Grazed 13C
	Proteobacteria	Amaricoccus	-7.33	P0 Grazed 13C
	Proteobacteria	Paludibacterium	-23.32	P0 Grazed 13C
	Firmicutes	Clostridium sensu stricto 9	6.76	P0 Cut 13C
	Actinobacteriota	Kibdelosporangium	-6.62	P0 Grazed 13C
	Verrucomicrobiota	FukuN18 freshwater group	3.67	P0 Cut 13C
	Abditibacteriota	Abditibacterium	2.46	P0 Cut 13C
	Bacteroidota	Fluviicola	-5.81	P0 Grazed 13C
	Firmicutes	Pelosinus	7.39	P0 Cut 13C
	Proteobacteria	Micropepsis	6.92	P0 Cut 13C
	Proteobacteria	Afipia	7.35	P0 Cut 13C
	Chloroflexi	Thermosporothrix	6.02	P0 Cut 13C
	Bacteroidota	Taibaiella	6.72	P0 Cut 13C
	Actinobacteriota	Phycicoccus	6.56	P0 Cut 13C
	Bdellovibrionota	Bacteriovorax	20.14	P0 Cut 13C
	Proteobacteria	Sulfuritalea	6.35	P0 Cut 13C
	Actinobacteriota	Longivirga	3.68	P0 Cut 13C
	Proteobacteria	Pseudorhodobacter	-5.60	P0 Grazed 13C

	Planctomycetota	Paludisphaera	6.74	P0 Cut 13C
	Verrucomicrobiota	Prosthecobacter	5.17	P0 Cut 13C
	Firmicutes	Tumebacillus	5.77	P0 Cut 13C
	Proteobacteria	Inquilinus	3.85	P0 Cut 13C
	Firmicutes	Fonticella	6.69	P0 Cut 13C
	Proteobacteria	[Aquaspirillum] arcticum group	5.54	P0 Cut 13C
	Actinobacteriota	Blastococcus	5.39	P0 Cut 13C
	Actinobacteriota	Streptacidiphilus	6.01	P0 Cut 13C
	Actinobacteriota	Arthrobacter	5.47	P0 Cut 13C
	Patescibacteria	TM7a	6.22	P0 Cut 13C
	Proteobacteria	Nevskia	5.31	P0 Cut 13C
	Chloroflexi	Ktedonobacter	3.70	P0 Cut 13C
	Myxococcota	Vulgatibacter	5.16	P0 Cut 13C
	Proteobacteria	Rhodobacter	5.28	P0 Cut 13C
	Proteobacteria	Rhodovastum	6.04	P0 Cut 13C
	Planctomycetota	Pir2 lineage	6.48	P0 Cut 13C
	Bacteroidota	Paludibacter	5.43	P0 Cut 13C
	Bdellovibrionota	OM27 clade	-4.12	P0 Grazed 13C

Table S49: Deseq2 analysis of P0 cut 13C vs P0 Slurry 13C.

P0 cut 13C vs P0 Slurry 13C	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	GOUTA6	-0.92	P0 Slurry 13C
	Proteobacteria	Rhodanobacter	-1.35	P0 Slurry 13C
	Verrucomicrobiota	Chthoniobacter	0.45	P0 Cut 13C
	Acidobacteriota	Vicinamibacter	-0.98	P0 Slurry 13C
	Acidobacteriota	Holophaga	-4.33	P0 Slurry 13C
	Proteobacteria	Paludibacterium	-22.92	P0 Slurry 13C

Table S50: Deseq2 analysis of P0 Cut Control vs P0 Grazed Control.

P0 Cut Control vs P0 Grazed Control	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	Pseudolabrys	-0.36	P0 Grazed Control
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.36	P0 Grazed Control
	Planctomycetota	Pir4 lineage	-0.86	P0 Grazed Control
	Actinobacteriota	Mycobacterium	0.36	P0 Cut Control
	Actinobacteriota	Gaiella	-0.81	P0 Grazed Control
	Bacteroidota	Puia	1.16	P0 Cut Control
	Nitrospirota	Nitrospira	-2.81	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrocosmicus	-1.10	P0 Grazed Control
	Proteobacteria	Reyranella	-0.29	P0 Grazed Control
	Proteobacteria	Rhodomicrobium	0.89	P0 Cut Control
	Proteobacteria	GOUTA6	-1.64	P0 Grazed Control
	Proteobacteria	Bradyrhizobium	-0.49	P0 Grazed Control
	Proteobacteria	Ellin6067	-0.50	P0 Grazed Control
	Actinobacteriota	Kribbella	-0.63	P0 Grazed Control

	Actinobacteriota	Nocardioides	-0.94	P0 Grazed Control
	Planctomycetota	Aquisphaera	1.50	P0 Cut Control
	Proteobacteria	Rhodanobacter	2.04	P0 Cut Control
	Proteobacteria	MND1	-3.13	P0 Grazed Control
	Proteobacteria	Roseiarcus	1.92	P0 Cut Control
	Bacteroidota	OLB12	0.90	P0 Cut Control
	Proteobacteria	Pedomicrobium	-1.94	P0 Grazed Control
	Acidobacteriota	Luteitalea	-2.07	P0 Grazed Control
	Spirochaetota	Spirochaeta 2	0.67	P0 Cut Control
	Acidobacteriota	Occallatibacter	2.15	P0 Cut Control
	Proteobacteria	Rhodoblastus	1.15	P0 Cut Control
	Acidobacteriota	RB41	-0.76	P0 Cut Control
	Proteobacteria	Acidibacter	-1.15	P0 Grazed Control
	Bacteroidota	Flavobacterium	-1.71	P0 Grazed Control
	Acidobacteriota	Candidatus Solibacter	0.77	P0 Cut Control
	Proteobacteria	Sphingomonas	1.43	P0 Cut Control
	Proteobacteria	Dongia	-1.97	P0 Grazed Control
	Verrucomicrobiota	Chthoniobacter	-1.28	P0 Grazed Control
	Actinobacteriota	Acidothermus	1.97	P0 Cut Control
	Bacteroidota	Parafilimonas	0.70	P0 Cut Control
	Proteobacteria	Rhodoplanes	-0.96	P0 Grazed Control
	Chloroflexi	UTCFX1	-6.38	P0 Grazed Control
	Desulfobacterota	Geobacter	-2.40	P0 Grazed Control
	Actinobacteriota	Streptomyces	-1.22	P0 Grazed Control
	Proteobacteria	mle1-7	-2.03	P0 Grazed Control
	Proteobacteria	Candidatus Alysiosphaera	-2.77	P0 Grazed Control
	Proteobacteria	Pseudorhodoplanes	-8.89	P0 Grazed Control

	Actinobacteriota	Conexibacter	1.12	P0 Cut Control
	Actinobacteriota	Actinocorallia	-1.79	P0 Grazed Control
	Myxococcota	Phaselicystis	-0.89	P0 Grazed Control
	Proteobacteria	Arenimonas	-1.01	P0 Grazed Control
	Bacteroidota	Edaphobaculum	1.06	P0 Grazed Control
	Bacteroidota	Mucilaginibacter	1.76	P0 Cut Control
	Planctomycetota	Pirellula	-1.65	P0 Grazed Control
	Proteobacteria	Bauldia	-1.25	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrososphaera	-6.20	P0 Grazed Control
	Actinobacteriota	Ilumatobacter	-3.22	P0 Grazed Control
	Proteobacteria	Devosia	0.42	P0 Cut Control
	Actinobacteriota	Rhodococcus	8.11	P0 Cut Control
	Myxococcota	Anaeromyxobacter	-0.88	P0 Grazed Control
	Actinobacteriota	Microlunatus	-2.93	P0 Grazed Control
	Acidobacteriota	Vicinamibacter	-1.38	P0 Grazed Control
	Verrucomicrobiota	Ellin517	-3.21	P0 Grazed Control
	Verrucomicrobiota	Ellin516	0.54	P0 Cut Control
	Fibrobacterota	possible genus 04	1.44	P0 Cut Control
	Bacteroidota	Chryseolinea	-9.09	P0 Grazed Control
	Acidobacteriota	Holophaga	3.18	P0 Cut Control
	Acidobacteriota	Geothrix	3.14	P0 Cut Control
	Bacteroidota	Sediminibacterium	1.44	P0 Cut Control
	Proteobacteria	Dokdonella	1.04	P0 Cut Control
	Bacteroidota	Heliimonas	0.98	P0 Cut Control
	Acidobacteriota	Acidipila	1.25	P0 Cut Control
	Proteobacteria	Leptothrix	1.09	P0 Cut Control
	Crenarchaeota	Candidatus Nitrosotalea	11.24	P0 Cut Control

	Planctomycetota	Schlesneria	1.08	P0 Cut Control
	Actinobacteriota	Dactylosporangium	-2.00	P0 Grazed Control
	Acidobacteriota	Candidatus Koribacter	2.95	P0 Cut Control
	Bacteroidota	Terrimonas	-2.43	P0 Grazed Control
	Planctomycetota	Gemmata	-0.37	P0 Grazed Control
	Proteobacteria	Sideroxydans	1.61	P0 Cut Control
	Proteobacteria	Microvirga	-3.99	P0 Grazed Control
	Proteobacteria	Nordella	-7.85	P0 Grazed Control
	Acidobacteriota	Granulicella	4.47	P0 Cut Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	4.94	P0 Cut Control
	Proteobacteria	Defluviicoccus	-2.47	P0 Grazed Control
	Proteobacteria	Pseudomonas	1.42	P0 Cut Control
	Proteobacteria	SWB02	-1.74	P0 Grazed Control
	Bacteroidota	Aurantisolimonas	-0.93	P0 Grazed Control
	Planctomycetota	Singulisphaera	1.27	P0 Cut Control
	Actinobacteriota	Rhizocola	-7.59	P0 Grazed Control
	Acidobacteriota	Bryobacter	1.08	P0 Cut Control
	Proteobacteria	Phenylobacterium	0.98	P0 Cut Control
	Firmicutes	Lysinibacillus	3.42	P0 Cut Control
	Actinobacteriota	CL500-29 marine group	-2.51	P0 Grazed Control
	Proteobacteria	Parablastomonas	-6.65	P0 Grazed Control
	Verrucomicrobiota	Roseimicrobium	-1.50	P0 Grazed Control
	Planctomycetota	SH-PL14	-3.71	P0 Grazed Control
	Bdellovibrionota	Bdellovibrio	0.70	P0 Cut Control
	Bacteroidota	Cytophaga	1.15	P0 Cut Control

	Acidobacteriota	Terracidiphilus	8.73	P0 Cut Control
	Planctomycetota	SM1A02	-1.14	P0 Grazed Control
	Proteobacteria	Tahibacter	-6.73	P0 Grazed Control
	Proteobacteria	Acidicaldus	1.87	P0 Cut Control
	Acidobacteriota	Subgroup 10	-2.24	P0 Grazed Control
	Proteobacteria	Polaromonas	-5.55	P0 Grazed Control
	Firmicutes	Turicibacter	7.71	P0 Cut Control
	Proteobacteria	Uliginosibacterium	6.90	P0 Cut Control
	Myxococcota	KD3-10	0.96	P0 Cut Control
	Proteobacteria	Altererythrobacter	1.12	P0 Cut Control
	Actinobacteriota	Lechevalieria	-8.42	P0 Grazed Control
	Actinobacteriota	Virgisporangium	-7.14	P0 Grazed Control
	Firmicutes	Sporosarcina	7.56	P0 Cut Control
	Proteobacteria	Polycyclovorans	-6.08	P0 Grazed Control
	Chloroflexi	Litorilinea	-6.00	P0 Grazed Control
	Firmicutes	Romboutsia	7.22	P0 Cut Control
	Firmicutes	Ammoniphilus	7.25	P0 Cut Control
	Proteobacteria	Rhizomicrobium	8.73	P0 Cut Control
	Bacteroidota	Ohtaekwangia	-6.94	P0 Grazed Control
	Proteobacteria	Rudaea	3.55	P0 Cut Control
	Actinobacteriota	Rugosimonospora	7.48	P0 Cut Control
	Proteobacteria	Pseudoduganella	-6.64	P0 Grazed Control
	Proteobacteria	Amaricoccus	-6.82	P0 Grazed Control
	Verrucomicrobiota	FukuN18 freshwater group	3.90	P0 Cut Control
	Proteobacteria	Asticcacaulis	4.16	P0 Cut Control
	Abditibacteriota	Abditibacterium	2.72	P0 Cut Control
	Bacteroidota	Fluviicola	-4.82	P0 Grazed Control
	Proteobacteria	Micropepsis	5.89	P0 Cut Control

	Bacteroidota	Sporocytophaga	6.80	P0 Cut Control
	Proteobacteria	Undibacterium	5.75	P0 Cut Control
	Planctomycetota	Paludisphaera	7.39	P0 Cut Control
	Firmicutes	Tumebacillus	6.23	P0 Cut Control
	Proteobacteria	Inquilinus	6.93	P0 Cut Control
	Firmicutes	Fonticella	5.97	P0 Cut Control
	Proteobacteria	Afipia	6.65	P0 Cut Control
	Proteobacteria	Alkanibacter	5.38	P0 Cut Control
	Proteobacteria	Acidisoma	7.21	P0 Cut Control
	Proteobacteria	Sulfurifustis	-5.53	P0 Grazed Control
	Chloroflexi	Ktedonobacter	5.94	P0 Cut Control
	Actinobacteriota	Kibdelosporangium	-6.93	P0 Grazed Control
	Firmicutes	Clostridium sensu stricto 12	5.53	P0 Cut Control
	Myxococcota	Vulgatibacter	6.32	P0 Cut Control
	Actinobacteriota	Longivirga	7.08	P0 Cut Control
	Proteobacteria	Alkanindiges	5.24	P0 Cut Control
	Proteobacteria	Nevskia	6.22	P0 Cut Control

Table S51: Deseq2 analysis of P0 cut control vs P0 Slurry control.

P0 cut control vs P0 Slurry control	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Verrucomicrobiota	Candidatus Udaeobacter	0.40	P0 Cut Control
	Proteobacteria	GOUTA6	-0.58	P0 Slurry Control
	Proteobacteria	Rhodanobacter	-1.54	P0 Slurry Control
	Acidobacteriota	Occallatibacter	-1.50	P0 Slurry Control
	Acidobacteriota	RB41	0.49	P0 Cut Control

	Verrucomicrobiota	Chthoniobacter	0.37	P0 Cut Control
	Proteobacteria	Candidatus Alysiosphaera	-0.71	P0 Slurry Control
	Proteobacteria	Bauldia	-0.47	P0 Slurry Control
	Crenarchaeota	Candidatus Nitrososphaera	-0.78	P0 Slurry Control
	Acidobacteriota	Vicinamibacter	-0.64	P0 Slurry Control
	Bacteroidota	Chryseolinea	0.61	P0 Cut Control
	Acidobacteriota	Holophaga	-8.04	P0 Slurry Control
	Bacteroidota	Terrimonas	0.47	P0 Cut Control
	Proteobacteria	Parablastomonas	-6.57	P0 Slurry Control
	Proteobacteria	Sideroxydans	-6.58	P0 Slurry Control

Table S52: Deseq2 analysis of P0 Cut Control vs P30 Cut Control.

P0 Cut Control vs P30 Cut Control	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	0.30	P0 Cut Control
	Crenarchaeota	Candidatus Nitrocosmicus	-0.66	P30 Cut Control
	Proteobacteria	Rhodanobacter	-1.11	P30 Cut Control
	Acidobacteriota	RB41	0.49	P0 Cut Control
	Verrucomicrobiota	Chthoniobacter	0.36	P0 Cut Control
	Actinobacteriota	Streptomyces	-0.53	P30 Cut Control
	Acidobacteriota	Holophaga	-8.62	P30 Cut Control
	Bacteroidota	Ferruginibacter	0.78	P0 Cut Control
	Verrucomicrobiota	Terrimicrobium	1.41	P0 Cut Control
	Bacteroidota	Sporocytophaga	6.70	P0 Cut Control

Table S53: Deseq2 analysis of P0 Grazed 13C vs P30 Grazed 13C.

P0 Grazed 13C vs P30 Grazed 13C	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	0.44	P0 Grazed 13C
	Proteobacteria	Pseudolabrys	0.39	P30 Grazed 13C
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.74	P30 Grazed 13C
	Planctomycetota	Pir4 lineage	0.87	P0 Grazed 13C
	Actinobacteriota	Mycobacterium	-0.49	P30 Grazed 13C
	Nitrospirota	Nitrospira	2.96	P0 Grazed 13C
	Proteobacteria	GOUTA6	1.46	P0 Grazed 13C
	Proteobacteria	Ellin6067	0.78	P0 Grazed 13C
	Planctomycetota	Aquisphaera	-0.40	P30 Grazed 13C
	Proteobacteria	Rhodanobacter	-2.85	P30 Grazed 13C
	Bacteroidota	OLB12	-0.51	P30 Grazed 13C
	Proteobacteria	Roseiarcus	-1.21	P30 Grazed 13C
	Proteobacteria	Pedomicrobium	1.16	P0 Grazed 13C
	Acidobacteriota	Occallatibacter	-0.53	P30 Grazed 13C
	Proteobacteria	Acidibacter	0.86	P0 Grazed 13C
	Proteobacteria	Sphingomonas	-1.06	P30 Grazed 13C
	Chloroflexi	UTCFX1	4.76	P0 Grazed 13C
	Actinobacteriota	Streptomyces	1.12	P0 Grazed 13C
	Proteobacteria	mle1-7	1.65	P0 Grazed 13C
	Actinobacteriota	Conexibacter	-0.66	P30 Grazed 13C
	Planctomycetota	Zavarzinella	1.17	P0 Grazed 13C
	Actinobacteriota	Actinocorallia	1.49	P0 Grazed 13C
	Proteobacteria	Arenimonas	0.92	P0 Grazed 13C
	Bacteroidota	Edaphobaculum	-0.53	P30 Grazed 13C

	Bacteroidota	Flavobacterium	0.96	P0 Grazed 13C
	Crenarchaeota	Streptomyces	7.17	P0 Grazed 13C
	Bdellovibrionota	Bdellovibrio	-0.67	P30 Grazed 13C
	Actinobacteriota	Nakamurella	0.62	P0 Grazed 13C
	Proteobacteria	Devosia	-1.19	P30 Grazed 13C
	Proteobacteria	Cellvibrio	4.01	P0 Grazed 13C
	Verrucomicrobiota	ADurb.Bin063-1	0.63	P0 Grazed 13C
	Bacteroidota	Mucilaginibacter	-1.80	P30 Grazed 13C
	Acidobacteriota	RB41	1.20	P0 Grazed 13C
	Actinobacteriota	Microlunatus	1.34	P0 Grazed 13C
	Verrucomicrobiota	Ellin517	1.60	P0 Grazed 13C
	Fibrobacterota	possible genus 04	-0.99	P30 Grazed 13C
	Acidobacteriota	Holophaga	-8.09	P30 Grazed 13C
	Acidobacteriota	Luteitalea	1.24	P0 Grazed 13C
	Bacteroidota	Sediminibacterium	-0.81	P30 Grazed 13C
	Proteobacteria	Dokdonella	-0.86	P30 Grazed 13C
	Planctomycetota	Pirellula	0.81	P0 Grazed 13C
	Gemmatimonadota	Gemmatimonas	-0.96	P30 Grazed 13C
	Bacteroidota	Heliimonas	-1.23	P30 Grazed 13C
	Crenarchaeota	Candidatus Nitrosotalea	-1.09	P30 Grazed 13C
	Planctomycetota	Schlesneria	-0.93	P30 Grazed 13C
	Acidobacteriota	Granulicella	-4.44	P30 Grazed 13C
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-1.85	P30 Grazed 13C
	Proteobacteria	MND1	1.89	P0 Grazed 13C
	Proteobacteria	966-1	1.16	P0 Grazed 13C
	Acidobacteriota	Bryobacter	0.30	P0 Grazed 13C

	Proteobacteria	Phenylobacterium	-0.84	P30 Grazed 13C
	Firmicutes	Lysinibacillus	-1.05	P30 Grazed 13C
	Proteobacteria	Parablastomonas	3.51	P0 Grazed 13C
	Actinobacteriota	Lysinimonas	-1.35	P30 Grazed 13C
	Bacteroidota	Cytophaga	-1.53	P30 Grazed 13C
	Acidobacteriota	Terracidiphilus	-1.62	P30 Grazed 13C
	Actinobacteriota	Cellulomonas	-1.26	P30 Grazed 13C
	Bacteroidota	Terrimonas	1.64	P0 Grazed 13C
	Actinobacteriota	Terrabacter	-7.52	P30 Grazed 13C
	Proteobacteria	Uliginosibacterium	-2.11	P30 Grazed 13C
	Myxococcota	KD3-10	-0.88	P30 Grazed 13C
	Proteobacteria	Altererythrobacter	-2.54	P30 Grazed 13C
	Acidobacteriota	JGI 0001001-H03	1.42	P0 Grazed 13C
	Proteobacteria	Mesorhizobium	-7.53	P30 Grazed 13C
	Proteobacteria	Rudaea	-2.14	P30 Grazed 13C
	Myxococcota	Pajaroellobacter	-0.88	P30 Grazed 13C
	Verrucomicrobiota	FukuN18 freshwater group	-3.70	P30 Grazed 13C
	Abditibacteriota	Abditibacterium	-2.56	P30 Grazed 13C
	Bacteroidota	UTBCD1	7.93	P0 Grazed 13C
	Proteobacteria	Micropepsis	-7.23	P30 Grazed 13C
	Proteobacteria	Aquicella	-1.25	P30 Grazed 13C
	Chloroflexi	Thermosporothrix	-6.33	P30 Grazed 13C
	Proteobacteria	Hirschia	-5.60	P30 Grazed 13C
	Bacteroidota	Taibaiella	-7.01	P30 Grazed 13C
	Actinobacteriota	Phycicoccus	-6.86	P30 Grazed 13C
	Planctomycetota	Paludisphaera	-7.06	P30 Grazed 13C
	Proteobacteria	Inquilinus	-7.07	P30 Grazed 13C
	Spirochaetota	Spirochaeta	5.66	P0 Grazed 13C

	Actinobacteriota	Blastococcus	-5.71	P30 Grazed 13C
	Actinobacteriota	Streptacidiphilus	-6.32	P30 Grazed 13C
	Myxococcota	Vulgatibacter	-5.47	P30 Grazed 13C
	Bacteroidota	Paludibacter	-5.74	P30 Grazed 13C
	Verrucomicrobiota	Candidatus Omnitrophus	-6.44	P30 Grazed 13C

Table S54: Deseq2 analysis of P0 Grazed Control vs P0 Slurry Control.

P0 Grazed Control vs P0 Slurry Control	Phylum	Genus	log2FoldChange	Enriched
	Planctomycetota	Pir4 lineage	0.81	P0 Grazed Control
	Actinobacteriota	Mycobacterium	-0.69	P0 Slurry Control
	Actinobacteriota	Gaiella	0.36	P0 Grazed Control
	Bacteroidota	Puia	-1.40	P0 Slurry Control
	Nitrospirota	Nitrospira	3.24	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrocosmicus	0.48	P0 Grazed Control
	Proteobacteria	Rhodomicrobium	-1.02	P0 Slurry Control
	Proteobacteria	GOUTA6	0.86	P0 Grazed Control
	Proteobacteria	Bradyrhizobium	0.52	P0 Grazed Control
	Actinobacteriota	Nocardioides	0.88	P0 Grazed Control
	Planctomycetota	Aquisphaera	-1.64	P0 Slurry Control
	Proteobacteria	Rhodanobacter	-3.80	P0 Slurry Control
	Proteobacteria	MND1	2.78	P0 Grazed Control
	Proteobacteria	Roseiarcus	-2.10	P0 Slurry Control
	Bacteroidota	OLB12	-1.23	P0 Slurry Control
	Proteobacteria	Pedomicrobium	1.63	P0 Grazed Control
	Acidobacteriota	Luteitalea	1.82	P0 Grazed Control

	Spirochaetota	Spirochaeta 2	-0.68	P0 Slurry Control
	Acidobacteriota	Occallatibacter	-3.87	P0 Slurry Control
	Proteobacteria	Rhodoblastus	-1.25	P0 Slurry Control
	Acidobacteriota	RB41	1.04	P0 Grazed Control
	Proteobacteria	Acidibacter	1.19	P0 Grazed Control
	Bacteroidota	Flavobacterium	1.25	P0 Grazed Control
	Acidobacteriota	Candidatus Solibacter	-0.90	P0 Slurry Control
	Proteobacteria	Sphingomonas	-1.30	P0 Slurry Control
	Proteobacteria	Dongia	1.79	P0 Grazed Control
	Verrucomicrobiota	Chthoniobacter	1.45	P0 Grazed Control
	Actinobacteriota	Acidothermus	-2.47	P0 Slurry Control
	Bacteroidota	Parafilimonas	-0.73	P0 Slurry Control
	Proteobacteria	Rhodoplanes	0.53	P0 Grazed Control
	Chloroflexi	UTCFX1	5.94	P0 Grazed Control
	Desulfobacterota	Geobacter	1.87	P0 Grazed Control
	Verrucomicrobiota	Lacunisphaera	-0.86	P0 Slurry Control
	Proteobacteria	Hyphomicrobium	-0.55	P0 Slurry Control
	Proteobacteria	mle1-7	1.77	P0 Grazed Control
	Proteobacteria	Candidatus Alysiosphaera	1.83	P0 Grazed Control
	Proteobacteria	Pseudorhodoplanes	9.22	P0 Grazed Control
	Actinobacteriota	Conexibacter	-1.41	P0 Slurry Control
	Actinobacteriota	Actinocorallia	2.02	P0 Grazed Control
	Actinobacteriota	Ilumatobacter	2.96	P0 Grazed Control
	Bacteroidota	Edaphobaculum	-1.02	P0 Slurry Control
	Bacteroidota	Mucilaginibacter	-2.10	P0 Slurry Control
	Planctomycetota	Pirellula	1.60	P0 Grazed Control
	Proteobacteria	Bauldia	0.56	P0 Grazed Control

	Crenarchaeota	Candidatus Nitrososphaera	5.19	P0 Grazed Control
	Proteobacteria	Devosia	-0.78	P0 Slurry Control
	Verrucomicrobiota	ADurb.Bin063-1	-0.40	P0 Slurry Control
	Actinobacteriota	Rhodococcus	-4.66	P0 Slurry Control
	Verrucomicrobiota	Ellin517	3.52	P0 Grazed Control
	Verrucomicrobiota	Ellin516	-0.92	P0 Slurry Control
	Fibrobacterota	possible genus 04	-1.59	P0 Slurry Control
	Bacteroidota	Chryseolinea	9.50	P0 Grazed Control
	Acidobacteriota	Holophaga	-11.42	P0 Slurry Control
	Acidobacteriota	Geothrix	-5.85	P0 Slurry Control
	Bacteroidota	Sediminibacterium	-1.72	P0 Slurry Control
	Proteobacteria	Dokdonella	-1.55	P0 Slurry Control
	Gemmatimonadota	Gemmatimonas	-0.70	P0 Slurry Control
	Actinobacteriota	Microlunatus	2.79	P0 Grazed Control
	Acidobacteriota	Bryobacter	-1.19	P0 Slurry Control
	Acidobacteriota	Acidipila	-2.53	P0 Slurry Control
	Proteobacteria	Leptothrix	-2.89	P0 Slurry Control
	Proteobacteria	Ellin6055	3.40	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrosotalea	-11.04	P0 Slurry Control
	Planctomycetota	Schlesneria	-1.28	P0 Slurry Control
	Acidobacteriota	Candidatus Koribacter	-3.19	P0 Slurry Control
	Bacteroidota	Terrimonas	2.69	P0 Grazed Control
	Proteobacteria	Sideroxydans	-8.39	P0 Slurry Control
	Proteobacteria	Microvirga	3.76	P0 Grazed Control
	Proteobacteria	Nordella	7.62	P0 Grazed Control
	Acidobacteriota	Granulicella	-4.22	P0 Slurry Control

	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-8.41	P0 Slurry Control
	Proteobacteria	SWB02	1.40	P0 Grazed Control
	Bacteroidota	Aurantisolimonas	1.20	P0 Grazed Control
	Planctomycetota	Singulisphaera	-1.52	P0 Slurry Control
	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	1.97	P0 Grazed Control
	Actinobacteriota	Rhizocola	6.88	P0 Grazed Control
	Proteobacteria	Phenylobacterium	-0.93	P0 Slurry Control
	Firmicutes	Lysinibacillus	-3.23	P0 Slurry Control
	Actinobacteriota	CL500-29 marine group	2.72	P0 Grazed Control
	Proteobacteria	Duganella	-1.81	P0 Slurry Control
	Proteobacteria	Asticcacaulis	-2.99	P0 Slurry Control
	Planctomycetota	SH-PL14	3.52	P0 Grazed Control
	Bdellovibrionota	Bdellovibrio	-1.20	P0 Slurry Control
	Bacteroidota	Cytophaga	-1.84	P0 Slurry Control
	Acidobacteriota	Terracidiphilus	-8.53	P0 Slurry Control
	Proteobacteria	Tahibacter	6.85	P0 Grazed Control
	Proteobacteria	Acidicaldus	-3.75	P0 Slurry Control
	Actinobacteriota	Nocardia	2.50	P0 Grazed Control
	Proteobacteria	Polaromonas	6.44	P0 Grazed Control
	Firmicutes	Turicibacter	-1.81	P0 Slurry Control
	Acidobacteriota	Stenotrophobacter	6.64	P0 Grazed Control
	Proteobacteria	Uliginosibacterium	-6.70	P0 Slurry Control
	Planctomycetota	Tundrisphaera	-6.41	P0 Slurry Control
	Myxococcota	KD3-10	-1.87	P0 Slurry Control

	Acidobacteriota	Subgroup 10	2.09	P0 Grazed Control
	Bacteroidota	Heliimonas	-2.03	P0 Slurry Control
	Actinobacteriota	Virgisporangium	5.90	P0 Grazed Control
	Firmicutes	Sporosarcina	-7.37	P0 Slurry Control
	Proteobacteria	Polycyclovorans	5.11	P0 Grazed Control
	Firmicutes	Ammoniphilus	-2.90	P0 Slurry Control
	Desulfobacterota	Citrifermentans	-6.75	P0 Slurry Control
	Proteobacteria	Rhizomicrobium	-8.53	P0 Slurry Control
	Proteobacteria	Plot4-2H12	-5.96	P0 Slurry Control
	Bacteroidota	Ohtaekwangia	7.44	P0 Grazed Control
	Proteobacteria	Rudaea	-7.42	P0 Slurry Control
	Actinobacteriota	Rugosimonospora	-7.28	P0 Slurry Control
	Proteobacteria	Amaricoccus	5.90	P0 Grazed Control
	Proteobacteria	BD1-7 clade	5.52	P0 Grazed Control
	Planctomycetota	Phycisphaera	-2.09	P0 Slurry Control
	Verrucomicrobiota	FukuN18 freshwater group	-7.14	P0 Slurry Control
	Abditibacteriota	Abditibacterium	-2.58	P0 Slurry Control
	Bacteroidota	Fluviicola	6.59	P0 Grazed Control
	Firmicutes	Pelosinus	-5.33	P0 Slurry Control
	Proteobacteria	Micropepsis	-5.69	P0 Slurry Control
	Chloroflexi	Thermosporothrix	-6.36	P0 Slurry Control
	Planctomycetota	Paludisphaera	-7.19	P0 Slurry Control
	Proteobacteria	Inquilinus	-6.73	P0 Slurry Control
	Verrucomicrobiota	Oikopleura	5.47	P0 Grazed Control
	Firmicutes	Fonticella	-5.77	P0 Slurry Control
	Proteobacteria	Afipia	-6.45	P0 Slurry Control
	Proteobacteria	Acidisoma	-7.01	P0 Slurry Control
	Chloroflexi	Ktedonobacter	-5.74	P0 Slurry Control

	Firmicutes	Clostridium sensu stricto 12	-5.33	P0 Slurry Control
	Myxococcota	Vulgatibacter	-6.13	P0 Slurry Control
	Actinobacteriota	Longivirga	-6.88	P0 Slurry Control
	Proteobacteria	Nevskia	-6.02	P0 Slurry Control

Table S55: Deseq2 analysis of P0 Grazed 13C vs P0 Slurry 13C.

P0 Grazed 13C vs P0 Slurry 13C	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Xiphinematobacter	0.41	P0 Grazed 13C
	Planctomycetota	Pir4 lineage	0.82	P0 Grazed 13C
	Actinobacteriota	Mycobacterium	-0.67	P0 Slurry 13C
	Bacteroidota	Puia	-1.19	P0 Slurry 13C
	Nitrospirota	Nitrospira	3.47	P0 Grazed 13C
	Crenarchaeota	Candidatus Nitrocosmicus	0.67	P0 Grazed 13C
	Proteobacteria	Ellin6067	0.66	P0 Grazed 13C
	Actinobacteriota	Nocardioides	0.60	P0 Grazed 13C
	Planctomycetota	Aquisphaera	-1.60	P0 Slurry 13C
	Proteobacteria	Rhodanobacter	-3.79	P0 Slurry 13C
	Bacteroidota	OLB12	-1.71	P0 Slurry 13C
	Proteobacteria	MND1	2.68	P0 Grazed 13C
	Proteobacteria	Roseiarcus	-2.51	P0 Slurry 13C
	Proteobacteria	Pedomicrobium	1.97	P0 Grazed 13C
	Acidobacteriota	Luteitalea	1.90	P0 Grazed 13C
	Spirochaetota	Spirochaeta 2	-0.85	P0 Slurry 13C
	Acidobacteriota	Occallatibacter	-3.40	P0 Slurry 13C

	Proteobacteria	Rhodoblastus	-0.47	P0 Slurry 13C
	Acidobacteriota	RB41	1.08	P0 Grazed 13C
	Proteobacteria	Acidibacter	1.02	P0 Grazed 13C
	Bacteroidota	Flavobacterium	1.26	P0 Grazed 13C
	Proteobacteria	Sphingomonas	-1.61	P0 Slurry 13C
	Proteobacteria	Dongia	1.36	P0 Grazed 13C
	Verrucomicrobiota	Chthoniobacter	1.40	P0 Grazed 13C
	Actinobacteriota	Acidothermus	-2.18	P0 Slurry 13C
	Bacteroidota	Parafilimonas	-0.71	P0 Slurry 13C
	Proteobacteria	Rhodoplanes	0.65	P0 Grazed 13C
	Chloroflexi	UTCFX1	6.84	P0 Grazed 13C
	Verrucomicrobiota	Lacunisphaera	-1.36	P0 Slurry 13C
	Proteobacteria	Hyphomicrobium	-0.59	P0 Slurry 13C
	Actinobacteriota	Streptomyces	0.92	P0 Grazed 13C
	Proteobacteria	mle1-7	1.83	P0 Grazed 13C
	Desulfobacterota	Geobacter	1.27	P0 Grazed 13C
	Proteobacteria	Candidatus Alysiosphaera	1.84	P0 Grazed 13C
	Acidobacteriota	Candidatus Solibacter	-0.93	P0 Slurry 13C
	Proteobacteria	Pseudorhodoplanes	9.58	P0 Grazed 13C
	Actinobacteriota	Solirubrobacter	1.48	P0 Grazed 13C
	Actinobacteriota	Conexibacter	-1.68	P0 Slurry 13C
	Actinobacteriota	Actinocorallia	1.87	P0 Grazed 13C
	Actinobacteriota	Ilumatobacter	2.92	P0 Grazed 13C
	Bacteroidota	Edaphobaculum	-1.19	P0 Slurry 13C
	Crenarchaeota	Candidatus Nitrososphaera	8.05	P0 Grazed 13C
	Proteobacteria	Devosia	-1.10	P0 Slurry 13C

	Actinobacteriota	Rhodococcus	-6.86	P0 Slurry 13C
	Bacteroidota	Mucilaginibacter	-2.74	P0 Slurry 13C
	Verrucomicrobiota	Ellin517	2.12	P0 Grazed 13C
	Verrucomicrobiota	Ellin516	-1.10	P0 Slurry 13C
	Fibrobacterota	possible genus 04	-1.53	P0 Slurry 13C
	Bacteroidota	Chryseolinea	9.94	P0 Grazed 13C
	Acidobacteriota	Holophaga	-7.84	P0 Slurry 13C
	Acidobacteriota	Geothrix	-7.78	P0 Slurry 13C
	Bacteroidota	Sediminibacterium	-1.65	P0 Slurry 13C
	Proteobacteria	Dokdonella	-1.96	P0 Slurry 13C
	Planctomycetota	Pirellula	1.40	P0 Grazed 13C
	Gemmatimonadota	Gemmatimonas	-0.82	P0 Slurry 13C
	Bacteroidota	Heliimonas	-2.31	P0 Slurry 13C
	Actinobacteriota	Microlunatus	2.19	P0 Grazed 13C
	Acidobacteriota	Acidipila	-2.50	P0 Slurry 13C
	Proteobacteria	Ellin6055	3.79	P0 Grazed 13C
	Crenarchaeota	Candidatus Nitrosotalea	-9.58	P0 Slurry 13C
	Bacteroidota	Terrimonas	1.61	P0 Grazed 13C
	Firmicutes	Clostridium sensu stricto 1	-9.48	P0 Slurry 13C
	Proteobacteria	alphaI cluster	1.52	P0 Grazed 13C
	Planctomycetota	Schlesneria	-1.55	P0 Slurry 13C
	Acidobacteriota	Candidatus Koribacter	-3.41	P0 Slurry 13C
	Proteobacteria	Sideroxydans	-9.26	P0 Slurry 13C
	Proteobacteria	Microvirga	4.08	P0 Grazed 13C
	Proteobacteria	Nordella	7.93	P0 Grazed 13C
	Acidobacteriota	Granulicella	-6.39	P0 Slurry 13C

	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-8.00	P0 Slurry 13C
	Verrucomicrobiota	Luteolibacter	2.57	P0 Grazed 13C
	Bacteroidota	Aurantisolimonas	1.79	P0 Grazed 13C
	Actinobacteriota	Rhizocola	7.30	P0 Grazed 13C
	Acidobacteriota	Bryobacter	-1.30	P0 Slurry 13C
	Proteobacteria	Phenylobacterium	-1.23	P0 Slurry 13C
	Firmicutes	Lysinibacillus	-2.82	P0 Slurry 13C
	Planctomycetota	Gemmata	-0.34	P0 Slurry 13C
	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.87	P0 Grazed 13C
	Verrucomicrobiota	Roseimicrobium	1.38	P0 Grazed 13C
	Proteobacteria	Duganella	-4.09	P0 Slurry 13C
	Proteobacteria	Asticcacaulis	-2.47	P0 Slurry 13C
	Planctomycetota	SH-PL14	1.91	P0 Grazed 13C
	Actinobacteriota	Lysinimonas	-2.94	P0 Slurry 13C
	Planctomycetota	Singulisphaera	-2.08	P0 Slurry 13C
	Firmicutes	Clostridium sensu stricto 13	-3.37	P0 Slurry 13C
	Bdellovibrionota	Bdellovibrio	-1.30	P0 Slurry 13C
	Bacteroidota	Cytophaga	-2.64	P0 Slurry 13C
	Acidobacteriota	Terracidiphilus	-8.31	P0 Slurry 13C
	Proteobacteria	Acidicaldus	-4.54	P0 Slurry 13C
	Planctomycetota	Candidatus Nostocoida	-9.22	P0 Slurry 13C
	Proteobacteria	Polaromonas	6.43	P0 Grazed 13C
	Firmicutes	Turicibacter	-7.06	P0 Slurry 13C

	Acidobacteriota	Stenotrophobacter	7.40	P0 Grazed 13C
	Proteobacteria	Uliginosibacterium	-1.62	P0 Slurry 13C
	Planctomycetota	Tundrisphaera	-3.36	P0 Slurry 13C
	Myxococcota	KD3-10	-2.85	P0 Slurry 13C
	Proteobacteria	Phyllobacterium	7.27	P0 Grazed 13C
	Actinobacteriota	Luedemannella	-6.59	P0 Slurry 13C
	Firmicutes	Sporosarcina	-6.62	P0 Slurry 13C
	Proteobacteria	Polycyclovorans	6.19	P0 Grazed 13C
	Desulfobacterota	Citrifermentans	-8.03	P0 Slurry 13C
	Chloroflexi	Litorilinea	4.56	P0 Grazed 13C
	Firmicutes	Ammoniphilus	-4.16	P0 Slurry 13C
	Proteobacteria	Rhizomicrobium	-7.86	P0 Slurry 13C
	Bacteroidota	Ohtaekwangia	6.73	P0 Grazed 13C
	Proteobacteria	Plot4-2H12	-6.68	P0 Slurry 13C
	Proteobacteria	Mesorhizobium	-3.16	P0 Slurry 13C
	Proteobacteria	Rudaea	-8.02	P0 Slurry 13C
	Actinobacteriota	Rugosimonospora	-6.47	P0 Slurry 13C
	Actinobacteriota	Kineosporia	-6.97	P0 Slurry 13C
	Acidobacteriota	Subgroup 10	1.28	P0 Grazed 13C
	Proteobacteria	BD1-7 clade	5.77	P0 Grazed 13C
	Firmicutes	Clostridium sensu stricto 9	-7.02	P0 Slurry 13C
	Bdellovibrionota	OM27 clade	5.03	P0 Grazed 13C
	Actinobacteriota	Catenulispora	-6.73	P0 Slurry 13C
	Verrucomicrobiota	FukuN18 freshwater group	-7.06	P0 Slurry 13C
	Proteobacteria	Amaricoccus	7.25	P0 Grazed 13C
	Abditibacteriota	Abditibacterium	-4.95	P0 Slurry 13C
	Verrucomicrobiota	Oikopleura	6.80	P0 Grazed 13C

	Proteobacteria	Bosea	6.09	P0 Grazed 13C
	Proteobacteria	Micropepsis	-7.16	P0 Slurry 13C
	Verrucomicrobiota	SH3-11	2.56	P0 Grazed 13C
	Proteobacteria	Afipia	-7.60	P0 Slurry 13C
	Bacteroidota	Sporocytophaga	-4.27	P0 Slurry 13C
	Planctomycetota	AKYG587	5.89	P0 Grazed 13C
	Chloroflexi	Thermosporothrix	-6.27	P0 Slurry 13C
	Bacteroidota	Taibaiella	-6.98	P0 Slurry 13C
	Actinobacteriota	Phycicoccus	-6.80	P0 Slurry 13C
	Proteobacteria	Sulfuritalea	-6.62	P0 Slurry 13C
	Actinobacteriota	Longivirga	-3.66	P0 Slurry 13C
	Planctomycetota	Paludisphaera	-6.98	P0 Slurry 13C
	Verrucomicrobiota	Prosthecobacter	-5.42	P0 Slurry 13C
	Firmicutes	Tumebacillus	-6.02	P0 Slurry 13C
	Proteobacteria	Inquilinus	-7.01	P0 Slurry 13C
	Firmicutes	Fonticella	-6.96	P0 Slurry 13C
	Actinobacteriota	Blastococcus	-5.63	P0 Slurry 13C
	Actinobacteriota	Streptacidiphilus	-6.26	P0 Slurry 13C
	Actinobacteriota	Arthrobacter	-5.70	P0 Slurry 13C
	Patescibacteria	TM7a	-6.46	P0 Slurry 13C
	Proteobacteria	Nevskia	-5.55	P0 Slurry 13C
	Chloroflexi	Ktedonobacter	-5.74	P0 Slurry 13C
	Myxococcota	Vulgatibacter	-5.39	P0 Slurry 13C
	Proteobacteria	Rhodobacter	-5.53	P0 Slurry 13C
	Planctomycetota	Pir2 lineage	-6.73	P0 Slurry 13C
	Bacteroidota	Paludibacter	-5.67	P0 Slurry 13C

Table S56: Deseq2 analysis of P0 Slurry 13C vs P30 Slurry 13C.

P0 Slurry 13C vs P30 Slurry 13C	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	Pedomicrobium	0.64	P0 Slurry 13C
	Spirochaetota	Spirochaeta 2	-0.70	P30 Slurry 13C
	Planctomycetota	Candidatus Nostocoida	8.59	P0 Slurry 13C
	Bacteroidota	Mucilaginibacter	-0.89	P30 Slurry 13C
	Acidobacteriota	Vicinamibacter	1.33	P0 Slurry 13C
	Proteobacteria	Ellin6055	1.09	P0 Slurry 13C
	Proteobacteria	Pseudomonas	1.17	P0 Slurry 13C

Table S57: Deseq2 analysis of P30 Slurry Control vs P0 Slurry Control.

P30 Slurry Control vs P0 Slurry Control	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	-0.52	P0 Slurry Control
	Proteobacteria	Pseudolabrys	-0.25	P0 Slurry Control
	Proteobacteria	Rhodomicrobium	-0.53	P0 Slurry Control
	Proteobacteria	GOUTA6	0.43	P30 Slurry Control
	Proteobacteria	Bradyrhizobium	-0.39	P0 Slurry Control
	Bacteroidota	Puia	-0.55	P0 Slurry Control
	Actinobacteriota	Nocardioides	0.29	P30 Slurry Control

	Proteobacteria	MND1	0.46	P30 Slurry Control
	Proteobacteria	Pedomicrobium	0.90	P30 Slurry Control
	Acidobacteriota	Luteitalea	0.70	P30 Slurry Control
	Spirochaetota	Spirochaeta 2	-0.78	P0 Slurry Control
	Proteobacteria	Devosia	-0.36	P0 Slurry Control
	Proteobacteria	Rhodoplanes	0.51	P30 Slurry Control
	Chloroflexi	UTCFX1	0.49	P30 Slurry Control
	Proteobacteria	Candidatus Alysiosphaera	0.50	P30 Slurry Control
	Bacteroidota	Parafilimonas	-0.76	P0 Slurry Control
	Actinobacteriota	Ilumatobacter	0.70	P30 Slurry Control
	Proteobacteria	Arenimonas	0.50	P30 Slurry Control
	Verrucomicrobiota	Ellin516	-0.59	P0 Slurry Control
	Verrucomicrobiota	Opitutus	-0.47	P0 Slurry Control
	Bacteroidota	Mucilaginibacter	-0.94	P0 Slurry Control
	Acidobacteriota	Vicinamibacter	1.09	P30 Slurry Control
	Proteobacteria	Ellin6055	1.45	P30 Slurry Control
	Proteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	-1.42	P0 Slurry Control
	Myxococcota	Pajaroellobacter	-0.68	P0 Slurry Control
	Actinobacteriota	Rhizocola	0.92	P30 Slurry Control
	Bacteroidota	Chitinophaga	-3.55	P0 Slurry Control
	Planctomycetota	SM1A02	0.84	P30 Slurry Control
	Proteobacteria	IS-44	5.34	P30 Slurry Control
	Sumerlaeota	Sumerlaea	5.34	P30 Slurry Control

Table S58: Deseq2 analysis of P0 Grazed Control vs P30 Grazed Control.

P0 Grazed Control vs P30 Grazed Control	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	Pseudolabrys	0.44	P0 Grazed Control
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.87	P30 Grazed Control
	Planctomycetota	Pir4 lineage	0.94	P0 Grazed Control
	Bacteroidota	Puia	-0.94	P30 Grazed Control
	Nitrospirota	Nitrospira	2.47	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrocosmicus	0.40	P0 Grazed Control
	Proteobacteria	GOUTA6	1.63	P0 Grazed Control
	Proteobacteria	Bradyrhizobium	0.66	P0 Grazed Control
	Proteobacteria	Rhodanobacter	-3.31	P30 Grazed Control
	Proteobacteria	Roseiarcus	-0.94	P30 Grazed Control
	Proteobacteria	Pedomicrobium	1.18	P0 Grazed Control
	Spirochaetota	Spirochaeta 2	-0.89	P30 Grazed Control
	Acidobacteriota	Occallatibacter	-1.13	P30 Grazed Control
	Proteobacteria	Acidibacter	0.68	P0 Grazed Control
	Proteobacteria	Sphingomonas	-1.32	P30 Grazed Control
	Proteobacteria	Rhodoplanes	0.66	P0 Grazed Control
	Chloroflexi	UTCFX1	3.77	P0 Grazed Control
	Desulfobacterota	Geobacter	1.86	P0 Grazed Control
	Verrucomicrobiota	Lacunisphaera	-0.97	P30 Grazed Control
	Proteobacteria	mle1-7	1.49	P0 Grazed Control

	Proteobacteria	Candidatus Alysiosphaera	1.23	P0 Grazed Control
	Planctomycetota	Zavarzinella	0.94	P0 Grazed Control
	Actinobacteriota	Actinocorallia	0.99	P0 Grazed Control
	Actinobacteriota	Ilumatobacter	1.47	P0 Grazed Control
	Proteobacteria	Arenimonas	0.85	P0 Grazed Control
	Bacteroidota	Mucilaginibacter	-1.71	P30 Grazed Control
	Bacteroidota	Flavobacterium	0.72	P0 Grazed Control
	Proteobacteria	Bauldia	1.21	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrososphaera	4.17	P0 Grazed Control
	Actinobacteriota	Nakamurella	0.91	P0 Grazed Control
	Proteobacteria	Devosia	-1.09	P30 Grazed Control
	Acidobacteriota	RB41	0.83	P0 Grazed Control
	Myxococcota	Anaeromyxobacter	0.99	P0 Grazed Control
	Actinobacteriota	Microlunatus	1.68	P0 Grazed Control
	Planctomycetota	Pirellula	1.08	P0 Grazed Control
	Fibrobacterota	possible genus 04	-1.35	P30 Grazed Control
	Acidobacteriota	Holophaga	-11.78	P30 Grazed Control
	Acidobacteriota	Luteitalea	0.66	P0 Grazed Control
	Bacteroidota	Sediminibacterium	-1.30	P30 Grazed Control
	Gemmatimonadota	Gemmatimonas	-0.84	P30 Grazed Control
	Bacteroidota	Heliimonas	-1.26	P30 Grazed Control
	Actinobacteriota	Pseudonocardia	0.93	P0 Grazed Control

	Planctomycetota	Gemmata	0.55	P0 Grazed Control
	Crenarchaeota	Candidatus Nitrosotalea	-1.19	P30 Grazed Control
	Planctomycetota	Schlesneria	-0.70	P30 Grazed Control
	Bacteroidota	Terrimonas	2.27	P0 Grazed Control
	Acidobacteriota	Granulicella	-4.64	P30 Grazed Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-2.17	P30 Grazed Control
	Verrucomicrobiota	Ellin517	2.08	P0 Grazed Control
	Proteobacteria	MND1	1.99	P0 Grazed Control
	Planctomycetota	Fimbriiglobus	0.85	P0 Grazed Control
	Proteobacteria	Phenylobacterium	-1.01	P30 Grazed Control
	Firmicutes	Lysinibacillus	-1.30	P30 Grazed Control
	Proteobacteria	Parablastomonas	5.86	P0 Grazed Control
	Acidobacteriota	Candidatus Koribacter	-0.50	P30 Grazed Control
	Proteobacteria	Asticcacaulis	-1.68	P30 Grazed Control
	Bdellovibrionota	Bdellovibrio	-1.12	P30 Grazed Control
	Bacteroidota	Cytophaga	-1.77	P30 Grazed Control
	Acidobacteriota	Terracidiphilus	-2.43	P30 Grazed Control
	Bacteroidota	Niastella	-0.93	P30 Grazed Control

	Proteobacteria	Uliginosibacterium	-3.01	P30 Grazed Control
	Myxococcota	KD3-10	-1.01	P30 Grazed Control
	Actinobacteriota	Luedemannella	6.77	P0 Grazed Control
	Proteobacteria	Altererythrobacter	-2.18	P30 Grazed Control
	Actinobacteriota	Nocardia	2.73	P0 Grazed Control
	Proteobacteria	Rudaea	-3.63	P30 Grazed Control
	Chloroflexi	Anaerolinea	2.63	P0 Grazed Control
	Planctomycetota	Phycisphaera	-1.63	P30 Grazed Control
	Verrucomicrobiota	FukuN18 freshwater group	-3.24	P30 Grazed Control
	Abditibacteriota	Abditibacterium	-1.50	P30 Grazed Control
	Bacteroidota	UTBCD1	7.37	P0 Grazed Control
	Actinobacteriota	CL500-29 marine group	2.95	P0 Grazed Control
	Proteobacteria	Micropepsis	-6.03	P30 Grazed Control
	Actinobacteriota	Hamadaea	6.30	P0 Grazed Control
	Chloroflexi	Thermosporothrix	-3.75	P30 Grazed Control
	Proteobacteria	Inquilinus	-7.10	P30 Grazed Control
	Proteobacteria	Tahibacter	5.55	P0 Grazed Control
	Proteobacteria	Acidisoma	-7.39	P30 Grazed Control
	Firmicutes	Solibacillus	-6.19	P30 Grazed Control

	Bacteroidota	Fluviicola	5.55	P0 Grazed Control

Table S59: Deseq2 analysis of P30 Cut Control vs P30 Slurry Control.

P30 Cut Control vs P30 Slurry Control	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	-0.40	P30 Slurry Control
	Planctomycetota	Pir4 lineage	0.25	P30 Cut Control
	Crenarchaeota	Candidatus Nitrocosmicus	0.59	P30 Cut Control
	Proteobacteria	Rhodomicrobium	-0.49	P30 Slurry Control
	Bacteroidota	Puia	-0.58	P30 Slurry Control
	Actinobacteriota	Nocardioides	0.39	P30 Cut Control
	Proteobacteria	Rhodanobacter	-1.07	P30 Slurry Control
	Proteobacteria	MND1	0.45	P30 Cut Control
	Proteobacteria	Pedomicrobium	0.53	P30 Cut Control
	Acidobacteriota	Luteitalea	0.56	P30 Cut Control
	Acidobacteriota	Occallatibacter	-1.25	P30 Slurry Control
	Bacteroidota	OLB12	-0.42	P30 Slurry Control
	Actinobacteriota	Acidothermus	-0.72	P30 Slurry Control
	Proteobacteria	Rhodoplanes	0.35	P30 Cut Control
	Chloroflexi	UTCFX1	0.57	P30 Cut Control
	Verrucomicrobiota	Lacunisphaera	-0.37	P30 Slurry Control
	Actinobacteriota	Streptomyces	0.42	P30 Cut Control
	Verrucomicrobiota	ADurb.Bin063-1	-0.57	P30 Slurry Control
	Bacteroidota	Parafilimonas	-0.69	P30 Slurry Control
	Proteobacteria	Pseudorhodoplanes	0.79	P30 Cut Control
	Actinobacteriota	Ilumatobacter	0.80	P30 Cut Control

	Verrucomicrobiota	Ellin516	-0.78	P30 Slurry Control
	Planctomycetota	Pirellula	0.27	P30 Cut Control
	Bacteroidota	Mucilaginibacter	-0.90	P30 Slurry Control
	Acidobacteriota	Vicinamibacter	0.79	P30 Cut Control
	Bacteroidota	Chryseolinea	0.83	P30 Cut Control
	Myxococcota	Sandaracinus	-0.59	P30 Slurry Control
	Bacteroidota	Ferruginibacter	-0.46	P30 Slurry Control
	Bacteroidota	Heliimonas	-1.86	P30 Slurry Control
	Acidobacteriota	Bryobacter	-0.51	P30 Slurry Control
	Acidobacteriota	Acidipila	-1.20	P30 Slurry Control
	Proteobacteria	Ellin6055	1.37	P30 Cut Control
	Proteobacteria	Microvirga	0.67	P30 Cut Control
	Proteobacteria	Nordella	0.90	P30 Cut Control
	Actinobacteriota	Rhizocola	0.99	P30 Cut Control
	Actinobacteriota	Lysinimonas	-2.90	P30 Slurry Control
	Bacteroidota	Cytophaga	-1.08	P30 Slurry Control
	Bacteroidota	Ohtaekwangia	1.15	P30 Cut Control
	Proteobacteria	Amaricoccus	2.86	P30 Cut Control
	Proteobacteria	Rudaea	-5.86	P30 Slurry Control
	Proteobacteria	IS-44	5.93	P30 Cut Control

Table S60: Deseq2 analysis of P30 Cut 13C vs P30 Slurry 13C.

P30 Cut 13C vs P30 Slurry 13C	Phylum	Genus	log2FoldChange	Enriched
	Nitrospirota	Nitrospira	0.52	P30 Cut 13C
	Proteobacteria	Rhodanobacter	-0.63	P30 Slurry 13C
	Proteobacteria	Pedomicrobium	0.89	P30 Cut 13C
	Spirochaetota	Spirochaeta 2	-0.81	P30 Slurry 13C
	Acidobacteriota	Occallatibacter	-1.83	P30 Slurry 13C
	Verrucomicrobiota	Lacunisphaera	-0.77	P30 Slurry 13C
	Planctomycetota	Zavarzinella	-0.76	P30 Slurry 13C
	Actinobacteriota	Ilumatobacter	0.79	P30 Cut 13C
	Verrucomicrobiota	Ellin516	-0.64	P30 Slurry 13C
	Bacteroidota	Mucilaginibacter	-1.60	P30 Slurry 13C
	Acidobacteriota	Vicinamibacter	1.06	P30 Cut 13C
	Bacteroidota	Chryseolinea	0.93	P30 Cut 13C
	Fibrobacterota	possible genus 04	-0.87	P30 Slurry 13C
	Bacteroidota	Sediminibacterium	-1.13	P30 Slurry 13C
	Bacteroidota	Heliimonas	-2.26	P30 Slurry 13C
	Proteobacteria	Ellin6055	1.35	P30 Cut 13C
	Acidobacteriota	Candidatus Koribacter	-1.55	P30 Slurry 13C
	Actinobacteriota	Lysinimonas	-7.15	P30 Slurry 13C
	Proteobacteria	Sideroxydans	-7.02	P30 Slurry 13C
	Bacteroidota	Cytophaga	-2.68	P30 Slurry 13C
	Acidobacteriota	Paludibaculum	1.29	P30 Cut 13C
	Proteobacteria	Legionella	-6.90	P30 Slurry 13C

Table S61: Deseq2 analysis of P30 Cut Control vs P30 Grazed Control.

P30 Cut Control vs P30 Grazed Control	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	0.25	P30 Cut Control
	Proteobacteria	Pseudolabrys	0.28	P30 Cut Control
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.99	P30 Grazed Control
	Planctomycetota	Pir4 lineage	0.46	P30 Cut Control
	Actinobacteriota	Gaiella	-0.41	P30 Grazed Control
	Bacteroidota	Puia	0.37	P30 Cut Control
	Proteobacteria	Rhodomicrobium	0.68	P30 Cut Control
	Proteobacteria	Bradyrhizobium	0.47	P30 Cut Control
	Planctomycetota	Aquisphaera	1.19	P30 Cut Control
	Proteobacteria	MND1	-0.85	P30 Grazed Control
	Proteobacteria	Roseiarcus	1.09	P30 Cut Control
	Bacteroidota	OLB12	0.94	P30 Cut Control
	Proteobacteria	Pedomicrobium	-0.85	P30 Grazed Control
	Acidobacteriota	Luteitalea	-1.36	P30 Grazed Control
	Acidobacteriota	Occallatibacter	1.48	P30 Cut Control
	Proteobacteria	Rhodoblastus	0.99	P30 Cut Control
	Bacteroidota	Flavobacterium	-0.85	P30 Grazed Control
	Acidobacteriota	Candidatus Solibacter	1.11	P30 Cut Control
	Proteobacteria	Dongia	-1.29	P30 Grazed Control
	Actinobacteriota	Nakamurella	0.66	P30 Cut Control

	Verrucomicrobiota	Chthoniobacter	-1.23	P30 Grazed Control
	Actinobacteriota	Acidothermus	1.87	P30 Cut Control
	Bacteroidota	Parafilimonas	0.68	P30 Cut Control
	Proteobacteria	Acidibacter	-0.51	P30 Grazed Control
	Chloroflexi	UTCFX1	-2.17	P30 Grazed Control
	Desulfobacterota	Geobacter	-0.45	P30 Grazed Control
	Verrucomicrobiota	Lacunisphaera	-0.44	P30 Grazed Control
	Proteobacteria	Candidatus Alysiosphaera	-0.97	P30 Grazed Control
	Proteobacteria	Pseudorhodoplanes	-4.62	P30 Grazed Control
	Actinobacteriota	Conexibacter	0.88	P30 Cut Control
	Actinobacteriota	Actinocorallia	-1.07	P30 Grazed Control
	Actinobacteriota	Ilumatobacter	-1.44	P30 Grazed Control
	Verrucomicrobiota	Opitutus	-0.64	P30 Grazed Control
	Bacteroidota	Edaphobaculum	0.88	P30 Cut Control
	Crenarchaeota	Candidatus Nitrososphaera	-1.70	P30 Grazed Control
	Actinobacteriota	Microlunatus	-1.01	P30 Grazed Control
	Planctomycetota	Pirellula	-0.34	P30 Grazed Control
	Verrucomicrobiota	Ellin517	-1.19	P30 Grazed Control

	Bacteroidota	Ferruginibacter	-0.73	P30 Grazed Control
	Bacteroidota	Chryseolinea	-5.88	P30 Grazed Control
	Proteobacteria	Dokdonella	0.98	P30 Cut Control
	Acidobacteriota	Bryobacter	1.03	P30 Cut Control
	Acidobacteriota	Acidipila	1.29	P30 Cut Control
	Actinobacteriota	Pseudonocardia	0.93	P30 Cut Control
	Proteobacteria	Ellin6055	-4.29	P30 Grazed Control
	Crenarchaeota	Candidatus Nitrosotalea	7.71	P30 Cut Control
	Planctomycetota	Schlesneria	0.93	P30 Cut Control
	Proteobacteria	Microvirga	-1.42	P30 Grazed Control
	Proteobacteria	Nordella	-7.43	P30 Grazed Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	6.81	P30 Cut Control
	Acidobacteriota	Vicinamibacter	-1.13	P30 Grazed Control
	Proteobacteria	Defluviicoccus	-0.78	P30 Grazed Control
	Planctomycetota	Singulisphaera	1.42	P30 Cut Control
	Actinobacteriota	Rhizocola	-7.37	P30 Grazed Control
	Firmicutes	Lysinibacillus	2.94	P30 Cut Control
	Acidobacteriota	Geothrix	3.05	P30 Cut Control
	Acidobacteriota	Candidatus Koribacter	2.38	P30 Cut Control

	Actinobacteriota	Rhodococcus	3.08	P30 Cut Control
	Firmicutes	Clostridium sensu stricto 13	1.78	P30 Cut Control
	Acidobacteriota	Terracidiphilus	6.68	P30 Cut Control
	Proteobacteria	Acidicaldus	1.88	P30 Cut Control
	Actinobacteriota	Terrabacter	-6.15	P30 Grazed Control
	Proteobacteria	Polaromonas	-6.17	P30 Grazed Control
	Firmicutes	Turicibacter	7.48	P30 Cut Control
	Acidobacteriota	Stenotrophobacter	-6.45	P30 Grazed Control
	Planctomycetota	SH-PL14	-1.58	P30 Grazed Control
	Proteobacteria	Rhizomicrobium	9.22	P30 Cut Control
	Actinobacteriota	Lechevalieria	-7.45	P30 Grazed Control
	Firmicutes	Sporosarcina	7.43	P30 Cut Control
	Proteobacteria	Polycyclovorans	-6.95	P30 Grazed Control
	Firmicutes	Romboutsia	6.61	P30 Cut Control
	Firmicutes	Ammoniphilus	6.87	P30 Cut Control
	Bacteroidota	Ohtaekwangia	-2.80	P30 Grazed Control
	Actinobacteriota	Rugosimonospora	6.61	P30 Cut Control
	Actinobacteriota	Kineosporia	6.59	P30 Cut Control
	Proteobacteria	Pseudoduganella	-6.38	P30 Grazed Control
	Chloroflexi	Anaerolinea	3.09	P30 Cut Control
	Bdellovibrionota	OM27 clade	-7.46	P30 Grazed Control

	Actinobacteriota	Arthrobacter	7.40	P30 Cut Control
	Bacteroidota	UTBCD1	8.05	P30 Cut Control
	Proteobacteria	Nevskia	4.83	P30 Cut Control
	Euryarchaeota	Methanobacterium	6.88	P30 Cut Control
	Proteobacteria	Afipia	6.15	P30 Cut Control
	Verrucomicrobiota	Oikopleura	-6.49	P30 Grazed Control
	Proteobacteria	Novosphingobium	-5.79	P30 Grazed Control
	Armatimonadota	Chthonomonas	6.03	P30 Cut Control
	Proteobacteria	Candidatus Ovatusbacter	-5.91	P30 Grazed Control

Table S62: Deseq2 analysis of P30 Cut 13C vs P30 Grazed 13C.

P30 Cut 13C vs P30 Grazed 13C	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	0.31	P30 Cut 13C
	Proteobacteria	Pseudolabrys	0.28	P30 Cut 13C
	Verrucomicrobiota	Candidatus Xiphinematobacter	-1.27	P30 Grazed 13C
	Actinobacteriota	Mycobacterium	0.44	P30 Cut 13C
	Bacteroidota	Puia	0.49	P30 Cut 13C

	Crenarchaeota	Candidatus Nitrocosmicus	-0.89	P30 Grazed 13C
	Proteobacteria	Rhodomicrobium	0.53	P30 Cut 13C
	Proteobacteria	GOUTA6	0.59	P30 Cut 13C
	Actinobacteriota	Nocardioides	-0.77	P30 Grazed 13C
	Planctomycetota	Aquisphaera	0.99	P30 Cut 13C
	Proteobacteria	MND1	-0.70	P30 Grazed 13C
	Proteobacteria	Roseiarcus	1.04	P30 Cut 13C
	Acidobacteriota	Luteitalea	-0.72	P30 Grazed 13C
	Acidobacteriota	Occallatibacter	1.96	P30 Cut 13C
	Proteobacteria	Rhodoblastus	0.97	P30 Cut 13C
	Bacteroidota	Flavobacterium	-0.87	P30 Grazed 13C
	Acidobacteriota	Candidatus Solibacter	1.24	P30 Cut 13C
	Proteobacteria	Dongia	-1.73	P30 Grazed 13C
	Verrucomicrobiota	Chthoniobacter	-0.99	P30 Grazed 13C
	Actinobacteriota	Acidothermus	1.45	P30 Cut 13C
	Bacteroidota	Parafilimonas	0.57	P30 Cut 13C
	Proteobacteria	Acidibacter	-0.71	P30 Grazed 13C
	Proteobacteria	Rhodoplanes	-0.56	P30 Grazed 13C
	Chloroflexi	UTCFX1	-2.38	P30 Grazed 13C
	Actinobacteriota	Iamia	-0.55	P30 Grazed 13C
	Actinobacteriota	Nakamurella	0.99	P30 Cut 13C
	Verrucomicrobiota	ADurb.Bin063-1	0.66	P30 Cut 13C
	Proteobacteria	Candidatus Alysiosphaera	-1.43	P30 Grazed 13C
	Proteobacteria	Pseudorhodoplanes	-4.24	P30 Grazed 13C
	Actinobacteriota	Solirubrobacter	-0.59	P30 Grazed 13C
	Actinobacteriota	Ilumatobacter	-2.57	P30 Grazed 13C

	Verrucomicrobiota	Opitutus	-0.79	P30 Grazed 13C
	Bacteroidota	Edaphobaculum	0.58	P30 Cut 13C
	Planctomycetota	Pirellula	-0.46	P30 Grazed 13C
	Crenarchaeota	Candidatus Nitrososphaera	-1.71	P30 Grazed 13C
	Actinobacteriota	Conexibacter	0.60	P30 Cut 13C
	Bdellovibrionota	Bdellovibrio	0.62	P30 Cut 13C
	Proteobacteria	Cellvibrio	2.78	P30 Cut 13C
	Actinobacteriota	Rhodococcus	5.14	P30 Cut 13C
	Actinobacteriota	Microlunatus	-0.71	P30 Grazed 13C
	Bacteroidota	Chryseolinea	-8.89	P30 Grazed 13C
	Acidobacteriota	Bryobacter	1.35	P30 Cut 13C
	Acidobacteriota	Acidipila	1.06	P30 Cut 13C
	Proteobacteria	Ellin6055	-7.58	P30 Grazed 13C
	Crenarchaeota	Candidatus Nitrosotalea	8.91	P30 Cut 13C
	Proteobacteria	Steroidobacter	-0.74	P30 Grazed 13C
	Proteobacteria	Sideroxydans	2.14	P30 Cut 13C
	Proteobacteria	Microvirga	-1.07	P30 Grazed 13C
	Proteobacteria	Pseudomonas	1.24	P30 Cut 13C
	Proteobacteria	Nordella	-4.48	P30 Grazed 13C
	Proteobacteria	Altererythrobacter	-2.53	P30 Grazed 13C
	Bacteroidota	Aurantisolimonas	-0.90	P30 Grazed 13C
	Planctomycetota	Singulisphaera	1.24	P30 Cut 13C
	Myxococcota	Pajaroellobacter	-1.20	P30 Grazed 13C
	Actinobacteriota	Rhizocola	-7.14	P30 Grazed 13C
	Acidobacteriota	Geothrix	1.81	P30 Cut 13C
	Proteobacteria	Parablastomonas	0.88	P30 Cut 13C

	Acidobacteriota	Candidatus Koribacter	2.45	P30 Cut 13C
	Proteobacteria	Duganella	6.90	P30 Cut 13C
	Actinobacteriota	Microbacterium	-1.12	P30 Grazed 13C
	Proteobacteria	Asticcacaulis	1.72	P30 Cut 13C
	Actinobacteriota	Demequina	-1.32	P30 Grazed 13C
	Planctomycetota	SH-PL14	-1.69	P30 Grazed 13C
	Acidobacteriota	Terracidiphilus	7.10	P30 Cut 13C
	Proteobacteria	Acidicaldus	2.15	P30 Cut 13C
	Proteobacteria	Tahibacter	-6.90	P30 Grazed 13C
	Verrucomicrobiota	Roseimicrobium	-1.05	P30 Grazed 13C
	Actinobacteriota	Terrabacter	-7.56	P30 Grazed 13C
	Acidobacteriota	JGI 0001001-H03	1.32	P30 Cut 13C
	Proteobacteria	Polaromonas	-6.37	P30 Grazed 13C
	Firmicutes	Turicibacter	7.15	P30 Cut 13C
	Acidobacteriota	Stenotrophobacter	-6.50	P30 Grazed 13C
	Proteobacteria	Rhizomicrobium	8.82	P30 Cut 13C
	Proteobacteria	Phyllobacterium	-6.09	P30 Grazed 13C
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	5.52	P30 Cut 13C
	Actinobacteriota	Lechevalieria	-5.41	P30 Grazed 13C
	Actinobacteriota	Agromyces	-5.73	P30 Grazed 13C
	Proteobacteria	Polycyclovorans	-6.34	P30 Grazed 13C
	Desulfobacterota	Citrifermentans	3.74	P30 Cut 13C
	Firmicutes	Ammoniphilus	6.20	P30 Cut 13C
	Bacteroidota	Ohtaekwangia	-3.78	P30 Grazed 13C
	Actinobacteriota	Rugosimonospora	6.46	P30 Cut 13C
	Actinobacteriota	Kineosporia	6.78	P30 Cut 13C

	Proteobacteria	Amaricoccus	-6.85	P30 Grazed 13C
	Firmicutes	Clostridium sensu stricto 9	5.93	P30 Cut 13C
	Actinobacteriota	Arthrobacter	6.87	P30 Cut 13C
	Actinobacteriota	Angustibacter	5.86	P30 Cut 13C
	Bacteroidota	UTBCD1	7.96	P30 Cut 13C
	Proteobacteria	Nevskia	6.97	P30 Cut 13C
	Patescibacteria	TM7a	7.23	P30 Cut 13C
	Planctomycetota	Pir2 lineage	7.31	P30 Cut 13C
	Spirochaetota	Spirochaeta	2.71	P30 Cut 13C
	Verrucomicrobiota	Oikopleura	-6.51	P30 Grazed 13C
	Bdellovibrionota	OM27 clade	-6.76	P30 Grazed 13C

Table S63: Deseq2 analysis of P30 Grazed 13C vs P30 Slurry 13C.

P30 Grazed 13C vs P30 Slurry 13C	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Verrucomicrobiota	Candidatus Udaeobacter	-0.53	P30 Slurry 13C
	Verrucomicrobiota	Candidatus Xiphinematobacter	1.33	P30 Grazed 13C
	Actinobacteriota	Gaiella	0.38	P30 Grazed 13C
	Bacteroidota	Puia	-0.86	P30 Slurry 13C
	Nitrospirota	Nitrospira	0.95	P30 Grazed 13C
	Crenarchaeota	Candidatus Nitrocosmicus	1.03	P30 Grazed 13C
	Actinobacteriota	Nocardioides	0.81	P30 Grazed 13C
	Planctomycetota	Aquisphaera	-1.16	P30 Slurry 13C

	Proteobacteria	Rhodanobacter	-0.93	P30 Slurry 13C
	Bacteroidota	OLB12	-1.39	P30 Slurry 13C
	Proteobacteria	MND1	1.02	P30 Grazed 13C
	Proteobacteria	Roseiarcus	-1.29	P30 Slurry 13C
	Proteobacteria	Pedomicrobium	1.61	P30 Grazed 13C
	Spirochaetota	Spirochaeta 2	-1.56	P30 Slurry 13C
	Acidobacteriota	Occallatibacter	-3.78	P30 Slurry 13C
	Proteobacteria	Acidibacter	0.45	P30 Grazed 13C
	Acidobacteriota	Candidatus Solibacter	-1.27	P30 Slurry 13C
	Proteobacteria	Sphingomonas	-0.62	P30 Slurry 13C
	Proteobacteria	Dongia	1.40	P30 Grazed 13C
	Verrucomicrobiota	Chthoniobacter	1.01	P30 Grazed 13C
	Actinobacteriota	Acidothermus	-2.04	P30 Slurry 13C
	Proteobacteria	Rhodoplanes	0.90	P30 Grazed 13C
	Chloroflexi	UTCFX1	2.74	P30 Grazed 13C
	Desulfobacterota	Geobacter	0.60	P30 Grazed 13C
	Verrucomicrobiota	Lacunisphaera	-0.76	P30 Slurry 13C
	Actinobacteriota	Iamia	0.47	P30 Grazed 13C
	Actinobacteriota	Nakamurella	-0.84	P30 Slurry 13C
	Acidobacteriota	Luteitalea	1.12	P30 Grazed 13C
	Verrucomicrobiota	ADurb.Bin063-1	-0.91	P30 Slurry 13C
	Proteobacteria	Candidatus Alysiosphaera	1.83	P30 Grazed 13C
	Bacteroidota	Parafilimonas	-1.03	P30 Slurry 13C
	Proteobacteria	Pseudorhodoplanes	4.60	P30 Grazed 13C
	Planctomycetota	Zavarzinella	-0.80	P30 Slurry 13C
	Actinobacteriota	Ilumatobacter	3.37	P30 Grazed 13C
	Verrucomicrobiota	Ellin516	-1.14	P30 Slurry 13C

	Verrucomicrobiota	Opitutus	0.50	P30 Grazed 13C
	Bacteroidota	Flavobacterium	0.95	P30 Grazed 13C
	Planctomycetota	Pirellula	0.75	P30 Grazed 13C
	Crenarchaeota	Candidatus Nitrososphaera	1.50	P30 Grazed 13C
	Actinobacteriota	Conexibacter	-1.31	P30 Slurry 13C
	Bdellovibrionota	Bdellovibrio	-0.98	P30 Slurry 13C
	Proteobacteria	Cellvibrio	-3.37	P30 Slurry 13C
	Actinobacteriota	Rhodococcus	-6.21	P30 Slurry 13C
	Bacteroidota	Mucilaginibacter	-1.69	P30 Slurry 13C
	Myxococcota	Anaeromyxobacter	-0.96	P30 Slurry 13C
	Actinobacteriota	Microlunatus	0.97	P30 Grazed 13C
	Acidobacteriota	Vicinamibacter	1.53	P30 Grazed 13C
	Bacteroidota	Chryseolinea	9.83	P30 Grazed 13C
	Bacteroidota	Sediminibacterium	-1.14	P30 Slurry 13C
	Bacteroidota	Heliimonas	-2.59	P30 Slurry 13C
	Acidobacteriota	Bryobacter	-1.22	P30 Slurry 13C
	Acidobacteriota	Acidipila	-3.36	P30 Slurry 13C
	Proteobacteria	Leptothrix	-1.87	P30 Slurry 13C
	Proteobacteria	Ellin6055	8.93	P30 Grazed 13C
	Crenarchaeota	Candidatus Nitrosotalea	-8.61	P30 Slurry 13C
	Fibrobacterota	possible genus 04	-0.99	P30 Slurry 13C
	Planctomycetota	Gemmata	-0.46	P30 Slurry 13C
	Proteobacteria	Sideroxydans	-9.17	P30 Slurry 13C
	Proteobacteria	Microvirga	1.66	P30 Grazed 13C
	Proteobacteria	Nordella	5.25	P30 Grazed 13C
	Proteobacteria	Altererythrobacter	3.38	P30 Grazed 13C
	Verrucomicrobiota	Luteolibacter	2.24	P30 Grazed 13C

	Planctomycetota	Singulisphaera	-1.35	P30 Slurry 13C
	Proteobacteria	Defluviicoccus	1.49	P30 Grazed 13C
	Myxococcota	Pajaroellobacter	1.01	P30 Grazed 13C
	Actinobacteriota	Rhizocola	7.32	P30 Grazed 13C
	Bacteroidota	Aurantisolimonas	1.18	P30 Grazed 13C
	Acidobacteriota	Geothrix	-3.81	P30 Slurry 13C
	Verrucomicrobiota	Roseimicrobium	1.48	P30 Grazed 13C
	Acidobacteriota	Candidatus Koribacter	-3.97	P30 Slurry 13C
	Actinobacteriota	Microbacterium	1.36	P30 Grazed 13C
	Proteobacteria	Asticcacaulis	-2.89	P30 Slurry 13C
	Planctomycetota	SH-PL14	1.78	P30 Grazed 13C
	Actinobacteriota	Lysinimonas	-6.57	P30 Slurry 13C
	Acidobacteriota	Terracidiphilus	-6.80	P30 Slurry 13C
	Proteobacteria	Tahibacter	6.71	P30 Grazed 13C
	Proteobacteria	Acidicaldus	-3.47	P30 Slurry 13C
	Bacteroidota	Cytophaga	-2.63	P30 Slurry 13C
	Proteobacteria	Polaromonas	6.67	P30 Grazed 13C
	Acidobacteriota	Paludibaculum	1.90	P30 Grazed 13C
	Acidobacteriota	Stenotrophobacter	7.01	P30 Grazed 13C
	Proteobacteria	Rhizomicrobium	-8.53	P30 Slurry 13C
	Proteobacteria	Phyllobacterium	7.51	P30 Grazed 13C
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-5.09	P30 Slurry 13C
	Actinobacteriota	Lechevalieria	6.47	P30 Grazed 13C
	Actinobacteriota	Agromyces	7.40	P30 Grazed 13C
	Proteobacteria	Polycyclovorans	5.74	P30 Grazed 13C
	Desulfobacterota	Citrifermentans	-7.78	P30 Slurry 13C

	Acidobacteriota	JGI 0001001-H03	-4.03	P30 Slurry 13C
	Bacteroidota	Ohtaekwangia	4.20	P30 Grazed 13C
	Actinobacteriota	Rugosimonospora	-6.16	P30 Slurry 13C
	Actinobacteriota	Kineosporia	-6.49	P30 Slurry 13C
	Proteobacteria	Hirschia	6.47	P30 Grazed 13C
	Proteobacteria	Rudaea	-6.00	P30 Slurry 13C
	Proteobacteria	BD1-7 clade	5.79	P30 Grazed 13C
	Firmicutes	Clostridium sensu stricto 9	-5.65	P30 Slurry 13C
	Actinobacteriota	Arthrobacter	-6.58	P30 Slurry 13C
	Proteobacteria	Amaricoccus	7.82	P30 Grazed 13C
	Verrucomicrobiota	Oikopleura	6.64	P30 Grazed 13C
	Bacteroidota	UTBCD1	-7.68	P30 Slurry 13C
	Proteobacteria	Noviherbaspirillum	6.18	P30 Grazed 13C
	Bacteroidota	Solitalea	5.48	P30 Grazed 13C
	Proteobacteria	Nevskia	-6.68	P30 Slurry 13C
	Firmicutes	Clostridium sensu stricto 12	8.15	P30 Grazed 13C
	Proteobacteria	Bosea	6.66	P30 Grazed 13C
	Bacteroidota	Fluviicola	4.25	P30 Grazed 13C
	Abditibacteriota	Abditibacterium	-2.71	P30 Slurry 13C
	Patescibacteria	TM7a	-2.08	P30 Slurry 13C
	Planctomycetota	Pir2 lineage	-2.62	P30 Slurry 13C
	Proteobacteria	Niveibacterium	6.25	P30 Grazed 13C
	Spirochaetota	Spirochaeta	-5.43	P30 Slurry 13C
	Myxococcota	Nannocystis	5.59	P30 Grazed 13C
	Bdellovibrionota	OM27 clade	7.32	P30 Grazed 13C
	Verrucomicrobiota	Candidatus Omnitrophus	5.60	P30 Grazed 13C

	Proteobacteria	Legionella	-6.77	P30 Slurry 13C
	Actinobacteriota	Catenulispora	-6.68	P30 Slurry 13C
	Proteobacteria	Dechloromonas	5.40	P30 Grazed 13C

Table S64: Deseq2 analysis of P30 Grazed Control vs P30 Slurry Control.

P30 Grazed Control vs P30 Slurry Control	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	-0.60	P30 Slurry Control
	Proteobacteria	Pseudolabrys	-0.39	P30 Slurry Control
	Verrucomicrobiota	Candidatus Xiphinematobacter	1.09	P30 Grazed Control
	Actinobacteriota	Gaiella	0.49	P30 Grazed Control
	Bacteroidota	Puia	-0.91	P30 Slurry Control
	Nitrospirota	Nitrospira	1.04	P30 Grazed Control
	Crenarchaeota	Candidatus Nitrocosmicus	0.49	P30 Grazed Control
	Proteobacteria	Rhodomicrobium	-1.12	P30 Slurry Control
	Proteobacteria	Bradyrhizobium	-0.44	P30 Slurry Control
	Actinobacteriota	Nocardioides	0.75	P30 Grazed Control
	Planctomycetota	Aquisphaera	-1.34	P30 Slurry Control
	Proteobacteria	Rhodanobacter	-1.04	P30 Slurry Control
	Proteobacteria	MND1	1.35	P30 Grazed Control
	Proteobacteria	Roseiarcus	-1.34	P30 Slurry Control
	Bacteroidota	OLB12	-1.33	P30 Slurry Control

	Proteobacteria	Pedomicrobium	1.44	P30 Grazed Control
	Spirochaetota	Spirochaeta 2	-0.48	P30 Slurry Control
	Acidobacteriota	Occallatibacter	-2.70	P30 Slurry Control
	Proteobacteria	Rhodoblastus	-1.41	P30 Slurry Control
	Acidobacteriota	RB41	0.51	P30 Grazed Control
	Proteobacteria	Acidibacter	0.49	P30 Grazed Control
	Acidobacteriota	Candidatus Solibacter	-1.29	P30 Slurry Control
	Proteobacteria	Dongia	1.48	P30 Grazed Control
	Actinobacteriota	Nakamurella	-0.82	P30 Slurry Control
	Verrucomicrobiota	Chthoniobacter	1.30	P30 Grazed Control
	Actinobacteriota	Acidothermus	-2.55	P30 Slurry Control
	Bacteroidota	Parafilimonas	-1.33	P30 Slurry Control
	Proteobacteria	Rhodoplanes	0.48	P30 Grazed Control
	Chloroflexi	UTCFX1	2.80	P30 Grazed Control
	Proteobacteria	Hyphomicrobium	-0.39	P30 Slurry Control
	Proteobacteria	mle1-7	0.53	P30 Grazed Control
	Acidobacteriota	Luteitalea	1.97	P30 Grazed Control
	Verrucomicrobiota	ADurb.Bin063-1	-0.73	P30 Slurry Control
	Proteobacteria	Candidatus Alysiosphaera	1.20	P30 Grazed Control

	Proteobacteria	Pseudorhodoplanes	5.52	P30 Grazed Control
	Actinobacteriota	Conexibacter	-1.18	P30 Slurry Control
	Planctomycetota	Zavarzinella	-0.92	P30 Slurry Control
	Actinobacteriota	Actinocorallia	1.13	P30 Grazed Control
	Actinobacteriota	Ilumatobacter	2.29	P30 Grazed Control
	Verrucomicrobiota	Ellin516	-1.07	P30 Slurry Control
	Bacteroidota	Mucilaginibacter	-1.23	P30 Slurry Control
	Bacteroidota	Flavobacterium	0.44	P30 Grazed Control
	Bacteroidota	Edaphobaculum	-0.79	P30 Slurry Control
	Crenarchaeota	Candidatus Nitrososphaera	1.64	P30 Grazed Control
	Myxococcota	Anaeromyxobacter	-0.61	P30 Slurry Control
	Actinobacteriota	Microlunatus	1.14	P30 Grazed Control
	Acidobacteriota	Vicinamibacter	1.98	P30 Grazed Control
	Planctomycetota	Pirellula	0.66	P30 Grazed Control
	Verrucomicrobiota	Ellin517	1.35	P30 Grazed Control
	Bacteroidota	Chryseolinea	6.77	P30 Grazed Control
	Myxococcota	Sandaracinus	-0.91	P30 Slurry Control
	Bacteroidota	Sediminibacterium	-0.83	P30 Slurry Control
	Proteobacteria	Dokdonella	-1.03	P30 Slurry Control
	Bacteroidota	Heliimonas	-2.07	P30 Slurry Control
	Acidobacteriota	Bryobacter	-1.49	P30 Slurry Control

	Acidobacteriota	Acidipila	-2.45	P30 Slurry Control
	Actinobacteriota	Pseudonocardia	-1.00	P30 Slurry Control
	Planctomycetota	Gemmata	-0.45	P30 Slurry Control
	Proteobacteria	Ellin6055	5.89	P30 Grazed Control
	Crenarchaeota	Candidatus Nitrosotalea	-10.33	P30 Slurry Control
	Actinobacteriota	Dactylosporangium	0.98	P30 Grazed Control
	Proteobacteria	Microvirga	2.13	P30 Grazed Control
	Proteobacteria	Nordella	8.38	P30 Grazed Control
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-6.73	P30 Slurry Control
	Proteobacteria	Altererythrobacter	2.10	P30 Grazed Control
	Verrucomicrobiota	Luteolibacter	2.02	P30 Grazed Control
	Planctomycetota	Schlesneria	-0.72	P30 Slurry Control
	Planctomycetota	Singulisphaera	-1.37	P30 Slurry Control
	Actinobacteriota	Rhizocola	8.40	P30 Grazed Control
	Firmicutes	Lysinibacillus	-2.99	P30 Slurry Control
	Acidobacteriota	Geothrix	-4.96	P30 Slurry Control
	Verrucomicrobiota	Roseimicrobium	0.99	P30 Grazed Control
	Acidobacteriota	Candidatus Koribacter	-2.88	P30 Slurry Control
	Actinobacteriota	Microbacterium	2.27	P30 Grazed Control

	Proteobacteria	Asticcacaulis	-2.85	P30 Slurry Control
	Actinobacteriota	Rhodococcus	-5.64	P30 Slurry Control
	Planctomycetota	SH-PL14	1.97	P30 Grazed Control
	Acidobacteriota	Terracidiphilus	-6.60	P30 Slurry Control
	Proteobacteria	Acidicaldus	-2.60	P30 Slurry Control
	Proteobacteria	Sideroxydans	-4.83	P30 Slurry Control
	Proteobacteria	Polaromonas	7.10	P30 Grazed Control
	Firmicutes	Turicibacter	-1.78	P30 Slurry Control
	Acidobacteriota	Stenotrophobacter	7.33	P30 Grazed Control
	Planctomycetota	Tundrisphaera	-2.75	P30 Slurry Control
	Proteobacteria	Rhizomicrobium	-9.14	P30 Slurry Control
	Proteobacteria	Phyllobacterium	6.37	P30 Grazed Control
	Actinobacteriota	Agromyces	2.54	P30 Grazed Control
	Actinobacteriota	Lechevalieria	7.98	P30 Grazed Control
	Actinobacteriota	Virgisporangium	5.04	P30 Grazed Control
	Firmicutes	Sporosarcina	-7.33	P30 Slurry Control
	Proteobacteria	Polycyclovorans	7.20	P30 Grazed Control
	Chloroflexi	Litorilinea	2.96	P30 Grazed Control
	Firmicutes	Ammoniphilus	-2.63	P30 Slurry Control
	Desulfobacterota	Citrifermentans	-4.15	P30 Slurry Control
	Bacteroidota	Ohtaekwangia	4.08	P30 Grazed Control

	Verrucomicrobiota	Pedosphaera	2.07	P30 Grazed Control
	Firmicutes	Bacillus	-6.93	P30 Slurry Control
	Proteobacteria	Plot4-2H12	-3.52	P30 Slurry Control
	Actinobacteriota	Rugosimonospora	-6.54	P30 Slurry Control
	Actinobacteriota	Kineosporia	-6.51	P30 Slurry Control
	Proteobacteria	Hirschia	2.84	P30 Grazed Control
	Proteobacteria	Amaricoccus	7.54	P30 Grazed Control
	Proteobacteria	BD1-7 clade	6.56	P30 Grazed Control
	Chloroflexi	Anaerolinea	-3.87	P30 Slurry Control
	Firmicutes	Clostridium sensu stricto 9	-6.66	P30 Slurry Control
	Bdellovibrionota	OM27 clade	7.92	P30 Grazed Control
	Actinobacteriota	Arthrobacter	-7.32	P30 Slurry Control
	Verrucomicrobiota	Oikopleura	6.38	P30 Grazed Control
	Bacteroidota	UTBCD1	-7.96	P30 Slurry Control
	Proteobacteria	Noviherbaspirillum	6.08	P30 Grazed Control
	Proteobacteria	Nevskia	-7.43	P30 Slurry Control
	Planctomycetota	CL500-3	5.66	P30 Grazed Control
	Planctomycetota	Pir2 lineage	-7.12	P30 Slurry Control
	Euryarchaeota	Methanobacterium	-6.80	P30 Slurry Control
	Proteobacteria	Afipia	-6.07	P30 Slurry Control
	Proteobacteria	Novosphingobium	5.29	P30 Grazed Control

	Proteobacteria	OM60(NOR5) clade	6.16	P30 Grazed Control
	Proteobacteria	Candidatus Accumulibacter	-5.85	P30 Slurry Control
	Bacteroidota	Taibaiella	-5.77	P30 Slurry Control
	Proteobacteria	IS-44	5.81	P30 Grazed Control

Table S65: Deseq2 analysis of P0 cut control vs P0 slurry control.

P0 cut control vs P0 slurry control	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Ascobolus	6.65	P0 cut control
	Ascomycota	Preussia	4.33	P0 cut control
	Ascomycota	unidentified_385	3.72	P0 cut control
	Ascomycota	Trichoglossum	-11.32	P0 slurry control
	Basidiomycota	Cotylidia	8.26	P0 cut control
	Ascomycota	Hypomyces	-6.96	P0 slurry control
	Ascomycota	Auxarthron	3.12	P0 cut control
	Ascomycota	Talaromyces	-6.75	P0 slurry control
	Basidiomycota	unidentified_144	8.38	P0 cut control
	Basidiomycota	Pholiotina	9.67	P0 cut control
	Ascomycota	Pseudogymnoascus	7.50	P0 cut control
	Ascomycota	Pseudorobillarda	8.63	P0 cut control
	Glomeromycota	Funneliformis	19.80	P0 cut control
	Ascomycota	Apodus	8.08	P0 cut control

Table S66: Deseq2 analysis of P0 cut control vs P0 slurry control.

P0 cut control vs P30 cut control	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Cladosporium	0.75	P0 cut control
	Basidiomycota	Apiotrichum	-2.28	P30 cut control
	Ascomycota	Striaticonidium	-2.94	P30 cut control
	Basidiomycota	Clavaria	-5.29	P30 cut control
	Basidiomycota	Delicatula	11.40	P0 cut control
	Ascomycota	unidentified_3145	1.65	P0 cut control
	Ascomycota	Trichoglossum	-7.76	P30 cut control
	Ascomycota	unidentified_385	2.15	P0 cut control
	Ascomycota	Acremonium	0.82	P0 cut control
	Basidiomycota	Tylospora	22.85	P0 cut control
	Basidiomycota	Parasola	4.05	P0 cut control
	Basidiomycota	Entoloma	-1.87	P30 cut control
	Ascomycota	Lachnum	6.43	P0 cut control
	Ascomycota	unidentified_4125	-10.10	P30 cut control
	Ascomycota	unidentified_63	-8.26	P30 cut control
	Ascomycota	unidentified_191	-4.06	P30 cut control
	Ascomycota	Schizothecium	8.35	P0 cut control
	Ascomycota	Oidiodendron	-1.51	P30 cut control
	Basidiomycota	unidentified_144	7.03	P0 cut control
	Ascomycota	Phaeosphaeria	8.43	P0 cut control
	Ascomycota	Lecythophora	-7.79	P30 cut control
	Basidiomycota	Auricularia	-22.83	P30 cut control

	Ascomycota	unidentified_3723	4.59	P0 cut control
	Basidiomycota	Cotylidia	10.13	P0 cut control
	Ascomycota	Petrakia	9.19	P0 cut control
	Ascomycota	unidentified_344	21.46	P0 cut control
	Olpidiomycota	unidentified_7719	4.63	P0 cut control
	Glomeromycota	unidentified_4690	-7.17	P30 cut control

Table S67: Deseq2 analysis of P0 grazed 13C vs P0 cut 13C.

P0 grazed 13C vs P0 cut 13C	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Cladosporium	1.36	P0 grazed 13C
	Basidiomycota	Apiotrichum	-2.22	P0 cut 13C
	Ascomycota	Dactylonectria	-1.83	P0 cut 13C
	Basidiomycota	Solicoccozyma	-1.27	P0 cut 13C
	Ascomycota	Preussia	5.42	P0 grazed 13C

	Ascomycota	Exophiala	-2.64	P0 cut 13C
	Basidiomycota	Clavaria	-6.18	P0 cut 13C
	Ascomycota	unidentified_5	2.74	P0 grazed 13C
	Basidiomycota	Delicatula	8.01	P0 grazed 13C
	Ascomycota	Leohumicola	4.06	P0 grazed 13C
	Ascomycota	unidentified_832	-10.65	P0 cut 13C
	Ascomycota	unidentified_3145	2.70	P0 grazed 13C
	Ascomycota	Pyrenochaeta	-4.47	P0 cut 13C
	Ascomycota	Trichoglossum	-13.37	P0 cut 13C
	Chytridiomycota	unidentified_957	-3.17	P0 cut 13C
	Ascomycota	Gremmenia	2.25	P0 grazed 13C
	Ascomycota	Acremonium	3.06	P0 grazed 13C
	Ascomycota	unidentified_20	5.22	P0 grazed 13C
	Ascomycota	Chrysosporium	3.98	P0 grazed 13C
	Basidiomycota	Tylospora	10.95	P0 grazed 13C
	Ascomycota	Belonium	12.56	P0 grazed 13C
	Basidiomycota	unidentified_2460	-8.00	P0 cut 13C
	Ascomycota	Microdochium	2.02	P0 grazed 13C
	Ascomycota	unidentified_388	7.25	P0 grazed 13C
	Chytridiomycota	Rhizophydium	3.69	P0 grazed 13C
	Basidiomycota	Serendipita	-2.26	P0 cut 13C
	Ascomycota	unidentified_51	-9.22	P0 cut 13C
	Ascomycota	unidentified_4125	-5.31	P0 cut 13C
	Ascomycota	unidentified_6793	8.44	P0 grazed 13C
	Ascomycota	Sagenomella	-9.24	P0 cut 13C
	Ascomycota	Neoascochyta	6.35	P0 grazed 13C
	Basidiomycota	Rhodotorula	-7.41	P0 cut 13C
	Ascomycota	Clohesyomyces	-9.55	P0 cut 13C
	Ascomycota	Gibberella	9.35	P0 grazed 13C

	Ascomycota	Podospora	1.71	P0 grazed 13C
	Ascomycota	Ilyonectria	2.29	P0 grazed 13C
	Rozellomycota	unidentified_73	8.96	P0 grazed 13C
	Ascomycota	unidentified_385	-3.78	P0 cut 13C
	Ascomycota	unidentified_3176	-8.85	P0 cut 13C
	Basidiomycota	unidentified_199	-8.14	P0 cut 13C
	Basidiomycota	Agaricus	22.61	P0 grazed 13C
	Ascomycota	Collembolispora	-2.78	P0 cut 13C
	Ascomycota	Chalara	8.31	P0 grazed 13C
	Ascomycota	Phialocephala	8.62	P0 grazed 13C
	Basidiomycota	Akenomyces	8.73	P0 grazed 13C
	Ascomycota	unidentified_457	6.92	P0 grazed 13C
	Ascomycota	Bimuria	6.14	P0 grazed 13C
	Ascomycota	Plectosphaerella	6.85	P0 grazed 13C
	Chytridiomycota	unidentified_40	-4.23	P0 cut 13C
	Basidiobolomycota	Basidiobolus	8.11	P0 grazed 13C
	Ascomycota	Thelebolus	5.02	P0 grazed 13C
	Basidiomycota	Hemimycena	-8.37	P0 cut 13C
	Basidiomycota	Atractiella	-10.31	P0 cut 13C
	Ascomycota	Fusidium	7.21	P0 grazed 13C
	Glomeromycota	unidentified_277	7.13	P0 grazed 13C
	Ascomycota	Myrmecridium	3.94	P0 grazed 13C
	Ascomycota	Alternaria	20.55	P0 grazed 13C
	Ascomycota	Talaromyces	-8.00	P0 cut 13C
	Ascomycota	Schizothecium	-7.18	P0 cut 13C
	Glomeromycota	Archaeospora	5.62	P0 grazed 13C

Table S68: Deseq2 analysis of P0 grazed control vs P0 cut control.

P0 grazed control vs P0 cut control	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Apiotrichum	-3.01	P0 cut control
	Ascomycota	Dactylonectria	-2.08	P0 cut control
	Ascomycota	Preussia	5.28	P0 grazed control
	Basidiomycota	unidentified_16	-3.49	P0 cut control
	Basidiomycota	Clavaria	-3.87	P0 cut control
	Ascomycota	unidentified_5	2.43	P0 grazed control
	Basidiomycota	Delicatula	11.52	P0 grazed control
	Ascomycota	Leohumicola	3.82	P0 grazed control
	Ascomycota	unidentified_3138	-5.28	P0 cut control
	Ascomycota	unidentified_832	-10.06	P0 cut control
	Ascomycota	unidentified_3145	3.15	P0 grazed control
	Ascomycota	Pyrenochaeta	-4.70	P0 cut control
	Ascomycota	Trichoglossum	-12.04	P0 cut control
	Chytridiomycota	unidentified_957	-2.42	P0 cut control
	Ascomycota	Gremmenia	3.37	P0 grazed control
	Ascomycota	Acremonium	2.02	P0 grazed control
	Ascomycota	Chrysosporium	4.62	P0 grazed control

	Basidiomycota	Tylospora	10.05	P0 grazed control
	Ascomycota	Belonium	7.48	P0 grazed control
	Basidiomycota	unidentified_2460	-4.43	P0 cut control
	Basidiomycota	Slooffia	-2.11	P0 cut control
	Ascomycota	unidentified_388	-8.06	P0 cut control
	Chytridiomycota	Rhizophydium	10.03	P0 grazed control
	Ascomycota	unidentified_51	-9.22	P0 cut control
	Ascomycota	unidentified_1321	8.44	P0 grazed control
	Ascomycota	unidentified_4125	-9.43	P0 cut control
	Ascomycota	Chaetomium	8.57	P0 grazed control
	Ascomycota	Sagenomella	-7.96	P0 cut control
	Ascomycota	Neoascochyta	4.56	P0 grazed control
	Basidiomycota	unidentified_199	-8.12	P0 cut control
	Ascomycota	Auxarthron	-6.46	P0 cut control
	Ascomycota	Talaromyces	-7.47	P0 cut control
	Basidiomycota	Rhodotorula	-7.00	P0 cut control
	Ascomycota	Clohesyomyces	-8.48	P0 cut control
	Basidiomycota	Claudopus	-4.15	P0 cut control
	Rozellomycota	unidentified_73	7.24	P0 grazed control
	Ascomycota	unidentified_3176	-7.55	P0 cut control
	Ascomycota	Mariannaea	-7.60	P0 cut control
	Basidiomycota	unidentified_39	-6.75	P0 cut control

	Glomeromycota	unidentified_276	4.28	P0 grazed control
	Basidiomycota	Agaricus	21.62	P0 grazed control
	Ascomycota	Phialocephala	4.75	P0 grazed control
	Ascomycota	Plectosphaerella	7.47	P0 grazed control
	Basidiomycota	unidentified_242	3.74	P0 grazed control
	Basidiobolomycota	Basidiobolus	8.17	P0 grazed control
	Ascomycota	Fusidium	7.51	P0 grazed control
	Basidiomycota	unidentified_21	22.66	P0 grazed control
	Glomeromycota	unidentified_277	7.92	P0 grazed control
	Ascomycota	Sarocladium	8.13	P0 grazed control
	Glomeromycota	Ambispora	8.58	P0 grazed control
	Ascomycota	Toxicocladosporium	6.76	P0 grazed control

Table S69: Deseq2 analysis of P0 grazed control vs P0 slurry control.

P0 grazed control vs	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**

P0 slurry control				
	Ascomycota	Dactylonectria	1.76	P0 grazed control
	Ascomycota	Ascobolus	3.49	P0 grazed control
	Ascomycota	Striaticonidium	-1.97	P0 slurry control
	Ascomycota	Preussia	-1.21	P0 slurry control
	Basidiomycota	unidentified_16	5.27	P0 grazed control
	Ascomycota	Exophiala	4.20	P0 grazed control
	Ascomycota	unidentified_5	-2.76	P0 slurry control
	Basidiomycota	Delicatula	-10.25	P0 slurry control
	Ascomycota	unidentified_3138	5.35	P0 grazed control
	Ascomycota	unidentified_832	9.55	P0 grazed control
	Ascomycota	unidentified_385	2.88	P0 grazed control
	Ascomycota	Pleurophragmium	1.23	P0 grazed control
	Ascomycota	unidentified_3145	-2.47	P0 slurry control
	Ascomycota	Pyrenochaeta	4.43	P0 grazed control
	Chytridiomycota	unidentified_957	1.57	P0 grazed control

	Ascomycota	Acremonium	-1.76	P0 slurry control
	Ascomycota	Leohumicola	-8.90	P0 slurry control
	Basidiomycota	Tylospora	-8.81	P0 slurry control
	Ascomycota	Belonium	-6.18	P0 slurry control
	Basidiomycota	unidentified_2460	4.92	P0 grazed control
	Basidiomycota	Slooffia	1.75	P0 grazed control
	Basidiomycota	Cotylidia	6.98	P0 grazed control
	Ascomycota	unidentified	-2.12	P0 slurry control
	Chytridiomycota	Rhizophydium	-5.77	P0 slurry control
	Ascomycota	Ilyonectria	-7.67	P0 slurry control
	Ascomycota	unidentified_9815	21.18	P0 grazed control
	Ascomycota	unidentified_51	8.90	P0 grazed control
	Ascomycota	unidentified_1321	-7.05	P0 slurry control
	Ascomycota	unidentified_88	9.86	P0 grazed control
	Ascomycota	Sagenomella	7.59	P0 grazed control
	Ascomycota	Neoascochyta	-3.10	P0 slurry control

	Mucoromycota	Mucor	-3.99	P0 slurry control
	Basidiomycota	unidentified_199	8.09	P0 grazed control
	Ascomycota	Auxarthron	9.30	P0 grazed control
	Basidiomycota	Mycena	-7.26	P0 slurry control
	Rozellomycota	unidentified_73	-7.81	P0 slurry control
	Glomeromycota	unidentified_276	-9.71	P0 slurry control
	Basidiomycota	Agaricus	-21.95	P0 slurry control
	Ascomycota	Phialocephala	-8.05	P0 slurry control
	Ascomycota	unidentified_3176	7.38	P0 grazed control
	Basidiomycota	Pholiotina	8.53	P0 grazed control
	Chytridiomycota	unidentified_40	7.14	P0 grazed control
	Basidiomycota	unidentified_242	-3.36	P0 slurry control
	Ascomycota	unidentified_4125	22.09	P0 grazed control
	Basidiomycota	unidentified_21	-23.00	P0 slurry control
	Glomeromycota	Ambispora	-7.22	P0 slurry control
	Glomeromycota	Funneliformis	20.34	P0 grazed control

Table S70: Deseq2 analysis of P0 grazed control vs P30 grazed control

P0 grazed control vs P30 grazed control	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Pseudeurotium	1.31	P0 grazed control
	Basidiomycota	unidentified_16	5.82	P0 grazed control
	Ascomycota	Dactylonectria	1.54	P0 grazed control
	Ascomycota	Pseudaleuria	13.50	P0 grazed control
	Ascomycota	Preussia	1.04	P0 grazed control
	Ascomycota	Pyrenochaetopsis	-1.70	P30 grazed control
	Basidiomycota	Delicatula	-11.62	P30 grazed control
	Ascomycota	unidentified_3138	6.67	P0 grazed control
	Ascomycota	unidentified_832	8.60	P0 grazed control
	Ascomycota	Penicillium	1.64	P0 grazed control
	Ascomycota	unidentified_59	2.15	P0 grazed control
	Ascomycota	unidentified_20	2.00	P0 grazed control

	Ascomycota	Leohumicola	-3.70	P30 grazed control
	Basidiomycota	Parasola	7.81	P0 grazed control
	Ascomycota	unidentified_388	9.34	P0 grazed control
	Glomeromycota	Claroideoglomus	3.52	P0 grazed control
	Ascomycota	unidentified_6793	6.66	P0 grazed control
	Ascomycota	Lasiosphaeria	23.59	P0 grazed control
	Basidiomycota	unidentified_199	6.44	P0 grazed control
	Ascomycota	unidentified_63	-9.70	P30 grazed control
	Basidiomycota	unidentified_682	8.52	P0 grazed control
	Basidiomycota	Rhodotorula	6.17	P0 grazed control
	Basidiomycota	Mycena	-8.62	P30 grazed control
	Ascomycota	unidentified_191	-1.33	P30 grazed control
	Rozellomycota	unidentified_73	-9.21	P30 grazed control
	Ascomycota	Valsonectria	3.86	P0 grazed control
	Basidiomycota	unidentified_39	7.50	P0 grazed control
	Ascomycota	Periconia	5.49	P0 grazed control

	Ascomycota	Trichophyton	7.26	P0 grazed control
	Basidiomycota	Clavaria	-3.66	P30 grazed control
	Ascomycota	unidentified_385	-4.84	P30 grazed control
	Basidiomycota	Filobasidiella	8.13	P0 grazed control
	Ascomycota	Phialocephala	-9.45	P30 grazed control
	Ascomycota	Tolypocladium	7.89	P0 grazed control
	Glomeromycota	Paraglomus	7.38	P0 grazed control
	Basidiomycota	unidentified_144	7.59	P0 grazed control
	Ascomycota	Fusidium	-7.59	P30 grazed control
	Chytridiomycota	Coralloidiomyces	5.18	P0 grazed control
	Glomeromycota	Funneliformis	18.28	P0 grazed control
	Glomeromycota	Ambispora	-8.59	P30 grazed control
	Ascomycota	Nadsonia	-5.88	P30 grazed control
	Ascomycota	Toxicocladosporium	-6.82	P30 grazed control

Table S71: Deseq2 analysis of P0 slurry 13C vs P0 cut 13C.

P0 slurry 13C vs P0 cut 13C	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Apiotrichum	-2.25	P0 cut 13C
	Ascomycota	Ascobolus	4.53	P0 slurry 13C
	Ascomycota	Preussia	4.87	P0 slurry 13C
	Basidiomycota	Clavaria	-3.12	P0 cut 13C
	Ascomycota	Pleurophragmium	2.03	P0 slurry 13C
	Ascomycota	Trichoglossum	-13.40	P0 cut 13C
	Basidiomycota	Parasola	8.27	P0 slurry 13C
	Basidiomycota	unidentified_7	3.80	P0 slurry 13C
	Ascomycota	unidentified_88	4.40	P0 slurry 13C
	Ascomycota	Pseudotaeniolina	-5.78	P0 cut 13C
	Ascomycota	Auxarthron	4.47	P0 slurry 13C
	Ascomycota	Clohesyomyces	-9.60	P0 cut 13C
	Ascomycota	unidentified_3176	-5.34	P0 cut 13C
	Ascomycota	Geoglossum	-23.78	P0 cut 13C
	Basidiomycota	Atractiella	-10.21	P0 cut 13C
	Basidiomycota	Mycena	-24.06	P0 cut 13C
	Ascomycota	unidentified_115	19.77	P0 slurry 13C
	Basidiomycota	unidentified_420	7.37	P0 slurry 13C
	Chytridiomycota	Paranamyces	5.77	P0 slurry 13C
	Glomeromycota	Archaeospora	6.51	P0 slurry 13C

Table S72: Deseq2 analysis of P0 slurry 13C vs P0 grazed 13C.

P0 slurry 13C vs P0 grazed 13C	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Dactylonectria	1.87	P0 slurry 13C
	Ascomycota	Ascobolus	4.36	P0 slurry 13C
	Ascomycota	Pseudaleuria	7.45	P0 slurry 13C
	Ascomycota	Striaticonidium	-1.58	P0 grazed 13C
	Ascomycota	Exophiala	2.70	P0 slurry 13C
	Ascomycota	unidentified_5	-2.59	P0 grazed 13C
	Basidiomycota	Delicatula	-13.43	P0 grazed 13C
	Ascomycota	Leohumicola	-7.59	P0 grazed 13C
	Ascomycota	unidentified_3138	3.66	P0 slurry 13C
	Ascomycota	unidentified_832	10.35	P0 slurry 13C
	Ascomycota	Pyrenochaetopsis	-2.05	P0 grazed 13C
	Ascomycota	unidentified_385	6.40	P0 slurry 13C
	Ascomycota	Pleurophragmium	2.35	P0 slurry 13C
	Chytridiomycota	unidentified_957	3.10	P0 slurry 13C

	Basidiomycota	Clavaria	2.91	P0 slurry 13C
	Ascomycota	Gremmenia	-2.70	P0 grazed 13C
	Ascomycota	Acremonium	-2.13	P0 grazed 13C
	Basidiomycota	Tylospora	-11.51	P0 grazed 13C
	Basidiomycota	Parasola	8.76	P0 slurry 13C
	Ascomycota	Belonium	-27.64	P0 grazed 13C
	Basidiomycota	unidentified_2460	8.96	P0 slurry 13C
	Basidiomycota	Cotylidia	6.51	P0 slurry 13C
	Ascomycota	unidentified	-2.51	P0 grazed 13C
	Basidiomycota	unidentified_7	6.30	P0 slurry 13C
	Ascomycota	Ilyonectria	-2.48	P0 grazed 13C
	Ascomycota	unidentified_9815	8.32	P0 slurry 13C
	Ascomycota	unidentified_51	8.32	P0 slurry 13C
	Ascomycota	unidentified_88	10.30	P0 slurry 13C
	Ascomycota	Sagenomella	8.37	P0 slurry 13C
	Ascomycota	Pseudotaeniolina	-6.18	P0 grazed 13C
	Basidiomycota	unidentified_199	8.95	P0 slurry 13C
	Ascomycota	Auxarthron	9.80	P0 slurry 13C
	Basidiomycota	Rhodotorula	8.20	P0 slurry 13C
	Basidiomycota	Mycena	-10.62	P0 grazed 13C
	Ascomycota	Gibberella	-4.37	P0 grazed 13C

	Ascomycota	unidentified_191	-2.52	P0 grazed 13C
	Rozellomycota	unidentified_73	-9.45	P0 grazed 13C
	Ascomycota	Schizothecium	7.95	P0 slurry 13C
	Glomeromycota	unidentified_276	-3.57	P0 grazed 13C
	Basidiomycota	unidentified_144	7.63	P0 slurry 13C
	Basidiomycota	Agaricus	-24.16	P0 grazed 13C
	Ascomycota	Chalara	-8.83	P0 grazed 13C
	Ascomycota	Phaeosphaeria	6.30	P0 slurry 13C
	Ascomycota	Phialocephala	-9.14	P0 grazed 13C
	Basidiomycota	Akenomyces	-9.19	P0 grazed 13C
	Basidiomycota	unidentified_242	-7.51	P0 grazed 13C
	Ascomycota	unidentified_7020	-5.90	P0 grazed 13C
	Ascomycota	Thelebolus	-5.52	P0 grazed 13C
	Basidiomycota	Pholiotina	7.14	P0 slurry 13C
	Ascomycota	Fusidium	-4.57	P0 grazed 13C
	Glomeromycota	Paraglomus	5.93	P0 slurry 13C
	Ascomycota	Pseudogymnoascus	3.73	P0 slurry 13C
	Basidiomycota	unidentified_1560	21.05	P0 slurry 13C
	Basidiomycota	unidentified_23	7.39	P0 slurry 13C

	Glomeromycota	unidentified_277	-7.69	P0 grazed 13C
	Ascomycota	Pseudopithomyces	20.86	P0 slurry 13C
	Basidiomycota	unidentified_420	7.83	P0 slurry 13C
	Ascomycota	unidentified_448	-6.50	P0 grazed 13C
	Chytridiomycota	Paranamyces	6.23	P0 slurry 13C

Table S73: Deseq2 analysis of P0 slurry 13C vs P30 slurry 13C.

P0 slurry 13C vs P30 slurry 13C	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Basidiomycota	Clavaria	-11.33	P30 slurry 13C
	Mucoromycota	Absidia	-3.33	P30 slurry 13C
	Ascomycota	Pseudogymnoascus	-6.38	P30 slurry 13C
	Ascomycota	unidentified_115	-20.84	P30 slurry 13C

Table S74: Deseq2 analysis of P30 cut 13C vs P0 cut 13C.

P30 cut 13C vs P0 cut 13C	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Apiotrichum	-3.24	P0 cut 13C
	Ascomycota	Striaticonidium	-2.17	P0 cut 13C
	Ascomycota	Trichoglossum	-9.75	P0 cut 13C
	Ascomycota	Acremonium	1.99	P30 cut 13C
	Basidiomycota	Cotylidia	8.38	P30 cut 13C
	Ascomycota	unidentified_388	20.70	P30 cut 13C
	Ascomycota	Lachnum	10.69	P30 cut 13C
	Ascomycota	unidentified_4125	-8.40	P0 cut 13C
	Ascomycota	unidentified_6793	8.11	P30 cut 13C
	Ascomycota	unidentified_63	-8.00	P0 cut 13C
	Basidiomycota	unidentified_420	26.10	P30 cut 13C
	Ascomycota	Gibberella	7.71	P30 cut 13C
	Ascomycota	unidentified_3723	9.19	P30 cut 13C
	Basidiomycota	Tubaria	24.74	P30 cut 13C
	Ascomycota	Geoglossum	-22.84	P0 cut 13C
	Basidiomycota	Atractiella	-9.33	P0 cut 13C
	Basidiomycota	Parasola	9.50	P30 cut 13C
	Basidiomycota	Mycena	-23.06	P0 cut 13C

	Basidiomycota	Trechispora	20.47	P30 cut 13C

Table S75: Deseq2 analysis of P30 cut 13C vs P30 grazed 13C.

P30 cut 13C vs P30 grazed 13C	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Striaticonidium	2.57	P30 cut 13C
	Ascomycota	Preussia	6.17	P30 cut 13C
	Basidiomycota	Delicatula	-8.40	P30 grazed 13C
	Ascomycota	unidentified_832	-2.05	P30 grazed 13C
	Ascomycota	unidentified_5	1.91	P30 cut 13C
	Ascomycota	unidentified_3145	3.00	P30 cut 13C
	Basidiomycota	Clavaria	-7.50	P30 grazed 13C
	Ascomycota	Pyrenochaeta	-4.27	P30 grazed 13C
	Ascomycota	unidentified_385	-8.38	P30 grazed 13C
	Ascomycota	Chrysosporium	4.01	P30 cut 13C
	Basidiomycota	Tylospora	9.11	P30 cut 13C
	Basidiomycota	unidentified_2460	-9.08	P30 grazed 13C
	Basidiomycota	Cotylidia	-8.60	P30 grazed 13C
	Basidiomycota	Coprinopsis	4.10	P30 cut 13C

	Ascomycota	unidentified_51	-7.50	P30 grazed 13C
	Ascomycota	unidentified_1321	9.15	P30 cut 13C
	Ascomycota	Sagenomella	-5.59	P30 grazed 13C
	Basidiomycota	unidentified_420	-27.19	P30 grazed 13C
	Ascomycota	Schizothecium	-22.07	P30 grazed 13C
	Ascomycota	unidentified_3176	-6.13	P30 grazed 13C
	Glomeromycota	unidentified_276	6.70	P30 cut 13C
	Ascomycota	Trichophyton	8.69	P30 cut 13C
	Basidiomycota	unidentified_9358	22.08	P30 cut 13C
	Rozellomycota	unidentified_1345	5.87	P30 cut 13C
	Ascomycota	unidentified_3723	-9.29	P30 grazed 13C
	Ascomycota	Plectosphaerella	6.46	P30 cut 13C
	Basidiomycota	Tubaria	-25.85	P30 grazed 13C
	Ascomycota	Thelebolus	6.37	P30 cut 13C
	Ascomycota	Dactylaria	6.52	P30 cut 13C
	Ascomycota	Westerdykella	6.06	P30 cut 13C
	Basidiomycota	Trechispora	-23.13	P30 grazed 13C
	Ascomycota	Minutisphaera	6.38	P30 cut 13C
	Ascomycota	Protomyces	19.95	P30 cut 13C
	Ascomycota	Fusarium	6.40	P30 cut 13C

Table S76: Deseq2 analysis of P30 cut 13C vs P30 slurry 13C.

P30 cut 13C vs P30 slurry 13C	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Preussia	4.11	P30 cut 13C
	Basidiomycota	Delicatula	-7.81	P30 slurry 13C
	Basidiomycota	Clavaria	-11.20	P30 slurry 13C
	Mucoromycota	Absidia	-2.93	P30 slurry 13C
	Ascomycota	Lachnum	-6.85	P30 slurry 13C
	Ascomycota	unidentified_4125	8.76	P30 cut 13C
	Ascomycota	Gibberella	-7.43	P30 slurry 13C
	Basidiomycota	Pholiotina	5.10	P30 cut 13C
	Basidiomycota	Tubaria	-25.78	P30 slurry 13C
	Basidiomycota	Hemimycena	20.50	P30 cut 13C

Table S77: Deseq2 analysis of P30 cut control vs P30 grazed control.

P30 cut control vs P30 grazed control	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Pseudeurotium	1.16	P30 cut control
	Basidiomycota	Solicoccozyma	-1.34	P30 grazed control
	Ascomycota	Ascobolus	2.35	P30 cut control
	Ascomycota	Pseudaleuria	14.02	P30 cut control
	Ascomycota	Striaticonidium	3.12	P30 cut control
	Ascomycota	Preussia	5.86	P30 cut control
	Basidiomycota	Delicatula	-11.44	P30 grazed control
	Ascomycota	unidentified_3138	1.59	P30 cut control
	Ascomycota	unidentified_832	-1.54	P30 grazed control
	Ascomycota	unidentified_52	-1.54	P30 grazed control
	Ascomycota	unidentified_3145	1.44	P30 cut control
	Ascomycota	unidentified_5	1.63	P30 cut control
	Ascomycota	Pyrenochaeta	-4.19	P30 grazed control
	Chytridiomycota	unidentified_957	-1.46	P30 grazed control

	Ascomycota	unidentified_385	-6.43	P30 grazed control
	Ascomycota	unidentified_20	4.66	P30 cut control
	Ascomycota	Chrysosporium	3.59	P30 cut control
	Basidiomycota	Parasola	-2.88	P30 grazed control
	Ascomycota	unidentified	1.06	P30 cut control
	Ascomycota	unidentified_82	2.00	P30 cut control
	Basidiomycota	Slooffia	-2.18	P30 grazed control
	Ascomycota	unidentified_9815	-9.29	P30 grazed control
	Basidiomycota	Coprinopsis	3.08	P30 cut control
	Ascomycota	unidentified_51	-9.08	P30 grazed control
	Ascomycota	unidentified_1321	9.68	P30 cut control
	Mucoromycota	Mucor	1.04	P30 cut control
	Ascomycota	Sagenomella	-6.15	P30 grazed control
	Ascomycota	Pseudotaeniolina	2.91	P30 cut control
	Ascomycota	Cadophora	-4.73	P30 grazed control
	Ascomycota	Lasiosphaeria	24.63	P30 cut control

	Basidiomycota	unidentified_199	-1.91	P30 grazed control
	Ascomycota	Auxarthron	-4.97	P30 grazed control
	Basidiomycota	Cortinarius	4.04	P30 cut control
	Basidiomycota	unidentified_682	9.09	P30 cut control
	Ascomycota	Talaromyces	-10.10	P30 grazed control
	Ascomycota	unidentified_191	4.20	P30 cut control
	Ascomycota	unidentified_3176	-5.01	P30 grazed control
	Chytridiomycota	Rhizophydium	4.74	P30 cut control
	Ascomycota	Trichophyton	7.84	P30 cut control
	Rozellomycota	unidentified_1345	8.10	P30 cut control
	Basidiomycota	Filobasidiella	4.83	P30 cut control
	Ascomycota	unidentified_3723	-7.79	P30 grazed control
	Ascomycota	Bimuria	7.67	P30 cut control
	Ascomycota	Plectosphaerella	6.68	P30 cut control
	Basidiomycota	Cotylidia	-10.15	P30 grazed control
	Glomeromycota	unidentified_276	5.11	P30 cut control

	Basidiobolomycota	Basidiobolus	8.32	P30 cut control
	Ascomycota	Thelebolus	7.05	P30 cut control
	Ascomycota	Dactylaria	7.38	P30 cut control
	Basidiomycota	unidentified_242	5.70	P30 cut control
	Ascomycota	Westerdykella	6.23	P30 cut control
	Ascomycota	unidentified_344	-22.93	P30 grazed control
	Glomeromycota	Funneliformis	21.06	P30 cut control
	Ascomycota	unidentified_448	-21.81	P30 grazed control
	Basidiomycota	unidentified_23	-7.37	P30 grazed control
	Ascomycota	Lipomyces	-7.14	P30 grazed control
	Ascomycota	Leptodontidium	-6.70	P30 grazed control

Table S78: Deseq2 analysis of P30 cut control vs P30 slurry control.

P30 cut control vs P30 slurry control	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Basidiomycota	Apiotrichum	1.02	P30 cut control

	Ascomycota	Ascobolus	4.49	P30 cut control
	Ascomycota	Striaticonidium	1.75	P30 cut control
	Ascomycota	Preussia	4.06	P30 cut control
	Basidiomycota	Delicatula	-10.36	P30 slurry control
	Basidiomycota	Clavaria	-8.69	P30 slurry control
	Basidiomycota	Tylospora	-23.06	P30 slurry control
	Ascomycota	Podospora	-2.22	P30 slurry control
	Basidiomycota	Entoloma	2.01	P30 cut control
	Ascomycota	unidentified_4125	10.48	P30 cut control
	Ascomycota	unidentified_3176	-9.03	P30 slurry control
	Ascomycota	Phaeosphaeria	-5.15	P30 slurry control
	Ascomycota	Petrakia	-8.20	P30 slurry control
	Ascomycota	unidentified_344	-21.70	P30 slurry control

Table S79: Deseq2 analysis of P30 grazed 13C VS P0 grazed 13C.

P30 grazed 13C VS P0 grazed 13C	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**

	Ascomycota	Pseudeurotium	1.01	P30 grazed 13C
	Ascomycota	Pseudaleuria	13.31	P30 grazed 13C
	Basidiomycota	Delicatula	-13.29	P0 grazed 13C
	Ascomycota	Leohumicola	-3.60	P0 grazed 13C
	Ascomycota	unidentified_3138	3.69	P30 grazed 13C
	Ascomycota	unidentified_832	8.20	P30 grazed 13C
	Ascomycota	unidentified_52	-1.56	P0 grazed 13C
	Chytridiomycota	unidentified_957	1.72	P30 grazed 13C
	Basidiomycota	Clavaria	-4.66	P0 grazed 13C
	Ascomycota	Acremonium	-1.27	P0 grazed 13C
	Basidiomycota	Parasola	10.58	P30 grazed 13C
	Ascomycota	unidentified_50	-2.12	P0 grazed 13C
	Basidiomycota	unidentified_7	4.59	P30 grazed 13C
	Ascomycota	unidentified_9815	22.72	P30 grazed 13C
	Glomeromycota	Claroideoglomus	2.37	P30 grazed 13C
	Ascomycota	Neoascochyta	-6.88	P0 grazed 13C

	Basidiomycota	unidentified_199	7.93	P30 grazed 13C
	Ascomycota	unidentified_63	-9.67	P0 grazed 13C
	Basidiomycota	Rhodotorula	5.23	P30 grazed 13C
	Basidiomycota	Mycena	-10.41	P0 grazed 13C
	Rozellomycota	unidentified_73	-9.32	P0 grazed 13C
	Ascomycota	Valsonectria	7.80	P30 grazed 13C
	Ascomycota	Trichophyton	9.28	P30 grazed 13C
	Basidiomycota	unidentified_144	8.29	P30 grazed 13C
	Basidiomycota	Agaricus	-24.06	P0 grazed 13C
	Ascomycota	Chalara	-8.64	P0 grazed 13C
	Basidiomycota	Filobasidiella	8.12	P30 grazed 13C
	Ascomycota	Phialocephala	-8.99	P0 grazed 13C
	Ascomycota	Tolypocladium	7.75	P30 grazed 13C
	Ascomycota	Lachnum	9.10	P30 grazed 13C
	Ascomycota	Coniochaeta	6.58	P30 grazed 13C
	Glomeromycota	unidentified_4690	-7.33	P0 grazed 13C

	Ascomycota	Myrmecridium	-7.01	P0 grazed 13C
	Ascomycota	Apodus	-5.80	P0 grazed 13C
	Ascomycota	Alternaria	-22.09	P0 grazed 13C
	Ascomycota	Protomyces	21.09	P30 grazed 13C
	Basidiomycota	Psathyrella	-5.71	P0 grazed 13C

Table S80: Deseq2 analysis of P30 grazed vs P30 slurry 13C.

P30 grazed vs P30 slurry 13C	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Ascomycota	Ascobolus	1.69	P30 grazed 13C
	Ascomycota	Preussia	-1.97	P30 slurry 13C
	Ascomycota	unidentified_832	2.31	P30 grazed 13C
	Ascomycota	unidentified_5	-2.07	P30 slurry 13C

	Ascomycota	unidentified_3145	-3.14	P30 slurry 13C
	Ascomycota	Pyrenochaeta	5.17	P30 grazed 13C
	Ascomycota	unidentified_385	7.63	P30 grazed 13C
	Ascomycota	Chrysosporium	-5.45	P30 slurry 13C
	Ascomycota	Leohumicola	-6.78	P30 slurry 13C
	Basidiomycota	Tylospora	-9.06	P30 slurry 13C
	Basidiomycota	Parasola	-3.85	P30 slurry 13C
	Basidiomycota	unidentified_2460	10.03	P30 grazed 13C
	Basidiomycota	Cotylidia	11.81	P30 grazed 13C
	Chytridiomycota	Rhizophydium	-6.46	P30 slurry 13C
	Mucoromycota	Absidia	-2.48	P30 slurry 13C
	Ascomycota	unidentified_51	7.90	P30 grazed 13C
	Ascomycota	unidentified_1321	-9.00	P30 slurry 13C
	Ascomycota	unidentified_4125	5.76	P30 grazed 13C
	Basidiomycota	unidentified_2191	-8.17	P30 slurry 13C
	Ascomycota	unidentified_88	6.94	P30 grazed 13C

	Ascomycota	unidentified_6793	5.62	P30 grazed 13C
	Ascomycota	Cadophora	4.21	P30 grazed 13C
	Basidiomycota	Cortinarius	-6.83	P30 slurry 13C
	Basidiomycota	unidentified_682	-3.84	P30 slurry 13C
	Ascomycota	Schizothecium	8.20	P30 grazed 13C
	Glomeromycota	unidentified_276	-6.68	P30 slurry 13C
	Ascomycota	Trichophyton	-8.54	P30 slurry 13C
	Basidiomycota	unidentified_9358	-23.46	P30 slurry 13C
	Basidiomycota	unidentified_4317	7.04	P30 grazed 13C
	Basidiomycota	Pholiotina	8.96	P30 grazed 13C
	Ascomycota	unidentified_7020	7.98	P30 grazed 13C
	Chytridiomycota	Spizellomyces	-7.79	P30 slurry 13C
	Ascomycota	Thelebolus	-6.36	P30 slurry 13C
	Basidiomycota	Hemimycena	21.07	P30 grazed 13C
	Ascomycota	Lachnum	-4.95	P30 slurry 13C
	Ascomycota	Petrakia	-7.70	P30 slurry 13C

	Ascomycota	Dactylaria	-6.48	P30 slurry 13C
	Ascomycota	Minutisphaera	-6.41	P30 slurry 13C
	Ascomycota	Protomyces	-20.46	P30 slurry 13C

Table S81: Deseq2 analysis of P30 grazed control vs P30 slurry control.

P30 grazed control vs P30 slurry control	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Apiotrichum	1.91	P30 grazed control
	Basidiomycota	Solicoccozyma	1.69	P30 grazed control
	Ascomycota	Ascobolus	2.55	P30 grazed control
	Ascomycota	Pseudaleuria	-12.94	P30 slurry control
	Ascomycota	Preussia	-1.41	P30 slurry control
	Ascomycota	Exophiala	4.06	P30 grazed control
	Ascomycota	unidentified_3138	-1.36	P30 slurry control
	Ascomycota	Ophiosphaerella	1.11	P30 grazed control
	Ascomycota	unidentified_832	2.34	P30 grazed control

	Ascomycota	Pleurophragmium	1.87	P30 grazed control
	Ascomycota	unidentified_3145	-1.73	P30 slurry control
	Ascomycota	unidentified_5	-2.09	P30 slurry control
	Basidiomycota	Clavaria	-5.91	P30 slurry control
	Ascomycota	Pyrenochaeta	5.00	P30 grazed control
	Chytridiomycota	unidentified_957	1.76	P30 grazed control
	Ascomycota	unidentified_385	7.74	P30 grazed control
	Ascomycota	Chrysosporium	-4.68	P30 slurry control
	Ascomycota	Leohumicola	-2.70	P30 slurry control
	Basidiomycota	Tylospora	-8.42	P30 slurry control
	Basidiomycota	Parasola	3.40	P30 grazed control
	Ascomycota	unidentified_82	-2.18	P30 slurry control
	Basidiomycota	Slooffia	1.85	P30 grazed control
	Basidiomycota	Cotylidia	8.78	P30 grazed control
	Ascomycota	Microdochium	-4.54	P30 slurry control
	Ascomycota	unidentified	-1.37	P30 slurry control

	Ascomycota	unidentified_9815	10.03	P30 grazed control
	Glomeromycota	Claroideoglomus	-1.53	P30 slurry control
	Ascomycota	unidentified_51	9.04	P30 grazed control
	Ascomycota	Trichoglossum	6.97	P30 grazed control
	Ascomycota	unidentified_1321	-8.49	P30 slurry control
	Basidiomycota	Entoloma	1.81	P30 grazed control
	Mucoromycota	Mucor	-1.25	P30 slurry control
	Ascomycota	unidentified_4125	4.96	P30 grazed control
	Basidiomycota	unidentified_2191	-8.42	P30 slurry control
	Ascomycota	Lasiosphaeria	-24.15	P30 slurry control
	Ascomycota	Gremmenia	-4.35	P30 slurry control
	Ascomycota	Auxarthron	5.58	P30 grazed control
	Basidiomycota	Cortinarius	-3.82	P30 slurry control
	Basidiomycota	unidentified_682	-5.63	P30 slurry control
	Basidiomycota	Claudopus	7.09	P30 grazed control
	Chytridiomycota	Rhizophydium	-6.81	P30 slurry control

	Ascomycota	Trichophyton	-6.69	P30 slurry control
	Rozellomycota	unidentified_1345	-3.67	P30 slurry control
	Basidiomycota	Filobasidiella	-7.48	P30 slurry control
	Basidiomycota	Auricularia	7.93	P30 grazed control
	Basidiomycota	unidentified_4317	4.11	P30 grazed control
	Ascomycota	unidentified_3723	7.11	P30 grazed control
	Ascomycota	Bimuria	-6.55	P30 slurry control
	Ascomycota	Plectosphaerella	-5.53	P30 slurry control
	Chytridiomycota	unidentified_40	3.37	P30 grazed control
	Basidiobolomycota	Basidiobolus	-7.16	P30 slurry control
	Ascomycota	Thelebolus	-5.89	P30 slurry control
	Basidiomycota	Pholiotina	8.67	P30 grazed control
	Ascomycota	Glarea	8.81	P30 grazed control
	Ascomycota	Dactylaria	-6.20	P30 slurry control
	Basidiomycota	unidentified_242	-7.22	P30 slurry control
	Chytridiomycota	Coralloidiomyces	-4.52	P30 slurry control

	Ascomycota	Coniochaeta	-6.07	P30 slurry control
	Ascomycota	Westerdykella	-5.04	P30 slurry control
	Basidiomycota	Pluteus	7.65	P30 grazed control
	Basidiomycota	Akenomyces	8.38	P30 grazed control
	Glomeromycota	Funneliformis	-20.82	P30 slurry control
	Ascomycota	Lipomyces	7.02	P30 grazed control
	Ascomycota	unidentified_448	19.65	P30 grazed control

Table S82: Deseq2 analysis of P30 slurry control vs P0 slurry control.

P30 slurry control vs P0 slurry control	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Clavaria	-11.55	P0 slurry control
	Ascomycota	unidentified_6793	7.66	P30 slurry control
	Ascomycota	Auxarthron	-2.12	P0 slurry control
	Basidiomycota	Cortinarius	-3.96	P0 slurry control
	Ascomycota	unidentified_3176	-7.36	P0 slurry control
	Basidiomycota	Marasmius	19.80	P30 slurry control
	Glomeromycota	Funneliformis	-21.72	P0 slurry control

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Table S83: Deseq2 analysis of VH Planted vs VH Fallow.

VH Planted vs VH Fallow	Phylum	Genus	log2FoldChange	Enriched
	Proteobacteria	MND1	-0.39	VH Fallow
	Crenarchaeota	Candidatus Nitrocosmicus	0.56	VH Planted
	Verrucomicrobiota	Lacunisphaera	-0.67	VH Fallow
	Bacteroidota	OLB12	-0.62	VH Fallow
	Bacteroidota	Flavobacterium	0.60	VH Planted
	Verrucomicrobiota	Ellin517	-0.69	VH Fallow
	Bacteroidota	Crocinitomix	-2.41	VH Fallow

Table S84: Deseq2 analysis of H Planted vs H Fallow.

H Planted vs H Fallow	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrosotalea	-0.92	H Fallow
	Bacteroidota	Flavobacterium	1.06	H Planted
	Proteobacteria	Pseudoduganella	1.80	H Planted
	Verrucomicrobiota	Diplosphaera	6.43	H Planted
	Bacteroidota	Crocinitomix	-7.00	H Fallow

Table S85: Deseq2 analysis of L Planted vs L Fallow.

L Planted vs L Fallow	Phylum	Genus	log2FoldChange	Enriched

	Crenarchaeota	Candidatus Nitrosotalea	-0.93	L Fallow
	Bacteroidota	Puia	0.38	L Planted
	Nitrospirota	Nitrospira	0.70	L Planted
	Proteobacteria	Rhodanobacter	-0.67	L Fallow
	Acidobacteriota	RB41	0.87	L Planted
	Acidobacteriota	Luteitalea	0.85	L Planted
	Verrucomicrobiota	Chthoniobacter	0.52	L Planted
	Proteobacteria	Pseudoduganella	3.38	L Planted
	Bacteroidota	UTBCD1	1.32	L Planted
	Proteobacteria	Undibacterium	7.00	L Planted
	Proteobacteria	Duganella	5.89	L Planted
	Verrucomicrobiota	Diplosphaera	6.40	L Planted
	Actinobacteriota	Lechevalieria	-21.75	L Fallow
	Proteobacteria	Massilia	6.76	L Planted
	Myxococcota	Pajaroellobacter	1.35	L Planted
	Proteobacteria	Methylocapsa	6.31	L Planted
	Proteobacteria	AAP99	5.10	L Planted
	Bdellovibrionota	Bacteriovorax	5.17	L Planted
	Planctomycetota	AKYG587	5.35	L Planted

Table S86: Deseq2 analysis of VL Planted vs VL Fallow.

VL Planted vs VL Fallow	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Bacteroidota	OLB12	0.83	VL Planted
	Fibrobacterota	possible genus 04	2.05	VL Planted
	Spirochaetota	Turneriella	2.93	VL Planted
	Proteobacteria	Undibacterium	7.38	VL Planted
	Proteobacteria	Alkanibacter	2.72	VL Planted
	Chloroflexi	Thermosporothrix	-1.59	VL Fallow
	Chloroflexi	1921-2	20.91	VL Planted

Table S87: Deseq2 analysis of H Planted vs VH Planted.

H Planted vs VH Planted	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	0.72	H Planted
	Crenarchaeota	Candidatus Nitrosotalea	-4.67	VH Planted
	Bacteroidota	Puia	-0.54	VH Planted
	Crenarchaeota	Candidatus Nitrocosmicus	1.23	H Planted
	Proteobacteria	Rhodanobacter	-1.05	VH Planted
	Proteobacteria	Rhodoblastus	-1.27	VH Planted
	Proteobacteria	Pedomicrobium	0.66	H Planted
	Verrucomicrobiota	Ellin516	-1.04	VH Planted
	Acidobacteriota	Candidatus Koribacter	-0.83	VH Planted
	Acidobacteriota	Candidatus Solibacter	-0.41	VH Planted
	Chloroflexi	UTCFX1	1.63	H Planted
	Acidobacteriota	Bryobacter	-0.78	VH Planted
	Actinobacteriota	Conexibacter	-0.91	VH Planted
	Bacteroidota	OLB12	-0.71	VH Planted
	Myxococcota	Phaselicystis	-0.67	VH Planted
	Actinobacteriota	Ilumatobacter	0.73	H Planted
	Acidobacteriota	Acidipila	-1.25	VH Planted
	Proteobacteria	Ellin6055	2.15	H Planted
	Planctomycetota	SH-PL14	1.14	H Planted
	Bacteroidota	UTBCD1	-2.23	VH Planted
	Acidobacteriota	Geothrix	-2.02	VH Planted
	Bacteroidota	Ohtaekwangia	1.74	H Planted
	Actinobacteriota	Rhizocola	4.91	H Planted
	Acidobacteriota	Stenotrophobacter	2.61	H Planted
	Planctomycetota	Planctomicrobium	4.49	H Planted
	Planctomycetota	AKYG587	3.34	H Planted
	Proteobacteria	Bosea	5.55	H Planted
	Actinobacteriota	Agromyces	5.91	H Planted
	Bacteroidota	Chryseobacterium	-21.57	VH Planted

Table S88: Deseq2 analysis of L Planted vs VH Planted.

L Planted vs VH Planted	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	-0.68	VH Planted
	Crenarchaeota	Candidatus Nitrosotalea	-7.04	VH Planted
	Bacteroidota	Puia	-1.03	VH Planted
	Nitrospirota	Nitrospira	0.92	L Planted
	Proteobacteria	MND1	0.98	L Planted
	Crenarchaeota	Candidatus Nitrocosmicus	0.95	L Planted
	Planctomycetota	Aquisphaera	-0.70	VH Planted
	Actinobacteriota	Acidothermus	-1.57	VH Planted
	Proteobacteria	Bradyrhizobium	-0.54	VH Planted
	Actinobacteriota	Conexibacter	-0.75	VH Planted
	Proteobacteria	Ellin6067	0.61	L Planted
	Proteobacteria	Rhodanobacter	-2.28	VH Planted
	Proteobacteria	Rhodoblastus	-1.27	VH Planted
	Proteobacteria	Pedomicrobium	0.95	L Planted
	Proteobacteria	Roseiarcus	-1.02	VH Planted
	Acidobacteriota	Holophaga	-3.71	VH Planted
	Proteobacteria	Acidibacter	0.47	L Planted
	Proteobacteria	Sphingomonas	-0.28	VH Planted
	Acidobacteriota	Occallatibacter	-2.62	VH Planted
	Verrucomicrobiota	Ellin516	-1.54	VH Planted
	Bacteroidota	OLB12	-1.74	VH Planted
	Verrucomicrobiota	Lacunisphaera	-0.81	VH Planted
	Bacteroidota	Parafilimonas	-0.85	VH Planted
	Acidobacteriota	Candidatus Koribacter	-1.06	VH Planted
	Acidobacteriota	Candidatus Solibacter	-0.68	VH Planted
	Chloroflexi	UTCFX1	1.57	L Planted
	Acidobacteriota	Bryobacter	-1.04	VH Planted
	Proteobacteria	Steroidobacter	0.89	L Planted
	Verrucomicrobiota	Chthoniobacter	0.38	L Planted
	Verrucomicrobiota	ADurb.Bin063-1	-0.57	VH Planted
	Actinobacteriota	Ilumatobacter	1.94	L Planted
	Planctomycetota	Zavarzinella	-1.10	VH Planted
	Actinobacteriota	Microlunatus	0.92	L Planted
	Proteobacteria	Candidatus Alysiosphaera	0.81	L Planted
	Planctomycetota	Gemmata	-0.38	VH Planted
	Proteobacteria	Rhodoplanes	1.20	L Planted
	Fibrobacterota	possible genus 04	-1.17	VH Planted
	Acidobacteriota	Acidipila	-1.51	VH Planted
	Bacteroidota	Flavobacterium	1.36	L Planted
	Proteobacteria	Dongia	1.44	L Planted
	Proteobacteria	Ellin6055	2.90	L Planted
	Actinobacteriota	Jatrophihabitans	-0.71	VH Planted

	Bacteroidota	Mucilaginibacter	-3.00	VH Planted
	Proteobacteria	Mesorhizobium	0.57	L Planted
	Proteobacteria	Pseudorhodoplanes	2.25	L Planted
	Proteobacteria	Dokdonella	-1.04	VH Planted
	Spirochaetota	Spirochaeta 2	-1.03	VH Planted
	Bacteroidota	Aurantisolimonas	0.78	L Planted
	Myxococcota	P3OB-42	1.27	L Planted
	Verrucomicrobiota	Luteolibacter	1.29	L Planted
	Acidobacteriota	Granulicella	-3.67	VH Planted
	Bacteroidota	Ferruginibacter	0.39	L Planted
	Spirochaetota	Turneriella	1.17	L Planted
	Proteobacteria	SWB02	0.86	L Planted
	Bacteroidota	Terrimonas	1.53	L Planted
	Planctomycetota	SH-PL14	3.13	L Planted
	Actinobacteriota	Catenulispora	-2.02	VH Planted
	Verrucomicrobiota	Terrimicrobium	-1.81	VH Planted
	Bacteroidota	UTBCD1	-2.11	VH Planted
	Acidobacteriota	Geothrix	-4.11	VH Planted
	Actinobacteriota	CL500-29 marine group	1.04	L Planted
	Gemmatimonadota	Gemmatimonas	-0.43	VH Planted
	Proteobacteria	Nordella	1.84	L Planted
	Proteobacteria	Altererythrobacter	2.10	L Planted
	Proteobacteria	Microvirga	1.42	L Planted
	Planctomycetota	Singulisphaera	-0.84	VH Planted
	Bacteroidota	Chryseolinea	2.39	L Planted
	Proteobacteria	Leptothrix	-3.28	VH Planted
	Verrucomicrobiota	Oikopleura	2.99	L Planted
	Myxococcota	Pajaroellobacter	1.13	L Planted
	Bacteroidota	Adhaeribacter	1.37	L Planted
	Bacteroidota	Ohtaekwangia	6.03	L Planted
	Firmicutes	Clostridium sensu stricto 9	-1.32	VH Planted
	Bacteroidota	Niastella	-1.10	VH Planted
	Proteobacteria	Polaromonas	4.50	L Planted
	Planctomycetota	Fimbriiglobus	1.15	L Planted
	Proteobacteria	Polycyclovorans	1.87	L Planted
	Verrucomicrobiota	Roseimicrobium	0.58	L Planted
	Planctomycetota	Schlesneria	-0.98	VH Planted
	Acidobacteriota	Vicinamibacter	3.91	L Planted
	Actinobacteriota	Rhizocola	7.90	L Planted
	Proteobacteria	Acidisoma	-6.52	VH Planted
	Bacteroidota	Cytophaga	-1.19	VH Planted
	Actinobacteriota	Microbacterium	1.29	L Planted
	Proteobacteria	Tahibacter	1.35	L Planted
	Bdellovibrionota	OM27 clade	3.38	L Planted

	Acidobacteriota	Paludibaculum	3.83	L Planted
	Planctomycetota	Planctopirus	1.25	L Planted
	Proteobacteria	Ahniella	5.32	L Planted
	Acidobacteriota	Subgroup 10	1.63	L Planted
	Bacteroidota	Heliimonas	-3.92	VH Planted
	Proteobacteria	BD1-7 clade	8.03	L Planted
	Actinobacteriota	Kineosporia	-6.28	VH Planted
	Proteobacteria	966-1	8.12	L Planted
	Planctomycetota	Planctomicrobium	7.49	L Planted
	Myxococcota	Nannocystis	8.76	L Planted
	Bacteroidota	Chitinophaga	-7.35	VH Planted
	Proteobacteria	Parablastomonas	-2.75	VH Planted
	Bacteroidota	Crocinitomix	6.24	L Planted
	Proteobacteria	Phyllobacterium	7.15	L Planted
	Proteobacteria	Duganella	-6.67	VH Planted
	Myxococcota	Polyangium	7.13	L Planted
	Proteobacteria	Hirschia	4.72	L Planted
	Planctomycetota	AKYG587	2.05	L Planted
	Proteobacteria	Amaricoccus	8.48	L Planted
	Proteobacteria	Ferrovibrio	6.22	L Planted
	Proteobacteria	Massilia	-7.56	VH Planted
	Proteobacteria	Asticcacaulis	-7.76	VH Planted
	Proteobacteria	Thermomonas	6.56	L Planted
	Bacteroidota	Lacibacter	5.82	L Planted
	Chloroflexi	Thermosporothrix	-5.97	VH Planted
	Proteobacteria	[Aquaspirillum] arcticum group	-7.28	VH Planted
	Armatimonadota	Chthonomonas	-6.31	VH Planted
	Proteobacteria	Bosea	3.68	L Planted
	Chloroflexi	Herpetosiphon	6.81	L Planted
	Verrucomicrobiota	ADurb.Bin118	3.91	L Planted
	Proteobacteria	IS-44	5.95	L Planted
	Proteobacteria	Candidatus Ovatusbacter	-3.78	VH Planted
	Firmicutes	Desulfosporosinus	-6.00	VH Planted
	Proteobacteria	Nevskia	-7.12	VH Planted
	Bacteroidota	Fluviicola	2.31	L Planted
	Proteobacteria	Dechloromonas	5.44	L Planted
	Actinobacteriota	Glycomyces	5.05	L Planted
	Proteobacteria	Chitinivorax	5.84	L Planted
	Planctomycetota	Rhodopirellula	6.05	L Planted
	Actinobacteriota	Virgisporangium	6.73	L Planted
	Proteobacteria	OM60(NOR5) clade	3.80	L Planted
	Bacteroidota	Arcticibacter	5.80	L Planted

Table S89: Deseq2 analysis of VH Planted vs VL Planted.

VH Planted vs VL Planted	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrosotalea	8.45	VH Planted
	Planctomycetota	Pir4 lineage	-1.86	VL Planted
	Bacteroidota	Puia	0.42	VH Planted
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.93	VL Planted
	Nitrospirota	Nitrospira	-2.59	VL Planted
	Crenarchaeota	Candidatus Nitrocosmicus	-2.46	VL Planted
	Planctomycetota	Aquisphaera	0.91	VH Planted
	Proteobacteria	Reyranella	-0.62	VL Planted
	Actinobacteriota	Acidothermus	2.74	VH Planted
	Proteobacteria	GOUTA6	-1.81	VL Planted
	Proteobacteria	MND1	-4.30	VL Planted
	Verrucomicrobiota	Ellin516	1.05	VH Planted
	Actinobacteriota	Nocardioides	-0.72	VL Planted
	Actinobacteriota	Conexibacter	1.64	VH Planted
	Proteobacteria	Ellin6067	-1.50	VL Planted
	Proteobacteria	Rhodoblastus	1.17	VH Planted
	Acidobacteriota	RB41	-2.34	VL Planted
	Proteobacteria	Pedomicrobium	-2.96	VL Planted
	Actinobacteriota	Streptomyces	-1.29	VL Planted
	Proteobacteria	Roseiarcus	1.66	VH Planted
	Acidobacteriota	Holophaga	6.27	VH Planted
	Acidobacteriota	Occallatibacter	3.20	VH Planted
	Actinobacteriota	Iamia	0.37	VH Planted
	Proteobacteria	Rhodanobacter	3.32	VH Planted
	Planctomycetota	Gemmata	0.66	VH Planted
	Bacteroidota	Parafilimonas	-1.00	VL Planted
	Acidobacteriota	Luteitalea	-2.83	VL Planted
	Proteobacteria	mle1-7	-2.26	VL Planted
	Acidobacteriota	Candidatus Koribacter	0.73	VH Planted
	Planctomycetota	Pirellula	-1.82	VL Planted
	Proteobacteria	Arenimonas	-4.25	VL Planted
	Bacteroidota	OLB12	1.51	VH Planted
	Myxococcota	Haliangium	-0.92	VL Planted
	Chloroflexi	UTCFX1	-7.72	VL Planted
	Acidobacteriota	Bryobacter	0.82	VH Planted
	Proteobacteria	Acidibacter	-3.17	VL Planted
	Actinobacteriota	Actinocorallia	-2.29	VL Planted
	Acidobacteriota	Candidatus Solibacter	0.40	VH Planted
	Proteobacteria	Steroidobacter	-2.10	VL Planted

	Proteobacteria	Bauldia	-1.68	VL Planted
	Verrucomicrobiota	Chthoniobacter	-1.91	VL Planted
	Actinobacteriota	Ilumatobacter	-6.14	VL Planted
	Planctomycetota	Zavarzinella	1.23	VH Planted
	Planctomycetota	Candidatus Nostocoida	1.50	VH Planted
	Actinobacteriota	Microlunatus	-2.71	VL Planted
	Proteobacteria	Candidatus Alysiosphaera	-3.14	VL Planted
	Proteobacteria	Rhodoplanes	-4.56	VL Planted
	Acidobacteriota	Granulicella	5.74	VH Planted
	Actinobacteriota	Terrabacter	1.22	VH Planted
	Acidobacteriota	Acidipila	1.44	VH Planted
	Proteobacteria	Ellin6055	-4.96	VL Planted
	Planctomycetota	Schlesneria	1.38	VH Planted
	Actinobacteriota	Jatrophihabitans	0.83	VH Planted
	Actinobacteriota	Solirubrobacter	-2.19	VL Planted
	Proteobacteria	Mesorhizobium	-2.85	VL Planted
	Proteobacteria	Pseudorhodoplanes	-9.49	VL Planted
	Proteobacteria	Pseudoduganella	-4.76	VL Planted
	Actinobacteriota	Pseudonocardia	-0.86	VL Planted
	Proteobacteria	Dokdonella	1.11	VH Planted
	Proteobacteria	Dongia	-2.55	VL Planted
	Verrucomicrobiota	Opitutus	-1.17	VL Planted
	Proteobacteria	Acidicaldus	4.43	VH Planted
	Desulfobacterota	Geobacter	-3.87	VL Planted
	Bacteroidota	Flavobacterium	-3.92	VL Planted
	Verrucomicrobiota	Ellin517	-5.03	VL Planted
	Firmicutes	Bacillus	1.20	VH Planted
	Bacteroidota	Aurantisolimonas	-4.41	VL Planted
	Myxococcota	P3OB-42	-2.42	VL Planted
	Verrucomicrobiota	Luteolibacter	-3.63	VL Planted
	Bacteroidota	Mucilaginibacter	3.95	VH Planted
	Spirochaetota	Turneriella	-2.23	VL Planted
	Proteobacteria	SWB02	-4.33	VL Planted
	Bacteroidota	Terrimonas	-6.38	VL Planted
	Planctomycetota	SH-PL14	-5.92	VL Planted
	Myxococcota	Anaeromyxobacter	-1.37	VL Planted
	Firmicutes	Lysinibacillus	0.87	VH Planted
	Actinobacteriota	Catenulispora	3.51	VH Planted
	Proteobacteria	Defluviicoccus	-2.20	VL Planted
	Actinobacteriota	Blastococcus	0.96	VH Planted
	Bacteroidota	Ferruginibacter	-2.58	VL Planted
	Proteobacteria	Nordella	-8.83	VL Planted
	Proteobacteria	Altererythrobacter	-2.84	VL Planted
	Actinobacteriota	Dactylosporangium	-1.82	VL Planted

	Proteobacteria	Microvirga	-9.07	VL Planted
	Bacteroidota	Chryseolinea	-9.24	VL Planted
	Proteobacteria	Aquicella	1.07	VH Planted
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	5.36	VH Planted
	Actinobacteriota	Streptacidiphilus	8.79	VH Planted
	Verrucomicrobiota	Roseimicrobium	-3.43	VL Planted
	Planctomycetota	Singulisphaera	1.24	VH Planted
	Firmicutes	Ammoniphilus	1.89	VH Planted
	Verrucomicrobiota	Oikopleura	-8.64	VL Planted
	Myxococcota	Pajaroellobacter	-1.78	VL Planted
	Proteobacteria	Labrys	-2.16	VL Planted
	Bacteroidota	Ohtaekwangia	-10.27	VL Planted
	Planctomycetota	Planctopirus	-4.02	VL Planted
	Actinobacteriota	CL500-29 marine group	-4.56	VL Planted
	Proteobacteria	Polaromonas	-8.05	VL Planted
	Proteobacteria	Polycyclovorans	-6.28	VL Planted
	Cyanobacteria	Nostoc PCC-73102	-2.01	VL Planted
	Acidobacteriota	Vicinamibacter	-7.75	VL Planted
	Actinobacteriota	Rhizocola	-7.52	VL Planted
	Proteobacteria	Acidisoma	8.44	VH Planted
	Acidobacteriota	JGI 0001001-H03	-1.98	VL Planted
	Acidobacteriota	Stenotrophobacter	-8.59	VL Planted
	Proteobacteria	Tahibacter	-6.99	VL Planted
	Bdellovibrionota	OM27 clade	-9.43	VL Planted
	Acidobacteriota	Paludibaculum	-7.51	VL Planted
	Planctomycetota	SM1A02	-2.69	VL Planted
	Proteobacteria	Ahniella	-8.44	VL Planted
	Actinobacteriota	Nonomuraea	-6.39	VL Planted
	Proteobacteria	BD1-7 clade	-7.66	VL Planted
	Actinobacteriota	Actinospica	7.65	VH Planted
	Acidobacteriota	Subgroup 10	-3.12	VL Planted
	Actinobacteriota	Kineosporia	6.66	VH Planted
	Abditibacteriota	Abditibacterium	1.20	VH Planted
	Proteobacteria	Alkanibacter	4.08	VH Planted
	Chloroflexi	Ktedonobacter	3.29	VH Planted
	Bacteroidota	Dinghuibacter	-6.83	VL Planted
	Proteobacteria	966-1	-7.74	VL Planted
	Planctomycetota	Fimbriiglobus	-4.45	VL Planted
	Proteobacteria	Pseudorhodobacter	-6.20	VL Planted
	Bacteroidota	Solitalea	-6.60	VL Planted
	Firmicutes	Psychrobacillus	7.07	VH Planted
	Bacteroidota	Adhaeribacter	-2.97	VL Planted
	Planctomycetota	Planctomicrobium	-7.11	VL Planted

	Myxococcota	Nannocystis	-8.38	VL Planted
	Planctomycetota	Tundrisphaera	2.08	VH Planted
	Proteobacteria	Rudaea	7.78	VH Planted
	Bacteroidota	Crocinitomix	-5.85	VL Planted
	Proteobacteria	Nevskia	6.80	VH Planted
	Bacteroidota	Heliimonas	4.33	VH Planted
	Verrucomicrobiota	DEV008	-4.55	VL Planted
	Proteobacteria	Phyllobacterium	-6.77	VL Planted
	Bdellovibrionota	Oligoflexus	-6.14	VL Planted
	Proteobacteria	Asticcacaulis	7.94	VH Planted
	Proteobacteria	Duganella	7.10	VH Planted
	Myxococcota	Polyangium	-6.75	VL Planted
	Proteobacteria	Hirschia	-5.36	VL Planted
	Planctomycetota	CL500-3	-6.23	VL Planted
	Verrucomicrobiota	Diplosphaera	-5.56	VL Planted
	Planctomycetota	AKYG587	-5.66	VL Planted
	Myxococcota	Sandaracinus	-2.20	VL Planted
	Proteobacteria	Novosphingobium	-7.34	VL Planted
	Proteobacteria	JTB255 marine benthic group	-6.34	VL Planted
	Armatimonadota	Armatimonas	-6.09	VL Planted
	Proteobacteria	Amaricoccus	-8.10	VL Planted
	Proteobacteria	Ferrovibrio	-5.84	VL Planted
	Sumerlaeota	Sumerlaea	-2.79	VL Planted
	Proteobacteria	Sulfurifustis	-3.16	VL Planted
	Verrucomicrobiota	SH3-11	-6.59	VL Planted
	Chloroflexi	1921-2	20.56	VH Planted
	Proteobacteria	Thermomonas	-6.17	VL Planted
	Chloroflexi	Litorilinea	-5.39	VL Planted
	Bacteroidota	Lacibacter	-5.44	VL Planted
	Proteobacteria	Candidatus Endoecteinascidia	5.97	VH Planted
	Firmicutes	Clostridium sensu stricto 12	6.28	VH Planted
	Armatimonadota	Chthonomonas	5.92	VH Planted
	Proteobacteria	Bosea	-5.90	VL Planted
	Chloroflexi	Herpetosiphon	-6.44	VL Planted
	Actinobacteriota	Geodermatophilus	-5.51	VL Planted
	Myxococcota	Vulgatibacter	-6.78	VL Planted
	Verrucomicrobiota	ADurb.Bin118	-5.71	VL Planted
	Proteobacteria	IS-44	-5.57	VL Planted
	Actinobacteriota	Agromyces	-6.26	VL Planted
	Actinobacteriota	Kitasatospora	6.66	VH Planted
	Bacteroidota	Fluviicola	-3.39	VL Planted

	Verrucomicrobiota	FukuN18 freshwater group	7.38	VH Planted
	Proteobacteria	Acidiphilium	5.63	VH Planted
	Chloroflexi	Thermosporothrix	7.88	VH Planted
	Proteobacteria	PMMR1	6.29	VH Planted
	Proteobacteria	Candidatus Ovatusbacter	4.36	VH Planted
	Bdellovibrionota	Bacteriovorax	-6.14	VL Planted
	Bacteroidota	Chitinophaga	6.90	VH Planted
	Proteobacteria	Inquilinus	6.21	VH Planted
	Proteobacteria	Chitinivorax	-5.47	VL Planted
	Firmicutes	Clostridium sensu stricto 10	5.98	VH Planted
	Planctomycetota	Rhodopirellula	-5.67	VL Planted
	Actinobacteriota	Virgisporangium	-6.35	VL Planted
	Proteobacteria	OM60(NOR5) clade	-5.61	VL Planted
	Bacteroidota	Arcticibacter	-5.43	VL Planted

Table S90: Deseq2 analysis of L Planted vs H Planted.

L Planted vs H Planted	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Crenarchaeota	Candidatus Nitrosotalea	2.42	L Planted
	Bacteroidota	Puia	0.53	L Planted
	Nitrospirota	Nitrospira	-0.56	H Planted
	Verrucomicrobiota	Ellin516	0.55	L Planted
	Proteobacteria	Rhodanobacter	1.28	L Planted
	Acidobacteriota	Holophaga	9.14	L Planted
	Acidobacteriota	Occallatibacter	1.77	L Planted
	Bacteroidota	OLB12	1.08	L Planted
	Verrucomicrobiota	Lacunisphaera	0.98	L Planted
	Bacteroidota	Parafilimonas	0.59	L Planted
	Myxococcota	Phaselicystis	-0.52	H Planted
	Actinobacteriota	Ilumatobacter	-1.17	H Planted
	Bacteroidota	Flavobacterium	-1.24	H Planted
	Proteobacteria	Dongia	-1.15	H Planted
	Bacteroidota	Mucilaginibacter	1.96	L Planted
	Proteobacteria	Pseudorhodoplanes	-1.54	H Planted
	Proteobacteria	Dokdonella	0.95	L Planted
	Acidobacteriota	Granulicella	4.05	L Planted
	Acidobacteriota	Geothrix	2.15	L Planted
	Proteobacteria	Altererythrobacter	-1.41	H Planted

	Proteobacteria	Leptothrix	3.99	L Planted
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	5.74	L Planted
	Planctomycetota	SH-PL14	-1.93	H Planted
	Bacteroidota	Ohtaekwangia	-4.33	H Planted
	Myxococcota	Sandaracinus	1.03	L Planted
	Proteobacteria	Acidisoma	5.70	L Planted
	Acidobacteriota	Paludibaculum	-2.92	H Planted
	Proteobacteria	Hirschia	-4.05	H Planted
	Bacteroidota	Heliimonas	7.17	L Planted
	Proteobacteria	Duganella	5.87	L Planted
	Myxococcota	Nannocystis	-7.21	H Planted
	Bdellovibrionota	OM27 clade	-2.14	H Planted
	Proteobacteria	Massilia	6.76	L Planted
	Bacteroidota	Flavisolibacter	5.49	L Planted
	Proteobacteria	[Aquaspirillum] arcticum group	6.48	L Planted
	Proteobacteria	BD1-7 clade	-6.90	H Planted
	Chloroflexi	HSB OF53-F07	6.38	L Planted
	Proteobacteria	Nevskia	6.32	L Planted
	Bacteroidota	Chryseobacterium	-21.86	H Planted
	Proteobacteria	Chitinimonas	-5.70	H Planted
	Bdellovibrionota	Peredibacter	-5.73	H Planted

Table S91: Deseq2 analysis of H Planted vs VL Planted.

H Planted vs VL Planted	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Verrucomicrobiota	Candidatus Udaeobacter	-0.35	VL Planted
	Crenarchaeota	Candidatus Nitrosotalea	4.10	H Planted
	Planctomycetota	Pir4 lineage	-1.27	VL Planted
	Verrucomicrobiota	Candidatus Xiphinematobacter	-0.62	VL Planted
	Nitrospirota	Nitrospira	-1.97	VL Planted
	Proteobacteria	MND1	-3.55	VL Planted
	Planctomycetota	Aquisphaera	0.76	H Planted
	Actinobacteriota	Acidothermus	2.20	H Planted
	Proteobacteria	GOUTA6	-1.77	VL Planted
	Actinobacteriota	Conexibacter	1.06	H Planted
	Proteobacteria	Ellin6067	-0.80	VL Planted
	Acidobacteriota	RB41	-2.33	VL Planted
	Proteobacteria	Pedomicrobium	-1.98	VL Planted

	Actinobacteriota	Streptomyces	-1.15	VL Planted
	Proteobacteria	Roseiarcus	1.19	H Planted
	Acidobacteriota	Holophaga	11.95	H Planted
	Proteobacteria	Acidibacter	-2.50	VL Planted
	Acidobacteriota	Occallatibacter	2.60	H Planted
	Proteobacteria	Rhodanobacter	2.58	H Planted
	Planctomycetota	Gemmata	1.18	H Planted
	Bacteroidota	Parafilimonas	-1.02	VL Planted
	Proteobacteria	Arenimonas	-4.02	VL Planted
	Acidobacteriota	Luteitalea	-2.97	VL Planted
	Proteobacteria	mle1-7	-1.62	VL Planted
	Planctomycetota	Pirellula	-1.40	VL Planted
	Bacteroidota	OLB12	1.09	H Planted
	Myxococcota	Haliangium	-0.82	VL Planted
	Chloroflexi	UTCFX1	-5.71	VL Planted
	Actinobacteriota	Actinocorallia	-1.94	VL Planted
	Proteobacteria	Bauldia	-1.51	VL Planted
	Verrucomicrobiota	Chthoniobacter	-1.43	VL Planted
	Crenarchaeota	Candidatus Nitrocosmicus	-0.91	VL Planted
	Actinobacteriota	Ilumatobacter	-5.14	VL Planted
	Planctomycetota	Candidatus Nostocoida	0.92	H Planted
	Actinobacteriota	Microlunatus	-2.14	VL Planted
	Proteobacteria	Candidatus Alysiosphaera	-2.33	VL Planted
	Proteobacteria	Rhodoplanes	-3.48	VL Planted
	Acidobacteriota	Granulicella	6.30	H Planted
	Actinobacteriota	Terrabacter	1.24	H Planted
	Proteobacteria	Ellin6055	-2.59	VL Planted
	Planctomycetota	Schlesneria	1.25	H Planted
	Actinobacteriota	Jatrophihabitans	0.98	H Planted
	Actinobacteriota	Solirubrobacter	-1.66	VL Planted
	Proteobacteria	Mesorhizobium	-2.86	VL Planted
	Proteobacteria	Pseudorhodoplanes	-8.50	VL Planted
	Proteobacteria	Pseudoduganella	-4.30	VL Planted
	Desulfobacterota	Geobacter	-3.66	VL Planted
	Proteobacteria	Dokdonella	1.27	H Planted
	Verrucomicrobiota	Opitutus	-0.77	VL Planted
	Proteobacteria	Acidicaldus	4.53	H Planted
	Bacteroidota	Flavobacterium	-3.53	VL Planted
	Bacteroidota	Aurantisolimonas	-3.71	VL Planted
	Verrucomicrobiota	Luteolibacter	-2.86	VL Planted
	Verrucomicrobiota	Ellin517	-4.78	VL Planted
	Bacteroidota	Mucilaginibacter	3.15	H Planted
	Bacteroidota	Ferruginibacter	-2.29	VL Planted
	Spirochaetota	Turneriella	-1.47	VL Planted

	Proteobacteria	SWB02	-3.64	VL Planted
	Actinobacteriota	Catenulispora	4.10	H Planted
	Bacteroidota	UTBCD1	-4.12	VL Planted
	Proteobacteria	Defluviicoccus	-1.75	VL Planted
	Actinobacteriota	CL500-29 marine group	-3.11	VL Planted
	Proteobacteria	Nordella	-7.52	VL Planted
	Proteobacteria	Nitrosospira	-2.87	VL Planted
	Bacteroidota	Terrimonas	-5.32	VL Planted
	Proteobacteria	Altererythrobacter	-1.83	VL Planted
	Actinobacteriota	Dactylosporangium	-1.70	VL Planted
	Proteobacteria	Microvirga	-8.31	VL Planted
	Proteobacteria	Dongia	-2.03	VL Planted
	Proteobacteria	Aquicella	1.12	H Planted
	Proteobacteria	Leptothrix	4.14	H Planted
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	9.23	H Planted
	Proteobacteria	alphaI cluster	-2.92	VL Planted
	Actinobacteriota	Streptacidiphilus	8.22	H Planted
	Planctomycetota	Singulisphaera	1.21	H Planted
	Firmicutes	Ammoniphilus	2.33	H Planted
	Verrucomicrobiota	Oikopleura	-7.38	VL Planted
	Planctomycetota	SH-PL14	-4.52	VL Planted
	Proteobacteria	Noviherbaspirillum	-3.33	VL Planted
	Proteobacteria	Labrys	-1.70	VL Planted
	Bacteroidota	Adhaeribacter	-1.70	VL Planted
	Bacteroidota	Ohtaekwangia	-8.24	VL Planted
	Planctomycetota	Planctopirus	-3.18	VL Planted
	Proteobacteria	Polaromonas	-6.61	VL Planted
	Planctomycetota	SM1A02	-2.21	VL Planted
	Planctomycetota	Fimbriiglobus	-3.32	VL Planted
	Bacteroidota	Chryseolinea	-7.27	VL Planted
	Acidobacteriota	Vicinamibacter	-5.43	VL Planted
	Proteobacteria	Acidisoma	7.92	H Planted
	Myxococcota	Pajaroellobacter	-1.27	VL Planted
	Acidobacteriota	Stenotrophobacter	-5.68	VL Planted
	Proteobacteria	Tahibacter	-6.75	VL Planted
	Verrucomicrobiota	Roseimicrobium	-3.15	VL Planted
	Acidobacteriota	Paludibaculum	-6.31	VL Planted
	Proteobacteria	Hirschia	-4.48	VL Planted
	Chloroflexi	Litorilinea	-4.95	VL Planted
	Actinobacteriota	Actinospica	7.10	H Planted
	Acidobacteriota	Subgroup 10	-1.60	VL Planted
	Abditibacteriota	Abditibacterium	1.98	H Planted
	Proteobacteria	Polycyclovorans	-5.19	VL Planted

	Proteobacteria	Alkanibacter	2.17	H Planted
	Bacteroidota	Dinghuibacter	-5.86	VL Planted
	Bacteroidota	Solitalea	-5.50	VL Planted
	Firmicutes	Psychrobacillus	6.50	H Planted
	Proteobacteria	Rudaea	7.25	H Planted
	Proteobacteria	Nevskia	6.22	H Planted
	Bacteroidota	Heliimonas	7.80	H Planted
	Bdellovibrionota	Oligoflexus	-5.81	VL Planted
	Acidobacteriota	Terracidiphilus	5.86	H Planted
	Proteobacteria	Duganella	6.54	H Planted
	Planctomycetota	CL500-3	-5.56	VL Planted
	Verrucomicrobiota	Diplosphaera	-6.79	VL Planted
	Myxococcota	Vulgatibacter	-6.11	VL Planted
	Myxococcota	Nannocystis	-6.57	VL Planted
	Proteobacteria	Novosphingobium	-6.23	VL Planted
	Proteobacteria	Sulfurifustis	-3.23	VL Planted
	Chloroflexi	1921-2	20.49	H Planted
	Proteobacteria	Ahniella	-6.03	VL Planted
	Proteobacteria	Candidatus Endoecteinascidia	5.38	H Planted
	Proteobacteria	BD1-7 clade	-6.25	VL Planted
	Actinobacteriota	Kitasatospora	6.08	H Planted
	Actinobacteriota	Nonomuraea	-6.23	VL Planted
	Bdellovibrionota	OM27 clade	-7.92	VL Planted
	Verrucomicrobiota	FukuN18 freshwater group	6.79	H Planted
	Chloroflexi	Thermosporothrix	4.31	H Planted
	Chloroflexi	HSB OF53-F07	6.37	H Planted
	Proteobacteria	PMMR1	5.71	H Planted
	Verrucomicrobiota	SH3-11	-5.83	VL Planted
	Proteobacteria	Inquilinus	5.63	H Planted
	Firmicutes	Clostridium sensu stricto 10	5.41	H Planted

Table S92: Deseq2 analysis of L Planted vs VL Planted.

L Planted vs VL Planted	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrosotalea	1.74	L Planted
	Planctomycetota	Pir4 lineage	-1.33	VL Planted
	Nitrospirota	Nitrospira	-1.36	VL Planted
	Crenarchaeota	Candidatus Nitrocosmicus	-1.19	VL Planted
	Planctomycetota	Aquisphaera	0.51	L Planted
	Actinobacteriota	Acidothermus	1.49	L Planted
	Proteobacteria	GOUTA6	-1.78	VL Planted
	Proteobacteria	MND1	-3.01	VL Planted
	Actinobacteriota	Conexibacter	1.22	L Planted
	Proteobacteria	Ellin6067	-0.57	VL Planted
	Proteobacteria	Rhodanobacter	1.37	L Planted
	Acidobacteriota	RB41	-2.12	VL Planted
	Proteobacteria	Pedomicrobium	-1.67	VL Planted
	Actinobacteriota	Streptomyces	-0.65	VL Planted
	Proteobacteria	Roseiarcus	0.96	L Planted
	Acidobacteriota	Holophaga	2.90	L Planted
	Proteobacteria	Acidibacter	-2.38	VL Planted
	Acidobacteriota	Occallatibacter	0.90	L Planted
	Planctomycetota	Gemmata	0.60	L Planted
	Verrucomicrobiota	Lacunisphaera	-1.00	VL Planted
	Bacteroidota	Parafilimonas	-1.54	VL Planted
	Proteobacteria	Arenimonas	-3.93	VL Planted
	Acidobacteriota	Luteitalea	-2.53	VL Planted
	Proteobacteria	mle1-7	-1.52	VL Planted
	Planctomycetota	Pirellula	-1.30	VL Planted
	Myxococcota	Haliangium	-0.66	VL Planted
	Chloroflexi	UTCFX1	-5.78	VL Planted
	Actinobacteriota	Actinocorallia	-1.73	VL Planted
	Proteobacteria	Bauldia	-1.39	VL Planted
	Actinobacteriota	Ilumatobacter	-3.95	VL Planted
	Planctomycetota	Candidatus Nostocoida	1.20	L Planted
	Actinobacteriota	Microlunatus	-1.49	VL Planted
	Proteobacteria	Candidatus Alysiosphaera	-2.07	VL Planted
	Proteobacteria	Rhodoplanes	-3.07	VL Planted
	Acidobacteriota	Granulicella	2.42	L Planted
	Bacteroidota	Flavobacterium	-2.25	VL Planted
	Planctomycetota	Schlesneria	0.73	L Planted
	Bacteroidota	Mucilaginibacter	1.27	L Planted
	Actinobacteriota	Solirubrobacter	-1.69	VL Planted
	Proteobacteria	Mesorhizobium	-1.98	VL Planted
	Proteobacteria	Pseudorhodoplanes	-6.91	VL Planted
	Proteobacteria	Pseudoduganella	-4.98	VL Planted

	Desulfobacterota	Geobacter	-3.29	VL Planted
	Verrucomicrobiota	Opitutus	-1.13	VL Planted
	Proteobacteria	Acidicaldus	2.12	L Planted
	Verrucomicrobiota	ADurb.Bin063-1	-0.60	VL Planted
	Verrucomicrobiota	Luteolibacter	-2.06	VL Planted
	Verrucomicrobiota	Ellin517	-4.58	VL Planted
	Proteobacteria	SWB02	-3.18	VL Planted
	Myxococcota	Anaeromyxobacter	-1.14	VL Planted
	Actinobacteriota	Catenulispora	1.82	L Planted
	Bacteroidota	UTBCD1	-3.98	VL Planted
	Acidobacteriota	Geothrix	-2.08	VL Planted
	Actinobacteriota	CL500-29 marine group	-3.22	VL Planted
	Proteobacteria	Microvirga	-7.35	VL Planted
	Bacteroidota	Terrimonas	-4.59	VL Planted
	Proteobacteria	Dongia	-0.81	VL Planted
	Planctomycetota	Singulisphaera	0.72	L Planted
	Proteobacteria	Aquicella	1.26	L Planted
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	3.51	L Planted
	Actinobacteriota	Streptacidiphilus	3.53	L Planted
	Verrucomicrobiota	Chthoniobacter	-1.21	VL Planted
	Firmicutes	Ammoniphilus	1.40	L Planted
	Proteobacteria	Labrys	-1.70	VL Planted
	Bacteroidota	Aurantisolimonas	-3.33	VL Planted
	Planctomycetota	SM1A02	-1.67	VL Planted
	Planctomycetota	Fimbriiglobus	-2.97	VL Planted
	Verrucomicrobiota	Roseimicrobium	-2.57	VL Planted
	Bacteroidota	Chryseolinea	-6.52	VL Planted
	Myxococcota	Sandaracinus	-2.27	VL Planted
	Bacteroidota	Ferruginibacter	-1.87	VL Planted
	Bacteroidota	Niastella	-1.63	VL Planted
	Proteobacteria	Acidisoma	2.24	L Planted
	Acidobacteriota	JGI 0001001-H03	-2.54	VL Planted
	Proteobacteria	Tahibacter	-5.33	VL Planted
	Planctomycetota	Planctopirus	-2.44	VL Planted
	Chloroflexi	Litorilinea	-4.90	VL Planted
	Proteobacteria	Nordella	-6.64	VL Planted
	Actinobacteriota	Actinospica	7.85	L Planted
	Abditibacteriota	Abditibacterium	1.23	L Planted
	Proteobacteria	Polycyclovorans	-4.07	VL Planted
	Proteobacteria	Alkanibacter	1.96	L Planted
	Bacteroidota	Dinghuibacter	-5.27	VL Planted
	Proteobacteria	Candidatus Paracaedibacter	4.67	L Planted

	Proteobacteria	Parablastomonas	-7.10	VL Planted
	Proteobacteria	Rudaea	2.94	L Planted
	Planctomycetota	CL500-3	-4.93	VL Planted
	Verrucomicrobiota	Diplosphaera	-6.37	VL Planted
	Proteobacteria	JTB255 marine benthic group	-4.93	VL Planted
	Bdellovibrionota	OM27 clade	-5.72	VL Planted
	Chloroflexi	1921-2	21.26	L Planted
	Verrucomicrobiota	DEV008	-3.32	VL Planted
	Proteobacteria	[Aquaspirillum] arcticum group	-6.46	VL Planted
	Proteobacteria	AAP99	-5.07	VL Planted
	Verrucomicrobiota	Oikopleura	-5.39	VL Planted
	Actinobacteriota	Kitasatospora	6.84	L Planted
	Actinobacteriota	Nonomuraea	-6.16	VL Planted
	Verrucomicrobiota	FukuN18 freshwater group	4.04	L Planted
	Chloroflexi	Thermosporothrix	2.21	L Planted
	Proteobacteria	PMMR1	6.47	L Planted
	Verrucomicrobiota	SH3-11	-5.11	VL Planted
	Proteobacteria	Caedibacter	6.25	L Planted
	Bdellovibrionota	Bacteriovorax	-5.13	VL Planted

Table S93: Deseq2 analysis of H Fallow vs VH Fallow.

H Fallow vs VH Fallow	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Verrucomicrobiota	Candidatus Udaeobacter	-0.79	VH Fallow
	Crenarchaeota	Candidatus Nitrosotalea	-4.64	VH Fallow

	Planctomycetota	Pir4 lineage	0.37	H Fallow
	Bacteroidota	Puia	-1.02	VH Fallow
	Nitrospirota	Nitrospira	0.51	H Fallow
	Proteobacteria	MND1	0.68	H Fallow
	Crenarchaeota	Candidatus Nitrocosmicus	1.06	H Fallow
	Proteobacteria	Rhodomicrobium	-0.58	VH Fallow
	Actinobacteriota	Acidothermus	-1.01	VH Fallow
	Actinobacteriota	Conexibacter	-0.72	VH Fallow
	Proteobacteria	Rhodanobacter	-1.15	VH Fallow
	Proteobacteria	Rhodoblastus	-1.11	VH Fallow
	Proteobacteria	Pedomicrobium	0.72	H Fallow
	Proteobacteria	Roseiarcus	-1.06	VH Fallow
	Acidobacteriota	Occallatibacter	-1.27	VH Fallow
	Verrucomicrobiota	Ellin516	-1.12	VH Fallow
	Bacteroidota	Parafilimonas	-0.57	VH Fallow
	Acidobacteriota	Candidatus Solibacter	-0.48	VH Fallow
	Chloroflexi	UTCFX1	1.45	H Fallow
	Acidobacteriota	Bryobacter	-0.58	VH Fallow
	Bacteroidota	OLB12	-0.63	VH Fallow
	Proteobacteria	Steroidobacter	0.73	H Fallow
	Verrucomicrobiota	ADurb.Bin063-1	-0.51	VH Fallow
	Bacteroidota	Flavobacterium	0.50	H Fallow
	Actinobacteriota	Ilumatobacter	0.80	H Fallow
	Proteobacteria	Ellin6055	1.97	H Fallow
	Proteobacteria	Pseudorhodoplanes	0.76	H Fallow
	Proteobacteria	Pseudoduganella	1.32	H Fallow
	Proteobacteria	SWB02	0.91	H Fallow
	Bacteroidota	Terrimonas	1.24	H Fallow
	Planctomycetota	SH-PL14	1.51	H Fallow
	Bacteroidota	UTBCD1	-1.81	VH Fallow
	Myxococcota	Nannocystis	3.87	H Fallow
	Acidobacteriota	Geothrix	-1.84	VH Fallow
	Proteobacteria	Nordella	1.85	H Fallow
	Proteobacteria	Altererythrobacter	1.01	H Fallow
	Proteobacteria	Microvirga	0.84	H Fallow
	Bacteroidota	Chryseolinea	1.99	H Fallow
	Bacteroidota	Ohtaekwangia	2.11	H Fallow
	Acidobacteriota	Vicinamibacter	2.41	H Fallow
	Actinobacteriota	Rhizocola	7.30	H Fallow
	Acidobacteriota	Stenotrophobacter	3.69	H Fallow
	Bdellovibrionota	OM27 clade	1.68	H Fallow
	Proteobacteria	Hirschia	2.35	H Fallow
	Proteobacteria	Ahniella	2.09	H Fallow
	Actinobacteriota	Kineosporia	-6.81	VH Fallow

	Acidobacteriota	Subgroup 10	2.11	H Fallow
	Proteobacteria	966-1	2.67	H Fallow
	Planctomycetota	Planctomicrobium	4.42	H Fallow
	Myxococcota	Polyangium	5.72	H Fallow
	Actinobacteriota	Lechevalieria	6.25	H Fallow
	Planctomycetota	AKYG587	1.72	H Fallow
	Proteobacteria	Amaricoccus	4.32	H Fallow
	Proteobacteria	Ferrovibrio	5.83	H Fallow
	Bacteroidota	Flavitalea	6.20	H Fallow
	Chloroflexi	Herpetosiphon	2.63	H Fallow
	Proteobacteria	OM60(NOR5) clade	5.09	H Fallow
	Proteobacteria	Flavimaricola	4.55	H Fallow
	Planctomycetota	Rhodopirellula	5.16	H Fallow

Table S94: Deseq2 analysis of L Fallow vs VH Fallow.

L Fallow vs VH Fallow	Phylum	Genus	log2FoldChange	Enriched
	Verrucomicrobiota	Candidatus Udaeobacter	-0.72	VH Fallow
	Crenarchaeota	Candidatus Nitrosotalea	-6.95	VH Fallow
	Proteobacteria	Pseudolabrys	-0.31	VH Fallow
	Actinobacteriota	Gaiella	-0.42	VH Fallow
	Planctomycetota	Pir4 lineage	0.38	L Fallow
	Bacteroidota	Puia	-0.97	VH Fallow
	Verrucomicrobiota	Candidatus Xiphinematobacter	0.41	L Fallow
	Nitrospirota	Nitrospira	1.59	L Fallow
	Actinobacteriota	Mycobacterium	-0.57	VH Fallow
	Proteobacteria	MND1	1.74	L Fallow
	Planctomycetota	Aquisphaera	-0.82	VH Fallow
	Actinobacteriota	Acidothermus	-1.79	VH Fallow
	Proteobacteria	Bradyrhizobium	-0.57	VH Fallow
	Actinobacteriota	Nocardioides	-0.50	VH Fallow
	Actinobacteriota	Conexibacter	-1.05	VH Fallow
	Proteobacteria	Ellin6067	0.73	L Fallow
	Proteobacteria	Rhodanobacter	-3.02	VH Fallow
	Proteobacteria	Rhodoblastus	-1.35	VH Fallow
	Acidobacteriota	RB41	0.96	L Fallow
	Proteobacteria	Pedomicrobium	1.07	L Fallow
	Proteobacteria	Roseiarcus	-1.48	VH Fallow
	Acidobacteriota	Holophaga	-6.29	VH Fallow
	Acidobacteriota	Occallatibacter	-3.31	VH Fallow

	Verrucomicrobiota	Ellin516	-1.33	VH Fallow
	Bacteroidota	OLB12	-1.67	VH Fallow
	Bacteroidota	Parafilimonas	-0.88	VH Fallow
	Acidobacteriota	Luteitalea	1.18	L Fallow
	Proteobacteria	mle1-7	0.79	L Fallow
	Acidobacteriota	Candidatus Koribacter	-0.85	VH Fallow
	Chloroflexi	UTCFX1	2.46	L Fallow
	Acidobacteriota	Bryobacter	-0.76	VH Fallow
	Proteobacteria	Acidibacter	0.74	L Fallow
	Proteobacteria	Steroidobacter	1.07	L Fallow
	Proteobacteria	Bauldia	0.54	L Fallow
	Verrucomicrobiota	Chthoniobacter	0.86	L Fallow
	Bacteroidota	Flavobacterium	1.34	L Fallow
	Actinobacteriota	Ilumatobacter	1.61	L Fallow
	Planctomycetota	Zavarzinella	-1.43	VH Fallow
	Planctomycetota	Candidatus Nostocoida	-1.39	VH Fallow
	Planctomycetota	Gemmata	-0.65	VH Fallow
	Proteobacteria	Rhodoplanes	1.50	L Fallow
	Acidobacteriota	Granulicella	-4.48	VH Fallow
	Actinobacteriota	Terrabacter	-1.37	VH Fallow
	Acidobacteriota	Acidipila	-1.59	VH Fallow
	Proteobacteria	Dongia	1.93	L Fallow
	Proteobacteria	Ellin6055	2.50	L Fallow
	Actinobacteriota	Jatrophihabitans	-0.80	VH Fallow
	Bacteroidota	Mucilaginibacter	-2.15	VH Fallow
	Bdellovibrionota	Bdellovibrio	0.88	L Fallow
	Proteobacteria	Mesorhizobium	1.03	L Fallow
	Proteobacteria	Pseudorhodoplanes	2.61	L Fallow
	Proteobacteria	Pseudoduganella	2.30	L Fallow
	Desulfobacterota	Geobacter	1.40	L Fallow
	Proteobacteria	Dokdonella	-1.18	VH Fallow
	Spirochaetota	Spirochaeta 2	-0.88	VH Fallow
	Verrucomicrobiota	Ellin517	1.85	L Fallow
	Myxococcota	P3OB-42	0.91	L Fallow
	Verrucomicrobiota	Luteolibacter	1.63	L Fallow
	Spirochaetota	Turneriella	1.29	L Fallow
	Proteobacteria	SWB02	1.91	L Fallow
	Bacteroidota	Terrimonas	2.28	L Fallow
	Planctomycetota	SH-PL14	2.96	L Fallow
	Actinobacteriota	Catenulispora	-5.56	VH Fallow
	Verrucomicrobiota	Terrimicrobium	-2.39	VH Fallow
	Proteobacteria	Defluviicoccus	0.77	L Fallow
	Myxococcota	Nannocystis	9.75	L Fallow
	Acidobacteriota	Geothrix	-2.43	VH Fallow

	Actinobacteriota	CL500-29 marine group	1.23	L Fallow
	Proteobacteria	Nordella	5.53	L Fallow
	Proteobacteria	Microvirga	1.95	L Fallow
	Planctomycetota	Singulisphaera	-1.06	VH Fallow
	Bacteroidota	Chryseolinea	2.64	L Fallow
	Verrucomicrobiota	Pedosphaera	3.68	L Fallow
	Proteobacteria	Rhodopila	-1.60	VH Fallow
	Myxococcota	Pajaroellobacter	2.32	L Fallow
	Firmicutes	Ammoniphilus	-1.59	VH Fallow
	Verrucomicrobiota	Oikopleura	5.37	L Fallow
	Bacteroidota	Ohtaekwangia	5.94	L Fallow
	Planctomycetota	Planctopirus	2.14	L Fallow
	Bacteroidota	Niastella	-0.82	VH Fallow
	Planctomycetota	Schlesneria	-1.16	VH Fallow
	Proteobacteria	Polaromonas	8.19	L Fallow
	Planctomycetota	SM1A02	0.73	L Fallow
	Proteobacteria	Polycyclovorans	2.87	L Fallow
	Acidobacteriota	Vicinamibacter	7.70	L Fallow
	Actinobacteriota	Rhizocola	5.64	L Fallow
	Bacteroidota	Sediminibacterium	-1.03	VH Fallow
	Bdellovibrionota	OM27 clade	3.86	L Fallow
	Acidobacteriota	Paludibaculum	5.00	L Fallow
	Proteobacteria	Hirschia	5.68	L Fallow
	Chloroflexi	Litorilinea	1.26	L Fallow
	Proteobacteria	Ahniella	4.44	L Fallow
	Bacteroidota	Heliimonas	-8.49	VH Fallow
	Proteobacteria	BD1-7 clade	7.41	L Fallow
	Actinobacteriota	Kineosporia	-8.01	VH Fallow
	Proteobacteria	Alkanibacter	-6.91	VH Fallow
	Gemmatimonadota	Roseisolibacter	5.52	L Fallow
	Acidobacteriota	Subgroup 10	1.75	L Fallow
	Proteobacteria	966-1	6.99	L Fallow
	Bacteroidota	Solitalea	3.98	L Fallow
	Planctomycetota	Planctomicrobium	7.56	L Fallow
	Actinobacteriota	Phycicoccus	-4.47	VH Fallow
	Bacteroidota	Chitinophaga	-7.86	VH Fallow
	Verrucomicrobiota	Candidatus Omnitrophus	1.24	L Fallow
	Bacteroidota	Crocinitomix	8.01	L Fallow
	Proteobacteria	Nevskia	-5.06	VH Fallow
	Actinobacteriota	Luedemannella	3.05	L Fallow
	Proteobacteria	Phyllobacterium	6.06	L Fallow
	Bacteroidota	Parasegetibacter	5.78	L Fallow
	Acidobacteriota	Terracidiphilus	-6.42	VH Fallow
	Chloroflexi	Thermosporothrix	-6.22	VH Fallow

	Myxococcota	Polyangium	6.12	L Fallow
	Myxococcota	Vulgatibacter	3.56	L Fallow
	Planctomycetota	AKYG587	7.08	L Fallow
	Proteobacteria	Asticcacaulis	-8.50	VH Fallow
	Proteobacteria	Amaricoccus	7.61	L Fallow
	Armatimonadota	Armatimonas	5.99	L Fallow
	Proteobacteria	Ferrovibrio	6.25	L Fallow
	Proteobacteria	Sulfurifustis	5.81	L Fallow
	Proteobacteria	Thermomonas	6.12	L Fallow
	Bacteroidota	Lacibacter	5.79	L Fallow
	Bacteroidota	Flavitalea	6.63	L Fallow
	Proteobacteria	Rhodovastum	4.85	L Fallow
	Proteobacteria	Bosea	5.54	L Fallow
	Chloroflexi	Herpetosiphon	6.65	L Fallow
	Actinobacteriota	Geodermatophilus	4.97	L Fallow
	Verrucomicrobiota	ADurb.Bin118	6.02	L Fallow
	Actinobacteriota	Agromyces	5.92	L Fallow
	Actinobacteriota	Aeromicrobium	5.65	L Fallow
	Proteobacteria	Novosphingobium	6.22	L Fallow
	Proteobacteria	OM60(NOR5) clade	5.50	L Fallow
	Actinobacteriota	Micromonospora	5.60	L Fallow
	Bacteroidota	Flavisolibacter	5.80	L Fallow
	Firmicutes	Anaerocolumna	-6.45	VH Fallow
	Proteobacteria	Qipengyuania	5.16	L Fallow
	Bdellovibrionota	Bacteriovorax	5.20	L Fallow
	Proteobacteria	Uliginosibacterium	-6.28	VH Fallow
	Bacteroidota	Fluviicola	3.45	L Fallow
	Spirochaetota	Leptospira	5.60	L Fallow
	Proteobacteria	AAP99	5.42	L Fallow
	Bacteroidota	Aureispira	5.35	L Fallow
	Proteobacteria	Flavimaricola	4.97	L Fallow
	Planctomycetota	Rhodopirellula	5.55	L Fallow
	Proteobacteria	Permianibacter	4.46	L Fallow

Table S95: Deseq2 analysis of L Fallow vs H Fallow.

L Fallow vs H Fallow	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrosotalea	2.41	L Fallow
	Actinobacteriota	Gaiella	0.52	L Fallow
	Nitrospirota	Nitrospira	-0.98	H Fallow
	Actinobacteriota	Mycobacterium	0.56	L Fallow

	Proteobacteria	MND1	-0.97	H Fallow
	Crenarchaeota	Candidatus Nitrocosmicus	0.83	L Fallow
	Actinobacteriota	Acidothermus	0.88	L Fallow
	Actinobacteriota	Nocardioides	0.75	L Fallow
	Proteobacteria	Ellin6067	-0.53	H Fallow
	Proteobacteria	Rhodanobacter	1.98	L Fallow
	Acidobacteriota	RB41	-1.06	H Fallow
	Acidobacteriota	Holophaga	10.10	L Fallow
	Proteobacteria	Acidibacter	-0.67	H Fallow
	Acidobacteriota	Occallatibacter	2.17	L Fallow
	Bacteroidota	OLB12	1.16	L Fallow
	Acidobacteriota	Luteitalea	-1.19	H Fallow
	Myxococcota	Phaselicystis	-0.61	H Fallow
	Bacteroidota	Flavobacterium	-0.74	H Fallow
	Planctomycetota	Gemmata	0.80	L Fallow
	Acidobacteriota	Granulicella	4.45	L Fallow
	Fibrobacterota	possible genus 04	1.08	L Fallow
	Acidobacteriota	Acidipila	1.13	L Fallow
	Myxococcota	Haliangium	-0.34	H Fallow
	Actinobacteriota	Jatrophihabitans	0.66	L Fallow
	Bacteroidota	Mucilaginibacter	1.87	L Fallow
	Bdellovibrionota	Bdellovibrio	-0.77	H Fallow
	Desulfobacterota	Geobacter	-1.37	H Fallow
	Verrucomicrobiota	Ellin517	-1.18	H Fallow
	Actinobacteriota	Catenulispora	2.06	L Fallow
	Verrucomicrobiota	Terrimicrobium	2.01	L Fallow
	Bacteroidota	Terrimonas	-0.92	H Fallow
	Proteobacteria	Dongia	-1.18	H Fallow
	Planctomycetota	Schlesneria	1.11	L Fallow
	Verrucomicrobiota	Oikopleura	-4.54	H Fallow
	Proteobacteria	Polaromonas	-7.16	H Fallow
	Planctomycetota	SH-PL14	-1.29	H Fallow
	Bacteroidota	Sediminibacterium	1.90	L Fallow
	Bacteroidota	Heliimonas	3.88	L Fallow
	Proteobacteria	Polycyclovorans	-1.95	H Fallow
	Proteobacteria	Alkanibacter	5.61	L Fallow
	Gemmatimonadota	Roseisolibacter	-4.80	H Fallow
	Bacteroidota	Ohtaekwangia	-3.64	H Fallow
	Actinobacteriota	Phycicoccus	6.27	L Fallow
	Proteobacteria	Nevskia	6.80	L Fallow
	Bacteroidota	Parasegetibacter	-5.70	H Fallow
	Myxococcota	Nannocystis	-5.75	H Fallow
	Proteobacteria	Asticcacaulis	4.40	L Fallow

	Proteobacteria	Sulfurifustis	-6.27	H Fallow
	Myxococcota	Pajaroellobacter	-1.57	H Fallow
	Bacteroidota	Crocinitomix	-7.02	H Fallow
	Proteobacteria	Candidatus Ovatusbacter	5.48	L Fallow
	Proteobacteria	Georgfuchsia	5.70	L Fallow
	Bdellovibrionota	Peredibacter	-3.89	H Fallow
	Actinobacteriota	Virgisporangium	-7.23	H Fallow

Table S96: Deseq2 analysis of H Fallow vs VL Fallow.

H Fallow vs VL Fallow	Phylum	Genus	log2FoldChange	Enriched
	Crenarchaeota	Candidatus Nitrosotalea	3.94	H Fallow
	Proteobacteria	Pseudolabrys	0.67	H Fallow
	Planctomycetota	Pir4 lineage	-0.85	VL Fallow
	Bacteroidota	Puia	0.67	H Fallow
	Nitrospirota	Nitrospira	-1.61	VL Fallow
	Actinobacteriota	Mycobacterium	0.59	H Fallow
	Proteobacteria	MND1	-3.37	VL Fallow
	Planctomycetota	Aquisphaera	1.39	H Fallow
	Actinobacteriota	Acidothermus	2.56	H Fallow
	Proteobacteria	GOUTA6	-1.26	VL Fallow
	Verrucomicrobiota	Ellin516	0.74	H Fallow
	Proteobacteria	Bradyrhizobium	0.87	H Fallow
	Actinobacteriota	Conexibacter	1.37	H Fallow
	Proteobacteria	Rhodoblastus	0.53	H Fallow
	Acidobacteriota	RB41	-2.62	VL Fallow
	Proteobacteria	Pedomicrobium	-1.21	VL Fallow
	Proteobacteria	Roseiarcus	1.52	H Fallow
	Acidobacteriota	Holophaga	12.05	H Fallow
	Proteobacteria	Acidibacter	-2.37	VL Fallow
	Acidobacteriota	Occallatibacter	2.63	H Fallow
	Planctomycetota	Gemmata	1.38	H Fallow
	Verrucomicrobiota	Lacunisphaera	-0.74	VL Fallow
	Bacteroidota	Parafilimonas	-0.92	VL Fallow
	Proteobacteria	Arenimonas	-4.57	VL Fallow
	Acidobacteriota	Luteitalea	-1.78	VL Fallow
	Proteobacteria	Rhodanobacter	2.81	H Fallow
	Proteobacteria	mle1-7	-1.61	VL Fallow
	Acidobacteriota	Candidatus Koribacter	0.70	H Fallow
	Planctomycetota	Pirellula	-1.01	VL Fallow
	Myxococcota	Haliangium	-0.97	VL Fallow

	Chloroflexi	UTCFX1	-8.44	VL Fallow
	Proteobacteria	Devosia	0.90	H Fallow
	Actinobacteriota	Actinocorallia	-2.63	VL Fallow
	Acidobacteriota	Candidatus Solibacter	0.72	H Fallow
	Myxococcota	Phaselicystis	-1.95	VL Fallow
	Proteobacteria	Steroidobacter	-1.56	VL Fallow
	Proteobacteria	Bauldia	-1.53	VL Fallow
	Verrucomicrobiota	Chthoniobacter	-1.04	VL Fallow
	Bacteroidota	Flavobacterium	-2.15	VL Fallow
	Actinobacteriota	Ilumatobacter	-5.51	VL Fallow
	Proteobacteria	Ellin6067	-0.47	VL Fallow
	Actinobacteriota	Microlunatus	-1.32	VL Fallow
	Proteobacteria	Candidatus Alysiosphaera	-2.61	VL Fallow
	Acidobacteriota	Granulicella	6.48	H Fallow
	Acidobacteriota	Acidipila	1.85	H Fallow
	Acidobacteriota	Bryobacter	1.03	H Fallow
	Planctomycetota	Schlesneria	1.87	H Fallow
	Actinobacteriota	Jatrophihabitans	1.33	H Fallow
	Bacteroidota	Mucilaginibacter	2.39	H Fallow
	Actinobacteriota	Solirubrobacter	-1.55	VL Fallow
	Proteobacteria	Mesorhizobium	-1.85	VL Fallow
	Proteobacteria	Pseudorhodoplanes	-8.30	VL Fallow
	Proteobacteria	Pseudoduganella	-7.05	VL Fallow
	Desulfobacterota	Geobacter	-4.19	VL Fallow
	Actinobacteriota	Pseudonocardia	-1.02	VL Fallow
	Firmicutes	Clostridium sensu stricto 13	1.10	H Fallow
	Gemmatimonadota	Gemmatimonas	0.70	H Fallow
	Verrucomicrobiota	Opitutus	-1.12	VL Fallow
	Proteobacteria	Acidicaldus	3.56	H Fallow
	Firmicutes	Bacillus	2.15	H Fallow
	Actinobacteriota	Marmoricola	0.86	H Fallow
	Bacteroidota	Aurantisolimonas	-2.72	VL Fallow
	Myxococcota	P3OB-42	-1.11	VL Fallow
	Fibrobacterota	possible genus 04	-1.91	VL Fallow
	Verrucomicrobiota	Luteolibacter	-4.39	VL Fallow
	Verrucomicrobiota	Ellin517	-6.28	VL Fallow
	Spirochaetota	Turneriella	-4.39	VL Fallow
	Proteobacteria	SWB02	-3.18	VL Fallow
	Myxococcota	Anaeromyxobacter	-1.73	VL Fallow
	Firmicutes	Lysinibacillus	2.52	H Fallow
	Actinobacteriota	Catenulispora	3.85	H Fallow
	Bacteroidota	UTBCD1	-3.67	VL Fallow
	Actinobacteriota	CL500-29 marine group	-3.53	VL Fallow

	Bacteroidota	Ferruginibacter	-2.26	VL Fallow
	Bacteroidota	Terrimonas	-3.65	VL Fallow
	Proteobacteria	Altererythrobacter	-3.10	VL Fallow
	Proteobacteria	Dongia	-1.70	VL Fallow
	Planctomycetota	Singulisphaera	1.87	H Fallow
	Proteobacteria	Rhodopila	1.66	H Fallow
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	4.28	H Fallow
	Proteobacteria	alphaI cluster	-7.14	VL Fallow
	Actinobacteriota	Streptacidiphilus	8.55	H Fallow
	Verrucomicrobiota	Roseimicrobium	-5.50	VL Fallow
	Firmicutes	Ammoniphilus	2.01	H Fallow
	Verrucomicrobiota	Oikopleura	-7.23	VL Fallow
	Planctomycetota	SH-PL14	-3.55	VL Fallow
	Bacteroidota	Adhaeribacter	-1.93	VL Fallow
	Proteobacteria	Aquicella	1.77	H Fallow
	Proteobacteria	Phenylobacterium	1.29	H Fallow
	Firmicutes	Clostridium sensu stricto 9	2.58	H Fallow
	Proteobacteria	Polaromonas	-7.27	VL Fallow
	Planctomycetota	SM1A02	-1.77	VL Fallow
	Proteobacteria	Microvirga	-5.00	VL Fallow
	Proteobacteria	Polycyclovorans	-7.71	VL Fallow
	Bacteroidota	Chryseolinea	-7.02	VL Fallow
	Myxococcota	Sandaracinus	-2.93	VL Fallow
	Proteobacteria	Acidisoma	7.91	H Fallow
	Firmicutes	Clostridium sensu stricto 1	2.48	H Fallow
	Acidobacteriota	JGI 0001001-H03	-4.30	VL Fallow
	Chloroflexi	1959-1	2.74	H Fallow
	Proteobacteria	Tahibacter	-6.79	VL Fallow
	Bdellovibrionota	OM27 clade	-7.15	VL Fallow
	Proteobacteria	Hirschia	-5.75	VL Fallow
	Chloroflexi	Litorilinea	-6.91	VL Fallow
	Proteobacteria	Ahniella	-6.07	VL Fallow
	Actinobacteriota	Actinospica	4.80	H Fallow
	Abditibacteriota	Abditibacterium	3.51	H Fallow
	Bacteroidota	Dinghuibacter	-5.68	VL Fallow
	Bacteroidota	Ohtaekwangia	-8.36	VL Fallow
	Proteobacteria	Candidatus Paracaedibacter	2.62	H Fallow
	Firmicutes	Psychrobacillus	6.59	H Fallow
	Proteobacteria	Plot4-2H12	2.33	H Fallow
	Actinobacteriota	Phycicoccus	6.15	H Fallow
	Firmicutes	Paenibacillus	2.49	H Fallow

	Proteobacteria	Rudaea	7.44	H Fallow
	Verrucomicrobiota	Cerasicoccus	-6.03	VL Fallow
	Proteobacteria	Nevskia	6.26	H Fallow
	Bacteroidota	Heliimonas	3.99	H Fallow
	Bacteroidota	Parasegetibacter	-5.75	VL Fallow
	Bdellovibrionota	Oligoflexus	-6.19	VL Fallow
	Acidobacteriota	Terracidiphilus	6.34	H Fallow
	Chloroflexi	Thermosporothrix	8.92	H Fallow
	Proteobacteria	Asticcacaulis	3.88	H Fallow
	Acidobacteriota	Edaphobacter	3.38	H Fallow
	Planctomycetota	Paludisphaera	2.32	H Fallow
	Myxococcota	Nannocystis	-5.85	VL Fallow
	Firmicutes	Clostridium sensu stricto 5	3.30	H Fallow
	Actinobacteriota	Kutzneria	6.63	H Fallow
	Verrucomicrobiota	FukuN18 freshwater group	6.64	H Fallow
	Proteobacteria	Sulfurifustis	-4.55	VL Fallow
	Verrucomicrobiota	SH3-11	-5.26	VL Fallow
	Proteobacteria	Candidatus Endoecteinascidia	6.05	H Fallow
	Firmicutes	Clostridium sensu stricto 12	3.23	H Fallow
	Bacteroidota	Crocinitomix	-7.09	VL Fallow
	Proteobacteria	Candidatus Berkiella	5.64	H Fallow
	Proteobacteria	Acidiphilium	5.49	H Fallow
	Proteobacteria	PMMR1	6.16	H Fallow
	Proteobacteria	Candidatus Ovatusbacter	6.68	H Fallow
	Bacteroidota	Fluviicola	-6.25	VL Fallow
	Proteobacteria	Rhizomicrobium	5.88	H Fallow
	Myxococcota	Vulgatibacter	-6.26	VL Fallow
	Proteobacteria	Inquilinus	5.96	H Fallow
	Bdellovibrionota	Peredibacter	-5.99	VL Fallow
	Firmicutes	Clostridium sensu stricto 10	5.86	H Fallow
	Actinobacteriota	Virgisporangium	-7.24	VL Fallow

Table S97: Deseq2 analysis of L Fallow vs VL Fallow.

L Fallow vs VL Fallow	**Phylum**	**Genus**	**log2FoldChange**	**Enriched**
	Crenarchaeota	Candidatus Nitrosotalea	1.47	L Fallow

	Planctomycetota	Pir4 lineage	-1.00	VL Fallow
	Bacteroidota	Puia	0.56	L Fallow
	Proteobacteria	Pseudolabrys	0.44	L Fallow
	Proteobacteria	MND1	-2.47	VL Fallow
	Proteobacteria	Rhodomicrobium	0.64	L Fallow
	Planctomycetota	Aquisphaera	0.86	L Fallow
	Actinobacteriota	Acidothermus	1.64	L Fallow
	Proteobacteria	Bradyrhizobium	0.61	L Fallow
	Actinobacteriota	Nocardioides	-0.46	VL Fallow
	Actinobacteriota	Conexibacter	0.90	L Fallow
	Acidobacteriota	RB41	-1.62	VL Fallow
	Proteobacteria	Roseiarcus	0.94	L Fallow
	Acidobacteriota	Holophaga	1.93	L Fallow
	Proteobacteria	Acidibacter	-1.74	VL Fallow
	Proteobacteria	Rhodanobacter	0.80	L Fallow
	Bacteroidota	OLB12	-1.13	VL Fallow
	Proteobacteria	Arenimonas	-4.57	VL Fallow
	Proteobacteria	mle1-7	-1.30	VL Fallow
	Planctomycetota	Pirellula	-1.29	VL Fallow
	Nitrospirota	Nitrospira	-0.70	VL Fallow
	Myxococcota	Haliangium	-0.67	VL Fallow
	Chloroflexi	UTCFX1	-7.62	VL Fallow
	Actinobacteriota	Actinocorallia	-2.62	VL Fallow
	Acidobacteriota	Candidatus Solibacter	0.69	L Fallow
	Myxococcota	Phaselicystis	-1.39	VL Fallow
	Crenarchaeota	Candidatus Nitrocosmicus	-0.64	VL Fallow
	Bacteroidota	Flavobacterium	-1.49	VL Fallow
	Actinobacteriota	Ilumatobacter	-4.89	VL Fallow
	Actinobacteriota	Microlunatus	-1.06	VL Fallow
	Proteobacteria	Candidatus Alysiosphaera	-2.85	VL Fallow
	Acidobacteriota	Granulicella	1.96	L Fallow
	Fibrobacterota	possible genus 04	-3.03	VL Fallow
	Bacteroidota	Parafilimonas	-1.39	VL Fallow
	Acidobacteriota	Acidipila	0.69	L Fallow
	Acidobacteriota	Bryobacter	0.71	L Fallow
	Planctomycetota	Schlesneria	0.73	L Fallow
	Actinobacteriota	Jatrophihabitans	0.64	L Fallow
	Bacteroidota	Mucilaginibacter	0.50	L Fallow
	Actinobacteriota	Solirubrobacter	-1.47	VL Fallow
	Proteobacteria	Mesorhizobium	-1.00	VL Fallow
	Proteobacteria	Pseudorhodoplanes	-6.58	VL Fallow
	Proteobacteria	Pseudoduganella	-6.18	VL Fallow
	Actinobacteriota	Pseudonocardia	-1.06	VL Fallow

	Firmicutes	Clostridium sensu stricto 13	0.94	L Fallow
	Verrucomicrobiota	Opitutus	-0.97	VL Fallow
	Proteobacteria	Acidicaldus	2.45	L Fallow
	Desulfobacterota	Geobacter	-2.92	VL Fallow
	Spirochaetota	Spirochaeta 2	-0.99	VL Fallow
	Actinobacteriota	Nocardia	-1.03	VL Fallow
	Firmicutes	Bacillus	1.42	L Fallow
	Myxococcota	P3OB-42	-0.94	VL Fallow
	Verrucomicrobiota	Luteolibacter	-3.53	VL Fallow
	Verrucomicrobiota	Ellin517	-5.21	VL Fallow
	Spirochaetota	Turneriella	-3.51	VL Fallow
	Proteobacteria	SWB02	-2.36	VL Fallow
	Myxococcota	Anaeromyxobacter	-1.29	VL Fallow
	Firmicutes	Lysinibacillus	0.94	L Fallow
	Actinobacteriota	Catenulispora	1.79	L Fallow
	Verrucomicrobiota	Terrimicrobium	-2.67	VL Fallow
	Verrucomicrobiota	Lacunisphaera	-1.18	VL Fallow
	Acidobacteriota	Geothrix	-3.83	VL Fallow
	Actinobacteriota	CL500-29 marine group	-3.01	VL Fallow
	Bacteroidota	Ferruginibacter	-1.67	VL Fallow
	Bacteroidota	Terrimonas	-2.82	VL Fallow
	Planctomycetota	Singulisphaera	0.81	L Fallow
	Proteobacteria	Burkholderia-Caballeronia-Paraburkholderia	1.94	L Fallow
	Proteobacteria	alphaI cluster	-7.28	VL Fallow
	Actinobacteriota	Streptacidiphilus	5.57	L Fallow
	Verrucomicrobiota	Chthoniobacter	-0.69	VL Fallow
	Firmicutes	Ammoniphilus	1.63	L Fallow
	Proteobacteria	Aquicella	1.63	L Fallow
	Planctomycetota	SM1A02	-1.37	VL Fallow
	Proteobacteria	Steroidobacter	-1.38	VL Fallow
	Bacteroidota	Chryseolinea	-6.57	VL Fallow
	Bacteroidota	Niastella	-1.21	VL Fallow
	Proteobacteria	Acidisoma	3.06	L Fallow
	Planctomycetota	SH-PL14	-2.33	VL Fallow
	Myxococcota	Pajaroellobacter	1.02	L Fallow
	Bacteroidota	Sediminibacterium	-2.32	VL Fallow
	Verrucomicrobiota	Roseimicrobium	-4.46	VL Fallow
	Proteobacteria	Altererythrobacter	-3.12	VL Fallow
	Chloroflexi	Litorilinea	-5.79	VL Fallow
	Actinobacteriota	Actinospica	4.99	L Fallow
	Abditibacteriota	Abditibacterium	1.68	L Fallow
	Proteobacteria	Polycyclovorans	-5.83	VL Fallow

	Actinobacteriota	Demequina	-4.99	VL Fallow
	Proteobacteria	Parablastomonas	-5.83	VL Fallow
	Myxococcota	Sandaracinus	-3.93	VL Fallow
	Proteobacteria	Rudaea	3.89	L Fallow
	Gemmatimonadota	Roseisolibacter	5.59	L Fallow
	Chloroflexi	Thermosporothrix	3.95	L Fallow
	Actinobacteriota	Lechevalieria	-21.10	VL Fallow
	Proteobacteria	Caulobacter	-6.26	VL Fallow
	Verrucomicrobiota	FukuN18 freshwater group	6.89	L Fallow
	Proteobacteria	Georgfuchsia	-6.13	VL Fallow
	Firmicutes	Desulfosporosinus	5.98	L Fallow
	Proteobacteria	Acidiphilium	5.75	L Fallow
	Proteobacteria	PMMR1	6.43	L Fallow
	Proteobacteria	Rhizomicrobium	6.16	L Fallow
	Bdellovibrionota	OM27 clade	-5.18	VL Fallow
	Proteobacteria	Inquilinus	6.21	L Fallow

Table S98: Deseq2 analysis of VH Planted vs VH Fallow.

Table S99: Deseq2 analysis of H Planted vs H Fallow.

H Planted vs H Fallow	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	unidentified_385	5.06	H Planted
	Basidiomycota	unidentified_420	-22.57	H Fallow
	Ascomycota	Torula	21.58	H Planted
	Ascomycota	unidentified_8	20.70	H Planted

Table S100: Deseq2 analysis of L Planted vs L Fallow.

L Planted vs L Fallow	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Cuphophyllus	9.20	L Planted

Table S101: Deseq2 analysis of H Planted vs VH Planted.

H Planted vs VH Planted	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Drechslera	-9.19	VH Planted
	Basidiomycota	unidentified_199	1.56	H Planted

	Basidiomycota	Conocybe	-2.67	VH Planted
	Ascomycota	Lachnum	-8.55	VH Planted
	Ascomycota	Fusidium	-2.23	VH Planted
	Basidiomycota	Minimedusa	22.08	H Planted
	Basidiomycota	unidentified_3513	21.05	H Planted
	Basidiomycota	unidentified_12	20.81	H Planted
	Chytridiomycota	Paranamyces	4.95	H Planted
	Ascomycota	unidentified_8	-21.15	VH Planted

Table S102: Deseq2 analysis of L Planted vs VH Planted.

L Planted vs VH Planted	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	unidentified_5	-3.27	VH Planted
	Ascomycota	Drechslera	-7.23	VH Planted
	Ascomycota	Gremmenia	-6.59	VH Planted
	Basidiomycota	Minimedusa	21.28	L Planted
	Ascomycota	Pyrenochaeta	4.54	L Planted
	Basidiomycota	Parasola	-6.36	VH Planted
	Basidiomycota	Auricularia	6.42	L Planted
	Chytridiomycota	unidentified_957	3.92	L Planted
	Ascomycota	Pseudopithomyces	18.37	L Planted
	Basidiomycota	Serendipita	4.02	L Planted
	Basidiomycota	Cuphophyllus	-8.50	VH Planted
	Basidiomycota	unidentified_3513	20.37	L Planted
	Chytridiomycota	Paranamyces	6.11	L Planted

Table S103: Deseq2 analysis of L Planted vs H Planted.

L Planted vs H Planted	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Pleurophragmium	-1.31	H Planted
	Chytridiomycota	Spizellomyces	2.46	L Planted
	Basidiomycota	Pholiotina	-7.41	H Planted
	Ascomycota	unidentified_385	-5.46	H Planted
	Basidiomycota	Conocybe	-5.73	H Planted
	Ascomycota	Fusidium	-2.04	H Planted
	Chytridiomycota	Rhizophydium	5.64	L Planted
	Basidiomycota	Parasola	5.22	L Planted
	Ascomycota	Gibberella	-5.46	H Planted
	Basidiomycota	Cuphophyllus	8.72	L Planted

	Ascomycota	Lipomyces	7.25	L Planted
	Ascomycota	unidentified_52	8.37	L Planted
	Ascomycota	unidentified_8	-20.79	H Planted

Table S104: Deseq2 analysis of L Planted vs H Planted.

H Fallow vs VH Fallow	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Minimedusa	22.66	H Fallow
	Ascomycota	unidentified_191	-7.45	VH Fallow
	Basidiomycota	unidentified_420	-23.65	VH Fallow

Table S105: Deseq2 analysis of L Fallow vs VH Fallow.

L Fallow vs VH Fallow	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	unidentified_191	-6.68	VH Fallow

Table S106: Deseq2 analysis of VL Fallow vs VH Fallow.

VL Fallow vs VH Fallow	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Saccharomyces	26.08	VL Fallow
	Ascomycota	unidentified_832	-3.26	VH Fallow
	Basidiomycota	Cryptococcus_1002	23.44	VL Fallow
	Ascomycota	unidentified_5	2.42	VL Fallow
	Basidiomycota	unidentified_242	-1.44	VH Fallow
	Ascomycota	Dactylonectria	-2.49	VH Fallow
	Ascomycota	Podospora	-3.69	VH Fallow
	Basidiomycota	Mycena	6.12	VL Fallow
	Ascomycota	Gremmenia	7.05	VL Fallow
	Ascomycota	unidentified_3138	-2.44	VH Fallow
	Ascomycota	Slopeiomyces	-6.00	VH Fallow
	Ascomycota	Ophiosphaerella	-3.96	VH Fallow
	Basidiomycota	unidentified_199	-9.62	VH Fallow
	Ascomycota	Penicillium	1.94	VL Fallow
	Basidiomycota	unidentified_2504	6.66	VL Fallow
	Ascomycota	Belonium	9.96	VL Fallow
	Chytridiomycota	Rhizophydium	5.05	VL Fallow
	Basidiomycota	Pholiotina	-9.39	VH Fallow
	Basidiomycota	Conocybe	-4.59	VH Fallow
	Ascomycota	Preussia	-6.02	VH Fallow
	Basidiomycota	Minimedusa	-23.53	VH Fallow

	Ascomycota	Pyrenochaeta	-4.92	VH Fallow
	Ascomycota	Vibrissea	23.70	VL Fallow
	Basidiomycota	Auricularia	-8.20	VH Fallow
	Ascomycota	unidentified_191	9.33	VL Fallow
	Ascomycota	unidentified_52	5.79	VL Fallow
	Basidiomycota	Cortinarius	7.21	VL Fallow
	Glomeromycota	Funneliformis	-7.84	VH Fallow
	Basidiomycota	Serendipita	-8.26	VH Fallow
	Basidiomycota	unidentified_2191	20.28	VL Fallow
	Basidiomycota	Arrhenia	6.81	VL Fallow
	Ascomycota	Scytalidium	6.18	VL Fallow

Table S107: Deseq2 analysis of L Fallow vs H Fallow.

L Fallow vs H Fallow	Phylum	Genus	log2FoldChange	Enriched
	Basidiomycota	Auricularia	-7.70	H Fallow
	Ascomycota	Pseudopithomyces	8.20	L Fallow
	Ascomycota	Pseudotaeniolina	5.53	L Fallow
	Basidiomycota	unidentified_420	-24.36	H Fallow
	Basidiomycota	unidentified_3513	8.78	L Fallow

Table S108: Deseq2 analysis of L Fallow vs VL Fallow.

L Fallow vs VL Fallow	Phylum	Genus	log2FoldChange	Enriched
	Ascomycota	Saccharomyces	26.94	L Fallow
	Ascomycota	unidentified_832	-3.17	VL Fallow
	Basidiomycota	Cryptococcus_1002	25.26	L Fallow
	Basidiomycota	unidentified_242	-1.99	VL Fallow
	Ascomycota	Dactylonectria	-1.75	VL Fallow
	Ascomycota	Drechslera	3.97	L Fallow
	Basidiomycota	Mycena	12.29	L Fallow
	Ascomycota	Gremmenia	5.57	L Fallow
	Ascomycota	unidentified_3138	-2.51	VL Fallow
	Ascomycota	Slopeiomyces	-4.92	VL Fallow
	Ascomycota	Ophiosphaerella	-3.67	VL Fallow
	Basidiomycota	Trechispora	6.91	L Fallow
	Basidiomycota	unidentified_199	-8.51	VL Fallow
	Ascomycota	Penicillium	1.32	L Fallow
	Ascomycota	Belonium	6.86	L Fallow
	Ascomycota	Lachnum	4.87	L Fallow

	Basidiomycota	Pholiotina	-21.49	VL Fallow
	Ascomycota	Preussia	-4.88	VL Fallow
	Basidiomycota	unidentified_2191	22.29	L Fallow
	Basidiomycota	Arrhenia	7.58	L Fallow
	Basidiomycota	unidentified_3513	-8.61	VL Fallow
	Glomeromycota	Funneliformis	-9.47	VL Fallow
	Ascomycota	Scytalidium	6.95	L Fallow
	Ascomycota	Collembolispora	8.01	L Fallow
	Mortierellomycota	unidentified_983	6.58	L Fallow