Linking soil pH and phosphorus management to potential N_2O emissions and nitrogen cycling microbial communities

Meritxell Grau Butinyac, BSc

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Supervisors:

Dr. Fiona Brennan, Dr. Karl Richards, Prof. Vincent O'Flaherty

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Declaration of Authorship

I, Meritxell Grau Butinyac, declare that this thesis entitled:

Linking soil pH and phosphorus management to potential N_2O emissions and nitrogen cycling microbial communities

and the work presented in it are my own and have been generated by me as a result of my own original research.

I confirm that:

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- × Except for such quotations, this thesis is my own work
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Date: 14th February 2022

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Abstract

Greenhouse gases (GHGs) in the atmosphere create the greenhouse gas effect, which allows maintenance of global temperatures. However, anthropogenic production of GHGs has caused dramatic increases of GHGs in the atmosphere, inducing global warming and resulting in substantial climatic changes. Mitigating GHGs emissions is thus a key priority to reduce the impact of climate change. Nitrous oxide (N_2O) is a potent greenhouse gas that is not only involved in global warming but that also causes damage to the ozone layer. Soils are one of the major sources of N_2O emissions. Excess application of nitrogen (N) on agricultural soils via synthetic fertiliser and manure can result in N losses due to leaching and N_2O emissions. The N transformations that facilitate these losses are driven by soil microbial communities that respond to changes in their environment. These changes such as availability of inorganic N, soil pH and phosphorus (P) levels can be a consequence of agricultural management practices.

Increases in soil pH have been linked to a reduction of N_2O emissions through impacts on the microbial community at the functional and structural level, making pH management a possible approach for mitigating emissions. However, there is a need to assess whether the impact of pH on microbial communities involved in N transformations is conserved across a wide range of agronomic scenarios. Soil pH also affects P availability in the soil, creating an interaction effect of these soil properties that could also dictate both N_2O emissions and microbial communities involved in the processes, but the role of P management, and of this interaction, on N_2O production rates, and N cycling microbial communities, is poorly understood. The overall aim of this thesis was to investigate the impact of soil pH on microbial community structure and functional communities involved in N cycling processes, and to assess if this relationship was maintained across a geoclimatic gradient and a wide range of soil types. Also, this thesis aimed to investigate the long-term interaction between soil pH and P on these same N cycling microbial communities, and associated processes, to better understand their possible role in reducing N_2O emissions from arable and grassland soils. This was achieved through qPCR quantification of functional, prokaryotic, and fungal communities, sequencing of prokaryotic and fungal communities, and laboratory incubations for the measurement of potential denitrification and nitrification.

The abundance of denitrifier and nitrifier functional communities, and of prokaryotic and fungal communities were strongly impacted by both geoclimatic region (including soil type) and pH treatment. This effect of pH treatment was primarily positive on the abundance of the microbial communities present across pH treatments; however, the relationships present sometimes varied between sites and between sampling times within a site. Potential N_2O emissions were influenced by pH treatment while potential nitrification rate was influenced by pH and P treatment interaction. This interaction effect was also observed on crenarchaea and denitrifier (*nirK*, *nirS* and *nosZII*) gene abundances. P treatment influenced fungal and nitrous oxide reductase (*nosZI*) gene abundances while pH treatment shaped the prokaryotic or fungal community structure.

Overall, these results demonstrate microbial communities are shaped by agricultural management, with soil pH being a strong factor determining the functional and structural community, but also indicating P has a role in influencing these same processes and microbes. Understanding the response of microbial communities to management practices will be key for future mitigation of greenhouse gas N_2O from agricultural soils and reducing the impact of agriculture on the environment.

CHAPTER 1

Introduction

Chapter 1. Introduction

1.1. Global warming and nitrous oxide (N_2O)

The atmosphere maintains global temperature by trapping solar energy. Gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are known as greenhouse gases (GHGs), build up in the atmosphere and form what is known as the "greenhouse effect", by absorbing the heat from the sun and reducing its loss back to space. The natural greenhouse effect is required for life on Earth, without it, temperature would drop to -18ºC since heat would not be retained (Casper, 2010). However, the atmosphere has been changing due to human activities. This has led to a more pronounced greenhouse effect due to the anthropogenic production of GHGs. The Industrial Revolution was the start point of anthropogenic emissions, with main GHGs CO_2, CH_4 and N_2O levels being increased by 40%, 150% and 20% respectively from pre-industrial levels (IPCC, 2014). This rise of GHGs in the atmosphere has induced global warming, a gradual increase in surface temperature caused by anthropogenic emissions (Meyer, 2012) and decreasing these emissions is a priority to avoid an acceleration of changes to climate.

Globally, agriculture contributes 40% of N_2O emissions (2007 – 2016), being one of the largest anthropogenic sources of N_2O (Masson-Delmotte, V. et al., 2021). 92.5% of N_2O emissions in Ireland also originate from agriculture (Environmental Protection Agency, 2021). Within agriculture, the main activity associated with the release of N_2O to the atmosphere is the intensive use of synthetic nitrogen fertiliser (Tian et al., 2020). N_2O emissions from agriculture have increased more than 45% since 1980s (IPCC, 2021). Relevant policies such as the Paris agreement from 2015 aimed to lower GHG emissions, while maintaining the same levels of food production. The significant increase of N_2O concentrations in the atmosphere, from 270 ppb pre-Industrial to concentrations of 332 ppb in 2019 (Masson-Delmotte, V. et al., 2021) emphasise the necessity to reduce these emissions since food demand will not diminish. Delivering this demand needs to be achieved while minimising GHGs emissions which is key to reduce the impact on global warming and greenhouse effect.

N_2O has an average lifetime of 116 years, and its global warming potential is between 265 to 298 times higher than CO_2 (IPCC, 2021). N_2O in the

atmosphere undergoes chemical reactions that produce nitric oxide (NO) and nitrogen dioxide (NO_2). These products can deplete the ozone layer (Ravishankara et al., 2009). These characteristics of N_2O justify the need to reduce its emissions in order to stabilise atmospheric build up and to avoid enhancing the greenhouse effect. Soils are the largest natural source of N_2O, with its emissions being produced from microbial processes such as nitrification and denitrification (Butterbach-Bahl et al., 2013). Of total N_2O emissions, 55% are attributed from natural sources, while 45% account from anthropogenic sources (Syakila and Kroeze, 2011). Studying the effect of nitrogen (N) application in soils, Liu and Greaver (2009) showed in a meta-analysis study that N addition caused a 216% increase in N_2O emissions across different soils. Different N_2O production outcomes across the diverse fertiliser formulations applied were observed, with nitrate (NO_3^-) being the product inducing highest N_2O emissions. It has been established that fertiliser type and application influences the production of N_2O (Bouwman et al., 2002). Even with a better understanding of the role N fertiliser application plays in inducing N_2O emissions, there are still gaps that need to be fully understood. More detailed knowledge of soil factors and agricultural practices changing N_2O emission rates will enable better estimates of impact N inputs have in the emissions of N_2O (Butterbach-Bahl et al., 2013). This understanding provides the opportunity of potential agricultural managements to be used for mitigation of N_2O emissions.

1.2. Nitrogen (N) cycle processes

Nitrogen (N) is required for all life since it is used for the formation of essential organic compounds including amino and nucleic acids, chitin and proteins (Butterbach-Bahl and Dannenmann, 2011). The most abundant N-form in the atmosphere is dinitrogen (N_2) gas (Canfield et al., 2010), but this form is not accessible to most organisms and needs to be reduced into reactive forms such as ammonium (NH_4^+), which is subsequently transformed into nitric oxide (NO), nitrite (NO_2^-) and nitrous oxide (N_2O) among others (Fowler et al., 2013). These N-transformations are dependent on reduction-oxidation (redox) reactions which are carried out by microorganisms (Canfield et al., 2010). The collection of these redox reactions form part of the nitrogen cycle which consists of a range of N transformation processes (Fig. 1.1) including nitrification, denitrification,

nitrogen fixation, ammonium assimilation, ammonification and anaerobic ammonium oxidation (anammox) (Kuypers et al., 2018).

Of the processes within the nitrogen cycle, nitrification and denitrification are two successional pathways involved in the production of N_2O emissions. A diverse range of N cycling microorganisms are involved in these processes including bacteria, archaea and fungi (discussed in the sections below) (Cabello et al., 2019; De Boer and Kowalchuk, 2001; Kuypers et al., 2018; Maeda et al., 2015).

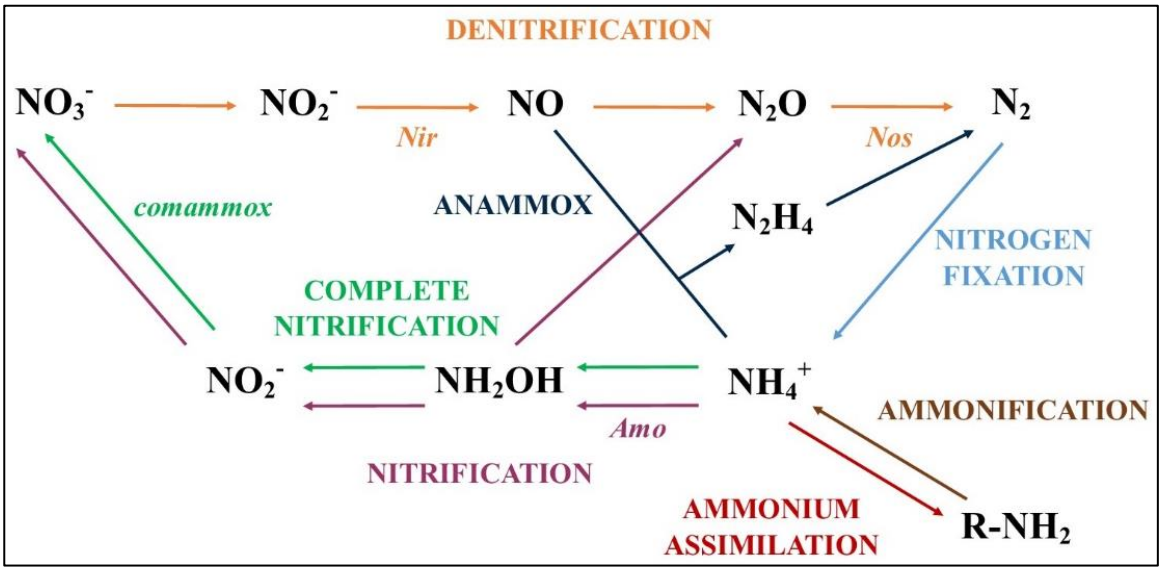

Figure 1.1. Schematic of major components of the nitrogen cycle adapted from (Cabello et al., 2019). Six main processes (in capitals) are represented in the diagram with arrows and corresponding colours showing each of the redox reactions and intermediate products. Enzymes (in italics) catalysing each of the reactions are placed next to their corresponding reaction. Nitrification in purple represents the two-step oxidation process, with *Amo* enzyme catalysing the first reaction. Nitrification in green represents both oxidation steps catalysed by a single enzyme COMAMMOX.

1.2.1. Nitrification

Nitrification is an aerobic process within the N cycle and produces N_2O as a by-product through biotic and abiotic reactions (Caranto and Lancaster, 2018; Liu et al., 2017). The process consists of two oxidation steps in which ammonia (NH_4^+) is oxidised into nitrite (NO_2^-) and then nitrate (NO_3^-). First step is catalysed by ammonia monooxygenase (*amoA*) present in ammonia-oxidising bacteria (AOB) including *Nitrosospira*, *Nitrosomonas*, *Nitrosococcus* and *Nitrosovibrio* and archaea (AOA) such as *Ca. Nitrosotalea devanaterra* (Drury et al., 2008; Nelson et al., 2016). NO_2^- is then subsequently converted to NO_3^- (Hu et al., 2015) by nitrite oxidising bacteria such as *Nitrobacter, Nitrosococcus* and *Nitrospira* genera (Drury et

al., 2008; Nelson et al., 2016). Complete ammonia-oxidising bacteria (COMAMMOX) are capable of carry out both oxidising steps in a single cell, "*Candidatus Nitrospira inopinata*" is an example (Kuypers et al., 2018). The production of N_2O within the process is carried out by ammonia oxidisers through the oxidation of hydroxylamine (NH_2OH/HNO) during the formation of NO_2^-, in which nitric oxide (NO) by-product can be oxidised to N_2O via NO reductases (Caranto and Lancaster, 2018; Tierling and Kuhlmann, 2018) or by chemical decomposition (Heil et al., 2016).

1.2.2. Denitrification

Denitrification is an anaerobic process within the N cycle and produces N_2O as either an intermediate or final product. The process consists of a series of reduction reactions in which nitrate (NO_3^-) and nitrite (NO_2^-) are subsequently reduced to nitric oxide (NO), N_2O or, if complete denitrification occurs, into dinitrogen (N_2) (Philippot et al., 2007). The key reactions are the reduction of NO_2^- into NO by nitrite reductase (NIR) enzyme, encoded by *nirK* or *nirS* genes (Zumft, 1997); while the last step of denitrification, in which the reduction of N_2O into N_2 is catalysed by N_2O reductase, is known as the only sink of N_2O in the biosphere, and is encoded by *nosZ* gene (Hallin et al., 2018). The gene encoding N_2O reductase can be grouped in either *nosZ* clade I (*nosZI*) or *nosZ* clade II (*nosZII*) (Jones et al., 2013).

Denitrification is a modular process, and not all microorganisms carry out every step of the pathway. For example, half of *nosZII* microorganisms lack *nir* genes while most of *nosZI* group possess either *nirK* or *nirS* gene (Graf et al., 2014). Also, microorganisms only harbouring *nosZ* genes are considered to be non-denitrifiers since these are only involved in the reduction and not production of N_2O (Hallin et al., 2018). Finally, another example is the lack of *nosZ* in fungal genomes, with *Trichoderma* and *Fusarium* (amongst other) genera as examples of N_2O producing fungi (Maeda et al., 2015). Bacterial phyla Actinobacteria and Acidobacteria also lack *nosZ* in their genomes (Kielak et al., 2016). Due to the modular nature of the denitrification process, the composition of the microbial community is important. The presence and abundances of these functional communities

is affected by a wide range of edaphic, environmental and management factors.

1.3. Factors impacting N cycle processes and microbes

Changes in the environment and edaphic factors influenced by agricultural management have an impact on the rates and end products of N transformations. These are caused by either a direct effect on the activity carried out by the microbial communities involved in the process or an indirect effect on the composition of these same communities.

1.3.1. Soil properties

A wide range of environmental, edaphic and management factors impact N cycling processes. For example, environmental factors include temperature and moisture (Butterbach-Bahl et al., 2013) which will be dependent on the climate the soil is exposed to. Oxygen (O_2) levels in the soil are regulated by soil moisture, and the presence or absence of O_2 will regulate microbial community activities (Butterbach-Bahl et al., 2013). Nitrification is sensitive to low O_2 levels since it is an aerobic pathway (Sahrawat, 2008), while on the other hand, denitrification is a facultative anaerobic pathway, therefore low O_2 will induce higher activity from this process (Giles et al., 2012). If anaerobic conditions induce denitrification activity, larger end-product of the pathway will be produced by the microbial community which is sensitive to those oxygen levels. However, in the case of denitrification, since N_2O reductase (*nosZ*) is sensitive to oxygen, low O_2 levels might lead to the final product being N_2O instead of N_2 due to the inhibiting effect O_2 has on *nosZ* protein synthesis. Soil edaphic factors such as soil texture or density which shape soil structure will also dictate other soil factors such availability of O_2, and therefore can also influence N cycling processes activities. Nutrient availabilities play a key role on the activity of the N cycling communities. Denitrification is prompted by the presence of NO_3^-, therefore the correct N substrate form is required for the processes to take place (Philippot et al., 2007). Readily available C sources are also needed for these pathways to occur (Mehnaz et al., 2019a; Spott et al., 2011). The presence and form of C has been reported to influence denitrification rates and products suggesting C regulates enzymes differently depending on

its concentration. Presence of C is a source of electrons for denitrifier enzymes, this highlights the requirement of C in soil for denitrification to occur, since the enzymes involved source electrons from the nutrient (Giles et al., 2012).

1.3.2. Soil pH

Soil pH is an edaphic condition that has been reported to regulate N cycle processes such as denitrification and nitrification (Baggs, 2011; Samad et al., 2016b; Šimek and Cooper, 2002). This soil property not only directly impacts the production of N_2O but can also indirectly influence N_2O emissions by shaping the structure of microbial communities involved in these processes (Čuhel and Šimek, 2011; Samad et al., 2016b). Soil pH can shift in a soil through a range of factors from natural consequences to human management. Soil type, climate and/or presence and absence of specific cations might naturally cause a soil to be acidic. However, in agricultural soils excessive application of N synthetic fertiliser and manure can result in acidification of soils, with different N-forms reducing soils buffering capacity and therefore resulting in their acidification (Tian and Niu, 2015).

Soil pH has been reported to correlate with N_2O emissions, with a negative linear relationship being observed in different experimental studies (Liu et al., 2010; Russenes et al., 2016; Samad et al., 2016a; Zurovec et al., 2021). The role pH has in influencing N_2O emissions starts at the molecular level with acidic soils causing a malfunction during the folding of N_2O reductase (Bergaust et al., 2010; Liu et al., 2014). A faulty enzyme cannot catalyse the reduction of N_2O to N_2, therefore the capacity of the microbial community to carry out complete denitrification is affected. However, soil pH can also shape the structural microbial community, and so the abundance of specific denitrifier and non-denitrifier (only harbouring *nosZ* genes) microorganisms might also be affected by the soil pH (Samad et al., 2016b). Changes in soil pH might select for microbial communities better adapted to the new environment. Shifts of the overall microbial community will also depend on availability of nutrients. Since soil pH impacts the presence of different elements in the soil (Penn and Camberato, 2019), changes in soil chemistry could lead to competition within the microbial community.

Liming is used as an agricultural management practice to increase soil pH (Holland et al., 2018). There are a range of lime types, and its application rate will vary depending on management plan. It has been reported application of lime decreases N_2O emissions from agricultural trials (Abalos et al., 2020; Zurovec et al., 2021). It could be suggested, increasing soil pH favours N-cycling communities capable of completing denitrification and therefore N_2O levels are reduced, but it is still not fully understood if the positive impact of increasing soil pH through lime application and the reduction of N_2O emissions is consistent throughout geoclimatic regions and their changing environmental and edaphic factors.

1.3.3. Soil phosphorus (P)

Phosphorus (P) nutrient availability has been reported to influence microbial communities involved in denitrification and nitrification processes (Jha et al., 2017; Wei et al., 2017). It has been considered P can be a limiting factor for rates of the N cycle processes (Cui et al., 2020). Availability of P can indirectly alter N transformations by restricting uptake and immobilisation of N or stimulating nitrification which leads to increased NO_3^- and therefore can cause loses through leaching (Mehnaz et al., 2019b). It can be suggested P availability will cause microbial communities to adapt and select for microorganisms that perform best at different P levels. For denitrification, correlations between P gradients and *nirK*, *nirS* and *nosZ* have been reported, with the strength of the correlation in this order respectively (Jha et al., 2017). Increased abundance of *nirK*, *nirS* and *nosZI* abundances after P application (Wei et al., 2017) supports the reported correlation between P availability and denitrifier functional communities. These correlations could be justified by the increase of available NO_3^- since nitrification is stimulated by P addition (Mehnaz et al., 2019b), which in turn could increase denitrification rates. Also, P nutrient is required for microbial growth, and C:N:P ratios are considered to regulate nutrient transformations in soil including N-cycling processes (Wei et al., 2017). The importance of P in relation to denitrifier functional genes is emphasised by the reported negative correlation between P limitation and denitrifier gene abundances (Cui et al., 2020). Not only the functional community but also the structure

of the microbial community can be shaped by availability of P. Soils with low P levels appear to be dominated by fungi (Chen, 2012). Since *nosZ* has not been detected in fungal genomes it might suggest P-poor soils will result in increased N_2O emissions. Bacteria have also been observed to be more dominant in soils with high P availability compared to low P soils in which arbuscular mycorrhizal fungi (AMF) colonisation was higher (Randall et al., 2019). The availability of P not only impacted the presence of the microbial community but also its activity. An increase of cumulative N_2O emissions after P addition has been reported (Mehnaz and Dijkstra, 2016). However, contradictory results have also been observed for example, low P levels have also been reported to lead to higher N_2O emissions (O'Neill et al., 2020).

Effect of P on nitrification functional communities is inconsistent. They have been negatively correlated with P limitation and AOB abundances have been reported to increase with P application while no effect has been observed on AOA (Cui et al., 2020; Wei et al., 2017). These results could be explained by the possible limiting effect P has on nitrification and P addition can relief this, suggesting N-cycling processes are dependent on P-acquiring enzymes (Deforest and Otuya, 2020). At this point in time, the effect of P availability on COMAMMOX abundances and community in agricultural soils has not been investigated. In forest soils, it has been observed the addition of P stimulated nitrification activity suggesting this might occur through the alleviation of P limitation in the community (Deforest and Otuya, 2020). Total nitrification has been observed to positively correlated with soil P (O'Neill et al., 2021). Further research on agricultural soils with P gradient experimental sites is required to understand the mechanism behind P availability influencing the abundance and activity of nitrification N cycling communities and the potential for an interaction between pH and P.

1.4. Methodology

Methodology used throughout the three chapters of this thesis consisted of molecular laboratory techniques including quantitative polymerase chain reaction (qPCR) and prokaryotic (bacterial and archaeal) and fungal amplicon sequencing using next generation Illumina MiSeq.

qPCR allows to quantify the number of gene copies of a gene of interest, either functional or taxonomical by using selected primers. This has become a culture-independent approach used in microbial ecology (Smith and Osborn, 2009). This technique focuses on the logarithmic phase of product accumulation, making it more accurate (Cooper and Rao, 2006). Over the years, primers have been developed to allow the quantification of N-cycling functional genes (Ma et al., 2019) including the design of primers that allow to quantify *nosZI* and *nosZII* clades separately (Henry et al., 2006; Jones et al., 2013). One of the major drawbacks of this methodology is while qPCR quantifies the size of the targeted functional or structural community, it cannot be known which portion of the community was alive, dormant, or dead. Other drawbacks include the influence of DNA extraction method since it affects final DNA yields as well as PCR inhibitors present in environmental samples (Smith and Osborn, 2009).

Amplicon sequencing was used to analyse prokaryotic (bacterial and archaeal) and fungal community composition. Next-generation sequencing (NGS) technology (described below was used). Compared to whole genome sequencing, amplicon sequencing is a targeted approach since a specific region of a gene is sequenced (Hugerth and Andersson, 2017). This methodology allows to assess the overall diversity of microorganisms present across treatments in the studies (chapters 3 and 4). It is an economical approach compared to whole genome sequencing and has the advantage even microorganisms that cannot be cultured will be present in the results (Gołębiewski and Tretyn, 2020).

NGS, described as short-read technologies consists in massive sequencing of short (250 – 800 bp) clonally amplified DNA molecules sequenced in parallel (T. Hu et al., 2021). The workflow includes library preparation and sequencing followed by data analysis. Within library preparation, throughout this project, Illumina Nextera was used to prepare DNA fragments. This technology includes DNA normalisation, fragmentation and size selection steps following a PCR to integrate adapters for sequencing and barcodes for sample indexing (T. Hu et al., 2021).

1.5. Thesis overview

N_2O emissions are enhanced from agricultural soils due to management and resulting changes on environmental and edaphic factors. These changes not only impact the N_2O rate production but also the N cycling microbial communities involved in the activities of these processes. Soil pH and P availability are two factors that play a role in shaping these emissions and the communities behind them, however their interaction effect on N cycling processes and communities is poorly understood. The overall aim of this thesis was to explore the role of management factors soil pH and P availability have on the microbial communities involved in the production and reduction of N_2O emissions across a range of soil types from different geoclimatic regions, and on medium- and long-term experimental trials to identify patterns and trends that can be applied at a larger management scale to reduce the impact of agriculture on the contribution of greenhouse gas production. The study aimed to address the following questions:

- What is the impact of soil pH on N cycling microbial communities? Is this effect consistent throughout geoclimatic regions and soil types?
- How does soil pH and P availability affect denitrification and nitrification activity on soils? What is the effect of their interaction on these N cycling process?
- How do microbial communities change across long-term pH and P treatments? Are functional and/or structural communities influenced by either factor or an interaction of both?

Chapter 2 aims to answer what is the impact soil pH has, and if this impact is the same across soil types and geoclimatic regions. This chapter will explore the distal effect soil pH has on the composition of microbial communities, to better understand if changes in soil pH shift the abundance of denitrifier functional genes that are favoured under specific environments, such as acidic soils having a smaller *nosZ* functional community. Also, soil type and climatic region which have an impact on soil properties, will allow to evaluate what other factors that differ across studied sites select for specific functional genes within the N cycling community. Chapter 3 and 4 aim to assess the impact of soil pH and P on medium- and long-term trials on both N cycling processes and microbial communities

involved. Both chapters explore the structure and composition of the prokaryotic and fungal communities across pH and P gradients. Changes in soil pH are expected to impact N cycling functional community, since its direct effect on protein synthesis of N_2O reductase will favour complete denitrifier communities in soils where the enzyme can be functional. Due to the stepwise nature of dentification, the abundance of specific functional genes will not only be determined by soil factors, but also by the environmental pressure of the community. Studying a gradient of P treatments will provide further information on the availability of the nutrient to induce nitrification and allow to evaluate how the N cycling community adapts to these changes, assuming higher P content will increase nitrification activity and therefore the size of the microbial community carrying out this process.

1.6. References

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CHAPTER 2

pH modulates soil nitrifier and denitrifier communities across soil type and geoclimatic gradients

Chapter 2. pH modulates soil nitrifier and denitrifier communities across soil type and geoclimatic gradients

2.1. Introduction

The denitrification pathway occurs under anaerobic conditions and consists of a series of sequential reduction steps transforming nitrate (NO_3^-) and nitrite (NO_2^-) to nitric oxide (NO), with either N_2O or dinitrogen (N_2) as final products (Philippot et al., 2007). These reactions are catalysed by four different reductase enzymes encoded by *narG*/*napA*, *nirK*/*nirS*, *norB* and *nosZ* genes (Zumft, 1997). Microorganisms containing either *nosZ* clade I (*nosZI*) or *nosZ* clade II (*nosZII*) (Jones et al., 2013), can reduce N_2O to N_2 (Hallin et al., 2018). These organisms are considered N_2O sinks and are of key importance in minimising emissions from soils. Nitrification, on the other hand, is an aerobic pathway where ammonia (NH_3) is oxidised to NO_3^- via NO_2^- (Hu et al., 2015). Ammonia oxidation ($NH_3 \Rightarrow NH_2OH/HNO \Rightarrow NO_2^-$), the first step of nitrification, is catalysed by ammonia monooxygenase (AMO), encoded by *amoA* gene found in bacteria (AOB) and archaea (AOA) (Hu et al., 2015); while complete ammonia oxidisers communities (COMAMMOX) can carry out both nitrification steps (Kuypers et al., 2018). In aerobic conditions, N_2O can be produced either as a bi-product of biotic processes such as nitrifier-denitrification (Kool et al., 2011) or as abiotic reactions between intermediate products from nitrification (Heil et al., 2016). Both, denitrification, and nitrification processes are dependent on environmental conditions. Of particular importance are carbon (C) and mineral nitrogen (N) availability (Senbayram et al., 2012) and aerobic status (Giles et al., 2012) but other soil characteristics such as temperature and soil pH are also known to impact the pathways (Saggar et al., 2013)**.**

Increased N_2O emissions at low soil pH, as well as a linear decrease of $N_2O/(N_2O+N_2)$ product ratio with increasing soil pH have been reported in both laboratory incubations (Liu et al., 2010; Samad et al., 2016a) and field trials (Abalos et al., 2020; Russenes et al., 2016; Zurovec et al., 2021). Decreased N_2O emissions have also been observed following liming of acidic soils (Hénault et al., 2019), with reductions in emissions considered to be a consequence of the microbial community response to shifts in soil pH (Brenzinger et al., 2015). The potential mechanisms involved in the reduction of N_2O emissions by soil pH are a direct impact on denitrifier enzymes or a shift in the microbial community, described respectively as a proximal and distal effects of soil pH (Čuhel et al., 2010). The pH-sensitivity of

nitrous oxide reductase (NosZ), a proximal effect, is considered to be caused by post-transcriptional impacts (Liu et al., 2010) or inhibition of protein assembly (Bergaust et al., 2010). The influence of pH on the structure of *nosZ* populations, an example of distal effect, has also been suggested to cause changes on N_2O emissions (Domeignoz-Horta et al., 2015; Jones et al., 2014a). Positive relationships have been reported between soil pH and the abundance of denitrifier target genes *nirK*, *nirS* and *nosZ* (Liu et al., 2010). While pH impacts N-cycling microbial communities through these proximal and distal effects, this soil property has been described as a "master variable" due to its chemical influence on the solubility of a variety of soil elements, including soil nutrients (Penn and Camberato, 2019). These chemical changes induced by soil pH will indirectly influence microbial communities since shifts in nutrient availability (such as C and N) not only can dictate the composition of the microbial community but also the rates and products of the different N cycling processes (Giles et al., 2012).

Changes in the size and composition of the microbial community will influence denitrifier and nitrifier populations and the genetic potential to transform N. For example, fungi lack *nosZ* gene (Hallin et al., 2018), therefore decreasing the capacity of the microbial community to reduce N_2O. The reported effects of soil pH on *nirK* and *nirS* populations are varied including studies reporting an effect on *nirK* or *nirS* only (Dandie et al., 2011; Enwall et al., 2010), and more recent analysis observing positive trends between community abundances of both, *nirK* and *nirS* and increasing pH (Herold et al., 2018). The discovery of *nosZII* (Jones et al., 2013; Sanford et al., 2012) led to studies focusing on how the occurrence of the two *nosZ* clades differed in the environment. Jones et al., (2014) reported that the abundance of *nosZII* was more strongly influenced by pH than that of *nosZI*, which was influenced to a greater extent by soil textural properties. Domeignoz-Horta et al., (2015) found *nosZII* to be more sensitive than *nosZI* with respect to pH. There are conflicting reports on the relationship between nitrifier genes and pH. While some investigators have observed a negative effect on AOA abundances and a positive effect on AOB abundances as soil pH increases (Nicol et al., 2008); other have reported a positive relationship between pH and AOA abundance with no effect on AOB abundances (Baolan et al., 2014). Investigation of how the recently discovered COMAMMOX populations (Hu and He, 2017) are shaped by pH has been limited but there are some indications of a negative relationship with their abundance and soil pH (Shi et al., 2018).

Soil pH has been observed as a strong predictor for community structure, diversity, and composition across multiple spatial scales (Kaminsky et al., 2017), but studies assessing how soil pH shapes functional communities involved in nitrification and denitrification have been largely constrained to single or few soil types, limited pH gradients, restricted geoclimatic ranges and shorter term liming trials. Knowledge of how communities involved in the transformation of N are impacted by pH on a broader scale and under a range of edaphic, climatic, and environmental conditions are critical to efforts to mitigate deleterious environmental N losses, including N_2O emissions. In this study we examined the relationship between pH and nitrifier/denitrifier communities range across nine different soils from seven different countries, incorporating a broad geoclimatic and pH (4.19 to 7.41) gradient. Relationships were investigated with pH gradients both within and across soil types, utilising medium- to long-term experimental pH trials. It was hypothesised that soil pH would be positively correlated with nitrifier and denitrifier populations. This hypothesis assumes less acidic environments will lead to larger overall microbial communities, therefore with a greater number of N-cycling functional communities present. Also, since soil pH can directly influence the post-transcription of *nosZ* enzyme, a strong increase of *nosZ* gene abundances with rising soil pH is expected across the geoclimatic gradient. This hypothesis is based on the impact soil environment has on microbial communities and how they adapt, assuming in low pH soils microbes lacking *nosZ* will be selected. Soil type and/or structure influence most soil properties, including pH. These differences of pH across the geoclimatic gradient will create niche environments for specific microbial communities. Soil pH creates selective pressure and competition within microbial communities and so, it was hypothesised while a relationship between soil pH and target genes would occur across all sites the strength of the effect on gene abundances might not be the same.

2.2. Methods

2.2.1. Sites and sampling

A total of 97 soil samples were collected in 2018 from 9 sites across 7 countries: Denmark (DK), Finland (FI), France (FRA1 & FRA2), Ireland (IE), New Zealand (NZ), Norway (NO), and Sweden (SE1 & SE2). Sites were chosen to encompass a wide pH range, ranging from 4.19 to 7.41, across a diversity of soil types and geoclimatic regions (Table 2.1). Plots sizes and management varied

across sites. To ensure consistency, the same soil sampling method was implemented across sites. At each plot, 15 to 20 cores were collected from 0 – 10 cm depth, in a "W" sampling pattern, and were homogenised to form a composite sample. Subsamples from each composite sample were flash frozen in the field for molecular analysis followed by storage at -80ºC prior to downstream analysis, while another subsample was dried at 40ºC for 48 hours and sieved to 2 mm for soil physiochemical analysis.

Table 2.1. Summary information of sites included in the study. Soil type described using World Reference Base (WRB). Climate shows mean annual temperature (ºC) and total annual rainfall (mm). Treatments vary across sites, including lime rate applications, lime application events or types of lime product. **Denmark** (n =3) 4T – 4 t/ha; 8T – 8 t/ha; 12T – t/ha. **Finland** (n = 4) 0L – Unlimed. **France 1** (n = 4) 0L – Unlimed. **France 2** (n = 4) 0L – Unlimed. **Ireland** (n = 4) L – 5 t/ha in 2011; H – 5 t/ha in 2014; VH – 5 t/ha in 2011 and 2014. **New Zeland** (n = 4) 0L – Unlimed. **Norway** (n = 4) D – Dolomite (Finely ground); LK – Larvikite; M – Marble; N – Norite; O – Olivine. **Sweden 1** (n =3) 10T – 10 t/ha; 20T – 20 t/ha. **Sweden 2** (n = 4) ML – Mixed lime (Slaked Lime and Calcium Carbonate); SL – Slaked Lime ($Ca(OH)_2$); and TL – Tunnel Kiln Slag.

Site	Soil Type (WRB)	Climate	pH range (H_2O)	Crop	Established Year	Treatments
Denmark (DK)	Arenosol (Orthic Haplohumod)	7.9 °C 995 mm	4.19 - 7.25	Barley	1942	0L, 4T, 8T, 12T
Finland (FI)	Halpic Gleysol (Acid Sulphate Soil)	3.5 °C 650 mm	4.98 - 5.08	Fallow	2018	0L
France 1 (FRA1)	Andosol	8.0 °C 1000 mm	5.14 - 5.50	Permanent Grassland	2018	0L
France 2 (FRA2)	Cambisol	7.8 °C 780 mm	5.85 - 6.05	Permanent Grassland	2018	0L
Ireland (IE)	Stagnic Cambisol	10 °C 1015 mm	4.67 - 6.17	Perennial Ryegrass	2011	0L, L, H, VH
New Zeland (NZ)	Fluvisoil	9 °C 700 mm	5.14 - 5.36	Pasture	2018	0L
Norway (NO)	Stagnosol (Fluvaquentic Humaquept)	6.2 °C 856 mm	4.79 - 7.41	Mixed grasses	2014	0L, D, LK, M, N, O
Sweden 1 (SE1)	Histosol (Fen Peat)	6 °C 558 mm	5.50 - 7.01	Timothy Grass	2017	0L, 10T, 20T
Sweden 2 (SE2)	Heavy Clay	6 °C 558 mm	5.60 - 6.47	Timothy Grass	2014	0L, ML, SL, TL

2.2.2. Soil physiochemical analysis

Soil pH was measured in a 1:2 ratio of soil:water (w/v) suspension using a Mettler Toledo glass calomel electrode (McCormack, 2002). Mehlich III extraction (Mehlich, 1984) was used for the extraction of macro- and micro-nutrients from 2 g of dried soil and analysed via inductively coupled plasma (ICP) optical emission spectrometry with Agilent 5100 ICP-OES spectrometer (Agilent Technologies, Mulgrave, Australia). Total soil carbon (TC) and total nitrogen (TN) were measured using elemental analyser (LECO TrueSpec CN elemental analyser, US) from 0.2 g of dried ball milled soil (Griffiths et al., 2012).

2.2.3. DNA extraction

Soil DNA extractions were performed on 0.25 g of frozen soil using DNeasy PowerSoil Kit (Qiagen, USA) following manufacturer's instructions. DNA concentration was measured using with Qubit™ dsDNA BR Assay Kit (Thermo Fisher, Ireland). Samples with DNA concentrations below 10 ng μl^{-1} were then quantified using Qubit™ dsDNA HS Assay Kit (Thermo Fisher, Ireland). DNA yields ranged between 0.4 - 130 ng μl^{-1}. DNA purity and quality were assessed with Nanodrop (Thermo Fisher, Ireland), considering both 260/280 and 260/230 ratios as well as by running agarose gels.

2.2.4. qPCR

Prior to qPCR analysis, DNA samples were normalised to 1 ng μl^{-1} DNA in UltraPure™ DEPC-Treated Water, which is prepared by incubating water with 0.1% diethylpyrocarbonate (DEPC) and is then autoclaved to remove DEPC (Thermo Fisher, Ireland). qPCR inhibition testing was done on all samples. A synthetic plasmid target, not naturally found in soil samples, which is amplified by primers T7F and M13R (Promega, Ireland) was added as a spike within the samples. Reduction in amplification compared to the positive control (DEPC water plus synthetic plasmid) was not detected, indicating inhibition was not present. qPCR was performed on all extractions to quantify bacterial (*16S rRNA* bacteria), crenarchaeal *(16S rRNA* crenarchaea) and fungal (*ITS*) phylogenetic genes, and functional genes involved in N cycling including *nirK*, *nirS*, *nosZI*, *nosZII*, AOA, AOB and COMAMMOX. Reactions were done with three technical replicates per sample using a CFX384 Touch Real-Time PCR Detection System (BIO-RAD, USA). From target genes and following manufacturer's protocols,

DNA standard curves were prepared using plasmid DNA (pGEM®-T) from Easy Vector System II kit (Promega, Ireland) and QIAprep Spin Miniprep Kit (Qiagen, Ireland). For each assay, serial dilutions corresponding to standard concentrations from 10^{10} to 10^{3} gene copies /µl were utilised to create the standard curve. Standards and negative controls, containing only DEPC water, were also analysed in technical triplicate. The qPCR mix was prepared using Takyon Low ROX SYBR 2X MasterMix blue dTTP (Eurogetee, Belgium). All reactions contained 5 µl MasterMix, 0.2 – 2.5 µM of primer (in Appendix 1, Table S2.1), 2 µl of normalised (1 ng μl^{-1}) template DNA and DEPC water to a final volume of 10 µl per reaction. qPCR mix without template was loaded automatically onto the plate using a multichannel pipette and automated robot arm (Integra, Ireland). DNA template was then manually added, using a multichannel pipette. A melt curve analysis was performed at the end of reactions to test for target specificity and confirm no amplification in the negative control.

2.2.5. Statistical analysis

Prior to statistical analysis, all gene copy numbers were averaged across qPCR technical replicates. Data analysis was performed with RStudio 4.2.0 (R Core Team, 2021). Data distribution and normality were evaluated statistically with Shapiro-Wilk normality test ($p > 0.05$) and data was transformed (log or square root), if required. If data was normally distributed, a linear model that included site was created. Homogeneity of variance (Levene's Test, $p > 0.05$) of the linear model were statistically tested. Since all sample groups fitted within a qPCR plate and consisted of more than two sample types, ANOVA testing was chosen for the analysis of the data (Ganger et al., 2017). If linear model assumptions were met, the effect of site on gene copy numbers was assessed with a parametric one-way ANOVA. A $p \leq 0.05$ was considered significantly different. Significant statistical results were further explored using Tukey's honestly significant differences (HSD) post hoc test. Non-parametric Kruskal-Wallis test was used to assess the effect of site on gene copy numbers where data did not meet the assumptions of ANOVA after transformation. Significant statistical results were further analysed with Dunn's post hoc test, function "dunnTest" ("FSA") with p values adjusted with the Benjamini-Hocberg (BH) method. The same statistical approach was followed for analysis of gene ratios.

Linear models, using function “lm” (“stats”) were used to evaluate the trends between gene abundance and soil pH. Adjusted R^2 was obtained from “summary” (“base”) of the linear model. Gene and soil properties correlations were assessed using “correlation” function (“correlation”) with Spearman’s rank correlation coefficient method. Correlation p values were adjusted with the BH method. To assess differences in soil physiochemical properties between sites, soil physiochemical properties from unlimed plots were compared using the same statistical approach as described above. Soil physiochemical properties from multiple pH treatments within a site were also statistically compared (Table 2.2). All soil measurements and not averages were used for correlation analysis Using “prcomp” function (“STATS”) principal component analysis (PCA) matrix assessed the relationship between samples and soil physiochemical properties, allowing to visualise groupings and report percentages of explained variance across samples.

Table 2.2. Average and standard deviation (within brackets) of soil chemical properties for each site included in the study. Capital letters indicate differences ($p \leq 0.05$) of soil properties across all sites using unlimed plots. X indicates all measurements were below detection limit of the machine (values below 0). TC - Total carbon, SOC – Soil organic carbon, TN - Total nitrogen. DK – Denmark (n = 3), FI – Finland (n = 4), FRA 1 – France 1 (n = 4), FRA 2 – France 2 (n = 4), IE – Ireland (n = 4), NO – Norway (n = 4), NZ – New Zealand (n = 4), SE1 – Sweden 1 (n = 3), SE2 – Sweden 2 (n = 4).

Site	TC		SOC		TN		pH		Al		Ca		Co		Cu	
DK	1.38(0.23)	A	0.96(0.29)	A	0.1(0.01)	A	4.25(0.05)	E	667.23(61.87)	AB	175.44(7.76)	A	0.02(0)	AB	1.36(0.1)	AB
FI	5.26(0.27)	BCD	5.04(0.28)	BC	0.48(0.03)	BCD	5.05(0.06)	AB	1228.35(18.26)	ABCD	1582.39(71.27)	BCDE	0.59(0.15)	C	1.27(0.15)	AB
FRA 1	12.28(0.76)	BC	11.63(0.21)	B	1.21(0.08)	BC	5.35(0.15)	AC	1766.04(203.82)	C	556.06(172.8)	AB	X		0.33(0.06)	A
FRA2	5.01(0.39)	BCDE	4.08(0.83)	ABC	0.5(0.04)	BCD	5.96(0.1)	D	1417.07(41.51)	CD	1088.46(63.88)	ABCD	0.08(0.02)	ABD	0.96(0.02)	A
IE	3.08(0.33)	ADE	2.32(0.14)	AC	0.34(0.03)	ABD	4.81(0.2)	B	761.41(41.5)	ABD	986.4(187.65)	ABC	0.17(0.07)	ABCD	4.84(1.41)	B
NO	3.28(0.52)	ABDE	2.89(0.41)	ABC	0.32(0.05)	AD	5.23(0.28)	AC	1209.48(131.6)	ACD	1441.94(71.11)	ABCDE	0.11(0.03)	ABCD	1.64(0.14)	AB
NZ	3.96(0.12)	ABCDE	3.52(0.41)	ABC	0.43(0.01)	ABCD	5.32(0.04)	AC	925.37(34.89)	ABCD	2497.16(40.87)	CDE	0.44(0.15)	CD	1.86(0.52)	AB
SE1	43.33(0.7)	C	48.37(0.45)	B	3.13(0.03)	C	5.59(0.06)	CD	209.34(175.36)	B	7186.62(2599.12)	E	0.13(0.01)	ABCD	1.27(0.47)	AB
SE2	2.33(0.29)	AE	2.24(0.32)	AC	0.28(0.03)	AD	5.83(0.17)	D	596.42(23)	AB	3456.19(406.53)	DE	0.24(0.04)	BCD	6.05(0.41)	B

Site	Fe		K		Mg		Mn		Na		P		S		Zn	
DK	509.32(46.63)	AB	26.19(1.36)	A	9.73(0.94)	A	5.06(0.76)	A	7.78(0.24)	BD	219.11(13.58)	A	18.57(3.33)	ABC	0.47(0.22)	A
FI	874.53(274.93)	A	361.94(32.7)	B	317.93(17.61)	BC	13.59(1.93)	BCD	49.64(6.54)	A	18.43(9.95)	B	87.22(4.26)	A	3.83(0.91)	B
FRA 1	90.24(8.31)	C	121.44(51.18)	ABC	80.24(23.18)	AD	13.34(2.73)	ABCD	6.3(3.38)	B	36.08(22.94)	BC	19.1(1.52)	BC	3.36(1.38)	BC
FRA2	118.43(17.68)	CD	129.16(76.53)	ABC	218.16(16.54)	BCD	10.08(2.08)	ABC	15.26(3.91)	CD	39.76(8.22)	B	14.41(0.63)	B	0.43(0.43)	A
IE	303.06(14.97)	BCD	60.29(2.48)	AC	102.04(18.47)	ABD	46.18(6.49)	D	21.83(3.95)	C	53.95(6.38)	ABC	21.41(0.87)	ABC	2.07(0.16)	ABC
NO	296.62(28.97)	BCD	71.25(12.44)	AC	75.03(18.3)	AD	10.52(1.76)	ABC	18.99(8.2)	CD	106.23(32.1)	AC	17.2(4.3)	BC	0.75(0.19)	AC
NZ	420.03(100.88)	ABD	189.25(14.31)	BC	184.29(15.94)	ABCD	27.75(4.6)	BD	45.92(1.85)	AE	61.81(4.02)	ABC	21.45(1.29)	ABC	2.5(0.49)	ABC
SE1	469.8(33.84)	ABD	173.52(51.01)	ABC	177.03(53.31)	ABCD	7.91(0.38)	AC	16.21(4.09)	CD	48.03(2.61)	ABC	115.86(40.87)	A	2.24(0.32)	ABC
SE2	322.13(33.1)	ABCD	153(21.33)	ABC	500.01(103.67)	C	9.52(2.64)	ABC	27.71(7.21)	CE	66.2(14.84)	ABC	30.33(9.64)	AC	2.18(0.24)	ABC

2.3. Results

2.3.1. Soil properties

Soil chemical analysis indicated that the unlimed soils had significantly different soil physiochemical properties. This was the case for most of the parameters measured including total carbon (TC) and total nitrogen (TN) amongst others (Table 2.2). Some of these differences were also observed between treatments within a site. For example, soil pH differed across corresponding treatments in each site, indicating lime applications were successful at rising the pH level to create a gradient. Other properties such as Ca also show different levels across treatments within a single site such as IE or NO (data not shown). All sites grouped independently (Fig. 2.1), except for IE and NO overlapping; as well as NO overlapping with FRA1 and FRA 2. A total of 59.8% variation was explained between axes PCA 1 (37.9%) and PCA 2 (21.9%). Some soil physiochemical properties significantly correlated with soil pH (Table 2.3). Soil pH was also positively correlated with both, denitrifier and nitrifier communities (Table 2.3). Other soil physiochemical properties also correlated with each other, with gene abundances and gene ratios (in Appendix 2, Table S2.2 – S2.4).

Figure 2.1. Principle component analysis (PCA) of soil chemical properties. Each colour represents a site. Ellipses show 95% confidence interval, with the exception of NZ due to only 3 samples being included in the analysis. Arrows indicate the impact of the soil factors on each of the sites, with the length of the arrow proportional to the strength of the effect. TC – Total carbon, TN – Total nitrogen, DK – Denmark, FI – Finland, FRA 1 – France 1, FRA 2 – France 2, IE – Ireland, NO – Norway, NZ – New Zealand, SE1 – Sweden 1, SE2 – Sweden 2.

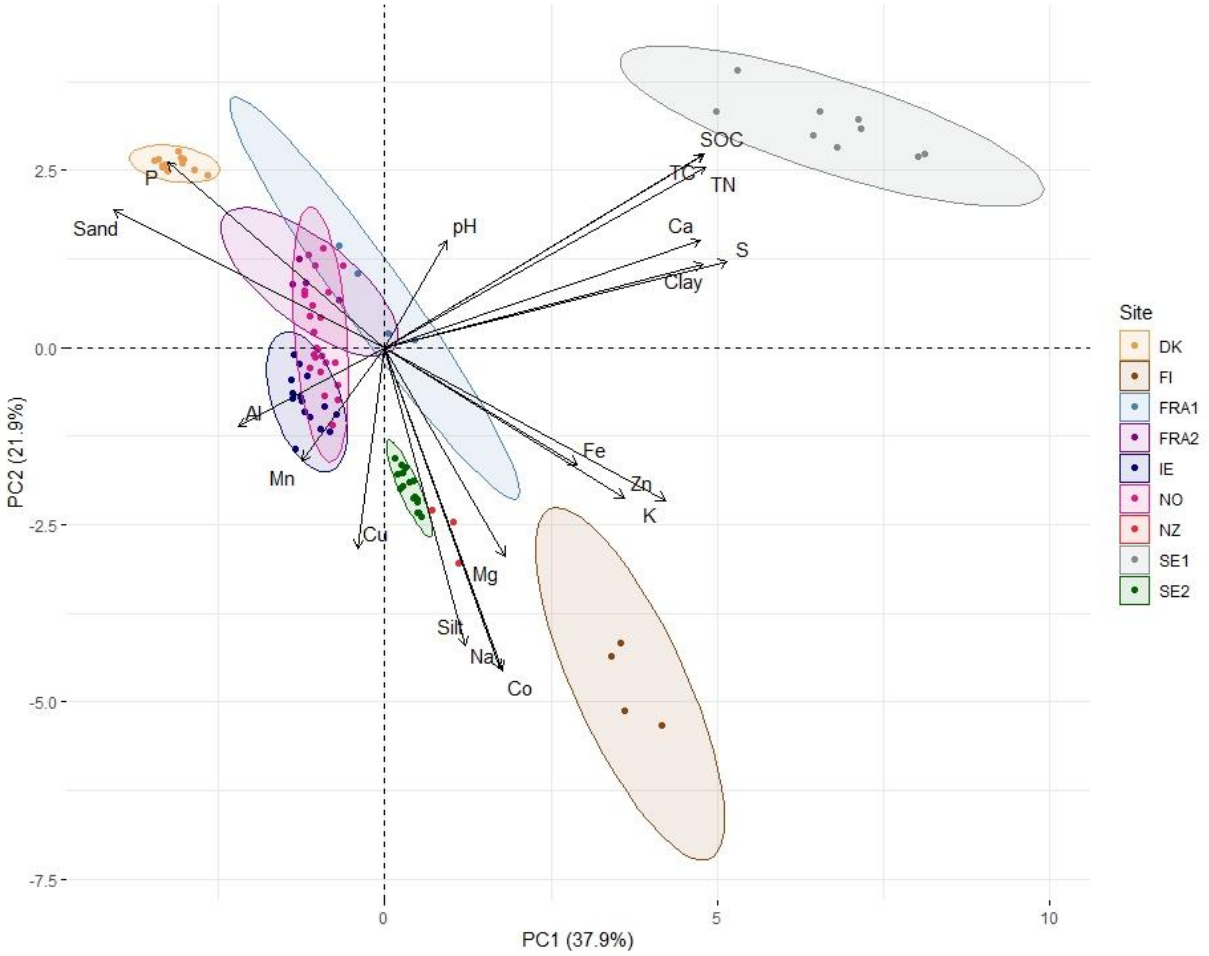


Table 2.3. Spearman's correlation coefficients of soil pH with soil properties, targeted gene abundances and gene ratios. Significant correlations highlighted in bold (***** $p \leq 0.05$; ****** $p \leq 0.01$; ******* $p \leq 0.001$). These results include both, limed and unlimed plots, from the overall data (all sites). TC - Total carbon, SOC - Soil organic carbon, TN - Total nitrogen.

	TC	SOC	TN	Sand	Silt	Clay	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
pH	0.2	0.19	0.2	0	-0.1	0.1	**-0.34 *****	**0.46 *****	**-0.23 ***	0.1	**-0.30 ****	0.1	**0.26 ***	**-0.28 ****	0	0	0.1	-0.1

	16S rRNA bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB	COMAMMOX	*ITS* fungi / *16S rRNA* bacteria	*nirK* / *nirS*	*nosZI* / *nosZII*
pH	**0.40 *****	0.04	0	**0.61 *****	**0.25 ***	**0.42 *****	**0.35 *****	**0.22 ***	**0.37 *****	**0.49 *****	**-0.29 ****	**-0.22 ***	**-0.31 ****

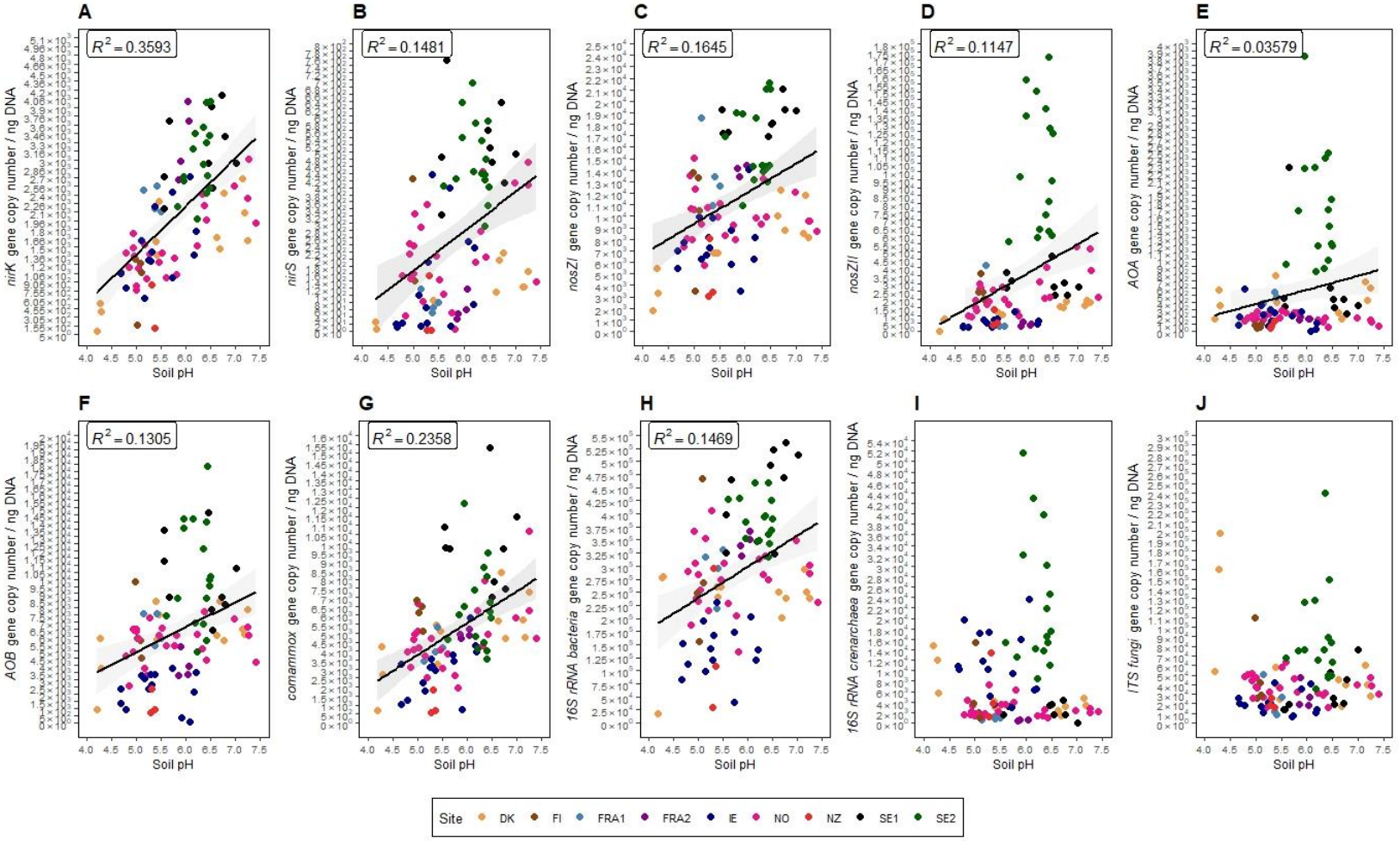
2.3.2. Impact of pH on denitrifier abundance

Denitrifier abundances (Fig. 2.2A – 2.2D) were positively correlated with soil pH (Table 2.3). *nirK* was more strongly correlated to soil pH than *nirS*. *nosZI* correlation with soil pH was stronger than *nosZII*. Soil pH was the soil factor most closely associated with *nirK* while K was for *nirS* abundances. TN was the soil factor most strongly correlated with *nosZI*, while it was Mg for *nosZII* (in Appendix 2, Tables S2.2). These observed correlations sometimes differed when analysed within a site with pH treatments (data not shown). For example, within DK site, same positive correlations were observed, however these were overall stronger. *nir* genes correlation strengths were similar (*nirK* Spearman's *rho* = 0.81, $p \leq 0.01$; and *nirS* Spearman's *rho* = 0.84, $p \leq 0.01$) while *nosZII* correlation with soil pH (Spearman's *rho* = 0.93, $p \leq 0.001$) was stronger than *nosZI* (Spearman's *rho* = 0.86, $p \leq 0.001$). Within IE, the only gene significantly correlated with soil pH was *nirK* (Spearman's *rho* = 0.56, $p \leq 0.05$), but this relationship is weaker when compared to the overall dataset. All denitrifier gene markers except for *nosZI* were also positively correlated with soil pH within NO site. Of both *nir* genes within NO site, *nirK* correlation with soil pH was stronger (Spearman's *rho* = 0.78, $p \leq 0.001$) than *nirS* (Spearman's *rho* = 0.44, $p \leq 0.05$) while correlation strength of *nosZII* with soil pH (Spearman's *rho* = 0.63, $p \leq 0.001$) appeared to be stronger than the overall dataset. SE1 and SE2 had no significant correlations between denitrifier genes and soil pH. The variations of strength and significance of correlations between denitrifier genes and soil pH in the overall dataset and specific sites might be caused due to the longevity of the experimental trials, with DK being the longest established showing strong correlations between the soil factor and gene abundances compared to more recently limed sites such as SE1 and SE2.

Site significantly impacted ($p \leq 0.01$) all denitrifier gene abundances when comparing unlimed plots (Table 2.4). Within sites DK, IE and NO, a significant effect of pH treatment on abundances can be seen (in Appendix 3, Table S2.5).

Figure 2.2. Scatter plots of gene abundances across soil pH range. Includes complete data with unlimed and lime plots from each site presented for each target gene. Gene abundances are grouped by site. Adjusted R-squared (R^2) from gene abundance x soil pH linear model present in graphs with a significant effec of soil pH on gene abundance. 95% coefficient intervals of linear trends present in plots in light grey. **2.2A.**

	16S rRNA bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosSZII*	AOA	AOB	COMAMMOX
DK	1.93E+05 ^{AB}	1.09E+04 ^{BCE}	1.37E+05 ^{B}	3.33E+02 ^{B}	1.56E+01 ^{C}	3.53E+03 ^{C}	5.04E+03 ^{A}	3.71E+02 ^{BC}	3.53E+03 ^{BDE}	2.54E+03 ^{AB}
FI	2.85E+05									6.14E+03 ^{AC}
FRA1	2.91E+05									4.23E+03 ^{ABC}
FRA2	3.44E+05									4.93E+03 ^{ABC}
IE	1.35E+05									1.95E+03 ^{B}
NO	2.94E+05									4.84E+03 ^{ABC}
NZ	1.44E+05									1.03E+03 ^{B}
SE1	3.96E+05									1.01E+04 ^{C}
SE2	3.98E+05									7.33E+03 ^{AC}



2.3.3. Impact of pH on nitrifier abundance

Nitrifier abundances (Fig, 2.2E – 2.2G) were positively correlated with soil pH (Table 2.3). AOB was more strongly correlated with pH than AOA, however the strongest relationship was observed with COMAMMOX (Table 2.3). Soil factors most closely associated with each nitrifer gene were Mg for AOA; Mn for AOB and Ca for COMAMMOX (in Appendix 2, Table S2.2). Positive correlation between soil pH and COMAMMOX was observed within DK, IE and NO sites. The strongest correlation between pH and COMAMMOX abundance occurred in DK (Spearman's *rho* = 0.73, $p \leq 0.01$). AOB was not correlated with soil pH within any of the sites with pH treatments, while AOA was negatively correlated with soil pH (Spearman's *rho* = -0.45, $p \leq 0.05$) in NO, opposite to the reported trend for the overall dataset.

Site significantly impacted ($p \leq 0.01$) all nitrifier gene abundances (Table 2.4). AOA abundances from site SE2 were an order of magnitude higher (10^3 gene copy number/ng DNA) than the rest of sites, except for SE1 (Table 2.4). Contrary to what was observed with the denitrifier community, the effect of pH treatment within a site only influenced the abundance of AOA and COMAMMOX nitrifier communities from NO site (in Appendix 2, Table S2.5).

2.3.4. Impact of pH on prokaryotic and fungal abundances

Soil pH was positively correlated ($p \leq 0.001$) with *16S rRNA* bacteria gene abundance (Fig. 2.2H). This significant correlation was not observed for any of the sites with pH treatments (Spearman's $p > 0.05$). Gene abundances for *16S rRNA* crenarchaea and *ITS* fungi were not significantly ($p > 0.05$) correlated with soil pH (Fig. 2.2I, 2.2J). This was not the case within DK site in which *16S rRNA* crenarchaea was more strongly negatively correlated (Spearman's *rho* = -0.72, $p \leq 0.01$) than *ITS* fungi (Spearman's *rho* = -0.65, $p \leq 0.05$) to pH. Within SE1, *ITS* fungi abundances were observed to be positively correlated with soil pH (Spearman's *rho* = 0.68, $p \leq 0.05$). The most closely associated property with bacterial gene abundances were Mn; Cu for crenarchaea gene abundances and Mg for fungal gene abundances (in Appendix 2, Table S2.2).

Site significantly impacted ($p \leq 0.01$) all three phylogenetic genes (Table 2.4). Overall community composition differed slightly across sites, with *16S rRNA* crenarchaea being most variable. Phylogenetic gene copy numbers were not

impacted by pH treatment apart from *16S rRNA* crenarchaea, decreasing with lime application within DK site (in Appendix 2, Table S2.5).

2.3.5. Gene ratios

Soil pH was negatively correlated with phylogenetic (*ITS* fungi/*16S rRNA* bacteria) and functional denitrifier gene ratios (*nirK*/*nirS* and *nosZI*/*nosZII*) but was not correlated with *NOS*/*NIR* ratio (Table 2.3). These significant correlations were also true within DK site for *ITS* fungi/*16S rRNA* bacteria (Spearman's rho = -0.60, $p \leq 0.05$) and *nirK*/*nirS* (Spearman's rho = -0.62, $p \leq 0.05$) ratios, both much stronger than the observed correlation in the overall dataset. Gene ratios from IE, SE1 and SE2 did not show significant correlations apart from *nosZI*/*nosZII* gene ratio in NO site, in which the strength of the correlation (Spearman's rho = -0.59, $p \leq 0.01$) was greater than in the overall dataset. The most closely associated property with *ITS* fungi/*16S rRNA* bacteria ratio was P and Ca for *nirK*/*nirS* ratio. Al and Mg correlations strengths were the same for *nosZI*/*nosZII* gene ratio with a positive and negative correlation respectively. NOS/NIR ratio was most closely associated with Co (in Appendix 2, Table S2.4).

Site significantly impacted ($p \leq 0.001$) all gene ratios (in Appendix 3, Table S2.6). Only *nosZI*/*nosZII* gene ratio within NO site was significantly influenced by pH treatment ($p \leq 0.05$), with ratio value being significantly higher in larvikite (LK) lime form treatment than in marble (M) lime form treatment.

2.4. Discussion

In this study the hypothesis that soil pH would positively correlate with nitrifier and denitrifier community abundances was tested and it was found this was true for all functional genes quantified in this study, as well as bacterial phylogenetic gene marker. It was tested if the same hypothesis applied to sites with pH treatments independently of their geoclimatic location and it was found the correlation between gene abundances and soil pH varied across sites. While the role of soil pH seems to be emphasised by these linear trends; the geoclimatic nature of the samples also had a significant impact on the composition of microbial communities. Significant differences between unlimed plots and treatments within a site, specifically for DK, indicate long-term pH management might be key to increase the presence of these functional communities.

Most sites grouped independently (Fig. 2.1) based on soil type and properties. These results demonstrate that there is a range of edaphic properties across sites to test the hypotheses across a wide gradient of geoclimatic regions. The range of edaphic properties demonstrated that variation across sites allowed to test the hypotheses of the study throughout a wide number of characteristics that could influence soil pH and its relationship with microbial community composition differently.

All denitrifier target genes were significantly influenced by site and positively correlated with soil pH (Fig 2.2A – 2.2D). Soil type and soil factors have been observed to impact gene abundances (Azziz et al., 2017; Jha et al., 2017; Juhanson et al., 2017), supporting the observed site effect on denitrifier *nir* and *nos* functional genes. Overall, *nirK* gene abundance was higher than *nirS* within an order of magnitude (10^3 versus 10^2 gene copy number/ng DNA). The dominant abundance of *nirK* was consistent with previous studies (Castellano-Hinojosa et al., 2018; Jones et al., 2014b). However, other studies have reported *nirS* abundance to be higher (Avşar and Aras, 2020; Graf et al., 2014; Krause et al., 2017). Different experimental designs across studies emphasise the possibility of niche specialisation of these two homologous genes (Enwall et al., 2010; Graf, 2015). This study would suggest *nirS* is more sensible to its environment, with more soil physiochemical properties correlating with the abundance of the denitrifier gene as well as lower presence in soils compared to *nirK*. Soil pH has been positively correlated with both *nir* genes (Herold et al., 2018), as seen in this study. Also, *nirS* abundances were correlated to activity in more alkaline soils and *nirK* abundances linked to activity in more acidic soils (Bowen et al., 2020). These correlations are an example of the distal effect soil pH has on N-cycling communities by selecting a functional gene over another. Also, *nirK* is a copper (Cu) based enzyme (Zumft, 1997). Cu availability in soils is influenced by soil pH with alkaline soils being limited in Cu compared to low pH soils. The correlation between *nirK* and acidic soils highlights soil pH is indirectly shaping the microbial community by impacting metal availability. The effect of treatment within site on *nir* abundances is in line with a positive correlation of soil pH with gene abundances. It emphasises the importance of soil pH to increase the presence of denitrifier microorganisms in soils as is seen across lime application rates in DK (in Appendix 3, Table S2.5). The denitrifier community composition might not only be impacted by the pH level in the soil but also the product used to raise the pH

in the soil. Three of the lime products used within NO site led to a significant increase of *nirK* gene copy numbers compared to the unlimed plot (in Appendix 3, Table S2.5).

Niche partitioning has also been suggested for *nosZI* and *nosZII*, showing gene abundances are not influenced by the same soil factors (Domeignoz-Horta et al., 2015). This was also evidenced in this study with correlations between clades and soil properties differing (in Appendix 2, Table S2.2). Correlations between soil pH and *nos* gene abundances were positive as previously reported (Domeignoz-Horta et al., 2018). The positive relationship between soil pH and N_2O reductase suggests more alkaline soils would tend to complete denitrification. While laboratory incubations and field gas campaigns were not carried for this study, previous publications including DK and IE sites have reported a decrease of N_2O levels from treatments with highest soil pH (Abalos et al., 2020; Zurovec et al., 2021). These observations suggest the positive correlation of *nosZ* and pH as well as a larger presence of complete denitrifiers within the microbial communities, resulting in decreased N_2O emissions. The effect of pH treatment on gene abundances within DK plots further supports as previously stated that an increase in soil pH by greater rates of lime application also increases the abundance of denitrifiers within the microbial community.

Site significantly affected nitrifier gene abundances (Table 2.4). In this study, only AOB was correlated with TN but there are other soil properties that show correlations with both nitrifier functional genes (in Appendix 2, Table S2.2), which could be co-dependent of soil pH. Both *amoA* gene abundances were positively correlated with soil pH, however, there appears to be a lot of contradictory trends of the relationship between soil pH and *amoA* gene abundances. A positive trend of the nitrifier AOA gene abundances had been previously reported (Baolan et al., 2014; Behnke et al., 2020). The contrary relationship has also been observed with AOA decreasing with increasing pH (Nicol et al., 2008). The reported positive correlation between soil pH and AOB does not align with previous results with no effect or clear trend of soil pH in relation bacterial *amoA* gene abundance (Baolan et al., 2014; Nicol et al., 2008) but could be compared to meta-analysis of field studies that reported an increase of AOB levels in soils with pH greater than 6 (Ouyang et al., 2018). Overall results show AOB gene abundance to be greater than AOA, which disagrees with observations of higher AOA abundances independently of environmental conditions, including soil pH (Waggoner et al., 2021) and ubiquity of *amoA* archaeal gene abundances present

throughout soil pH ranges (Prosser and Nicol, 2012). While geoclimatic region impacted the nitrifier microbial community composition across the sites, soil pH treatments did not influence the abundances of *amoA* target genes greatly (in Appendix 3, Table S2.5). Only within NO site, AOA gene abundances differed depending on the lime product applied. The correlation of nitrifier markers with pH might be related to the soil environment the microbial community is found in instead of a direct effect for the selection of nitrifier functional genes as suggested for the denitrifier functional community.

Relationships between the nitrifier community and soil pH might also be important to better understand how pathways within the N cycle are dependent on one another and what possible role they might have in reducing N_2O emissions. This complex network not only of soil factors influencing the selection of functional communities within the microbial community composition can be emphasised by the significant positive correlations between all denitrifier and nitrifier gene abundances targeted in this study (in Appendix 2, Table S2.3).

Reduction of ammonia to nitrate can occur within a single organism if harbouring COMAMMOX gene (Hu and He, 2017). This study is one of the firsts in literature to quantify COMAMMOX abundances in such a wide pH gradient across sites from the northern and southern hemispheres. The significant effect of site (Table 2.4) might be related to the positive correlation of bacterial *amoA* and COMAMMOX gene abundances with TC and TN, these properties also differ across sites in the study (Table 2.2), which has been previously reported (Li et al., 2021; Shi et al., 2018). This effect is also in line with reports of COMAMMOX distribution being significantly different across different climates and habitats (J. Hu et al., 2021) as well as to gene abundance being significantly correlated to mean annual precipitation (Li et al., 2021); further justifying the variation across the geoclimatic regions included in this study. Ammonia oxidiser COMAMMOX was positively correlated with soil pH (Table 2.3), this is the opposite trend to previous reports that observed a decline of COMAMMOX abundance with increasing pH (J. Hu et al., 2021; Shi et al., 2018) but no effect has also been reported (Li et al., 2021). Positive correlation of soil pH with COMAMMOX gene abundances has not been previously observed. Like archaeal *amoA*, the effect of treatment on the nitrifier functional gene COMAMMOX was only observed within NO site, in which the different lime products applied lead to significantly different abundances within the nitrifier composition of the microbial

community (in Appendix 3, Table S2.5). The novelty of this functional gene requires further studies to explore which soil factors might favour the presence of COMAMMOX within the microbial communities.

Site had a significant effect (Table 2.4) on overall microbial community composition for *16S rRNA* bacteria, *16S rRNA* crenarchaea and *ITS* fungi, in line with observations of microbial community structures being influenced by soil factors (Thomson et al., 2015), since the measured soil properties significantly differ across geoclimatic regions (Table 2.2). Soil pH was only positively correlated with bacterial gene abundance, as previously reported (Rousk et al., 2010). There is a poor consensus on the role soil pH has on *16S rRNA* crenarchaea gene abundances, negative correlations between the soil property and specific crenarchaea groups have been observed, but no clear trends for the phylogenetic gene haven been seen (Lehtovirta et al., 2009). Optimal fungal growth across a wider soil pH range (Rousk et al., 2010) could explain the lack of correlation of the soil property with gene copy numbers. For overall microbial community composition, it appears pH might not be as key of a factor as it is for N-cycling functional communities. There was no observed treatment effect on phylogenetic gene abundances in relation to lime applications for a shift in pH except for DK site where increasing pH resulted in decreased *16S rRNA crenarchaea* compared to the unlimed plots abundances (in Appendix 3, Table S2.5).

Fungal to bacterial ratio (*ITS* fungi/*16S rRNA* bacteria) was negatively correlated with soil pH (Table 2.3), indicating an increase on soil pH influences microbial community composition, in this case with less fungi being present as soil pH rises. This ratio was also negatively correlated with denitrifier *nosZI* abundances (in Appendix 2, Table S2.3). Fungi lack *nosZ* gene (Hallin et al., 2018), supporting the negative correlation between the microbial community proportions and denitrifier abundances. This in turn will impact N_2O emissions as microbial communities with a larger fungal presence will not be able to act as N_2O sinks due to the lack of NOS within the microbial community.

The experimental sites in this study created a wide pH gradient across a variety of soil types and geoclimatic regions in which analyse the microbial gene abundances of key communities directly (denitrifiers) or indirectly (nitrifiers) involved in the production and reduction of N_2O. Previous studies focusing on the role of soil pH lacked a diversity of geoclimatic regions (Rousk et al., 2010) or a narrow pH range ranging from 4.91 to 5.65 (Dandie et al., 2011). In contrast, our study had 9 unique

soil types, with a pH range of 4.19 to 7.41 across a geoclimatic gradient. Given this, the diversity of sites and soil pH values allowed us to test the role of soil pH across a wide range of soil types.

In conclusion, our results indicate soil pH can influence the microbial community composition for functional genes involved in both, denitrifier and nitrifier pathways. This effect might be post-transcriptional (Bakken et al., 2012), suggesting the proximal role pH plays on protein synthesis might have led to positive relationships between the soil property and gene abundances, assuming the microbial community selects for N-cycling functional genes in pH ranges where the synthesised protein will be functional. However, this post-transcriptional effect cannot be analysed based on qPCR quantification. However, it is important to emphasise the strong effect site has on the quantified gene copy numbers and the different correlation between gene abundances and soil physiochemical properties. This effect can be justified by the different soil types across the geoclimatic regions. These create unique environments, including soil structure and pH; and therefore a shift in the structure and composition of the microbial communities. The site effect on gene abundances can be linked to the "master variable" property of soil pH. Since each geoclimatic region has its own pH range, the nutrient profile of the soil is influenced by pH. The availability or limitation of different elements will also determine the composition of the N-cycling microbial community. Not only the factors shaping the environment of the microbial community are important but also the positive correlations between denitrifier and nitrifier gene abundances. While it may be possible to improve soil pH management to reduce N_2O emissions, more detailed studies exploring the complex network between soil environmental factors and microbial communities and niche specialisations created by a combination of both will be key to improve the N_2O sink capacity of soils.

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CHAPTER 3

pH and phosphorus management effects on nitrifier and denitrifier communities in grassland soils

Chapter 3. pH and phosphorus management effects on nitrifier and denitrifier communities in grassland soils

3.1. Introduction

Nitrous oxide (N_2O) is potent greenhouse gas, with a global warming potential 273 times greater than carbon dioxide (CO_2) (Masson-Delmotte, V. et al., 2021), and it also damages the ozone layer (Ravishankara et al., 2009). Mostly, N_2O emissions are emitted from soils, with agriculture being one of its main sources (Butterbach-Bahl et al., 2013). Overall, the contribution of agriculture to anthropogenic N_2O emissions is enhanced by nitrogen (N) fertiliser and manure application which has led to a total global emission average of 3.8 TgN N_2O yr^{-1} (Masson-Delmotte, V. et al., 2021). Nitrification and denitrification are the main sources of these emissions, primarily due to soil microbial processes within the N cycle (Kool et al., 2011).

Nitrification is an aerobic process where ammonia (NH_3) is oxidised to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-) (Hu et al., 2015). The process is catalysed by ammonia monooxygenase (*amoA*) encoded by bacterial (AOB) and archaeal (AOA) genes (Hu et al., 2015). Both oxidising steps within the nitrification process can also be catalysed by a single enzyme, encoded by functional gene COMAMMOX (Kuypers et al., 2018). Denitrification is an anaerobic stepwise respiratory process in which NO_3^- is sequentially reduced to NO_2^-, nitric oxide (NO), N_2O and N_2 (Saggar et al., 2013). The final reduction step where N_2O is reduced to N_2 is catalysed by nitrous oxide reductase (N_2OR) and behaves as an N_2O sink (Hallin et al., 2018). There are two clades present in the environment, *nosZ* clade I (*nosZI*) and *nosZ* clade II (*nosZII*) (Jones et al., 2013). The denitrifier microbial community can also be quantified in soils with functionally equivalent genes *nirK* and *nirS*, which encode the enzymes catalysing nitrite (NO_2^-) to nitric oxide (NO) reduction (Zumft, 1997). Since denitrification is a modular, partial and complete denitrifiers, harbouring some or all functional genes occur, with different gene occurrence and co-occurrence patterns observable across taxa (Hallin et al., 2018).

As living organisms, microbial communities are influenced by their environment. Management of agricultural soils shape their environments, and consequently can shift the function and genetic potential of the microbial communities, thereby favouring processes best suited for these new conditions. Environmental factors driving those changes in microbial communities involved in N transformations include carbon (C) and N availability (Senbayram et al., 2012); oxygen availability

(Giles et al., 2012) or soil pH (Saggar et al., 2013). Another soil factor influencing microbial communities is phosphorus (P) nutrient availability. Denitrifier gene abundances have been reported to be correlated with P gradients across 10 New Zealand dairy farm soils, with *nirK* being the most strongly correlated, followed by *nirS* and *nosZ* (Jha et al., 2017). This correlation is further supported by the observed increase of denitrifier gene abundances of *nirS*, *nirK* and *nosZI* after P application (Wei et al., 2017) and a negative correlation between these denitrifier gene abundances and P limitation (Cui et al., 2020). For nitrification functional genes, AOB abundance was increased after P application while no effect was observed for AOA (Wei et al., 2017). As reported for denitrifier genes, bacterial and archaeal *amoA* abundances were negatively correlated with P limitation (Cui et al., 2020). To our knowledge, the relationship between available P and COMAMMOX abundances has not been investigated previously. Soil P also influences N cycling processes these communities are involved in. Nitrous oxide emissions were observed to increase after P addition (Mehnaz and Dijkstra, 2016). In contrast, a number of studies have reported that low P levels led to higher N_2O emissions in long term P fertilisation studies (O'Neill et al., 2020). In addition to impacts on functional communities, the broader microbial community structure has also been reported to be influenced by P availability, with low P soils being dominated by fungi (Chen, 2012) and having a higher arbuscular mycorrhizal fungi (AMF) colonisation (Randall et al., 2019) compared to soils with high P availability, which are dominated by bacteria.

While the reported impacts of P on N_2O emissions are conflicting, soil pH is known to strongly influence N_2O production, and negative correlations between N_2O and $N_2O/(N_2O+ N_2)$ products and this soil property have been reported in both laboratory (Samad et al., 2016a) and field studies, including the same field trial samples from this study were collected from (Zurovec et al., 2021). The abundance of nitrifier functional genes appear to be variable in relation to pH, with archaeal *amoA* gene abundances increasing with soil pH in some studies and reducing in others (Baolan et al., 2014; Nicol et al., 2008), while bacterial *amoA* abundances show positive or no correlation at all with soil pH (Baolan et al., 2014; Nicol et al., 2008). The response of recently discovered complete nitrification functional gene COMAMMOX so far has appeared to be negatively correlated with soil pH (Shi et al., 2018), however positive correlations between COMAMMOX abundances and soil pH we reported in chapter 2. All denitrifier functional genes have been reported to be

correlated with soil pH, with studies observing positive increasing trends of *nirK* and *nirS* abundances with soil pH (Herold et al., 2018). This same trend has also been observed for *nosZ* (Liu et al., 2010) and an increase of denitrifier abundances has been reported after lime application for all denitrifier genes (Jha et al., 2020). The pH-sensitivity of NosZ, especially for *nosZII* (Domeignoz-Horta et al., 2015) highlights the link between the soil factor and *nosZ* abundances. Broader phylogenetic community abundances have also been correlated with soil pH. Overall bacterial abundances were reported to be positively correlated to soil pH (Rousk et al., 2010), while fungal and crenarchaeal abundances do not appear to be impacted by soil pH (Lehtovirta et al., 2009; Rousk et al., 2010). The structure of the microbial community will determine the abundance of functional genes within the microbial community. For example, denitrifying fungi lack *nosZ* gene encoding for nitrous oxide reductase (Hallin et al., 2018), which may cause larger N_2O emissions in soils where there is a larger fungal component within the microbial community, as denitrification cannot be completed. The impact of soil pH on the functional denitrifier communities also dictates N_2O emissions due to direct and indirect impacts of soil pH on the denitrifier community (Čuhel et al., 2010). Protein assembly inhibition (Bergaust et al., 2010) or post-transcriptional issues (Liu et al., 2010) of the enzyme and shifts of the structural microbial community composition caused by soil pH (Jones et al., 2014a) will in turn dictate an increase or reduction of N_2O emissions from soils.

In this study, we investigated the impact of P availability and soil pH on denitrifier and nitrifier functional communities in grassland soil, and the resultant potential N_2O emissions. The study aimed to further explore the role pH plays in shifting the microbial community and analyse if those changes were translated in different N_2O production potentials across the pH treatments. The study also aimed to better understand if increasing soil P availability through P treatment increased denitrification activity, the size of its community and if it induced complete denitrification (N2 as the final product) or not, since P is required for microbial activity. The impact of the interaction of these factors was studied on microbial community structure, functional community abundances and potential N_2O production. We hypothesised that soil pH treatments would impact structural (bacterial and fungal) and functional denitrifier (*nirK*, *nirS*, *nosZI* and *nosZII*) and nitrifier (AOA, AOB and COMAMMOX) abundance. The potential N_2O production was tested across soil pH and P treatment combinations, and it was hypothesised that

lower pH and P availability would increase N_2O emissions arising from larger fungal communities and smaller abundances of denitrifier (*nosZ*) functional genes.

3.2. Methods

3.2.1. Site description and sampling

Samples were collected from a long-term grassland trial in Teagasc Johnstown Castle, Ireland (52◦17′47″N 6◦30′25″W). The site, established in 2011 consists of lime and phosphorus (P) treatments under a perennial ryegrass (*Lolium perenne*) monoculture, where the soil is classified as a moderately drained brown earth (Zurovec et al., 2021). The trial was comprised of a randomised block design with four biological replicates per treatment (Fig. 3.1). Using four lime (ground limestone, $CaCO_3$) and P (triple super phosphate, 16% P) application rates a total of 16 treatment combinations were created as outlined in Table 3.1. P fertiliser was applied annually in March at the relevant application rate. Soil pH ranged from 4.82 to 7.07 and average P content (Mehlich III extraction) was 37.34 mg kg^{-1}, 50.86 mg kg^{-1}, 65.46 mg kg^{-1} and 74.24 mg kg^{-1} for P0, P20, P40 and P60 treatments respectively. In Ireland, soil P concentrations are based on Morgan's soil P extraction and the results are categorised into 4 indices (1 to 4) with index 1 having lowest P content and having a definite response to P fertiliser application and index 4 soils expected to show no response (Coulter and Lalor., 2008). P treatments in this experiment aimed to recreate all four indices with P40 representation optimal P agricultural management and soil P concentration. All plots received 300 kg ha^{-1} of N fertiliser (Sulfa CAN - calcium ammonium nitrate with sulfur, 26% – N; 5% – S) across 8 applications throughout the growing season (February 2019 – October 2019). Potassium fertiliser (MOP, 50% K) application was 250 mg kg^{-1} for all plots between two splits.

Soil sampling took place in June 2019 before grass harvesting and N fertiliser application (four weeks after last application in May 2019). Following a "W" shape across each experimental plot, the top 10 cm soil was sampled using a soil corer, bulked, and homogenised to form a composite sample. Subsamples for molecular analysis were flash frozen with liquid nitrogen in the field and stored in the -80°C freezer prior to further analysis. Another subsample for soil physiochemical analysis was taken, dried at 40°C and sieved to 2 mm. The rest

of the composite sample was stored at 4°C for downstream laboratory incubation analysis.

Table 3.1. Treatment summary of experimental site. Soil pH treatments were created by ground limestone ($CaCO_3$) application rate throughout different years since the establishment of the trial. VL – Very Low; L – Low; H – High; VH – Very High. Phosphorus treatment gradients were established by application of yearly P (triple super phosphate) rates.

Treatment	Ground limestone rate
VL	Unlimed control
L	5 t ha^{-1} applied in 2011
H	5 t ha^{-1} applied in 2014 + 1.5 t ha^{-1} applied in 2019
VH	5 t ha^{-1} applied in 2011, 2014 and 2019
Treatment	**Phosphorus rate**
P0	0 kg P ha^{-1} yr^{-1}
P20	20 kg P ha^{-1} yr^{-1}
P40	40 kg P ha^{-1} yr^{-1}
P60	60 kg P ha^{-1} yr^{-1}

Figure 3.1. Schematic diagram of randomised block design from experimental site. Each row represents a biological replicate (n = 4). Each pH and P treatment combination represents an experimental plot within the biological replicate (n = 16). pH treatments are represented in orange. P application rates (ha^{-1} yr^{-1}) are represented in green. VL – Very Low; L – Low; H – High; VH – Very High.

P	20	20	0	0	40	40	0	60	20	40	60	60	40	20	0	60
pH	VH	VL	VL	VH	VH	VL	L	H	L	L	VH	L	H	H	H	VL
P	0	40	40	0	60	20	40	60	60	40	20	0	60	20	20	0
pH	VH	VH	VL	L	H	L	L	VH	L	H	H	H	VL	VH	VL	VL
P	60	20	40	60	60	40	20	0	60	20	20	0	0	40	40	0
pH	H	L	L	VH	L	H	H	H	VL	VH	VL	VL	VH	VH	VL	L
P	60	40	20	0	60	20	20	0	0	40	40	0	60	20	40	60
pH	L	H	H	H	VL	VH	VL	VL	VH	VH	VL	L	H	L	L	VH

3.2.2. Soil physiochemical analysis

Gravimetric water content (GWC) and water holding capacity (WHC) percentage were determined using Jones et al., (2014b). Briefly, GWC was calculated by weighing 20 g of fresh soil and drying overnight at 105°C. The weight difference between fresh and dry soil provided GWC value. For WHC, fresh soils were sieved to 4 mm and 25 g weighed inside a centrifuge tube. The end of the tube had been previously cut and mesh (tule fabric) held with rubber bands secured at the end. Tubes were soaked in deionised water overnight. The saturated soils were then weighed and dried overnight at 105°C. The weight difference between the saturated and dried soil provided WHC percentage values of samples.

Mehlich III extraction (Mehlich, 1984) was used on 2 g of dried soil for the extraction of macro- and micro-nutrients. Mehlich III extractants were analysed using an inductively coupled plasma (ICP) optical emissions spectrometry (Agilent 5100 ICP-OES spectrometer; Agilent Technologies, Mulgrave, Australia). Soil pH was measured from a 1:2 soil:water (w/v) ratio suspension using a Mettler Toledo glass calomel electrode (McCormack, 2002). Total soil Carbon (TC) and total soil Nitrogen (TN) were measured from 0.2 g of ball milled soils dried at 40°C for 48 hours (Griffiths et al., 2012) and analysed by an elemental analyser (LECO TrueSpec CN elemental analyser, USA).

3.2.3. Potential denitrification assays (PDAs)

Potential denitrification was measured using acetylene (C_2H_2) inhibition method (adapted from Yoshinari et al., 1977). 20 g sieved (4 mm) fresh soil was placed in 0.16 L serum glass flasks. Soils were incubated overnight at 15°C. Each experimental plot had two paired flasks, incubated with Helium (He) or 10% C_2H_2 headspaces, respectively. Deionised water was added to achieve 70% WHC. Flasks were sealed with septa and crimped lids to avoid gas leaks. Headspace was flushed three times with He to remove any present Oxygen and create an anaerobic environment. Background sample (0 h) was taken before replacing 10% of flask's headspace with C_2H_2, followed by the injection of 2 mL nutrient solution from 100 mL stock solution containing 75 mM Potassium Nitrate (KNO_3), 25 mM D-Glucose ($C_6H_{12}O_6$), 7.5 mM Sodium Acetate ($C_2H_3NaO_2$) and 75 mM Disodium Succinate ($C_4H_4Na_2O_4$). Flasks were

incubated at 15°C for 5 hours, sampling every 60 minutes. The sampling syringe was flushed with headspace gas three times before collection of 10 mL sample. Following each sampling point, the headspace was refilled to minimise loss of pressure within serum flask. Gas samples were analysed for N_2O using gas chromatography (GC-2 Bruker Scion 456, USA) connected to a Combi-Pal autosampler (CTC Analysis, Switzerland). N_2O was measured on an electron capture detector (ECD). N_2O fluxes were calculated from the slope of the linear regression line of N_2O concentration versus time. N_2O measurements received in parts per million (ppm) were converted to ng N_2O-N g^{-1} dry soil min^{-1}.

3.2.4. DNA extraction

DNA extraction was performed on 0.25 g of frozen soil using DNeasy PowerSoil Kit (Qiagen, USA) following manufacturer's instructions. DNA concentration was measured using with Qubit™ dsDNA BR Assay Kit (Thermo Fisher, Ireland). DNA yields ranged between 11.3 – 27.8 ng μl^{-1}. DNA purity and quality were assessed with Nanodrop (Thermo Fisher, Ireland), considering both 260/280 and 260/230 ratios, and agarose gel of the DNA product.

3.2.5. qPCR

DNA samples were normalised to 1 ng μl^{-1} with UltraPure™ DEPC-Treated Water (Thermo Fisher, Ireland). Testing of qPCR inhibition was done on all samples as described on des Roseaux *et al.*, (2020). Correct amplification of plasmid with presence of sample indicated inhibitors were not present in samples. Phylogenetic bacteria (*16S rRNA* bacteria), *Thaumarchaea* (*16S rRNA* group 1 crenarchaea) and fungi (*ITS*) gene abundances were quantified. Functional denitrifier (*nirK*, *nirS*, *nosZI* and *nosZII*) and nitrifier genes (AOA, AOB and COMAMMOX) were also quantified by qPCR. Three technical replicates (except for *16S rRNA* bacteria and *Thaumarchaea* which only had two technical replicates) per sample were quantified in 384-well plates using a CFX384 Touch Real-Time PCR Detection System (BIO-RAD, USA). DNA standard curves were prepared from target genes inserted into a plasmid and linearised with the appropriate restriction enzyme following manufacturer's protocol using pGEM-T Easy Vector System II kit (Promega, Ireland) and QIAprep Spin Miniprep Kit (Qiagen, Ireland). Each target gene had its corresponding standard curve, in which the template consisted of a serial dilution from 10^8 or 10^7 to 10^2 gene

copies μl^{-1}. Standards, negative control (DEPC Water) and positive controls (in Appendix 4, Table S3.1) were run in triplicate in the qPCR run. MasterMix prepared for qPCR included 5µl Takyon Low ROX SYBR 2X MasterMix blue dTTP (Eurogetee, Belgium); 0.2 – 5 µl of primer (in Appendix 4, Table S3.1); 2 µl of normalised DNA template and DEPC water to adjust the final reaction volume to 10 µl. Details of the primers, primer concentrations, cycle conditions and assay efficiencies are provided in in Appendix 4, Table S3.1 All targeted genes consisted of the same MasterMix except for *nirS* which also required 1 µM bovine serum albumin (BSA). MasterMix without template was automatically loaded using a multichannel pipette and automated robot arm (Integra, Ireland). DNA template was manually added, using an electronic multichannel. qPCR cycles differed across targeted genes (in Appendix 4, Table S3.1). Melt curve analysis was performed at the end of qPCR cycle run to test for specificity and confirm lack of amplification of negative control.

3.2.6. Library preparation

Extracted DNA from soil was normalised to 5 ng/µL. Two-step PCR was the chosen procedure. Considering pros and cons of this procedure, including reduced cross-contamination by not using tag primers in the first PCR, use of bead purification steps and lack of inter-sample chimeras (Bohmann et al., 2022), two-step PCR, compared to one-step or tagged PCR, appeared to be the most suited method. Also, each sample consisted of a unique index combination to reduce misassignment of library indices.

The standard protocol for library preparation was carried out as follows. Normalised DNA was amplified through PCR1 (in Appendix 4, Table S3.2) for both *16S rRNA* bacteria and *ITS* fungi. PCR 1 product was then clean-up using Agencourt AMPure XP beads (Beckman Coulter Genomics, Ireland) to purify amplicon product, ensuring it is from free primers and primer dimers. The clean PCR1 product was run on an agarose gel to check clean-up was successful, followed by PCR2 which attaches indexes and Illumina sequencing adapters using Nextera XT Index Kit (Illumina, Ireland). Clean-up was also done on PCR2 product, which was then quantified using Qubit™ dsDNA HS Assay Kit (Thermo Fisher, Ireland); if concentration was above 15-20 ng/µl it was repeated using Qubit™ dsDNA BR Assay Kit (Thermo Fisher, Ireland). For all samples,

amplification products for *16S rRNA* bacteria and *ITS* fungi, were pooled together for a single run and were between 580 bp to 500 bp respectively; so, there would not be a bias in sequencing depth. The sample pool quality was checked using DNA 1000 Kit on 2100 Bioanalyser (Agilent, Ireland). The final libraries were then sent off for sequencing using Illumina MiSeq Platform (Illumina, Ireland) at Teagasc Moorepark Sequencing Facility which used the MiSeq Reagent kit v3, providing 40 million paired reads, a sequencing depth of 30000 base pairs (bp) and a final output of 13.2-15 Gb.

3.2.7. Statistical analysis

3.2.7.1. Metadata

Prior to statistical analysis, all gene copy numbers were averaged across qPCR technical replicates. All statistical analysis was carried out using RStudio (4.1.1, R Core Team, 2021). Data distribution and normality were statistically tested with Shapiro-Wilk normality test ($p \leq 0.05$) and if normality assumption failed data was transformed accordingly (log or square rooted if the transformation met normality assumption). Linear models with soil pH, soil P and their interaction were created for all potential gas fluxes and gene abundances. Homogeneity of variance (Levene's Test, $p \leq 0.05$) assumption was tested. If met, the effect of treatments on potential gas fluxes and gene abundances was tested using a parametric two-way ANOVA. A $p \leq 0.05$ was considered significant. If a statistically significant effect was reported, further analysis was done using parametric post-hoc Tukey's honestly significant differences (HSD) test. If data or linear model assumptions were not met, a non-parametric Kruskal-Wallis test was used to assess significant effects of treatments and a non-parametric post-hoc Dunn's test with adjusted p values (Benjamini-Hocberg method) using "dunnTest" function ("FSA") to report statistical differences across treatments. Soil physiochemical properties and their grouping according to pH treatments were assessed with principal component analysis (PCA) matrix using "prcomp" function ("STATS"), which allowed visualisation of any possible grouping of samples based on treatment and reported the percentages of explained variation across samples. Soil physiochemical measurements were statistically analysed in the same manner as gene copy numbers to detect any significant differences across

treatments. Soil physiochemical factors, potential fluxes and gene abundances correlations were assessed using "rcorr" function ("HMISC") with Spearman's rank correlation coefficient method.

3.2.7.2. Sequencing

Raw sequencing reads were processed using DADA2 (Callahan et al., 2016) through R (4.0, R Core Team, 2020) within the server. DADA2 allowed pre-processing including filtering and trimming reads, merging of forward and reverse paired reads, construction of the sequence table, removal of chimeras, and assignment of taxonomy. Taxonomic assignments were done with Silva v138 database for *16S rRNA* bacteria and *16S rRNA* archaeal and UNITE v2020 database for *ITS* fungi reads. Dataset was then ready for downstream analysis in RStudio (4.1.1, R Core Team, 2021).

Amplicon sequence variants (ASV) approach was chosen for the analysis of the data because ASVs are exact sequences, generated without clustering, compared to operating taxonomic units (OUT). OTUs are a cluster of multiple similar sequences. Instead, a gene sequence will generate the same ASV. A single base change in a gene sequence will lead to the generation of a separate ASV. This provides the advantage of more specific identification of microbes, allows a more detailed picture of the microbial diversity within a sample, and provides the opportunity to compare ASVs across studies using the same target region.

Data was not rarefied as justified by Gloor *et al.*, 2017, instead Centred Log Ratio (CLR) transformation was done by using "transform" function ("MICROBIOME). After data transformation, vectors were extracted to allow the "VEGAN" package to read and access the transformed data for downstream analysis. A phyloseq object containing taxa, OTU and tree tables and metadata (including soil physiochemical analysis, potential gas fluxes and qPCR gene abundances) was created using functions from "PHYLOSEQ" package. Permutational multivariate analysis (PERMANOVA), with permutation number equal to 999 and selecting Euclidean method, using the "adonis" function ("PAIRWISEADONIS") was used to analyse and identify what treatments, if any, were significantly influencing the structure of the microbial communities; followed by

principal component analysis (PCA) plots to visually represent the grouping of communities based on treatment. To further explore which microorganisms differed across pairwise treatments statistical tests, DESeq2 analysis using "DESeq" function ("DESeq2") was carried out to identify which bacterial and fungal communities were enriched in either of the treatments within a pair of treatments.

Redundancy analysis (RDA) was carried out on transformed data to identify environmental predictors driving the structural microbial community. RDA model was created using "rda" function ("VEGAN"). A series of models were created, starting by including all possible predictors (soil physiochemical properties, potential gas fluxes and gene abundances). The significance of the overall model was tested using "anova.cca" function ("VEGAN"), if significant ($p \leq 0.05$) the RDA model was further statistically tested to identify which predictors within the model were significantly relevant. Based on significance and variance inflation factors (VIF) scores, the RDA model was reduced until a final model was generated with only relevant environmental predictors.

3.3. Results

3.3.1. Soil properties

Soil pH increased along a gradient from VL plots (average pH 5.11) to L plots (average pH 5.48) to H plots (average pH 6.11) to finally VH plots (average pH 6.86). Each pH treatment was significantly different from each other except for VL and L (Table 3.2). Total nitrogen (TN), soil organic carbon (SOC), Al, Ca, Fe, K, Mg, Mn, S and Zn also differed significantly between pH treatments. Available P differed significantly between P treatment plots as it did for TN and Fe. PCA plot (Fig. 3.2) of the physiochemical soil properties, which accounted for 50.4% of the variation, shows that VL and VH pH treatments cluster away from each other, while the other treatments are overlapping. Soil pH and P were not correlated (Table 3.3). However, soil pH did positively correlate with Ca and negatively correlated with TN, Al, Co, Fe, K, Mn, S and Zn (in Appendix 5, Table S3.4). Soil P did not positively correlate with any other soil property but negatively correlated with TC and TN (Table 3.3).

Table 3.2. Average and standard deviation (within brackets) of soil chemical properties for each site included in the study. Lowercase letters indicate differences ($p \leq 0.05$) of soil properties across pH treatments. TC - Total carbon, SOC – Soil organic carbon, TN - Total nitrogen. VL – Very Low.

pH treatment	pH	TC	SOC	TN	Al	Ca	Co	Cu
		(%)	(%)	(%)	(mg/ha)	(mg/ha)	(mg/ha)	(mg/ha)
VL	5.11(0.2) [a]	3.44(0.29) [a]	8(0.47) [b]	0.35(0.03) [b]	814.82(47.08) [a]	1019.88(211.56) [a]	0.16(0.04) [a]	4.27(0.78) [a]
L	5.48(0.29) [a]	3.39(0.21) [a]	7.66(0.38) [ab]	0.35(0.02) [ab]	735.74(55.75) [b]	1477.31(364.08) [b]	0.14(0.03) [a]	4.58(1.07) [a]
H	6.11(0.28) [b]	3.25(0.23) [a]	7.59(0.36) [a]	0.33(0.02) [a]	683.88(58.83) [bc]	1908.08(251.69) [c]	0.14(0.04) [a]	4.61(0.92) [a]
VH	6.86(0.15) [c]	3.37(0.23) [a]	7.82(0.3) [ab]	0.34(0.02) [ab]	566.77(141.22) [c]	2629.89(368.91) [d]	0.12(0.04) [a]	4.55(0.73) [a]

pH treatment	Fe	K	Mg	Mn	P	S	Zn
	(mg/ha)	(mg/ha)	(mg/ha)	(mg/ha)	(mg/ha)	(mg/ha)	(mg/ha)
VL	302.84(22.9) [d]	82.54(11.01) [b]	103.84(29.01) [a]	53.63(5.32) [b]	53.94(14.13) [a]	28.56(3.69) [b]	2.5(0.58) [c]
L	262.35(21.45) [a]	63.9(9.56) [a]	93.69(22.25) [ab]	49.64(5.43) [ab]	56.16(17.53) [a]	24.31(3.34) [a]	1.94(0.37) [a]
H	234.27(21.8) [b]	68.43(9.15) [a]	98.7(33.75) [ab]	45.3(4.05) [ac]	59.49(16.16) [a]	27.61(6.22) [ab]	1.81(0.49) [ab]
VH	203.3(26.53) [c]	65.5(9.66) [a]	84.48(16.67) [b]	44.27(5.74) [c]	58.3(18.84) [a]	25.52(3.81) [ab]	1.49(0.27) [b]

Figure 3.2. Principal component analysis (PCA) of soil physiochemical properties grouped by pH treatment. Ellipses represent 95% confidence. Arrows indicate how soil properties related to samples, with the length of the arrow being proportional to the strength of the effect. Variability across samples is explained by a total of 50.4% between axes PCA 1 and PCA 2. SOC – Soil organic carbon, TC - Total carbon, TN – Total nitrogen.

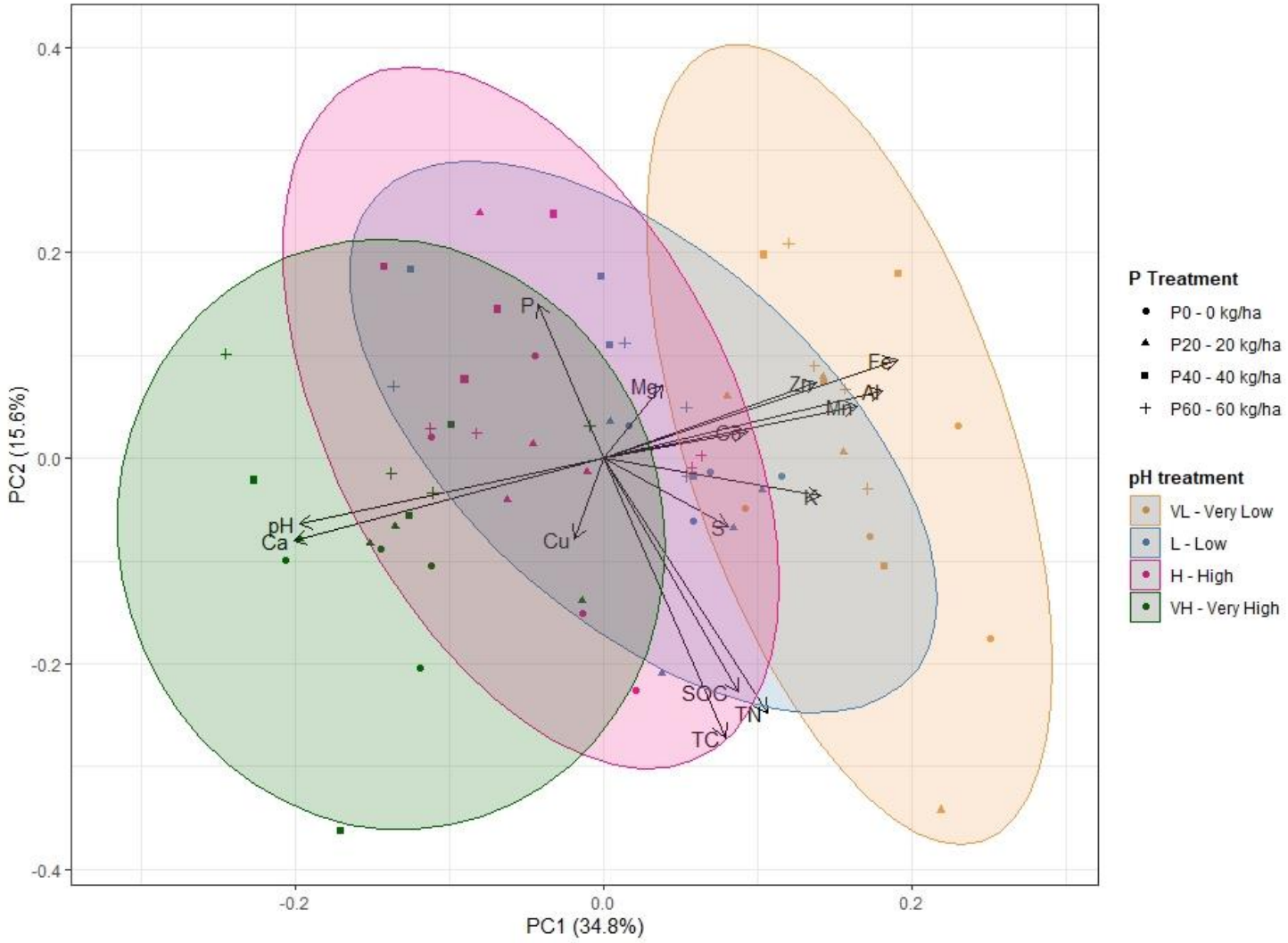


Table 3.3. Spearman's correlation coefficients of soil pH and phosphorus with soil physiochemical properties, potential gas fluxes, gene abundances and gene ratios. Significant correlations highlighted in bold (***** $p \leq 0.05$; ****** $p \leq 0.01$; ******* $p \leq 0.001$). TC - Total carbon, TN - Total nitrogen, SOC - Soil organic carbon.

	P	TC	TN	SOC	Al	Ca	Co	Cu	Fe	K	Mg	Mn	S	Zn
pH	0.17	-0.2	**-0.31***	-0.17	**-0.86*****	**0.94*****	**-0.32****	0.01	**-0.82*****	**-0.39****	-0.24	**-0.65*****	**-0.27***	**-0.56*****
P		**-0.28***	**-0.38****	-0.16	-0.05	0.16	-0.18	-0.1	0.12	-0.16	0.03	-0.07	-0.11	0.12

	Potential N_2O	Total Denitrification ($N_2O + N_2$)	Potential N_2	Product Ratio (N_2O/N_2O+N_2)
pH	-0.07	-0.03	0.02	-0.09
P	-0.08	-0.13	-0.08	0.06

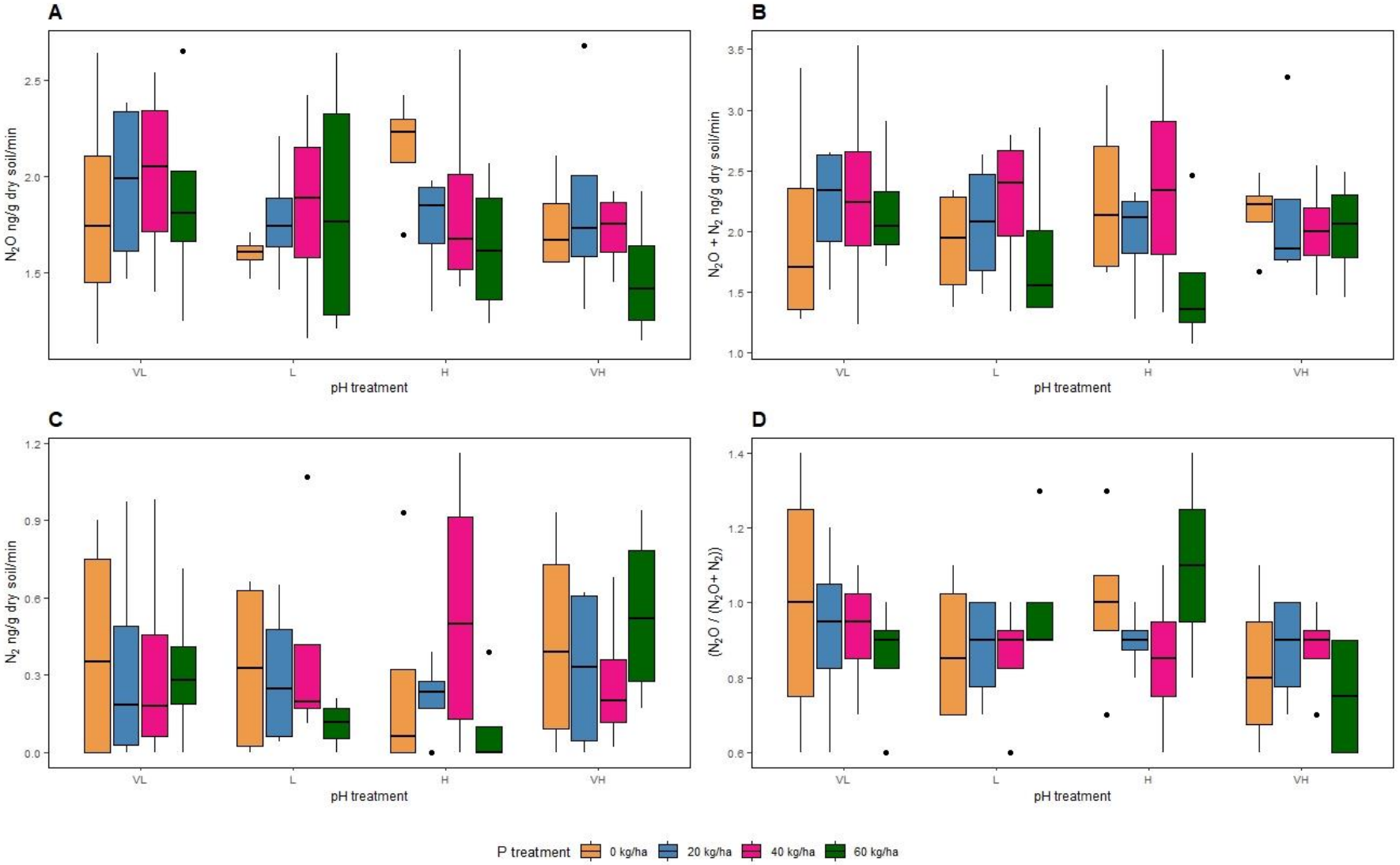
	16S rRNA bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB	COMAMMOX	*ITS* fungi/*16S rRNA* bacteria	*nirK / nirS*	*nosZI / nosZII*	*NOS / NIR*
pH	**0.40****	**-0.68*****	**0.32****	**0.69*****	**0.62*****	**0.47*****	**0.49*****	**-0.45*****	**0.58*****	**0.65*****	0.07	**0.40****	**-0.30***	-0.06
P	0.12	-0.16	**0.38****	0.2	0.11	**0.27***	**0.27***	0.24	0.22	0.17	**0.35****	0.16	-0.25	**0.29***

3.3.2. Potential denitrification fluxes

Potential N_2O fluxes across all treatments ranged between 1.13 to 2.68 g^{-1} dry soil min^{-1}; for total denitrification rate (N_2O + N_2) fluxes ranged between 1.07 to 3.53 g^{-1} dry soil min^{-1}while potential N_2 fluxes were between 0 and 1.16 g^{-1} dry soil min^{-1}. The product ratio (N_2O / (N_2O+N_2) ranged between 0.6 to 1.4. No significant effect of soil pH or P treatments (assessed with ANOVA) was observed on potential denitrification fluxes ($p > 0.05$).

Potential denitrification products were not significantly correlated with soil pH or P (Table 3.3) but significant correlations between them and with other soil properties were observed (in Appendix 5, Table S3.3). Potential product ratio (N_2O/N_2O+N_2) was negatively correlated with TC ($p \leq 0.05$) and SOC ($p \leq 0.05$), while potential N_2 was positively correlated with TC ($p \leq 0.05$) and SOC ($p \leq 0.05$). Potential N_2O flux was negatively correlated with Cu ($p \leq 0.001$). Large variation across all gas measurements can be seen across all treatments but trends of decreasing N_2O are still visible (Figure 3.3A – 3.3D). The denitrification product ratio was also positively correlated with *16S rRNA* bacteria and AOA gene abundances ($p \leq 0.001$), while potential N_2 was negatively correlated with AOA gene copy numbers ($p \leq 0.01$). Potential N_2O and potential total dentification were not correlated with any functional or phylogenetic target genes.

Figure 3.3. Potential gas fluxes grouped by soil pH treatment (VL – Very Low; L – Low; H – High; VH – Very High) in the X axis and P treatments (in colour). **3.2A.** Potential Nitrous Oxide (N_2O); **3.2B.** Potential total denitrification ($N_2O + N_2$); **3.2C.** Potential Dinitrogen (N_2); **3.2D.** Potential product ratio (N_2O / $N_2O + N_2$).



3.3.3. Impact of pH and P on denitrifier abundance

The highest average abundance of denitrifier genes was for *nosZI* (1.66 x 10^4 gene copy number / ng DNA), followed by *nosZII*, *nirS* and *nirK* genes within the same order of magnitude (4.36 x 10^3, 1.26 x 10^3 and 1.22 x 10^3 gene copy number / ng DNA). Soil pH treatment significantly impacted *nir* ($p \leq 0.001$), with VL having lower *nirK* abundances than H and VH treatments (Table 3.4). *nirS* abundances were one order of magnitude significantly lower in VL treatment compared to the rest of treatments (Table 3.4). Soil pH treatments also influenced *nosZ* abundances ($p \leq 0.05$). Abundance of both, *nosZI* and *nosZII*, were significantly lower in VL compared to VH treatment (Table 3.4). Soil P treatment only had a significant effect on *nosZI* gene abundance ($p \leq 0.05$). There was an interaction between pH and P with *nir* genes ($p \leq 0.05$). *nirK* abundances were only impacted by the treatment interaction at P0, with VL treatment having a significantly lower abundance than VH. At P0 and P20 treatments, *nirS* abundances were significantly lower at VL compared to H and VH treatments. At P60, *nirS* abundances were significantly lower at VL compared to H treatment only.

Soil pH was positively correlated with all denitrifier functional genes (Fig. 3.4A – 3.4D). The strength of correlation with pH was similar between *nir* genes, with *nirK* being slightly higher than *nirS* (Spearman's *rho* = 0.69, $p \leq 0.001$; Spearman's *rho* = 0.62, $p \leq 0.001$). Soil pH was correlated with both *nosZI* and *nosZII* genes (Table 3.3). A positive weak correlation was found between *nosZI* and P availability ($p \leq 0.05$). There was a strong negative correlation between *nir* genes and soil Al (in Appendix 5, Table S3.3), which might be a consequence of the link between soil pH and different metals present in soil.

3.3.4. Impact of pH and P on nitrifier abundance

Bacterial *amoA* and COMAMMOX nitrifer genes abundances, were higher, 1.86 x 10^3 and 1.48 x 10^3 gene copy number / ng DNA respectively, than average of archaeal *amoA* gene abundances at 9.07 x 10^2 gene copy number / ng DNA across all plots. There was a significant pH treatment effect on AOA ($p \leq 0.01$), AOB ($p \leq 0.001$) and COMAMMOX ($p \leq 0.001$) abundances (Table 3.4). Archaeal *amoA* abundance was significantly higher in VL treatment compared to H and VH; while the opposite was observed for bacterial *amoA*, with a significant

increase of AOB abundance on VH treatment compared to VL and L. In VL pH treatment, COMAMMOX abundance was significantly lower than in the rest of treatments. No P treatment effect on nitrifier gene abundances was observed.

Soil pH was negatively correlated with AOA nitrifier gene and positively correlated with AOB and COMAMMOX (Fig. 3.4E – 3.4G), while soil P availability was not correlated with any nitrifier abundances (Table 3.3). Soil pH was the physiochemical property most strongly correlated with AOB (Spearman's *rho* = 0.58, $p \leq 0.001$). For COMAMMOX, soil pH and Ca shared the same correlation strength (Spearman's *rho* = 0.65, $p \leq 0.001$).

Soil properties with the strongest correlation with AOA were Fe and Zn (Spearman's *rho* = 0.52, $p \leq 0.001$), these correlations and Ca sharing the same correlation strength with soil pH for COMAMMOX abundances could be a consequence of co-linearity between soil pH and metals.

3.3.5. Impact of pH and P on prokaryotic and fungal abundances

The most abundant genes amplified were bacterial *16S rRNA* genes, with an average of 3.45×10^5 gene copy number/ng DNA across all plots, followed by fungal gene abundances with an average of 1.16×10^4 gene copy number/ng DNA and finally crenarchaea abundances averaging at 6.17×10^3 gene copy number/ng DNA. Soil pH treatment significantly impacted *16S rRNA* crenarchaea ($p \leq 0.001$), with VL treatment showing the highest abundance (Table 3.4). No effect was observed for *16S rRNA* bacteria or *ITS* fungi ($p > 0.05$) copy numbers (Table 3.4). P treatment influenced *ITS* fungi ($p \leq 0.05$) gene abundance, with P0 showing a significantly lower fungal abundance than P60 (Table 3.4). *16S rRNA* crenarchaea gene copy number was also influenced by a soil pH and soil P treatment interaction ($p \leq 0.01$).

Soil pH was positively correlated with *16S rRNA* bacterial and *ITS* fungal abundances, and negatively correlated with *16S rRNA* crenarchaeal abundances (Fig. 3.4H – 3.4J). Fungal abundances showed the weakest correlation with soil pH (Spearman's *rho* = 0.32, $p \leq 0.01$) and was most strongly correlated with P (Spearman's *rho* = 0.52, $p \leq 0.001$). For crenarchaea abundances, its strongest correlation was with soil pH (Spearman's *rho* = -0.68, $p \leq 0.001$). Bacterial abundances were correlated with soil pH (Spearman's *rho* = 0.40, $p \leq 0.01$). In some cases, besides soil pH and P, other soil properties had strong correlation

with gene abundances (in Appendix 5, Table S3.3), this might be due a link between metals and soil pH.

Figure 3.4. Gene copy numbers / ng DNA of all targeted genes. pH treatment represented by colour, and P treatment represented by point shape. R-squared (R^2) value included in graphs when soil pH was significantly correlated with gene abundances. **3.3A.** *nirK*; **3.3B.** *nirS*; **3.3C.** *nosZI*; **3.3D.** *nosZII*; **3.3E.** AOA; **3.3F.** AOB; **3.3G.** COMAMMOX; **3.3H.** *16S rRNA* bacteria; **3.3I.** *16S rRNA* crenarchaea; **3.3J.** *ITS* fungi.

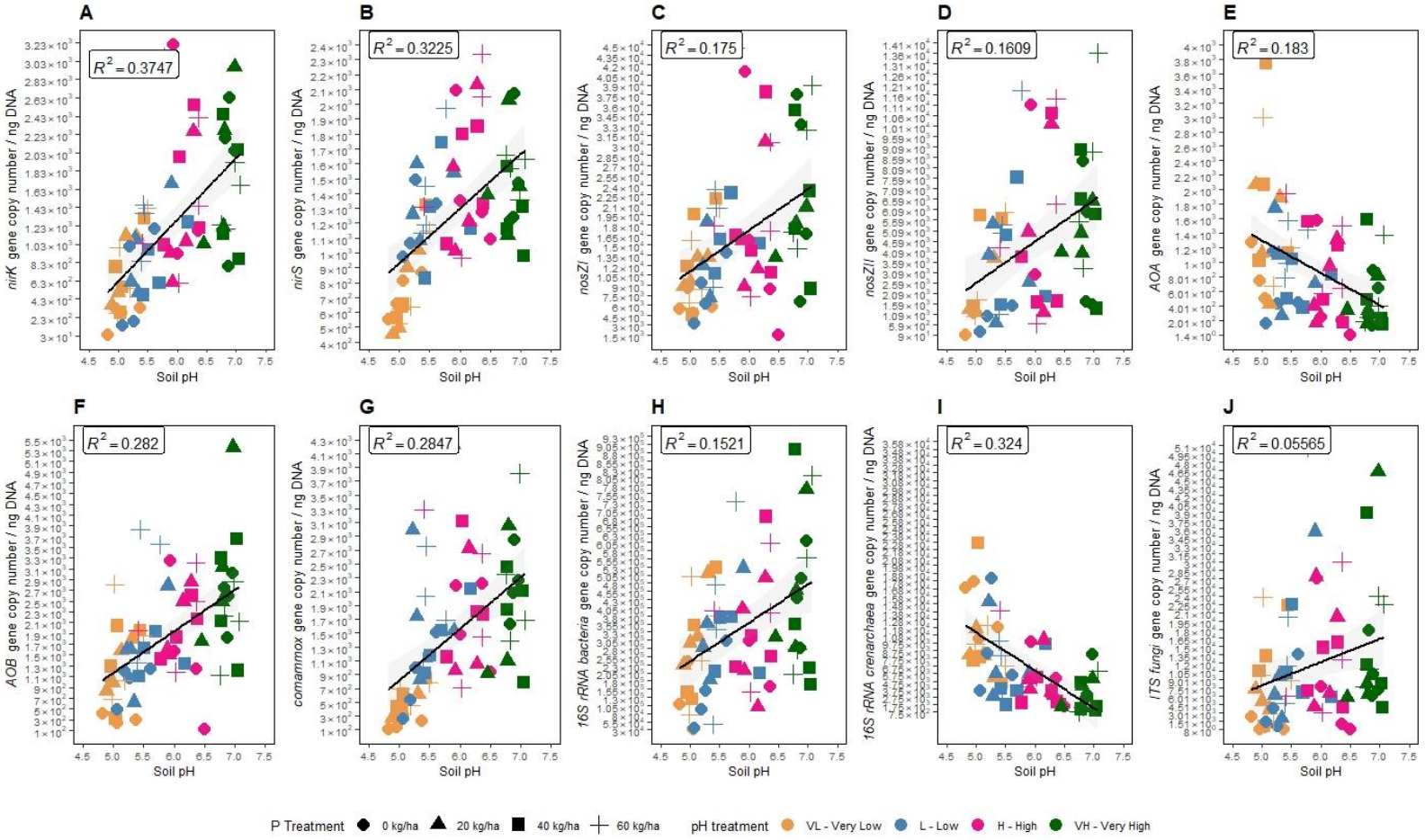


Table 3.4. Average of gene copy number / ng DNA of each target gene. Significance differences of gene abundances across pH treatments indicated by lowercase letters ($p \leq 0.05$). VL – Very Low; L – Low; H – High; VH – Very High.

pH level	*16S rRNA* bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB	COMAMMOX
VL	2.72E+05 [a]	1.22E+04 [a]	7.42E+03 [a]	6.40E+02 [a]	7.41E+02 [a]	1.11E+04 [b]	2.64E+03 [b]	1.46E+03 [b]	1.19E+03 [a]	4.43E+02 [b]
L	2.93E+05 [a]	6.74E+03 [b]	1.28E+04 [a]	1.03E+03 [ab]	1.32E+03 [b]	1.55E+04 [ab]	3.63E+03 [ab]	8.46E+02 [ab]	1.68E+03 [a]	1.48E+03 [a]
H	3.73E+05 [a]	5.04E+03 [bc]	1.17E+04 [a]	1.50E+03 [bc]	1.53E+03 [b]	1.78E+04 [ab]	5.46E+03 [ab]	8.09E+02 [a]	1.97E+03 [ab]	1.98E+03 [a]
VH	4.44E+05 [a]	2.81E+03 [c]	1.47E+04 [a]	1.73E+03 [c]	1.45E+03 [b]	2.19E+04 [a]	5.70E+03 [a]	5.10E+02 [a]	2.60E+03 [b]	2.03E+03 [a]

3.3.6. Gene ratios

Soil pH treatment significantly influenced *nirK*/*nirS* ratio ($p \leq 0.05$). Soil P treatment significantly influenced *ITS* fungi/*16S rRNA* bacteria and NOS/NIR ratios ($p \leq 0.05$). Soil pH was positively and negatively correlated with *nirK*/*nirS* and *nosZI*/*nosZII* ratios respectively (Spearman's *rho* = 0.40, $p \leq 0.01$; Spearman's *rho* = -0.30, $p \leq 0.05$). Soil P availability was the only soil property correlated with *ITS* fungi/*16S rRNA* bacteria (Spearman's *rho* = 0.35, $p \leq 0.01$) and was also correlated with NOS/NIR ratio (Spearman's *rho* = 0.29, $p \leq 0.05$).

Al, Co and Zn shared the strongest correlations with gene ratios (in Appendix 5, Table S3.3) which might be due to the link between soil pH and soil metals.

3.3.7. Impact of pH and P on prokaryotic community structure

A total of 10583 ASVs were obtained across all samples after removing singletons. PCA plot (Fig. 3.5A) for *16S rRNA* bacterial shows that communities largely cluster within their pH treatments, creating a gradient in which VL and VH communities do not overlap. A total variation of 18.68% was explained between PCA1 axis (12.42%) and PCA2 axis (6.26%). Soil pH significantly influenced bacterial microbial community ($p \leq 0.001$) but there was no significant effect of soil P ($p > 0.05$). Microbial communities in each pH level were highly significantly different ($p \leq 0.001$) from all other pH levels, except for L vs H ($p \leq 0.05$). Relevant genera that were significantly enriched in specific pH treatments were identified (in Appendix 6, Table S3.5 – S3.10). On average across all pH treatment pairs 32 genera were enriched, with comparison between VL and VH treatment having a total of 71 genera enriched, with 38 genera present in VH treatment. For example, *Bryobacter* genus has been associated with denitrification (Nelson et al., 2016), and was always significantly enriched in VL treatment. *Geobacter*, a genus that has been associated with both, nitrification and denitrification (Nelson et al., 2016), was significantly enriched in soil pH treatments L and H when compared to VL, but was not significantly enriched between VL and VH treatments. Other examples are the denitrifying genera *Nocardioides, Streptomyces, Polaromonas*; the nitrifying genus *Nitrospira* and the genus *Nitrosospira* (associated to both N pathways), which were significantly enhanced in the VH treatment compared to VL. Environmental factors driving

the microbial community structure in this study include soil pH, K, Co, Mn and *nirS* as shown in RDA (Fig. 3.6A) but not P. Inverse Simpson index and richness of bacterial alpha diversity was not significantly affected by either soil pH or P ($p > 0.05$).

3.3.8. Impact of pH and P on fungal community structure

A total of 4125 ASVs were obtained across all samples after removing singletons. PCA plot (Fig. 3.5B) explained a total variation of 13.17% fungal community structure due to pH treatments between axis 1 (8.13%) and axis 2 (5.04%). There was a significant effect of soil pH treatment on bacterial microbial community ($p \leq 0.001$) but not soil P treatment ($p > 0.05$). Pairwise Adonis test statistically indicated that fungal communities across pH treatments significantly differed, with VL treatment versus H and VH being highly significant ($p \leq 0.001$) as well as comparisons between VL versus L and VH versus H ($p \leq 0.01$) while fungal communities between L and H were not significantly different ($p > 0.05$). On average, 17 genera were enriched across all pH treatment pairs (in Appendix 7, Table S3.11 – S3.15), with a total of 40 genera being enriched in treatment comparison VL versus VH, with 21 genera increasing in VH treatment. The class *Glomeromycete*, which the arbuscular mycorrhizal fungi (AMF) belong to was also observed to be enriched throughout the pH treatments. Inverse Simpson index and richness of fungal alpha diversity was not significantly affected by either pH or P treatment ($p > 0.05$). Environmental factors driving the fungal community structure were soil pH, K, and Mg as shown in RDA (Fig. 3.6B) but not P. Inverse Simpson index and richness of fungal alpha diversity was not significantly affected by either soil pH or P ($p > 0.05$).

Figure 3.5. PCA plots of prokaryotic and fungal community structures CLR-transformed data grouped by pH treatment. Ellipses represent 95 % confidence intervals. **3.4A.** Prokaryotic (bacterial and archaeal) community structure. **3.4B.** Fungal community structure. VL – Very low; L – Low; H – High; VH – Very High.

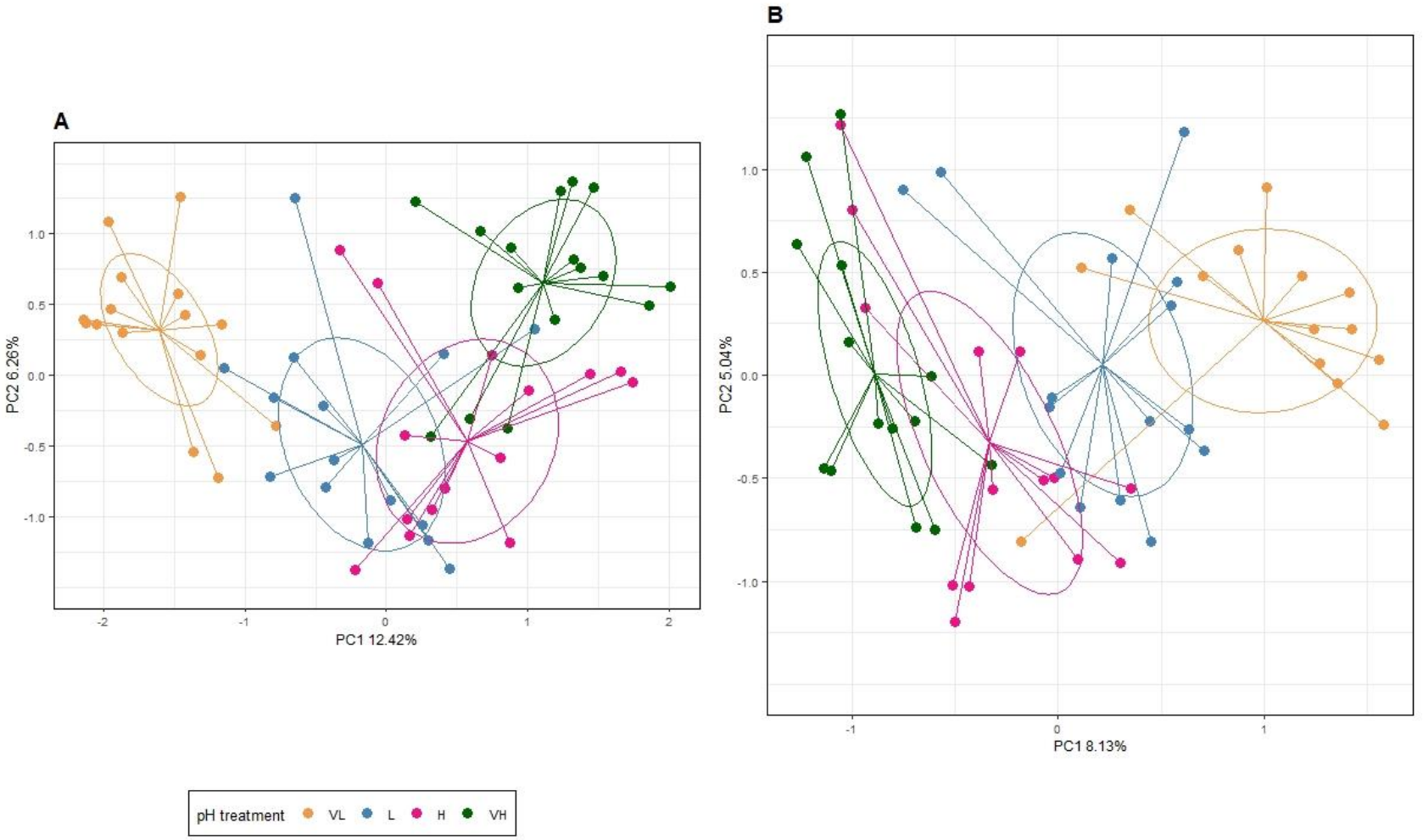
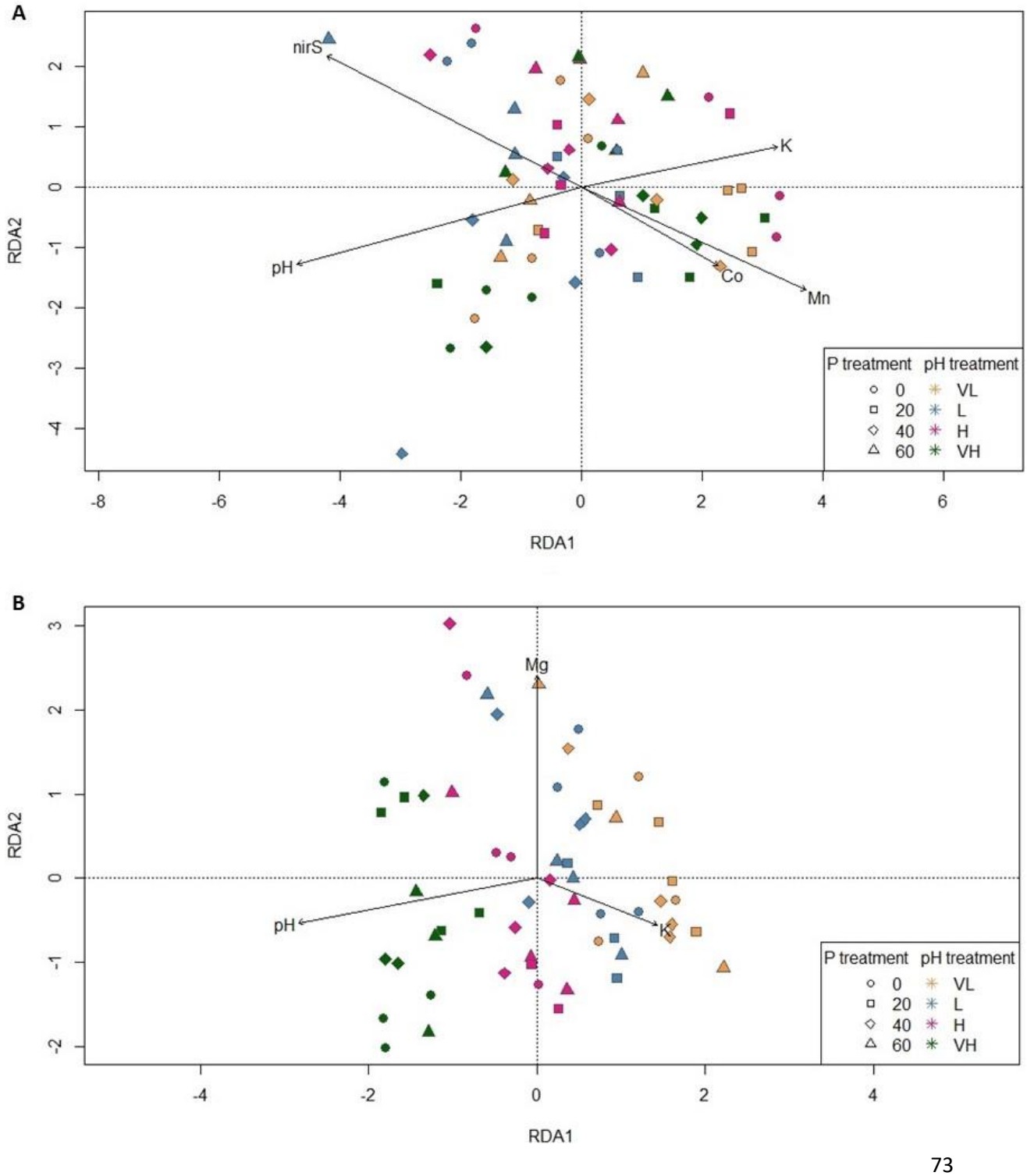


Figure 3.6. Redundancy analysis **(**RDA) plots. Ellipses represent 95 % confidence intervals. Black arrows correspond to significant ($p \leq 0.05$) fitted environmental variables. Symbols correspond to P treatment and colours correspond to soil pH treatment. **3.5A.** Prokaryotic (bacterial and archaeal) structural community. **3.5B.** Fungal structural community. VL – Very low; L – Low; H – High; VH – Very High; 0 – 0 kg/ha P application rate; 20 – 20 kg/ha P application rate; 40 – 40 kg/ha P application rate; 60 – 60 kg/ha P application rate.



Soil P had no effect on PDA fluxes ($p > 0.05$) but a decreasing trend of N_2O with increasing P application can be seen for pH treatment H (Fig. 3.3A). This reduction of N_2O with the highest P application treatment (P60) is also visible for VL and VH treatments, suggesting relief of P limitation might lead to more efficient denitrification in soils, since low nutrient environments limit microbial metabolism and P limitation has been negatively correlated with denitrifier and nitrifier gene abundances (Cui et al., 2020). Studies have reported relief of P shortage by P addition stimulated N_2O (Mehnaz and Dijkstra, 2016) while other have observed a decrease of N_2O emissions (O'Neill et al., 2020). Other soil physiochemical properties were correlated with fluxes, such as Cu with potential N_2O (in Appendix 5, Table S3.3). This negative correlation could suggest Cu, which is contained in nitrite reductase NirK enzyme (Zumft, 1997), would link *nirK* gene to microbial communities prone to complete denitrification (reduction of N_2O to N_2), however co-occurrence of *nosZ* gene, encoding for nitrous oxide reductase enzyme, has been observed much more often with *nirS* when comparing microbial genomes across 18 different phyla which was the case for both *nosZ* clades (Graf et al., 2014). Both, SOC and TC were negatively correlated with potential product ratio and positively correlated with potential N_2 fluxes (in Appendix 5, Table S3.3). While these correlations align with reports of C availability decreasing product ratio and therefore justifying the positive correlation with potential N_2 (Saggar et al., 2013), in PDAs, C is added within the nutrient solution therefore these correlations might not be a real representation of the link between soil C and denitrification rates.

3.4.2. Effect of pH and P on denitrifier functional community

Positive correlations between denitrifier abundances and soil properties pH and P availability have been reported. Quantifying those functional genes across pH and P treatments to confirm previously reported trends and better understand how the response to their environment will influence the production and reduction of N_2O emissions. All four denitrifier genes were positively correlated with pH (Table 3.3), and other soil nutrients including Al, Ca, Co and Mn (in Appendix 5, Table S3.3). These functional genes were also significantly influenced by pH treatment. An increase of denitrifier genes across a soil pH gradient has been previously reported (Herold et al., 2018; Samad et al., 2016b)

and aligns with the hypothesis of the study. These linear correlations between gene abundances and soil pH could suggest a larger complete denitrifier community present in soils with higher pH levels might lead to lower N_2O fluxes. This significant effect emphasises the impact pH has at the molecular level on the community and suggests rising the pH allows for selection of functional communities better adapted to the environment, in this microbes harbouring *nosZ* since the enzyme is functional. Soil pH was not the only factor correlated with denitrifier abundances, *nosZI* was positively correlated with soil P (Table 3.3). The correlation between the soil factor and the denitrifier gene explains the significant effect of P treatment on *nosZI* abundance, with a significant increase ($p \leq 0.05$) between no P application (P0) and highest P application rate (P60). An influence of soil P on abundance of denitrifier genes, including *nosZI*, has been previously observed with positive correlations (Jha et al., 2017) and increase in numbers after P application (Wei et al., 2017). The effect suggests denitrifier microbial community could be hindered by P limitation by lowering its metabolism and so an increase on soil P benefits the functional denitrifier community. Since this effect was not observed on *nosZII* it suggests niche specialisation and differences on sensitivity towards their environment. While *nir* genes were not affected by P treatment or showed a correlation with P availability in soil, a significant interaction ($p \leq 0.05$) of both treatments on both *nir* genes was observed. This is due a significant difference ($p \leq 0.05$) of gene abundances for either *nirK* or *nirS* across pH treatments within a P treatment. For example, *nirK* abundances significantly increase between VL and VH pH treatments within the P0 treatment, while this significant trend is not observed at any other P treatment. Instead, for *nirS* abundances, the significant increase is observed between VL, H and VH pH treatments within P0 and P20 while in P60, *nirS* abundances are significantly different between VL and H pH treatments only. Even though, P treatment did not have an impact on *nir* abundances overall, it appears, specific P application rates link with pH levels and dictate an increase of part of the denitrifier community.

3.4.3. Effect of pH and P on nitrifier functional community

The final product of nitrification feeds into the denitrification process. Quantifying the abundances of this functional community could indicate if pH

and P treatments influence the nitrifer community in a similar manner to denitrifiers. Nitrifier target genes were correlated with soil pH (Table 3.3). Archaeal *amoA* was the only nitrifier gene which was negatively correlated, as previously reported (Nicol et al., 2008) since it is expected for archaeal communities to be present in lower pH environments. However, positive correlations between AOA abundances and soil pH have also been observed, but in the same study AOA communities were present in sites below pH 6 (Baolan et al., 2014). *Ca. Nitrosotalea devanaterra* is an obligately acidophilic AOA, growing at a pH range of 4.0 to 5.5 (Lehtovirta-Morley et al., 2016), and in this study it has been observed to always be enriched in VL treatment when compared to L, H and VH; with the exception of this ammonia oxidiser archaea that appears to be enriched in treatment VH when compared to L (in appendix 6, Table S3.5 – S3.10). Bacterial *amoA* positive correlation aligns with previous results in which the same trend has been observed (Baolan et al., 2014; Scarlett et al., 2021) but lack of correlation between its abundances and pH has also been reported (Nicol et al., 2008; Shi et al., 2018). Since the discovery of COMAMMOX, the only reported correlation between nitrifier abundance and soil pH has been negative in studies with soil pH ranging from 4.37 to 9.48 and 4.47 to 5.38 (J. Hu et al., 2021; Shi et al., 2018), however in this study we observe the opposite trend, with COMAMMOX abundance increasing with increasing soil pH (Fig. 3.4G). This was also seen in chapters 2 and 4 of this thesis. These findings are in line with (Blum et al., 2018), who observed that COMAMMOX prefer slightly more alkaline environments but contradicted the reports of negative correlations with soil pH and COMAMMOX abundances. Different mechanisms can be considered for this nitrifier community to adapt to its environment in relation to soil pH, including ammonia affinity, substrate transporters and/or pH homeostasis (Xu et al., 2020). The increase of soil pH in this case might favour these mechanisms, justifying the increase of the functional nitrifier gene.

P treatment did not have an effect on nitrifier abundances, nor P availability was correlated with them. These lack of effects do not align with reports in the literature which have observed increased AOB abundance after P application (Wei et al., 2017) as well as P limitation negatively correlating with AOA and AOB abundances (Cui et al., 2020).

3.4.4. Effect of pH and P on prokaryotic and fungal communities

Soil pH was correlated with all phylogenetic gene targets. Bacterial and fungal abundances were positively correlated with the soil property while crenarchaea was negatively correlated (Table 3.3). Previous reports suggest that the size of the overall bacterial community will increase within a rising pH gradient (4.0 – 8.3), while no effect was observed on fungal or crenarchaeal abundance (Lehtovirta et al., 2009; Rousk et al., 2010).

While all phylogenetic genes were correlated with pH, crenarchaea was the only target gene significantly influenced by pH treatment ($p \leq 0.001$). The observed drop of crenarchaeal abundance across pH treatments (Table 3.4) appears to align with the decrease in abundances observed in a pH range of 4.7 to 5.2 (Bengtson et al., 2012). This could be linked to the observed negative correlation between soil pH and AOA abundances as well as the linear trends of the quantified genes across the pH gradient (Fig. 3.4E and 3.4I). However, Bengtson, *et al* (2012), also reported an almost 150-fold increase of archaeal abundance from pH 5.2 up to pH 8.0, which they justified by niche specialisation and competition between archaea, bacteria, and fungi. Noting the differences of the experimental site from the referenced study, including the larger pH range (4.0 – 8.0) created by chalk application; soil type and management (no fertiliser application) is important since these factors might lead to different effects and therefore abundances of the targeted gene.

Fungal abundances were positively correlated with soil P (Table 3.3) and this correlation was also translated as a P treatment effect ($p \leq 0.05$) on the fungal community with P0 having significantly lower fungal abundances than P60 (Dunn's test, $p \leq 0.05$). This is contrary to studies where fungi have been reported to be present in low P conditions (Chen, 2012; Randall et al., 2019). This quantification does not differentiate between groups and it might be possible that the fungal community adapted to poor soil conditions is overall smaller than other fungal communities that thrive in highly managed soils (considered in section 3.4.5). Fungal abundances can play a key role in increasing N_2O emissions from soils as they lack *nosZ* genes (Hallin et al., 2018), therefore more studies to confirm the relationship between P and fungal abundances could provide a management tool to limit N_2O emissions from fungal sources. P treatment did not influence crenarchaea abundances, but a significant interaction of pH and P

treatments ($p \leq 0.01$) on them was observed. As described for *nir* genes, within a P treatment, crenarchaea abundances were significantly different across pH levels. For P0, P40 and P60 crenarchaea copy numbers were significantly higher ($p \leq 0.05$) at VL compared to VH treatments. This interaction was not significant at P20 treatment. These interactions emphasise multiple factors can influence the microbial community and the cumulative result of prevailing conditions will determine the selective pressure that drives the emergence of the most abundant organisms.

3.4.5. Effect of pH and P on prokaryotic and fungal community structure

Not only the size of the community will change, but also the presence of different species within the community. The enrichment of specific genera on treatment pairs will determine if specific functional communities are favoured or not, and so dictate the potential of the soil to denitrify.

As mentioned above, soil pH treatment significantly impacted crenarchaeal abundances, with VL having a significantly higher crenarchaeal abundance than the rest of pH treatments (Table 3.4). These differences and the correlations between structural (*16S rRNA* crenarchaea) and functional (AOA) archaeal gene abundances and soil pH can be further explored by looking in which pH treatments archaeal genera are enriched (in Appendix 6, Table S3.5 – S3.10). A total of two nitrifiers genera *Candidatus Nitrocosmicus* and *Candidatus Nitrosotalea* were enriched throughout the pH treatment pairs. *Candidatus Nitrosotalea* related sequences have been reported to be more dominant in soils below pH 6.0 and the percentage of sequence for its cluster was negatively correlated with soil pH (Baolan et al., 2014). In this study, this genus is observed to be enriched in VL treatment when compared to L, H and VH; as well as enriched in treatment VH when compared to L. When compared to *Candidatus Nitrocosmicus*, in its characterisation it was reported this archaeal genus grows in pH 6.0 – 8.5, and the source of the strain was from an agricultural soil at pH 7.5 (Lehtovirta-Morley et al., 2016). In this study, this genus is observed to be enriched in H and VH when compared to VL treatment. Since structural and functional archaeal gene abundances were negatively correlated with soil pH and the highest quantities were detected at VL treatment, the enrichment of *Candidatus Nitrosotalea* in this acidic pH treatment could justify

the larger abundance of quantified target genes since this genus optimum pH for growth is 5.2 (Prosser and Nicol, 2016). The lower abundances of archaeal target genes detected in H and VH treatment might be due to the enrichment of *Candidatus Nitrocosmicus* as this archaeal genus is present in more neutral soils (Lehtovirta-Morley et al., 2016).

A range of bacterial genera can be observed to be enriched throughout all pH treatments (in Appendix 6, Table S3.5 – S3.10). Proteobacteria and Bacteroidetes phyla use NIR and NosZ enzymes (Kuypers et al., 2018). Genera from these phyla are listed and enriched throughout pH treatments. Acidobacteria and Actinobacteria phyla lack *nosZ* in their genomes (Hallin et al., 2018). In this study, *Bryobacter* genus (belonging to Acidobacteria phylum) is only enriched in VL treatment when compared to L, H and VH (in Appendix 6, Table S3.5 – S3.10). It could be speculated organisms lacking *nosZ* in their genome would be favoured in more acidic environments, however this might not be the case as *Acidothermus* genus (belonging to Actinobacteria phylum) while it is also enriched in the same treatments as *Bryobacter*, it is enriched in H pH treatment when compared to L and VH (in Appendix 6, Table S3.5 – S3.10). The bacterial structural community was not influenced by P treatment ($p > 0.05$).

While denitrifying genera appear to be enriched throughout pH treatments, nitrifier genus *Nitrospira* was enriched in L, H and VH treatments compared to VL (in Appendix 6, Table S3.5 – S3.10). *Nitrospira* can perform complete ammonia oxidation (Cabello et al., 2019), its enrichment in pH treatments that are not acidic in this study aligns with the positive correlation between comammox gene abundances and soil pH, as well as the pH treatment effect on these abundances. It has been reported the ammonia monooxygenase (AMO) present in COMAMMOX *Nitrospira* are novel types (Pjevac et al., 2017), therefore the correlation of soil pH with abundances of nitrifier gene AOB in this case cannot be justified by enrichment of nitrifier genera from this study.

Fungal communities were significantly different across all pH treatments except for L and H. Fungi are involved in the production of N_2O since they lack nosZ gene (Hallin et al., 2018). In this study, classes *Sordariomycetes* and *Dothideomycetes* were observed to be enriched throughout all pH treatments (Table S5), these consist of a 46% and 8% respectively of the Ascomycota phylum capable of producing N_2O (Mothapo et al., 2015). The wide enrichment

of fungal genera involved in N_2O emissions throughout pH treatments might explain part of the large variation observed of N_2O potential production of the lab incubations from this study. For example, genus *Trichoderma* was enriched in VH treatment when compared to VL, this genus has been reported to be more abundant than other isolated fungal taxa from agroecosystems and it is known to produce N_2O since it belongs to the *Sordariomycete* class in which this trait is widely spread (Maeda et al., 2015; Mothapo et al., 2015). Another genus enriched throughout pH treatments VL, L and H is *Glomus* (in Appendix 7, Table S3.11 – S3.15) which are part of arbuscular mycorrhizal fungi (AMF) and play a dominant role throughout different grassland conditions (Frac et al., 2018). This genus has been described as P-tolerant due to its large ecological plasticity and being dominant in intensively managed sites (Melo et al., 2014). However not all fungi respond similarly to soil P availability and application. In this study *Claroideoglomeraceae sp.* (belonging to *Glomeromycetes* class) was observed to be enriched in P0 treatment versus P60, justifying a reported decrease of *Glomeromycetes* after N and P additions in soils (Frac et al., 2018). The opposite was observed for species *Helotiaceaea sp.* (belonging to class *Leotiomycetes*) and *Lasiosphaeriaceae sp.* (belonging to class *Sordariomycetes*) which were both enriched in P40 treatment compared to P20 and P0 respectively. This fits with the behaviour of its phylum Ascomycota which has been observed to increase after N and P addition in soil (Frac et al., 2018). The enrichment of *Sordariomycetes* species after P application might be key for a better understanding of the role soil P availability play in influencing N_2O emissions since this fungal class represent 46% of N_2O-producing fungi within the Ascomycota phylum (Mothapo et al., 2015).

3.4.6. Conclusion

Soil pH strongly shaped nitrifier and denitrifier functional communities, with their abundances being correlated with the soil property and pH treatment impacting their numbers present in the soil. The observed effect of P treatment on denitrifier *nosZI* abundance suggest P availability might also shape the denitrifier functional community and therefore the final product rate of the process. The possible role of this nutrient in dictating the microbial community is emphasised by the observed interaction effect of both, pH and P treatment on *nir* denitrifier gene abundances. Not

only the size of the functional communities has been observed to be dictated by their environment in this study but also the overall community structure, with soil pH treatments showing clear separate clustering of each of their communities. We found genera involved in denitrification but lacking *nosZ* in their genome to be enriched in specific pH treatments suggesting management of soil pH could be key in favouring microbial communities capable of carrying out complete denitrification and therefore reduce N_2O emissions. Finally, the lack of effect of pH and/or P treatment on potential denitrification fluxes suggest laboratory incubations might not represent the events at field scale, since in the same site samples from this study were collected, a decrease in N_2O emissions was observed from increasing soil pH through lime application (Zurovec et al., 2021). This study highlights the importance of exploring the complexity of the network formed between microbial communities and their environment to further progress in developing agricultural management techniques that will allow to reduce the production of potent greenhouse gases such as N_2O.

3.5. References

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CHAPTER 4

Soil pH and available phosphorus impact denitrification and nitrification potential and shape N cycling microbial communities

Chapter 4. Soil pH and available phosphorus impact denitrification and nitrification potential and shape N cycling microbial communities

4.1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential 273 times higher than carbon dioxide (CO_2) (Masson-Delmotte, V. et al., 2021) but it is also involved in the damage of the ozone layer (Ravishankara et al., 2009). One of its major sources is agricultural soils, with N_2O emissions being enhanced due to the excessive application of nitrogen (N) synthetic fertiliser and manure (Butterbach-Bahl et al., 2013; Masson-Delmotte, V. et al., 2021). The use of fertilisers impacts microbial processes within the N cycle such as denitrification and nitrification, which are main N_2O production sources (Kool et al., 2011). Another negative outcome of the extensive application of fertilisers is soil acidification (Tian and Niu, 2015), with decreased soil pH being linked to higher N_2O emissions (Qu et al., 2014).

Denitrification is an anaerobic process in which nitrate (NO^-_3) is reduced to nitrite (NO^-_2), nitric oxide (NO), N_2O and dinitrogen (N_2) in a series of stepwise reductions (Saggar et al., 2013). Soils can act as N_2O sinks where the presence of nitrous oxide reductase (NOS) enzyme allows the reduction of N_2O to N_2 (Hallin et al., 2018). The denitrifier community can be quantified with *nirK* and *nirS* genes encoding for nitrite reductase (NIR) enzymes, catalysing the reduction of NO^-_2 to NO (Zumft, 1997); and/or *nosZ* clade I (*nosZI*) and clade II (*nosZII*) for assessing the capacity for complete denitrification (Jones et al., 2013). Nitrification, in contrast, is an aerobic process in which ammonia (NH^+_4) is oxidised to NO^-_2, catalysed by ammonia monooxygenase (*amoA*), which is present in archaea (AOA) and bacteria (AOB) (Hu et al., 2015). NO^-_2 oxidation is then oxidised to nitrification's final product NO^-_3 (Hu et al., 2015). Complete ammonia oxidisers (COMAMMOX) can catalyse both oxidation steps (Kuypers et al., 2018).

The abundances of functional genes involved in N cycling can be influenced by soil properties including pH and P availability. Changes in the composition of these microbial communities may impact the production rates and products of N cycling processes denitrification and nitrification. To reduce the impact of agricultural management on soil pH as well as its N_2O production, lime application has been suggested (Russenes et al., 2016; Šimek and Cooper, 2002).

Increasing soil pH has been reported to reduce N_2O levels from soils as well as being positively correlated with functional microbial communities involved in the production and reduction of this potent greenhouse gas (Baggs et al., 2010; Samad et al., 2016b). Another nutrient affected by agricultural management is phosphorus (P). Like soil pH, this soil factor and its availability has been described to influence N_2O emissions and the abundance of functional microorganisms involved in N cycling processes nitrification and denitrification (Cui et al., 2020; O'Neill et al., 2020). For example, functional denitrifier communities were observed to increase after P application, with a rise of *nirK*, *nirS* and *nosZI* gene abundances (Wei et al., 2017). These genes were also positively correlated with P gradients (Jha et al., 2017) and negatively correlated with P limitation (Cui et al., 2020). Following P application bacterial but not archaeal *amoA* increased in abundance (Wei et al., 2017) and these negatively correlated with (Cui et al., 2020). It appears the relationship, if any, between COMAMMOX and P availability has not been explored yet. In additional to the functional community, the overall microbial community structure has also been reported to change based on P availability in soils. Low P levels are associated with higher arbuscular mycorrhizal fungi (AMF) colonisation (Randall et al., 2019) and dominated by fungi (Chen, 2012). Consequently, higher N_2O emissions from poor-P soils can be expected since fungi lack *nosZ* (Hallin et al., 2018). The influence of P availability is not only visible on the composition of the microbial communities, but their response is also observed on the outcome of their activities. For denitrification products there is conflicting evidence of P effect. Low P levels have been reported to lead to higher N_2O emissions; but cumulative N_2O emissions have been observed to increase after P addition; also no P effect on N_2O emissions from a grassland trial have been reported (Mehnaz and Dijkstra, 2016; O'Neill et al., 2020; Zurovec et al., 2021). Nitrification rates increased after P application in forest soils (Deforest and Otuya, 2020) and total nitrification has been positively correlated with soil P (O'Neill et al., 2021).

Soil P is not the only factor that influences the rates of these microbial processes. The effect of soil pH has been seen in laboratory experiments and field campaigns with significant reductions of N_2O emissions between control and limed plots (Abalos et al., 2020; Samad et al., 2016a; Zurovec et al., 2021). In line with the effect soil pH has on the production of N_2O, studies have reported a

positive correlation between denitrifier functional community abundances and soil pH. These observations include *nirK* and *nirS* abundances (Herold et al., 2018) as well as *nosZI* (Liu et al., 2010) and *nosZII* (Domeignoz-Horta et al., 2015). An increase of denitrifier abundance has also been reported after lime application (Jha et al., 2020). An increase in the *nosZ* denitrifier community might partially explain the reduction of N_2O emissions from soils with adjusted soil pH. Nitrous oxide reductase (NosZ) is sensitive to pH, with acidic pH inhibiting protein assembly or interfering at the post-transcriptional level (Bergaust et al., 2010; Liu et al., 2010). Therefore, adjusting soil pH would ensure the functionality of the NosZ enzyme. Soil pH appears not to influence overall abundances of fungal and archaeal communities in the soil (Lehtovirta et al., 2009; Rousk et al., 2010). However, positive correlation between soil pH and bacterial abundances (Rousk et al., 2010) indicate less acidic conditions benefit and increase the size of the community present in the soil. The rise on the size of the overall microbial community will in consequence influence the abundance of functional communities in the soil.

In the case of nitrifier communities, they have been reported to respond differently to soil pH across studies. Archaeal *amoA* were observed to decrease and increase in separate studies; while bacterial *amoA* were observed to be both positively and to lack correlation with pH in the same studies (Baolan et al., 2014; Nicol et al., 2008). So far, COMAMMOX abundances have been reported to negatively correlated with soil pH (Shi et al., 2018) but there has been limited investigation the relationship of comammox communities with pH to date.

While it is known that low pH will generally result in higher N_2O emissions, the trends between soil pH and microbial abundances involved in N cycling processes vary across studies. This is also true for P availability and how its role on N_2O production and shaping microbial communities is less understood. It is important to note changes in pH will influence P availability. Soil acidification can result in loss of plant-available P (Bowman et al., 2008). The possibility of an interaction between those two factors which controls N_2O emissions and/or N cycling communities instead of a single soil property cannot be ignored. Analysing the capacity of the soils to carry out denitrification and nitrification as well as their functional community in a long-term (almost 80 years)

trial will allow to identify possible trends and patterns from a well-adapted and established microbial community.

In this study, the long-term effect of pH and P treatments, and/or their interaction, on N cycling processes rates and associated microbial communities were analysed. We hypothesised soil pH treatment would impact structure and diversity of prokaryotic and fungal communities, and the abundance of prokaryotic, fungal, and N cycling functional communities based on proximal (causing enzyme inhibition through affection protein synthesis of NosZ) and distal (selecting for functional communities better adapted to specific soil environments) effects on microbial communities. Denitrification potentials were hypothesised to be influenced by soil pH, with higher N_2O emissions expected from acidic low P soils compared to limed soils. A linear increase of nitrification rate across pH treatments was also expected as well as a positive relationship with soil P since availability of this nutrient affects uptake and immobilisation of N and can stimulate nitrification.

4.2. Methods

4.2.1. Soil sampling

Soil samples were collected from experimental site (54˚54′ N, 09˚07′ E) in Denmark, established in 1942, with P treatments starting in 1944. As described in Abalos et al., (2020), this long-term site consists of a coarse sand (*Orthic Haplohumod*) with a total of 16 pH and phosphorus treatment combinations (Table 4.1 and Fig. 4.1). In Denmark, P application is restricted to 30 kg ha^{-1} per year and further restrictions based in European legislation are implemented to allow or stop further P application based on P Olsen levels and soil type (Amery & Schoumans., 2014). There are three biological replicates of each treatment. Since 1985 plots have been sown in April with spring barley (*Hordeum vulgare L.*); Nitrogen fertiliser as Calcium Ammonium Nitrate form (50 kg NO^-_3-N and 50 kg NH^+_4-N ha^{-1}) was applied 2 – 3 weeks after sowing and crop was harvested in August (with straw removal). Soil sampling for this study took place in August 2020 after harvesting. Topsoil was sampled (0 – 10 cm) using a soil corer, following a "W" shape across each plot and this was bulked and homogenised in the field to create composite samples. Subsamples were taken and flash frozen

using liquid nitrogen and stored at –80°C until further analysis. The rest of the composite sample was stored at 4°C until further analysis.

Table 4.1. Treatment summary of experimental site. Soil pH treatments were created by applying indicated rate of dolomite ($CaMg(CO_3)_2$) every 5 – 9 years, leading to the presented average pH level at time of sampling. Super-phosphate rates were first applied in 1944 (Start P) and from 1945 onwards P was applied (Annual P) to create the 4 P treatment levels.

Dolomite ($CaMg(CO_3)_2$) rate		
Treatment	Rate	Average pH
0	0 Mg ha^{-1}	4.17
4	4 Mg ha^{-1}	5.59
8	8 Mg ha^{-1}	6.68
12	12 Mg ha^{-1}	7.24
Super-phosphate rate		
Treatment	Start P	Annual P
I	0 kg ha^{-1}	0 kg ha^{-1}
II	156 kg ha^{-1}	0 kg ha^{-1}
III	0 kg ha^{-1}	15.6 kg ha^{-1}
IV	156 kg ha^{-1}	15.6 kg ha^{-1}

Figure 4.1. Schematic diagram of block design from experimental site. Each pair of columns represents a biological replicate (n = 3). pH treatment, in orange, is indicated by lime application rate (Mg ha^{-1}). Refer to Table 4.1. for P treatment, in green, descriptions and application rates.

pH	P		pH	P
8	I		0	I
8	III		0	III
8	II		0	II
8	IV		0	IV
12	I		4	I
12	III		4	III
12	II		4	II
12	IV		4	IV

pH	P		pH	P
8	I		0	I
8	III		0	III
8	II		0	II
8	IV		0	IV
12	I		4	I
12	III		4	III
12	II		4	II
12	IV		4	IV

pH	P		pH	P
8	I		0	I
8	III		0	III
8	II		0	II
8	IV		0	IV
12	I		4	I
12	III		4	III
12	II		4	II
12	IV		4	IV

4.2.2. Soil physiochemical properties

Water holding capacity (WHC), gravimetric water content (GWC), soil pH and Mehlich II extractions were carried out as described in chapter 3.

4.2.3. Potential nitrification assays (PNAs)

Potential nitrification assays (PNA) enabled measurement of the capacity soils to carry out nitrification under optimal conditions (Drury et al., 2008) and was carried out as follows. Fresh soil was sieved through 4 mm. 15 g of soil was weighed into Erlenmeyer flasks and 100mL of nutrient solution (adjusted to pH 7.2), consisting of 0.2 M potassium monobasic phosphate (KH_2PO_4); 0.2 M potassium dibasic phosphate (K_2HPO_4) and 50 mM ammonium sulphate ($(NH_4)_2SO_4$), was added to create soil slurries. These were placed in a shaker at 20°C to incubate for 24 hours. Flasks were sampled four times (at 2h, 6h, 21h and 24h), with sample solution being filtered through Whatman paper nº 2 and analysed for NH^+_4, NO^-_2 and NO^-_3 in Aquakem 600A Photometric analyser (Thermo Scientific, USA). Potential nitrification rate was calculated based on linear regression of the NO^-_3 concentration (Drury et al., 2008). Final potential nitrification rate result was reported as mg N g^{-1} day^{-1}.

4.2.4. Potential denitrification assays (PDAs)

Potential denitrification was assessed by means of the acetylene (C_2H_2) inhibition method (adapted from Yoshinari et al., 1977), as described in chapter 3.

4.2.5. DNA extraction

DNA extraction was performed as described in chapter 3. DNA yields ranged between 1.05 – 8.62 ng μl^{-1}. DNA purity and quality were assessed with Nanodrop (Thermo Fisher, Ireland), considering both 260/280 and 260/230 ratios and agarose gel of the DNA product.

4.2.6. qPCR

DNA sample preparation, qPCR inhibition and targeted genes were analysed as described in chapter 3 were normalised to 1 ng μl^{-1} with UltraPure™ DEPC-Treated Water (Thermo Fisher, Ireland). Testing of qPCR inhibition was done on all samples as described in chapter 3.

4.2.7. Library preparation

Library preparation was carried out as described in chapter 3.

4.2.8. Statistical analysis

4.2.8.1. Metadata

All statistical analysis including incubations, qPCR and soil physiochemical properties, followed the same steps as described in chapter 3.

4.2.8.2. Sequencing

Data processing and analysis of sequencing data was done as described in chapter 3.

4.3. Results

4.3.1. Soil physiochemical properties

Soil physiochemical analysis indicated average measured soil pH was significantly different ($p \leq 0.001$) across each pH treatment except for the 8 Mg ha^{-1} and 12 Mg ha^{-1} treatments (Table 4.2). Average measured P showed treatments PI and PII were significantly different ($p \leq 0.001$) from PIII and PIV, but not significantly different from each other ($p > 0.05$) (in Appendix 9, Fig. S4.1). Soil pH and P negatively correlated with each other (Table 4.3). Ca, Cu, K, Mg, Mn and SOC positively correlated with pH, while Fe negatively correlated with it (Table 4.3). P positively correlated with Fe, and negatively correlated with Mg (Table 4.3). The negative relationship between P and pH was also observed at the treatment level. pH and P treatment had a significant interaction effect on soil P availability ($p \leq 0.001$). Further statistical analysis (post-hoc test) indicated P availability was significantly higher ($p \leq 0.01$) in the unlimed (0 Mg ha^{-1}) pH treatment compared to the limed treatments in all P treatments (in Appendix 9, Fig. S4.1).

A PCA plot of physiochemical properties shows separate grouping of 0 Mg ha^{-1} and 4 Mg ha^{-1} pH treatments from 8 Mg ha^{-1} and 12 Mg ha^{-1} which overlap (Fig. 4.2). Total of 56% variation was explained between axes PCA 1 (37.5%) and PCA 2 (18.5%). Most soil physiochemical factors appear to be clustering on higher pH treatments except for Fe, P and S which are appearing closer to lower pH levels.

Table 4.2. Averages and standard deviation, within brackets, of soil physiochemical properties for each pH treatment (lime application rate Mg ha^{-1}). Lowercase letters indicate the differences across pH treatments of each soil physiochemical property ($p \leq 0.05$). SOC – Soil organic carbon, TC – Total carbon, TN – Total nitrogen.

pH treatment	pH	Al (mg/ha)	Ca (mg/ha)	Co (mg/ha)	Cu (mg/ha)	Fe (mg/ha)	K (mg/ha)	Mg (mg/ha)
0	4.17(0.12) a	558.49(79.76) a	66.66(15.6) a	0(0.01) a	1.32(0.34) a	517.42(34.76) a	22.89(2.69) a	7.87(1.78) a
4	5.59(0.14) b	810.97(118.95) a	405.75(56.43) b	0.02(0.01) b	1.44(0.15) a	279.28(26.37) b	40.29(4.27) b	17.34(2.03) b
8	6.68(0.13) c	672.23(48.07) a	983.94(121.45) c	0.02(0.01) bc	1.79(0.57) a	213.48(25.3) c	46.2(3.16) c	51.98(5.71) c
12	7.24(0.1) c	558.77(87.65) a	1094.42(134.74) c	0.01(0.01) ac	1.65(0.17) a	203.56(26.34) c	45.81(3.6) c	90.45(7.73) c

pH treatment	Mn (mg/ha)	Na (mg/ha)	P (mg/ha)	S (mg/ha)	TC (%)	TN (%)	SOC (%)
0	4.39(0.72) a	4.77(0.98) a	158.91(34.62) a	13.1(1.94) a	1.27(0.12) a	0.08(0.01) a	2.33(0.13) a
4	8.46(1.45) b	8.47(0.83) a	124.17(33.63) a	12.89(2.13) a	1.21(0.15) a	0.15(0.22) a	2.38(0.17) a
8	12.26(3.33) c	7.58(0.76) a	122.52(34.77) b	11.49(1.19) a	1.29(0.16) a	0.09(0.01) a	2.4(0.2) a
12	12.47(2.58) c	6.43(0.69) a	120.6(32.58) b	11.86(1.12) a	1.29(0.15) a	0.14(0.18) a	2.41(0.16) a

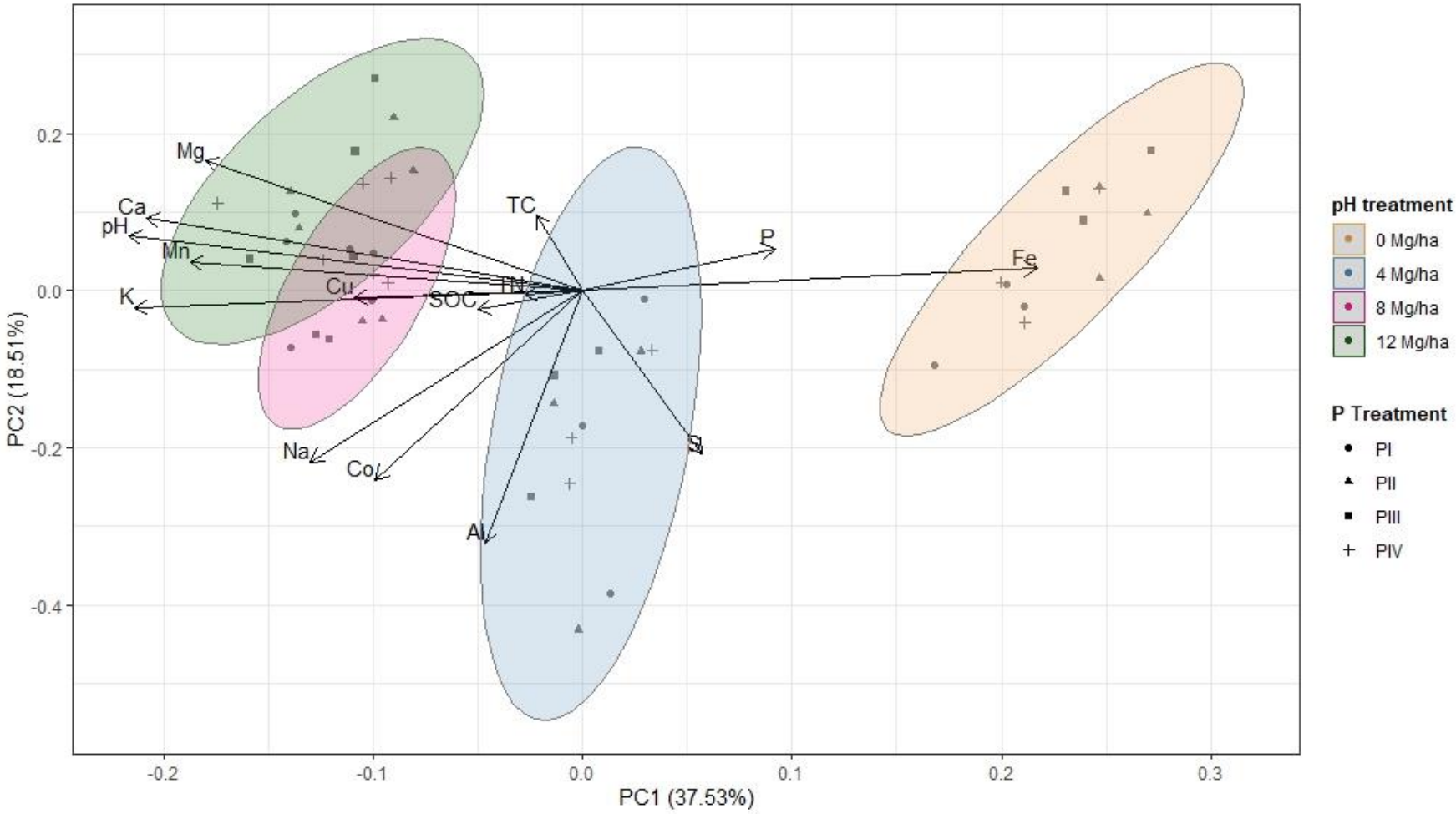
Table 4.3. Spearman's correlation coefficients of soil pH and phosphorus against soil physiochemical properties, PDAs, PNAs, target gene abundances and gene ratios. Significant correlations highlighted in bold (***** $p \leq 0.05$; ****** $p \leq 0.01$; ******* $p \leq 0.001$). PNA – Potential nitrification assay, SOC – Soil organic carbon, TC – Total carbon, TN – Total nitrogen.

	P	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	S	TC	TN	SOC
pH	**-0.29***	-0.03	**0.92*****	0.21	**0.56*****	**-0.87*****	**0.74*****	**0.96*****	**0.84*****	0.24	-0.28	0.09	**0.42****	0.14
P		-0.09	-0.23	-0.12	-0.13	**0.48*****	-0.27	**-0.33***	-0.16	-0.2	-0.07	0.06	-0.23	0.03

	Potential N_2O	Total Denitrification ($N_2O + N_2$)	Potential N_2	Product Ratio (N_2O/N_2O+N_2)	PNA
pH	-0.05	-0.03	0.17	-0.11	**0.85*****
P	0.11	0.05	-0.12	0.15	-0.28

	16S rRNA bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB	COMAMMOX	*ITS* fungi / *16S rRNA* bacteria	*nirK / nirS*	*nosZI / nosZII*	*NOS / NIR*
pH	0.04	**-0.69*****	**-0.65*****	**0.60*****	**0.37****	0.02	**0.66*****	-0.12	**0.30***	**0.61*****	**-0.83*****	**0.32***	**-0.73*****	-0.13
P	0.12	**0.36***	**0.30***	-0.26	-0.18	0.1	-0.18	-0.05	-0.28	-0.02	**0.32***	-0.09	**0.34***	0.26

Figure 4.2. Principal component analysis (PCA) of soil physiochemical properties grouped by pH. Ellipses represent 95% confidence. Arrows indicate how soil properties related to samples, with the length of the arrow being proportional to the strength of the effect. SOC – Soil organic carbon, TC - Total carbon, TN – Total nitrogen.



4.3.2. Potential nitrification rates

There was a significant interaction effect of pH and P treatments on nitrification rates ($p \leq 0.001$). While potential nitrification rates show a linear increase with pH treatment, the interaction effect indicates slight variations of each pH level within a P treatment (Fig. 4.3). Potential nitrification rate was positively correlated with soil pH (Spearman's *rho* = 0.85, $p \leq 0.001$) but similar strength positive correlations of potential nitrification rate with Ca and Mg and negatively correlations with Fe were observed (in Appendix 10, Table S4.3).

Figure 4.3. Potential nitrification rates grouped by P level on the X axis and pH treatments within each P treatment. Uppercase letters show significant difference of nitrification rate at P treatment only ($p \leq 0.05$). Lowercase letters indicate significant difference across potential nitrification rates across pH treatments within a P level ($p \leq 0.05$).

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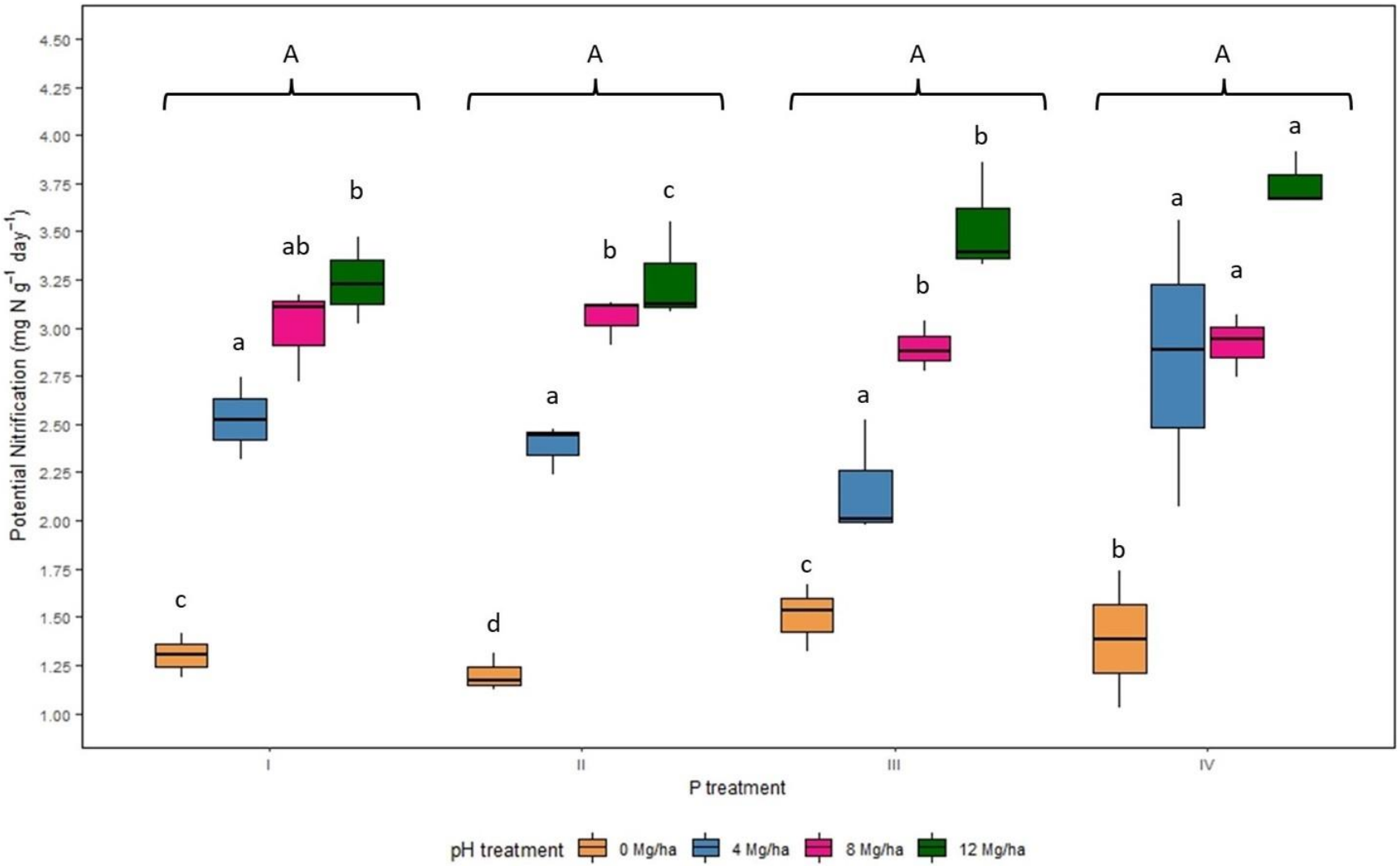
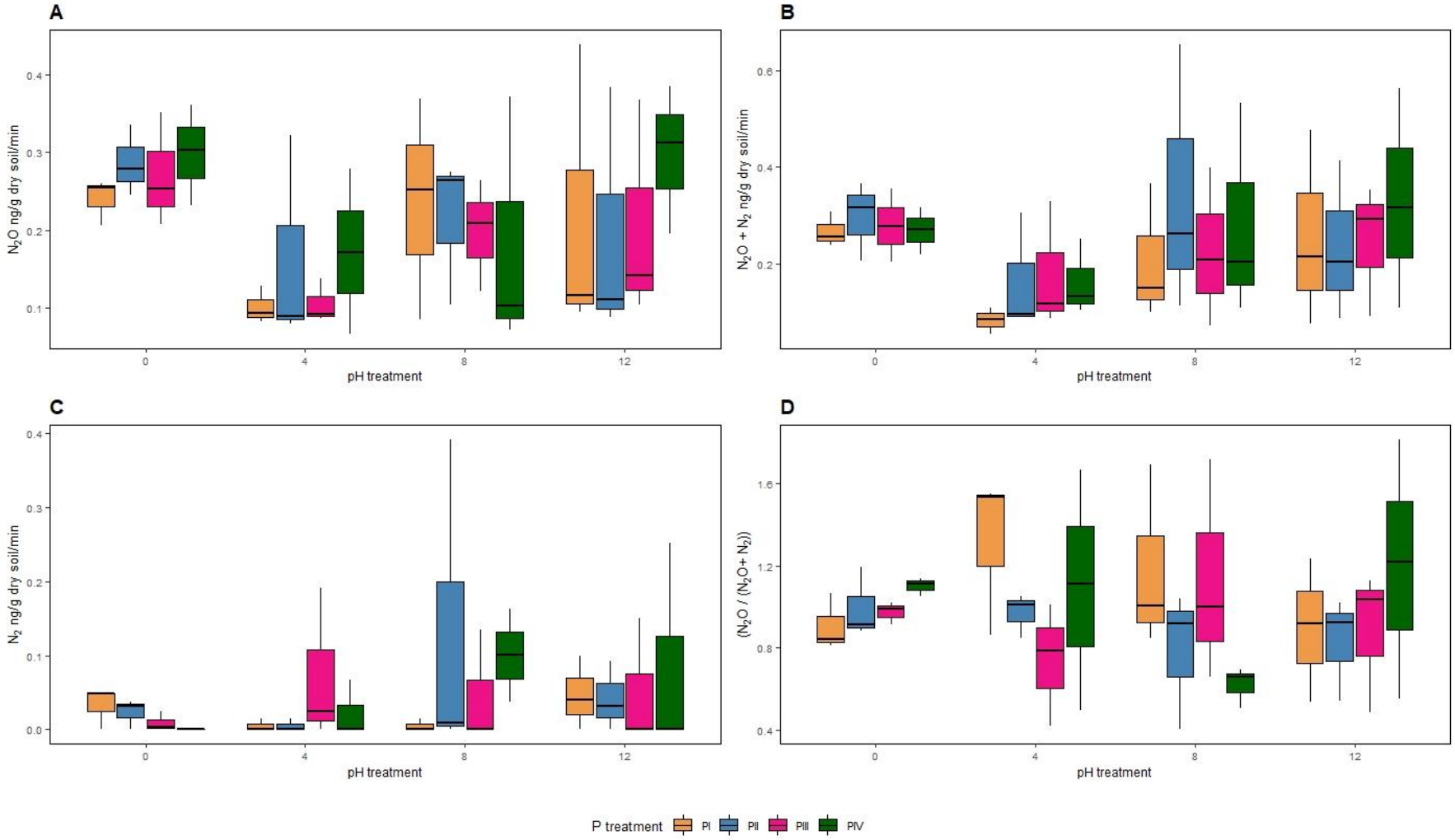


4.3.3. Potential denitrification fluxes

Large variation can be seen on all denitrification potential fluxes (Fig. 4.4), especially within the pH treatment 12 Mg ha^{-1}. Even though flux variation was so large, pH treatment had a significant effect on potential N_2O emissions ($p \leq 0.05$), with pH treatment 0 Mg ha^{-1} being significantly higher than treatment 4 Mg ha^{-1}. The other pH treatments did not appear to be significantly different. None of the other potential fluxes were significantly influenced by either pH, P, or the treatment interaction.

Potential denitrification fluxes did not correlate with soil pH or P (Table 4.3) but were correlated with each other and other soil properties (in Appendix 10, Table S3.4). Potential N_2O and total denitrification rate ($N_2O + N_2$) were negatively correlated with a range of soil physiochemical properties (in appendix 10, Table S3.4), but Na had the strongest correlation (Spearman's *rho* = -0.54, $p \leq 0.001$). Potential N_2 strongest correlation was with TC (Spearman's *rho* = 0.37, $p \leq 0.01$) while product ratio was negatively correlated with it (Spearman's *rho* = -0.35, $p \leq 0.05$).

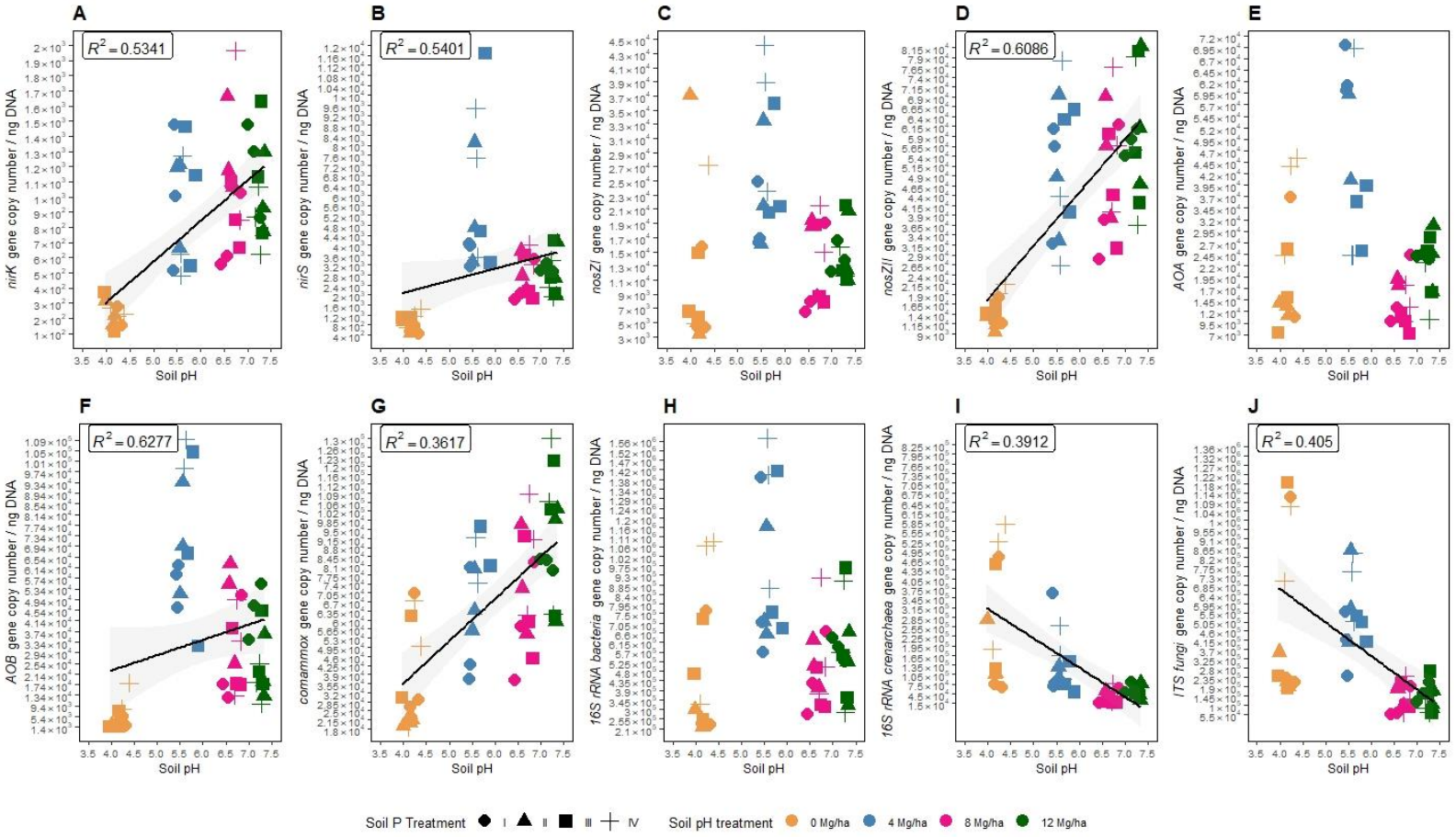
Figure 4.4. Potential denitrification fluxes grouped by pH level on X axis with P treatment (colour) within each pH level. **4.4A.** Potential N_2O fluxes. **4.4B.** Total denitrification (TD) rate ($N_2O + N_2$). **4.4C.** Potential N_2 fluxes. **4.4D.** Potential product ration (N_2O / ($N_2O + N_2$)).



4.3.4. Impact of pH and P on denitrifier abundance

Overall, the most abundant denitrifier gene was *nosZII* (4.38 x 10^4 gene copy number/ng DNA), followed by *nosZI* (1.73 x 10^4 copy number/ng DNA), *nirS* (3.01 x 10^3 copy number/ng DNA) and *nirK* (8.46 x 10^2 copy number/ng DNA). A pH and P treatment interaction effect was observed for *nosZII* ($p \leq 0.05$). Post-hoc statistical analysis indicated *nosZII* abundance from unlimed pH treatment in PII was significantly lower than in pH treatments 8 Mg ha^{-1} and 12 Mg ha^{-1} ($p \leq 0.05$), but this significant difference was not observed between pH treatments 0 Mg ha^{-1} and 4 Mg ha^{-1} (in Appendix 11, Fig. S4.2A). Within PIII, *nosZII* abundances in unlimed pH treatment were significantly lower ($p \leq 0.05$) than in pH treatment 4 Mg ha^{-1} and 12 Mg ha^{-1} but not pH treatment 8 Mg ha^{-1}. For PI and PIV levels, *nosZII* abundances were not significantly different across pH treatments. Also, pH treatment significantly influenced ($p \leq 0.001$) of all denitrifier gene abundances (Table 4.4), but no significant effect of P treatment was observed ($p > 0.05$). pH was positively correlated with all denitrifier genes except for *nosZI* (Fig. 4.5A – 4.5D). Between *nir* genes, *nirK* had the strongest correlation with soil pH. None of the denitrifier genes were correlated with P availability (Table 4.3). Other soil physiochemical properties were correlated with denitrifier abundances (in Appendix 10, Table S4.4), with Mg having the strongest correlation with nirK (Spearman's rho = 0.63, $p \leq 0.001$); and Na with *nirS* (Spearman's rho = 0.76, $p \leq 0.001$). For *nosZI*, Al had the strongest correlation (Spearman's rho = 0.55, $p \leq 0.001$), while soil pH and Mg had the same correlation strength (Spearman's rho = 0.66, $p \leq 0.001$) for *nosZII* abundances.

Figure 4.5. Gene copy numbers/ng DNA for all genes. Trendline indicates significant correlation (Spearman's, $p \leq 0.05$) between soil pH and gene abundances. **4A.** *nirK*; **4B.** *nirS*; **4C.** *nosZI*; **4D.** *nosZII*; **4E.** AOA; **4F.** AOB; **4G.** COMAMMOX; **4H.** *16S rRNA* bacteria; **4I.** *16S rRNA* crenarchaea; **4J.** *ITS* fungi.



4.3.5. Impact of pH and P on nitrifier abundance

All nitrifier gene abundances were within the same order of magnitude, with COMAMMOX having the highest abundance (8.19×10^4 gene copy number/ng DNA), followed by AOB (4.73×10^4 gene copy number/ng DNA) and then AOA (2.83×10^4 gene copy number/ng DNA). There was a pH treatment effect ($p \leq 0.001$) on all nitrifier genes but not a P treatment effect ($p > 0.05$). The treatment interaction effect was not significant for AOA and AOB abundances (p value = 0.07), however further statistical analysis showed these nitrifier abundances differed across pH treatments within a P level. For AOA abundances, the interaction effect occurred at PI, PII and PIII treatments, with AOA abundances from pH treatment 4 Mg ha^{-1} being significantly higher than in pH treatments 0 Mg ha^{-1} and 8 Mg ha^{-1} within PI ($p \leq 0.01$); 0 Mg ha^{-1}, 8 Mg ha^{-1} and 12 Mg ha^{-1} within PII ($p \leq 0.01$) and 8 Mg ha^{-1} within PIII ($p \leq 0.05$), respectively (in Appendix 11, Fig. S4.2B). For AOB abundances, the interaction effect was observed at each P treatment (in Appendix 11, Fig. S4.2C). In all P treatments, AOB abundances from pH treatment 0 Mg ha^{-1} was significantly lower than AOB abundances from pH treatment 4 Mg ha^{-1}. pH treatment 4 Mg ha^{-1} had the highest AOB abundances within each P treatment, showing a significantly higher abundance than pH treatment 12 Mg ha^{-1} within PII and PIV levels. Nitrifier gene abundances (Fig. 4.5E – 4.5G), bacterial *amoA* and COMAMMOX were positively correlated with soil pH, with COMAMMOX having the strongest correlation, but were not correlated with soil P (Table 4.2). Archaeal *amoA* was not correlated with soil pH or P (Table 4.2), or any other soil physiochemical property (in Appendix 10, Table S4.4). AOB abundance strongest correlation was with Na (Spearman's *rho* = 0.75, $p \leq 0.001$) while for COMAMMOX it was soil pH (Spearman's *rho* = 0.61, $p \leq 0.001$).

4.3.6. Impact of pH and P on prokaryotic and fungal abundances

Bacterial, crenarchaeal and fungal abundances were present within the same order of magnitude, with bacteria being the most abundant (8.19×10^5 gene copy number/ng DNA), followed by fungal (5.07×10^5 gene copy number/ng DNA) and crenarchaeal (1.73×10^5 gene copy number/ng DNA) genes. As reported for denitrifier and nitrifier abundances, pH treatment significantly

influenced abundances of all phylogenetic marker genes ($p \leq 0.001$), while there was no significant effect of P treatment effect or interaction ($p > 0.05$).

Crenarchaeal and fungal abundances were negatively correlated with soil pH but positively correlated with soil P. Bacterial abundances were not correlated with either pH or soil P but with other soil properties (in appendix 10, Table S4.4). The strongest correlation of bacterial abundances with soil physiochemical properties was Al (Spearman's *rho* = 0.51, $p \leq 0.001$); for crenarchaeal abundances the strongest correlation was with Fe (Spearman's *rho* = 0.72, $p \leq 0.001$), while fungal abundance strongest correlation was negative and equal between Ca and Mg (Spearman's *rho* = -0.66, $p \leq 0.001$).

4.3.7. Gene ratios

An effect of pH treatment was observed for NIR ratio ($p \leq 0.05$) and fungal *ITS*/*16S rRNA* bacteria marker genes ($p \leq 0.001$). A significant interaction effect of pH and P treatment was reported for *nosZI*/*nosZII* gene ratio ($p \leq 0.01$) and NOS/NIR functional ratio (p value 0.05). Soil pH was negatively correlated with *ITS*/*16S rRNA* bacteria gene ratio and *nosZI*/*nosZII* ratio, while these same ratios were positively correlated with soil P (Table 4.2). Soil pH was the factor with the strongest correlation for both ratios (Table 4.2) along with Mg (Spearman's *rho* = -0.84, $p \leq 0.001$; Spearman's *rho* = -0.78, $p \leq 0.001$). *nirK*/*nirS* ratio was positively correlated with pH but not soil P (Table 4.2). However, its strongest correlation physiochemical property was S (Spearman's *rho* = -0.39, $p \leq 0.01$) (in appendix 11, Table S4.5). The denitrifier functional ratio, NOS/NIR was not correlated with pH or P (Table 4.2), and Na (Spearman's *rho* = -0.73, $p \leq 0.001$) had the strongest correlation with the denitrifier gene ratio (in Appendix 11, Table S4.5).

4.3.8. Impact of pH and P on prokaryotic and fungal community structures

For bacterial structure community, a total of 14991 ASVs were obtained. The bacterial community composition is clearly different across pH treatments, with a PCA plot showing separate grouping of the communities from each treatment (Fig. 4.6A). In total, 43.85 % of variation of the data was explained between axis PCA 1 (27.8%) and axis PCA 2 (16.05%). There was a significant effect of pH treatment on bacterial structural composition ($p \leq 0.001$) but not for P treatment ($p > 0.05$). Composition of bacterial community across pH treatments

was significantly different from each other at all four pH levels. Relevant genera, involved in denitrification and nitrification and significantly enriched across pH treatments were identified (in Appendix 12, Table S4.6 – S4.11). Denitrifier genus *Acidothermus* was observed to be enriched only in pH treatments 0 Mg ha^{-1} and 4 Mg ha^{-1}. Treatment was not the only factor influencing the structural composition of the bacterial community, with soil pH, Al as well as potential N_2O fluxes and total denitrification rate (TD) driving the microbial community (Fig. 4.7A).

The total ASVs count for fungal structural community was 3218 ASVs. As reported for bacterial community structure, pH treatment had a significant effect on the composition of the community ($p \leq 0.001$), as seen by the separate fungal community grouping of each pH treatment (Fig. 4.6B). The total variation across pH treatments was 34.7% across axis PCA 1 (25.79%) and PCA 2 (8.91%). Structure of fungal community was significantly different at each pH treatment (in Appendix 13, Table S4.12 – S4.17). For fungal communities, not only pH treatment but Al, soil pH as well as soil P drove the community structure (Fig. 4.7B)

Figure 4.6. PCA plots of prokaryotic and fungal community structures CLR-transformed data grouped by pH treatment. Ellipses represent 95 % confidence intervals. **4.6A.** Prokaryotic (bacterial and archaeal) community structure. **4.6B.** Fungal community structure.

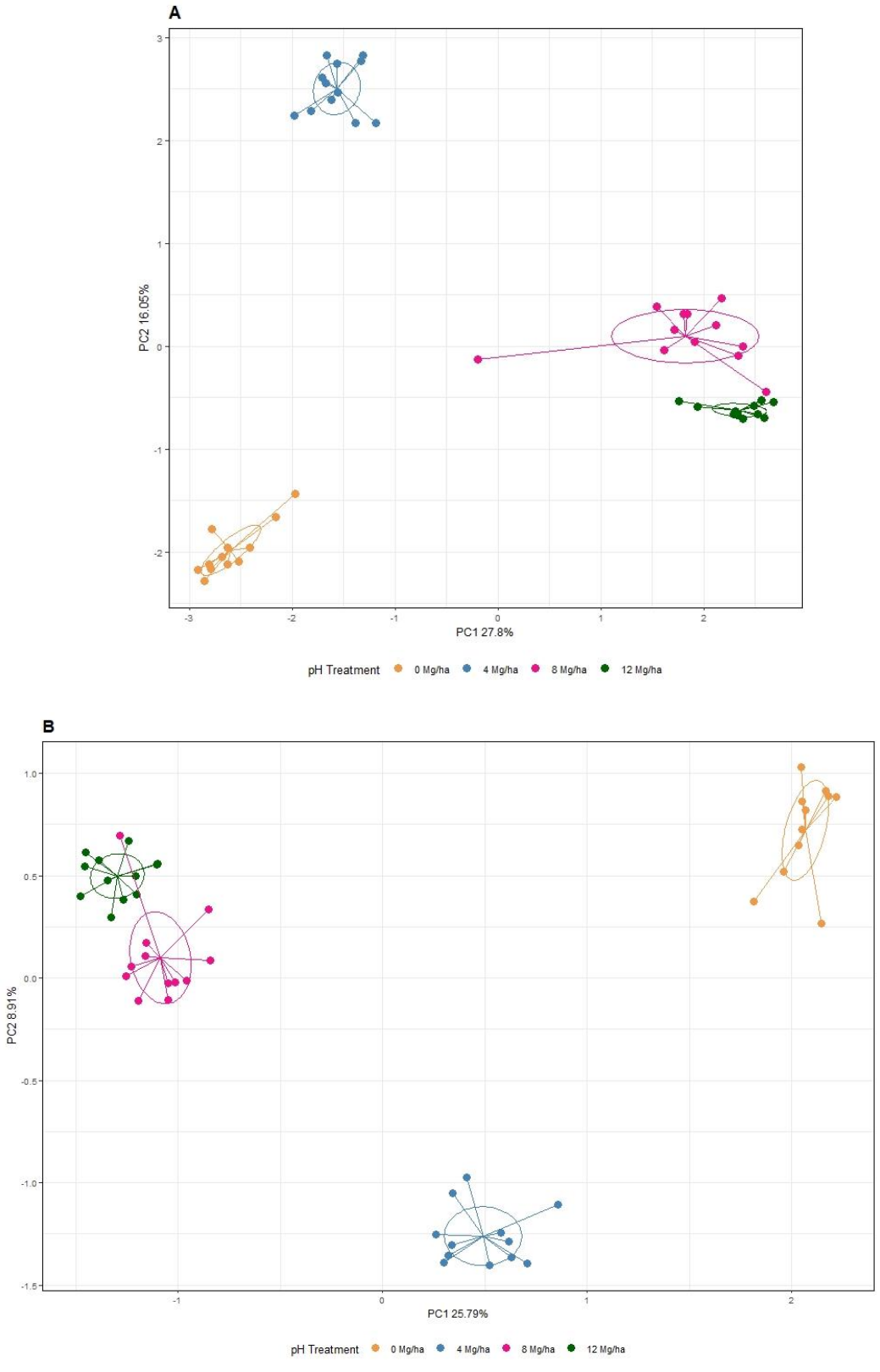
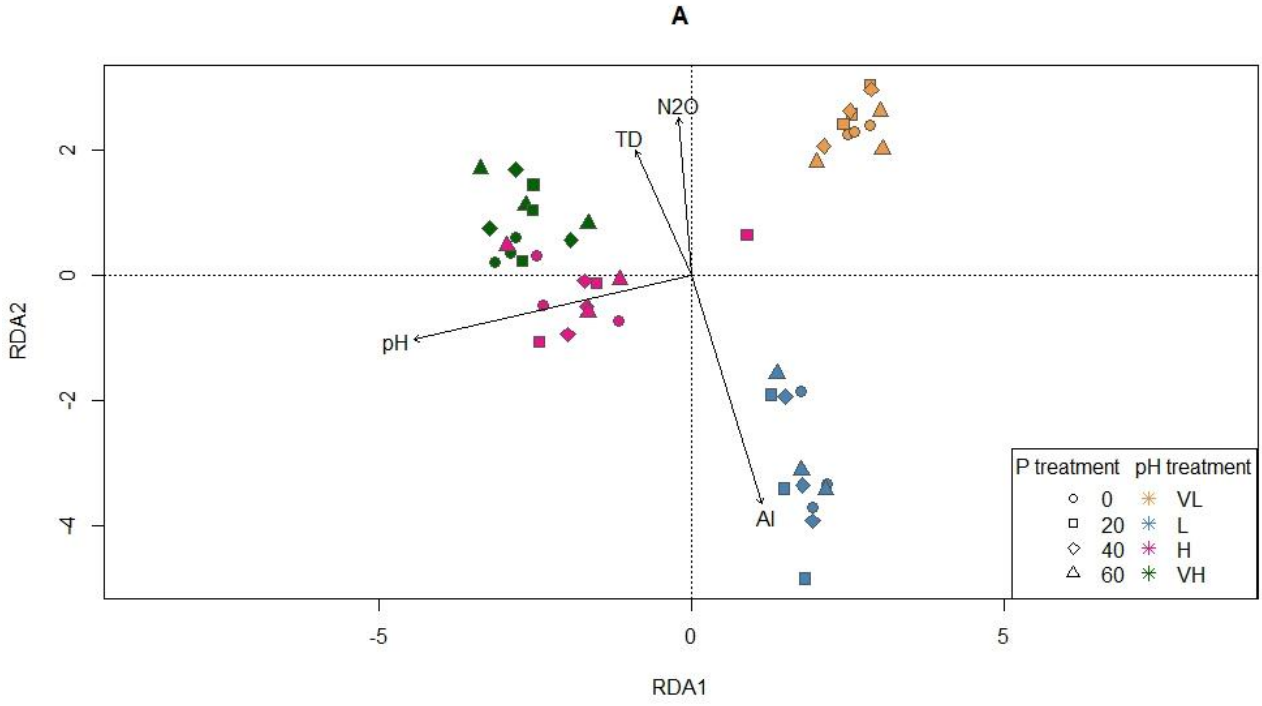
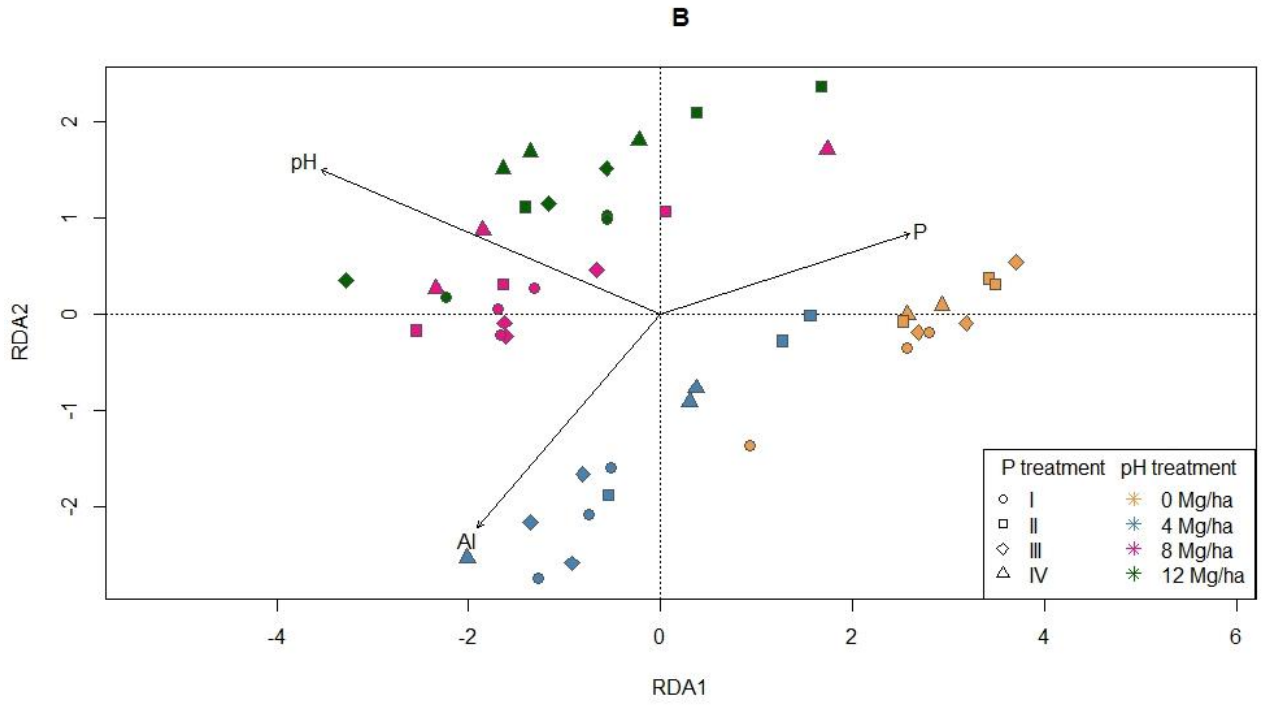


Figure 4.7. Redundancy analysis **(**RDA) plots. Ellipses represent 95 % confidence intervals. Black arrows correspond to significant ($p \leq 0.05$) fitted environmental variables. Symbols correspond to P treatment and colours correspond to soil pH treatment. **4.6A.** Prokaryotic (bacterial and archaeal) structural community. **4.6B.** Fungal structural community.



long-term P application, including if increased P application induced denitrifier



As for denitrification potentials, it was expected for nitrification rate to be influenced by soil pH, while little is understood about P and if it has a role on the production levels of this N cycling process. Interestingly, a significant interaction effect of both treatments on PNA rates was observed in this study. Within each P treatment there is a clear increase of PNA rate across pH treatments (Fig. 4.3). Potential nitrification rate has been reported to increase with increasing pH (Nadeem et al., 2020) which is also true for the results in this study. P has been positively correlated with total nitrification (O'Neill et al., 2021), which was not the case in this study, however the P treatment effect indicates the availability of the nutrient influences N transformations and induces nitrification activity. The interaction of both soil factors and their impact on nitrification have been reported in forest soils with increasing P stimulating nitrification but that same addition reduced the impact of pH management on nitrification (Deforest and Otuya, 2020). This could also be true for the results reported here, since at PII level PNA rates from each pH treatment are significantly different from each other, and instead at PIV, the only PNA rates significantly different from each other are from 0 Mg ha^{-1} and 12 Mg ha^{-1} pH treatments (Fig. 4.3).

4.4.2. Effect of pH and P on denitrifier functional community

To better understand how soil properties such as soil pH and P influence the production and reduction of N_2O emissions, it is important to assess the N cycling communities carrying out these processes and analyse how they are impacted by changes in their environment, especially by those same soil factors.

None of the denitrifier functional genes were correlated with soil P (Table 4.3) but all were positively correlated with soil pH, except for *nosZI* (Fig. 4.5A – 4.5D). All denitrifier gene abundances were significantly influenced by the pH treatments. The positive relationship between pH and denitrifier functional genes has been previously reported (Herold et al., 2018; Samad et al., 2016b). Overall, *nirS* abundances were an order of magnitude (10^4 gene copy number/ng DNA) higher than *nirK* (10^3 gene copy number/ng DNA), aligning with previous observations (Avşar and Aras, 2020; Graf et al., 2014; Krause et al., 2017). However this was not true for chapter 2 and other studies in which *nirK* abundance was dominant over *nirS* (Castellano-Hinojosa et al., 2018; Jones et al., 2014b). The consistent differences of *nir* dominance within functional

communities across studies suggest the possibility environmental sensibility and of niche differentiation between *nirK* and *nirS* (Enwall et al., 2010; Graf, 2015). Both clades of *nosZ* genes were within the same abundance range (10^4 gene copy number/ng DNA). The lack of correlation between soil pH and *nosZI* might be due to the large variability of gene copy numbers across acidic soils in this study. Also, from both *nosZ* clades, *nosZII* has been reported to be more sensitive to its environment (Domeignoz-Horta et al., 2015), while this could justify the lack of correlation between pH and denitrifier *nosZI* it is difficult to ignore the observed correlations between soil pH correlations and *nosZI* abundances in chapters 2 and 3 within this thesis, as well as in other studies (Domeignoz-Horta et al., 2018; Liu et al., 2010). Niche specialisation has also been suggested for *nosZ* denitrifier genes (Domeignoz-Horta et al., 2015). The results of this study might suggest this is the case since *nosZI* abundances appear to peak at pH treatment 4 Mg ha^{-1} followed by a decrease of its abundances on 8 Mg ha^{-1} and 12 Mg ha^{-1} pH treatments (Fig. 4.5C), while *nosZII* abundances appear to benefit from lime application with a clear increase of gene copy numbers across the pH gradient (Fig. 4.5D). The positive correlation between soil pH and denitrifier functional genes suggests increasing soil pH might be beneficial for decreasing N_2O emissions, as a reduction of potential N_2O was also observed in this study from treatments with higher lime rates (Fig. 4.4A), which is also in line with field studies (Zurovec et al., 2021) including the same experimental site (Abalos et al., 2020). This result also supports the distal role soil pH plays in shaping the overall composition of microbial communities. It could be assumed denitrifier functional communities harbouring *nosZ* are selected in more neutral pH environments since folding of the enzyme is not impaired by pH acidity and therefore N2O is reduced to denitrification final product N_2. The unexpected high N_2O flux at 12 Mg ha^{-1} pH treatment within PIV, does not align with the high abundance of *nosZII* making it difficult to draw any conclusions on why N_2O fluxes peaked at that treatment and the role the N cycling functional community have in producing and reducing this greenhouse gas.

4.4.3. Effect of pH and P on nitrifier functional community

The interaction effect pH and P had on nitrification rates reported in this study suggests the functional community behind the N cycling process is also

impacted by both these soil properties. Assessing the impact pH and P had on the abundances of nitrifier functional genes provides insight into how PNA rates are influenced by these soil properties. Since soil P availability can influence N transformations and induce nitrification, different P treatments might lead to changes in the microbial community, selecting for organisms that benefit from increased P availability.

Bacterial *amoA* and COMAMMOX were positively correlated with soil pH (Table 4.3) but not with soil P. Archaeal *amoA* was not correlated with either soil property. All nitrifier functional abundances were significantly influenced by pH treatment ($p \leq 0.001$) but not by P treatment. The effect of pH treatment on AOA abundances appears to imply 4 Mg ha^{-1} treatment is optimal for archaeal *amoA* as abundances peak in this treatment (Fig. 4.5E). This peak is also observed for AOB (Fig. 4.5F), with lower abundances in 8 Mg ha^{-1} and 12 Mg ha^{-1} pH treatments. A positive correlation between soil pH and AOB abundances has been previously reported (Baolan et al., 2014), but appears to be really weak in this study. A strong positive correlation between pH and COMMAMOX abundances (Fig. 4.5G) as well as a pH treatment effect have been observed in this study. Previously, COMAMMOX abundances have been negatively correlated with soil pH (J. Hu et al., 2021; Shi et al., 2018). However more alkaline soils appear to favour COMAMMOX (Blum et al., 2018); positive correlations between soil pH and its abundances have been observed in chapters 2 and 3, including a significant pH treatment effect in COMAMMOX abundances in chapter 3.

4.4.4. Effect of pH and P on prokaryotic and fungal communities

N cycling processes are modular, and not all microorganisms harbour each of the genes encoding the required enzymes to catalyse each reaction within the process. Therefore, the abundance of prokaryotic and fungal microorganisms will influence the quantity of functional nitrifier and denitrifier communities. The environment that microbial communities are present in will directly dictate their abundance and size.

For phylogenetic gene abundances, crenarchaea and fungal gene markers were negatively correlated with soil pH but positively correlated with soil P (Table 4.3). The positive correlation with soil P might be explained by the

capability of increased P availability to induce nitrification (Mehnaz et al., 2019b) since crenarchaea harbour *amoA* nitrifier functional gene. Bacterial gene abundances did not show any significant correlations with either soil factor. pH treatment significantly influenced ($p \leq 0.001$) all three phylogenetic gene abundances (Table 4.4) but there was no P treatment effect ($p > 0.05$). The effect of pH on crenarchaea gene abundances is in contrast to findings reported elsewhere in which no effect of pH was observed (Lehtovirta et al., 2009). pH treatment effect on fungal abundances (Table 4.4), with gene abundances decreasing along the pH gradient (Fig. 4.5J) has been reported in previous studies where fungal abundances were higher in acidic soils (Behnke et al., 2020; Chen et al., 2014). This could also justify higher N_2O emissions from acidic soils since fungal communities lack denitrifier functional *nosZ* (Hallin et al., 2018). The positive correlation of fungal abundance and soil P is opposite to previous reports were a decrease of fungi has been observed in soils treated with P (Chen, 2012; Randall et al., 2019). This same trend was observed at the quantification of fungal abundances in chapter 3. There was no P treatment effect on the abundances of the fungal marker gene.

4.4.5. Effect of pH and P on prokaryotic and fungal community structures

Not only the abundance of the different phylogenetic gene markers and functional genes will be shaped by soil pH and P but also the structure and diversity of the prokaryotic and fungal communities. Both were significantly influenced by soil pH ($p \leq 0.001$) with each pH treatment having a different structure than the other. pH has previously been reported to correlate to microbial populations (Samad et al., 2016b) and this effect was also reported in chapter 3 of this thesis. Two nitrifier archaeal genera *Candidatus Nitrocosmicus* and *Candidatus Nitrosotalea* were enriched throughout pH treatment pairs (in Appendix 12, Table S4.6 – S4.11). If both archaeal genera were within a pH treatment comparison, *Candidatus Nitrosotalea* was enriched in the lower pH treatment compared *Candidatus Nitrocosmicus*. These genera were also observed in chapter 3, and the same enrichment patters occur, with *Candidatus Nitrocosmicus* being enriched in higher pH treatments and *Candidatus Nitrosotalea* dominating lower pH treatments. In previous reports, pH growth range of *Candidatus Nitrocosmicus* was between 6.0 – 8.5 (Lehtovirta-Morley et

al., 2016) while *Candidatus Nitrosotalea* has been detected in soils with pH lower than 6.0 (Baolan et al., 2014), justifying the opposite enrichment trends between them.

A great variety of bacterial genera, including denitrifiers and nitrifiers are enriched throughout all pH treatments (in Appendix 12, Table S4.6 – S4.11). Denitrifiers such as *Streptomyces and Acidothermus* belong to Actinobacteria phylum and *Bryobacter* belongs to Acidobacteria phylum, all of which lack *nosZ* in their genomes (Hallin et al., 2018). Nitrifier genus *Nitrospira* is enriched throughout all pH treatments, this genus harbours COMAMMOX (Cabello et al., 2019). The enrichment of this genus across all pH treatments aligns with the quantified abundances of the nitrifier functional gene.

The effect of pH treatment on the structure of fungal communities can be seen at each pH level. Arbuscular mycorrhizal fungi (AMF) within class *Glomeromycetes* are enriched only in 8 Mg ha^{-1} and 12 Mg ha^{-1} pH treatments (in Appendix 13, Table S4.12 – S4.17). The influence of pH treatment on the fungal community structure emphasises the need to better understand the interactions between soil pH and P availability since the main function of AMF is to increase root P uptake (Smith and Read, 2010). Other fungal communities affected by pH treatment are those within *Sordariomycetes* class. Fungi within that class that belong to the *Hypocreales* order, are involved in the production of N_2O (Maeda et al., 2015). In this study, from the *Sordariomycetes* class it can be seen *Trichoderma* is enhanced in 0 Mg ha^{-1} pH treatment when compared to 12 Mg ha^{-1} pH treatment, which could justify arguments of N_2O production from acidic soils being enhanced by larger fungal communities, while *Dactylonectria* is mostly enhanced in pH treatments where limed treatments (in Appendix 13, Table S4.12 – S4.17).

4.4.6. Conclusion

This study shows long-term pH and P treatment application significantly impacts the composition and structure of the microbial communities present in the soil, including their function on N cycling processes denitrification and nitrification. PNAs rates indicated this process is significantly affected by an interaction of pH and P treatments, which is also reflected on the AOA and AOB functional abundances. The impact of pH treatment was also observed on potential

N_2O fluxes and abundances of denitrifier genes. Interestingly, *nosZII* abundances were impacted by a treatment interaction effect, while none of the potential fluxes were affected by this interaction. Finally, the prokaryotic and fungal abundances as well structural composition were strongly shaped by pH treatment but not P, further dictating the size and presence of communities capable of producing and reducing N_2O emissions. These results provide insight into the role soil pH and P have in relation to N cycling processes and microbial communities, with the necessity to further explore the mechanisms between both soil factors, microbial communities and greenhouse emissions for better agricultural managements and reduce its impact on the environment.

4.5. References

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CHAPTER 5
Final discussion

Chapter 5. Final discussion

5.1. Discussion

Since N_2O has a global warming potential around 300 times higher than CO_2 (Masson-Delmotte, V. et al., 2021), and soils are one of its main sources (Butterbach-Bahl et al., 2013), the role agriculture, and its management, has on the emissions of this gas are of great importance in efforts to reduce the impact of N_2O on the environment and climate change. An understanding of how microbial communities involved in N-transformations produce and reduce N_2O is established in literature. However, how these microbial communities respond to environmental changes that affect the processes within the N cycle is much more complex than a single soil property dictating the overall production rate of N_2O emissions, or the composition and abundance of functional microbial communities carrying out these activities. It is known that soil pH impacts N cycling communities and their activities but many gaps in knowledge remain. This thesis aimed to assess if the relationship between pH and functional N cycling microbial communities is maintained across geoclimatic regions and across soil types. It also aimed to assess the effect of pH management on potential N_2O fluxes and communities from medium- and long-term trials, and to better understand the impact of soil pH on recently discovered nitrifier COMAMMOX. Additionally, it aimed to explore the role of P availability on the production and reduction of N_2O from agricultural soils, by studying the effect and impact long-term P treatments on microbial community composition and functionality, and how this was associated with N cycling processes potentials of denitrification and nitrification. These experimental trials utilised in this thesis enabled assessment of the interaction of soil pH and P on these same N cycling microbial communities and their potential activity rates.

In chapter 2, functional and phylogenetic gene abundance were quantified using qPCR across 9 soil types, a gradient of geoclimatic regions and a wide soil pH range. While throughout literature it has been reported pH was positively correlated with the abundance of denitrifier and nitrifier communities (Baolan et al., 2014; Domeignoz-Horta et al., 2015; Herold et al., 2018; Liu et al., 2010), each study has its unique experimental set-up, including pH treatment (type and rate of lime applied), soil type, longevity of the experimental trial and/or small pH ranges, among others. The study aimed to compare the abundance of phylogenetic and functional N cycling genes across soil types to test if soil pH had the same effect on these communities throughout all experimental sites. It also

provided insight on how different soil pH managements affected the abundances of these target genes and if the same outcome was occurring throughout.

Site, which incorporates soil type that will vary based on the geoclimatic region of the sample, had the strongest impact on all phylogenetic and functional gene abundances. Soil pH was observed to positively correlate with all denitrifier and nitrifier gene abundances as well the abundance of prokaryotes when these were analysed throughout the complete pH range. Fungal and crenarchaeal phylogenetic gene abundances did not correlated with soil pH. However, correlations between pH and all N cycling communities were not observed in each individual site, only occurring in DK, IE and NO sites. The communities that were positively correlated with pH also differed between sites. In the case of NO site a negative correlation was observed between soil pH and AOA abundance, which contrasts with the findings of the overall dataset. The differing correlations when comparing gene abundances overall to individual sites suggest while it is true increasing soil pH is beneficial for functional and phylogenetic microbial abundances, differences in edaphic factors changing the soil environments due to soil type and geoclimatic location dictate the abundance of these communities, and therefore a rule of thumb in which rising soil pH will increase abundance of N cycling functional genes, and therefore one type of agricultural management, will fit all cannot be assumed.

While the study from chapter 2 provides insight on the relationship between soil pH and abundance of prokaryotic, fungal, and functional genes, the effect of pH treatment differed within sites. Treatments from DK and NO sites had the most impact on targeted gene abundances. Treatments from IE and SE 2 only impacted on AOB and crenarchaea gene abundances respectively. Since lime form and application rate differed across sites, it cannot be determined if the treatment effect might be caused by the change in pH. This emphasises the importance to understand what mechanisms lay behind soil pH shaping the microbial community and what lime products and application rates will be more favourable for the reduction of N_2O emissions from soils.

Experiments from chapter 3 and chapter 4 were done on samples from two of the sites included in chapter 2. These experimental sites were chosen due to their medium- and long-term, around 10 and 80 years respectively, set-up. Besides their longevity, these sites differed in soil type, allowing to assess the interaction effect of pH and P interaction

and the effects of long-term management on N cycling processes denitrification and nitrification as well as the impact on the microbial communities involved. Chapter 3 study was carried out on grassland samples from IE site and chapter 4 study was carried out on arable samples from DK site, allowing to test these relationships under contrasting conditions.

Separate sampling events occurred for each study with soils used in chapter 2 being collected in winter of 2017; soils from chapter 3 (IE) being collected in June 2019 and soils from chapter 4 (DK) being collected in August 2020. Both sites not had four pH treatments but also four P treatments that had not been assessed in the chapter 2 study. In chapters 3 and 4 qPCR was used to quantify phylogenetic and N cycling functional communities, as well as sequencing to analyse prokaryotic (bacterial and archaeal) and fungal structural community to explore the impact of those treatment combinations on microbial communities involved in N_2O production and reduction. Additionally, potential denitrification assays (PDAs) were used in both chapters to assess the capacity of each soil to perform denitrification under optimal conditions. In chapter 4, potential nitrification assays (PNAs) were also used to measure the potential nitrification rate of soils under optimal conditions. Both studies aimed to gain insight of N cycling processes and the microbial communities involved in response to long-term changes of soil pH and phosphorus availability.

In chapter 3, N_2O was the primary gaseous N product from potential denitrification incubations. Large variation of potential N_2O fluxes across treatments was observed with no significant effect of pH treatment, however decreasing N_2O fluxes associated with increased lime addition can be seen. This lack of correlation with pH and of the pH treatment effect was also true for P availability, P treatment and interaction of pH and P treatments. This contrasts with what has been reported in in-situ field trials from this site as Zurovec et al., (2021) found that N_2O emissions were reduced with the Very High (VH) pH treatment. It is important to note, lab incubations and PDAs do not recreate field conditions. PDAs represent the genetic potential of the community to denitrify under a prescribed set of conditions and therefore the same results might not be observed measurements in the field represent actual emissions. While the potential of the soil to denitrify appeared not be influenced by soil pH or its treatments, the microbial community composition and abundance were. At the structural level, assessed by PERMANOVA analysis, bacterial and fungal community structure differed across pH treatments, but not

between P treatments. At the abundance level, all target genes, phylogenetic and functional were correlated with soil pH. This was not the case when compared to chapter 2 within IE site results, suggesting sampling time (therefore season and temperature) might be another required factor to consider when exploring the impact of soil edaphic factors on the composition and abundance of microbial communities. All gene abundances were positively correlated with soil pH except for crenarchaeal and AOA communities. The impact of pH treatment from the same site (IE) on functional and crenarchaeal abundances differed between sampling times (i.e. between chapter 2 and chapter 3). On the other hand, P availability positively correlated with the abundances of fungal and denitrifier *nosZI* genes. It is interesting to note, that denitrifier abundances of *nirK*, *nirS* and *nosZI* were negatively correlated to P availability on the overall dataset from chapter 2. As suggested by soil pH, opposite trends in different sites emphasises the complexity of the role soil edaphic factors play in shaping the abundance and structure of microbial communities but also how management of a single aspect might not be the best solution to reduce the impact of agriculture in the environment. Finally, significant effect of pH and P treatment interaction were observed in the abundances of crenarchaeal and *nirK* and *nirS* denitrifier communities and once again, these results show soil pH as well as P are factors that influence the microbial communities present in the soil. While, in this case no effect on potential denitrification fluxes were observed, it is safe to assume shifts in the microbial community will consequently impact the activity of these N cycling processes. The results in this study bring insight into the complexity of the environment in which soil microbial communities are exposed to and how it is not a single factor which impacts the community.

Chapter 4 study followed the same workflow as chapter 3, with the addition of potential nitrification assays (PNAs), aiming to explore once again the impact of soil pH and P availability on a long-term experimental trial on N cycling processes and the communities involved in carrying out these activities. While the aim of the PNAs was not to measure the potential N_2O by-product, both N cycling process occur sequentially, with nitrification final product feeding into the denitrification pathway. However, no correlation between potential nitrification rate and potential denitrification fluxes was found. PNAs rate was positively correlated with soil pH. On the other hand, none of the PDAs fluxes were correlated with soil pH, however, soil pH treatment did have a significant effect on potential N_2O flux. In agreement with what was observed in chapter

3, the bacterial and fungal community structures were significantly influenced by pH treatment. With respect to the abundance of communities, all functional genes were positively correlated with pH except for *nosZI* and AOA, while in the overall dataset from chapter 2 all functional genes were correlated with soil pH apart from *16S rRNA* crenarchaea and *ITS* fungi. It is important to note that DK samples from chapter 2 only had one the highest P treatment level (PIV) and four pH treatments, while in this chapter all pH and P treatment levels were included in the analysis. The inclusion of the P management element and seasonal differences may play a role in the differential response to what was observed in chapter 2. pH treatment did have a significant effect on abundances of every phylogenetic, denitrifier and nitrifier targeted gene. These results differ from chapter 2 within DK site abundances in which bacterial, fungal and nitrifier gene abundances were not significantly impacted by pH treatment. Including a grassland and arable site in chapters 3 and 4 enabled to examine the relationships of soil pH, P and their interaction across a range of conditions, including soil type, longevity of the experimental trial and management application rate, allowing to detect which gene abundances behave similarly and which are not. For example, bacterial and fungal marker gene abundances were not significantly impacted by pH treatment in chapter 3, but they were in chapter 4. Availability of P nutrient was not correlated with either denitrification or nitrification potential assays. Only abundances of crenarchaea and fungal phylogenetic markers were influenced by P, but the structural composition of bacterial and fungal communities were not influenced by P treatment, and neither were abundances of functional and phylogenetic genes. These results suggest in this case, P does not have a key role in shaping the microbial N cycling communities but a significant interaction effect of pH and P treatment on *nosZII* abundance as well as nitrification rate were observed. Briefly, the results from this study show the role soil pH can play in favouring microbial communities involved in N cycling processes and influence their activity. These results also bring insight into the possible impact P availability can also have in these processes and the functional communities behind the reactions.

Since experimental trials from chapters 3 and 4 consisted of a similar design, it is important to take into consideration the differences in results. PNA incubations were not done in chapter 3, however the results could have provided further insight into the interaction between soil pH and P availability assuming the same results would have been observed. If this had been the case it could have been suggested both soil factors have a

strong influence on the activity of the pathway since it would have occurred in two different soil types and agricultural management (grassland and barley, as well as treatment rate application). If different PNA trends had been observed between chapters 3 and 4 it might have been possible to interpret the results to identify the factors controlling nitrification activity and what differences between experimental trials had led to different outcomes.

Denitrification activity was assessed in both chapters. Potential N_2O emissions were only significantly influenced by pH treatment in the experimental trial from chapter 4. The lack of treatment effect in measurements from chapter 3 in relation to pH treatment as well as no effect at all of P treatment on either chapter could be interpret as these factors playing a minor role in denitrifier activity. However, this was not the case. A drop in N_2O production from unlimed to limed plots in chapter 4 lead to a significant pH treatment effect in chapter 4, suggesting pH management does induce N_2O reduction. This could be justified by a larger presence of denitrifier functional communities capable of completing the pathway being more abundant in less acidic soils since N_2O reductase is functional. While this was the only statistically significant result, visual decreasing N_2O trends across pH treatments in both chapters do emphasise pH management could be implemented at larger scale to reduce the contribution of agriculture on N_2O emissions. The results from these chapters indicate while different practices might be needed to best fit each environment (crop, soil type, application rate), it can be suggested an increase of soil pH should induce N_2O reduction. As discussed in the respective chapters, the reported denitrifier activity and final products do not align with field campaigns in which an impact of pH treatment in fluxes was reported. PDA incubations do not aim to recreate field conditions but instead expose soil to optimal conditions for denitrification to occur. It is possible the treatment effect is override by the addition of nutrients, providing microbial communities with excessive N. This extreme N availability could justify the lack of P treatment effect on denitrifier activity, while soil P availability has been reported to influence N transformations, nutrient stoichiometry that will influence activity at field scale might be overridden during the incubation.

Quantifying the same functional genes across the three experimental chapters, allowed to evaluate if the same patterns and trends across pH ranges and gene abundances occurred independently of the different environments. Firstly, denitrifier functional genes are significantly influenced by pH treatment across all experimental chapters. This effect

was emphasised by the positive correlation between *nir* and *nos* genes and the pH gradient. These results emphasise the role soil pH plays in shaping the denitrifier functional community and how influences on the quantity of these functional genes will impact the denitrification rate as well as the final product of the pathway. However, it is interesting to note, while treatment had a significant effect on *nosZI* abundance in chapter 4, it did not have a positive correlation with soil pH. This result highlights the complexity of the interactions between microbial communities and their environment. The lack of a correlation is justified by the treatment effect on *nosZI*, with treatment 4 Mg ha^{-1} having the highest *nosZI* abundance. This result shows a "rule of thumb" cannot be applied, assuming increasing soil pH will lead to a larger denitrifier community. The experimental trial sampled in chapter 4 was also included in chapter 2. In that study, *nosZI* abundances showed a different trend, while pH treatment still had a significant effect on gene abundance, 4 Mg ha^{-1} treatment did not have the highest *nosZI* gene copy numbers, instead a significant increase across pH treatments was observed. It could be suggested, the lack of correlation between the functional N_2O reductase and soil pH might be due to clade I being less sensitive to its environment, but the different trends across the study from the same sampling site imply instead, environmental aspects such as seasons which cannot be controlled through agricultural management might have to be taken into consideration in future studies when analysing the role pH has in shaping the abundance and composition of denitrifier communities.

For nitrifier functional genes, pH treatment was observed for all three functional genes in chapters 3 and 4. Also, AOA and COMAMMOX were significantly influenced by pH treatment in site NO from chapter 2. These significant effects were not directly translated to a correlation between soil pH and nitrifier gene abundances. For AOA, abundances were negatively correlated with pH in chapter 3 but showed no correlation in chapter 4. This could be due to the peak of AOA copy numbers present in 4 Mg ha^{-1} treatment. Once again, these results indicate different soil types and environments will impact differently the same functional gene. Both sites were included in chapter 2 but neither showed pH treatment to influence AOA abundances at that sampling time. For AOB and COMMAMOX positive correlations with soil pH were reported, but at pH treatment level AOB does not have the same patterns between chapter 3 and 4. In the Irish experimental site, AOB abundances increase with rising soil pH. However, in chapter 4, the highest AOB abundance is in treatment 4 Mg ha^{-1}. Soil pH from treatment 4 Mg ha^{-1}

averaged around 5.59, while the lowest pH in experimental trial from chapter 3 was 5.11. These results could suggest different microorganisms harbouring the same functional gene are better adapted to different soil pH levels and a possible optimal pH for these microorganisms to thrive might differ based on other factors including soil type or agricultural management (a grassland and spring barley in this case). For COMAMMOX abundances, a positive correlation with pH was observed in both chapters. In this case, while the quantification of the functional gene appears to be the same independently of changes in soil type and other factors, pH treatment effect was also observed for this nitrifier gene in NO site from chapter 2. This is an important result to highlight since COMAMMOX is a new identified nitrifier gene and in that specific site different lime types (and not rates) were applied. Therefore, pH treatment effect in that setting suggests while soil pH could be considered for the management of nitrifier abundances, the type of lime being used could become an extra added factor to consider which will impact gene copy numbers not only by creating more favourable pH values but also benefit (or not) the abundance of the functional gene based on its composition.

For overall bacterial gene abundance, pH treatment only appeared to be significant in quantified genes from chapter 4. This effect explains the lack of correlation between the soil property and the gene copy numbers since treatment 4 Mg ha^{-1} had the highest bacterial abundance. Soil from experimental chapter 4 is a sandy soil which is associated with smaller microbial communities due to lower soil aggregates as well as less nutrients. These results could suggest in this specific experimental trial, pH level induced by treatment creates an optimal pH for the overall size of the bacterial community, however it is important to note, qPCR does not differentiate between dead and alive microorganisms. Also, soil type and soil pH will impact the DNA yield from extraction which in turn will affect gene quantification. Since pH treatment effect was not observed for the same site when included within chapter 2 data set, parameters that might impact gene abundances could justify the different results reported in chapters 2 and 3 since a positive correlation between bacterial abundance and pH was observed. Crenarchaea gene abundances showed a pH treatment effect throughout all experimental chapters with the same trend, as pH increased, gene abundance decreased. It is known crenarchaea prefers acidic environments and these results show this is true across soil types, management and seasons since the same effect was observed for the Danish site in chapter 2 and 4. Within chapter 2, crenarchaeal gene abundances were also significantly impacted by pH

treatment in NO site. This provides a new knowledge gap in which the lime type, rather than the application rate of the product will also need to be taken into consideration when evaluating the role of pH since these different products might lead to different outcomes in relation to N cycling pathway activity if for each lime type the microbial community responds differently. Finally, for fungal abundances, different results were also observed throughout the chapters. Chapter 2 did not report any pH treatment effect or correlation between soil pH and fungal abundances, while chapter 3 and 4 showed different correlations. In the Irish site, fungal abundances were not affected by pH treatment but showed an increasing trend with increasing pH while in the Danish site, pH treatment effect led to higher fungal abundances in treatments 0 Mg ha^{-1} and 4 Mg ha^{-1} compared to higher application rates and showed a negative correlation. Since these two experimental designs consisted of a grassland and a barley field and soil type was different it is possible for different fungal communities to be present which better adapt to each of the conditions provided by the environment and that could justify the opposite trends of the same target gene abundances. Plant type might play a role in selecting different fungal species due to the formation of symbiotic relationships. Fungi have been reported to be less sensitive to their environment and can perform under harsher soil conditions (low nutrient levels) therefore it could be suggested based on these results that fungal gene abundances are influenced by pH treatment because different species are being favoured by the plant present in the field as well as the changing parameters across both experimental sites.

Comparing all qPCR results highlights the complexity of microbial communities present in soil. These results emphasise microbial ecology requires in depth research and studies and while overall trends might be visible throughout different soil types or environments, in most case a single rule cannot be widely applied. In this thesis it has been shown soil pH does have an important role in dictating functional and phylogenetic gene abundance, however, reporting different results in relation to pH treatment for the same experimental sites in separate chapters (Irish and Danish sites were included in chapter 2 dataset) suggest pH management might have to be adapted to seasonal changes, amongst other cases. While it was not explored the opportunity to analyse results in which the lime type instead of application rate was considered a pH treatment a new knowledge gap appears. If the aim is to expand the understanding on how soil pH can be used in agriculture to reduce N_2O emissions, it is important to study the response the microbial communities involved in the production and reduction of the greenhouse gas will respond

to the product applied and which is best suited for each specific microbial community and its composition. The ecology of these communities is further emphasised in this thesis by reported interactions between soil pH and soil P and a lack of great knowledge on how this nutrient is influencing those N cycling pathways.

Overall, the results from this thesis show a strong effect of soil pH on nearly all nitrifier and denitrifier community abundances, indicating pH management could be used to shift and influence the presence of these N cycling communities and therefore reduce the impact agriculture has in the production and emission of N_2O. However, a single management might not be suitable for all soils and more in depth and specific designs will be required to fit different geoclimatic regions. Moreover, the results from this thesis suggest that other soil edaphic factors such as P nutrient availability might also impact abundances of N cycling microbial communities when it interacts with soil pH treatments, but the mechanisms are still poorly understood. Insight in the complexity of the soil microbial network, and how all edaphic factors balance and interact with each to effect denitrifier and phylogenetic communities, and associated N transformations, can provide clearer guidance on new agricultural managements that will have a greater impact on the reduction of N_2O emissions from soils.

5.2. Future work

All three experiments within this thesis highlight the importance soil edaphic factors and geoclimatic region on the composition and structure of soil microbial communities involved in N cycling processes. However, as hypotheses are tested further gaps in knowledge appear.

In chapter 2, while a wide pH range was accessible for the experimental set-up thanks to the variety of geoclimatic region and treatment regimens applied in the sites, each treatment was unique. While the effect of pH could still be explored throughout all plots that did not receive any lime application, it was difficult to determine what exact effect pH had due to different lime products used, different application rates and different liming timelines. Studies of this scale are a challenge to create, however the possibility of carrying out a study with such a diverse number of soils that has received the same amount and type of lime would allow to better understand if the role soil pH has at shaping those microbial communities is strong enough to overcome a range of different soil edaphic factors and geoclimatic regions, bringing better insight to agricultural

management techniques that can be applied more widely for reducing N_2O emissions. As done in chapters 3 and 4, N_2O fluxes or in situ measurements would have provided great knowledge on how not only the microbial communities but their activity of N cycling processes was influenced by soil pH, to gain better understanding on how this soil factor can be managed across geoclimatic regions to achieve the same outcome of lower N_2O emissions.

Being able to use sites from chapter 2 in the following studies of the thesis, raised the question of the influence sampling time can have on the observed results, independently of the hypothesis being tested. Seasonal sampling and repetition of each of the methodology throughout a year could be considered as a potential future study.

Gaps that remain in the understanding of the activity and communities involved in these N cycling processes include the role P availability play in influencing N_2O emissions. Recent studies (Cui et al., 2020; O'Neill et al., 2020) have suggested P influences N_2O production, and our results emphasise this might be reflected on the abundance of functional microbial communities. The same way the mechanism by which acidic soils (low pH) affect the folding of N_2O reductase enzyme at the molecular level is understood, the knowledge on the possible mechanisms behind the levels of P in soil need to be explored. Future experiments would require considering other soil edaphic factors that could also indirectly influence P availability and in turn the role this soil nutrient plays.

Finally, amplicon sequence of N communities, as done by Jones et al., (2014) and further work on the genetic make-up of the microbial communities in soil might be suggested as a future experiment. While quantification of functional and phylogenetic markers provides information on the size of the microbial community; and prokaryotic and fungal sequencing provides insight into their structure, providing identification of specific taxa, it cannot be known which of these communities are active at a specific moment in time. Due to the vast diversity of microorganisms in soils, little is known about which specific phyla, genera or family might harbour any of the relevant genes to increase the capacity of the soil to act as an N_2O sink and reduce the emissions of this greenhouse gas.

5.3. References

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CHAPTER 6

Appendices

Appendix 1

Table S2.1. qPCR cycles information, including forward and reverse primers; primer concentration; starting standard concentration for standard curve; positive control used; complete cycle conditions; and final qPCR efficiency run.

Gene	Primers	Primer Sequences (5' - 3')	Primer Concentration (µM)	Standard concentration	Positive Control	Cycle Conditions	Efficiency (%)	R^2	References
16S rRNA bacteria	341F	CCTACGGGNGGCWGCAG	1.5	2.99×10^9	*Pseudomonas Aeruginosa*	95 °C 5 minutes Denaturation step 95 °C 40 seconds 55.6 °C 1 minute 72 °C 30 seconds 80 °C 3 seconds Data acquisition 40 cycles	81.5	0.981	Klindworth et al., 2013
	785R	GACTACHVGGGTATCTAATCC				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Klindworth et al., 2013
16S rRNA crenarchaea	771F	ACGGTGAGGGATGAAAGCT	0.2	3.83×10^7		95 °C 5 minutes Denaturation step 95 °C 30 seconds 54 °C 1 minute 72 °C 1 minute 82 °C 3 seconds Data acquisition 40 cycles	91.5	0.999	Ochsenreiter et al., 2003
	957R	CGGCGTTGACTCCAATTG				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Ochsenreiter et al., 2003
ITS fungi	ITS86F	GTGAATCATCGAATCTTTGAA	0.2	3.79×10^7		95 °C 2 minutes Denaturation step 95 °C 30 seconds 54 °C 1 minute 72 °C 1 minute Data acquisition 40 cycles	81.6	0.999	De Beeck et al., 2014
	ITS4R	TCCTCCGCTTATTGATATGC				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			De Beeck et al., 2014
nirK	nirK1040F	GCCTCGATCAGRTTRTGGTT	0.2	3.22×10^9	*Ensifer melloti*	95 °C 10 minutes Denaturation step 95 °C 10 seconds 60 °C 30 seconds 72 °C 10 seconds 85 °C 2 seconds Data acquisition 40 cycles	80.6	0.999	Hallin et al., 2009
	nirK876R	ATYGGCGGVCAYGGCGA				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Hallin et al., 2009
nirS	cd3AF	GTSAACGTSAAGGARACSGG	1	1.63×10^{10}	*Pseudomonas Aeruginosa*	95 °C 10 minutes Denaturation step 95 °C 10 seconds 66 °C 20 seconds 72 °C 20 seconds 80 °C 2 seconds Data acquisition 40 cycles	89.2	0.999	Throback et al., 2004; Yergeau et al., 2004
	R3cd	GASTTCGGRTGSGTCTTGA				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Throback et al., 2004
nosZI	nosZ2F	WCSYTGTTCMTCGACAGCCAG	0.2	0.5×10^8	*Pseudomonas Aeruginosa*	95 °C 15 minutes Denaturation step 95 °C 15 seconds 65 °C 30 seconds Decrease temperature by -1°C 72 °C 1 minute 6 cycles 95 °C 15 seconds 60 °C 30 seconds 72 °C 1 minute Data acquisition 29 cycles	77.2	0.993	Henry et al., 2006
	nosZ2R	ATGTCGATCARCTGUKCRTTYTC				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Henry et al., 2006
nosZII	nosZ II F	CTIGGICCIYTKCAYAC	2	6.56×10^7		95 °C 5 minutes Denaturation step 95 °C 30 seconds 62 °C 30 seconds 72 °C 30 seconds 85 °C 2 seconds Data acquisition 49 cycles	83.2	0.996	Jones et al.; 2013
	nosZ II R	GCIGARCARAAITCBGTR C				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Jones et al.; 2013
AOA	crenamoA23F	ATGGTCTGGCTWAGACG	0.2	4.61×10^7		95 °C 5 minutes Denaturation step 95 °C 30 seconds 55.6 °C 40 seconds 72 °C 1 minute 78 °C 2 seconds Data acquisition 39 cycles	78.0	0.998	Tourna et al., 2008
	crenamoA616R	GCCATCCATCTGTATGTCCA				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Tourna et al., 2008
AOB	amoA1F	GGGGTTTCTACTGGTGGT	1	1.05×10^8		94 °C 5 minutes Denaturation step 94 °C 30 seconds 55 °C 45 seconds 72 °C 45 seconds 82 °C 2 seconds Data acquisition 39 cycles	84.4	0.997	Rothhauwe et al.,1998
	amoA2R	CCCCTCBGSAAAVCCTTCTTC				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Hornek et al., 2006
COMAMMOX	Ntsp_amoA_162F	GGATTTCTGGNTSGATTGGA	1	4.40×10^8		95 °C 5 minutes Denaturation step 95 °C 30 seconds 56 °C 30 seconds 72 °C 45 seconds 82 °C 2 seconds Data acquisition 39 cycles	87.1	0.998	Fowler et al., 2018
	Ntsp_amoA_359R	WAGTTNGACCACCASTACCA				95 °C 5 seconds 65 °C 5 seconds 95 °C Data acquisition Melt Curve			Fowler et al., 2018

Appendix 2

Table S2.2. Spearman's correlation coefficients of soil properties, targeted gene abundances and gene ratios. Significant correlations highlighted in bold (***** p value ≤ 0.05; ****** p value ≤ 0.01; ******* p value ≤ 0.001). TC - Total carbon, SOC - Soil organic carbon, TN - Total nitrogen.

	TC	SOC	TN	Sand	Silt	Clay	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
SOC	**1.00 *****																	
TN	**1.00 *****	**0.99 *****																
Sand	**-0.46 *****	**-0.45 *****	**-0.49 *****															
Silt	-0.18	-0.18	-0.13	**-0.73 *****														
Clay	**0.84 *****	**0.83 *****	**0.84 *****	**-0.76 *****	0.12													
Al	**-0.44 *****	**-0.46 *****	**-0.40 *****	-0.04	**0.22 ***	-0.16												
Ca	**0.82 *****	**0.83 *****	**0.80 *****	**-0.50 *****	0.03	**0.71 *****	**-0.63 *****											
Co	-0.14	-0.13	-0.13	**-0.43 *****	**0.51 *****	0.14	-0.01	0										
Cu	**-0.28 ****	**-0.27 ****	**-0.28 ****	0.02	**0.39 *****	**-0.39 *****	**-0.49 *****	0.07	**0.25 ***									
Fe	**0.27 ***	**0.27 ****	**0.24 ***	**-0.29 ****	0.06	**0.36 *****	-0.19	**0.23 ***	**0.67 *****	-0.13								
K	**0.41 *****	**0.41 *****	**0.42 *****	**-0.55 *****	**0.33 ****	**0.49 *****	-0.08	**0.48 *****	**0.60 *****	-0.04	**0.62 *****							
Mg	-0.01	0	-0.01	**-0.38 *****	**0.56 *****	0.03	-0.21	**0.29 ****	**0.40 *****	**0.52 *****	0.14	**0.46 *****						
Mn	**-0.23 ***	**-0.24 ***	**-0.21 ***	0.16	-0.01	**-0.23 ***	-0.07	**-0.28 ****	0.16	**0.36 *****	-0.1	**-0.26 ***	**-0.33 ****					
Na	-0.14	-0.14	-0.13	**-0.44 *****	**0.52 *****	0.15	-0.02	0.06	**0.84 *****	**0.29 ****	**0.50 *****	**0.54 *****	**0.42 *****	**0.25 ***				
P	**-0.33 ****	**-0.32 ****	**-0.38 *****	**0.66 *****	**-0.55 *****	**-0.43 *****	0.04	**-0.32 ****	**-0.41 *****	**-0.25 ***	-0.13	**-0.54 *****	**-0.33 ****	**-0.30 ****	**-0.46 *****			
S	**0.87 *****	**0.88 *****	**0.86 *****	**-0.49 *****	-0.08	**0.78 *****	**-0.42 *****	**0.84 *****	0.16	-0.19	**0.56 *****	**0.67 *****	0.14	**-0.24 ***	0.16	**-0.34 *****		
Zn	**0.40 *****	**0.39 *****	**0.43 *****	**-0.41 *****	**0.33 ****	**0.28 ****	-0.15	**0.38 *****	**0.44 *****	0.18	**0.42 *****	**0.71 *****	**0.31 ****	0.13	**0.41 *****	**-0.63 *****	**0.54 *****	
16S rRNA bacteria	**0.47 *****	**0.48 *****	**0.47 *****	**-0.40 *****	0.19	**0.41 *****	**-0.24 ***	**0.59 *****	-0.05	0.02	0.03	**0.44 *****	**0.46 *****	**-0.64 *****	-0.07	-0.18	**0.46 *****	0.2
16S rRNA crenarchaea	**-0.23 ***	**-0.22 ***	**-0.23 ***	0.02	**0.34 ****	**-0.34 *****	**-0.36 *****	0.05	**0.22 ***	**0.70 *****	0.02	0.05	**0.54 *****	0.09	**0.26 ***	-0.19	-0.15	0.2
ITS fungi	-0.16	-0.15	-0.18	0.11	0.11	**-0.26 ***	-0.2	0.03	0.04	**0.26 ***	0.09	0.05	**0.38 *****	**-0.36 *****	0.01	0.18	-0.07	0.01
nirK	**0.36 *****	**0.37 *****	**0.37 *****	**-0.22 ***	0.13	0.2	**-0.33 ****	**0.54 *****	-0.18	**0.26 ***	**-0.29 ****	**0.26 ***	**0.47 *****	**-0.35 *****	-0.12	**-0.32 ****	**0.28 ****	0.17
nirS	**0.30 ****	**0.31 ****	**0.29 ****	**-0.45 *****	**0.34 ****	**0.32 ****	**-0.32 ****	**0.47 *****	**0.34 ****	0.18	**0.37 *****	**0.62 *****	**0.48 *****	**-0.32 ****	**0.39 *****	**-0.31 ****	**0.49 *****	**0.34 ****
nosZI	**0.51 *****	**0.51 *****	**0.52 *****	**-0.46 *****	**0.28 ****	**0.40 *****	**-0.28 ****	**0.60 *****	-0.08	0.13	-0.07	**0.45 *****	**0.49 *****	**-0.45 *****	-0.06	**-0.36 *****	**0.46 *****	**0.33 ****
nosZII	-0.05	-0.04	-0.05	-0.24 *	**0.49 *****	-0.11	**-0.33 ****	**0.30 ****	0.2	**0.57 *****	0	**0.25 ***	**0.74 *****	**-0.37 *****	0.18	-0.16	0.03	0.18
AOA	0.01	0.02	0.01	-0.07	**0.30 ****	-0.18	**-0.37 *****	**0.25 ***	0.08	**0.55 *****	-0.07	0.16	**0.62 *****	**-0.28 ****	0.05	-0.14	0.03	**0.22 ***
AOB	**0.34 ****	**0.35 *****	**0.33 ****	**-0.25 ***	0.19	0.18	**-0.32 ****	**0.46 *****	-0.05	0.16	0.07	**0.32 ****	**0.53 *****	**-0.58 *****	-0.08	-0.07	**0.33 ****	**0.23 ***
COMAMMOX	**0.57 *****	**0.58 *****	**0.55 *****	**-0.30 ****	0	**0.44 *****	**-0.36 *****	**0.65 *****	-0.09	-0.05	0.15	**0.40 *****	**0.31 ****	**-0.47 *****	-0.06	-0.14	**0.58 *****	0.2

Table S2.3. Spearman's correlation coefficients of targeted gene abundances and gene ratios. Significant correlations highlighted in bold (***** p value ≤ 0.05; ****** p value ≤ 0.01; ******* p value ≤ 0.001).

	16S rRNA bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB	COMAMMOX
16S rRNA crenarchaea	0.12									
ITS fungi	**0.32 ****	**0.52 *****								
nirK	**0.71 *****	0.2	0.11							
nirS	**0.64 *****	**0.21 ***	0.2	**0.37 *****						
nosZI	**0.80 *****	0.11	0.11	**0.73 *****	**0.54 *****					
nosZII	**0.50 *****	**0.68 *****	**0.56 *****	**0.48 *****	**0.47 *****	**0.50 *****				
AOA	**0.45 *****	**0.76 *****	**0.54 *****	**0.44 *****	**0.37 *****	**0.41 *****	**0.84 *****			
AOB	**0.66 *****	**0.32 ****	**0.47 *****	**0.50 *****	**0.46 *****	**0.61 *****	**0.75 *****	**0.65 *****		
COMAMMOX	**0.64 *****	0.11	**0.25 ***	**0.51 *****	**0.54 *****	**0.58 *****	**0.46 *****	**0.41 *****	**0.78*****	
ITS fungi / *16S rRNA* bacteria	**-0.30 ****	**0.21 ***	**0.34 ****	**-0.26 ***	-0.1	**-0.31 ****	0.02	0.05	-0.11	-0.2
nirK / nirS	**-0.37 *****	-0.08	-0.07	-0.19	**-0.54 *****	**-0.29 ****	**-0.31 ****	-0.17	**-0.30 ****	**-0.38 *****
nosZI / nosZII	**-0.33 ****	-0.14	**-0.28 ****	-0.12	**-0.48 *****	-0.2	**-0.44 *****	**-0.27 ***	**-0.44 *****	**-0.33 ****
NOS / NIR	0.02	**0.38 *****	**0.31 ****	-0.2	0.17	-0.02	**0.49 *****	**0.34 ****	**0.30 ****	0.12

Table S2.4. Spearman's correlation coefficients of targeted gene abundances and gene ratios. Significant correlations highlighted in bold (***** p value ≤ 0.05; ****** p value ≤ 0.01; ******* p value ≤ 0.001).

	TC	SOC	TN	Sand	Silt	Clay	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	S	Zn	*ITS* fungi / *16S rRNA* bacteria	*nirK / nirS*	*nosZI / nosZII*
ITS fungi / *16S rRNA* bacteria	-0.2	-0.2	-0.2	**0.30 ****	-0.2	**-0.26 ***	-0.1	-0.17	-0.05	0.01	0.2	-0.14	-0.06	-0.1	-0.07	**0.34 *****	-0.13	-0.1			
nirK / nirS	-0.1	-0.2	-0.1	**0.24 ***	-0.2	-0.15	**0.23 ***	**-0.28 ****	**-0.22 ***	-0.1	**-0.21 ***	**-0.25 ***	-0.2	0.21	**-0.24 ***	0	**-0.21 ***	-0.2	0.16		
nosZI / nosZII	0	0	0	**0.24 ***	**-0.26 ***	-0.1	**0.32 ****	**-0.27 ***	**-0.31 ****	**-0.24 ***	-0.21	-0.15	**-0.32 ****	**0.24 ***	**-0.25 ***	-0.13	-0.15	0	**0.42 *****	**0.49 *****	
NOS / NIR	-0.1	-0.1	-0.1	**-0.23 ***	**0.38 *****	-0.02	-0.03	0.02	**0.60 *****	0.16	**0.55 *****	**0.32 ****	**0.38 *****	-0.17	**0.37 *****	-0.11	0.03	0.21	**0.28 ****	-0.2	**-0.41 *****

Appendix 3

Table S2.5. Average of gene abundances for each treatment within sites. Lower case letters indicate significant differences ($p \leq 0.05$) of gene abundances across treatments within a site. Treatments vary across sites, unless indicated otherwise finely ground dolomite was applied. 0L - unlimed plots. **DK** 4T – 4 t/ha; 8T – 8 t/ha; 12T – t/ha. **IE** L – 5 t/ha in 2011; H – 5 t/ha in 2014; VH – 5 t/ha in 2011 and 2014. **NO** D – Dolomite (Finely ground); LK – Larvikite; M – Marble; N – Norite; O – Olivine. **SE1** 10T – 10 t/ha; 20T – 20 t/ha. **SE2** ML – Mixed lime (Slaked Lime and Calcium Carbonate); SL – Slaked Lime ($Ca(OH)_2$); and TL – Tunnel Kiln Slag.

Site	Treatment	*16S rRNA* bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosSZII*	AOA	AOB	COMAMMOX
DK	0L	1.93E+05 [a]	1.09E+04 [a]	1.37E+05 [a]	3.33E+02 [a]	1.56E+01 [a]	3.53E+03 [a]	5.04E+03 [b]	3.71E+02 [a]	3.53E+03 [a]	2.54E+03 [a]
	4T	2.54E+05 [a]	3.35E+03 [b]	4.07E+04 [a]	1.76E+03 [b]	7.55E+01 [b]	6.81E+03 [ab]	1.07E+04 [ab]	5.88E+02 [a]	7.27E+03 [a]	4.61E+03 [a]
	8T	2.30E+05 [a]	2.32E+03 [b]	3.42E+04 [a]	1.86E+03 [b]	1.75E+02 [b]	1.03E+04 [b]	1.63E+04 [ac]	2.21E+02 [a]	6.77E+03 [a]	6.24E+03 [a]
	12T	2.61E+05 [a]	3.61E+03 [b]	4.27E+04 [a]	2.13E+03 [b]	1.37E+02 [b]	9.49E+03 [b]	1.79E+04 [c]	5.72E+02 [a]	6.86E+03 [a]	5.92E+03 [a]
IE	0L	1.35E+05 [a]	1.45E+04 [a]	2.06E+04 [a]	1.13E+03 [b]	3.82E+01 [a]	7.52E+03 [a]	4.10E+03 [a]	3.10E+02 [a]	1.98E+03 [a]	1.95E+03 [a]
	L	1.60E+05 [a]	7.76E+03 [a]	2.34E+04 [a]	1.38E+03 [a]	1.78E+02 [a]	7.51E+03 [a]	5.62E+03 [a]	1.76E+02 [a]	2.88E+03 [a]	2.73E+03 [a]
	H	1.11E+05 [a]	9.98E+03 [a]	1.15E+04 [a]	1.57E+03 [a]	1.46E+02 [a]	7.60E+03 [a]	7.58E+03 [a]	2.53E+02 [a]	3.58E+03 [a]	3.60E+03 [a]
	VH	1.55E+05 [a]	1.31E+04 [a]	2.54E+04 [a]	1.95E+03 [a]	1.86E+02 [a]	7.99E+03 [a]	2.77E+03 [a]	1.05E+02 [a]	1.50E+03 [a]	3.70E+03 [a]
NO	0L	2.94E+05 [a]	3.54E+03 [a]	4.51E+04 [a]	1.08E+03 [c]	2.55E+02 [ab]	1.05E+04 [a]	2.49E+04 [ab]	2.45E+02 [b]	6.27E+03 [a]	4.84E+03 [ab]
	D	2.98E+05 [a]	1.62E+03 [a]	2.68E+04 [a]	2.03E+03 [ab]	2.81E+02 [ab]	1.06E+04 [a]	2.82E+04 [ab]	1.36E+02 [a]	5.87E+03 [a]	5.85E+03 [a]
	LK	2.41E+05 [a]	2.92E+03 [a]	3.95E+04 [a]	1.19E+03 [c]	1.40E+02 [ab]	1.05E+04 [a]	1.70E+04 [ab]	1.88E+02 [ab]	5.09E+03 [a]	4.30E+03 [ab]
	M	2.92E+05 [a]	2.29E+03 [a]	3.88E+04 [a]	2.46E+03 [ab]	3.57E+02 [a]	1.00E+04 [a]	4.10E+04 [b]	1.40E+02 [a]	6.06E+03 [a]	6.86E+03 [a]
	N	3.06E+05 [a]	1.52E+03 [a]	3.57E+04 [a]	1.41E+03 [bc]	1.93E+02 [ab]	9.02E+03 [a]	1.51E+04 [a]	1.76E+02 [ab]	4.58E+03 [a]	3.70E+03 [ab]
	O	2.30E+05 [a]	4.01E+03 [a]	4.35E+04 [a]	1.25E+03 [c]	1.02E+02 [b]	9.58E+03 [a]	1.75E+04 [a]	2.47E+02 [b]	6.02E+03 [a]	2.94E+03 [b]
SE1	0L	3.96E+05 [a]	2.12E+03 [a]	1.59E+04 [a]	2.85E+03 [a]	5.20E+02 [a]	1.79E+04 [a]	3.18E+04 [a]	1.02E+03 [a]	1.11E+04 [a]	1.01E+04 [a]
	10T	4.46E+05 [a]	2.83E+03 [a]	3.25E+04 [a]	3.13E+03 [a]	5.13E+02 [a]	1.77E+04 [a]	3.23E+04 [a]	4.56E+02 [a]	9.67E+03 [a]	1.01E+04 [a]
	20T	5.06E+05 [a]	2.00E+03 [a]	4.76E+04 [a]	3.49E+03 [a]	5.14E+02 [a]	1.98E+04 [a]	2.69E+04 [a]	3.53E+02 [a]	9.28E+03 [a]	9.55E+03 [a]
SE2	0L	3.98E+05 [a]	2.79E+04 [a]	8.61E+04 [a]	3.11E+03 [a]	5.01E+02 [a]	1.64E+04 [a]	1.11E+05 [a]	2.17E+03 [a]	1.10E+04 [a]	7.33E+03 [a]
	ML	3.72E+05 [a]	2.17E+04 [a]	8.26E+04 [a]	2.97E+03 [a]	4.22E+02 [a]	1.57E+04 [a]	8.29E+04 [a]	1.34E+03 [a]	1.04E+04 [a]	5.94E+03 [a]
	SL	3.81E+05 [a]	1.62E+04 [a]	5.93E+04 [a]	3.50E+03 [a]	4.32E+02 [a]	1.75E+04 [a]	1.12E+05 [a]	1.59E+03 [a]	1.03E+04 [a]	6.23E+03 [a]
	TL	4.00E+05 [a]	2.63E+04 [a]	1.28E+05 [a]	2.92E+03 [a]	5.60E+02 [a]	1.53E+04 [a]	1.07E+05 [a]	1.68E+03 [a]	9.52E+03 [a]	6.16E+03 [a]

Table S2.6. Average of gene ratios for each site included in the study. Lowercase letters indicate differences ($p \leq 0.05$) of gene abundances across all sites using unlimed plots. Gene ratios with significant differences are highlighted in bold. Denmark, FI – Finland, FRA 1 – France 1, FRA 2 – France 2, IE – Ireland, NO – Norway, NZ – New Zealand, SE1 – Sweden 1, SE2 – Sweden 2.

Site	*ITS* fungi / *16S rRNA* bacteria		*nirK* / *nirS*		*nosZI* / *nosZII*		*NOS* / *NIR*	
DK	0.26	**c**	23.49	a	0.60	**ab**	15.09	**ab**
FI	0.24	**ab**	1.84	a	0.40	**ab**	57.37	**a**
FRA1	0.08	**ab**	32.10	a	2.77	**a**	10.85	**ab**
FRA2	0.08	**ab**	59.83	a	3.53	**b**	5.52	**b**
IE	0.14	**ab**	30.49	a	1.69	**ab**	9.29	**ab**
NO	0.15	**ab**	16.46	a	0.50	**ab**	20.12	**a**
NZ	0.12	**abc**	15.00	a	0.64	**ab**	50.21	**a**
SE1	0.07	**a**	6.24	a	0.64	**ab**	13.76	**ab**
SE2	0.23	**bc**	7.04	a	0.18	**a**	34.18	**a**

Appendix 4

Table S3.1. qPCR cycle summary for all genes, including primers, primer sequencing and concentration; standard starting concentration; cycle summary; final qPCR run efficiency and references.

Gene	Primers	Primer Sequences (5' - 3')	Primer Concentration (µM)	Standard concentration (gene copy / µl)	Positive Control	Cycle Conditions				Efficiency (%)	R^2	References
16S rRNA bacteria	341F	CCTACGGGNGGCWGCAG	0.2	3.35 x 10^7	*Pseudomonas Aeruginosa*	95 °C	5 minutes	Denaturation step		79.7%	0.986	Klindworth et al., 2013
						95 °C	40 seconds		39 cycles			
						55.6 °C	1 minute					
						72 °C	30 seconds					
						80 °C	3 seconds	Data acquisition				
	785R	GACTACHVGGGTATCTAATCC				95 °C	5 seconds					Klindworth et al., 2013
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
16S rRNA crenarchaea	771F	ACGGTGAGGGATGAAAGCT	0.2	3.83 x 10^7		95 °C	5 minutes	Denaturation step		85.2%	1.000	
						95 °C	30 seconds		39 cycles			Ochsenreiter et al., 2003
						54 °C	1 minute					
						72 °C	1 minute					
						82 °C	3 seconds	Data acquisition				
	957R	CGGCGTTGACTCCAATTG				95 °C	5 seconds					Ochsenreiter et al., 2003
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
ITS fungi	ITS86F	GTGAATCATCGAATCTTTGAA	0.2	3.79 x 10^7		95 °C	2 minutes	Denaturation step		85.2%	0.999	
						95 °C	30 seconds		39 cycles			De Beeck et al., 2014
						54 °C	30 seconds					
						72 °C	30 seconds	Data acquisition				
	ITS4R	TCCTCCGCTTATTGATATGC				95 °C	5 seconds					De Beeck et al., 2014
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
nirK	nirK1040F	GCCTCGATCAGRTTRTGGTT	0.2	2.08 x 10^8	*Ensifer melloti*	95 °C	10 minutes	Denaturation step		88.5%	0.999	
						95 °C	10 seconds		39 cycles			Hallin et al., 2009
						60 °C	30 seconds					
						72 °C	10 seconds					
						85 °C	2 seconds	Data acquisition				
	nirK876R	ATYGGCGGVCAYGGCGA				95 °C	5 seconds					Hallin et al., 2009
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
nirS	cd3AF	GTSAACGTSAAGGARACSGG	0.5	1.63 x 10^7	*Pseudomonas Aeruginosa*	95 °C	15 minutes	Denaturation step		84.8%	0.998	
						95 °C	15 seconds		6 cycles			Throback et al., 2004; Yergeau et al., 2004
						63 °C	30 seconds					
						Decrease temperature by -1°C						
						72 °C	30 seconds					
						80 °C	30 seconds					
	R3cd	GASTTCGGRTGSGTCTTGA				95 °C	15 seconds		40 cycles			Throback et al., 2004
						58 °C	30 seconds					
						72 °C	30 seconds					
						80 °C	30 seconds	Data acquisition				
						95 °C	5 seconds					
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
nosZI	nosZ2F	WCSYTGTTCMTCGACAGCCAG	1.5	7.50 x 10^7		95 °C	5 minutes	Denaturation step		92.1%	1.00	
						95 °C	30 seconds		6 cycles			Henry et al., 2006
						65 °C	30 seconds					
						Decrease temperature by -1°C						
						72 °C	30 seconds					
	nosZ2R	ATGTCGATCARCTGUKCRTTYTC				95 °C	30 seconds		39 cycles			Henry et al., 2006
						65 °C	30 seconds					
						72 °C	20 seconds					
						85 °C	3 seconds	Data acquisition				
						95 °C	5 seconds					
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
nosZII	nosZ II F	CTIGGICCIYTKCAYAC	2	6.56 x 10^7		95 °C	5 minutes	Denaturation step		72.0%	0.996	
						95 °C	30 seconds		49 cycles			Jones et al.; 2013
						62 °C	30 seconds					
						72 °C	30 seconds					
						85 °C	2 seconds	Data acquisition				
	nosZ II R	GCIGARCARAAITCBGTR C				95 °C	5 seconds					Jones et al.; 2013
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
AOA	crenamoA23F	ATGGTCTGGCTWAGACG	0.2	4.61 x 10^7		95 °C	5 minutes	Denaturation step		80.4%	0.999	
						95 °C	30 seconds		39 cycles			Tourna et al., 2008
						55.6 °C	40 seconds					
						72 °C	1 minute					
						78 °C	2 seconds	Data acquisition				
	crenamoA616R	GCCATCCATCTGTATGTCCA				95 °C	5 seconds					Tourna et al., 2008
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
AOB	amoA1F	GGGGTTTCTACTGGTGGT	1	4.89 x 10^7		94 °C	5 minutes	Denaturation step		82.4%	0.999	
						94 °C	30 seconds		39 cycles			Rothhauwe et al.,1998
						55 °C	45 seconds					
						72 °C	45 seconds					
						82 °C	2 seconds	Data acquisition				
	amoA2R	CCCCTCBGSAAAVCCTTCTTC				95 °C	5 seconds					Hornek et al., 2006
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				
COMAMMOX	Ntsp_amoA_162F	GGATTTCTGGNTSGATTGGA	1	1.74 x 10^8		95 °C	5 minutes	Denaturation step		81.9%	0.999	
						95 °C	30 seconds		39 cycles			Fowler et al., 2018
						56 °C	30 seconds					
						72 °C	45 seconds					
						82 °C	2 seconds	Data acquisition				
	Ntsp_amoA_359R	WAGTTNGACCACCASTACCA				95 °C	5 seconds					Fowler et al., 2018
						65 °C	5 seconds		Melt Curve			
						95 °C		Data acquisition				

Table S3.2. Illumina sequencing library preparation PCR information including primers with overhangs for PCR1, primer concentrations and cycle information. For PCR2, only cycle conditions are included since INDEX primers with unique barcodes were used.

PCR 1

Gene	Primers	Primer Sequences (5' - 3')	Primer Concentration (µM)	Cycle Conditions			
16S rRNA bacteria	515F	Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGYCAGCMGCCGCGGTAA] Reverse overhang: 5'	0.2	94 °C	3 minutes	Denaturation step	
				94 °C	45 seconds		25 cycles
				50 °C	45 seconds		
				72 °C	1 minute		
	926R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [CCGYCAARTYMTTTRAGTTT]		72 °C	5 minutes	Elongation step	
				15 °C	Hold at		
ITS fungi	ITS86F	Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGAATCATCGAATCTTTGAA] Reverse overhang: 5'	0.2	95 °C	3 minutes	Denaturation step	
				95 °C	30 seconds		25 cycles
				54 °C	30 seconds		
				72 °C	30 seconds		
	ITS4R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [TCCTCCGCTTATTGATATGC]		72 °C	5 minutes	Elongation step	
				15 °C	Hold at		

PCR 2

Gene	Primer Concentration (µM)	Cycle Conditions			
16S rRNA bacteria	0.2	95 °C	3 minutes	Denaturation step	
		95 °C	30 seconds		7 cycles
		55 °C	30 seconds		
		72 °C	30 seconds		
		72 °C	5 minutes	Elongation step	
ITS fungi	0.2	95 °C	3 minutes	Denaturation step	
		95 °C	30 seconds		7 cycles
		55 °C	30 seconds		
		72 °C	30 seconds		
		72 °C	5 minutes	Elongation step	

Appendix 5

Table S3.3. Spearman's correlation coefficients of potential denitrification fluxes with soil physiochemical properties, target gene abundances and gene ratios. Significant correlations highlighted in bold (* p value < 0.05; ** p value < 0.01; *** p value < 0.001). TC - Total carbon, TN - Total nitrogen, SOC - Soil organic carbon.

	TC	TN	SOC	Al	Ca	Co	Cu	Fe	K	Mg	Mn	S	Zn	Total Denitrification ($N_2O + N_2$)	Potential N_2O	Product Ratio (N_2O/N_2O+N_2)	Potential N_2
($N_2O + N_2$)	0.2	0.05	0.19	-0.06	0.04	0.12	-0.15	0.01	-0.01	-0.01	0.09	0.1	0.08				
Potential N_2O	0	0.04	-0.04	0	-0.07	0.15	**-0.43*** **	0.11	0.07	-0.01	0.05	0.01	0.11	**0.66*** **			
(N_2O/N_2O+N_2)	**-0.28***	0.03	**-0.29***	0.12	-0.17	0	-0.22	0.14	0.11	0.09	-0.01	-0.1	0.08	**-0.67*** **	0.05		
Potential N_2	**0.28***	0.02	**0.31***	-0.07	0.1	0.05	0.13	-0.06	-0.06	-0.04	0.07	0.12	0.01	**0.77*** **	0.11	**-0.96*** **	
16S bacteria	**-0.27***	-0.18	-0.17	**-0.42*** **	**0.38****	**-0.53*** **	-0.19	-0.15	-0.19	**0.33****	**-0.42*** **	-0.24	0.09	-0.08	0.08	**0.29***	-0.24
16S crenarchaea	0	0.16	0.11	**0.58*** **	**-0.65*** **	0.14	-0.05	**0.54*** **	**0.43*** **	**0.31***	**0.42*** **	0.1	**0.48*** **	-0.13	-0.05	0.17	-0.14
ITS fungi	-0.22	-0.18	-0.17	**-0.33****	**0.31***	**-0.35****	-0.21	-0.06	-0.2	0.2	**-0.30***	**-0.25***	0.04	-0.01	0.08	0.13	-0.13
nirK	-0.21	-0.19	-0.16	**-0.72*** **	**0.71*** **	**-0.43*** **	-0.1	**-0.48*** **	**-0.36****	0.14	**-0.63*** **	**-0.39****	-0.16	-0.03	-0.06	0.02	-0.03
nirS	**-0.28***	-0.2	**-0.29***	**-0.68*** **	**0.66*** **	**-0.49*** **	0.05	**-0.48*** **	**-0.42*** **	0.13	**-0.64*** **	**-0.40****	-0.23	-0.15	-0.15	0.09	-0.12
nosZI	-0.24	-0.2	-0.12	**-0.54*** **	**0.49*** **	**-0.46*** **	-0.12	-0.23	**-0.30***	**0.26***	**-0.50*** **	**-0.25***	0.05	-0.03	-0.05	0.05	-0.04
nosZII	-0.22	-0.17	-0.17	**-0.54*** **	**0.49*** **	**-0.48*** **	-0.08	-0.2	-0.25	**0.32***	**-0.52*** **	-0.26	0.09	-0.05	-0.04	0.11	-0.05
AOA	-0.23	-0.02	-0.17	**0.35****	**-0.44*** **	**0.05**	-0.19	**0.52*** **	0.15	**0.42*** **	**0.26***	-0.03	**0.52*** **	-0.19	0	**0.41*** **	**-0.33****
AOB	-0.23	-0.16	-0.14	**-0.55*** **	**0.57*** **	**-0.36****	-0.07	**-0.33****	**-0.26***	0.09	**-0.43*** **	**-0.26***	-0.19	0.07	0.08	0.04	-0.05
COMAMMOX	-0.21	-0.19	**-0.28***	**-0.55*** **	**0.65*** **	**-0.48*** **	0.29*	**-0.56*** **	**-0.41*** **	-0.22	**-0.51*** **	-0.2	**-0.47*** **	-0.17	-0.24	0.02	-0.09
ITS fungi/16S bacteria	-0.08	-0.08	-0.12	-0.05	0.03	0	-0.18	0.04	-0.09	0.07	-0.03	-0.15	-0.09	-0.09	0.05	0.11	-0.14
nirK / nirS	-0.1	-0.08	0.04	**-0.42*** **	**0.40****	**-0.29***	-0.17	-0.2	-0.12	0.16	**-0.37****	-0.22	0.02	0.15	0.09	-0.1	0.12
nosZI / nosZII	0.16	0.09	0.12	**0.31***	**-0.31***	**0.37****	0	0.04	0.19	-0.18	**0.35****	0.21	-0.04	0.13	-0.02	-0.24	0.18
NOS / NIR	-0.15	-0.12	0.07	0	-0.06	-0.14	-0.22	**0.25***	0.03	0.22	0.02	0.11	**0.32****	0.05	0.09	0.09	-0.01

Table S3.3. Spearman's correlation coefficients of targeted gene abundances and gene ratios. Significant correlations highlighted in bold (***** p value < 0.05; ****** p value < 0.01; ******* p value < 0.001).

	16S rRNA bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB	*ITS* fungi/*16S rRNA* bacteria	*nirK / nirS*	*nosZI / nosZII*	*NOS / NIR*
16S rRNA crenarchaea	-0.21												
ITS fungi	**0.71*****	**-0.32****											
nirK	**0.74*****	**-0.44*****	**0.66*****										
nirS	**0.63*****	**-0.41*****	**0.46*****	**0.75*****									
nosZI	**0.83*****	**-0.37****	**0.76*****	**0.84*****	**0.67*****								
nosZII	**0.78*****	**-0.43*****	**0.69*****	**0.73*****	**0.68*****	**0.93*****							
AOA	**0.30***	**0.50*****	**0.31***	0.05	-0.08	**0.28***	0.19						
AOB	**0.76*****	**-0.37****	**0.72*****	**0.84*****	**0.64*****	**0.82*****	**0.71*****	0.17					
COMAMMOX	**0.46*****	**-0.35****	**0.37****	**0.66*****	**0.74*****	**0.57*****	**0.48*****	-0.1	**0.65*****				
ITS fungi/*16S rRNA* bacteria	0.08	**-0.26***	**0.69*****	0.24	0.06	**0.27***	0.08	0.12	**0.31***	0.06			
nirK / nirS	**0.63*****	-0.18	**0.63*****	**0.82*****	**0.31***	**0.71*****	**0.51*****	0.21	**0.73*****	**0.36****	**0.29***		
nosZI / nosZII	**-0.51*****	**0.43****	**-0.41****	**-0.33***	**-0.49*****	**-0.58*****	**-0.81*****	-0.17	**-0.40****	**-0.32***	0.05	-0.05	
NOS / NIR	**0.51*****	-0.09	**0.56*****	**0.25***	0.04	**0.67*****	**0.55*****	**0.50*****	**0.39****	0.04	0.23	**0.43*****	**-0.41****

Appendix 6

Table S3.5. DESeq2 pairwise L vs VL treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

L vs VL	Class	Genus	Log2FoldChange	Enriched
	Acidobacteriae	Bryobacter *	-0.956761129	VL
	Acidobacteriae	Candidatus Solibacter	-0.718472891	VL
	Acidobacteriae	Occallatibacter	-1.9511978	VL
	Actinobacteria	Acidothermus *	-1.398408676	VL
	Actinobacteria	Actinocorallia	1.829641943	L
	Alphaproteobacteria	Rhodomicrobium	-0.573013317	VL
	Alphaproteobacteria	SWB02	4.414543245	L
	Anaerolineae	UTCFX1	5.378311079	L
	Bacteroidia	Mucilaginibacter	-1.374163089	VL
	Bacteroidia	Terrimonas	2.68270122	L
	Blastocatellia	RB41	1.625878578	L
	Desulfuromonadia	Geobacter ***	1.475529488	L
	Gammaproteobacteria	Acidibacter	0.807552211	L
	Gammaproteobacteria	GOUTA6	1.015588424	L
	Gammaproteobacteria	MND1	1.966952779	L
	Gammaproteobacteria	Rhodanobacter	-0.852001661	VL
	Nitrososphaeria	Candidatus Nitrosotalea **	-1.578885721	VL
	Nitrospiria	Nitrospira **	1.467978667	L
	Planctomycetes	Pir4 lineage	0.707113066	L
	Planctomycetes	Pirellula	0.677689391	L
	Planctomycetes	Singulisphaera	-4.483247863	VL
	Thermoleophilia	Conexibacter *	-0.637137499	VL
	Verrucomicrobiae	Ellin516	-1.021428315	VL
	Verrucomicrobiae	Ellin517	1.577396129	L

Table S3.6. DESeq2 pairwise L vs VL treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Acidobacteriae	Bryobacter *	-1.689818905	VL
	Acidobacteriae	Candidatus Koribacter	-1.793832118	VL
	Acidobacteriae	Candidatus Solibacter	-0.974114866	VL
	Acidobacteriae	Granulicella	-7.86943899	VL
	Acidobacteriae	Occallatibacter	-3.819874917	VL
	Acidobacteriae	Terracidiphilus	-5.096386412	VL
	Actinobacteria	Acidothermus *	-2.271666009	VL
	Actinobacteria	Actinocorallia	1.820783809	H
	Actinobacteria	Catenulispora *	-3.058147745	VL
	Actinobacteria	Kineosporia	-3.364701799	VL
	Actinobacteria	Streptacidiphilus	-5.969419145	VL
	Alphaproteobacteria	Asticcacaulis	-2.059119304	VL
	Alphaproteobacteria	Pedomicrobium	1.731728721	H
	Alphaproteobacteria	Rhodomicrobium	-0.909460111	VL
	Alphaproteobacteria	Roseiarcus	-1.213294293	VL
	Alphaproteobacteria	Sphingomonas	-1.138794416	VL
	Anaerolineae	UTCFX1	5.335242241	H
	Bacteroidia	Mucilaginibacter	-2.7824143	VL
	Bacteroidia	Ohtaekwangia	3.182368007	H
	Bacteroidia	OLB12	-1.228520597	VL
	Bacteroidia	Puia	-1.11898186	VL
	Bacteroidia	Terrimonas	3.169177341	H
	Blastocatellia	RB41	1.512826715	H
	Desulfuromonadia	Geobacter ***	1.563910321	H
	Gammaproteobacteria	Acidibacter	1.105193011	H
	Gammaproteobacteria	Dokdonella	-2.247549039	VL
	Gammaproteobacteria	GOUTA6	1.123494472	H
	Gammaproteobacteria	mle1-7	1.174308332	H
	Gammaproteobacteria	MND1	2.326188868	H
	Gammaproteobacteria	Polycyclovorans	3.51718829	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Gammaproteobacteria	Rhodanobacter	-1.585509955	VL
	Gemmatimonadetes	Gemmatimonas *	-0.925467766	VL
	Holophagae	Holophaga	-7.082043838	VL
	Ktedonobacteria	Thermosporothrix	-5.216590803	VL
	Leptospirae	Turneriella	4.757517029	H
	Nitrososphaeria	Candidatus Nitrocosmicus **	0.750098629	H
	Nitrososphaeria	Candidatus Nitrosotalea **	-2.363737053	VL
	Nitrospiria	Nitrospira **	1.854451382	H
	Planctomycetes	Aquisphaera	-0.935974542	VL
	Planctomycetes	Pir4 lineage	0.969419522	H
	Planctomycetes	Pirellula	1.06171475	H
	Planctomycetes	Schlesneria	-1.965949087	VL
	Polyangia	Haliangium	-0.820506426	VL
	Polyangia	Phaselicystis	0.596026755	H
	Thermoleophilia	Conexibacter *	-1.189663357	VL
	Verrucomicrobiae	ADurb.Bin063-1	-0.556878316	VL
	Verrucomicrobiae	Ellin516	-1.888511749	VL
	Verrucomicrobiae	Ellin517	1.919714729	H
	Verrucomicrobiae	Lacunisphaera	-1.042405643	VL
	Vicinamibacteria	Luteitalea	1.734257803	H

Table S3.7. DESeq2 pairwise VH vs VL treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Acidimicrobiia	Ilumatobacter	2.884461763	VH
	Acidobacteriae	Bryobacter *	-2.272256376	VL
	Acidobacteriae	Candidatus Koribacter	-3.047286809	VL
	Acidobacteriae	Candidatus Solibacter	-1.798239404	VL
	Acidobacteriae	Edaphobacter	-5.362118381	VL
	Acidobacteriae	Granulicella	-7.571445572	VL
	Acidobacteriae	Occallatibacter	-5.300880102	VL
	Acidobacteriae	Terracidiphilus	-6.336834806	VL
	Actinobacteria	Acidothermus *	-3.350106147	VL
	Actinobacteria	Actinocorallia	2.559682193	VH
	Actinobacteria	Catenulispora *	-4.912847871	VL
	Actinobacteria	Kineosporia	-5.775534712	VL
	Actinobacteria	Lechevalieria	5.586833958	VH
	Actinobacteria	Nocardioides *	0.882673697	VH
	Actinobacteria	Rhizocola	4.139430839	VH
	Actinobacteria	Streptacidiphilus	-5.616289114	VL
	Actinobacteria	Streptomyces *	1.391715898	VH
	Actinobacteria	Virgisporangium	4.897332617	VH
	Alphaproteobacteria	Acidicaldus	-3.748334732	VL
	Alphaproteobacteria	Candidatus Alysiosphaera	2.580164821	VH
	Alphaproteobacteria	Devosia	-1.63729367	VL
	Alphaproteobacteria	Ellin6055	4.436966033	VH
	Alphaproteobacteria	Microvirga	5.081791693	VH
	Alphaproteobacteria	Pedomicrobium	1.540359536	VH
	Alphaproteobacteria	Pseudolabrys	-0.433796924	VL
	Alphaproteobacteria	Pseudorhodoplanes	5.580164915	VH
	Alphaproteobacteria	Rhodomicrobium	-1.105058026	VL
	Alphaproteobacteria	Roseiarcus	-2.492106239	VL
	Alphaproteobacteria	Sphingomonas	-2.040478024	VL
	Alphaproteobacteria	SWB02	4.294657082	VH

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Anaerolineae	UTCFX1	6.197392527	VH
	Bacteroidia	Chryseolinea	4.802003619	VH
	Bacteroidia	Crocinitomix	4.378379609	VH
	Bacteroidia	Mucilaginibacter	-4.313651309	VL
	Bacteroidia	Ohtaekwangia	4.745891628	VH
	Bacteroidia	Puia	-2.784782135	VL
	Bacteroidia	Terrimonas	3.3583364	VH
	Bdellovibrionia	Bdellovibrio *	-1.316201868	VL
	Bdellovibrionia	OM27 clade	3.616979082	VH
	Blastocatellia	RB41	0.992763591	VH
	Gammaproteobacteria	Acidibacter	1.396764871	VH
	Gammaproteobacteria	Arenimonas	1.499390839	VH
	Gammaproteobacteria	MND1	2.308197112	VH
	Gammaproteobacteria	Nitrosospira ***	2.443359846	VH
	Gammaproteobacteria	Polaromonas *	4.651642082	VH
	Gammaproteobacteria	Polycyclovorans	3.401282359	VH
	Gammaproteobacteria	Rhodanobacter	-3.053101225	VL
	Gemmatimonadetes	Gemmatimonas *	-0.851505904	VL
	Holophagae	Holophaga	-6.789864129	VL
	Ktedonobacteria	Thermosporothrix	-5.789271754	VL
	Leptospirae	Turneriella	5.946204048	VH
	Myxococcia	Anaeromyxobacter ***	-1.397139147	VL
	Nitrososphaeria	Candidatus Nitrocosmicus **	1.268113317	VH
	Nitrososphaeria	Candidatus Nitrosotalea **	-5.153427554	VL
	Nitrospiria	Nitrospira **	1.720545238	VH
	Phycisphaerae	CL500-3	3.723582611	VH
	Phycisphaerae	Phycisphaera	-2.635367541	VL
	Planctomycetes	Aquisphaera	-1.349930275	VL
	Planctomycetes	Pir4 lineage	0.927292707	VH
	Planctomycetes	Pirellula	1.087556806	VH
	Planctomycetes	SH-PL14	4.92822865	VH
	Polyangia	Haliangium	-1.006286504	VL
	Polyangia	Nannocystis	4.222120505	VH
	Thermoanaerobaculia	Subgroup 10	2.645940284	VH

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Thermoleophilia	Conexibacter *	-1.025403223	VL
	Thermoleophilia	Solirubrobacter	1.906545956	VH
	Verrucomicrobiae	ADurb.Bin063-1	-1.521478214	VL
	Verrucomicrobiae	Candidatus Udaeobacter	-0.466786843	VL
	Verrucomicrobiae	Ellin516	-3.421127186	VL
	Verrucomicrobiae	Ellin517	2.145236573	VH
	Verrucomicrobiae	Pedosphaera	2.016954365	VH

Table S3.8. DESeq2 pairwise H vs L treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

L vs H	Class	Genus	Log2FoldChange	Enriched
	Actinobacteria	Acidothermus *	-0.747028318	H
	Alphaproteobacteria	Pedomicrobium	0.880551988	L
	Bacteroidia	Ohtaekwangia	5.67404899	L
	Bacteroidia	OLB12	-0.895625312	H
	Polyangia	Haliangium	-0.486227913	H
	Polyangia	Phaselicystis	0.501151175	L
	Verrucomicrobiae	Ellin516	-0.747104981	H

Table S3.9. DESeq2 pairwise VH vs H treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

VH vs H	Class	Genus	Log2FoldChange	Enriched
	Acidobacteriae	Candidatus Solibacter	-0.724040052	H
	Actinobacteria	Acidothermus *	-1.001877896	H
	Actinobacteria	Actinocorallia	0.824164537	VH
	Actinobacteria	Lechevalieria	5.788327888	VH
	Actinobacteria	Nocardioides *	0.948535737	VH
	Alphaproteobacteria	Ellin6055	3.484437422	VH
	Bacteroidia	Puia	-1.580631838	H
	Gammaproteobacteria	GOUTA6	-0.853771345	H
	Gammaproteobacteria	Rhodanobacter	-1.364865973	H
	Myxococcia	Anaeromyxobacter ***	-1.419307815	H
	Polyangia	Nannocystis	5.221526461	VH
	Verrucomicrobiae	ADurb.Bin063-1	-0.897442797	H
	Verrucomicrobiae	Candidatus Udaeobacter	-0.34657113	H

Table S3.10. DESeq2 pairwise VH vs H treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

L vs VH	Class	Genus	Log2FoldChange	Enriched
	Acidobacteriae	Candidatus Koribacter	-2.457053461	VH
	Acidobacteriae	Candidatus Solibacter	-0.834564161	VH
	Acidobacteriae	Occallatibacter	-3.094898076	VH
	Actinobacteria	Acidothermus	-1.698109335	VH
	Actinobacteria	Actinocorallia	0.952425304	L
	Actinobacteria	Lechevalieria	5.642261774	L
	Actinobacteria	Streptomyces *	1.278834764	L
	Alphaproteobacteria	Candidatus Alysiosphaera	1.428937914	L
	Alphaproteobacteria	Ellin6055	5.512642623	L
	Alphaproteobacteria	Hirschia	5.561008493	L
	Alphaproteobacteria	Pedomicrobium	0.797806177	L
	Alphaproteobacteria	Roseiarcus	-1.678146369	VH
	Bacteroidia	Chryseolinea	6.19413717	L
	Bacteroidia	Mucilaginibacter	-2.650180084	VH
	Bacteroidia	Ohtaekwangia	7.337546107	L
	Bacteroidia	Puia	-2.106078252	VH
	Bdellovibrionia	OM27 clade	3.138155655	L
	Gammaproteobacteria	Acidibacter *	0.877077124	L
	Gammaproteobacteria	MND1	0.563234454	L
	Gammaproteobacteria	Polaromonas *	4.621694337	L
	Gammaproteobacteria	Rhodanobacter	-1.962147837	VH
	Leptospirae	Turneriella	2.746785968	L
	Myxococcia	Anaeromyxobacter ***	-1.788735848	VH
	Nitrososphaeria	Candidatus Nitrosotalea **	-3.278141688	VH
	Phycisphaerae	SM1A02	-1.11310936	VH
	Planctomycetes	Pir4 lineage	0.466565852	L
	Planctomycetes	Pirellula	0.667573294	L
	Polyangia	Haliangium	-0.560974151	VH
	Polyangia	Nannocystis	3.867984344	L

L vs VH	Class	Genus	Log2FoldChange	Enriched
	Verrucomicrobiae	ADurb.Bin063-1	-1.016064139	VH
	Verrucomicrobiae	Ellin516	-2.183161032	VH

Table S3.11. DESeq2 pairwise L vs VL treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

L vs VL	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	Akenomyces	9.922084575	L
	Agaricomycetes	Minimedusa	24.58837372	L
	Agaricomycetes	unidentified_3513	-23.05138251	VL
	Dothideomycetes	Ophiosphaerella	1.941759592	L
	Leotiomycetes	Belonium	-8.42752144	VL
	Sordariomycetes	Microdochium	-2.212784895	VL

Table S3.12. DESeq2 pairwise L vs VH treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

L vs VH	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	Cotylidia	-5.39797247	VH
	Dothideomycetes	Drechslera	-6.57499319	VH
	Dothideomycetes	Ophiosphaerella	1.006153117	L
	Dothideomycetes	Pyrenophora	4.500836235	L
	Dothideomycetes	unidentified_6793	7.053306507	L
	Glomeromycetes	Glomus	2.929040339	L
	Glomeromycetes	unidentified_4174	9.573837749	L
	Leotiomycetes	Collembolispora	-3.439394678	VH
	Leotiomycetes	Glarea	23.50358458	L
	Leotiomycetes	Lachnum	-4.069054274	VH
	Leotiomycetes	Mollisia	-4.428031882	VH
	Leotiomycetes	unidentified_5	-2.287112717	VH
	Microbotryomycetes	Slooffia	-3.281254611	VH
	Olpidiomycetes	unidentified_7719	3.408359322	L
	Sordariomycetes	Dactylonectria	0.756009692	L

Table S3.13. DESeq2 pairwise H vs VL treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	Akenomyces	7.755155296	H
	Agaricomycetes	Auricularia	6.645340289	H
	Agaricomycetes	Cotylidia	-4.162463263	VL
	Agaricomycetes	Pholiotina	7.291535022	H
	Agaricomycetes	Serendipita	5.279464067	H
	Agaricomycetes	Tylospora	-23.84146248	VL
	Agaricomycetes	unidentified_3513	-23.47141761	VL
	Archaeosporomycetes	unidentified_276	-3.746345552	VL
	Dothideomycetes	Ophiosphaerella	2.019075279	H
	Glomeromycetes	Funneliformis	3.415438251	H
	Leotiomycetes	Belonium	-10.21080393	VL
	Leotiomycetes	Collembolispora	-2.967028852	VL
	Leotiomycetes	Gremmenia	-4.420956483	VL
	Leotiomycetes	Mollisia	-4.708197483	VL
	Leotiomycetes	unidentified_5	-2.486047319	VL
	Leotiomycetes	Vibrissea	-4.979668005	VL
	Rhizophydiomycetes	Rhizophydium	-7.246633652	VL
	Sordariomycetes	unidentified_832	1.592574337	H
	unidentified	unidentified	-1.550281239	VL

Table S3.14. DESeq2 pairwise H vs VH treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

H vs VH	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	Parasola	-6.214671066	VH
	Glomeromycetes	Glomus	2.253879472	H
	Glomeromycetes	unidentified_4174	8.741560497	H
	Leotiomycetes	Glarea	23.43687993	H
	Leotiomycetes	Pseudeurotium	-1.079857666	VH
	Sordariomycetes	Microdochium	-2.663552619	VH
	Tremellomycetes	Apiotrichum	-2.122835178	VH

Table S3.15. DESeq2 pairwise VL vs VH treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

VL vs VH	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	Akenomyces	6.104795638	VL
	Agaricomycetes	Cotylidia	-6.064531459	VH
	Agaricomycetes	Parasola	-4.77610876	VH
	Agaricomycetes	Pholiotina	8.816088847	VL
	Agaricomycetes	Tylospora	-23.73321384	VH
	Agaricomycetes	unidentified_90	-3.108813241	VH
	Archaeosporomycetes	unidentified_276	-4.846882353	VH
	Dothideomycetes	Drechslera	-8.122014102	VH
	Dothideomycetes	Ophiosphaerella	2.779896047	VL
	Dothideomycetes	unidentified_6793	4.040159959	VL
	Exobasidiomycetes	Tilletiaria	-4.235050035	VH
	Glomeromycetes	Claroideoglomus	2.932429638	VL
	Glomeromycetes	Funneliformis	3.663778933	VL
	Glomeromycetes	Glomus	4.163068889	VL
	Glomeromycetes	unidentified_4174	9.295710363	VL
	Glomeromycetes	unidentified_77	-1.467314739	VH
	Leotiomycetes	Belonium	-9.662873481	VH
	Leotiomycetes	Collembolispora	-4.638517699	VH
	Leotiomycetes	Glarea	23.15567672	VL
	Leotiomycetes	Lachnum	-2.838525867	VH
	Leotiomycetes	Mollisia	-6.546152272	VH
	Leotiomycetes	Pseudeurotium	-0.663455834	VH
	Leotiomycetes	unidentified_5	-2.342016968	VH
	Leotiomycetes	Vibrissea	-4.977710167	VH
	Microbotryomycetes	Slooffia	-2.15689217	VH
	Microbotryomycetes	unidentified_199	3.415626168	VL
	Mucoromycetes	Mucor	-2.5099534	VH
	Rhizophydiomycetes	Rhizophydium	-4.90765541	VH
	Sordariomycetes	Dactylonectria	0.871116107	VL
	Sordariomycetes	Microdochium	-3.260815589	VH

VL vs VH	Class	Genus	Log2FoldChange	Enriched
	Sordariomycetes	Pleurophragmium	2.023871372	VL
	Sordariomycetes	Trichoderma *	-2.569820553	VH
	Sordariomycetes	unidentified_3145	-1.609818467	VL
	Sordariomycetes	unidentified_832	1.757995532	VH
	Tremellomycetes	Apiotrichum	-1.888930342	VL
	Tremellomycetes	Cryptococcus	-2.683871136	VL
	Tremellomycetes	Papiliotrema	-2.795703681	VL
	Tremellomycetes	Solicoccozyma	-1.269736981	VL
	Tremellomycetes	Vishniacozyma	-2.327834325	VL
	unidentified	unidentified	-1.549310707	VL

Appendix 8

Table S4.1. qPCR cycle summary for all genes, including primers, primer sequencing and concentration; standard starting concentration; cycle summary; final qPCR run efficiency and references.

Gene	Primers	Primer Sequences (5' - 3')	Primer Concentration (µM)	Standard concentration	Positive Control	Cycle Conditions			Efficiency (%)	R^2	References
						95 °C 5 minutes	Denaturation step				
	341F	CCTACGGGNGGCWGCAG				95 °C 40 seconds 55.6 °C 1 minute 72 °C 30 seconds 80 °C 3 seconds	Data acquisition	39 cycles			Klindworth et al., 2013
16S rRNA bacteria			1.5	0.5×10^8	*Pseudomonas Aeruginosa*				91.9%	0.997	
	785R	GACTACHVGGGTATCTAATCC				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Klindworth et al., 2013
						95 °C 5 minutes	Denaturation step				
	771F	ACGGTGAGGGATGAAAGCT				95 °C 30 seconds 54 °C 1 minute 72 °C 1 minute 82 °C 3 seconds	Data acquisition	39 cycles			Ochsenreiter et al., 2003
16S rRNA crenarchaea			0.2	0.5×10^8					88.6%	0.999	
	957R	CGGCGTTGACTCCAATTG				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Ochsenreiter et al., 2003
						95 °C 2 minutes	Denaturation step				
	ITS86F	GTGAATCATCGAATCTTTGAA				95 °C 30 seconds 54 °C 30 seconds 72 °C 30 seconds	Data acquisition	39 cycles			De Beeck et al., 2014
ITS fungi			0.2	0.5×10^8					98.7%	0.992	
	ITS4R	TCCTCCGCTTATTGATATGC				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			De Beeck et al., 2014
						95 °C 10 minutes	Denaturation step				
	nirK1040F	GCCTCGATCAGRTTRTGGTT				95 °C 10 seconds 60 °C 30 seconds 72 °C 10 seconds 85 °C 2 seconds	Data acquisition	39 cycles			Hallin et al., 2009
nirK			0.2	3.22×10^9	*Ensifer melloti*				89.1%	0.999	
	nirK876R	ATYGGCGGVCAYGGCGA				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Hallin et al., 2009
						95 °C 15 minutes	Denaturation step				
	cd3AF	GTSAACGTSAAGGARACSGG				95 °C 15 seconds 63 °C 30 seconds Decrease temperature by -1°C 72 °C 30 seconds 80 °C 30 seconds		6 cycles			Throback et al., 2004; Yergeau et al., 2004
nirS			0.5	0.5×10^8	*Pseudomonas Aeruginosa*				84.7%	0.997	
	R3cd	GASTTCGGRTGSGTCTTGA				95 °C 15 seconds 58 °C 30 seconds 72 °C 30 seconds 80 °C 30 seconds	Data acquisition	40 cycles			Throback et al., 2004
						95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			
						95 °C 15 minutes	Denaturation step				
	nosZ2F	WCSYTGTTCMTCGACAGCCAG				95 °C 15 seconds 65 °C 30 seconds Decrease temperature by -1°C 72 °C 30 seconds		6 cycles			Henry et al., 2006
nosZI			0.2	0.5 x 108	*Pseudomonas Aeruginosa*				84.0%	0.993	
	nosZ2R	ATGTCGATCARCTGUKCRTTYTC				95 °C 15 seconds 60 °C 30 seconds 72 °C 1 minute	Data acquisition	29 cycles			Henry et al., 2006
						95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			
						95 °C 15 minutes	Denaturation step				
	nosZ II F	CTIGGICCIYTKCAYAC				95 °C 15 seconds 54 °C 30 seconds 72 °C 30 seconds 80 °C 30 seconds	Data acquisition	39 cycles			Jones et al.; 2013
nosZII			2	5.38×10^9	*Guarianthe Aurantiaca*				81.5%	1.000	
	nosZ II R	GCIGARCARAAITCBGTR C				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Jones et al.; 2013
						95 °C 10 minutes	Denaturation step				
	crenamoA23F	ATGGTCTGGCTWAGACG				94 °C 45 seconds 50 °C 45 seconds 72 °C 45 seconds 80 °C 15 seconds	Data acquisition	39 cycles			Tourna et al., 2008
AOA			1	0.5×10^8	*Nitrosocosmicus Franklandus*				85.0%	0.995	
	crenamoA616R	GCCATCCATCTGTATGTCCA				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Tourna et al., 2008
						94 °C 5 minutes	Denaturation step				
	amoA1F	GGGGTTTCTACTGGTGGT				94 °C 30 seconds 55 °C 45 seconds 72 °C 45 seconds 82 °C 2 seconds	Data acquisition	39 cycles			Rothhauwe et al.,1998
AOB			1	4.89×10^7					89.6%	0.997	
	amoA2R	CCCCTCBGSAAAVCCTTCTTC				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Hornek et al., 2006
						95 °C 5 minutes	Denaturation step				
	Ntsp_amoA_162F	GGATTTCTGGNTSGATTGGA				95 °C 30 seconds 56 °C 30 seconds 72 °C 45 seconds 82 °C 2 seconds	Data acquisition	39 cycles			Fowler et al., 2018
COMAMMOX			1	0.5×10^8					88.1%	0.999	
	Ntsp_amoA_359R	WAGTTNGACCACCASTACCA				95 °C 5 seconds 65 °C 5 seconds 95 °C	Data acquisition	Melt Curve			Fowler et al., 2018

Table S4.2. Illumina sequencing library preparation PCR information including primers with overhangs for PCR1, primer concentrations and cycle information. For PCR2, only cycle conditions are included since INDEX primers with unique barcodes were used.

PCR 1

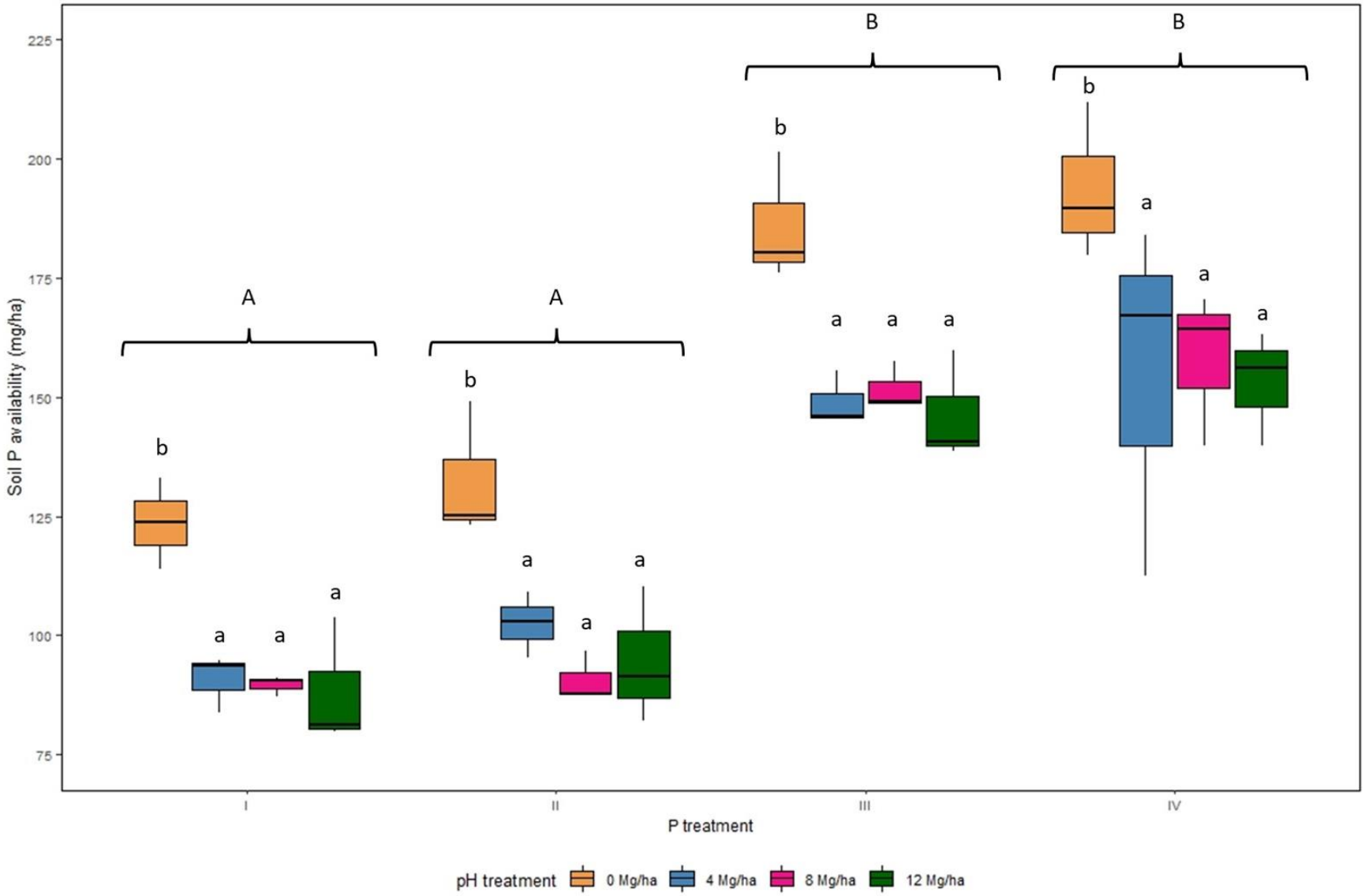
Gene	Primers	Primer Sequences (5' - 3')	Primer Concentration (µM)	Cycle Conditions			
16S rRNA bacteria	515F	Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGYCAGCMGCCGCGGTAA]	0.2	94 °C	3 minutes	Denaturation step	
				94 °C	45 seconds		25 cycles
				50 °C	45 seconds		
				72 °C	1 minute		
	926R	Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [CCGYCAARTYMTTTRAGTTT]		72 °C	5 minutes	Elongation step	
				15 °C	Hold at		
ITS fungi	ITS86F	Forward overhang: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG [GTGAATCATCGAATCTTTGAA]	0.2	95 °C	3 minutes	Denaturation step	
				95 °C	30 seconds		25 cycles
				54 °C	30 seconds		
				72 °C	30 seconds		
	ITS4R	Reverse overhang: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [TCCTCCGCTTATTGATATGC]		72 °C	5 minutes	Elongation step	
				15 °C	Hold at		

PCR 2

Gene	Primer Concentration (µM)	Cycle Conditions		
16S rRNA bacteria	0.2	95 °C	3 minutes	Denaturation step
		95 °C	30 seconds	7 cycles
		55 °C	30 seconds	
		72 °C	30 seconds	
		72 °C	5 minutes	Elongation step
ITS fungi	0.2	95 °C	3 minutes	Denaturation step
		95 °C	30 seconds	7 cycles
		55 °C	30 seconds	
		72 °C	30 seconds	
		72 °C	5 minutes	Elongation step

Appendix 9

Figure S4.1. Boxplot to present pH and P treatment effect on soil P availability. P availability is grouped by P treatment on the X axis, with all four pH treatment (in colour) within each P level. Uppercase letters indicate significant difference (p value ≤ 0.05) of P availability across P treatments only. Lowercase letters indicate significant difference (p value ≤ 0.05) of pH treatments within a P treatment.



Appendix 10

Table S4.3. Spearman's correlation coefficients of potential denitrification fluxes, potential nitrification rate with soil physiochemical properties. Significant correlations highlighted in bold (* p value < 0.05; ** p value < 0.01; *** p value < 0.001). TC - Total carbon, TN - Total nitrogen, SOC - Soil organic carbon, PNA – Potential nitrification assay.

	Potential N_2O	Total Denitrification ($N_2O + N_2$)	Potential N_2	Product Ratio (N_2O/N_2O+N_2)	PNA	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	S	TC	TN
($N_2O + N_2$)	**0.84*** **																
Potential N_2	0	**0.47*** **															
(N_2O/N_2O+N_2)	0.13	**-0.39****	**-0.91*** **														
PNA	-0.12	-0.08	0.15	-0.1													
Al	**-0.46****	**-0.38****	0.04	-0.13	-0.03												
Ca	-0.11	-0.05	0.26	-0.18	**0.82*** **	0.12											
Co	**-0.32***	**-0.34***	-0.11	0.01	0.21	**0.71*** **	0.2										
Cu	-0.2	-0.12	0.26	-0.16	**0.67*** **	0.12	**0.65*** **	0.12									
Fe	0.1	0.06	-0.12	0.15	**-0.76*** **	-0.08	**-0.83*** **	**-0.31***	**-0.44****								
K	-0.23	-0.13	**0.29***	-0.27	**0.73*** **	0.24	**0.85*** **	**0.31***	**0.66*** **	**-0.73*** **							
Mg	-0.07	-0.03	0.2	-0.14	**0.88*** **	-0.07	**0.92*** **	0.14	**0.65*** **	**-0.82*** **	**0.80*** **						
Mn	0.05	0.02	0.03	0	**0.77*** **	0.09	**0.77*** **	**0.51*** **	**0.48*** **	**-0.79*** **	**0.66*** **	**0.79*** **					
Na	**-0.54*** **	**-0.44****	0.07	-0.11	0.26	**0.74*** **	**0.35***	**0.53*** **	**0.33***	-0.25	**0.42****	0.24	0.26				
S	-0.18	-0.18	0.08	-0.07	-0.2	**0.37****	-0.18	0.12	0.1	0.29*	-0.12	-0.23	**-0.33***	0.06			
TC	0.05	0.22	**0.37****	**-0.35***	0.17	-0.08	0.19	-0.15	**0.35***	0	0.18	0.17	-0.02	-0.04	0.17		
TN	-0.08	0	0.19	-0.21	**0.40****	0.07	**0.50*** **	0.04	**0.47*** **	**-0.38****	**0.56*** **	0.50***	0.27	0.23	-0.02	**0.74*** **	
SOC	-0.18	-0.07	0.26	-0.22	**0.31***	0.12	0.21	0.06	**0.39****	-0.06	0.23	0.19	0.03	0.27	0.18	**0.36***	0.25

Table S4.4. Spearman's correlation coefficients of target genes with potential denitrification fluxes, potential nitrification rate with soil physiochemical properties. Significant correlations highlighted in bold (* p value < 0.05; ** p value < 0.01; *** p value < 0.001). TC - Total carbon, TN - Total nitrogen, SOC - Soil organic carbon, PNA – Potential nitrification assay.

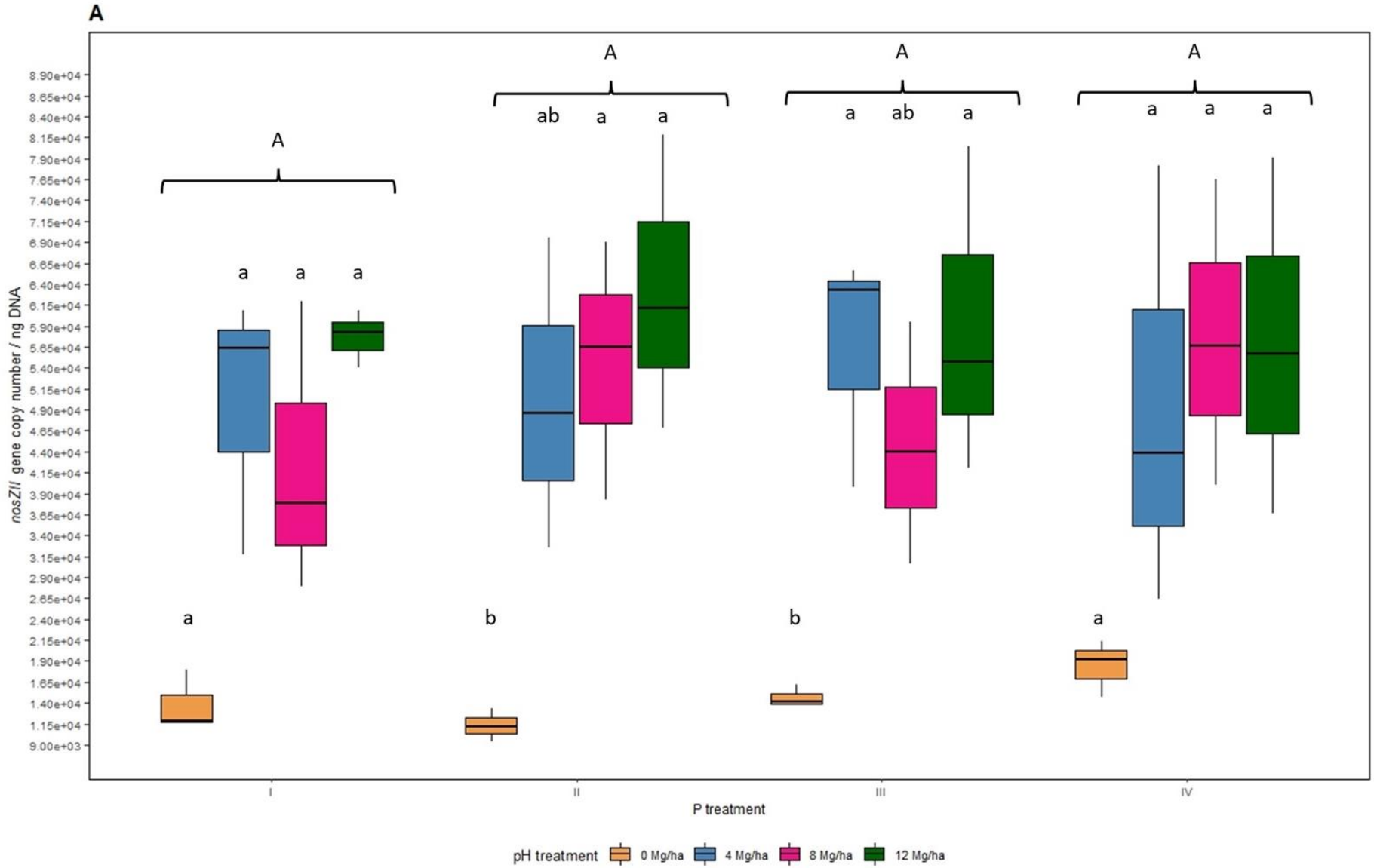
	Potential N_2O	Total Denitrification ($N_2O + N_2$)	Potential N_2	Product Ratio (N_2O/N_2O+N_2)	PNA	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	S	TC	TN	SOC	*16S rRNA* bacteria	*16S rRNA* crenarchaea	*ITS* fungi	*nirK*	*nirS*	*nosZI*	*nosZII*	AOA	AOB
16S rRNA bacteria	**-0.32***	**-0.35***	-0.21	0.15	0.07	**0.51*****	0.03	**0.34***	0.11	0.02	0.01	0.01	0.05	**0.48*****	0.2	-0.05	0.01	0.05									
16S rRNA crenarchaea	-0.09	-0.18	**-0.30***	0.23	**-0.61*****	0.04	**-0.69*****	-0.14	**-0.45****	**0.72*****	**-0.67*****	**-0.69*****	**-0.67*****	-0.2	**0.39****	-0.09	**-0.38****	-0.14	**0.46*****								
ITS fungi	-0.17	-0.21	-0.28	0.18	**-0.61*****	**0.29***	**-0.66*****	0.05	**-0.42****	**0.63*****	**-0.58*****	**-0.66*****	**-0.56*****	0.04	**0.29***	-0.19	**-0.36***	-0.18	**0.61*****	**0.84*****							
nirK	-0.24	-0.23	-0.01	0.01	**0.56*****	0.2	**0.62*****	0.18	**0.40****	**-0.55*****	**0.60*****	**0.63*****	**0.54*****	**0.53*****	**-0.40****	-0.14	**0.30***	0.07	**0.31***	**-0.40****	-0.2						
nirS	**-0.51*****	**-0.49*****	-0.16	0.06	**0.40****	**0.56*****	**0.35***	**0.56*****	0.2	**-0.36***	**0.37****	**0.35***	**0.42****	**0.76*****	-0.06	-0.16	0.13	0.07	**0.72*****	0.01	0.21	**0.67*****					
nosZI	-0.28	**-0.30***	-0.18	0.1	0.01	**0.55*****	0	**0.44****	-0.04	0	0.04	-0.03	0.11	**0.44****	0.19	-0.19	-0.08	-0.03	**0.83*****	**0.42****	**0.58*****	**0.31***	**0.71*****				
nosZII	-0.25	-0.25	-0.05	0.05	**0.54*****	0.18	**0.63*****	0.27	**0.33***	**-0.58*****	**0.57*****	**0.66*****	**0.64*****	**0.46*****	**-0.41****	-0.21	0.23	-0.05	**0.42****	**-0.34***	-0.13	**0.90*****	**0.73*****	**0.42****			
AOA	-0.21	-0.28	-0.24	0.19	-0.14	0.28	-0.18	0.17	-0.15	0.19	-0.17	-0.11	-0.16	0.26	0.13	-0.12	0.01	-0.03	**0.69*****	**0.50*****	**0.62*****	0.26	**0.49*****	**0.61*****	**0.37****		
AOB	**-0.45****	**-0.45****	-0.14	0.06	**0.31***	**0.66*****	**0.32***	**0.62*****	0.14	**-0.34***	**0.37***	**0.29***	**0.36***	**0.75*****	0.04	-0.18	0.13	0.07	**0.69*****	0.02	0.27	**0.62*****	**0.92*****	**0.70*****	**0.67*****	**0.52*****	
COMAMMOX	-0.24	-0.2	-0.02	0	**0.56*****	0.2	**0.55*****	0.28	**0.37****	**-0.47*****	**0.46****	**0.59*****	**0.56*****	**0.44****	-0.13	0.04	0.22	0.05	**0.63*****	-0.12	0	**0.62*****	**0.74*****	**0.54*****	**0.73*****	0.28	**0.63*****

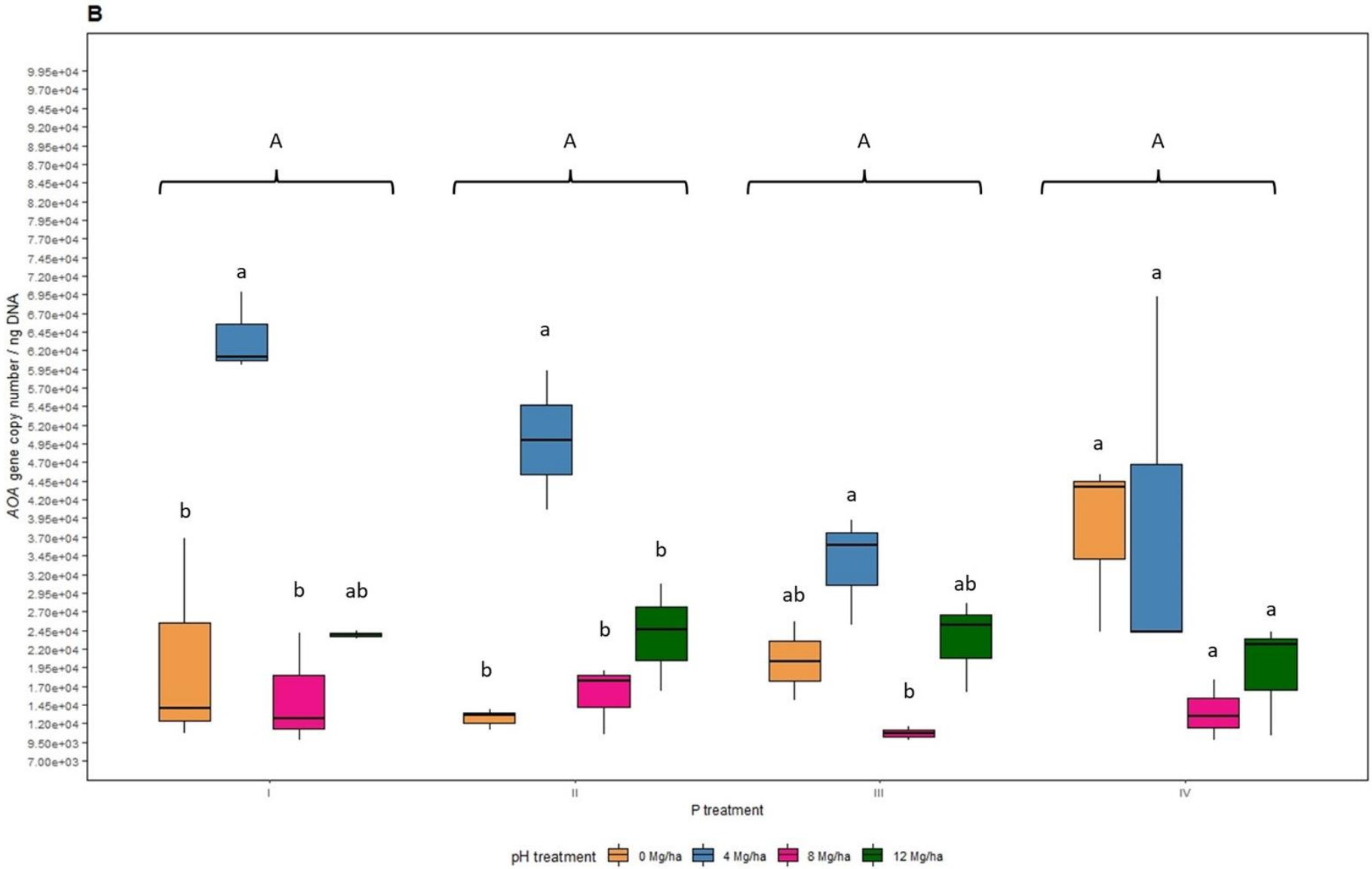
Table S4.5. Spearman's correlation coefficients of target genes with potential denitrification fluxes, potential nitrification rate with soil physiochemical properties. Significant correlations highlighted in bold (* p value < 0.05; ** p value < 0.01; *** p value < 0.001). TC - Total carbon, TN - Total nitrogen, SOC - Soil organic carbon, PNA – Potential nitrification assay.

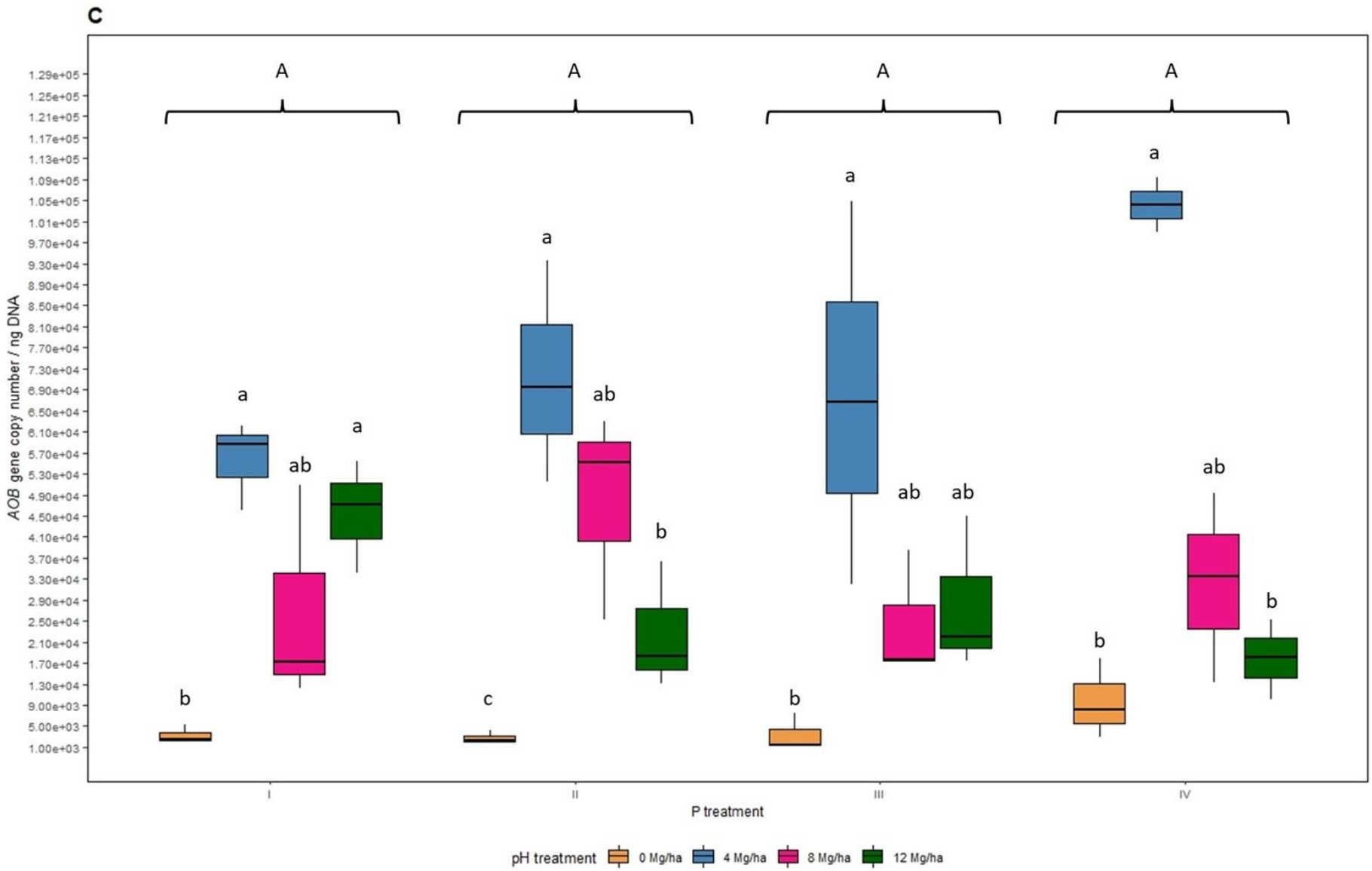
	Potential N_2O	Total Denitrification ($N_2O + N_2$)	Potential N_2	Product Ratio (N_2O/N_2O+N_2)	PNA	Al	Ca	Co	Cu	Fe	K	Mg	Mn	Na	S	TC	TN	SOC	*ITS* fungi/*16S rRNA* bacteria	*nirK / nirS*	*nosZI / nosZII*
ITS fungi/*16S rRNA* bacteria	0.01	-0.02	-0.2	0.13	**-0.84*****	0.05	**-0.82*****	-0.17	**-0.56*****	**0.79*****	**-0.73*****	**-0.84*****	**-0.73*****	-0.26	0.22	-0.16	**-0.46****	-0.24			
nirK / nirS	0.15	0.21	0.22	-0.11	**0.31***	**-0.29***	**0.38****	**-0.33***	**0.33***	-0.22	**0.30***	**0.38****	0.19	-0.02	**-0.39****	0	0.17	0.14	**-0.34***		
nosZI / nosZII	-0.07	-0.1	-0.22	0.11	**-0.65*****	0.26	**-0.74*****	0.12	**-0.52*****	**0.65*****	**-0.65*****	**-0.78*****	**-0.58*****	-0.13	**0.45****	-0.14	**-0.45****	-0.15	**0.76*****	**-0.54*****	
NOS / NIR	**0.50*****	**0.46*****	0.1	0.01	**-0.32***	**-0.51*****	-0.22	**-0.38****	-0.23	0.24	**-0.31***	-0.16	-0.13	**-0.73*****	0.03	-0.07	-0.26	**-0.30***	0.28	0.03	0.13

Appendix 11

Figure S4.2. Boxplot to present pH and P treatment effect on gene abundances. **S4.2A.** Interaction effect on *nosZII* abundances. **S4.2B.** Interaction effect on AOA abundances. **S4.2C.** Interaction effect on AOB abundances. Gene abundances are grouped by P treatment on the X axis, with all four pH treatment (in colour) within each P level. Uppercase letters indicate significant difference (p value ≤ 0.05) of P availability across P treatments only. Lowercase letters indicate significant difference (p value ≤ 0.05) of pH treatments within a P treatment.




B
AOA gene copy number / ng DNA
9.95e+04
9.70e+04
9.45e+04
9.20e+04
8.95e+04
8.70e+04
8.45e+04
8.20e+04
7.95e+04
7.70e+04
7.45e+04
7.20e+04
6.95e+04
6.70e+04
6.45e+04
6.20e+04
5.95e+04
5.70e+04
5.45e+04
5.20e+04
4.95e+04
4.70e+04
4.45e+04
4.20e+04
3.95e+04
3.70e+04
3.45e+04
3.20e+04
2.95e+04
2.70e+04
2.45e+04
2.20e+04
1.95e+04
1.70e+04
1.45e+04
1.20e+04
9.50e+03
7.00e+03
A
A
A
A
a
b
b
ab
b
a
b
b
ab
a
b
ab
a
a
a
a
I
II
III
IV
P treatment
pH treatment
0 Mg/ha
4 Mg/ha
8 Mg/ha
12 Mg/ha


C
A
A
A
A
a
ab
a
b
a
ab
b
c
a
ab
ab
b
a
b
ab
b
AOB gene copy number / ng DNA
1.29e+05
1.25e+05
1.21e+05
1.17e+05
1.13e+05
1.09e+05
1.05e+05
1.01e+05
9.70e+04
9.30e+04
8.90e+04
8.50e+04
8.10e+04
7.70e+04
7.30e+04
6.90e+04
6.50e+04
6.10e+04
5.70e+04
5.30e+04
4.90e+04
4.50e+04
4.10e+04
3.70e+04
3.30e+04
2.90e+04
2.50e+04
2.10e+04
1.70e+04
1.30e+04
9.00e+03
5.00e+03
1.00e+03
I
II
III
IV
P treatment
pH treatment
0 Mg/ha
4 Mg/ha
8 Mg/ha
12 Mg/ha

Appendix 12

Table S4.6. DESeq2 pairwise VL vs L treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Acidimicrobiia	Iamia	5.48	VL
	Acidimicrobiia	CL500-29 marine group	6.52	VL
	Acidobacteriae	Occallatibacter	-3.75	L
	Acidobacteriae	Candidatus Solibacter	1.63	VL
	Acidobacteriae	Granulicella	-3.60	L
	Acidobacteriae	Acidipila	-2.89	L
	Acidobacteriae	Edaphobacter	-5.29	L
	Acidobacteriae	Terracidiphilus	-4.55	L
	Acidobacteriae	Candidatus Koribacter	1.76	VL
	Acidobacteriae	Telmatobacter	-9.10	L
	Actinobacteria	Catenulispora *	-4.07	L
	Actinobacteria	Dactylosporangium	4.96	VL
	Actinobacteria	Actinocorallia	3.90	VL
	Actinobacteria	Mycobacterium	-0.81	L
	Actinobacteria	Kutzneria	5.98	VL
	Actinobacteria	Nakamurella	2.87	VL
	Actinobacteria	Pseudonocardia	7.36	VL
	Actinobacteria	Flexivirga	-6.41	L
	Actinobacteria	Nocardioides *	3.53	VL
	Actinobacteria	Kineosporia	6.17	VL
	Actinobacteria	Actinospica	-4.28	L
	Actinobacteria	Amycolatopsis	6.82	VL
	Actinobacteria	Streptomyces *	8.83	VL
	Actinobacteria	Aeromicrobium	5.02	VL
	Actinobacteria	Acidothermus *	-2.99	L
	Actinobacteria	Terrabacter	3.47	VL

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Alphaproteobacteria	Roseiarcus	-3.87	L
	Alphaproteobacteria	Acidicaldus	-0.84	L
	Alphaproteobacteria	Altererythrobacter	4.63	VL
	Alphaproteobacteria	Sphingomonas	1.96	VL
	Alphaproteobacteria	Bradyrhizobium	7.73	VL
	Alphaproteobacteria	SWB02	6.93	VL
	Alphaproteobacteria	PMMR1	-5.04	L
	Alphaproteobacteria	Phenylobacterium	-1.59	L
	Alphaproteobacteria	Dongia	7.59	VL
	Alphaproteobacteria	Nordella	7.02	VL
	Alphaproteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	5.60	VL
	Alphaproteobacteria	Candidatus Alysiosphaera	3.55	VL
	Alphaproteobacteria	Mesorhizobium	4.49	VL
	Alphaproteobacteria	1174-901-12	-4.52	L
	Alphaproteobacteria	Labrys	6.68	VL
	Alphaproteobacteria	Devosia	1.51	VL
	Alphaproteobacteria	Methylovirgula	-6.61	L
	Alphaproteobacteria	Endobacter	-5.26	L
	Alphaproteobacteria	Hirschia	6.33	VL
	Alphaproteobacteria	Pseudolabrys	2.57	VL
	Alphaproteobacteria	Pedomicrobium	8.19	VL
	Alphaproteobacteria	Methylocapsa	5.44	VL
	Alphaproteobacteria	Reyranella	8.88	VL
	Alphaproteobacteria	Hyphomicrobium	3.57	VL
	Alphaproteobacteria	Acidiphilium	1.72	VL
	Alphaproteobacteria	Bauldia	4.88	VL
	Anaerolineae	UTCFX1	4.35	VL
	Bacteroidia	Parafilimonas	4.43	VL

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Bacteroidia	Chitinophaga	6.02	VL
	Bacteroidia	Terrimonas	8.77	VL
	Bacteroidia	UTBCD1	6.01	VL
	Bacteroidia	Mucilaginibacter	1.36	VL
	Bacteroidia	Chryseolinea	6.82	VL
	Bacteroidia	Ferruginibacter	5.62	VL
	Bacteroidia	Pseudoflavitalea	4.72	VL
	Bacteroidia	OLB12	5.20	VL
	Bacteroidia	Ohtaekwangia	6.35	VL
	Bacteroidia	Niastella	7.47	VL
	Blastocatellia	JGI 0001001-H03	5.28	VL
	Blastocatellia	RB41	7.57	VL
	Chloroflexia	Nitrolancea	-7.59	L
	Chthonomonadetes	Chthonomonas	4.77	VL
	Deinococci	Deinococcus	-2.24	L
	Fibrobacteria	possible genus 04	5.77	VL
	Gammaproteobacteria	Candidatus Ovatusbacter	-4.63	L
	Gammaproteobacteria	Pseudomonas	4.03	VL
	Gammaproteobacteria	Burkholderia-Caballeronia-Paraburkholderia	1.18	VL
	Gammaproteobacteria	Polycyclovorans	5.77	VL
	Gammaproteobacteria	IS-44	7.16	VL
	Gammaproteobacteria	Chujaibacter	-10.26	L
	Gammaproteobacteria	Nevskia	1.96	VL
	Gammaproteobacteria	Acidibacter	-0.60	L
	Gammaproteobacteria	Leptothrix	3.81	VL
	Gammaproteobacteria	MND1	8.25	VL
	Gammaproteobacteria	Dokdonella	8.52	VL
	Gammaproteobacteria	Ellin6067	6.00	VL
	Gammaproteobacteria	Cellvibrio	4.01	VL
	Gammaproteobacteria	mle1-7	6.05	VL

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Gammaproteobacteria	Tahibacter	5.29	VL
	Gammaproteobacteria	Rhodanobacter	-2.25	L
	Gammaproteobacteria	GOUTA6	8.02	VL
	Gammaproteobacteria	Nitrosospira	7.89	VL
	Gammaproteobacteria	Steroidobacter	5.80	VL
	Gammaproteobacteria	Arenimonas	4.72	VL
	Gemmatimonadetes	Gemmatimonas *	2.29	VL
	Holophagae	Holophaga	3.18	VL
	Holophagae	Geothrix	4.50	VL
	Ktedonobacteria	Thermosporothrix	-4.12	L
	Ktedonobacteria	FCPS473	-2.58	L
	Ktedonobacteria	JG30a-KF-32	-4.77	L
	Ktedonobacteria	1921-3	6.22	VL
	Ktedonobacteria	G12-WMSP1	4.56	VL
	Ktedonobacteria	Ktedonobacter	-5.41	L
	Leptospirae	Turneriella	5.02	VL
	Myxococcia	KD3-10	5.31	VL
	Myxococcia	Anaeromyxobacter	1.68	VL
	Nitrospiria	Nitrospira **	1.52	VL
	Phycisphaerae	SM1A02	5.81	VL
	Phycisphaerae	Phycisphaera	4.49	VL
	Planctomycetes	Candidatus Nostocoida	-1.60	L
	Planctomycetes	Pir4 lineage	9.47	VL
	Planctomycetes	Zavarzinella	5.65	VL
	Planctomycetes	Gemmata	4.69	VL
	Planctomycetes	SH-PL14	5.02	VL
	Planctomycetes	Planctopirus	5.52	VL
	Planctomycetes	Pirellula	7.70	VL
	Planctomycetes	Schlesneria	1.80	VL
	Planctomycetes	Aquisphaera	-3.18	L
	Planctomycetes	Fimbriiglobus	4.67	VL
	Polyangia	Phaselicystis	5.67	VL

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Polyangia	Sandaracinus	5.68	VL
	Polyangia	Pajaroellobacter	-1.87	L
	Polyangia	Haliangium	2.11	VL
	Spirochaetia	Spirochaeta	5.29	VL
	Spirochaetia	Spirochaeta 2	6.83	VL
	Thermoanaerobaculia	Subgroup 10	5.95	VL
	Thermoleophilia	Solirubrobacter	5.51	VL
	Thermoleophilia	Gaiella	1.58	VL
	Thermoleophilia	Conexibacter *	-2.32	L
	Verrucomicrobiae	ADurb.Bin063-1	3.62	VL
	Verrucomicrobiae	Luteolibacter	3.88	VL
	Verrucomicrobiae	Candidatus Xiphinematobacter	4.14	VL
	Verrucomicrobiae	Roseimicrobium	4.88	VL
	Verrucomicrobiae	Chthoniobacter	1.25	VL
	Verrucomicrobiae	DEV008	4.75	VL
	Verrucomicrobiae	Ellin516	1.24	VL
	Verrucomicrobiae	Candidatus Udaeobacter	10.10	VL
	Verrucomicrobiae	Ellin517	7.12	VL
	Verrucomicrobiae	Opitutus	7.96	VL
	Vicinamibacteria	Luteitalea	7.68	VL

Table S4.7. DESeq2 pairwise H vs VL treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Acidimicrobiia	Ilumatobacter	4.95	H
	Acidimicrobiia	Iamia	5.51	H
	Acidimicrobiia	CL500-29 marine group	8.98	H
	Acidobacteriae	Occallatibacter	-11.85	VL
	Acidobacteriae	Candidatus Koribacter	-4.79	VL
	Acidobacteriae	Granulicella	-10.83	VL
	Acidobacteriae	Acidipila	-10.22	VL
	Acidobacteriae	Bryobacter *	-2.04	VL
	Acidobacteriae	Edaphobacter	-9.70	VL
	Acidobacteriae	Terracidiphilus	-11.62	VL
	Acidobacteriae	Telmatobacter	-9.78	VL
	Actinobacteria	Catenulispora *	-9.32	VL
	Actinobacteria	Lysinimonas	4.33	H
	Actinobacteria	Dactylosporangium	4.68	H
	Actinobacteria	Pseudonocardia	7.43	H
	Actinobacteria	Streptomyces *	7.88	H
	Actinobacteria	Microlunatus	5.42	H
	Actinobacteria	Actinocorallia	7.15	H
	Actinobacteria	Mycobacterium	-1.63	VL
	Actinobacteria	Lechevalieria	7.07	H
	Actinobacteria	Kribbella	3.72	H
	Actinobacteria	Nakamurella	2.29	H
	Actinobacteria	Jatrophihabitans	-2.12	VL
	Actinobacteria	Microbacterium	3.71	H
	Actinobacteria	Modestobacter	3.67	H
	Actinobacteria	Blastococcus	-1.23	VL
	Actinobacteria	Flexivirga	-7.11	VL
	Actinobacteria	Nocardioides *	3.49	H
	Actinobacteria	Cellulomonas	4.25	H
	Actinobacteria	Friedmanniella	3.83	H
	Actinobacteria	Actinospica	-10.43	VL
	Actinobacteria	Amycolatopsis	5.09	H
	Actinobacteria	Crossiella	-5.48	VL
	Actinobacteria	Aeromicrobium	6.64	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Actinobacteria	Acidothermus *	-9.25	VL
	Actinobacteria	Terrabacter	2.93	H
	Alphaproteobacteria	Rhodomicrobium	5.45	H
	Alphaproteobacteria	Methylobacterium-Methylorubrum	-2.30	VL
	Alphaproteobacteria	Roseiarcus	-12.75	VL
	Alphaproteobacteria	Sphingomonas	0.98	H
	Alphaproteobacteria	Acidicaldus	-7.13	VL
	Alphaproteobacteria	Pseudorhodoplanes	3.72	H
	Alphaproteobacteria	Hyphomicrobium	2.91	H
	Alphaproteobacteria	Reyranella	7.98	H
	Alphaproteobacteria	Ensifer	4.95	H
	Alphaproteobacteria	Bradyrhizobium	6.06	H
	Alphaproteobacteria	SWB02	6.83	H
	Alphaproteobacteria	PMMR1	-5.02	VL
	Alphaproteobacteria	Pedomicrobium	10.02	H
	Alphaproteobacteria	Phenylobacterium	-3.63	VL
	Alphaproteobacteria	Alsobacter	-2.62	VL
	Alphaproteobacteria	Caulobacter	4.62	H
	Alphaproteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	5.37	H
	Alphaproteobacteria	Asticcacaulis	-3.59	VL
	Alphaproteobacteria	Telmatospirillum	-5.23	VL
	Alphaproteobacteria	Nordella	8.65	H
	Alphaproteobacteria	Acidiphilium	-5.89	VL
	Alphaproteobacteria	Candidatus Alysiosphaera	5.51	H
	Alphaproteobacteria	Microvirga	6.79	H
	Alphaproteobacteria	Mesorhizobium	4.48	H
	Alphaproteobacteria	1174-901-12	-5.31	VL
	Alphaproteobacteria	Dongia	7.45	H
	Alphaproteobacteria	Labrys	6.85	H
	Alphaproteobacteria	Ellin6055	2.18	H
	Alphaproteobacteria	Methylovirgula	-7.31	VL
	Alphaproteobacteria	Endobacter	-5.95	VL
	Alphaproteobacteria	Pseudolabrys	1.10	H
	Alphaproteobacteria	Bauldia	5.21	H
	Alphaproteobacteria	Altererythrobacter	5.06	H
	Alphaproteobacteria	Hirschia	8.02	H
	Alphaproteobacteria	Amaricoccus	3.84	H
	Alphaproteobacteria	Skermanella	5.96	H
	Anaerolineae	UTCFX1	7.98	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Bacteroidia	Spirosoma	-5.06	VL
	Bacteroidia	Segetibacter	-1.86	VL
	Bacteroidia	Terrimonas	9.58	H
	Bacteroidia	Niastella	5.54	H
	Bacteroidia	Chitinophaga	5.55	H
	Bacteroidia	Ohtaekwangia	7.28	H
	Bacteroidia	Pseudoflavitalea	4.73	H
	Bacteroidia	Ferruginibacter	7.68	H
	Bacteroidia	Parafilimonas	4.59	H
	Bacteroidia	Flavitalea	1.91	H
	Bacteroidia	OLB12	3.79	H
	Bacteroidia	Sporocytophaga	-5.03	VL
	Bacteroidia	Chryseolinea	7.17	H
	Bacteroidia	Flavobacterium	2.64	H
	Bacteroidia	Puia	-3.20	VL
	Bdellovibrionia	OM27 clade	4.68	H
	Bdellovibrionia	Bdellovibrio	-1.32	VL
	Blastocatellia	Aridibacter	4.49	H
	Blastocatellia	RB41	9.68	H
	Blastocatellia	JGI 0001001-H03	5.76	H
	Chloroflexia	Nitrolancea	-8.32	VL
	Chthonomonadetes	Chthonomonas	2.66	H
	Deinococci	Deinococcus	-5.53	VL
	Fibrobacteria	possible genus 04	4.58	H
	Gammaproteobacteria	Cellvibrio	3.78	H
	Gammaproteobacteria	Ellin6067	5.70	H
	Gammaproteobacteria	Pseudomonas	3.99	H
	Gammaproteobacteria	Pseudoduganella	5.24	H
	Gammaproteobacteria	Ahniella	3.55	H
	Gammaproteobacteria	Aquicella	-1.51	VL
	Gammaproteobacteria	Chujaibacter	-11.01	VL
	Gammaproteobacteria	Polycyclovorans	7.79	H
	Gammaproteobacteria	Alkanibacter	-4.19	VL
	Gammaproteobacteria	Acidibacter	-1.49	VL
	Gammaproteobacteria	Legionella	-2.00	VL
	Gammaproteobacteria	JTB255 marine benthic group	4.52	H
	Gammaproteobacteria	Dyella	-6.94	VL
	Gammaproteobacteria	IS-44	7.75	H
	Gammaproteobacteria	Nitrosospira	6.92	H
	Gammaproteobacteria	Dokdonella	6.77	H
	Gammaproteobacteria	Arenimonas	6.29	H
	Gammaproteobacteria	Luteimonas	4.30	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Gammaproteobacteria	Permianibacter	5.33	H
	Gammaproteobacteria	Steroidobacter	6.67	H
	Gammaproteobacteria	mle1-7	7.72	H
	Gammaproteobacteria	Rhodanobacter	-6.44	VL
	Gammaproteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-2.12	VL
	Gammaproteobacteria	MND1	4.80	H
	Gammaproteobacteria	Tahibacter	5.89	H
	Ktedonobacteria	Thermosporothrix	-8.52	VL
	Ktedonobacteria	FCPS473	-7.28	VL
	Ktedonobacteria	JG30a-KF-32	-11.58	VL
	Ktedonobacteria	Ktedonobacter	-6.89	VL
	Leptospirae	Leptospira	3.30	H
	Leptospirae	Turneriella	5.28	H
	Myxococcia	P3OB-42	-1.41	VL
	Nitrososphaeria	Candidatus Nitrosotalea **	-5.99	VL
	Nitrososphaeria	Nitrosarchaeum	4.75	H
	Nitrososphaeria	Candidatus Nitrososphaera	7.17	H
	Nitrososphaeria	Candidatus Nitrosotenuis	7.59	H
	Nitrospiria	Nitrospira **	1.86	H
	Oligoflexia	Oligoflexus	-4.05	VL
	Phycisphaerae	AKYG587	5.55	H
	Phycisphaerae	SM1A02	5.65	H
	Planctomycetes	Candidatus Nostocoida	-9.67	VL
	Planctomycetes	Pir4 lineage	10.13	H
	Planctomycetes	Gemmata	3.56	H
	Planctomycetes	Pirellula	8.06	H
	Planctomycetes	Planctopirus	5.67	H
	Planctomycetes	Fimbriiglobus	4.66	H
	Planctomycetes	Aquisphaera	-5.32	VL
	Planctomycetes	SH-PL14	6.47	H
	Planctomycetes	Singulisphaera	-1.32	VL
	Polyangia	Phaselicystis	5.32	H
	Polyangia	Pajaroellobacter	-3.86	VL
	Polyangia	Haliangium	1.40	H
	Polyangia	Nannocystis	4.08	H
	Polyangia	Sandaracinus	3.92	H
	Spirochaetia	Spirochaeta	3.58	H
	Spirochaetia	Spirochaeta 2	5.06	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Thermoanaerobaculia	Subgroup 10	5.55	H
	Thermoleophilia	Solirubrobacter	6.86	H
	Thermoleophilia	Gaiella	1.30	H
	Thermoleophilia	Conexibacter *	-5.38	VL
	Verrucomicrobiae	Candidatus Udaeobacter	9.90	H
	Verrucomicrobiae	Chthoniobacter	1.13	H
	Verrucomicrobiae	Ellin517	6.51	H
	Verrucomicrobiae	DEV008	4.82	H
	Verrucomicrobiae	Luteolibacter	4.57	H
	Verrucomicrobiae	Opitutus	7.00	H
	Verrucomicrobiae	Roseimicrobium	5.39	H
	Verrucomicrobiae	Ellin516	-1.54	VL
	Verrucomicrobiae	Pedosphaera	-4.81	VL
	Vicinamibacteria	Luteitalea	8.02	H
	Vicinamibacteria	Vicinamibacter	6.89	H

Table S4.8. DESeq2 pairwise H vs L treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

H vs L	Class	Genus	Log2FoldChange	Enriched
	Acidimicrobiia	Ilumatobacter	5.65	H
	Acidimicrobiia	CL500-29marine group	2.69	H
	Acidobacteriae	Terracidiphilus	-7.01	L
	Acidobacteriae	Granulicella	-7.06	L
	Acidobacteriae	Bryobacter *	-1.90	L
	Acidobacteriae	Occallatibacter	-8.01	L
	Acidobacteriae	Acidipila	-7.20	L
	Acidobacteriae	Candidatus Solibacter	-1.70	L
	Acidobacteriae	Candidatus Koribacter	-6.41	L
	Actinobacteria	Lysinimonas	5.01	H
	Actinobacteria	Actinocorallia	2.87	H
	Actinobacteria	Mycobacterium	-0.59	L
	Actinobacteria	Lechevalieria	2.96	H
	Actinobacteria	Geodermatophilus	5.44	H
	Actinobacteria	Phycicoccus	-1.04	L
	Actinobacteria	Kribbella	4.49	H
	Actinobacteria	Streptacidiphilus	-6.58	L
	Actinobacteria	Kutzneria	-7.11	L
	Actinobacteria	Microbacterium	4.44	H
	Actinobacteria	Crossiella	-5.10	L
	Actinobacteria	Actinoplanes	-6.10	L
	Actinobacteria	Blastococcus	-0.95	L
	Actinobacteria	Kineosporia	-7.29	L
	Actinobacteria	Actinospica	-6.00	L

H vs L	Class	Genus	Log2FoldChange	Enriched
	Actinobacteria	Amycolatopsis	-1.49	L
	Actinobacteria	Acidothermus *	-6.05	L
	Actinobacteria	Jatrophihabitans	-2.57	L
	Alphaproteobacteria	Rhodomicrobium	4.41	H
	Alphaproteobacteria	Plot4-2H12	-5.03	L
	Alphaproteobacteria	Phenylobacterium	-1.83	L
	Alphaproteobacteria	Roseiarcus	-8.74	L
	Alphaproteobacteria	Rhizomicrobium	-6.83	L
	Alphaproteobacteria	Pseudorhodoplanes	4.42	H
	Alphaproteobacteria	Sphingomonas	-0.76	L
	Alphaproteobacteria	Hyphomicrobium	-0.43	L
	Alphaproteobacteria	Reyranella	-0.70	L
	Alphaproteobacteria	Ensifer	5.68	H
	Alphaproteobacteria	Bradyrhizobium	-1.46	L
	Alphaproteobacteria	Pedomicrobium	2.04	H
	Alphaproteobacteria	Phyllobacterium	4.25	H
	Alphaproteobacteria	Nordella	1.87	H
	Alphaproteobacteria	Candidatus Alysiosphaera	2.10	H
	Alphaproteobacteria	Microvirga	3.12	H
	Alphaproteobacteria	Acidicaldus	-6.12	L
	Alphaproteobacteria	Devosia	-0.58	L
	Alphaproteobacteria	Methylobacterium-Methylorubrum	-1.32	L
	Alphaproteobacteria	Pseudolabrys	-1.34	L
	Alphaproteobacteria	Bauldia	0.44	H
	Alphaproteobacteria	Rhodoplanes	0.29	H
	Alphaproteobacteria	Acidiphilium	-7.36	L
	Alphaproteobacteria	Hirschia	1.87	H
	Alphaproteobacteria	Amaricoccus	4.57	H
	Alphaproteobacteria	Skermanella	2.64	H
	Anaerolineae	UTCFX1	3.87	H

H vs L	Class	Genus	Log2FoldChange	Enriched
	Bacteroidia	Spirosoma	-4.55	L
	Bacteroidia	Terrimonas	1.05	H
	Bacteroidia	UTBCD1	-3.82	L
	Bacteroidia	Ferruginibacter	2.26	H
	Bacteroidia	Puia	-3.12	L
	Bacteroidia	Mucilaginibacter	-2.10	L
	Bacteroidia	OLB12	-1.26	L
	Bacteroidia	Niastella	-1.83	L
	Bacteroidia	Edaphobaculum	-1.87	L
	Bdellovibrionia	OM27 clade	5.42	H
	Blastocatellia	Aridibacter	3.33	H
	Blastocatellia	RB41	2.34	H
	Chloroflexia	Herpetosiphon	3.78	H
	Chthonomonadetes	Chthonomonas	-2.01	L
	Fibrobacteria	possible genus 04	-1.08	L
	Gammaproteobacteria	Pseudoduganella	5.97	H
	Gammaproteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-3.05	L
	Gammaproteobacteria	Rhodanobacter	-3.99	L
	Gammaproteobacteria	Ramlibacter	-4.43	L
	Gammaproteobacteria	IS-44	0.82	H
	Gammaproteobacteria	Methylotenera	-5.39	L
	Gammaproteobacteria	Nevskia	-4.57	L
	Gammaproteobacteria	Alkanibacter	-3.12	L
	Gammaproteobacteria	Polycyclovorans	2.27	H
	Gammaproteobacteria	Leptothrix	-7.52	L
	Gammaproteobacteria	JTB255 marine benthic group	5.25	H
	Gammaproteobacteria	Dokdonella	-1.55	L
	Gammaproteobacteria	Arenimonas	1.80	H
	Gammaproteobacteria	Luteimonas	5.02	H

H vs L	Class	Genus	Log2FoldChange	Enriched
	Gammaproteobacteria	Permianibacter	6.08	H
	Gammaproteobacteria	Steroidobacter	1.09	H
	Gammaproteobacteria	mle1-7	1.89	H
	Gammaproteobacteria	GOUTA6	-4.25	L
	Gammaproteobacteria	Nitrosospira	-0.76	L
	Gemmatimonadetes	Roseisolibacter	-6.01	L
	Gemmatimonadetes	Gemmatimonas *	-1.12	L
	Holophagae	Holophaga	-4.17	L
	Holophagae	Geothrix	-5.60	L
	Ktedonobacteria	JG30a-KF-32	-6.58	L
	Ktedonobacteria	FCPS473	-4.53	L
	Ktedonobacteria	HSB OF53-F07	-5.72	L
	Ktedonobacteria	1921-3	-7.30	L
	Ktedonobacteria	G12-WMSP1	-6.91	L
	Myxococcia	KD3-10	-2.02	L
	Myxococcia	Anaeromyxobacter	-2.45	L
	Nitrososphaeria	Candidatus Nitrosotalea **	-6.34	L
	Nitrososphaeria	Nitrosarchaeum	5.48	H
	Nitrososphaeria	Candidatus Nitrososphaera	7.91	H
	Nitrososphaeria	Candidatus Nitrosotenuis	4.18	H
	Nitrospiria	Nitrospira **	0.55	H
	Omnitrophia	Candidatus Omnitrophus	-1.00	L
	Phycisphaerae	AKYG587	3.86	H
	Phycisphaerae	Phycisphaera	-5.55	L
	Planctomycetes	Candidatus Nostocoida	-7.94	L
	Planctomycetes	Pir4 lineage	0.90	H
	Planctomycetes	Zavarzinella	-3.52	L

H vs L	Class	Genus	Log2FoldChange	Enriched
	Planctomycetes	Gemmata	-0.92	L
	Planctomycetes	Pirellula	0.51	H
	Planctomycetes	SH-PL14	1.69	H
	Planctomycetes	Schlesneria	-1.69	L
	Polyangia	Haliangium	-0.48	L
	Polyangia	Nannocystis	4.79	H
	Polyangia	Pajaroellobacter	-1.81	L
	Spirochaetia	Spirochaeta	-1.45	L
	Spirochaetia	Spirochaeta 2	-1.56	L
	Thermoleophilia	Solirubrobacter	1.55	H
	Thermoleophilia	Conexibacter *	-2.82	L
	Verrucomicrobiae	ADurb.Bin063-1	-3.15	L
	Verrucomicrobiae	Candidatus Xiphinematobacter	-2.29	L
	Verrucomicrobiae	Lacunisphaera	-0.80	L
	Verrucomicrobiae	Ellin516	-2.56	L
	Verrucomicrobiae	Pedosphaera	-5.75	L
	Vicinamibacteria	Vicinamibacter	3.15	H

Table S4.9. DESeq2 pairwise VH vs VL treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Acidimicrobiia	Ilumatobacter	6.46	VH
	Acidimicrobiia	CL500-29 marine group	9.73	VH
	Acidimicrobiia	Iamia	6.78	VH
	Acidobacteriae	Occallatibacter	-11.59	VL
	Acidobacteriae	Candidatus Solibacter	-0.89	VL
	Acidobacteriae	Paludibaculum	5.08	VH
	Acidobacteriae	Candidatus Koribacter	-7.28	VL
	Acidobacteriae	Granulicella	-10.41	VL
	Acidobacteriae	Acidipila	-9.97	VL
	Acidobacteriae	Bryobacter *	-2.09	VL
	Acidobacteriae	Edaphobacter	-9.24	VL
	Acidobacteriae	Terracidiphilus	-11.33	VL
	Acidobacteriae	Telmatobacter	-9.46	VL
	Actinobacteria	Catenulispora *	-8.69	VL
	Actinobacteria	Streptacidiphilus	-7.76	VL
	Actinobacteria	Dactylosporangium	4.80	VH
	Actinobacteria	Microlunatus	6.12	VH
	Actinobacteria	Actinocorallia	7.54	VH
	Actinobacteria	Rhizocola	3.94	VH
	Actinobacteria	Virgisporangium	4.30	VH
	Actinobacteria	Mycobacterium	-1.37	VL
	Actinobacteria	Geodermatophilus	3.14	VH
	Actinobacteria	Streptomyces *	8.35	VH
	Actinobacteria	Nocardioides *	4.25	VH
	Actinobacteria	Nakamurella	2.88	VH
	Actinobacteria	Pseudonocardia	8.15	VH
	Actinobacteria	Jatrophihabitans	-7.84	VL
	Actinobacteria	Flexivirga	-6.69	VL
	Actinobacteria	Aeromicrobium	7.17	VH
	Actinobacteria	Cellulomonas	4.69	VH
	Actinobacteria	Friedmanniella	3.95	VH
	Actinobacteria	Actinospica	-9.97	VL
	Actinobacteria	Crossiella	-8.84	VL

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Actinobacteria	Lechevalieria	7.67	VH
	Actinobacteria	Acidothermus *	-10.46	VL
	Actinobacteria	Microbacterium	4.73	VH
	Actinobacteria	Terrabacter	2.63	VH
	Alphaproteobacteria	Rhodomicrobium	6.94	VH
	Alphaproteobacteria	Methylobacterium-Methylorubrum	-1.74	VL
	Alphaproteobacteria	Roseiarcus	-12.42	VL
	Alphaproteobacteria	Acidicaldus	-9.62	VL
	Alphaproteobacteria	Pseudorhodoplanes	5.44	VH
	Alphaproteobacteria	Hyphomicrobium	3.44	VH
	Alphaproteobacteria	Sphingomonas	1.49	VH
	Alphaproteobacteria	Reyranella	7.83	VH
	Alphaproteobacteria	Ensifer	5.97	VH
	Alphaproteobacteria	Bradyrhizobium	5.98	VH
	Alphaproteobacteria	PMMR1	-3.10	VL
	Alphaproteobacteria	Pedomicrobium	10.62	VH
	Alphaproteobacteria	Phenylobacterium	-2.89	VL
	Alphaproteobacteria	Labrys	7.08	VH
	Alphaproteobacteria	Aminobacter	4.95	VH
	Alphaproteobacteria	Inquilinus	-4.95	VL
	Alphaproteobacteria	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	5.24	VH
	Alphaproteobacteria	Asticcacaulis	-5.87	VL
	Alphaproteobacteria	Telmatospirillum	-4.88	VL
	Alphaproteobacteria	Nordella	8.91	VH
	Alphaproteobacteria	Acidiphilium	-5.31	VL
	Alphaproteobacteria	Candidatus Alysiosphaera	6.08	VH
	Alphaproteobacteria	Microvirga	7.62	VH
	Alphaproteobacteria	Mesorhizobium	4.43	VH
	Alphaproteobacteria	1174-901-12	-4.86	VL
	Alphaproteobacteria	Amaricoccus	5.52	VH
	Alphaproteobacteria	Phyllobacterium	5.00	VH
	Alphaproteobacteria	Ellin6055	3.18	VH
	Alphaproteobacteria	Methylovirgula	-6.99	VL
	Alphaproteobacteria	Endobacter	-5.51	VL
	Alphaproteobacteria	Pseudolabrys	0.91	VH
	Alphaproteobacteria	Bauldia	5.72	VH
	Alphaproteobacteria	Altererythrobacter	6.38	VH
	Alphaproteobacteria	Dongia	8.20	VH
	Alphaproteobacteria	SWB02	7.02	VH

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Alphaproteobacteria	Hirschia	8.96	VH
	Alphaproteobacteria	Devosia	1.17	VH
	Alphaproteobacteria	Skermanella	6.76	VH
	Anaerolineae	UTCFX1	9.24	VH
	Armatimonadia	Armatimonas	3.54	VH
	Bacteroidia	Spirosoma	-3.26	VL
	Bacteroidia	Segetibacter	-1.95	VL
	Bacteroidia	Adhaeribacter	4.34	VH
	Bacteroidia	Terrimonas	10.22	VH
	Bacteroidia	Niastella	6.04	VH
	Bacteroidia	Chitinophaga	4.59	VH
	Bacteroidia	Ohtaekwangia	8.65	VH
	Bacteroidia	Ferruginibacter	8.57	VH
	Bacteroidia	OLB12	2.49	VH
	Bacteroidia	Pseudoflavitalea	5.82	VH
	Bacteroidia	Parafilimonas	4.47	VH
	Bacteroidia	Flavitalea	2.49	VH
	Bacteroidia	Chryseolinea	8.12	VH
	Bacteroidia	Aurantisolimonas	3.57	VH
	Bacteroidia	UTBCD1	3.95	VH
	Bacteroidia	Flavobacterium	3.26	VH
	Bacteroidia	Puia	-4.03	VL
	Bdellovibrionia	Bdellovibrio	-1.05	VL
	Bdellovibrionia	OM27 clade	4.79	VH
	Blastocatellia	Aridibacter	6.29	VH
	Blastocatellia	RB41	10.40	VH
	Blastocatellia	JGI 0001001-H03	6.55	VH
	Chloroflexia	Herpetosiphon	3.42	VH
	Chloroflexia	Nitrolancea	-7.90	VL
	Deinococci	Deinococcus	-3.32	VL
	Fibrobacteria	possible genus 04	4.58	VH
	Gammaproteobacteria	Ellin6067	6.15	VH
	Gammaproteobacteria	GOUTA6	3.75	VH
	Gammaproteobacteria	Aquicella	-1.58	VL
	Gammaproteobacteria	Ahniella	5.33	VH
	Gammaproteobacteria	Nevskia	-4.67	VL
	Gammaproteobacteria	Chujaibacter	-10.55	VL
	Gammaproteobacteria	Polycyclovorans	8.36	VH
	Gammaproteobacteria	Alkanibacter	-5.72	VL
	Gammaproteobacteria	Acidibacter	-0.79	VL
	Gammaproteobacteria	JTB255 marine benthic group	4.74	VH
	Gammaproteobacteria	Dyella	-8.68	VL

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Gammaproteobacteria	Pseudomonas	3.80	VH
	Gammaproteobacteria	IS-44	8.10	VH
	Gammaproteobacteria	Nitrosospira	7.18	VH
	Gammaproteobacteria	Dokdonella	6.04	VH
	Gammaproteobacteria	Arenimonas	6.78	VH
	Gammaproteobacteria	Luteimonas	3.71	VH
	Gammaproteobacteria	Permianibacter	4.75	VH
	Gammaproteobacteria	Lysobacter	5.48	VH
	Gammaproteobacteria	Steroidobacter	7.31	VH
	Gammaproteobacteria	mle1-7	8.20	VH
	Gammaproteobacteria	Rhodanobacter	-11.59	VL
	Gammaproteobacteria	Nitrosomonas	5.84	VH
	Gammaproteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-4.80	VL
	Gammaproteobacteria	MND1	4.69	VH
	Gammaproteobacteria	Tahibacter	5.81	VH
	Holophagae	Holophaga	-6.32	VL
	Ktedonobacteria	Thermosporothrix	-8.24	VL
	Ktedonobacteria	FCPS473	-6.97	VL
	Ktedonobacteria	JG30a-KF-32	-11.21	VL
	Ktedonobacteria	Ktedonobacter	-8.08	VL
	Leptospirae	Turneriella	5.59	VH
	Myxococcia	P3OB-42	-2.08	VL
	Nitrososphaeria	Candidatus Nitrocosmicus **	0.66	VH
	Nitrososphaeria	Candidatus Nitrosotalea **	-11.72	VL
	Nitrososphaeria	Nitrosarchaeum	6.11	VH
	Nitrososphaeria	Candidatus Nitrososphaera	7.87	VH
	Nitrososphaeria	Candidatus Nitrosotenuis	7.27	VH
	Nitrospiria	Nitrospira **	2.60	VH
	Omnitrophia	Candidatus Omnitrophus	-1.72	VL
	Phycisphaerae	SM1A02	5.31	VH
	Phycisphaerae	AKYG587	5.54	VH
	Planctomycetes	Rhodopirellula	5.14	VH
	Planctomycetes	Candidatus Nostocoida	-9.30	VL
	Planctomycetes	Pirellula	8.72	VH
	Planctomycetes	Pir4 lineage	11.10	VH
	Planctomycetes	SH-PL14	8.03	VH
	Planctomycetes	Gemmata	4.29	VH

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Planctomycetes	Planctomicrobium	5.48	VH
	Planctomycetes	Fimbriiglobus	6.23	VH
	Planctomycetes	Bythopirellula	4.78	VH
	Planctomycetes	Blastopirellula	4.09	VH
	Planctomycetes	Planctopirus	6.37	VH
	Planctomycetes	Aquisphaera	-7.60	VL
	Polyangia	Phaselicystis	5.58	VH
	Polyangia	Pajaroellobacter	-3.56	VL
	Polyangia	Haliangium	1.38	VH
	Polyangia	Nannocystis	5.17	VH
	Spirochaetia	Spirochaeta 2	5.01	VH
	Thermoanaerobaculia	Subgroup 10	5.42	VH
	Thermoleophilia	Solirubrobacter	7.57	VH
	Thermoleophilia	Conexibacter *	-5.58	VL
	Thermoleophilia	Gaiella	1.73	VH
	Verrucomicrobiae	Roseimicrobium	5.74	VH
	Verrucomicrobiae	Candidatus Udaeobacter	9.71	VH
	Verrucomicrobiae	Luteolibacter	4.89	VH
	Verrucomicrobiae	Ellin517	6.65	VH
	Verrucomicrobiae	Opitutus	6.97	VH
	Verrucomicrobiae	Ellin516	-1.29	VL
	Verrucomicrobiae	Pedosphaera	-7.81	VL
	Verrucomicrobiae	Chthoniobacter	1.84	VH
	Vicinamibacteria	Vicinamibacter	8.64	VH
	Vicinamibacteria	Luteitalea	6.88	VH

Table S4.10. DESeq2 pairwise VH vs L treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

VH vs L	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	unidentified_3513	22.64	VH
	Agaricomycetes	Inocybe	7.46	VH
	Agaricomycetes	unidentified_90	22.91	VH
	Agaricomycetes	Coprinopsis	5.69	VH
	Archaeosporomycetes	unidentified_276	-7.49	L
	Dothideomycetes	Devriesia	-1.24	L
	Dothideomycetes	Preussia	4.65	VH
	Dothideomycetes	unidentified_82	1.96	VH
	Dothideomycetes	Fusicladium	-2.71	L
	Eurotiomycetes	Talaromyces	-3.28	L
	Eurotiomycetes	Cladophialophora	-3.94	L
	Glomeromycetes	Dominikia	7.05	VH
	Glomeromycetes	Claroideoglomus	2.85	VH
	Glomeromycetes	Diversispora	7.28	VH
	GS37	unidentified_6982	-2.39	L
	Lecanoromycetes	unidentified_332	1.18	VH
	Leotiomycetes	Thelebolus	2.11	VH
	Leotiomycetes	Gyoerffyella	-7.60	L
	Leotiomycetes	Meliniomyces	-7.81	L
	Leotiomycetes	unidentified_5	-1.10	L
	Leotiomycetes	Chalara	7.96	VH
	Leotiomycetes	Xenopolyscytalum	6.18	VH
	Leotiomycetes	Calyptrozyma	-6.97	L
	Leotiomycetes	Hyalodendriella	6.06	VH
	Leotiomycetes	Tetracladium	5.97	VH
	Microbotryomycetes	Leucosporidium	5.99	VH
	Microbotryomycetes	Udeniozyma	-6.02	L
	Microbotryomycetes	Sporobolomyces	-1.43	L
	Mucoromycetes	Rhizopus	4.15	VH
	Olpidiomycetes	unidentified_7719	5.23	VH
	Olpidiomycetes	Olpidium	4.03	VH
	Orbiliomycetes	unidentified_448	6.18	VH
	Saccharomycetes	Candida	2.72	VH
	Sordariomycetes	Fusidium	-2.49	L
	Sordariomycetes	Gaeumannomyces	5.32	VH
	Sordariomycetes	Cordana	8.22	VH

VH vs L	Class	Genus	Log2FoldChange	Enriched
	Sordariomycetes	Coniochaeta	-6.29	L
	Sordariomycetes	Falciphora	1.62	VH
	Sordariomycetes	Metarhizium	-1.99	L
	Sordariomycetes	Conlarium	-3.65	L
	Sordariomycetes	unidentified_50	1.41	VH
	Sordariomycetes	unidentified_1582	4.57	VH
	Sordariomycetes	Lecythophora	1.77	VH
	Sordariomycetes	unidentified_63	-3.47	L
	Sordariomycetes	unidentified_115	9.57	VH
	Sordariomycetes	Dactylonectria	2.67	VH
	Sordariomycetes	unidentified_3145	-5.62	L
	Sordariomycetes	Chloridium	-3.19	L
	Sordariomycetes	Acremonium	1.67	VH
	Sordariomycetes	unidentified_52	-9.62	L
	Tremellomycetes	unidentified_67	-2.44	L
	Tremellomycetes	Solicoccozyma	-0.97	L
	Umbelopsidomycetes	Umbelopsis	-4.10	L
	unidentified	unidentified	0.86	VH
	unidentified_96	unidentified_40	4.51	VH

Table S4.11. DESeq2 pairwise H vs VH treatment test reporting which archaeal and bacterial genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Nelson et al., (2016)).

H vs VH	Class	Genus	Log2FoldChange	Enriched
	Acidobacteriae	Bryobacter *	-0.43	VH
	Acidobacteriae	Candidatus Solibacter	-1.01	VH
	Actinobacteria	Jatrophihabitans	-6.22	VH
	Alphaproteobacteria	Reyranella	-0.54	VH
	Alphaproteobacteria	Aminobacter	5.75	H
	Alphaproteobacteria	Pseudolabrys	-0.52	VH
	Alphaproteobacteria	Bauldia	0.28	H
	Alphaproteobacteria	Hirschia	0.56	H
	Anaerolineae	UTCFX1	0.88	H
	Armatimonadia	Armatimonas	4.41	H
	Bacteroidia	Adhaeribacter	2.87	H
	Bacteroidia	Ohtaekwangia	1.01	H
	Bacteroidia	OLB12	-1.56	VH
	Blastocatellia	Aridibacter	1.40	H
	Blastocatellia	RB41	0.33	H
	Gammaproteobacteria	Pseudomonas	-0.87	VH
	Gammaproteobacteria	Pseudoduganella	-6.55	VH
	Gammaproteobacteria	Burkholderia-Caballeronia-Paraburkholderia	-3.11	VH
	Gammaproteobacteria	Rhodanobacter	-5.59	VH
	Gammaproteobacteria	Dokdonella	-1.14	VH
	Gammaproteobacteria	Nitrosomonas	2.50	H
	Holophagae	Holophaga	-5.51	VH
	Nitrososphaeria	Candidatus Nitrocosmicus **	0.44	H
	Nitrososphaeria	Candidatus Nitrosotalea **	-6.01	VH
	Nitrospiria	Nitrospira **	0.33	H
	Planctomycetes	Pir4 lineage	0.59	H
	Planctomycetes	SH-PL14	1.17	H
	Planctomycetes	Pirellula	0.36	H
	Planctomycetes	Planctomicrobium	3.32	H
	Planctomycetes	Bythopirellula	5.69	H
	Polyangia	Haliangium	-0.46	VH
	Spirochaetia	Spirochaeta	-2.59	VH

H vs VH	Class	Genus	Log2FoldChange	Enriched
	Verrucomicrobiae	Candidatus Udaeobacter	-0.59	VH
	Verrucomicrobiae	Candidatus Xiphinematobacter	-2.82	VH
	Verrucomicrobiae	ADurb.Bin063-1	-1.08	VH
	Vicinamibacteria	Vicinamibacter	1.40	H
	Vicinamibacteria	Luteitalea	-1.59	VH

Appendix 13

Table S4.12. DESeq2 pairwise VL vs L treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	Psathyrella	-3.6367078	L
	Agaricomycetes	Tylospora	1.2195861	VL
	Agaricomycetes	unidentified_12	7.1713311	VL
	Agaricomycetes	unidentified_364	-22.5675632	L
	Agaricomycetes	Hyphodontia	6.4761286	VL
	Agaricomycetes	Mycena	-6.9454514	L
	Cystobasidiomycetes	Microsporomyces	-5.3562890	L
	Dothideomycetes	unidentified_6793	4.9943500	VL
	Dothideomycetes	unidentified_15	4.2431477	VL
	Dothideomycetes	Pyrenochaetopsis	2.7228208	VL
	Dothideomycetes	Paraphoma	6.8454732	VL
	Dothideomycetes	unidentified_82	2.0590687	VL
	Dothideomycetes	Ophiosphaerella	7.1192594	VL
	Dothideomycetes	Ramularia	4.8881868	VL
	Dothideomycetes	Fusicladium	9.3586211	VL
	Dothideomycetes	Bipolaris	3.6235811	VL
	Dothideomycetes	Neoascochyta	3.4915881	VL
	Dothideomycetes	Aureobasidium	-2.5864811	L
	Eurotiomycetes	Exophiala	3.8834530	VL
	Eurotiomycetes	Talaromyces	-4.0594910	L
	Eurotiomycetes	Sagenomella	-2.8570885	L
	Eurotiomycetes	Cladophialophora	-1.3430909	L
	Glomeromycetes	Claroideoglomus	8.5951758	VL
	Glomeromycetes	unidentified_4174	5.8740051	VL
	GS37	unidentified_6982	3.2406777	VL
	Lecanoromycetes	unidentified_332	4.0727831	VL

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Leotiomycetes	unidentified_191	-5.9269259	L
	Leotiomycetes	Meliniomyces	6.2725237	VL
	Leotiomycetes	Alatospora	5.6583975	VL
	Leotiomycetes	Phialocephala	-4.4191374	L
	Leotiomycetes	Pseudogymnoascus	-2.6223955	L
	Leotiomycetes	Cryptosporiopsis	7.0236896	VL
	Microbotryomycetes	unidentified_2460	7.6146629	VL
	Microbotryomycetes	Leucosporidium	-4.2584705	L
	Microbotryomycetes	Slooffia	-1.8854695	L
	Saccharomycetes	Candida	4.1440881	VL
	Saccharomycetes	Lipomyces	-5.0405214	L
	Sordariomycetes	Robillarda	3.1152884	VL
	Sordariomycetes	Fusidium	8.2049448	VL
	Sordariomycetes	unidentified_832	-5.0942316	L
	Sordariomycetes	unidentified_50	3.7778924	VL
	Sordariomycetes	Clonostachys	-1.4766904	L
	Sordariomycetes	Coniochaeta	7.0809373	VL
	Sordariomycetes	Falciphora	9.9904756	VL
	Sordariomycetes	Lecythophora	8.8167292	VL
	Sordariomycetes	unidentified_63	7.3562477	VL
	Sordariomycetes	Dactylonectria	4.7984878	VL
	Sordariomycetes	Podospora	8.8860606	VL
	Sordariomycetes	Chaetosphaeria	5.6595435	VL
	Sordariomycetes	unidentified_3145	4.9216652	VL
	Sordariomycetes	Fusicolla	1.8857701	VL
	Sordariomycetes	Pleurophragmium	3.1793557	VL
	Sordariomycetes	Gibberella	3.6894321	VL
	Sordariomycetes	Chloridium	5.8641073	VL
	Sordariomycetes	Acremonium	6.9385202	VL
	Sordariomycetes	unidentified_52	10.3656169	VL
	Spizellomycetes	Powellomyces	7.9235983	VL
	Spizellomycetes	Spizellomyces	9.7271369	VL

VL vs L	Class	Genus	Log2FoldChange	Enriched
	Tremellomycetes	Apiotrichum	5.0419152	VL
	Tremellomycetes	Goffeauzyma	-10.1053642	L
	Tremellomycetes	Tetragoniomyces	3.9767722	VL
	Tremellomycetes	Bullera	-7.8223288	L
	Tremellomycetes	Naganishia	-3.7932376	L
	Tremellomycetes	Solicoccozyma	-0.9295218	L
	Tremellomycetes	Vishniacozyma	-2.0149192	L
	Umbelopsidomycetes	Umbelopsis	-0.8215914	L
	unidentified	unidentified	2.5653048	VL

Table S4.13. DESeq2 pairwise VL vs L treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	unidentified_7	7.89	H
	Agaricomycetes	Hypholoma	4.55	H
	Agaricomycetes	unidentified_3513	22.62	H
	Agaricomycetes	Inocybe	6.90	H
	Agaricomycetes	unidentified_682	9.38	H
	Agaricomycetes	Hyphodontia	7.99	H
	Agaricomycetes	Lycoperdon	7.59	H
	Agaricomycetes	Mycena	-7.65	VL
	Agaricomycetes	Tomentella	6.82	H
	Agaricomycetes	Serendipita	10.38	H
	Agaricomycetes	Minimedusa	3.81	H
	Aphelidiomycetes	unidentified_5840	4.51	H
	Archaeosporomycetes	unidentified_276	-6.10	VL
	Cystobasidiomycetes	Microsporomyces	-4.70	VL
	Dothideomycetes	unidentified_3723	6.11	H
	Dothideomycetes	Paraphoma	10.51	H
	Dothideomycetes	unidentified_15	4.53	H
	Dothideomycetes	Stemphylium	-3.34	VL
	Dothideomycetes	Pyrenochaetopsis	3.62	H
	Dothideomycetes	unidentified_82	3.63	H
	Dothideomycetes	Ophiosphaerella	6.70	H
	Dothideomycetes	Ramularia	4.52	H
	Dothideomycetes	Paraphaeosphaeria	1.10	H
	Dothideomycetes	Fusicladium	7.07	H
	Dothideomycetes	Bipolaris	4.80	H
	Dothideomycetes	Neoascochyta	3.33	H
	Dothideomycetes	Aureobasidium	-3.57	VL
	Dothideomycetes	unidentified_6793	6.48	H
	Eurotiomycetes	Aspergillus	-23.28	VL
	Eurotiomycetes	Exophiala	4.80	H
	Eurotiomycetes	Talaromyces	-5.46	VL
	Eurotiomycetes	Sagenomella	-2.85	VL
	Eurotiomycetes	Cladophialophora	-2.58	VL
	Glomeromycetes	Glomus	8.28	H
	Glomeromycetes	Diversispora	10.04	H
	Glomeromycetes	Claroideoglomus	11.67	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Glomeromycetes	unidentified_77	8.94	H
	Glomeromycetes	Rhizophagus	8.53	H
	Glomeromycetes	Dominikia	7.51	H
	Glomeromycetes	unidentified_410	8.39	H
	Glomeromycetes	Pacispora	6.78	H
	Glomeromycetes	unidentified_4174	9.32	H
	GS37	unidentified_6982	2.72	H
	Kickxellomycetes	Ramicandelaber	6.89	H
	Lecanoromycetes	unidentified_332	4.73	H
	Leotiomycetes	Acephala	-6.85	VL
	Leotiomycetes	unidentified_191	-5.55	VL
	Leotiomycetes	Alatospora	5.75	H
	Leotiomycetes	Thelebolus	1.98	H
	Leotiomycetes	Gyoerffyella	-8.87	VL
	Leotiomycetes	Calyptrozyma	-7.97	VL
	Leotiomycetes	Hyalodendriella	7.24	H
	Leotiomycetes	Xenopolyscytalum	9.26	H
	Leotiomycetes	Phialocephala	-10.70	VL
	Leotiomycetes	unidentified_8	-4.12	VL
	Leotiomycetes	Chalara	23.93	H
	Leotiomycetes	Cryptosporiopsis	7.12	H
	Leotiomycetes	Tetracladium	10.64	H
	Microbotryomycetes	unidentified_2460	7.29	H
	Microbotryomycetes	Udeniozyma	-3.25	VL
	Microbotryomycetes	Slooffia	-3.26	VL
	Mucoromycetes	Rhizopus	7.22	H
	Olpidiomycetes	Olpidium	7.00	H
	Orbiliomycetes	unidentified_448	8.51	H
	Pezizomycetes	unidentified_20	7.18	H
	Rhizophlyctidomycetes	Sonoraphlyctis	-2.15	VL
	Rozellomycotina_cls_Incertae_sedis	unidentified_1345	4.78	H
	Saccharomycetes	Candida	6.23	H
	Saccharomycetes	Lipomyces	-5.26	VL
	Sordariomycetes	Dichotomopilus	6.96	H
	Sordariomycetes	Ramophialophora	6.60	H
	Sordariomycetes	unidentified_52	8.64	H
	Sordariomycetes	Fusidium	6.80	H
	Sordariomycetes	Cercophora	6.78	H
	Sordariomycetes	Magnaporthiopsis	6.35	H
	Sordariomycetes	Gaeumannomyces	8.40	H
	Sordariomycetes	Clonostachys	-0.95	VL
	Sordariomycetes	Cordana	7.59	H

H vs VL	Class	Genus	Log2FoldChange	Enriched
	Sordariomycetes	Coniochaeta	6.63	H
	Sordariomycetes	Falciphora	12.25	H
	Sordariomycetes	Conlarium	-2.02	VL
	Sordariomycetes	unidentified_50	4.19	H
	Sordariomycetes	Fusarium	6.13	H
	Sordariomycetes	unidentified_1582	7.34	H
	Sordariomycetes	Lecythophora	10.48	H
	Sordariomycetes	unidentified_115	6.71	H
	Sordariomycetes	Dactylonectria	8.16	H
	Sordariomycetes	Podospora	11.52	H
	Sordariomycetes	Chaetosphaeria	5.93	H
	Sordariomycetes	unidentified_88	1.74	H
	Sordariomycetes	Fusicolla	2.99	H
	Sordariomycetes	Pleurophragmium	4.09	H
	Sordariomycetes	Chloridium	5.44	H
	Sordariomycetes	Acremonium	8.94	H
	Spizellomycetes	unidentified_2246	6.89	H
	Spizellomycetes	Spizellomyces	11.01	H
	Tremellomycetes	Holtermanniella	1.98	H
	Tremellomycetes	Apiotrichum	3.98	H
	Tremellomycetes	Goffeauzyma	-5.06	VL
	Tremellomycetes	Tetragoniomyces	2.68	H
	Tremellomycetes	Rhynchogastrema	5.21	H
	Tremellomycetes	Filobasidium	-1.69	VL
	Tremellomycetes	unidentified_67	-3.01	VL
	Tremellomycetes	Bullera	-6.32	VL
	Tremellomycetes	Naganishia	-4.91	VL
	Tremellomycetes	Solicoccozyma	-0.98	VL
	Tremellomycetes	Vishniacozyma	-1.64	VL
	Umbelopsidomycetes	Umbelopsis	-3.14	VL
	unidentified	unidentified	3.54	H
	unidentified_143	unidentified_62	2.33	H
	unidentified_96	unidentified_40	7.35	H

Table S4.14. DESeq2 pairwise VH vs VL treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	unidentified_682	6.99	VH
	Agaricomycetes	unidentified_7	7.88	VH
	Agaricomycetes	Hypholoma	4.41	VH
	Agaricomycetes	unidentified_3513	22.49	VH
	Agaricomycetes	unidentified_12	7.81	VH
	Agaricomycetes	Inocybe	8.15	VH
	Agaricomycetes	unidentified_364	-22.28	VL
	Agaricomycetes	unidentified_1560	6.05	VH
	Agaricomycetes	Hyphodontia	7.43	VH
	Agaricomycetes	Lycoperdon	6.21	VH
	Agaricomycetes	Mycena	-7.98	VL
	Agaricomycetes	unidentified_90	7.13	VH
	Agaricomycetes	Serendipita	9.07	VH
	Agaricomycetes	Minimedusa	3.90	VH
	Agaricomycetes	Coprinopsis	6.75	VH
	Aphelidiomycetes	unidentified_5840	4.54	VH
	Archaeosporomycetes	Ambispora	6.36	VH
	Archaeosporomycetes	unidentified_276	-6.38	VL
	Cystobasidiomycetes	Microsporomyces	-4.80	VL
	Dothideomycetes	Devriesia	-0.74	VL
	Dothideomycetes	unidentified_15	3.78	VH
	Dothideomycetes	Stemphylium	-3.50	VL
	Dothideomycetes	Pyrenochaetopsis	3.66	VH
	Dothideomycetes	Preussia	4.71	VH
	Dothideomycetes	unidentified_3723	7.63	VH
	Dothideomycetes	Phaeosphaeria	4.11	VH
	Dothideomycetes	Paraphoma	9.06	VH
	Dothideomycetes	unidentified_82	3.79	VH
	Dothideomycetes	Ophiosphaerella	6.98	VH
	Dothideomycetes	Ramularia	4.92	VH
	Dothideomycetes	Paraphaeosphaeria	1.32	VH
	Dothideomycetes	Fusicladium	6.87	VH
	Dothideomycetes	Bipolaris	4.56	VH
	Dothideomycetes	Neoascochyta	3.85	VH
	Dothideomycetes	Cladosporium	1.97	VH
	Dothideomycetes	Aureobasidium	-3.40	VL

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Eurotiomycetes	unidentified_318	5.12	VH
	Eurotiomycetes	Aspergillus	-21.54	VL
	Eurotiomycetes	Exophiala	4.73	VH
	Eurotiomycetes	Talaromyces	-7.05	VL
	Eurotiomycetes	Sagenomella	-3.12	VL
	Eurotiomycetes	Cladophialophora	-5.17	VL
	Glomeromycetes	Glomus	6.95	VH
	Glomeromycetes	unidentified_77	9.57	VH
	Glomeromycetes	Dominikia	7.76	VH
	Glomeromycetes	Claroideoglomus	11.54	VH
	Glomeromycetes	Rhizophagus	7.27	VH
	Glomeromycetes	Funneliformis	6.72	VH
	Glomeromycetes	Diversispora	9.30	VH
	Glomeromycetes	unidentified_4174	7.40	VH
	Lecanoromycetes	unidentified_332	4.91	VH
	Leotiomycetes	Acephala	-7.09	VL
	Leotiomycetes	unidentified_191	-6.06	VL
	Leotiomycetes	Alatospora	5.55	VH
	Leotiomycetes	Thelebolus	2.40	VH
	Leotiomycetes	Gyoerffyella	-9.10	VL
	Leotiomycetes	unidentified_5	-1.55	VL
	Leotiomycetes	Chalara	8.45	VH
	Leotiomycetes	Xenopolyscytalum	12.30	VH
	Leotiomycetes	Calyptrozyma	-7.20	VL
	Leotiomycetes	Hyalodendriella	7.69	VH
	Leotiomycetes	Phialocephala	-10.96	VL
	Leotiomycetes	Pseudogymnoascus	-3.00	VL
	Leotiomycetes	unidentified_8	-4.62	VL
	Leotiomycetes	Cryptosporiopsis	6.23	VH
	Leotiomycetes	Tetracladium	10.52	VH
	Microbotryomycetes	unidentified_2460	7.80	VH
	Microbotryomycetes	Udeniozyma	-6.69	VL
	Microbotryomycetes	Slooffia	-4.08	VL
	Mucoromycetes	Rhizopus	8.71	VH
	Olpidiomycetes	unidentified_7719	4.92	VH
	Olpidiomycetes	Olpidium	8.47	VH
	Orbiliomycetes	unidentified_448	10.49	VH
	Pezizomycetes	Ascobolus	6.36	VH
	Rhizophlyctidomycetes	Sonoraphlyctis	-5.74	VL
	Saccharomycetes	Candida	6.33	VH
	Saccharomycetes	Lipomyces	-5.64	VL
	Sordariomycetes	Fusidium	4.60	VH
	Sordariomycetes	Fusarium	5.53	VH

VH vs VL	Class	Genus	Log2FoldChange	Enriched
	Sordariomycetes	Cercophora	5.60	VH
	Sordariomycetes	unidentified_832	-6.24	VL
	Sordariomycetes	Gaeumannomyces	10.40	VH
	Sordariomycetes	Cladorrhinum	6.56	VH
	Sordariomycetes	Cordana	8.85	VH
	Sordariomycetes	unidentified_63	3.91	VH
	Sordariomycetes	Falciphora	11.76	VH
	Sordariomycetes	Metarhizium	-1.48	VL
	Sordariomycetes	Conlarium	-4.19	VL
	Sordariomycetes	unidentified_50	4.11	VH
	Sordariomycetes	unidentified_1582	7.82	VH
	Sordariomycetes	Lecythophora	10.72	VH
	Sordariomycetes	unidentified_115	10.13	VH
	Sordariomycetes	Dactylonectria	7.56	VH
	Sordariomycetes	Trichoderma *	-1.73	VL
	Sordariomycetes	Podospora	10.50	VH
	Sordariomycetes	Fusicolla	2.91	VH
	Sordariomycetes	Pleurophragmium	3.96	VH
	Sordariomycetes	Ramophialophora	5.38	VH
	Sordariomycetes	Acremonium	8.79	VH
	Spizellomycetes	unidentified_2246	6.68	VH
	Spizellomycetes	Powellomyces	5.69	VH
	Spizellomycetes	unidentified_4017	6.04	VH
	Spizellomycetes	Spizellomyces	10.06	VH
	Tremellomycetes	Apiotrichum	4.22	VH
	Tremellomycetes	Goffeauzyma	-5.17	VL
	Tremellomycetes	Cystofilobasidium	5.75	VH
	Tremellomycetes	Tausonia	5.81	VH
	Tremellomycetes	Filobasidium	-1.70	VL
	Tremellomycetes	unidentified_67	-2.97	VL
	Tremellomycetes	Bullera	-7.43	VL
	Tremellomycetes	Naganishia	-7.15	VL
	Tremellomycetes	Solicoccozyma	-1.74	VL
	Tremellomycetes	Vishniacozyma	-1.75	VL
	Umbelopsidomycetes	Umbelopsis	-4.85	VL
	unidentified	unidentified	3.49	VH
	unidentified_143	unidentified_62	1.88	VH
	unidentified_96	unidentified_40	8.28	VH

Table S4.15. DESeq2 pairwise H vs L treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

H vs L	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	unidentified_3513	21.95	H
	Agaricomycetes	unidentified_12	-4.31	L
	Agaricomycetes	Inocybe	6.03	H
	Agaricomycetes	Lycoperdon	6.69	H
	Agaricomycetes	Tomentella	5.95	H
	Archaeosporomycetes	unidentified_276	-7.64	L
	Dothideomycetes	Devriesia	-1.02	L
	Dothideomycetes	Paraphoma	3.21	H
	Dothideomycetes	unidentified_82	1.06	H
	Dothideomycetes	Fusicladium	-2.69	L
	Eurotiomycetes	Cladophialophora	-1.75	L
	Glomeromycetes	Diversispora	7.87	H
	Glomeromycetes	Claroideoglomus	2.70	H
	Glomeromycetes	Dominikia	6.48	H
	Glomeromycetes	unidentified_410	7.36	H
	Lecanoromycetes	unidentified_332	1.59	H
	Leotiomycetes	Gyoerffyella	-7.74	L
	Leotiomycetes	Meliniomyces	-5.98	L
	Leotiomycetes	Calyptrozyma	-8.14	L
	Leotiomycetes	Hyalodendriella	5.32	H
	Leotiomycetes	Chalara	7.90	H
	Leotiomycetes	Tetracladium	5.79	H
	Microbotryomycetes	Leucosporidium	4.20	H
	Microbotryomycetes	Sporobolomyces	-1.75	L
	Saccharomycetes	Candida	2.38	H
	Sordariomycetes	Dichotomopilus	5.97	H
	Sordariomycetes	Fusidium	-1.75	L
	Sordariomycetes	Cercophora	5.15	H
	Sordariomycetes	Cordana	6.80	H
	Sordariomycetes	Falciphora	1.89	H
	Sordariomycetes	Metarhizium	-1.97	L
	Sordariomycetes	Conlarium	-2.02	L
	Sordariomycetes	unidentified_50	1.18	H
	Sordariomycetes	unidentified_1582	3.78	H
	Sordariomycetes	unidentified_63	-4.25	L
	Sordariomycetes	Dactylonectria	3.01	H

H vs L	Class	Genus	Log2FoldChange	Enriched
	Sordariomycetes	unidentified_3145	-5.02	L
	Sordariomycetes	Acremonium	1.54	H
	Spizellomycetes	unidentified_2246	4.87	H
	Tremellomycetes	Goffeauzyma	4.56	H
	Tremellomycetes	Filobasidium	-1.90	L
	Tremellomycetes	unidentified_67	-2.96	L
	Tremellomycetes	Solicoccozyma	-0.51	L
	Umbelopsidomycetes	Umbelopsis	-2.73	L

Table S4.16. DESeq2 pairwise H vs VH treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

H vs VH	Class	Genus	Log2FoldChange	Enriched
	Eurotiomycetes	Cladophialophora	-2.26	VH
	Leotiomycetes	unidentified_5	-1.24	VH
	Leotiomycetes	Xenopolyscytalum	3.59	H
	Sordariomycetes	unidentified_52	-7.33	VH
	unidentified_64	unidentified_23	3.82	H

Table S4.17. DESeq2 pairwise VH vs L treatment test reporting which fungal genera are significantly enriched across each pH treatment pair. Log2fold change indicates which genera has either increased or decreased in treatment listed in the enriched column. * Denitrifier genera; ** Nitrifier genera; *** Denitrifier and Nitrifier genera (all these according to Maeda et al., (2015)).

VH vs L	Class	Genus	Log2FoldChange	Enriched
	Agaricomycetes	unidentified_3513	22.64	VH
	Agaricomycetes	Inocybe	7.46	VH
	Agaricomycetes	unidentified_90	22.91	VH
	Agaricomycetes	Coprinopsis	5.69	VH
	Archaeosporomycetes	unidentified_276	-7.49	L
	Dothideomycetes	Devriesia	-1.24	L
	Dothideomycetes	Preussia	4.65	VH
	Dothideomycetes	unidentified_82	1.96	VH
	Dothideomycetes	Fusicladium	-2.71	L
	Eurotiomycetes	Talaromyces	-3.28	L
	Eurotiomycetes	Cladophialophora	-3.94	L
	Glomeromycetes	Dominikia	7.05	VH
	Glomeromycetes	Claroideoglomus	2.85	VH
	Glomeromycetes	Diversispora	7.28	VH
	GS37	unidentified_6982	-2.39	L
	Lecanoromycetes	unidentified_332	1.18	VH
	Leotiomycetes	Thelebolus	2.11	VH
	Leotiomycetes	Gyoerffyella	-7.60	L
	Leotiomycetes	Meliniomyces	-7.81	L
	Leotiomycetes	unidentified_5	-1.10	L
	Leotiomycetes	Chalara	7.96	VH
	Leotiomycetes	Xenopolyscytalum	6.18	VH
	Leotiomycetes	Calyptrozyma	-6.97	L
	Leotiomycetes	Hyalodendriella	6.06	VH
	Leotiomycetes	Tetracladium	5.97	VH
	Microbotryomycetes	Leucosporidium	5.99	VH
	Microbotryomycetes	Udeniozyma	-6.02	L
	Microbotryomycetes	Sporobolomyces	-1.43	L
	Mucoromycetes	Rhizopus	4.15	VH
	Olpidiomycetes	unidentified_7719	5.23	VH
	Olpidiomycetes	Olpidium	4.03	VH
	Orbiliomycetes	unidentified_448	6.18	VH
	Saccharomycetes	Candida	2.72	VH
	Sordariomycetes	Fusidium	-2.49	L
	Sordariomycetes	Gaeumannomyces	5.32	VH
	Sordariomycetes	Cordana	8.22	VH

VH vs L	Class	Genus	Log2FoldChange	Enriched
	Sordariomycetes	Coniochaeta	-6.29	L
	Sordariomycetes	Falciphora	1.62	VH
	Sordariomycetes	Metarhizium	-1.99	L
	Sordariomycetes	Conlarium	-3.65	L
	Sordariomycetes	unidentified_50	1.41	VH
	Sordariomycetes	unidentified_1582	4.57	VH
	Sordariomycetes	Lecythophora	1.77	VH
	Sordariomycetes	unidentified_63	-3.47	L
	Sordariomycetes	unidentified_115	9.57	VH
	Sordariomycetes	Dactylonectria	2.67	VH
	Sordariomycetes	unidentified_3145	-5.62	L
	Sordariomycetes	Chloridium	-3.19	L
	Sordariomycetes	Acremonium	1.67	VH
	Sordariomycetes	unidentified_52	-9.62	L
	Tremellomycetes	unidentified_67	-2.44	L
	Tremellomycetes	Solicoccozyma	-0.97	L
	Umbelopsidomycetes	Umbelopsis	-4.10	L
	unidentified	unidentified	0.86	VH
	unidentified_96	unidentified_40	4.51	VH