Imprint of management on microbial communities in arable soil

Implications for N_2O emissions

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Abstract

Arable soils are a major source of the greenhouse gas nitrous oxide (N_2O), emissions of which are directly linked to increased use of N fertilizers. Microbial communities that drive N cycle processes in soil ultimately determine the fate of reactive N inputs and control N_2O emissions. Under anoxic conditions, two processes compete for nitrate (NO_3^-): denitrification, the stepwise reduction of NO_3^- to N_2O or atmospheric nitrogen, and dissimilatory nitrate reduction to ammonium (DNRA), in which NO_3^- is reduced to ammonium (NH_4^+). The reduction of N_2O by denitrifiers and non-denitrifying N_2O reducers is the only known biological sink for N_2O.

The aim of this thesis was to investigate how edaphic factors that are altered by soil management practices influence the diversity, structure and activity of the soil microbiota regulating anaerobic N cycling, and thereby N_2O emissions. We hypothesised that N replete conditions and long-term fertilization promote incomplete denitrifiers, whereas high C content increases the abundance of DNRA bacteria and N_2O reducing organisms, enhancing soil N_2O sink capacity. Inoculation of soil microcosms with a non-denitrifying N_2O reducing strain confirmed that increased abundance of these organisms can mitigate soil N_2O emissions. A survey of long-term fertilization trials showed a consistent increase in the relative abundance of taxonomic groups previously inferred from genomic evidence to produce or consume N_2O in fertilized soil. Nevertheless, the abundance of organisms comprising a truncated denitrification remained dominant, concomitant with increased potential N_2O emissions. Another field study including fertilization and different crop rotations suggested that changes in soil C/N ratio due to cropping system influenced the competition between DNRA and denitrification, with higher C/N promoting DNRA and N_2O reducing community abundance and activity. This was confirmed in controlled manipulations of C/N in a microcosm study, suggesting that soils covering higher C/N ratios sustain a greater abundance of DNRA and N_2O reducing bacteria and therefore have a lower N_2O emission potential.

Keywords: denitrification, arable soils, nitrous oxide, microbial communities, *nosZ*, DNRA, fertilization, C/N ratio

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Sammanfattning

Jordbruksmark är den enskilt största källan av växthusgasen lustgas, vilket är direkt kopplat till det ökade användandet av kvävegödsel. Mikrobsamhällen i marken avgör vad som händer med tillsatt kväve och styr lustgasproduktionen. Under syrefria förhållanden påverkas nitrat i marken av två processer: denitrifikation, som stegvis omvandlar nitrat till lustgas eller till atmosfäriskt kväve, och dissimilatorisk nitratreduktion till ammonium (DNRA), som omvandlar det till ammonium. Denitrifierande bakterier, och icke - denitrifierande lustgasreducerande bakterier, är den enda kända biologiska lustgassänkan.

Syftet med denna avhandling var att undersöka hur brukandet av jorden påverkar mångfalden, strukturen och aktiviteten hos de marklevande mikroorganismer som reglerar de dominerande anaeroba kväveomvandlingsprocesserna i mark, och därigenom lustgasproduktionen. Hypotesen var att kväverika förhållanden och långvarig gödsling gynnar icke fullständiga denitrifierare, medan kolrika förhållanden ökar mängden DNRA-bakterier och lustgasreducerande organismer, vilket stärker jordens lustgassänkande kapacitet. Vi inokulerade jord med icke denitrifierande lustgasreducerande bakterier, vilket bekräftar ett samband mellan antalet lustgasrducerande bakterier och minskad lustgasproduktionen. I en studie med långliggande gödslingförsök fann vi att kvävegödsling konsekvent ökade förekomsten av organismer med genetiska förutsättningar för att både producera och konsumera lustgas. Trots detta dominerades jordarna av ofullständigt denitrifierande organismer, vilket leder till ökad potential för lustgasproduktion. En annan fältstudie med gödsling i växtföljder med och utan vall indikerade att växtföljden orsakar förändringar i jordens kol-kväve-förhållande, vilket påverkar balansen mellan DNRA och denitrifikation: högre kol-kväve-kvot ger mer DNRA och ett mikrobsamhälle med bättre förmåga att reducera lustgas. Detta bekräftades i kontrollerade försök där kol-kväve-förhållandet i olika jordar manipulerades.

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Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Domeignoz-Horta L.A., Putz M., Spor A., Bru D., Breuil M.C., Hallin S. Philippot L. (2016). Non-denitrifying nitrous oxide-reducing bacteria – An effective N_2O sink in soil. *Soil Biology and Biochemistry*, 103, 376-379

II Putz M., Jones C.M., Emmerich M., Hallin S. Fertilizer-induced N_2O production is consistently linked to changes in soil microbial community composition and community functions controlling N_2O. (manuscript)

III Putz M., Schleusner P., Rütting T., Hallin S. Relative abundance of denitrifying and DNRA bacteria and their activity determine nitrogen retention or loss in agricultural soil (*Soil Biology and Biochemistry*, accepted)

IV Putz M., Maheshwari A., Jones C.M., Hallin S. Legacy effects of N fertilization and soil C:N ratio controls microbial communities and N2O emissions under anoxic conditions. (manuscript)

The contribution of Martina Putz to the papers included in this thesis was as follows:

I Contributed to molecular work and analysis of results

II Performed some of the field work the majority of the molecular work, data analysis and writing

III Performed field work, contributed to the experimental work and did the molecular work, half of the data analysis and writing

IV Contributed to planning of the work, and performed the experimental and molecular work, data analysis and writing

Abbreviations

DNRA	dissimilatory nitrate reduction to ammonium
ITS	internal transcribed spacer
N_2O	nitrous oxide
NH_4^+	ammonium
nirK	gene encoding copper nitrite reductase
nirS	gene encoding heme nitrite reductase
NO_2^-	nitrite
NO_3^-	nitrate
nosZ	gene encoding nitrous oxide reductase
OTU	operational taxonomic unit
WHC	water holding capacity

1 Introduction

1.1 Background

Nitrogen (N) is the main component of our atmosphere and one of the most abundant elements on earth. The ability to artificially fix N, by the so-called Haber-Bosch process, and its use as fertilizer in agriculture has more than quadrupled global crop production and feeds more than half of the world's population. Adversely, large quantities of reactive N have become one of the main threats to the environment and human health (Sutton *et al.*, 2011). High amounts of N are lost from arable fields through microbial activities. Denitrification, together with other processes producing nitrous oxide (N_2O), resulted in increased N_2O emissions. Consequently, agriculture contributes 62 % to global N_2O emissions (Thomson *et al.*, 2012). Nitrous oxide is a potent greenhouse gas and also has a strong depletion effect on the atmospheric ozone layer (Ravishankara *et al.*, 2009). The high increase in atmospheric N_2O since the massive application of mineral N as fertilizer has, therefore, contributed to the acceleration of global warming (Davidson & Kanter, 2014).

Microorganisms control the interchange between N_2 in the atmosphere and reactive N, which is a product of metabolisms or growth and are the conducting unit of biogeochemical processes (Stein & Klotz, 2016). Nitrous oxide is naturally produced by soil bacteria, archaea and fungi by several different processes. The main pathways responsible for soil N_2O emissions are nitrification and denitrification (Butterbach-Bahl *et al.*, 2013). Nitrification occurs under oxic conditions and is either a two-step oxidation of ammonia (NH_3) to nitrite (NO_2^-) and further to nitrate (NO_3^-) by two different organisms, or proceeds as a one-step process in the so called comammox process (Daims *et*

al., 2015; van Kessel *et al.*, 2015). Ammonia oxidation, is carried out by two distinct groups of microorganisms, ammonia oxidizing archaea and ammonia oxidizing bacteria. Niche partitioning between the two groups has been suggested and there is evidence for higher abundance of ammonium oxidizing bacteria in fertilized soils (Hink *et al.*, 2017; Verhamme *et al.*, 2011; Di *et al.*, 2010). Nitrous oxide is produced during ammonia oxidation and more N_2O is produced, when bacteria perform the process and to a lesser extent by the archaeal counterpart (Hink *et al.*, 2018). The importance of comammox for N_2O production is not yet known.

Denitrification is an anaerobic respiration process with the sequential reduction of NO_3^- or NO_2^- to gaseous N compounds (NO, N_2O and N_2) and considered as the main source of N_2O (Butterbach-Bahl *et al.*, 2013). The last step, the reduction of N_2O to N_2, is also a N_2O sink. However, denitrification is characterized as a modular pathway (Hallin *et al.*, 2018; Graf *et al.*, 2014) implying a proportion of denitrifiers, including all fungal denitrifiers, do not have the genetic potential to reduce N_2O. In addition, non-denitrifying N_2O reducers also exist and most of them belong to the recently discovered group (Jones *et al.*, 2013; Sanford *et al.*, 2012), which is suggested to be an important N_2O sink in soil (Samad *et al.*, 2016; Domeignoz-Horta *et al.*, 2015; Jones *et al.*, 2014). A yet understudied process in soils that counteracts the loss of N, is the dissimilatory ammonification of NO_3^- and NO_2^- under anoxic conditions. While NO_3^- is highly mobile in soils and the starting point of denitrification, dissimilatory nitrate reduction to ammonium (DNRA) is retaining N. Ammonium (NH_4^+) is less mobile due to the positive interaction with the soil matrix and the contribution of DNRA to N_2O emissions is minor (Stremińska *et al.*, 2012; Rütting *et al.*, 2011a). Thus, DNRA does not only have the advantage of resulting in higher retention, but also circumvents negative environmental impacts such as N_2O emissions compared to denitrification.

The dissimilatory processes denitrification, N_2O reduction and DNRA are found amongst a wide variety of microorganisms that are not phylogenetically related. Hence, taxonomy-based approaches are not appropriate to assess the communities that perform these processes. Instead, these organisms are grouped into functional guilds, defined as a group of taxonomically unrelated organisms sharing the same function. To study these groups, genes encoding catalytic enzymes were developed early on as an alternative to phylogenetic marker genes like 16S rRNA for PCR-based tools for quantification and sequencing (Hallin & Lindgren, 1999; Scala & Kerkhof, 1999; Braker *et al.*, 1998). Based on molecular tools, it has been estimated that the proportion of denitrifiers and N_2O reducers account for up to 5 % of the total bacterial community in soil (Jones *et al.*, 2013; Hallin *et al.*, 2009). Estimates for DNRA bacteria suggest an

abundance of 3 - 4 % of the total soil microbial community (Bárta *et al.*, 2017; Brenzinger *et al.*, 2017), but these numbers are to some degree uncertain, since molecular tools were developed only very recently and there are few studies of DNRA in soil.

The abundances, diversity, community composition and functionality of these functional guilds in arable soils are influenced by environmental factors and agricultural practices in particular (Juhanson *et al.*, 2017; Tatti *et al.*, 2017; Domeignoz-Horta *et al.*, 2015; Hartmann *et al.*, 2015). Fundamental knowledge about the importance and interplay of soil management and biotic factors driving these processes and communities is needed to undertake actions for proper N management in soils and development of N_2O mitigation strategies.

1.2 Objectives of the thesis

This thesis was dedicated to investigate the ecology of communities involved in dissimilatory NO_3^- reduction in agricultural soils. The loss of N from applied fertilizers along with N_2O emissions are main environmental issues in modern agriculture, thus the aim was to identify factors influencing the activity and structure of microbial communities driving N turnover processes. We specifically aimed at gaining better understanding of the role of the abundance and diversity of microorganisms carrying out denitrification, N_2O reduction and DNRA. To reach this goal, soil microcosm incubations as well as utilization of long-term fertilization field experiments were used.

In **paper I** we hypothesized that a higher abundance of a non-denitrifying N_2O reducing strain effectively enhances the net N_2O reduction capacity of arable soils by using N_2O produced by the indigenous soil community under anoxic conditions. An assumed causal link between higher proportions of a non-denitrifying organism featuring the *nosZ* gene to the proportion of N_2O as denitrification end-products was assessed.

Paper II focused on the effects of long-term N fertilization on bacterial taxa and if there are predictive trends of fertilization on the composition of the overall bacterial communities and the functional guilds of N_2O reducers. We hypothesised that fertilizer-induced N_2O emissions are determined by changes in the relative abundance of specific bacterial taxa, which controls the genetic capacity underpinning net N_2O production caused by heterotrophic denitrification and N_2O reduction.

In **paper III** we investigated if different crop rotations and fertilization regimes leading to different soil properties, influence the soil microbial community steering the fate of NO_3^-. We hypothesised that short term grasslands favour primarily the soil organic matter content in relation to NO_3^- and thus DNRA over denitrification activity. Shifts in the relative importance of both processes consequently altered N_2O emissions. Moreover, a potential effect of the relative importance of both processes and consequences for N loss and N retention was expected.

In **paper III** we investigated if crop rotations with and without short-term grasslands and different fertilization regimes leading to different soil properties, primarily the soil organic matter content in relation to NO_3^- influence the soil microbial community steering the fate of NO_3^-. We hypothesised that short-term grasslands increase the soil organic matter content in relation to NO_3^- and thus DNRA over denitrification activity. Consequently, shifts in the relative importance of both processes should alter N_2O emissions. Moreover, a potential effect of the relative importance of both processes and consequences for N loss and N retention was expected.

Based on results from **papers I-III**, indicating the importance of the soil C/N ratio for N_2O reduction, a microcosm experiment was set up to determine mechanistic effects of altered C/N ratios on the bacterial communities (**paper IV**). The hypothesis was that a high C/N ratio favours DNRA and a low C/N ratio not only promotes higher denitrification potential, but also fosters a truncated denitrification pathway without N_2O reduction capacity. The effects of C/N ratio on the relative importance of both processes, and on N_2O reducers, was expected to provide insight into the mechanisms influencing the balance of the N_2O budget. Another objective was to test if legacy effects of long-term fertilization affect the response of the microbial community to short-term availability of resources under anoxic conditions.

2 Microbial mediated N_2O fluxes from soils under anoxic conditions

2.1 Agriculture and climate change

Soils are a fundamental resource for human civilisations as they are an integral component of food, feed and fibre production. Before the invention of artificial N_2 fixation, reactive N was generated by microorganisms, but today the anthropogenic creation of reactive N exceeds all the natural terrestrial sources combined (Galloway *et al.*, 2004; Vitousek *et al.*, 1997). Although the N use efficiency increased over the past decades in many parts of the world, the amount of applied N that is recovered in major crops in the US is still below 40 – 50 %, implying high losses of all forms of reactive N from agricultural fields (Cavigelli *et al.*, 2012; Venterea *et al.*, 2012). A waste of fertilizer does not only mean an economic loss, but also has detrimental and expensive consequences for the environment. Agricultural soils emit 4.3-5.8 Tg N_2O y^{-1} (Butterbach-Bahl *et al.*, 2013). Besides being a strong greenhouse gas, N_2O is removed from the stratosphere through photodissociation, where NO_x is generated which reacts with ozone causing its depletion (Ravishankara *et al.*, 2009).

2.2 Anaerobic N transformation processes in soils

Processes leading to N retention and loss

There are microbial anaerobic processes resulting in retention of soil N, whereas other processes result in losses (Fig. 1). In addition to assimilation, DNRA has been recognized as a process conserving N in ecosystems including arable soils

(Inselsbacher *et al.*, 2010). It can be important for N conservation since the positively charged NH_4^+ molecule is adsorbed to negatively charged surfaces, *e.g.* clay minerals and, thus becomes more stable in the soil compared to water soluble NO_3^-. In tropical forest soils, DNRA was shown to be three times higher than denitrification rates (Silver *et al.*, 2001).

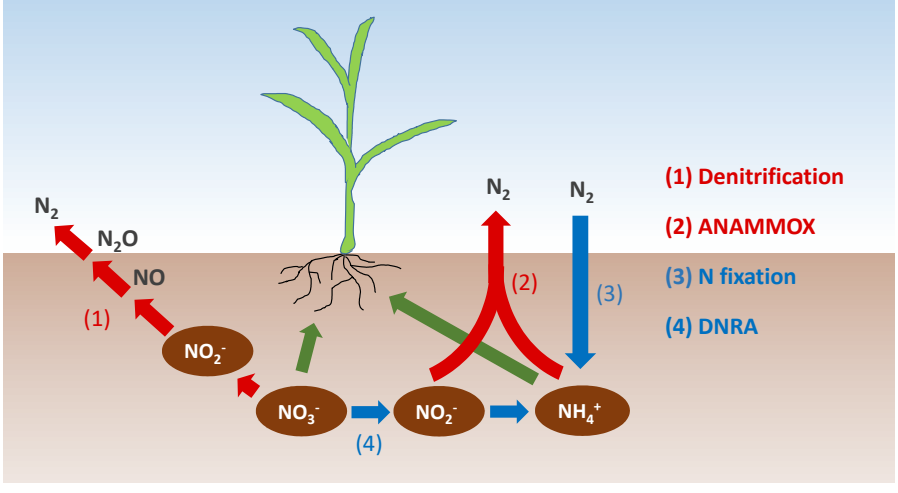

Figure 1. Simplified schematic representation of major anaerobic N transformations performed by microorganisms in soils. Pathways in red colour indicate loss of N and blue arrows indicate N retaining pathways. In addition plant uptake is indicated by green arrows (Elaborated from yara.com).

Denitrification is the main N-loss process in upland soil (Zhu *et al.*, 2018) and for a long time it was considered the only pathway contributing to N loss from the biosphere to the atmosphere. Another pathway occurring under anoxic conditions that directly returns fixed N to the atmosphere is anaerobic ammonia oxidation (anammox). Anammox combines NO from NO_2^- reduction and NH_4^+ to hydrazine (N_2H_4), which is further oxidized to N_2. This pathway contributed to 1% - 14 % loss of N from agricultural upland soil (Zhu *et al.*, 2018; Long *et al.*, 2013).

Fate of nitrate – competition between denitrification and DNRA

The reduction of NO_3^- is a shared starting point of three anaerobic N-transformation pathways – DNRA, denitrification, and anammox (Kraft *et al.*, 2011). The ecological significance of all three processes is different. Denitrification and anammox are leading to a loss of reactive N from the ecosystem to the atmosphere. While denitrification is the main contributor to

N_2O, anammox bacteria do not possess the ability to reduce NO further to N_2O (Strous *et al.*, 2006). Compared to denitrification the contribution to N loss is marginal and anammox bacteria are not known to emit N_2O, facilitated by a tolerance of high NO levels (Zhu *et al.*, 2018; Strous *et al.*, 2006). Since, anammox has a subordinate part of N loss and N_2O production in agricultural soils, the focus of this thesis was on NO_3^- reduction by denitrification or DNRA.

A principal factor influencing DNRA compared to denitrification is the soil oxidation state (Matheson *et al.*, 2002). Under fluctuating redox conditions, DNRA can be more competitive and act as a significant and sometimes dominant process of NO_3^- loss (Pett-Ridge *et al.*, 2006). Both, denitrification and DNRA occur under anoxic conditions and require N-oxides as terminal electron acceptor and suitable electron donors such as organic carbon (C) . Based on calculations of the Gibbs free energy, each molecule of NO_3^- allows microorganisms to gain significantly more free energy by denitrification compared to DNRA (Strohm *et al.*, 2007; Tiedje *et al.*, 1982). Nevertheless, based on cultivations the actual biomass yield was shown to be lower for denitrification than for DNRA (van den Berg *et al.*, 2015; Strohm *et al.*, 2007). Under shortage of electron acceptor like NO_3^-, and strongly reducing conditions, DNRA is suggested to have an advantage over denitrification, as more electrons are transferred during DNRA compared to denitrification (Kraft *et al.*, 2014; Tiedje *et al.*, 1982). Thus the C/NO_3^- ratio has been shown to be an environmental factor regulating NO_3^- partitioning between denitrification and DNRA.

2.3 Dissimilatory nitrate reduction to ammonium

Dissimilatory nitrate reduction to ammonium occurs mainly under anoxic conditions (Morley & Baggs, 2010) and is predominant in reductant-rich environments such as wetlands, forest soils, peatlands and mangrove sediments (Fernandes *et al.*, 2012; Davis *et al.*, 2008; Huygens *et al.*, 2007). Typically more than 90 % of the end product of DNRA is NH_4^+ (Stremińska *et al.*, 2012; Bleakley & Tiedje, 1982). Although in small amounts, DNRA is the third biological process which was discovered to produce N_2O as a by-product (Rütting *et al.*, 2011a). The ability to perform DNRA is spread among bacterial and fungal phyla (Rütting *et al.*, 2011a; Simon, 2002) with many different lifestyles (Welsh *et al.*, 2014; Polcyn & Podeszwa, 2009).

The process itself consists of two independent steps: the reduction of NO_3^- to NO_2^-, and further the reduction of NO_2^- to NH_4^+. The first step, the reduction of

NO_2^- is catalysed by enzymes encoded in *nar* or *nap* genes depending on if the process takes place in the cytoplasm or the periplasm (Simon, 2002). Reduction of NO_2^- is carried out by the reductase NrfA. The gene *nrfA* is used as the marker gene to target organisms potentially mediating this step (Welsh *et al.*, 2014; Moreno-Vivián & Ferguson, 1998). Interestingly, *nrfA* sequences that can be assigned to specific organisms are also characterized by the capability of N_2O reduction (Sanford *et al.*, 2012). However, in DNRA N_2O is not an intermediate product like in denitrification, but a by-product.

2.4 Denitrification

Denitrification is a facultative heterotrophic pathway and mainly occurs under low O_2 partial pressure, as most denitrifying organisms prefer O_2 respiration due to the higher energy gain, but denitrification under the presence of oxygen has been also reported (Patureau *et al.*, 2000; Robertson & Kuenen, 1984). Complete denitrification comprises a four-step reduction of the soluble anion NO_3^- to NO_2^- and further to gaseous compounds NO, N_2O and N_2. Nitrous oxide is not only an intermediate product, prone to volatilization. It can as well be the end product of denitrification (Zumft, 1997). The reduction of N_2O to N_2, which is the final step of complete denitrification, is the only biological sink of N_2O (Conrad, 1996). The step NO_3^- reduction to NO_2^- is not necessarily associated with denitrification, but could also be involved in anammox, DNRA or simply a process in itself (Stein & Klotz, 2016). Denitrification is a trait within 19 phyla of bacteria, archaea and also found among certain fungi (Graf *et al.*, 2014; Spott & Stange, 2011). So far, no fungal denitrifiers are known to perform the last step of denitrification and terminate with N_2O (Maeda *et al.*, 2015; Laughlin & Stevens, 2002).

Nitrate reduction is performed by a periplasmic or membrane-associated NO_2^- reductase encoded by *napA* or *narG* genes, respectively. The key step of denitrification is the reduction of NO_2^- to NO because it transforms soluble NO_2^- into a gaseous N compound. Nitrite reduction takes place in the periplasm and can be catalysed by one of the two non-homologous enzymes encoded by *nirS* or *nirK* genes. The *nirK* gene encodes the copper binding NO_2^- reductase, while *nirS* encodes the heme-binding reductase. Even though both enzymes are considered to be mutually exclusive, a few NO_2^- reducers were found to have both *nir* genes (Sánchez & Minamisawa, 2018; Vaccaro *et al.*, 2016; Graf *et al.*, 2014). To assess the abundance and diversity of denitrifiers in an environment, the *nirK* and *nirS* abundance are often used in combination as molecular markers. Niche partitioning of the two *nir* genes has been suggested several

times (Yuan *et al.*, 2012; Enwall *et al.*, 2010; Jones & Hallin, 2010). Arable soils are commonly characterized by a higher abundance of *nirS* than *nirK* (Graf *et al.*, 2014) including most soils studied in this thesis (**paper II-IV**).

Not every denitrifying organism performs all four steps of the denitrification pathway (Zumft, 1997). In an analysis of denitrification gene co-occurrences across 652 genomes, each with at least one denitrification gene, Graf *et al.* (2014) demonstrated that 64 % of organisms lack the *nosZ* gene and are therefore incapable of reducing N_2O. Moreover, a difference in the co-occurrence of *nir* and *nosZ* genes was apparent. While 80 % of *nirS* denitrifiers possess the *nosZ* gene, 70 % of *nirK* organisms lack it. On the other hand, 23 % were shown to harbour the *nosZ* gene, but lack other genes associated with denitrification (Graf *et al.*, 2014; Simon *et al.*, 2004; Sanford *et al.*, 2002). In order to assess the potential for N_2O reduction, and at the same time estimate complete denitrification, the abundance and diversity of *nosZ* is usually determined. Nitrous oxide is neither known to cause inhibition of any other enzyme in denitrification, nor is it involved in regulation unlike the cytotoxic NO (Carreira *et al.*, 2017). However, high concentrations of N_2O can have negative effects by causing destruction of vitamin B_{12} (Sullivan *et al.*, 2013).

2.5 Nitrous oxide reducers

Nitrous oxide reduction is not only a step in the denitrification pathway as there are many organisms only reducing N_2O (Jones *et al.*, 2013) and negative N_2O fluxes in soils have been reported and shown to be of biological nature (Wu *et al.*, 2013; Chapuis-Lardy *et al.*, 2007). Hence, N_2O reduction can be considered as a trait on its own.

It has been recently discovered that the gene *nosZ*, encoding the N_2O-reductase, can be divided in two phylogenetic clades (I and II) (Jones *et al.*, 2013; Sanford *et al.*, 2012). Both genes are abundant in a wide range of environments implying a relative ecological relevance (Jones *et al.*, 2013). Contrasting abundance patterns and ecological differences between *nosZ*I and *nosZ*II were observed in soils, but also in marine sediments and the rhizosphere (Juhanson *et al.*, 2017; Graf *et al.*, 2016; Wittorf *et al.*, 2016). The abundance of *nosZ*I has been reported to correlate with specific soil factors like clay content, N, C or C/N ratio (Juhanson *et al.*, 2017; Domeignoz-Horta *et al.*, 2015). Clade I harbours mainly Proteobacteria, whereas clade II consists of a diverse range of denitrifying taxa. There is also a difference in the co-occurrence with other denitrification genes; *e.g.* while 17% of *nosZ*I organisms lack *nirK* and *nirS*, about 52% of *nosZ*II do not possess the ability to denitrify (Graf *et al.*, 2014).

The enzyme N_2O reductase is sensitive to oxygen, and low pH might also affect the N_2O reductase (Blum *et al.*, 2018; Liu *et al.*, 2014; Bergaust *et al.*, 2010; Zumft & Kroneck, 2006; Šimek & Cooper, 2002). Thus, even if microorganisms have the genetic ability to possess the last step of denitrification, it might be impaired by unfavourable environmental conditions and high amounts of N_2O are emitted.

Yoon *et al.* (2016) reported a lower half-saturation constant (*Ks)* for *nosZ*II which would result in an advantage under N_2O limiting conditions. Under low dilution rates *nosZ*II dominates over *nosZ*I but at faster growth rates *nosZ*I outcompetes *nosZ*II independent of N_2O availability (Conthe *et al.*, 2018b). Conthe *et al.* (2018a) found that N_2O reduction kinetics and stoichiometric yields do not distinguish between *nosZ*I and *nosZ*II, which contradicts reports of higher growth yields of *nosZ*II during growth on N_2O as sole electron acceptor. The authors conclude that differences in N_2O affinity might be taxa-dependent rather than related to the clade of (Conthe *et al.*, 2018a). Furthermore it has been reported that organisms within *nosZ*I reduce N_2O at different rates (Cavigelli & Robertson, 2001).

2.6 Determining NO_3^- reduction activities

Denitrification is responsible for high losses of N, therefore a great interest in the development of several methods to assess the denitrification activity has evolved. Calculation of the N balance budget is the simplest method to quantify loss of N. However, it is not possible to distinguish between denitrification and N leaching when using this approach. Also, this method does not provide information about the proportion of N_2O produced. Another, more specific approach is the acetylene inhibition method, developed in the 1970s (Yoshinari *et al.*, 1977; Balderston & Payne, 1976). Thereby, the N_2O reductase is inhibited by the presence of the gas acetylene and total N loss by denitrification can be quantified as N_2O. Due to its toxicity, NO emissions are very low and the production of N_2 and N_2O is considered as a good estimator of denitrification (Meyer *et al.*, 2010). Acetylene inhibition methods have been applied to soil cores, slurries and *in situ* in the field using closed chambers (Ryden & Dawson, 1982). To assess the potential denitrification and potential N_2O production of a soil, samples are incubated for a short time under non-limiting denitrification conditions with- or without the presence of acetylene and the rate of N_2O is measured (Pell *et al.*, 1996). This method cannot be used to determine field rates, however the potential for N_2O emissions can be quantified and compared between samples.

Another approach to determine the denitrification activity is based on addition of the stable isotope ^{15}N. Subsequently transformations of $^{15}NO_3^-$ to $^{15}N_2O$ or $^{15}N_2$ can be traced by analysis of the isotopic ratio of denitrification products. Application of a ^{15}N-tracing model was the method of choice to compare the activity of denitrification and DNRA in **paper III** (Rütting *et al.*, 2011a). Addition and tracing of $^{15}NH_4^+$ and $^{15}NO_3^-$ provides insights in soil N cycling rates, representative for the actual field conditions since only small amounts of N compounds need to be added if the N-components are highly enriched in ^{15}N. In this way, an increase of the soil N and water content, which consequently alters C/N ratio and enhances microbial activity can be avoided (Rütting *et al.*, 2011b). When using ^{15}N labelling approaches, modelling can be used to trace simultaneously occurring N transformations (Müller *et al.*, 2014).

2.7 Linking soil microorganisms to processes

Although the importance of microorganisms for N cycling in soil is known, the relevance of the microbial community composition and abundance of functional groups of microorganisms to predict process rates is under debate (Graham *et al.*, 2014; Nemergut *et al.*, 2014). Evidence of a positive relation between soil diversity and ecosystem functioning has been shown (Wagg *et al.*, 2014), with variation between different ecosystems and processes (Graham *et al.*, 2016). For nutrient cycling processes, positive effects of higher diversity have been suggested to depend on community composition rather than species richness (Bardgett & van der Putten, 2014) and three out of four models predicting denitrification were improved by 16S rRNA community diversity but less by considering functional gene abundances (Graham *et al.*, 2016). Both *nirK* and *nirS* OTUs from a study with agricultural soils have been positively, but also negatively correlated to N_2O emissions, relativizing the significance of solely functional gene abundance (Bent *et al.*, 2016). Petersen *et al.* (2012) suggested that gene abundances might not be a good predictor for relatively small changes in activity, but a good predictor when large fluctuation is observed. Variation partitioning revealed that the genetic potential of N_2O reduction is an important variable describing N_2O emissions occurring at high levels, while basal N_2O is governed by soil properties (Domeignoz-Horta *et al.*, 2018).

A lack of observed correlations between N_2O emissions and gene-based abundance of denitrifiers could be due to the facultative nature of the process, regulation at the gene or protein level or because of limited coverage of the high phylogenetic diversity (Bonilla-Rosso *et al.*, 2016; Rocca *et al.*, 2015; Wei *et al.*, 2015). Another considerable factor may be a lack of complete knowledge of

N turnover processes. For example the recent findings of *nosZ*II (Jones *et al.*, 2013; Sanford *et al.*, 2012) or comammox (Daims *et al.*, 2015; van Kessel *et al.*, 2015) point towards an incomplete picture of microbial driven N processes.

Relationships of N_2O reducing bacteria to N_2O emissions

A high N_2O emission potential of soils was characterized by a low *nos/nir* ratio of the native soil microbial communities throughout **papers II-VI**. Manipulations of the *nos/nir* ratio were shown to have implications for the denitrification end-product ratio. In a study *Agrobacterium tumefaciens* was inoculated to a variety of soils and an increased denitrification activity as well as changes in the denitrification end product ratio were observed (Philippot *et al.*, 2011). In **paper I**, the non-denitrifying N_2O-reducing strain *Dyadobacter fermentans* was added to 11 different soils, which significantly reduced the denitrification end-product ratio by 51 % on average and some soils even became N_2O sinks. Due to the short incubation time, we cannot speculate on growth of the amended N_2O reducing strain, which has been shown to be a limiting factor of a successful application of N_2O reducing strains (Gao *et al.*, 2017) but we showed that there is a causal relationship between the abundance of non-denitrifying N_2O reducers and the net N_2O emissions. In **paper I**, we identified soil factors that influence the effectiveness of *D. fermentans.* The strains N_2O sink capacity was associated with pH, C/N ratio and also biotic factors like the *nirK/nirS* and *nosZ*I abundance (Table 1). This study demonstrated that the microbial community composition is ultimately controlling the extent of N_2O reduction.

Table 1. *Contribution of abiotic and biotic factors in percent to differences in the effectiveness of D. fermentans reducing N_2O emissions. Results are based on a model selection analysis, significant variables are indicated (p<0.05*, p<0.01**) and the direction of the correlation is indicated (-) or (+).*

Variable	Sink capacity
pH	10.8** (+)
Clay (%)	0.1
Loam (%)	2.5
C/N	19.4** (+)
16S rRNA	3.8
nirK/*nirS*	17.3* (+)
*nosZ*I (%)	6.4* (+)
*nosZ*II (%)	1.5
Residuals	36.8

Not only is the relative abundance of denitrifiers possessing a truncated pathway relevant for N_2O emissions, but also the abundance of solely N_2O reducing bacteria. In agreement, statistical modelling indicate that the abundance of *nosZ* clade II directly affects the soil N_2O sink capacity when comparing a range of different soils (Jones *et al.*, 2014). Considering denitrification as a shared pathway is relevant for balancing N_2O emissions. Recent investigations suggest that denitrification in soils might be performed by an assembly of different populations (Orellana *et al.*, 2018). Potential interactions between denitrifiers with a truncated denitrification pathway and lineages of *nosZ* acting as N_2O consumers could be an important mechanism of N_2O emission mitigation of soil ecosystems (Juhanson *et al.*, 2017). Besides the abundance of N_2O reducers, the diversity of N_2O reducing communities has been shown to be important for N_2O emissions in situ (Domeignoz-Horta *et al.*, 2018). The community structure of *nosZ*I and *nosZ*II correlated negatively with the denitrification end-product ratio in **paper II**. The diversity of the *nosZ*II community is important for the soil N_2O sink capacity while not contributing to higher potential denitrification (Domeignoz-Horta *et al.*, 2015; Jones *et al.*, 2014).

3 Fertilization affects functional microbial groups accounting for N_2O emissions

3.1 Influence of fertilization on the soil microbiome

Fertilization has long lasting effects on soil properties (Kätterer *et al.*, 2014; Rousk *et al.*, 2011), crop yields (Zhang *et al.*, 2009; Steiner *et al.*, 2007) and the soil microbial community (Cassman *et al.*, 2016; Hartmann *et al.*, 2015; Lauber *et al.*, 2013; Clark *et al.*, 2012; Hallin *et al.*, 2009). Long-term field experiments are useful resources when assessing the impact of management practices on various ecosystem services of arable fields since major impacts of soil management on the microbial community are detectable only after several years. In this thesis, a total of 15 long term field trials across Sweden were used to test the impact of fertilization regimes on the soil microbial communities and their N processing functionality. These field trials were set up during the years 1956-1998 and are well suited for exploration of the long-term effects of fertilization on the soil microbiome.

Fertilization and the soil microbiome

In general, fertilization leads to a higher microbial biomass but if the applied N fertilizer lowers the soil pH, the microbial biomass decreases (Geisseler & Scow, 2014; Börjesson *et al.*, 2011). In **papers II** and **IV**, we found higher bacterial 16S rRNA gene abundances in fertilized fields compared to unfertilized control fields. All fertilized fields exclusively received mineral fertilizer which did not alter the soil pH and the fertilizer N was simply an input of resources. **Paper III** revealed a higher abundance of fungi in fertilized fields, which we suspected was due to a growth of Ascomycota (Leff *et al.*, 2015; Nemergut *et al.*, 2008), a

fungal phylum that was shown to be predominant in agro-ecosystems (Lienhard *et al.*, 2014).

The observed increase in bacterial abundance after fertilization (**paper II**) coincided with a change of the microbial community structure. After six months incubation of these soils with or without NO_3^- and C addition (**paper IV**), this contrast was still evident indicating persistent major structural changes in the microbial community composition (Cassman *et al.*, 2016; Francioli *et al.*, 2016; Börjesson *et al.*, 2011; Allison & Martiny, 2008). Changes in the microbial community structure were apparent in the shifts in relative abundance of bacterial groups at high and low taxonomic levels (Figure 2; **paper II**). Certain phylogenetic taxa like Actinobacteria or Acidobacteria have been assigned to a specific lifestyle based on consistent response to fertilization (Fierer, 2017). Results in **paper II** confirm the suggested *r*-strategy lifestyle of Actinobacteria, which are characterized by rapid growth rates in conditions of high nutrient availability (Fierer *et al.*, 2012). Adversely, independent of pH, the abundance of Acidobacteria decreased with fertilization typically for a *k*-strategy lifestyle, exposing low growth rates and being favoured under nutrient limitation (Leff *et al.*, 2015; Cederlund *et al.*, 2014; Fierer *et al.*, 2012; Ramirez *et al.*, 2012). Moreover, consistent effects on a lower taxonomic level were observed (Figure 2). The highest change in relative abundance was observed for taxa with a known function in the N cycle, like the N–fixing Cyanobacteria, which were characterized by a strong decrease in fertilized soils, or NH_3 oxidizing Nitrosomonadales, which increased the most in relative terms (**paper II**).

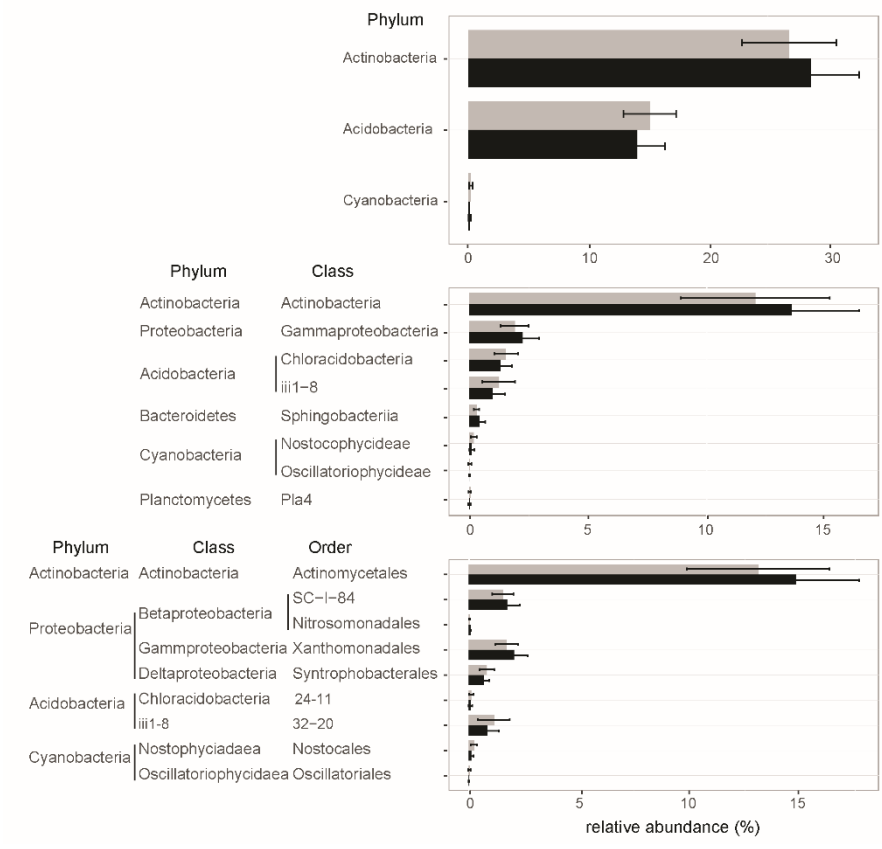

Figure 2. Bacterial taxonomic groups with significant response to fertilization ($p < 0.05$). Relative abundances in unfertilized (grey bars) and fertilized field plots (black bars) are expressed as percent of the total bacterial community.

The change in microbial community composition was not reflected in the alpha diversity in **paper II**. However, short term NO_3^- application to the same soils from the field experiments led to a decrease in Shannon's diversity (**paper IV**). Several studies reported that application of fertilizer was either related to a decrease of bacterial richness and diversity (Samad *et al.*, 2017; Zeng *et al.*, 2016; Koyama *et al.*, 2014; Campbell *et al.*, 2010) or shown to not affect Shannon's diversity index (Coolon *et al.*, 2013). While mineral fertilizer are chemically simply structured and activate biomass growth, other, more complex fertilizers, like manure have been shown to stimulate a broader range of organisms (Francioli *et al.*, 2016). It has been suggested that N availability could be a predictor for the loss of soil bacterial diversity (Zeng *et al.*, 2016). However, contradictive results and distinct effects of different types of N-fertilizer on the

bacterial diversity limit general conclusions from N application to the soil microbial diversity.

Fertilization and N_2O emissions

The application of N fertilizer is known to enhance N_2O emissions (Charles *et al.*, 2017; Buckingham *et al.*, 2014; Lebender *et al.*, 2014; Shcherbak *et al.*, 2014; Coolon *et al.*, 2013). Increased N_2O release from soils due to denitrification is observed as high emissions during a short period of time – so-called 'hot-moments' – which commonly occur after N application followed by heavy rainfall, or frequent freeze-thaw cycles in spring (Congreves *et al.*, 2017; Domeignoz-Horta *et al.*, 2018; Abalos *et al.*, 2016; Liang *et al.*, 2016). A combination of high water content, leading to anaerobic conditions, and high availability of N in the soil promotes denitrification (Enwall *et al.*, 2005). In agreement with the literature, we observed that fertilized soils had a higher potential denitrification activity and also a higher N_2O/N_2 denitrification end product ratio which consequently is associated with higher N_2O emissions (Figure 3A; **paper II**). Nitrification and the abundance of nitrifying organisms has been shown to increase after the application of N fertilizer (Hink *et al.*, 2017; Zhou *et al.*, 2015; Fisk & Schmidt, 1996), thus accelerating N loss with high N loads. Various types of fertilizers can have secondary effects like acidification of the soil which might be a factor increasing the denitrification end-product ratio. Consequently, not only the amount of N application but the type of fertilizer affects N_2O emissions (Shcherbak *et al.*, 2014; Šimek & Cooper, 2002).

3.2 Effects of fertilization on NO_3^- reducing communities

Fertilization effects on denitrifiers

The abundance of N_2O producing microbes is often positively correlated with the N_2O emissions (Cui *et al.*, 2016; Morales *et al.*, 2010), suggesting that the quantity of N_2O generating enzymes can be rate limiting for N_2O production. Shared response of denitrifier communities to an ecosystem disturbance such as fertilization might be less frequent due to the facultative nature of denitrification and the high metabolic versatility of most microorganisms. It has been shown several times that denitrifiers increase not only in activity, but also in abundance after N fertilization (Yang *et al.*, 2015; Hallin *et al.*, 2009), however this relationship was not always observed (Wu *et al.*, 2017; Yin *et al.*, 2014).

Despite the mainly higher abundance of *nirS* compared to *nirK* in soils, higher responsiveness of *nirK* to N loads is observed in **paper II** (*e.g.* Figure 2B). This confirmed the proposed promotion of *nirK* denitrifiers by fertilization compared to *nirS* (Coyotzi *et al.*, 2017; Maeda *et al.*, 2017; Brenzinger *et al.*, 2015; Clark *et al.*, 2012; Hallin *et al.*, 2009), although this may depend on the type of fertilizer (Cui *et al.*, 2016). Similarly, a strong increase of *nirK* abundance during the growing season, or under high cattle impact has also been observed (Schulz *et al.*, 2017; Philippot *et al.*, 2009).

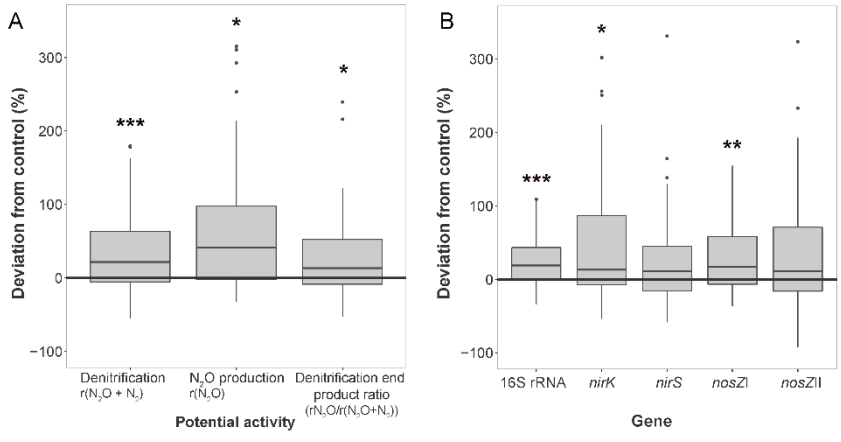

Figure 3. Deviation of gene abundance and potential activities in fertilized arable soils compared to unfertilized control soils. (A) Relative change of potential denitrification, N_2O production and denitrification end product ratio and (B) gene copy numbers per g soil. Significant increases are indicated by asterisks (* p <0.05, ** p < 0.01, *** p < 0.001).

In **paper II**, both *nirK* and *nirS* were positively correlated with the denitrification activity, but only *nirS* was positively correlated to the potential N_2O production rate. A high abundance of *nirK* was correlated with a low denitrification end-product ratio and a high abundance of *nosZ*I. Higher levels of *nirK* and *nosZ*I were associated with lower net N_2O production. Despite a higher probability of *nirS* to co-occur with a *nosZ* gene (Graf *et al.*, 2014), organisms harbouring *nirS* potentially contribute to net N_2O emissions. This finding is in line with observations of direct influence of *nirS* organisms on N_2O emissions from an arable soil (Cui *et al.*, 2016).

Bacteroidetes increased marginally after fertilization (**paper II**). Nevertheless, Bacteroidetes and subgroups within this phylum were associated with lower N_2O emissions, potentially because several members of this phylum are non-denitrifying N_2O reducers (Graf *et al.*, 2014). These results in **paper II**

highlight that the taxonomic composition of microbial communities may have an important impact on N_2O emissions, due to the non-random distributional patterns of N-cycle genes amongst different taxonomic groups.

Across multiple sites, it was shown that fertilization caused shifts of the abundance of microbial taxa. The adaptation of the microbial community to N fertilization occurred with higher denitrification activity in fertilized soils in annual cereal crop rotations compared to unfertilized treatment (**paper II** and **III**). Long-term fertilization effects on denitrifiers was further shown to impact the response to additional nutrient input. In **paper IV** we observed legacy effects of long-term fertilization on the abundance of functional genes including *nirS* which was higher in incubated soils from fertilized field plots. The distinct response of *nirS* gene abundance based on previous fertilization, significantly influences the genetic potential of microbial communities controlling N_2O emissions.

Fertilization effects on DNRA activity and abundance

Several studies have outlined the importance of DNRA over denitrification in N poor environments (Franklin *et al.*, 2017; Kim *et al.*, 2016; Song *et al.*, 2014; Fernandes *et al.*, 2012), but less is known about the effects of high N loads on organisms conducting DNRA. In **paper III** we could not detect a response of fertilization on the abundance of the *nrfA* gene. However, *nrfA* was correlated to C and N and thus higher abundance was observed in the ley cropping soils compared to the annual cereal crop rotation, likely related to the higher C/NO_3^- ratio in the ley (see chapter 4). Within the annual crop rotation system, fertilized soils were characterized by a lower reduction rate of NO_3^- to NH_4^+ suggesting that soils with regular mineral N input, retain less N despite the lack of captured differences in soil properties and genetic abundances. It can be hypothesized that the difference in DNRA rates could result from differences in the relative abundance of *nrfA* community induced by long-term fertilization.

Fertilization had no effect on the relative abundance of *nrfA/nir* due to high levels of both NO_3^- reductase genes in fertilized fields (**paper III** and **IV**). However, short-term NO_3^- application in microcosm incubations decreased the *nrfA/nir* ratio. It can be speculated that a reduced spatial heterogeneity and wet conditions in the microcosms, enable higher nutrient mobility and consequently increase the direct competition between denitrifiers and DNRA bacteria. Under the tested conditions with NO_3^- levels corresponding to 200 and 1000 kg N/ha denitrifiers appear to be more competitive for NO_3^-. This finding is in line with results from pure culture and enrichment studies, where DNRA was repeatedly shown to be favoured under limitation of electron acceptor, such as NO_3^- and NO_2^- (van den Berg *et al.*, 2015; Yoon *et al.*, 2015).

3.3 N input influencing N_2O reducing communities

A dominance of *nir* genes over the genes encoding the N_2O reductase was characteristic for all arable soils and not affected by long-term fertilization (**paper II**). After 6 months of incubation with or without added NO_3^-, the *nos/nir* ratio in previously fertilized soils decreased (**paper IV**), while it increased in the unfertilized soils. This increase was mainly driven by an increase in *nosZ*II abundance. The abundance of *nosZ*II also increased in the previously fertilized soils, although to a lesser extent, suggesting that a higher N_2O reduction potential develops under constant denitrifying conditions. Six months after NO_3^- application to the microcosm incubations, the N_2O concentration in the headspace with soils of low C/ NO_3^- ratio was still high. This high production of N_2O might allow certain soil organisms to grow on N_2O produced by other organisms, which could be another reason why growth of *nosZ*II organisms was observed. About 50 % of *nosZ*II organisms are solely N_2O reducers (Graf *et al.*, 2014), and in **paper I**, we demonstrated that soil organisms without the capacity to produce N_2O could grow on medium with N_2O as sole electron acceptor. Moreover, *nosZ*II organisms can be favoured over *nosZ*I organisms (i.e. denitrifiers) when N_2O is the only electron acceptor (Conthe *et al.*, 2018a).

In an environmental study, it has been recognized that fertilization regimes affect organisms of the two *nosZ* clades differently (Krause *et al.*, 2017). Distinct spatial distributions of *nosZ*I and *nosZ*II in organic and integrated cropping systems as well as a correlation of *nosZ*I but not *nosZ*II and soil organic N has been reported (Juhanson *et al.*, 2017). In **paper II** and **IV**, N_2O reducing bacteria with *nosZ*I more often increased in abundance after higher nutrient application in a long and short-term N application. Higher correlations of *nosZ*I with NO_3^- and N_2O emissions, but not *nosZ*II have been observed previously. Thereupon, it has been suggested that organisms harbouring *nosZ*I grow faster than those with *nosZ*II in fertilized soils due to a more widespread genetic ability to process NO_3^- and intermediate products of denitrification (Krause *et al.*, 2018). In agreement, *nosZ*I organisms were shown to dominate over *nosZ*II at high dilution rates in chemostat experiments and the most abundant *nosZ*II organisms had the capacity for full denitrification (Conthe *et al.*, 2018b). Similarly, Yoon *et al.* (2016) concluded that *nosZ*I organisms were *r*-strategists, whereas organisms harbouring *nosZ*II were *k*-strategists based on their apparent affinities for N_2O. However, the current literature on niche differentiation of *nosZ*I and *nosZ*II and potential functional differences with respect to N_2O reduction are too limited to draw general and robust conclusions. More effort in classifying the ecophysiology of both clades is needed, but might be impeded by the high phylogenetic diversity of *nosZ* clade II.

While the *nosZ*I community was increasing in abundance after fertilization, the *nosZ*II community structure changed and the diversity increased (**paper II**). Input of additional N might benefit specific *nosZ*II organisms, which affects *nosZ*II species richness and phylogenetic diversity. A stronger susceptibility of the *nosZ*II community to agricultural practices has been suggested previously (Domeignoz-Horta *et al.*, 2015).

4 C/N ratio as determining factor of N_2O

4.1 Effect of C/N ratio on soil microbial community structure and diversity

Agricultural management practices can influence the soil C/N ratio. For example, incorporation of crop residues counteracts the loss of C from agricultural soils with additional beneficial effects such as erosion control, increased nutrient recycling and improved soil structure, which could stimulate plant growth (Liu *et al.*, 2015; Poeplau *et al.*, 2015).

Changes in the microbial community according to C/N may not only have consequences for C cycling in soils, but can also affect microbial mediated N turnover (Mooshammer *et al.*, 2014). The microbial community structure in a range of arable soils across Sweden were significantly influenced by the soil C/N ratio (**paper II**), as microbial diversity increased with increasing soil C/N (**paper II** and **IV**). This effect of C/N has been observed in relation to change of specific taxonomic groups. Bacterial taxa like Chloroflexi and Firmicutes were negatively correlated to C/N ratio, whereas Planctomycetes and Acidobacteria were positively correlated to C/N (Delgado-Baquerizo *et al.*, 2017). Specific correlations of certain taxa with C/N ratio are in line with lowered C/N by fertilization (Leff *et al.*, 2015; Fierer *et al.*, 2012). Delgado-Baquerizo *et al.*, (2017) suggested that the preference for high or low C availability is a result of an adaptation during early evolution of specific taxa to geologically old mineral soils, or organic soils emerging later on.

4.2 C/NO_3^- ratio determining the fate of NO_3^-

Under anoxic conditions denitrification and DNRA compete for NO_3^- as electron acceptor. It has been suggested that the relative importance of DNRA versus denitrification is controlled by the C/NO_3^- ratio as means of ratio of electron-donor to electron acceptor (Schmidt *et al.*, 2011; Tiedje *et al.*, 1982). This has been supported by pure culture studies of a strain of *Shewenella loihica*, which is capable of both denitrification and DNRA. This strain reduced NO_3^- to N_2 when growing on a medium with a low C/NO_3^- ratio while ammonifying NO_3^- under high C/NO_3^- conditions (Yoon *et al.*, 2015). Moreover, chemostat studies have shown that high C/NO_3^- ratios allow for enrichment of DNRA populations (van den Berg *et al.*, 2015). In **paper IV**, the effect of C/N ratio on complex native soil communities was tested utilizing soil microcosm incubations with straw and KNO_3^- addition to determine causal and general effects of altering the C/N ratio in soils with different edaphic factors and microbial communities. The consequence of modified C/N ratio was a shift in the NO_3^- reducing communities, with the *nrfA/nir* gene abundance ratio positively correlated with soil C/N. Straw addition to soil incubations promoted *nrfA* over denitrification genes, whereas when straw was added in combination with NO_3^-, the *nrfA/nir* ratio was not affected or decreased. A possible explanation could be the advantage of DRNA bacteria under electron acceptor limiting conditions, since NO_2^- reduction to NH_4^+ accepts eight instead of five electrons per nitrite (Kraft *et al.*, 2014; Tiedje *et al.*, 1982).

The predominance of DNRA over denitrification was also observed in **paper III**, in which the C/NO_3^- ratio of field plots that were altered by 45 years of different cropping systems. Here, we could directly link the activities of denitrification and DNRA with both C/NO_3^- and *nrfA/nir* ratio (Figure 3). It is important to recognize that total N and NO_3^- was higher in the ley (L) soils compared to the annual cereal rotation (AC), indicating that a high N level does not necessarily promote N loss from soils, and that a high activity of DNRA over the years can foster N conservation.

Besides effects on the DNRA bacteria, the soil C/N ratio also affects denitrifiers with direct links to N_2O emissions (Yuan *et al.*, 2017; Bent *et al.*, 2016). Therefore the balance between the two NO_3^- reduction processes govern N_2O emissions, as also shown in **paper III**.

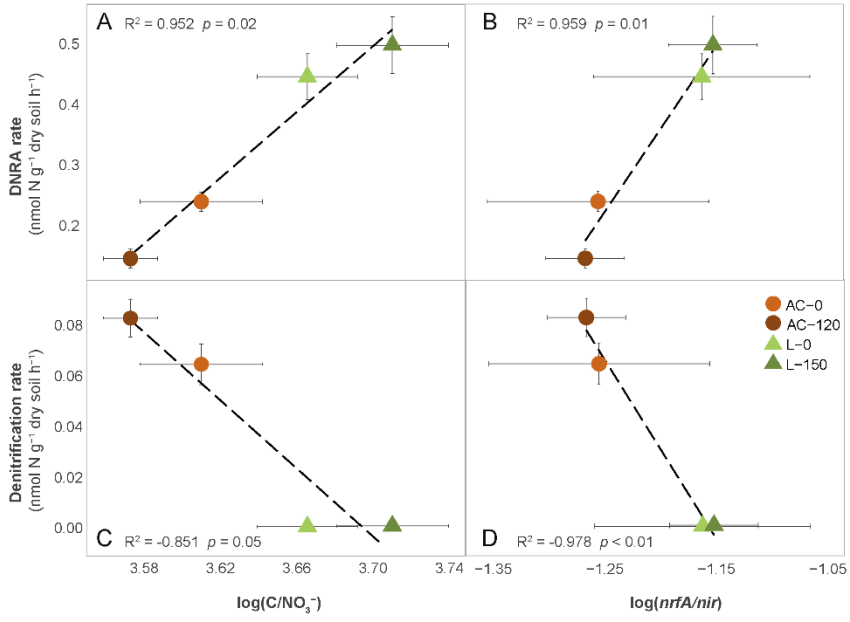

Figure 3. Regression analysis for illustrating the relationship between NO_3^- reducing activities DNRA (A, B) and denitrification (C, D) with explanatory variables soil C/NO_3^- ratio (A, C) and *nrfA/nir* gene abundance ratio (B, D). Different colors and shapes of data points correspond to annual cereal crop rotation (AC) or ley rotation (L) and the level of N fertilization (0 or 120, 150 kg N ha^{-1} yr^{-1}).

The N_2O emission pattern in the headspace of microcosms in **paper IV** hints towards a differential response of soils to straw and NO_3^- application, depending on the fertilization history. As we observed legacy effects of N fertilization, a possible adaption of the soil microbial community might also be relevant for various soil management practices with the aim to raise the C content. The strong interdependence of C and N cycling in soils suggests that long-term manipulation of the soil C content, effects as well N_2O emissions.

4.3 C/N influencing N_2O reduction

Across the studies the effect of different agricultural management systems on the functional microbial communities with impact on N_2O emissions were evident. Initiated adaptations of microbial regulated N use efficiency has impact on N conservation and N losses with implementation on the N_2O budget. In **paper III-IV** we observed significant correlations between N_2O production and soil C/NO_3^- ratio, underscoring how management strategies that directly

influence C/N in soils can result in lower N_2O emissions (Domeignoz-Horta *et al.*, 2018; Abalos *et al.*, 2016; Gelfand *et al.*, 2016; Thompson *et al.*, 2016).

It has been suggested that for the availability of electron donor (C) in relation to NO_3^- (electron acceptor) has consequences for the extent of N_2O reduction (Richardson *et al.*, 2009). Accordingly the last step of denitrification, the reduction of N_2O demands twice as much electrons compared to NO_2^- reduction. A high availability of electron donor consequently promoted the N_2O reduction activity of a denitrifying bacterium (Felgate *et al.*, 2012). This is in line with the finding of C/N ratio being a main factor for effectivity of *D. fertmentans*, a N_2O-reducing strain, in the inoculated soils (**paper I**). While we found a higher abundance of N_2O reducers, expressed as *nos/nir* gene ratio in soils with high C/NO_3^- ratios in **paper III**, a negative correlation of the *nos/nir* ratio and C/N was determined in **paper IV**. Consequently, the relation to N_2O emissions was inconclusive. The C/N ratio reportedly affects the abundance and community of *nosZ* organisms (Krause *et al.*, 2018; Juhanson *et al.*, 2017; Domeignoz-Horta *et al.*, 2015). Thereby disparate effects of altered C/N ratio on the *nosZ*I and *nosZ*II communities can be expected. In **paper II** and **III**, higher C/N ratio was correlated with a higher *nosZ*I/*nosZ*II ratio. The gene abundance of *nosZ*I has been correlated to C/N in arable soils but not *nosZ*II (Juhanson *et al.*, 2017; Domeignoz-Horta *et al.*, 2015), which further signals that under N limiting conditions, denitrifiers with a complete pathway have an advantage (Felgate *et al.*, 2012). In a field study utilizing straw incorporation, the only *nosZ* OTU negatively correlated with N_2O emissions was assigned to Betaproteobacteria, highly abundant in high C/N ratio fields (Bent *et al.*, 2016). The level of *nosZ*I abundance or *nosZ*I transcripts can be increased by crop residue incorporation or biochar amendment (Krause *et al.*, 2018; Németh *et al.*, 2014). Attempts to raise the C/N ratio by adding a C source may also lead to higher denitrification activity and emissions of N_2O, depending on the N amendment (Köbke *et al.*, 2018). If C is added to fertilized plots, no effect on the *nosZ*I abundance was shown, whereas C addition under shortage of NO_3^- promotes *nosZ*I and N_2O reduction to N_2 (Fracetto *et al.*, 2017).

Further, the effect of crop residue incorporation on N_2O emissions is highly affected by synthetic fertilizer application (Liu *et al.*, 2015; Shan & Yan, 2013). It has been observed, that in soils receiving organic amendment in combination with N fertilizer, the emission factor, modulated by the C/N, soil texture and precipitation rises (Charles *et al.*, 2017).

In a lab-experiment of **paper IV**, we observed that straw addition without NO_3^- addition promoted the gene abundance for *nosZ*I and *nosZ*II. However, if straw was applied in combination with NO_3^- we observed no or suppressing effects compared to the treatment without straw addition. This affected the

pattern for the gene abundance ratios of *nos/nir* ratio (Figure 4), which serves as an experimental evidence of higher amounts of electron donor enhance the potential for N_2O reduction. Here, *nosZ*I and *nosZ*II contributed to a higher N_2O reduction in relation to C/N ratio. In **paper III**, *nosZ*II was the only gene correlated with the C/NO_3^- ratio. It has also been shown that the *nosZ*II community structure is correlated to C/N ratio, and both factors *nosZ*II community and C/N ratio are important explanatory factors for N_2O emissions (Juhanson *et al.*, 2017; Domeignoz-Horta *et al.*, 2015; Jones *et al.*, 2014).

The strong correlation of the *nosZ*II and *nrfA* abundance in **paper IV** and similar response to various treatments suggests a significant amount of organisms harbouring both genes, *nosZ*II and *nrfA* (Mania *et al.*, 2014; Sanford *et al.*, 2012). The co-occurrence of *nosZ*II and *nrfA* might be common as N_2O can be produced as a side product of DNRA and thus organisms might use it to gain energy. Organisms with this asset have high potential in retaining N in soils, and mitigate N_2O emissions and further research should be focused on the ecology of this trait.

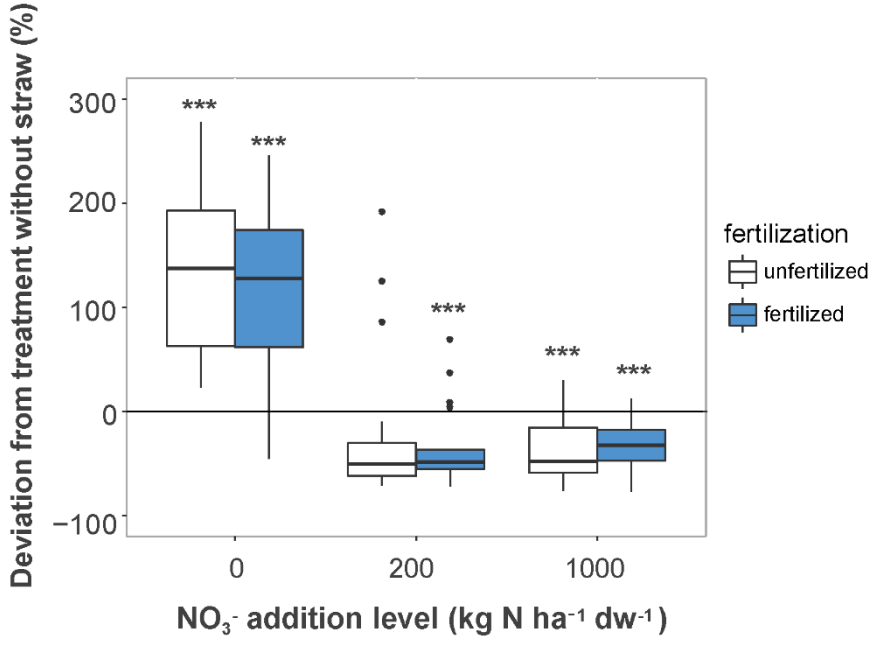

Figure 4. Difference of the *nos/nir* gene abundance ratio after application of straw to microcosms at different levels of NO_3^- application. The different colours of the boxplot indicate the fertilization treatment prior to microcosm incubation. Significant deviations are indicated by asterisks (*p <0.05, **p < 0.01, ***p < 0.001).

5 General conclusions and future perspectives

There is a growing interest in understanding the functions of soil microbial communities in relation to ecosystem functioning as it will be needed to meet the pressing challenges to reduce agriculture's impact on climate warming. This thesis showed the complexity of functions within the soil microbial communities that affect N_2O emissions under anoxic conditions. Across **papers I-IV** we found evidence that the soil C/N and C/NO_3^- ratio respectively were important factors influencing the microbial communities and net N_2O emissions.

As a proof of concept, the abundance of non-denitrifying bacteria harbouring the *nosZ* gene was shown to reduce N_2O emitted by the native soil microbial community in **paper I**. Moreover, the effectivity of nitrous oxide reduction was correlated with higher C/N ratio of soils.

A field study (**paper III**) revealed that crop rotations with ley (short-term grasslands) leading to higher C/NO_3^- ratio promoted not only a higher relative abundance of potential N_2O reduction, but more important also higher rates of DNRA which can contribute significantly to soil N retention. At the same time, denitrifying bacteria were less favoured than DNRA bacteria and subsequently less N_2O was produced. We found that ley had a stronger effect on soil C/NO_3^- ratio compared to fertilization, as grasslands retained C and N content in soils with possible implications for higher crop yields.

In the manipulation experiment in **paper IV**, the effects of altered C/N ratio on the relative abundance of DNRA versus denitrifiers within complex soil communities was observed. The DNRA process and N_2O reduction were favoured under high C/N ratios and were shown to reduce N_2O emissions to a great extent. In both studies, **paper III** and **IV**, lower C/N or C/NO_3^- ratios, respectively were associated with higher N_2O emissions. Determined higher N_2O emissions and denitrification end-product ratios were associated with lower *nos/nir* ratios (**paper II – IV**) demonstrating the relevance of the genetic basis

for actual N_2O emissions. Changes in the *nos/nir* ratio were mainly driven by an increase of *nosZ*I bacteria at higher N levels (**paper II - IV**). Consistent effects of fertilization on the functional microbial groups of denitrifiers and N_2O reducers were demonstrated and we could identify taxonomic groups likely involved in regulating net N_2O emission by affecting N_2O production and reduction. Moreover, we observed a distinct response of both *nosZ* clades to N fertilization, including higher susceptibility of *nosZ*II, although community structure of both clades were correlated with N_2O reduction (**paper II**).

Future perspectives

The N cycle is still characterized by high uncertainties and the microbial N transformation are not yet fully understood. Recent discoveries of processes and genes possessing steps of the N cycle like archaeal NH_3 oxidation (Francis *et al.*, 2005; Könneke *et al.*, 2005; Treusch *et al.*, 2005; Venter *et al.*, 2004), codenitrification (Laughlin & Stevens, 2002; Shoun *et al.*, 1992; Tanimoto *et al.*, 1992) or the recent discovery of comammox organisms (Daims *et al.*, 2015; van Kessel *et al.*, 2015) illustrates our evolving view on the N cycle. A combination of better methods to analyse the soil microbiome and quantification of the bacterial N turnover provides improved possibilities to develop nutrient management options by incorporating soil microbial communities. The observed correlations between the soil microbial diversity, C/N ratio and the *nrfA* abundance with implications for N_2O emissions reported in this thesis are a first step towards that goal, but better characterization of the soil microorganisms possessing NO_3^- reduction to NH_4^+ and their abilities in N_2O reduction is required.

A threat of enhanced production of N_2O when more C is sequestered in soils highlights the urgency to study the interaction of C and N cycling in arable soils. The demonstrated importance of the C/N ratio on the N turnover of soils, clarifies that integrated nutrient cycling is a key in the mitigation of greenhouse gases to meet future challenges of a climate friendly agriculture. A future challenge in agricultural research is also incorporation of knowledge on functional microbial guilds, which could allow for better predictions of soil N cycling under different management practices. This thesis points at an important mitigation potential by using the *nosZ* community. Experimental testing of the suggested niche differentiation between *nosZ*I and *nosZ*II is needed to be able to adjust agronomic practices to reach high efficiency of microbial N_2O reduction. Moreover, although N_2O reducers are important, we conclude that the balance between DNRA and denitrification can be more determinant for net N_2O emissions. Knowledge on the ecology of DNRA organisms in arable soils is still very limited and more research within this area is therefore needed. Furthermore,

studies focusing on soil microbiota, nutrients and the interaction with plants have potential to increase our understanding on how interactions between different trophic levels contribute to N cycling in soil. A deeper understanding of the soil microbial complexity in respect to agronomic practices and climate change are indispensable steps towards reaching resource and climate conservation agriculture and technologies to foster a soil microbial community that supports high N use efficiency and high crop yields is needed.

References

Abalos, D., Brown, S.E., Vanderzaag, A.C., Gordon, R.J., Dunfield, K.E. & Wagner-Riddle, C. (2016). Micrometeorological measurements over 3 years reveal differences in N_2O emissions between annual and perennial crops. *Global Change Biology*, 22(3), pp. 1244-55.

Allison, S.D. & Martiny, J.B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, 105(1), pp. 11512-11519.

Balderston, W. & Payne, W. (1976). Inhibition of methanogenesis in salt marsh sediments and whole-cell suspensions of methanogenic bacteria by nitrogen oxides. *Applied and Environmental Microbiology*, 32(2), pp. 264-269.

Bardgett, R.D. & van der Putten, W.H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, 515(7528), p. 505.

Bárta, J., Tahovská, K., Šantrůčková, H. & Oulehle, F. (2017). Microbial communities with distinct denitrification potential in spruce and beech soils differing in nitrate leaching. *Scientific Reports*, 7(1), pp. 9738.

Bent, E., Németh, D., Wagner-Riddle, C. & Dunfield, K. (2016). Residue management leading to higher field-scale N_2O flux is associated with different soil bacterial nitrifier and denitrifier gene community structures. *Applied Soil Ecology*, 108, pp. 288-299.

Bergaust, L., Mao, Y., Bakken, L.R. & Frostegård, Å. (2010). Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrogen oxide reductase in Paracoccus denitrificans. *Applied and Environmental Microbiology*, 76(19), pp. 6387-6396.

Bleakley, B.H. & Tiedje, J.M. (1982). Nitrous oxide production by organisms other than nitrifiers or denitrifiers. *Applied and Environmental Microbiology*, 44(6), pp. 1342-1348.

Blum, J.M., Su, Q., Ma, Y., Valverde-Pérez, B., Domingo-Félez, C., Jensen, M.M. & Smets, B.F. (2018). The pH dependency of N-converting enzymatic processes, pathways and microbes: effect on net-N_2O production. *Environmental Microbiology*, 20(5), pp. 1632-1640.

Bonilla-Rosso, G., Wittorf, L., Jones, C.M. & Hallin, S. (2016). Design and evaluation of primers targeting genes encoding NO-forming nitrite reductases: implications for ecological inference of denitrifying communities. *Scientific Reports*, 6, p. 39208.

Börjesson, G., Menichetti, L., Kirchmann, H. & Kätterer, T. (2011). Soil microbial community structure affected by 53 years of nitrogen fertilisation and different organic amendments. *Biology and Fertility of Soils*, 48(3), pp. 245-257.

Braker, G., Fesefeldt, A. & Witzel, K.-P. (1998). Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples. *Applied and Environmental Microbiology*, 64(10), pp. 3769-3775.

Brenzinger, K., Dorsch, P. & Braker, G. (2015). pH-driven shifts in overall and transcriptionally active denitrifiers control gaseous product stoichiometry in growth experiments with extracted bacteria from soil. *Frontiers in Microbiology*, 6, p. 961.

Brenzinger, K., Kujala, K., Horn, M.A., Moser, G., Guillet, C., Kammann, C., Müller, C. & Braker, G. (2017). Soil conditions rather than long-term exposure to elevated CO_2 affect soil microbial communities associated with N-cycling. *Frontiers in Microbiology*, 8, p. 1976.

Buckingham, S., Anthony, S., Bellamy, P.H., Cardenas, L.M., Higgins, S., McGeough, K. & Topp, C.F.E. (2014). Review and analysis of global agricultural N_2O emissions relevant to the UK. *Science of the Total Environment*, 487, pp. 164-172.

Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos Trans R Soc Lond B Biol Sci*, 368(1621), p. 20130122.

Campbell, B.J., Polson, S.W., Hanson, T.E., Mack, M.C. & Schuur, E.A.G. (2010). The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environmental Microbiology*, 12(7), pp. 1842-1854.

Carreira, C., Pauleta, S.R. & Moura, I. (2017). The catalytic cycle of nitrous oxide reductase - The enzyme that catalyzes the last step of denitrification. *Journal of Inorganic Biochemistry*, 177, pp. 423-434.

Cassman, N.A., Leite, M.F., Pan, Y., de Hollander, M., van Veen, J.A. & Kuramae, E.E. (2016). Plant and soil fungal but not soil bacterial communities are linked in long-term fertilized grassland. *Scientific Reports*, 6, p. 23680.

Cavigelli, M. & Robertson, G. (2001). Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biology and Biochemistry*, 33(3), pp. 297-310.

Cavigelli, M.A., Del Grosso, S.J., Liebig, M.A., Snyder, C.S., Fixen, P.E., Venterea, R.T., Leytem, A.B., McLain, J.E. & Watts, D.B. (2012). US agricultural nitrous oxide emissions: context, status, and trends. *Frontiers in Ecology and the Environment*, 10(10), pp. 537-546.

Cederlund, H., Wessén, E., Enwall, K., Jones, C.M., Juhanson, J., Pell, M., Philippot, L. & Hallin, S. (2014). Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. *Applied Soil Ecology*, 84, pp. 62-68.

Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J.L. & Bernoux, M. (2007). Soils, a sink for N_2O? A review. *Global Change Biology*, 13(1), pp. 1-17.

Charles, A., Rochette, P., Whalen, J.K., Angers, D.A., Chantigny, M.H. & Bertrand, N. (2017). Global nitrous oxide emission factors from agricultural soils after addition of organic amendments: A meta-analysis. *Agriculture, Ecosystems & Environment*, 236, pp. 88-98.

Clark, I.M., Buchkina, N., Jhurreea, D., Goulding, K.W. & Hirsch, P.R. (2012). Impacts of nitrogen application rates on the activity and diversity of denitrifying bacteria in the Broadbalk Wheat Experiment. *Philos Trans R Soc Lond B Biol Sci*, 367(1593), pp. 1235-44.

Congreves, K.A., Brown, S.E., Németh, D.D., Dunfield, K.E. & Wagner-Riddle, C. (2017). Differences in field-scale N_2O flux linked to crop residue removal under two tillage systems in cold climates. *GCB Bioenergy*, 9(4), pp. 666-680.

Conrad, R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiological reviews*, 60(4), pp. 609-640.

Conthe, M., Wittorf, L., Kuenen, J.G., Kleerebezem, R., Hallin, S. & van Loosdrecht, M. (2018a). Growth yield and selection of *nosZ* clade II-types in a continuous enrichment culture of N_2O respiring bacteria. *Environmental microbiology reports*.

Conthe, M., Wittorf, L., Kuenen, J.G., Kleerebezem, R., Loosdrecht, M.C. & Hallin, S. (2018b). Life on N_2O: deciphering the ecophysiology of N_2O respiring bacterial communities in a continuous culture. *ISME Journal*, 12(4), pp. 1142-1153.

Coolon, J.D., Jones, K.L., Todd, T.C., Blair, J.M. & Herman, M.A. (2013). Long-term nitrogen amendment alters the diversity and assemblage of soil bacterial communities in tallgrass prairie. *PLoS ONE*, 8(6), p. e67884.

Coyotzi, S., Doxey, A.C., Clark, I.D., Lapen, D.R., Van Cappellen, P. & Neufeld, J.D. (2017). Agricultural soil denitrifiers possess extensive nitrite reductase gene diversity. *Environmental Microbiology*, 19(3), pp. 1189-1208.

Cui, P., Fan, F., Yin, C., Song, A., Huang, P., Tang, Y., Zhu, P., Peng, C., Li, T. & Wakelin, S.A. (2016). Long-term organic and inorganic fertilization alters temperature sensitivity of potential N_2O emissions and associated microbes. *Soil Biology and Biochemistry*, 93, pp. 131-141.

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J. & Bulaev, A. (2015). Complete nitrification by Nitrospira bacteria. *Nature*, 528(7583), p. 504.

Davidson, E.A. & Kanter, D. (2014). Inventories and scenarios of nitrous oxide emissions. *Environmental Research Letters*, 9(10), p. 105012.

Davis, J.H., Griffith, S.M., Horwath, W.R., Steiner, J.J. & Myrold, D.D. (2008). Denitrification and nitrate consumption in an herbaceous riparian area and perennial ryegrass seed cropping system. *Soil Science Society of America Journal*, 72(5), pp. 1299-1310.

Delgado-Baquerizo, M., Reich, P.B., Khachane, A.N., Campbell, C.D., Thomas, N., Freitag, T.E., Abu Al-Soud, W., Sørensen, S., Bardgett, R.D. & Singh, B.K. (2017). It is elemental: soil nutrient stoichiometry drives bacterial diversity. *Environmental Microbiology*, 19(3), pp. 1176-1188.

Di, H.J., Cameron, K.C., Shen, J.-P., Winefield, C.S., O'Callaghan, M., Bowatte, S. & He, J.-Z. (2010). Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology*, 72(3), pp. 386-394.

Domeignoz-Horta, L.A., Philippot, L., Peyrard, C., Bru, D., Breuil, M.C., Bizouard, F., Justes, E., Mary, B., Leonard, J. & Spor, A. (2018). Peaks of in situ N_2O emissions are influenced by N_2O-producing and reducing microbial communities across arable soils. *Global Change Biology*, 24(1), pp. 360-370.

Domeignoz-Horta, L.A., Spor, A., Bru, D., Breuil, M.C., Bizouard, F., Leonard, J. & Philippot, L. (2015). The diversity of the N_2O reducers matters for the N_2O:N_2 denitrification end-product ratio across an annual and a perennial cropping system. *Frontiers in Microbiology*, 6, p. 971.

Enwall, K., Philippot, L. & Hallin, S. (2005). Activity and composition of the denitrifying bacterial community respond differently to long-term fertilization. *Applied and Environmental Microbiology*, 71(12), pp. 8335-8343.

Enwall, K., Throbäck, I.N., Stenberg, M., Soderstrom, M. & Hallin, S. (2010). Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. *Applied and Environmental Microbiology*, 76(7), pp. 2243-50.

Felgate, H., Giannopoulos, G., Sullivan, M.J., Gates, A.J., Clarke, T.A., Baggs, E., Rowley, G. & Richardson, D.J. (2012). The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways. *Environmental Microbiology*, 14(7), pp. 1788-1800.

Fernandes, S.O., Bonin, P.C., Michotey, V.D., Garcia, N. & LokaBharathi, P.A. (2012). Nitrogen-limited mangrove ecosystems conserve N through dissimilatory nitrate reduction to ammonium. *Scientific Reports*, 2, p. 419.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology*, 15(10), p. 579.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A. & Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME Journal*, 6(5), pp. 1007-17.

Fisk, M.C. & Schmidt, S.K. (1996). Microbial responses to nitrogen additions in alpine tundra soil. *Soil Biology and Biochemistry*, 28(6), pp. 751-755.

Fracetto, F.J.C., Fracetto, G.G.M., Bertini, S.C.B., Cerri, C.C., Feigl, B.J. & Siqueira Neto, M. (2017). Effect of agricultural management on N_2O emissions in the Brazilian sugarcane yield. *Soil Biology and Biochemistry*, 109, pp. 205-213.

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F. & Reitz, T. (2016). Mineral vs. Organic Amendments: Microbial Community Structure, Activity and Abundance of Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies. *Frontiers in Microbiology*, 7, p. 16.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E. & Oakley, B.B. (2005). Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Science of the United States of America*, 102(41), pp. 14683-14688.

Franklin, R.B., Morrissey, E.M. & Morina, J.C. (2017). Changes in abundance and community structure of nitrate-reducing bacteria along a salinity gradient in tidal wetlands. *Pedobiologia*, 60, pp. 21-26.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P. & Holland, E.A. (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry*, 70(2), pp. 153-226.

Gao, N., Shen, W., Camargo, E., Shiratori, Y., Nishizawa, T., Isobe, K., He, X. & Senoo, K. (2017). Nitrous oxide (N_2O)-reducing denitrifier-inoculated organic fertilizer mitigates N_2O emissions from agricultural soils. *Biology and Fertility of Soils*, 53(8), pp. 885-898.

Geisseler, D. & Scow, K.M. (2014). Long-term effects of mineral fertilizers on soil microorganisms – A review. *Soil Biology and Biochemistry*, 75, pp. 54-63.

Gelfand, I., Shcherbak, II, Millar, N., Kravchenko, A.N. & Robertson, G.P. (2016). Long-term nitrous oxide fluxes in annual and perennial agricultural and unmanaged ecosystems in the upper Midwest USA. *Global Change Biology*, 22(11), pp. 3594-3607.

Graf, D.R., Jones, C.M. & Hallin, S. (2014). Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. *PLoS ONE*, 9(12), p. e114118.

Graf, D.R.H., Zhao, M., Jones, C.M. & Hallin, S. (2016). Soil type overrides plant effect on genetic and enzymatic N_2O production potential in arable soils. *Soil Biology and Biochemistry*, 100, pp. 125-128.

Graham, E.B., Knelman, J.E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Beman, J., Abell, G., Philippot, L. & Prosser, J. (2016). Microbes as engines of ecosystem function: when does community structure enhance predictions of ecosystem processes? *Frontiers in Microbiology*, 7, p. 214.

Graham, E.B., Wieder, W.R., Leff, J.W., Weintraub, S.R., Townsend, A.R., Cleveland, C.C., Philippot, L. & Nemergut, D.R. (2014). Do we need to understand microbial communities to predict ecosystem function? A comparison of statistical models of nitrogen cycling processes. *Soil Biology and Biochemistry*, 68, pp. 279-282.

Hallin, S., Jones, C.M., Schloter, M. & Philippot, L. (2009). Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *ISME Journal*, 3(5), pp. 597-605.

Hallin, S. & Lindgren, P.E. (1999). PCR detection of genes encoding nitrile reductase in denitrifying bacteria. *Applied and Environmental Microbiology*, 65(4), pp. 1652-1657.

Hallin, S., Philippot, L., Löffler, F.E., Sanford, R.A. & Jones, C.M. (2018). Genomics and ecology of novel N_2O-reducing microorganisms. *Trends in Microbiology*, 26(1), pp. 43-55.

Hartmann, M., Frey, B., Mayer, J., Mader, P. & Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *ISME Journal*, 9(5), pp. 1177-1194.

Hink, L., Gubry-Rangin, C., Nicol, G.W. & Prosser, J.I. (2018). The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. *ISME Journal*, 12(4), pp. 1084-1093.

Hink, L., Nicol, G.W. & Prosser, J.I. (2017). Archaea produce lower yields of N_2O than bacteria during aerobic ammonia oxidation in soil. *Environmental Microbiology*, 19(12), pp. 4829-4837.

Huygens, D., Rütting, T., Boeckx, P., Van Cleemput, O., Godoy, R. & Müller, C. (2007). Soil nitrogen conservation mechanisms in a pristine south Chilean Nothofagus forest ecosystem. *Soil Biology and Biochemistry*, 39(10), pp. 2448-2458.

Inselsbacher, E., Umana, N.H.-N., Stange, F.C., Gorfer, M., Schüller, E., Ripka, K., Zechmeister-Boltenstern, S., Hood-Novotny, R., Strauss, J. & Wanek, W. (2010). Short-term competition between crop plants and soil microbes for inorganic N fertilizer. *Soil Biology and Biochemistry*, 42(2), pp. 360-372.

Jones, C.M., Graf, D.R.H., Bru, D., Philippot, L. & Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *ISME Journal*, 7(2), pp. 417-426.

Jones, C.M. & Hallin, S. (2010). Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. *ISME Journal,* 4(5), pp. 633-641.

Jones, C.M., Spor, A., Brennan, F.P., Breuil, M.-C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S. & Philippot, L. (2014). Recently identified microbial guild mediates soil N_2O sink capacity. *Nature Climate Change,* 4(9), pp. 801-805.

Juhanson, J., Hallin, S., Söderström, M., Stenberg, M. & Jones, C.M. (2017). Spatial and phyloecological analyses of nosZ genes underscore niche differentiation amongst terrestrial N_2O reducing communities. *Soil Biology and Biochemistry,* 115, pp. 82-91.

Kätterer, T., Börjesson, G. & Kirchmann, H. (2014). Changes in organic carbon in topsoil and subsoil and microbial community composition caused by repeated additions of organic amendments and N fertilisation in a long-term field experiment in Sweden. *Agriculture, Ecosystems & Environment,* 189, pp. 110-118.

Kim, H., Bae, H.-S., Reddy, K.R. & Ogram, A. (2016). Distributions, abundances and activities of microbes associated with the nitrogen cycle in riparian and stream sediments of a river tributary. *Water Research,* 106, pp. 51-61.

Köbke, S., Senbayram, M., Pfeiffer, B., Nacke, H. & Dittert, K. (2018). Post-harvest N_2O and CO_2 emissions related to plant residue incorporation of oilseed rape and barley straw depend on soil NO_3-content. *Soil and Tillage Research,* 179, pp. 105-113.

Könneke, M., Bernhard, A.E., José, R., Walker, C.B., Waterbury, J.B. & Stahl, D.A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature,* 437(7058), p. 543.

Koyama, A., Wallenstein, M.D., Simpson, R.T. & Moore, J.C. (2014). Soil bacterial community composition altered by increased nutrient availability in Arctic tundra soils. *Frontiers in Microbiology,* 5, p. 516.

Kraft, B., Strous, M. & Tegetmeyer, H.E. (2011). Microbial nitrate respiration--genes, enzymes and environmental distribution. *Journal of Biotechnology,* 155(1), pp. 104-17.

Kraft, B., Tegetmeyer, H.E., Sharma, R., Klotz, M.G., Ferdelman, T.G., Hettich, R.L., Geelhoed, J.S. & Strous, M. (2014). Nitrogen cycling. The environmental controls that govern the end product of bacterial nitrate respiration. *Science,* 345(6197), pp. 676-9.

Krause, H.-M., Hüppi, R., Leifeld, J., El-Hadidi, M., Harter, J., Kappler, A., Hartmann, M., Behrens, S., Mäder, P. & Gattinger, A. (2018). Biochar affects community composition of nitrous oxide reducers in a field experiment. *Soil Biology and Biochemistry,* 119, pp. 143-151.

Krause, H.-M., Thonar, C., Eschenbach, W., Well, R., Mäder, P., Behrens, S., Kappler, A. & Gattinger, A. (2017). Long term farming systems affect soils potential for N_2O production and reduction processes under denitrifying conditions. *Soil Biology and Biochemistry,* 114, pp. 31-41.

Lauber, C.L., Ramirez, K.S., Aanderud, Z., Lennon, J. & Fierer, N. (2013). Temporal variability in soil microbial communities across land-use types. *ISME Journal,* 7(8), pp. 1641-1650.

Laughlin, R.J. & Stevens, R.J. (2002). Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. *Soil Science Society of America Journal,* 66(5), pp. 1540-1548.

Lebender, U., Senbayram, M., Lammel, J. & Kuhlmann, H. (2014). Impact of mineral N fertilizer application rates on N_2O emissions from arable soils under winter wheat. *Nutrient Cycling in Agroecosystems,* 100(1), pp. 111-120.

Leff, J.W., Jones, S.E., Prober, S.M., Barberan, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schutz, M., Steenbock, C., Stevens, C.J. & Fierer, N. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America*, 112(35), pp. 10967-72.

Liang, L.L., Grantz, D.A. & Jenerette, G.D. (2016). Multivariate regulation of soil CO_2 and N_2O pulse emissions from agricultural soils. *Global Change Biology*, 22(3), pp. 1286-98.

Lienhard, P., Terrat, S., Prévost-Bouré, N.C., Nowak, V., Régnier, T., Sayphoummie, S., Panyasiri, K., Tivet, F., Mathieu, O. & Levêque, J. (2014). Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. *Agronomy for sustainable development*, 34(2), pp. 525-533.

Liu, B., Frostegård, Å. & Bakken, L.R. (2014). Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of *nosZ*. *MBio*, 5(3), pp. e01383-14.

Liu, G., Yu, H., Ma, J., Xu, H., Wu, Q., Yang, J. & Zhuang, Y. (2015). Effects of straw incorporation along with microbial inoculant on methane and nitrous oxide emissions from rice fields. *Science of the Total Environment*, 518-519, pp. 209-16.

Long, A., Heitman, J., Tobias, C., Philips, R. & Song, B. (2013). Co-occurring anammox, denitrification, and codenitrification in agricultural soils. *Applied and Environmental Microbiology*, 79(1), pp. 168-176.

Maeda, K., Spor, A., Edel-Hermann, V., Heraud, C., Breuil, M.-C., Bizouard, F., Toyoda, S., Yoshida, N., Steinberg, C. & Philippot, L. (2015). N_2O production, a widespread trait in fungi. *Scientific Reports*, 5, p. 9697.

Maeda, K., Toyoda, S., Philippot, L., Hattori, S., Nakajima, K., Ito, Y. & Yoshida, N. (2017). Relative contribution of *nirK*- and *nirS*-bacterial denitrifiers as well as fungal denitrifiers to nitrous oxide production from dairy manure compost. *Environmental Science & Technology*, 51(24), pp. 14083-14091.

Mania, D., Heylen, K., van Spanning, R.J.M. & Frostegard, A. (2014). The nitrate-ammonifying and *nosZ*-carrying bacterium Bacillus vireti is a potent source and sink for nitric and nitrous oxide under high nitrate conditions. *Environmental Microbiology*, 16(10), pp. 3196-3210.

Matheson, F., Nguyen, M., Cooper, A., Burt, T. & Bull, D. (2002). Fate of ^{15}N-nitrate in unplanted, planted and harvested riparian wetland soil microcosms. *Ecological Engineering*, 19(4), pp. 249-264.

Meyer, C., Heiss, C., Drexhage, C., Kehmeier, E.S., Balzer, J., Mühlfeld, A., Merx, M.W., Lauer, T., Kühl, H. & Floege, J. (2010). Hemodialysis-induced release of hemoglobin limits nitric oxide bioavailability and impairs vascular function. *Journal of the American College of Cardiology*, 55(5), pp. 454-459.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M. & Wild, B. (2014). Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling. *Nature Communications*, 5, p. 3694.

Morales, S.E., Cosart, T. & Holben, W.E. (2010). Bacterial gene abundances as indicators of greenhouse gas emission in soils. *ISME Journal*, 4(6), p. 799-808.

Moreno-Vivián, C. & Ferguson, S.J. (1998). Definition and distinction between assimilatory, dissimilatory and respiratory pathways. *Molecular microbiology*, 29(2), pp. 664-666.

Morley, N. & Baggs, E. (2010). Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. *Soil Biology and Biochemistry*, 42(10), pp. 1864-1871.

Müller, C., Laughlin, R.J., Spott, O. & Rütting, T. (2014). Quantification of N_2O emission pathways via a ^{15}N tracing model. *Soil Biology and Biochemistry*, 72, pp. 44-54.

Nemergut, D.R., Shade, A. & Violle, C. (2014). When, where and how does microbial community composition matter? *Frontiers in Microbiology*, 5, p. 497.

Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman, W.D., Schadt, C.W., Weintraub, M.N. & Schmidt, S.K. (2008). The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. *Environmental Microbiology*, 10(11), pp. 3093-105.

Németh, D.D., Wagner-Riddle, C. & Dunfield, K.E. (2014). Abundance and gene expression in nitrifier and denitrifier communities associated with a field scale spring thaw N_2O flux event. *Soil Biology and Biochemistry*, 73, pp. 1-9.

Orellana, L.H., Chee-Sanford, J.C., Sanford, R.A., Löffler, F.E. & Konstantinidis, K.T. (2018). Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. *Applied and Environmental Microbiology*, 84(2), pp. e01646-17.

Patureau, D., Bernet, N., Delgenes, J. & Moletta, R. (2000). Effect of dissolved oxygen and carbon–nitrogen loads on denitrification by an aerobic consortium. *Applied Microbiology and Biotechnology*, 54(4), pp. 535-542.

Pell, M., Stenberg, B., Stenström, J. & Torstensson, L. (1996). Potential denitrification activity assay in soil - With or without chloramphenicol? *Soil Biology and Biochemistry*, 28(3), pp. 393-398.

Petersen, D.G., Blazewicz, S.J., Firestone, M., Herman, D.J., Turetsky, M. & Waldrop, M. (2012). Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. *Environmental Microbiology*, 14(4), pp. 993-1008.

Pett-Ridge, J., Silver, W.L. & Firestone, M.K. (2006). Redox fluctuations frame microbial community impacts on N-cycling rates in a humid tropical forest soil. *Biogeochemistry*, 81(1), pp. 95-110.

Philippot, L., Andert, J., Jones, C.M., Bru, D. & Hallin, S. (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. *Global Change Biology*, 17(3), pp. 1497-1504.

Philippot, L., Bru, D., Saby, N.P., Cuhel, J., Arrouays, D., Simek, M. & Hallin, S. (2009). Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. *Environmental Microbiology*, 11(12), pp. 3096-104.

Poeplau, C., Kätterer, T., Bolinder, M.A., Börjesson, G., Berti, A. & Lugato, E. (2015). Low stabilization of aboveground crop residue carbon in sandy soils of Swedish long-term experiments. *Geoderma*, 237-238, pp. 246-255.

Polcyn, W. & Podeszwa, J. (2009). Coordinate induction of dissimilatory ammonification and fermentative pathways in rhizobia. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 96(1), pp. 79-87.

Ramirez, K.S., Craine, J.M. & Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, 18(6), pp. 1918-1927.

Ravishankara, A., Daniel, J.S. & Portmann, R.W. (2009). Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. *Science*, 326(5949), pp. 123-125.

Richardson, D., Felgate, H., Watmough, N., Thomson, A. & Baggs, E. (2009). Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle - could enzymic regulation hold the key? *Trends Biotechnol*, 27(7), pp. 388-97.

Robertson, L. & Kuenen, J. (1984). Aerobic denitrification—old wine in new bottles? *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 50(5-6), pp. 525-544.

Rocca, J.D., Hall, E.K., Lennon, J.T., Evans, S.E., Waldrop, M.P., Cotner, J.B., Nemergut, D.R., Graham, E.B. & Wallenstein, M.D. (2015). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. *ISME Journal*, 9(8), pp. 1693-1699.

Rousk, J., Brookes, P.C. & Bååth, E. (2011). Fungal and bacterial growth responses to N fertilization and pH in the 150-year 'Park Grass' UK grassland experiment. *FEMS Microbiology Ecology*, 76(1), pp. 89-99.

Rütting, T., Boeckx, P., Müller, C. & Klemedtsson, L. (2011a). Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences*, 8(7), pp. 1779-1791.

Rütting, T., Huygens, D., Staelens, J., Müller, C. & Boeckx, P. (2011b). Advances in ^{15}N-tracing experiments: new labelling and data analysis approaches. *Biochemical Society Transactions*, 39(1), pp. 279-83.

Ryden, J.C. & Dawson, K.P. (1982). Evaluation of the acetylene-inhibition technique for the measurement of denitrification in grassland soils. *Journal of the Science of Food and Agriculture*, 33(12), pp. 1197-1206.

Samad, M.D., Biswas, A., Bakken, L.R., Clough, T.J., de Klein, C.A., Richards, K.G., Lanigan, G.J. & Morales, S.E. (2016). Phylogenetic and functional potential links pH and N_2O emissions in pasture soils. *Scientific Reports*, 6, p. 35990.

Samad, M.S., Johns, C., Richards, K.G., Lanigan, G.J., de Klein, C.A., Clough, T.J. & Morales, S.E. (2017). Response to nitrogen addition reveals metabolic and ecological strategies of soil bacteria. *Molecular Ecology*, 26, pp. 5500-5514.

Sánchez, C. & Minamisawa, K. (2018). Redundant roles of Bradyrhizobium oligotrophicum Cu-type (NirK) and cd 1-type (NirS) nitrite reductase genes under denitrifying conditions. *FEMS Microbiology Letters*, 365(5), p. fny015.

Sanford, R.A., Cole, J.R. & Tiedje, J.M. (2002). Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl-halorespiring facultative anaerobic myxobacterium. *Applied and Environmental Microbiology*, 68(2), pp. 893-900.

Sanford, R.A., Wagner, D.D., Wu, Q., Chee-Sanford, J.C., Thomas, S.H., Cruz-Garcia, C., Rodriguez, G., Massol-Deya, A., Krishnani, K.K., Ritalahti, K.M., Nissen, S., Konstantinidis, K.T. & Löffler, F.E. (2012). Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *Proceedings of the National Academy of Sciences of the United States of America*, 109(48), pp. 19709-14.

Scala, D.J. & Kerkhof, L.J. (1999). Diversity of nitrous oxide reductase (*nosZ*) genes in continental shelf sediments. *Applied and Environmental Microbiology*, 65(4), pp. 1681-1687.

Schmidt, C.S., Richardson, D.J. & Baggs, E.M. (2011). Constraining the conditions conducive to dissimilatory nitrate reduction to ammonium in temperate arable soils. *Soil Biology and Biochemistry*, 43(7), pp. 1607-1611.

Schulz, S., Kolbl, A., Ebli, M., Buegger, F., Schloter, M. & Fiedler, S. (2017). Field-Scale Pattern of Denitrifying Microorganisms and N_2O Emission Rates Indicate a High Potential for Complete Denitrification in an Agriculturally Used Organic Soil. *Microbial Ecology*, 74(4). pp. 765-770.

Shan, J. & Yan, X. (2013). Effects of crop residue returning on nitrous oxide emissions in agricultural soils. *Atmospheric Environment*, 71, pp. 170-175.

Shcherbak, I., Millar, N. & Robertson, G.P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America*, 111(25), pp. 9199-204.

Shoun, H., Kim, D.H., Uchiyama, H. & Sugiyama, J. (1992). Denitrification by fungi. *FEMS Microbiology Letters*, 94(3), pp. 277-281.

Silver, W.L., Herman, D.J. & Firestone, M.K. (2001). Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology*, 82(9), pp. 2410-2416.

Šimek, M. & Cooper, J. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, 53(3), pp. 345-354.

Simon, J. (2002). Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiology Reviews*, 26(3), pp. 285-309.

Simon, J., Einsle, O., Kroneck, P.M. & Zumft, W.G. (2004). The unprecedented nos gene cluster of Wolinella succinogenes encodes a novel respiratory electron transfer pathway to cytochrome c nitrous oxide reductase. *FEBS Letters*, 569(1-3), pp. 7-12.

Song, B., Lisa, J.A. & Tobias, C.R. (2014). Linking DNRA community structure and activity in a shallow lagoonal estuarine system. *Frontiers in Microbiology*, 5, p. 460.

Spott, O. & Stange, F.C. (2011). Formation of hybrid N_2O in a suspended soil due to co-denitrification of NH2OH. *Journal of Plant Nutrition and Soil Science*, 174(4), pp. 554-567.

Stein, L.Y. & Klotz, M.G. (2016). The nitrogen cycle. *Current Biology*, 26(3), pp. R94-R98.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macêdo, J.L.V., Blum, W.E. & Zech, W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, 291(1-2), pp. 275-290.

Stremińska, M.A., Felgate, H., Rowley, G., Richardson, D.J. & Baggs, E.M. (2012). Nitrous oxide production in soil isolates of nitrate-ammonifying bacteria. *Environmental Microbiology Reports*, 4(1), pp. 66-71.

Strohm, T.O., Griffin, B., Zumft, W.G. & Schink, B. (2007). Growth yields in bacterial denitrification and nitrate ammonification. *Applied and Environmental Microbiology*, 73(5), pp. 1420-1424.

Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M.W., Horn, M., Daims, H., Bartol-Mavel, D. & Wincker, P. (2006). Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature*, 440(7085), p. 790.

Sullivan, M.J., Gates, A.J., Appia-Ayme, C., Rowley, G. & Richardson, D.J. (2013). Copper control of bacterial nitrous oxide emission and its impact on vitamin B_{12}-dependent metabolism. *Proceedings of the National Academy of Sciences*, 110(49), pp. 19926-19931.

Sutton, M.A., Oenema, O., Erisman, J.W., Leip, A., van Grinsven, H. & Winiwarter, W. (2011). Too much of a good thing. *Nature*, 472(7342), p. 159.

Tanimoto, T., Hatano, K.-i., Kim, D.-h., Uchiyama, H. & Shoun, H. (1992). Co-denitrification by the denitrifying system of the fungus Fusarium oxysporum. *FEMS Microbiology Letters*, 93(2), pp. 177-180.

Tatti, E., Goyer, C., Zebarth, B.J., Wertz, S., Burton, D.L., Chantigny, M., Filion, M. & Zeng, J. (2017). Over-winter dynamics of soil bacterial denitrifiers and nitrite ammonifiers influenced by crop residues with different carbon to nitrogen ratios. *Applied Soil Ecology*, 110, pp. 53-64.

Thompson, K.A., Bent, E., Abalos, D., Wagner-Riddle, C. & Dunfield, K.E. (2016). Soil microbial communities as potential regulators of in situ N_2O fluxes in annual and perennial cropping systems. *Soil Biology and Biochemistry*, 103, pp. 262-273.

Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M., & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society B*, 367, pp. 1157-1168.

Tiedje, J.M., Sexstone, A.J., Myrold, D.D. & Robinson, J.A. (1982). Denitrification – ecological niches, competition and survival. *Antonie Van Leeuwenhoek Journal of Microbiology*, 48(6), pp. 569-583.

Treusch, A.H., Leininger, S., Kletzin, A., Schuster, S.C., Klenk, H.P. & Schleper, C. (2005). Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environmental Microbiology*, 7(12), pp. 1985-1995.

Vaccaro, B.J., Thorgersen, M.P., Lancaster, W.A., Price, M.N., Wetmore, K.M., Poole, F.L., Deutschbauer, A., Arkin, A.P. & Adams, M.W. (2016). Determining roles of accessory genes in denitrification by mutant fitness analyses. *Applied and Environmental Microbiology*, 82(1), pp. 51-61.

van den Berg, E.M., van Dongen, U., Abbas, B. & van Loosdrecht, M.C. (2015). Enrichment of DNRA bacteria in a continuous culture. *ISME Journal*, 9(10), pp. 2153-2161.

van Kessel, M.A., Speth, D.R., Albertsen, M., Nielsen, P.H., den Camp, H.J.O., Kartal, B., Jetten, M.S. & Lücker, S. (2015). Complete nitrification by a single microorganism. *Nature*, 528(7583), p. 555.

Venter, J.C., Remington, K., Heidelberg, J.F., Halpern, A.L., Rusch, D., Eisen, J.A., Wu, D., Paulsen, I., Nelson, K.E. & Nelson, W. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, 304(5667), pp. 66-74.

Venterea, R.T., Halvorson, A.D., Kitchen, N., Liebig, M.A., Cavigelli, M.A., Del Grosso, S.J., Motavalli, P.P., Nelson, K.A., Spokas, K.A. & Singh, B.P. (2012). Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. *Frontiers in Ecology and the Environment,* 10(10), pp. 562-570.

Verhamme, D.T., Prosser, J.I. & Nicol, G.W. (2011). Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *ISME Journal,* 5(6), p. 1067.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997). Human domination of Earth's ecosystems. *Science,* 277(5325), pp. 494-499.

Wagg, C., Bender, S.F., Widmer, F. & van der Heijden, M.G. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences,* 111(14), pp. 5266-5270.

Wei, W., Isobe, K., Shiratori, Y., Nishizawa, T., Ohte, N., Ise, Y., Otsuka, S. & Senoo, K. (2015). Development of PCR primers targeting fungal nirK to study fungal denitrification in the environment. *Soil Biology and Biochemistry,* 81, pp. 282-286.

Welsh, A., Chee-Sanford, J.C., Connor, L.M., Löffler, F.E. & Sanford, R.A. (2014). Refined NrfA phylogeny improves PCR-based nrfA gene detection. *Applied and Environmental Microbiology,* 80(7), pp. 2110-2119.

Wittorf, L., Bonilla-Rosso, G., Jones, C.M., Bäckman, O., Hulth, S. & Hallin, S. (2016). Habitat partitioning of marine benthic denitrifier communities in response to oxygen availability. *Environmental microbiology reports,* 8(4), pp. 486-492.

Wu, D., Dong, W., Oenema, O., Wang, Y., Trebs, I. & Hu, C. (2013). N_2O consumption by low-nitrogen soil and its regulation by water and oxygen. *Soil Biology and Biochemistry,* 60, pp. 165-172.

Wu, X., Liu, H.F., Fu, B.J., Wang, Q., Xu, M., Wang, H.M., Yang, F.T. & Liu, G.H. (2017). Effects of land-use change and fertilization on N_2O and NO fluxes, the abundance of nitrifying and denitrifying microbial communities in a hilly red soil region of southern China. *Applied Soil Ecology,* 120, pp. 111-120.

Yang, C., Hamel, C. & Gan, Y. (2015). Incongruous variation of denitrifying bacterial communities as soil N level rises in Canadian canola fields. *Applied Soil Ecology,* 89, pp. 93-101.

Yin, C., Fan, F., Song, A., Li, Z., Yu, W. & Liang, Y. (2014). Different denitrification potential of aquic brown soil in Northeast China under inorganic and organic fertilization accompanied by distinct changes of *nirS*- and *nirK*-denitrifying bacterial community. *European Journal of Soil Biology,* 65, pp. 47-56.

Yoon, S., Cruz-García, C., Sanford, R., Ritalahti, K.M. & Löffler, F.E. (2015). Denitrification versus respiratory ammonification: environmental controls of two competing dissimilatory NO_3^-/NO_2^- reduction pathways in *Shewanella loihica* strain PV-4. *ISME Journal,* 9(5), p. 1093.

Yoon, S., Nissen, S., Park, D., Sanford, R.A. & Löffler, F.E. (2016). Nitrous oxide reduction kinetics distinguish bacteria harboring clade I *nosZ* from those harboring clade II *nosZ*. *Applied and Environmental Microbiology,* 82(13), pp. 3793-800.

Yoshinari, T., Hynes, R. & Knowles, R. (1977). Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. *Soil Biology and Biochemistry*, 9(3), pp. 177-183.

Yuan, Q., Liu, P. & Lu, Y. (2012). Differential responses of nirK-and nirS-carrying bacteria to denitrifying conditions in the anoxic rice field soil. *Environmental Microbiology Reports*, 4(1), pp. 113-122.

Yuan, Y., Chen, H., Yuan, W., Williams, D., Walker, J.T. & Shi, W. (2017). Is biochar-manure co-compost a better solution for soil health improvement and N 2 O emissions mitigation? *Soil Biology and Biochemistry*, 113, pp. 14-25.

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C. & Chu, H. (2016). Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry*, 92, pp. 41-49.

Zhang, H.-M., Bo-Ren, W., Ming-Gang, X. & Ting-Lu, F. (2009). Crop yield and soil responses to long-term fertilization on a red soil in southern China. *Pedosphere*, 19(2), pp. 199-207.

Zhou, X., Fornara, D., Wasson, E.A., Wang, D., Ren, G., Christie, P. & Jia, Z. (2015). Effects of 44 years of chronic nitrogen fertilization on the soil nitrifying community of permanent grassland. *Soil Biology and Biochemistry*, 91, pp. 76-83.

Zhu, G., Wang, S., Li, Y., Zhuang, L., Zhao, S., Wang, C., Kuypers, M.M., Jetten, M.S. & Zhu, Y. (2018). Microbial pathways for nitrogen loss in an upland soil. *Environmental Microbiology*, 20(5), pp. 1723-1738.

Zumft, W.G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews*, 61(4), pp. 533-616.

Zumft, W.G. & Kroneck, P.M. (2006). Respiratory transformation of nitrous oxide (N_2O) to dinitrogen by bacteria and archaea. *Advances in Microbial Physiology*, 52, pp. 107-227.

Popular science summary

Nitrogen (N) is an essential element for life on earth. Air consists to 78% of N and bioavailable forms of N, like nitrate and ammonium, are important for production of food, feed and fibre. In 1909, the so called Haber-Bosch method was invented, which enables the production of artificial fertilizer. Since then, the global application of fertilizer has increased dramatically and has become "too much of a good thing". Nitrogen fertilizer, often applied in form of nitrate, escape the agricultural fields and pollute aquatic systems. It may also spread to the atmosphere as ammonia or as the powerful greenhouse gas N_2O (laughing gas), known to accelerate global warming. Excessive fertilizer use has increased the concentration of N_2O in the atmosphere by 20% since agricultural intensification began around 1930. Despite the known warming potential of N_2O, which is about 300 times stronger than carbon dioxide, decreasing N fertilization is problematic, as current food production relies on high inputs of N fertilizer and the demand is predicted to even increase with a growing world population.

Nitrogen in the soil can be converted by a wide range of microorganisms. Certain bacteria have the ability to transform nitrate to atmospheric N or N_2O while other bacteria are able to convert nitrate to ammonium, which is more stable in the soil. Additionally there has been a group of bacteria described recently that is able to produce atmospheric N out of N_2O. In that case N is still lost from the soil and not available to plants, but at least not in form of a dangerous greenhouse gas.

The aim of the thesis was to understand how different soil factors, like nitrogen or carbon content, affect the soil microbial community. These soil factors don't only differ between different soils, but are influenced by different agricultural management practices, like fertilization or different crop rotations. We measured that a high abundance of bacteria able to convert N_2O is effectively reducing the greenhouse gas emissions from the soils by ~50%. These bacteria are not able to produce N_2O by themselves and thus effectively reduce the gas

produced by other bacteria. This is important, as they may provide a potential way to mitigate N_2O emissions. We observed bacteria reducing N_2O were present in fertilized fields, but bacteria converting nitrate to N_2O were even more prevalent. This was in connection with greater loss of N and also higher N_2O emissions from fertilized fields than from unfertilized. The abundance of N_2O converting bacteria was dependent on content of soil carbon compared to N. A comparison between a cereal crop rotation and a four year ley rotation revealed that choice of crop had stronger effects on the soil N and carbon content, and on the bacterial community, than did fertilization. During 40 years of an annual cereal crop rotation, the soil lost carbon and N, while the ley soil retained both nutrients. We could see that a high amount of bacteria with the ability to convert nitrate to ammonium were active in the ley soils. This type of bacteria and their role in agricultural fields are poorly studied, but our results suggest that they thrive on carbon-rich soils. To further test this hypothesis, we conducted a lab experiment where naturally carbon rich straw and nitrate was added in different amounts to soil, to simulate fertilization. This showed that the ratio between carbon and N was of importance for bacteria controlling N_2O emissions. We could prove that the straw addition helped retaining nitrogen in soils, but not if nitrate was added.

In this study we showed that the ratio of carbon to N in soils was a driving factor influencing the microbial community. A high ratio fostered the abundance and activity of bacteria retaining nitrogen. Not only bacteria involved in nitrogen loss are active in agricultural soils, but also less-studied bacteria important for nitrogen retention and N_2O emissions. Bacteria reducing N_2O were shown to be sensitive to agricultural management practices and are important to decrease emissions from fertilized fields.

Populärvetenskaplig sammanfattning

Kväve (N) är en förutsättning för allt liv på jorden. Luften består till 78 % av kväve, och kväveföreningar som nitrat och ammonium är viktiga för produktion av mat, foder och annat växtriket ger oss. År 1909 uppfanns den så kallade Haber-Bosch-metoden, som gör det möjligt att producera kvävebaserat konstgödsel. Sedan dess har det globala användandet av gödsel ökat dramatiskt och blivit ett problem. Gödslets kväve, som ofta appliceras i form av nitrat, kan vandra från åkrarna och förorena vattendrag. Det kan också hamna i atmosfären som ammonium eller som lustgas (N_2O), som är känd för att bidra till växthusteffekten. Vidlyftigt gödslande har ökat koncentrationen av lustgas i atmosfären med 20 % sedan jordbruket började intensifieras runt 1930. Trots att lustgas är en 300 gånger starkare växthusgas än koldioxid är det svårt att dra ner på användandet av gödsel. Dagens matproduktion bygger på höga tillsatser av kvävegödning och efterfrågan på mat förväntas dessutom öka, eftersom världens befolkning ökar.

Kväve i jorden kan omvandlas av en mängd olika mikroorganismer. Vissa bakterier kan omvandla nitrat till atmosfäriskt kväve eller lustgas, medan andra kan omvandla det till den i jorden stabilare formen ammonium. Nyligen har dessutom beskrivits en grupp av bakterier som kan omvandla lustgas till atmosfäriskt kväve, vilket visserligen fortfarande gör kvävet otillgängligt för växterna men åtminstone inte i form av en farlig växthusgas.

Målet med denna avhandling var att förstå hur olika jordfaktorer, som mängden kväve och kol, påverkar mikrobsamhället. Dessa faktorer varierar inte bara mellan olika jordar, utan påverkas också av hur jorden brukas, som mängd tillsatt gödsel eller val av växtföljd. Vi noterade att jordar med hög förekomst av bakterier som kan omvandla lustgas släpper ut ~50% mindre växthusgaser. Sådana bakterier producerar inte själva lustgas utan minskar den mängd som producerats av andra bakterier. Detta är viktigt, eftersom de därmed kan begränsa lustgasproduktionen gödslandet ger upphov till. Även om vi observerade att dessa bakterier förekom i gödslade jordar, var bakterier som

producerar lustgas ännu vanligare. Gödslade jordar förlorar alltså kväve och producerar lustgas snabbare än icke gödslade. Mängden bakterier som omvandlar lustgas påverkades av förhållandet mellan kol och kväve i jorden. En jämförelse mellan en jord med växlande spannmålsgrödor och en med vallgrödor avslöjade att valet av gröda hade större inverkan på kol/kväve-förhållandet och mikrobsamhället än vad gödslingen hade. Jorden som i 40 år producerade spannmål tappade både kväve och kol, medan valljordarna kunde behålla båda. Valljordarna innehöll också många bakterier som omvandlar nitrat till ammonium. Dessa typer av bakterier och deras roll i jordbruket har inte studerats ingående, men våra resultat antyder att de trivs på kolrika jordar. För att testa den hypotesen ytterligare gjorde vi ett experiment där jord berikades i olika mängder med naturligt kolrika växtdelar och nitrat, för att simulera gödsling. Detta visade att förhållandet mellan kol och kväve var av betydelse för bakterier som påverkar lustgasutsläppet. Vi såg att växtdelarna bidrog till att fixera kvävet i jorden, men bara om man inte samtidigt tillsatte nitrat.

I denna avhandling har vi visat att förhållandet mellan kol och kväve i jorden hade en avgörande inverkan på mikrobsamhället. Mer kol gynnade förekomst och aktivitet av bakterier som fixerar kväve. Bakterier som minskar kvävet i jorden är inte de enda som verkar i jordbruksjord; här finns också mindre studerade bakterier viktiga för kvävefixering och lustgasutsläpp. Bakterier som minskar dessa utsläpp har visat sig vara känsliga för hur jorden brukas och är viktiga för att minska utsläppen från gödslade åkrar.

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