Nitrogen Cycling Communities Associated to Roots of Arable Crops in Relation to Management

Plant, Intercropping and Soil Effects

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Kväveomsättande mikrobsamhällen associerade med rötter hos jordbruksgrödor i förhållande till brukningsåtgärder. Växt-, samodlings- och markeffekter

Sammanfattning

Att ta hand om markens kväve (N) har identifierats som en viktig utmaning i för ett hållbart jordbruk. Omvandlingar av oorganiskt N utförs i huvudsak av olika mikrobiella funktionella grupper som reglerar om N stannar eller lämnar systemet. Brukningsåtgärder kan påverka mångfald, sammansättning och funktion hos de N-omvandlande mikrobiella samhällena. Syftet med avhandlingen var att definiera effekter av markegenskaper, grödor och samodling på N-omvandlande mikrobiella samhällen associerade med växtrötter och i jord, med särskilt fokus på den genetiska och enzymatiska potentialen för denitrifikation och reduktion av lustgas (N_2O).

Ett växthusförsök där samodling av gräset hundäxing och baljväxten lusern jämfördes med odling i renbestånd visade att växtart och samodling hade stor påverkan på abundansen av rotassocierade samhällen som styr retention eller förlust av N, vilket tyder på förändrade växt-mikrob-interaktioner och/eller mikrob-mikrob-interaktioner. Tillsats av rötrester som gödningsmedel förändrade inte effekten av samodling. En högre produktionshastighet av N_2O konstaterades hos rotassocierade mikrobiella samhällen i hundäxing under samodling jämfört med hundäxing i renbestånd, vilket sammanföll med minskad genetisk potential för reduktion av N_2O hos organismer inom *nosZ*II-kladen. Sekvensering visade att dessa N_2O reducerande mikroorganismer var besläktade med Ignavibacteria, som har en trunkerad denitrifikationskedja och saknar den genetiska förmågan att producera N_2O.

Jordtyp hade också en stark inverkan på de rotassocierade N-omvandlande samhällen. I ett fullfaktoriellt experiment med två jordtyper och två olika grödor (korn och solros) visade sig jordtyp ha större betydelse än gröda när det gäller både genetisk och enzymatisk potential för denitrifikation. Således bestämmer jordens fysikaliska och kemiska egenskaper snarare än växtarternas egenskaper denitrifikations- och N_2O produktionshastighet hos de rotassocierade mikrobsamhällena.

Den genetiska potentialen för de olika N-omvandlande samhällena skiljde sig mellan jord och rötter, vilket tyder på att olika N-omvandlande funktionella organismer gynnades i olika delar av jord-rot-miljön. De N_2O reducerande mikroorganismerna som har *nosZ*I hade en affinitet för växtrötter, medan de med *nosZ*II gynnades i jorden vilket indikerar en möjlig differentiering av nischer mellan N_2O reducerare från de två kladerna.

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Abstract

Management of terrestrial nitrogen (N) has been identified as a key challenge in the implementation of sustainable agricultural practices. Transformations of inorganic N are mainly performed by microbial functional guilds that regulate the retention or loss of N. Soil management may affect the diversity, composition, and functioning of N-cycling microbial communities. The aim was to define the influence of soil properties, crops and intercropping on root- and soil-associated N-cycling communities with a special focus on the genetic and enzymatic potential for denitrification and nitrous oxide (N_2O) reduction.

A greenhouse experiment comparing intercropped cocksfoot and lucerne with sole cropping practices showed that plant species and intercropping significantly affected the abundances of root associated communities that drive the retention or loss of N, suggesting altered plant-microbial and/or microbial-microbial interactions. Addition of biogas digestate as fertilizer did not alter the intercropping effects. A higher N_2O production rate was found in root-associated microbial communities in cocksfoot during intercropping, which coincided with decreased genetic potential for N_2O reduction by organisms within *nosZ* clade II compared to sole cropped cocksfoot. Sequencing revealed that these N_2O reducers were related to Ignavibacteria, which have a truncated denitrification pathway that lacks the genetic capacity to produce N_2O.

Soil type also had a strong influence on root-associated N-cycling communities. In a full-factorial experiment with two soil types and two different crops (barley and sunflower), soil type overrode crop effects regarding both genetic and enzymatic potential for denitrification. Thus, soil physical and chemical properties rather than plant species determine the denitrification and N_2O production rates of the root-associated communities.

The genetic potential for the various N-cycling communities differed between bulk soil and roots, indicating that N-cycling functional organisms were favored in different compartments in the soil-root environment. The N_2O reducing organisms carrying *nosZ*I were shown to have an affinity to plant roots, whereas those with *nosZ*II prefer the bulk soil thus indicating a possible niche differentiation between the two clades.

Keywords: nitrogen cycling, agricultural management practices, microbial community, nitrogen fixation, nitrification, denitrification, nitrous oxide, plant, intercropping, soil, fertilizer

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Dedication

To my family.

Life lies in diligence, no pains, no gains.
Zhang Heng (A.D.78-139)

人生在勤，不索何获

张衡

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 Zhao M., Jones, C.M., Meijer J., Lundquist P.O., Carlsson G., Hallin S. Intercropping affects genetic potential for inorganic nitrogen cycling by root-associated microorganisms in *Medicago sativa* and *Dactylis glomerata*. (manuscript)

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

III Graf D.R.H, Zhao, M., Carlsson, G., Jones, C.M. and Hallin, S. Composition and activity of N_2O-reducing communities associated with roots of *Medicago sativa* and *Dactylis glomerata* during intercropping. (manuscript)

The contribution of Ming Zhao to the papers included in this thesis was as follows:

I Performed all of the laboratory work and the analysis. Wrote the manuscript with support from the co-authors.

II Participated in the planning and execution of the experiment, performed half of the lab work and statistical analysis, and a minor part of and writing of the manuscript.

III Performed half of the lab work and statistical analysis, participated in the writing of the manuscript.

In addition to the papers within this thesis, the author has contributed to the following paper within the timeframe of the thesis work:

Graf D.R.H, Jones, C.M., Zhao, M. and Hallin, S. Community assembly of N_2O-reducing microorganisms on roots of annual crops is governed by priority effects and competitive exclusion. (manuscript)

Abbreviations

Anammox	anaerobic ammonia oxidation
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
AOM	ammonia-oxidizing microorganisms
AMO	ammonia monooxygenase
DNRA	dissimilatory nitrate reduction to ammonium
N_2O	nitrous oxide
N_2OR	nitrous oxide reductase
NAP	periplasmic nitrate reductase
NAR	respiratory nitrate reductase
NH_4^+	ammonium
NH_3	ammonia
NIR	nitrite reductase
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NOB	nitrite-oxidizing bacteria
NOR	nitric oxide reductase

1 Introduction

Nitrogen (N) is an essential nutrient for plants. Cultivated soils are usually N fertilized to support plant development and high crop yield. It has been estimated that the total N-fertilizer demand increased by 1.3 percent annually during 2012 to 2016 globally to meet the increasing demand in food production. However, significant amounts of N are lost from agriculture and the N-use efficiency in Sweden year 2008 was 64% based on the data from Food and Agricultural Organization of the United Nations (FAO) (2010) and International Fertilizer Association (IFA) (2010) statistics (Brentrup and Pallière, 2010). Nitrogen-use efficiency for the plant is defined as the dry mass productivity per unit N taken up from soil (Hirose, 2011), and with respect to fertilization in agriculture, it is the ratio between the amount of fertilizer N taken up by the crop and the amount of fertilizer N applied in the field.

Nitrogen loss from arable soils is a major constraint causing economic losses. In addition, N losses also impact human health, the environment and global climate change. Excess N loading in aquatic ecosystems causes both groundwater pollution and eutrophication, as well as toxicity to fish. Losses in the form of emissions of nitrous oxide (N_2O) effects climate change since N_2O is a potent greenhouse gas and destroys the ozone layer (IPCC, 2013). Thus, management of terrestrial N is critical for dealing with the challenges between crop yield, food security, water pollution, environmental degradation and climate change (Zhang et al., 2015b, Tilman et al., 2002). Understanding how different cropping systems and soil management practices affect N-cycling is a research priority (Galloway et al., 2008, Sutton et al.,(Eds.) 2011).

1.1 Nitrogen loss and retention in arable soil

Soil microbial communities are the principal drivers of N-cycling in arable soils, with distinct functional guilds regulating retention or loss of N. Atmospheric N_2 that is converted to ammonia (NH_3) by the activity of free-

living or plant-associated N_2-fixing bacteria is retained in soil as organic matter. However, inorganic N, derived from organic matter mineralization or applied as fertilizer, may be lost due to nitrification, a process in which NH_3 is oxidized to nitrate (NO_3^-) that can leach into nearby waterways. Additionally, NO_3^- is a substrate for denitrification, a facultative anaerobic respiratory pathway in which NO_3^- is sequentially reduced to N_2O or N_2. This pathway not only leads to N-loss via emission of gaseous N into the atmosphere, but also is a potential source or sink for N_2O, as denitrifying organisms vary in their genetic capacity to produce or consume N_2O (Graf *et al.*, 2014; Jones *et al.*, 2014). Gaseous N losses in the form of N_2O are of special concern due to the severe effects this gas has on climate change. The gas has a global warming potential 310 times greater than that of the equivalent amount of CO_2, and N_2O is the main substance causing ozone depletion in the stratosphere (Ravishankara et al., 2009). Over 70% of total N_2O emissions come from soil (http://www3.epa.gov/climatechange/ghgemissions/gases/n2o.html), but the only biological mechanism for N_2O consumption is also in the soil (Firestone and Davidson, 1989, Reay et al., 2012). If soil N in the form of NO_3^- is not reduced by denitrification, it may also be retained in soils through dissimilatory nitrate reduction to ammonia (DNRA). This is another anaerobic respiratory pathway that utilizes NO_3^- but instead results in NH_4^+, which is largely immobile in soil. Thus, differences in the diversity, abundance or composition of various N-cycling communities have a direct consequence on the fate of N in arable soil.

Soil management practices may affect the diversity, composition, and functioning of N-cycling microbial communities in soil, either directly or indirectly due to altered soil abiotic factors (Xue et al 2013). Soil fertility amendments, including manure, composted organic waste and synthetic fertilizers, affect soil N-cycling and microbial activity, such as respiration, ammonia oxidation, denitrification, N mineralization and N_2O emissions (Hallin et al., 2009, Niboyet et al., 2010, Odlare et al., 2011). Many studies have investigated N-fertilizer effects on soil denitrification, especially in relation to greenhouse gas N_2O emissions (Mulvaney et al., 1997, Skiba, 2000, Akiyama et al., 2004).

Plants and cropping systems can also have an effect on soil microbial communities involved in N cycling, and the key ecosystem processes they perform. The composition and diversity of plants have been shown to influence the levels of inorganic N in soils and N_2O emissions (Epstein et al., 1998, Sun et al., 2013, Whitmore and Schroder, 2007, Manevski et al., 2015). Soil NO_3^- concentration typically increases with decreasing plant diversity (Zak *et al.*, 2003; Niklaus *et al.*, 2006; Mueller *et al.*, 2013). Higher N_2O emissions have

also been observed in sole crop systems compared to those with mixed plants species (Niklaus *et al.*, 2006; Sun *et al.*, 2013). Thus, intercropping practices that combine two or more crops in the same field could be a way to manage N and increase N-use efficiency. Interactions between plants and microbes in the rhizosphere, the small zone of soil that is influenced by plant roots, may also be of importance for managing soil N. Different plant species can either stimulate or inhibit of nitrification activity in the rhizosphere, whereas the rhizosphere has been commonly referred to as a 'hotspot' for denitrification, as the influence of roots on oxygen, carbon, and nitrogen availability tend to promote denitrification (Philippot *et al.*, 2013). However, this affect may also vary among plant species (Philippot *et al.*, 2009), and whether the rhizosphere of different plants select for soil denitrifying communities that produce N_2O, or those that are capable of reducing N_2O to N_2, has yet to be determined.

2 Aims and research questions

The overall aim is to define the influence of soil properties and crops on root- and soil-associated N-cycling microbial communities, with special emphasis on the genetic and enzymatic potential for denitrification and N_2O reduction. The specific questions addressed in this thesis are:

1) *Intercropping effects on soil activity, abundance and composition of N-cycling microbial communities (**Papers I and III**).* Intercropping, the practice of growing two or more crops together at the same time and in the same field has been suggested as a means of managing soil N through increasing N-use efficiency. This would lower the losses of N through leaching or gaseous losses. However, both increased and decreased N_2O emissions have been observed in intercropped systems. The aim of **Paper I** was to compare the effect of intercropped and sole crop practices on the abundances of root associated and soil inhabiting N-cycling communities that drive the retention or loss of N. Since an increase in soil N can cancel the positive effects of intercropping on plant N use, we also addressed the effects of fertilization by amendment with biogas digestate on the abundance of root associated and soil inhabiting N-cycling communities with or without intercropping. In **Paper III**, we specifically examined the activity and structure of microbial communities that reduce N_2O to N_2 to determine the effect of plant species and intercropping on potential N_2O emission.

2) *Relative importance of soil type and plant type on potential N_2O production and abundance N_2O reducing microbial communities (**Paper II**).* The importance of the recently described *nosZ* Clade II

(Jones et al., 2013, Sanford et al., 2012) for net N_2O emissions in the rhizosphere is not known. Since soil type can exert a strong influence on the root-associated microbial communities, the aim of **Paper II** was to determine the relative influence of soil and plant type on the abundances of nitrous oxide reducers and the genetic and enzymatic N_2O emission potential.

3 Background

3.1 Soil nitrogen cycling and the microbial communities involved

The N cycle is one of the key biogeochemical cycles that support life on earth. It consists of pathways that add or retain N in the biosphere, and those that return N to the atmosphere (Figure 1).

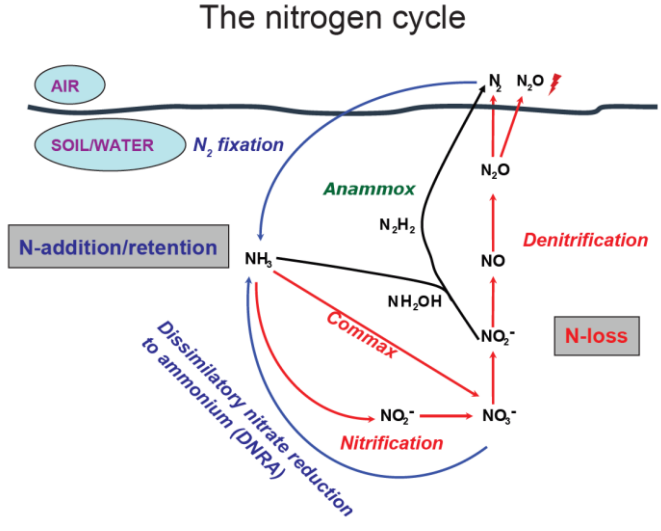

Figure 1. Nitrogen cycle.

3.1.1 Nitrogen fixation

Nitrogen gas (N_2) makes up about 78% of the earth's atmosphere, which is a major reservoir of N that in the absence of chemical or enzymatic activity would be unavailable to organisms. Biological input of N to the soil is performed by the process of N_2 fixation which converts N to ammonia (NH_3)

by N_2-fixing microorganisms and is an extremely energy expensive process. The main N_2-fixing organisms are bacteria, some of which are symbiotic while others are free-living. All N_2-fixers employ a special enzyme called nitrogenase, which is capable of catalysing N_2 to ammonium. Symobiotic nitrogen fixing bacteria, such as the rhizobia, are able to sense flavonoids secreted by the certain host legume roots, then enter through the root hairs and establish the symbiotic relationship with host roots by forming nodules, where N_2-fixation occurs (Schultze and Kondorosi, 1998). This relationship greatly benefits both the host plants and bacteria by the bacteria providing N when it is of limited availability in the environment, while the plant supports N_2-fixing microorganisms with carbon compounds (electron donors).

The symbiotic N_2-fixing bacteria can also exist as free-living organisms in soil, in addition other free-living N_2-fixing bacteria, such as species from the genera *Azospirillum* and *Azotobacter* (Abdelmal, 1971, Oyaizumasuchi and Komagata, 1988). Free-living N_2-fixing bacteria are diverse, as they can be photosynthetic or non-photosynthetic, aerobic or anaerobic. Free-living, non-photosynthetic N_2-fixing bacteria obtain energy from soil organic matter. The oxygen level in soil can strongly affect aerobic free-living N_2-fixation since most of the nitrogenases are irreversibly inactivated by oxygen (O_2). Thus, a mechanism to protect the nitrogenase is required. This is not a problem for anaerobic free-living N_2-fixers such as *Clostridium*, which predominate in waterlogged soils as well as in anaerobic niches in grasslands (Rice et al., 1967, Drozd and Postgate, 1970).

3.1.2 Nitrification

Ammonia in equilibrium with ammonium (NH_4^+), formed as a result of N_2-fixation, is retained in the soil biomass, soil organic matter or bound to negatively charged soil particles, e.g. clay. Ammonium is one form of N that is utilized by higher plants, however many species prefer NO_3^-. Ammonium is reactive and readily oxidized by microorganisms. The oxidation of NH_3 to NO_3^- in the soil occurs through a two-step aerobic microbial process called nitrification, in which NH_3 is oxidized to NO_2^- and then to NO_3^- to gain energy. It was generally accepted that each step in nitrification was carried out by two phylogenetically distinct clades of microorganisms; the ammonia oxidizing bacterial (AOB) that oxidize ammonia to nitrite, and the nitrite oxidizing bacteria (NOB) that further oxidize nitrite to nitrate (Winogradsky, 1890). In 2006, ammonia-oxidizing archaea (AOA) within the phylum *Thaumarchaeota* were discovered in various marine and terrestrial ecosystems supplemental to AOB (Nicol and Schleper, 2006). It has been suggested that *Thaumarchaeota* may be the most abundant ammonia-oxidizer in soil ecosystems (Hatzenpichler,

2012, Leininger et al., 2006, Caffrey et al., 2007). The known ammonia oxidizers and NOB have been regarded as two different functional groups, as none of these organisms have all enzymes needed to oxidize both NH_3 and NO_2^-. However, recent studies have characterized nitrifiers from the bacterial genus *Nitrospira* that have all enzymes necessary for complete ammonia oxidiation to nitrate and this process has been coined comammox, complete ammonia oxidation (van Kessel et al., 2015, Daims et al., 2015).

Nitrifying organisms play the important role in regulating NO_3^- content in the soil and controlling the availability of N as a plant nutrient. Moreover, NO_3^- is soluble and may be lost from soil through leaching. The leached NO_3^- can flow through the hydrologic system to rivers and lakes, and some eventually reaches the oceans where it can be returned to N_2 by denitrification or anaerobic ammonium oxidation (anammox; see below).

3.1.3 Denitrification

Nitrate can be depleted from soil through denitrification mediated by denitrifying microbial communities. Denitrification is a facultative respiratory pathway by which denitrifying organisms perform a stepwise reduction of NO_3^- or NO_2^- to nitric oxide (NO), N_2O and eventually to N_2. Hence, this pathway leads to N-loss via emission of gaseous N into the atmosphere. Nitrous oxide can either be an intermediate or the end product of denitrification, making this pathway a potential source or sink for N_2O (Jones et al., 2014, Philippot et al., 2011, Graf et al., 2014). The generation of N_2O by terrestrial denitrifiers contributes a large portion of the total N_2O emissions on the earth (Canfield et al., 2010).

Denitrifying microorganisms are found among a broad range of bacterial phyla (Zumft and Korner, 1997, Philippot et al., 2007), but archaeal and fungal denitrifiers also exist although less is known about their ecology and importance (Kobayashi et al., 1996, Dodsworth et al., 2011, Shoun, 1992, Philippot et al., 2002). The majority of characterized denitrifiers belong to the phylum *Proteobacteria* (Graf et al. 2014). The first step in denitrification is the reduction of NO_3^- to NO_2^-, catalysed by membrane bound or periplasmic nitrate reductases (NAP or NAR, respectively). This step is not specific to denitrification, as the resulting NO_2^- can be used in other N-cycle pathways. The second step is the reduction of NO_2^- to NO by the nitrite reductase (NIR), which is regarded as defining step of denitrification. Nitric oxide has cytotoxic properties and needs to be reduced to N_2O, performed by the nitric oxide reductase (NOR). Nitrous oxide can be emitted directly into the air as a terminal product if the denitrifying microorganism do not contain the enzyme

nitrous oxide reductase (N_2OR), which reduces N_2O to dinitrogen (N_2) (Zumft and Kroneck, 2007). Many denitrifying organisms do not possess the enzymes to conduct all steps of denitrification (Zumft, 1997, Graf et al., 2014) and microorganisms possessing a complete or partial denitrification pathway exist across the microbial tree of life (Jones et al., 2008, Graf et al., 2014).

Denitrifying populations and communities vary in their tolerances to environmental conditions and stress (Hallin et al., 2012, Cavigelli and Robertson, 2000, Holtan-Hartwig et al., 2000). For example, Yin et al. (2015) indicated changes of denitrifier community structure and abundance were associated with soil pH, total available N, organic matter and phosphorus. Temperature and seasonal variations also affect denitrifiers. The abundance of denitrifiers was lower in summer compared to other seasons in an acid soil of a Norway spruce forest (Mergel et al., 2001) and many studies have revealed that soil N_2O emissions show seasonal variation (Bremner et al., 1980). Freeze-thaw events in particular contribute to substantial emissions (Müller et al., 2003, Goodroad and Keeney, 1984, Cui et al., 2016), which could be caused by the presence of degraded substrate derived from dead microbial biomass providing extra N and energy (Guckland et al., 2010, Matzner and Borken, 2008). Soil pH is a well-known controller of N_2O production by denitrifiers (Firestone et al., 1980) and plenty of studies have shown increased N_2O emissions in soils with low pH (Odlare et al., 2012, Baggs et al., 2010, Chao, 1995, Obia et al., 2015, Liu et al., 2014). However, the composition of the microbial community also plays a role (Jones et al., 2014, Philippot et al., 2011).

3.1.4 Dissimilatory nitrate reduction to ammonia (DNRA)

Another pathway that utilizes NO_3^- is the dissimilatory NO_3^- reduction to ammonium (DNRA), in which NO_3^- is converted to ammonium and can be considered the reverse process of nitrification. In this pathway, NO_2^- produced by the reduction of NO_3^- is converted to NH_4^+ by the cytochrome C nitrite reductase (NrfA) (Simon, 2002). Thus, the key difference with denitrification is that NO_2^- is reduced in the process of respiratory ammonification, rather than being converted to NO. This process can contribute to retention of soil N due to the formation of NH_4^+, which can be immobilized in the soil (Rütting et al., 2011).

Similar to denitrification, respiratory ammonification is performed under anaerobic soil conditions (Silver et al., 2001), and competition between respiratory ammonification and denitrification may occur depending on environmental conditions (Silver et al., 2005, Kraft et al., 2014). For example,

DNRA has been found to be the dominant pathway for NO_3^- reduction in estuarine and coastal sediments (Kelly-Gerreyn et al., 2001) and the relative contribution of DNRA/respiratory ammonification in soils can be significant (Rütting et al., 2011). Conditions of high ratios of dissolved organic carbon and NO_3^- have been found to favor DNRA over denitrification (Porubsky et al., 2009, Schmidt et al., 2011), as do high levels of sulfide (Brunet and GarciaGil, 1996, Behrendt et al., 2013), elevated temperature (Dong et al., 2011), salinity (An and Gardner, 2002) and soil pH (Schmidt et al., 2011). Organisms performing respiratory ammonification have been shown to be significantly overrepresented in rhizosphere compared to the surrounding bulk soil (Li et al., 2011, Nijburg and Laanbroek, 1997).

3.1.5 Anaerobic ammonium oxidation (anammox)

Nitrite can also be reduced through the process anaerobic ammonium oxidation (anammox), which converts NO_2^- and NH_4^+ directly into N_2 gas (Mulder et al., 1995, Strous et al., 1999). All anammox bacteria discovered so far to belong to the phylum *Planctomycetes* (Kuenen, 2008, Lam and Kuypers, 2011). Anammox may be responsible for producing 30-50% of the N_2 gas in the oceans (Devol, 2015). Thus, it is an important process for removing fixed N at the global scale. Apart from marine ecosystems, anammox is also occurring in wetlands and rice paddy soils (Zhu et al., 2011, Erler et al., 2008), and peat soils (Hu et al., 2011). In deciduous forest soil, anammox has been shown to contribute 0.5% to 14.4% of the total N_2 production (Xi et al., 2016). Anammox bacteria have been detected in arable soils and grasslands (Humbert et al., 2010), but there is not much known about their importance in these types of soils.

3.2 Nitrogen cycling genes as functional markers

Many of the genes coding for the key enzymes in N cycling were introduced as functional markers for use in PCR-based assays 10-20 years ago (Throbäck, 2004, Henry et al., 2004, Poly et al., 2001, Rotthauwe et al., 1997, Braker et al., 1998, Hallin and Lindgren, 1999, Scala and Kerkhof, 1999), and new marker genes have been added along with the revision of several primers since the first introduction. They have been widely used as molecular markers to predict the potential functional traits and their communities in the environment (Correa-Galeote et al., 2013, Hallin et al., 2012, Lyautey et al., 2013, Trias et al., 2012, Wessén et al., 2011, Andersson et al., 2014). The N-cycling microbial communities, such as N_2-fixing microbes and N_2O reducers, show

high diversity in soils, (Duc et al., 2009, Sanford et al., 2012, Jones et al., 2014, Domeignoz-Horta et al., 2015). Closely related denitrification genes may be distributed across distantly related taxa (Philippot et al., 2002), and some closely related microorganisms may or may not have the same functional traits. For example, a large fraction of denitrifiers do not possess the *nosZ* gene (Jones et al., 2008, Graf et al., 2014). Thus, studies of N-cycling microbial communities need to employ functional genes as molecular markers, and cannot rely on taxonomic markers such as the 16 rRNA gene. Due to the high degree of sequence polymorphism in the N-cycling genes, primer design to reach sufficient coverage and specificity is often difficult and primer bias and non-specificity have been pointed out recurrently (Penton et al., 2013, Helen et al., 2016).

Quantitative PCR is commonly used to examine the gene abundance, for example to interpret the size of the microbial community performing specific steps of N cycling or the genetic potential for a process (Zhang et al., 2013, Ding et al., 2015, Petersen et al., 2012, Cheneby et al., 2009). To address diversity, microbial community structure and composition, high-throughput sequencing technologies integrated with bioinformatics analysis is widely used (Larose et al., 2013, Shu et al., 2016, Xu et al., 2014), either by amplicon sequencing or the direct genetic analysis of genomes within an environmental sample, which provides information about the organisms and functions in a given environment (Thomas et al., 2012, Eisen, 2007, Logares et al., 2012).

The conversion of atmospheric N_2 to NH_3 by the nitrogenase enzyme is encoded by the *nifH* gene, which is highly conserved in microbial organisms and therefore used as a molecular marker for N_2 fixation (Poly et al., 2001, Gaby and Buckley, 2012). The *nifH* gene is in general more abundant in rhizosphere than bulk soil (Poly et al., 2001). For nitrifiers, it has been most common to target the genes encoding the ammonia monooxygenase (AMO) found in ammonia oxidizers performing the first step in the nitrification process, such as the bacterial *amoA* (Rotthauwe et al., 1997) and archaeal *amoA* genes (Tourna et al., 2008). Bacterial *amoA* genes are widely distributed in soils, sediments and fresh-water environments, whereas archaeal *amoA* genes are ubiquitously found and dominate in the ocean (Francis et al., 2005). The key step in respiratory ammonification is catalyzed by nitrite reductase encoded by *nrfA* gene (Mohan et al., 2004), and primers for this gene were recently updated (Welsh et al., 2014). The other reactions resulting in gaseous N-loss are catalysed by several enzymes involved in the denitrification pathway, and the key genes *nirK* and *nirS* that encoded two different nitrite reductases (NirK and NirS) are commonly used markers for deitrificiation (Braker et al., 1998, Hallin and Lindgren, 1999, Henry et al., 2004, Throbäck,

2004, Wei et al., 2015). The N_2O reductase, encoded by the *nosZ* gene, two different variants known as *nosZ* Clade I (*nosZ*I) and the recently described *nosZ* Clade II (*nosZ*II), and primers have been developed that target both groups of this gene (Jones et al., 2013, Throbäck, 2004, Henry et al., 2006).

3.3 Management of soil nitrogen cycling

Management of terrestrial N is practiced worldwide to ensure economical profit; however, there are many problems that are gradually being realized. The efficiency of fertilizer N by major grain crops is often less than 50% (Zhang et al., 2015b). The fate and transformations of N in the soil system is complex, and the consequences of soil N-loss by improper practices may exceed environmental tolerance. The environmental problems have led to re-evaluations of some management strategies. The most common practices can be roughly divided into soil management, which includes tillage, fertilization and soil amendments, and crop management, which can include practices such as crop rotation, intercropping, catch crops and cover crops grown for mulching (green manure). However, external factors are also influencing and are influenced by the extent of N surplus from the farm system (Figure 2).

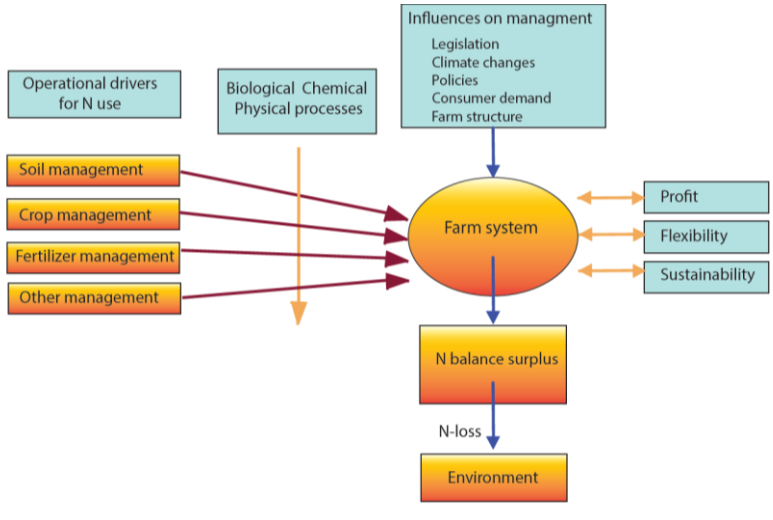

Figure 2. Management and N flows at the farm scale. (Figure modified from Jarvis S. et al. (2011))

3.3.1 Soil type

The physical, chemical and biological characteristics of a soil differ with geology, geographical location, soil depth, vegetation type and management practices, which together control soil texture, pH, organic matter and other edaphic factors. It is well known that soil type (texture and geological origin) exerts a strong effect on the soil microbial community composition (Buyer et al., 1999, Gelsomino and Azzellino, 2011, Singh et al., 2007, Zechmeister-Boltenstern et al., 2011). Girvan (2003) investigated three geographically separated agricultural sites, and found that soil type was the primary determinant of bacterial community composition.

Soil type also exerts a strong effect on the rhizosphere community (Berg and Smalla, 2009). This has been investigated in detail for *Arabidopsis thaliana* (Bulgarelli et al., 2012, Lundberg et al., 2012). However, the relative contribution of the soil and the selective effects of different crops on the rhizosphere community have also been examined and several studies show that soil type play an important role in shaping the microbial community structure in general (Buyer et al., 2002, Bulgarelli et al., 2015, Edwards et al., 2015). However, less is known about the N-cycling microbial communities, especially the newly described N_2O- reducing microorganisms.

3.3.2 Soil management

Soil physical-chemical properties might be disturbed or changed by long-term land management. Tillage with mouldboard ploughing mixes the soil, whereas no-till regimes create a stratification of the soil profile in addition to compaction of the soil. Both stratification and compaction can affect the soil microbial community. A higher overall diversity has often been observed in no-till regimes due to physical separation (Sipila et al., 2012) and different tillage regimes can also alter the relative abundance of different functional groups (Fan, 1997). For example, denitrification and abundance of denitrifiers can increase if the soil is more compacted and thereby less aerated (Melero et al., 2011), which would increase N losses. In agreement, a study investigating spatial patterns of denitrifiers communities across organic and integrated farming systems showed that the size of the denitrifying community was negatively correlated to soil parameters like pore volume and soil infiltration capacity, which indicates that soil compaction favours denitrifiers (Enwall et al. 2010). However, structure, size and activity were governed by soil-based parameters rather than land management in this case.

The application of fertilizers and organic amendments usually have a strong impact on soil microbial communities, including those involved in N cycling

(Wessen et al., 2010, Hallin et al., 2009, Abubaker et al., 2013). Fertilizers increase soil fertility and ensure crop productivity (Blackmer, 1997, Diacono and Montemurro, 2010), which directly and indirectly influence soil microbial communities (Bulluck et al., 2002). This depends on the type of fertilizer and the effects can differ on soil organisms mediating various N-cycling process. For example, the activity of N_2-fixing microorganisms may be suppressed by N fertilization, and abundance and composition of N_2-fixing bacteria can therefore change in response to long-term N-fertilizer application (Romero et al., 2012). In contrast, many studies have shown that the losses of N, such as nitrate leaching and N_2O emissions, were strongly associated with fertilization (Venterea et al., 2011, Schroder et al., 2010, Gheysari et al., 2009, Maharjan et al., 2014, Odlare et al., 2012). Nitrogen fertilizers typically promote denitrification in agricultural soil, and of the added N about 50-75% of the annual loss is through denitrification (Philippot et al., 2007, Mulvaney et al., 1997, Kaiser et al., 1998). Fertilization can also affect the N_2O to N_2 ratio from denitrification, and global N_2O emissions are increasing due to an increased input of mineral N fertilizers (IPCC 2013). Organic fertilizers, like manure, compost and biogas digestate, differ profoundly from mineral fertilizers by contributing to the overall microbial biomass in the soil, which in turn can result in increased biomass of certain soil functional microbial groups as well as the accumulation of organic C and N (Hallin et al., 2009, Jin et al., 2012, Jannoura et al., 2014, Wessen et al., 2010).

3.3.3 Plants and plant type

Plant species can impact ecosystem N cycling due to the differences in several important traits that affect either N inputs or losses, which are directly or indirectly caused by inherent differences in N uptake ability, produced biomass per unit N, and interactions with soil microbial decomposers, N_2-fixers, herbivores etc. (Knops et al., 2002). For example, legumes can employ symbiotic bacteria for N_2-fixation, which is preferably chosen as intercropping or crop rotation management regimes to enhance soil fertility. Besides symbiotic N_2-fixation, several studies have reported non-symbiotic N_2-fixation by grasses or forbs that also contribute to soil N input (Abbadie et al., 1992, Brejda et al., 1994, Mckone and Biesboer, 1986), but the knowledge of non-symbiotic N_2-fixation and plant control phenomenon is limited.

The rhizosphere is the zone surrounding the plant roots and influenced by plant roots, whereas the rhizoplane refers to root surface zone. Microorganisms can physically attach to root surface structures. Different plant species create physically or chemically different rhizosphere and rhizoplane environments for soil microorganisms (Philippot et al., 2013). This means that

there can be plant species-specific effects on soil microbial communities involved in N cycling in proximity to roots (Kilian and Werner, 1996, Bakken, 1988, Osanai et al., 2012). Plant roots produce chemical compounds and signalling molecules that are released into the rhizosphere (Philippot et al., 2013) and these compounds have a considerable effect on the soil microbial community and the processes they mediate (Insam and Domsch, 1988, Quispel, 1988). Root exudates include a variety of substances such as ions, enzymes, mucilage, free oxygen and diverse array of carbon (C) or N-containing compounds (Carvalhais et al., 2011, Walker et al., 2003). Roots also produce inhibitory or signalling substances. A well-known example of the latter is when rhizobia recognize and nodulate on their host legume root due to specific flavonoids released from legume roots (Bais et al., 2006).

The rhizosphere is often referred to as hot spot for denitrification. In agreement, a higher proportion of denitrifiers relative to other heterotrophic organisms is generally detected in proximity to roots (Vonberg and Bothe, 1992, Clays-josserand et al., 1995, Hamonts et al., 2013). The C rich environment is one factor explaining why denitrifiers are promoted in the rhizosphere. Fisk et al. (2015) showed the short-term plant-residue C decreased the risk of N losses due to microbial decomposers consuming amino acid-C as a C source and peptide-C for energy production in a semi-arid soil, but long-term root residue additions had no effect. Different types of exudates have been shown to affect denitrification rates differently (Henry et al., 2008). Depending on the plant type, free oxygen can also be exuded from the roots (Bodelier et al., 1996), whereas root respiration lowers the oxygen concentration in the proximity of roots. This means that oxygen concentrations fluctuate in proximity to the roots. It has been suggested that the ability to grow by respiring nitrogenous compounds when oxygen is limited could be a selective advantage for denitrifiers in the rhizosphere since they are facultative. Indeed, denitrification rates were increased up to 22-fold in the rhizosphere compared to the surrounding soil (Philippot et al., 2009, Philippot et al., 2013). This may contribute to increased N_2O emissions. A range of studies showed that N_2O emissions are higher in cropped soils when compared to bulk soils, thus indicating a significant influence of plants (Ding et al., 2007; Hénault et al., 1998; Højberg et al., 1996; Klemedtsson et al., 1987; Ni et al., 2012; Sey et al., 2010; Verma et al., 2006).

Plants affect several factors that influence nitrification and there are plant species effects (Patra et al., 2006, Philippot et al., 2009). An experiment comparing two plant species (*Eucalyptus camaldulensis* vs *Arundo donax*) has shown that plant species strongly affect potential nitrification rate due to the direct effects of ammonia oxidizer abundance and activity in effluent irrigated

land (Tsiknia et al., 2013). Enwall et al. (2007) showed that nitrification was promoted in an unfertilized cropped soil in comparison to a bare soil, which could be due to increased soil organic matter that provides NH_3 when mineralized. However, it could also an effect of the lower pH in the bare soil since pH determines availability of NH_3 for the ammonia oxidizers. Nitrification is often suppressed in the rhizosphere, likely because of competition with plants for N or due to competition with fast-growing heterotrophic bacteria for other resources. Several studies report nitrification can be inhibited in the rhizosphere (Boudsocq et al., 2009, Sylvesterbradley et al., 1988, Munro, 1966). It has been discovered that certain plants can naturally inhibit microbial nitrification via release of root exudates (Subbarao et al., 2013, Subbarao et al., 2006). Subbarao (2009) identified the compound brachialactone from roots of a tropical pasture grass *Brachiaria humidicola*, which can suppress nitrification in the rhizosphere by blocking enzymatic pathways in nitrifying bacteria (Subbarao et al., 2015), effectively curbing N_2O emission from soil (Subbarao et al., 2009). Another example is the tissues of Brassica plants that contain many secondary compounds, which when degraded form, iso-thiocyanates (ITCs) and other toxic volatile sulfuric compounds that can inhibit nitrification (Bending and Lincoln, 1999). Overall, these discoveries open the way for new approaches to manage N cycling by enhancing biological nitrification inhibitors in major crops.

In wetland soils, oxygen-releasing plants develop aerenchyma tissue for root respiration in order to adapt anoxic conditions (Armstrong et al., 1994, Laan et al., 1989), which benefits ammonia- and nitrite-oxidizing bacteria by providing oxygen in rhizosphere. It is reported that radial diffusion of oxygen from aerenchyma tissue is associated with the respiratory activity of the roots, which is influenced by temperature and age of the root (Moorhead and Reddy, 1988, Caffrey and Kemp, 1991). Several studies have indirectly reported a stimulation of nitrification by oxygen-releasing plant in sediments and wetland (Herrmann et al., 2008, Engelaar et al., 1995, Kirk and Kronzucker, 2005, Reddy et al., 1989).

3.3.4 Crop rotation and intercropping

The relationship between plant community diversity and the composition of the rhizosphere microbiota is more complex than for single plant species. Previous studies have described the change of soil microbial community biomass, activity and composition as being strongly associated with greater plant diversity, and likewise influenced microbial mediating processes such as soil C and N cycling (Zak et al., 2003, Hooker and Stark, 2008).

Crop rotation is a common practice in agriculture. Crop rotation involves growing two or more crops one after the other to increase yield and decrease pest pressure. Crop rotation can also change soil microbial community structure and activity, which are likely related to changes in soil organic matter accumulation (Tiemann et al., 2015). The diversity of crops can also be increased through intercropping. Intercropping is a multiple cropping practice which more than one crop in grown simultaneously on the same local, and it has been shown to improve space use and result in greater yield compared to sole crops (Brooker et al., 2015). Intercropping relies on key ecological concepts to increase aboveground plant biomass, such as efficient use of resources via niche complementarity (Brooker et al., 2015), increased resistance to diseases and pests (Risch, 1983) , and enhanced soil fertility, particularly in regards to soil N (Cong et al., 2015, Stern, 1993). Intercropping therefore often includes a legume to provide symbiotically fixed N to the system. In cereal-legume intercropping systems, biological N_2 fixation by bacteria associated with the legume increases plant available N compared to unfertilized non-legume crops. Uptake of soil N by the intercropped cereal results in depletion of N in the rhizosphere of the legume, which in turn stimulates the N_2 fixation activity (Afza et al., 1987, Ehrmann and Ritz, 2014, Hauggaard-Nielsen et al., 2001). This will reduce the input of fertilizer and thereby the production costs.

A large number of studies have shown aboveground effects of intercropping in relation to N management, such as increased biomass N-content. Fewer studies have examined the effects of intercropping on the soil microbial communities that drive belowground N cycling, although interactions between roots and soil organisms have been shown to enhance crop resource use efficiency (Ehrmann and Ritz, 2014, Shen et al., 2013, Li et al., 2014). Several studies have shown that soil NO_3^- concentration increases with decreasing plant diversity (Mueller et al., 2013, Niklaus et al., 2006, Zak et al., 2003). Recent work suggests that intercropping may reduce the abundance of ammonia-oxidizers in the rhizosphere, which could explain this (Zhang et al., 2015a). In addition, intercropping has been identified to effectively reduce both NO_3^- leaching and N_2O emission due to more efficient use of N in comparison with sole crops (Whitmore and Schroder, 2007, Pappa et al., 2011, Sanchez-Martin et al., 2010). However increased emissions of N_2O have also been observed during intercropping (Sun et al., 2013), indicating that intercropping influences the activity and possibly also the composition of microbial communities either producing or reducing N_2O. The mechanisms controlling net N_2O emission are complex and controlled by numerous factors, such as

availability of mineral N, temperature, moisture, texture and microbial community properties (Skiba, 2000).

4 Result and discussion

Two separate experiments were performed to assess the effect of plant species, cropping practices, fertilization and soil type on N-cycling microbial communities. The plant and intercropping experiment (**Papers I** and **III**) were set up in rhizoboxes with lucerne (*Medicago sativa*) and cocksfoot (*Dactylis glomerata*) grown either as single crops or intercropped in an agricultural soil from Alnarp, Sweden (Figure 3). To compare the effect of plant species versus edaphic factors on the abundance, diversity and functioning of denitrifying and N_2O reducing communities (**Paper II**), an experiment was established in growth chambers growing a monocot plant barley (*Hordeum vulgare*) and a dicot plant sunflower (*Helianthus anuus*) in two agricultural soils in Uppsala, Sweden (Figure 4).

Figure 3. The growth rhizobox in greenhouse. A rhizobox with intercropped cocksfoot (*Medicago sativa*) and lucerne (*Dactylis glomerata*) taken at the soil and root sampling occasion (left), The overview of experiment setups (right down). Sketch of a rhizobox indicating the dimension (right up). (Photo: Georg Carlsson).

Figure 4. The pot experiment with barely and sunflower as well as unplanted soil randomized on a tray in a growth chamber kept at 20°C during day time and 15°C during night with 18h day length (Photo: Daniel Graf).

4.1 Plant (Papers I, II, III)

In all three studies, the effect of plants on the root and soil associated microbial communities was studied. A plant effect was observed on the genetic potential of the targeted N-cycling communities associated with root samples, whereas no significant plant effects on genetic potential of N-cycling communities were found in the bulk soil (Table 1, **Paper I**). The lack of a significant plant effect in the bulk soil was also seen in **Paper II**, as the abundance of denitrifiers did not differ significantly between plant species within the same soil type. In addition, there was no effect of plant species in the bulk soil with regards to the N_2O reducing microbial community structure and composition (**Paper III**). This was also previously reported for microbial community structure in general in the rhizosphere of other plant species (Berg and Smalla, 2009; Bulgarelli *et*

al., 2012; Lundberg *et al.*, 2012; Edwards *et al.*, 2015; Prasse *et al.*, 2015). Based on the results in this thesis, plant effects on the soil microbial community can be ascribed to plant-microbial interactions restricted to the communities in the root compartment and not influencing those inhabiting the bulk soil.

4.1.1 Genetic potential and enzymatic activity

The two forage plants *Medicago sativa* (lucerne) and *Dactylis glomerata* (cocksfoot) performed differently in affecting the abundance of root-associated N-cycling communities, which can be ascribed to plant species-specific effects (Figure 1, **Paper I**). Plant species can differ in root exudates and root morphology, which influence root-associated and rhizosphere microbiota (Philippot et al., 2013). The small and finer root structure of cocksfoot creates a larger surface for the root-associated microorganisms compared to lucerne, which may be a reason that cocksfoot in general supported a higher abundance of total bacteria and all functional groups on roots. The abundances of *nirS* and *nirK*, representing the genetic potential for denitrification, were similar between *Hordeum vulgare* (barely) and *Helianthus annuus* (sunflower), but denitrification activity on sunflower roots was below detection limit (Figure 1, **Paper II**). The relationship between denitrification activity and gene abundance is not always correlated, which may be due to the facultative nature of denitrifiers (Graham et al., 2016). Plant effects on the activity of functional communities in rhizosphere may also result from differences in root exudates and possibly oxygen at the root-soil interface of different plant species (Henry et al., 2008, Cao et al., 2015, Prade and Trolldenier, 1988).

The work in this thesis has also shown that N_2O reducing organisms carrying *nosZ*I have an affinity to plant roots, whereas those with *nosZ*II prefer the bulk soil (Figure 2, Paper II) thus indicating a possible niche differentiation between the two clades. Organisms harbouring *nosZ*I are typically denitrifiers with a complete pathway and found among Alpha-, Beta- and Gammaproteobacteria (Graf et al., 2014). A higher proportion of denitrifiers relative to other heterotrophic organisms is generally detected in proximity to roots (Clays-josserand et al., 1995, Vonberg and Bothe, 1992). In accordance with our findings, Hamonts et al. (2013) recently found *nosZ*I to be in higher abundance in proximity to roots compared to surrounding bulk soil, although they did not determine the preference of *nosZ* II in their study. Recent work on denitrifying microorganisms in pure cultures has suggested that species with a complete denitrification pathway may be more competitive in nitrate limited environments, having the capacity to utilize all electron acceptors available from the reduction of nitrate (Felgate et al., 2012).

Principle component analysis (PCA) and multi-response permutation procedures (MRPP) illustrated a distinct separation of samples from lucerne roots, cocksfoot roots and soil (Figure 1 **Paper I),** irrespective of intercropping and fertilization ($P<0.001$), due to higher abundance of the *nifH* and denitrifier genes (*nirS*, *nirK nosZ*I) in the root samples, and the bacterial and archaeal *amoA* genes in the bulk soil. In addition, the genetic potential for denitrifiers (*nirK*, *nosZ*I, *nosZ*II) abundance in **Paper II** also showed a significant difference of soil and root samples within the same soil type (Figure 2, **Paper II**). Overall, the results from the studies in this thesis indicate that different N-cycling organisms were favoured in different compartments in the soil-root environment.

4.1.2 N_2O reducing community composition

When focusing on the N_2O reducing community composition, we could observe that the community composition of both *nosZ* clade I and clade II differed significantly between root and soil samples, thus indicating selective pressure by the plants (PERMANOVA, $P<0.001$; **Paper III**). Moreover, the structure of *nosZ* clade I communities in soil also differed significantly between plant types, whereas only *nosZ* clade II root-associated communities were affected by plant type (PERMANOVA, $P<0.05$). Significant plant effects on potential denitrification and N_2O production rates were also observed on roots in both **Paper II** and **III**, whereas no difference was observed in soils regarding plant effects.

Many *nosZ* clade I root associated organisms were associated with *Bradyrhizobium*, which are typical rhizosphere bacteria known to harbour this gene variant (Graf et al., 2014) and to be able to denitrify (Philippot et al., 2007). The *nosZ* clade II root communities predominantly belonged to the Gemmatimonadaceae and Ignavibacteria. The two known Ignavibacteria genomes harbouring *nosZ* genes, *Ignavibacterium album* and *Melioribacter roseus*, both possess the *nosZ* Clade II gene, but no *nir* or *nor* genes (Graf et al., 2014). Moreover both possess *nrfA* genes encoding the formate-dependent nitrite reductase (Sanford et al., 2012, Song et al., 2014), catalysing nitrite in reduction respiratory ammonification. Thus, we postulate that N_2O reducers within clade II were "DNRA" bacteria rather than denitrifiers in this system (Sanford et al., 2012).

While conditions promoting denitrification and respiratory ammonification are similar as they both occur in anaerobic environments, compete for NO_3^- and require organic C, respiratory ammonification is generally believed to out-compete denitrification in highly reduced environments with high C:N ratios (Nogaro and Burgin, 2014, Schmidt et al., 2011, Song et al., 2014). Schmidt et

al. (2011) hypothesized that respiratory ammonification may be promoted in the rhizosphere due to the transient O_2 conditions, production of low molecular weight carbon compounds, and competition for NO_3^- by the roots. This could explain the increased abundance of *nosZ* clade II copy numbers and sequences associated with organisms that potentially perform respiratory ammonification on the roots of non-legume plants such as cocksfoot, which has been shown to strongly compete for NO_3^- as well as produce large amounts low molecular weight carbon compounds (Danso et al., 1987, Ehrmann and Ritz, 2014). Nevertheless, our results show that the genetic potential for respiratory ammonification was one order of magnitude lower than that of denitrification in the root-associated communities suggesting that denitrifiers have been favoured (**Paper I**). The importance of respiratory ammonification for N retention in arable soils in general, and in relation to crops and during intercropping in particular, is not known.

4.2 Intercropping (Papers I, III)

4.2.1 Genetic potential and enzymatic activity

Intercropping significantly affected the abundance of total bacterial community and several N-cycling functional groups on roots that are associated with the retention and loss of N, (Table 1, **Paper I**), but did not affect the structure of the N_2O reducing communities (**Paper III**). The legume exerted a strong effect on the abundance of the root-associated N-cycling communities on the grass roots, which indicates altered plant-microbial or microbial-microbial interactions during intercropping. The abundance of the non-symbiotic N_2-fixing community decreased (**Paper I**), which likely resulted from the changes in the level of available resources. The introduction of lucerne should alter the level of available resources for free-living microorganisms since symbiotic N_2-fixing bacteria would be well supported by the legume. Resource competition may be particularly relevant for available C, which fits with our results showing significant intercropping effects on heterotrophic rather than autotrophic root-associated microorganisms. In agreement, intercropping also significantly affected the abundance of "DNRA" bacteria and *nirS* type denitrifers (**Paper I**).

Several studies have shown that intercropping results in enhanced NO_3^- depletion (Doltra and Olesen, 2013), and can effectively reduce soil NO_3^- leaching (Nie et al., 2012, Whitmore and Schroder, 2007). Given that organisms performing respiratory ammonification and denitrification processes compete for NO_3^- under anaerobic conditions, rapid depletion of NO_3^- may limit its availability to microbial communities that perform either process. The

genetic potential for denitrification was higher than that for respiratory ammonification by one order of magnitude in the root-associated communities, which indicates that the denitrifier community was favoured in the present experiment. Inhibition of nitrification or a decrease in abundance of nitrifiers also contributes the control of NO_3^- leaching (Zhang et al., 2015a), but we did not observe an intercropping effect on the genetic potential for ammonia oxidation.

Legume-mixed intercropping contributes to increased N availability through N fixation, presenting a risk of increased N_2O emissions if not managed properly (Herridge et al., 2008, Epie et al., 2015). On the other hand, intercropping may reduce N_2O emissions by increased plant N uptake (Sun et al., 2013). A lower abundance of N_2O reducing *nosZ* clade II community was found in intercropped cocksfoot root comparing to sole cropped cocksfoot root (Figure 3b, **Paper III**), and slightly higher N_2O production rates were detected on intercropped cocksfoot roots compared to sole cropped cocksfoot roots grown in non-fertilized soil (Figure 2, **Paper III**). Thus, the ratio of N_2O production and denitrification was lower in sole cropped cocksfoot root than for intercropped cocksfoot, indicating a higher potential N_2O emission from intercropped cocksfoot roots. In cereal-legume intercropping systems, the cereal competes for soil N resulting in depletion of N in the rhizosphere of the legume, which stimulates N-fixation by legume associated N_2-fixing bacteria (Danso et al., 1987; Ehrmann and Ritz, 2013; Hauggaard-Nielsen et al., 2001). This in turn may decrease the C:N ratio, and from our results it appears plausible that heterotrophic microorganisms associated with lucerne roots become C limited due to growth stimulation by high N availability. In turn, competition for available C, produced by the cocksfoot roots, may have resulted in lower abundance of DNRA organisms and thus *nosZ* Clade II during intercropping. Altogether, we hypothesize that such C: N dynamics and decreased abundance of organisms performing respiratory ammonification lead to an increase of the potential N_2O emission rate on intercropped cocksfoot roots.

Fertilization has been shown to suppress biological N_2 fixation (Gulden and Vessey, 1998, Fan et al., 2006, Stern, 1993), although this can be alleviated depending on the intercropped species (Fan et al., 2006, Li et al., 2009). The intercropping experiment used biogas digestate as biofertilizer amendment. It did not show any obvious effects on the N-cycling community, except for a significantly increased abundance of *nirS* gene on roots, but this was only observed in sole crops. This suggests that biogas digestate did not alter the intercropping effects on the genetic potential for denitrification in this study. The biogas digestate amendment corresponded to about 50 kg N and 500 kg C

per ha. This low fertilization rate could explain the lack of fertilizer effects. The average N-fertilizer application rate in the European Union countries is much higher with approximately over 100 kg per ha (Sutton et al., 2011).

4.3 Soil type (Paper II)

The relative contribution of soil type and plant effects on soil microbial communities involved in denitrification and N_2O reduction was investigated (**Paper II**). Two agricultural soils, Ekhaga and Kungshamn, were used in a pot experiment and planted with either barley or sunflower. The results showed that soil type rather than plant species affected potential denitrification, N_2O production rates and genetic potential for denitrification and N_2O production in both root-associated and soil borne microorganisms.

Lower denitrification rates and higher N_2O emissions were observed in Kungshamn soil compared to Ekhaga soil, which might be caused by low soil pH (Figure 1 and Table 1, **Paper II**) (Van den Heuvel et al., 2011, Firestone et al., 1980). An additional explanation for higher N_2O emissions from Kungshamn soil can be drawn from the genetic composition of the N_2O reducing community, with Kungshamn soil displaying lower *nosZ*II gene abundance than Ekhaga soil. The high *nirS* abundance in relation to overall lower genetic potential for N_2O reduction in Kungshamn might also contribute to the observed difference in N_2O emission ratios.

5 Conclusion and perspectives

5.1 Conclusions

During recent decades, management strategies in agriculture have positively contributed economic growth and even the human way of life, but also affected the environment negatively. With time, we might have to rethink certain ways of managing our agroecosystems. This thesis explored the effects of soil type, plant type, and intercropping on the activity, abundance and community structure of soil microorganisms involved in N cycling, especially soil microbial community mediating N losses since these processes pose a major impact on the environment. The results are summarized as follows.

- Plant species was the key factor determining the genetic potential of root-associated N-cycling functional groups in relation to intercropping or soil organic amendments (**Paper I** and **III**). However, when comparing specific plant species cultivated in different soils, soil type was overriding plant effects on the genetic potential (**Paper II**). The abundance of soil microbial communities on roots between a non-legume plant (cocksfoot) and a legume (lucerne) was significantly different (**Paper I**), but this was not the case when comparing two non-legume plants (barley and sunflower; **Paper II**).
- Plant effects on the soil microbial community were ascribed to plant-microbial interactions restricted to the root compartment and not influencing those inhabiting the bulk soil (**Papers I-III**).
- Soil type was overriding plant effects on enzymatic N_2O production potential in arable soils (**Paper II**). Thus, the soil physical and chemical properties rather than plant species

determine the denitrification and N_2O production rates of the root-associated communities.

- Legume-grass intercropping affected the abundance and activity of root-associated N-cycling communities, likely due to altered plant-microbial and/or microbial-microbial interactions (**Paper I** and **III**). Lucerne intercropped with cocksfoot reduced the genetic potential for N_2O reduction by N_2O reducers belonging to *nosZ* clade II on roots, and increased the N_2O-production/ denitrification ratio.
- Addition of biogas digestate did not alter the intercropping effects on the genetic potential for N cycling (**Paper I**), however, this could probably differ depending on the type, source and amount of biogas digestate used.

5.2 Future perspectives

Intercropping effects on the crop have been widely studied, but the effects on the below-ground biota are lagging behind. We examined intercropping effects at one-time point in the current thesis, but vegetation period may have different influences on soil N-cycling microbial functional groups. Zhang et al., (2015a) showed that intercropping effects on ammonia oxidizers were different in relation to the plant growth phase, and how it would differ during the vegetation period for other functional groups in the N-cycle requires investigation. The knowledge of intercropping effects on soil N-cycling communities is incomplete regarding plant type. Most studies have focused on intercropping with a grass and a legume, whereas other combinations, for example in cereal crops, vegetables and trees have not been well studied. Moreover, different plant types, such as C3 vs C4 plants, monocot vs dicot plants, plants with different root traits, plants differing in nutrient acquisition strategies etc., also warrants further research. A knowledge-based selection of crop combinations may save time and labour to improve N-use efficiency and reduce soil NO_3^- leaching or N_2O emissions.

Besides the work presented in this thesis, additional management practices such as tillage, crop rotation etc., effects on soil N-cycling microbial community, as well as the ecosystem processes and services they mediate also need to be further explored.

References

Abbadie, L., Mariotti, A. & Menaut, J. C. 1992. Independence of savanna grasses from soil organic-matter for their nitrogen supply. *Ecology,* 73**,** 608-613.

Abdelmal, Y. 1971. Free-living nitrogen-fixing bacteria in egyptian soils and their possible contribution to soil fertility. *Plant and Soil***,** 423.

Abubaker, J., Odlare, M. & Pell, M. 2013. Nitrous oxide production from soils amended with biogas residues and cattle slurry. *Journal of Environmental Quality,* 42**,** 1046-1058.

Afza, R., Hardarson, G., Zapata, F. & Danso, S. K. A. 1987. Effects of delayed soil and foliar N fertilization on yield and N_2 fixation of soybean. *Plant and Soil,* 97**,** 361-368.

Akiyama, H., McTaggart, I. P., Ball, B. C. & Scott, A. 2004. N_2O, NO, and NH_3 emissions from soil after the application of organic fertilizers, urea and water. *Water Air and Soil Pollution,* 156**,** 113-129.

An, S. M. & Gardner, W. S. 2002. Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). *Marine Ecology Progress Series,* 237**,** 41-50.

Andersson, B., Sundback, K., Hellman, M., Hallin, S. & Alsterberg, C. 2014. Nitrogen fixation in shallow-water sediments: Spatial distribution and controlling factors. *Limnology and Oceanography,* 59**,** 1932-1944.

Armstrong, W., Brandle, R. & Jackson, M. B. 1994. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica,* 43**,** 307-358.

Baggs, E. M., Smales, C. L. & Bateman, E. J. 2010. Changing pH shifts the microbial sourceas well as the magnitude of N_2O emission from soil. *Biology and Fertility of Soils,* 46**,** 793-805.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S. & Vivanco, J. M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology,* 57**,** 233-66.

Bakken, L. R. 1988. Denitrification under different cultivated plants: effects of soil moisture tension, nitrate concentration, and photosynthetic activity. *Biology and Fertility of Soils,* 6**,** 271-278.

Behrendt, A., de Beer, D. & Stief, P. 2013. Vertical activity distribution of dissimilatory nitrate reduction in coastal marine sediments. *Biogeosciences,* 10**,** 7509-7523.

Bending, G. D. & Lincoln, S. D. 1999. Characterisation of volatile sulphur-containing compounds produced during decomposition of *Brassica juncea* tissues in soil. *Soil Biology & Biochemistry,* 31**,** 695-703.

Berg, G. & Smalla, K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology,* 68**,** 1-13.

Blackmer, A. M., R.D. Voss, and A.P. Mallarino 1997. Nitrogen fertilizer recommendations for corn in Iowa. *PM-1714. Iowa State Univ. Ext., Ames*.

Bodelier, P. L. E., Libochant, J. A., Blom, C. W. P. M. & Laanbroek, H. J. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. *Applied and Environment Microbiololgy,* 62**,** 4100-4107.

Boudsocq, S., Lata, J. C., Mathieu, J., Abbadie, L. & Barot, S. 2009. Modelling approach to analyse the effects of nitrification inhibition on primary production. *Functional Ecology,* 23**,** 220-230.

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**,** 3769-3775.

Brejda, J. J., Kremer, R. J. & Brown, J. R. 1994. Indications of associative nitrogen fixation in eastern gamagrass. *Journal of Range Management,* 47**,** 192-195.

Bremner, J. M., Robbins, S. G. & Blackmer, A. M. 1980. Seasonal variability in emission of nitrous oxide from soil. *Geophysical Research Letters,* 7**,** 641-644.

Brentrup, F. & Pallière, C. Nitrogen use efficiency as an agro-environmental indicator. Proceedings of the OECD Workshop on Agrienvironmental Indicators, March, 2010. 23-26.

Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., McKenzie, B. M., Pakeman, R. J., Paterson, E., Schob, C., Shen, J. B., Squire, G., Watson, C. A., Zhang, C. C., Zhang, F. S., Zhang, J. L. & White, P. J. 2015. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist,* 206**,** 107-117.

Brunet, R. C. & GarciaGil, L. J. 1996. Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiology Ecology,* 21**,** 131-138.

Bulgarelli, D., Garrido-Oter, R., Munch, P. C., Weiman, A., Droge, J., Pan, Y., McHardy, A. C. & Schulze-Lefert, P. 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host & Microbe,* 17**,** 392-403.

Bulgarelli, D., Rott, M., Schlaeppi, K., van Themaat, E. V. L., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T. & Schulze-Lefert, P. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature,* 488**,** 91-95.

Bulluck, L. R., Brosius, M., Evanylo, G. K. & Ristaino, J. B. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology,* 19**,** 147-160.

Buyer, J. S., Roberts, D. P. & Russek-Cohen, E. 2002. Soil and plant effects on microbial community structure. *Canadian Journal of Microbiology,* 48**,** 955-964.

Caffrey, J. M., Bano, N., Kalanetra, K. & Hollibaugh, J. T. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME Journal,* 1**,** 660-662.

Caffrey, J. M. & Kemp, W. M. 1991. Seasonal and spatial patterns of oxygen production, respiration and root rhizome release in *Potamogeton-perfoliatus* L. and *Zostera-marina* L. *Aquatic Botany,* 40**,** 109-128.

Canfield, D. E., Glazer, A. N. & Falkowski, P. G. 2010. The evolution and future of earth's nitrogen cycle. *Science,* 330**,** 192-196.

Cao, J., Lee, J. W., Six, J. A., Yan, Y., Zhang, F. S. & Fan, M. S. 2015. Changes in potential denitrification-derived N_2O emissions following conversion of grain to greenhouse vegetable cropping systems. *European Journal of Soil Biology,* 68**,** 94-100.

Carvalhais, L. C., Dennis, P. G., Fedoseyenko, D., Hajirezaei, M. R., Borriss, R. & von Wiren, N. 2011. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *Journal of Plant Nutrition and Soil Science,* 174**,** 3-11.

Cavigelli, M. A. & Robertson, G. P. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology,* 81**,** 1402-1414.

Chao, W. L. 1995. Effects of acid-rain on the nitrifying activity of copper amended soil. *Letters in Applied Microbiology,* 21**,** 23-24.

Cheneby, D., Brauman, A., Rabary, B. & Philippot, L. 2009. Differential responses of nitrate reducer community size, structure, and activity to tillage systems. *Applied and Environmental Microbiology,* 75**,** 3180-3186.

Clays-josserand, A., Lemanceau, P., Philippot, L. & Lensi, R. 1995. Influence of two plant pecies (flax and tomato) on the distribution of nitrogen dissimilative abilities within fluorescent *Pseudomonas* spp. *Applied and Environmental Microbiology,* 61**,** 1745-1749.

Cong, W. F., Hoffland, E., Li, L., Six, J., Sun, J. H., Bao, X. G., Zhang, F. S. & van der Werf, W. 2015. Intercropping enhances soil carbon and nitrogen. *Global Change Biology,* 21**,** 1715-1726.

Correa-Galeote, D., Marco, D. E., Tortosa, G., Bru, D., Philippot, L. & Bedmar, E. J. 2013. Spatial distribution of N-cycling microbial communities showed complex patterns in constructed wetland sediments. *FEMS Microbiology Ecology,* 83**,** 340-351.

Cui, Q., Song, C. C., Wang, X. W., Shi, F. X., Wang, L. L. & Guo, Y. D. 2016. Rapid N_2O fluxes at high level of nitrate nitrogen addition during freeze-thaw events in boreal peatlands of Northeast China. *Atmospheric Environment,* 135**,** 1-8.

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H. & Wagner, M. 2015. Complete nitrification by *Nitrospira* bacteria. *Nature,* 528**,** 504-523.

Danso, S. K. A., Zapata, F., Hardarson, G. & Fried, M. 1987. Nitrogen fixation in fababeans as affected by plant population density in sole or intercropped systems with barley. *Soil Biology & Biochemistry,* 19**,** 411-415.

Devol, A. H. 2015. Denitrification, anammox, and N_2 production in marine sediments. *Annual Review of Marine Science, Vol 7,* 7**,** 403-423.

Diacono, M. & Montemurro, F. 2010. Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development,* 30**,** 401-422.

Ding, K., Zhong, L., Xin, X. P., Xu, Z. H., Kang, X. M., Liu, W. J., Rui, Y. C., Jiang, L. L., Tang, L. & Wang, Y. F. 2015. Effect of grazing on the abundance of functional genes associated with N cycling in three types of grassland in Inner Mongolia. *Journal of Soils and Sediments,* 15**,** 683-693.

Dodsworth, J. A., Hungate, B. A. & Hedlund, B. P. 2011. Ammonia oxidation, denitrification and dissimilatory nitrate reduction to ammonium in two US Great Basin hot springs with abundant ammonia-oxidizing archaea. *Environmental Microbiology,* 13**,** 2371-2386.

Doltra, J. & Olesen, J. E. 2013. The role of catch crops in the ecological intensification of spring cereals in organic farming under Nordic climate. *European Journal of Agronomy,* 44**,** 98-108.

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,* 6:971.

Dong, L. F., Sobey, M. N., Smith, C. J., Rusmana, I., Phillips, W., Stott, A., Osborn, A. M. & Nedwell, D. B. 2011. Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries. *Limnology and Oceanography,* 56**,** 279-291.

Drozd, J. & Postgate, J. R. 1970. Interference by oxygen in the acetylene-reduction test for aerobic nitrogen-fixing bacteria. *Journal of General Microbiology* 60**,** 427-9.

Duc, L., Noll, M., Meier, B. E., Burgmann, H. & Zeyer, J. 2009. High diversity of diazotrophs in the forefield of a receding alpine glacier. *Microbial Ecology,* 57**,** 179-190.

Edwards, J., Johnson, C., Santos-Medellin, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A. & Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the United States of America,* 112**,** E911-E920.

Ehrmann, J. & Ritz, K. 2014. Plant: soil interactions in temperate multi-cropping production systems. *Plant and Soil,* 376**,** 1-29.

Eisen, J. A. 2007. Environmental shotgun sequencing: Its potential and challenges for studying the hidden world of microbes. *PLoS Biology,* 5**,** 384-388.

Engelaar, W. M. H. G., Symens, J. C., Laanbroek, H. J. & Blom, C. W. P. M. 1995. Preservation of nitrifying capacity and nitrate availability in waterlogged soils by radial oxygen loss from roots of wetland plants. *Biology and Fertility of Soils,* 20**,** 243-248.

Enwall, K., Nyberg, K., Bertilsson, S., Cederlund, H., Stenstrom, J. & Hallin, S. 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. *Soil Biology & Biochemistry,* 39**,** 106-115.

Epie, K. E., Saikkonen, L., Santanen, A., Jaakkola, S., Makela, P., Simojoki, A. & Stoddard, F. L. 2015. Nitrous oxide emissions from perennial grass-legume intercrop for bioenergy use. *Nutrient Cycling in Agroecosystems,* 101**,** 211-222.

Epstein, H. E., Burke, I. C. & Mosier, A. R. 1998. Plant effects on spatial and temporal patterns of nitrogen cycling in shortgrass steppe. *Ecosystems,* 1**,** 374-385.

Erler, D. V., Eyre, B. D. & Davison, L. 2008. The contribution of anammox and denitrification to sedirnent N_2 production in a surface flow constructed wetland. *Environmental Science & Technology,* 42**,** 9144-9150.

Fan, F. L., Zhang, F. S., Song, Y. N., Sun, J. H., Bao, X. G., Guo, T. W. & Li, L. 2006. Nitrogen fixation of faba bean (*Vicia faba* L.) interacting with a non-legume in two contrasting intercropping systems. *Plant and Soil,* 283**,** 275-286.

Fan, M. X. M., A.F.; Abbott M;Cadrin F 1997. Denitrification estimateds in monoculture and rotation corn as influenced by tillage and nitrogen fertilizer. *Canadian Journal of Soil Science,* 1997,77(3).

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**,** 1788-1800.

Firestone, M. K. & Davidson, E. A. 1989. Microbiological basis of NO and N_2O production and consumption in soil. *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere,* 47**,** 7-21.

Firestone, M. K., Firestone, R. B. & Tiedje, J. M. 1980. Nitrous oxide from soil denitrification: Factors controlling its biological production. *Science,* 208**,** 749-751.

Fisk, L. M., Barton, L., Jones, D. L., Glanville, H. C. & Murphy, D. V. 2015. Root exudate carbon mitigates nitrogen loss in a semi-arid soil. *Soil Biology & Biochemistry,* 88**,** 380-389.

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 Sciences of the United States of America,* 102**,** 14683-14688.

Gaby, J. C. & Buckley, D. H. 2012. A comprehensive evaluation of PCR primers to amplify the *nifH* gene of nitrogenase. *PLoS One,* 7.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z. C., Freney, J. R., Martinelli, L. A., Seitzinger, S. P. & Sutton, M. A. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science,* 320**,** 889-892.

Gelsomino, A. & Azzellino, A. 2011. Multivariate analysis of soils: microbial biomass, metabolic activity, and bacterial-community structure and their relationships with soil depth and type. *Journal of Plant Nutrition and Soil Science,* 174**,** 381-394.

Gheysari, M., Mirlatifi, S. M., Homaee, M., Asadi, M. E. & Hoogenboom, G. 2009. Nitrate leaching in a silage maize field under different irrigation and nitrogen fertilizer rates. *Agricultural Water Management,* 96**,** 946-954.

Girvan, M. S., Bullimore, J., Pretty, J. N., Osborn, A. M. & Ball, A. S. 2003. Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Applied and Environmental Microbiology,* 69**,** 1800-1809.

Goodroad, L. L. & Keeney, D. R. 1984. Nitrous oxide emissions from soils during thawing. *Canadian Journal of Soil Science,* 64**,** 187-194.

Graf, D. R. H., 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.

Graham, E. B., Knelman, J. E., Schindlbacher, A., Siciliano, S., Breulmann, M., Yannarell, A., Bemans, J. M., Abell, G., Philippot, L., Prosser, J., Foulquier, A., Yuste, J. C., Glanville, H. C., Jones, D. L., Angel, F., Salminen, J., Newton, R. J., Burgmann, H., Ingram, L. J., Hamer, U., Siljanen, H. M. P., Peltoniemi, K., Potthast, K., Baneras, L., Hartmann, M., Banerjee, S., Yu, R. Q., Nogaro, G., Richter, A., Koranda, M., Castle, S. C., Goberna, M., Song, B., Chatterjee, A., Nunes, O. C., Lopes, A. R., Cao, Y. P., Kaisermann, A., Hallin, S., Strickland, M. S., Garcia-Pausas, J., Barba, J., Kang, H., Isobe, K., Papaspyrou, S., Pastorelli, R., Lagomarsino, A., Lindstrom, E. S., Basiliko, N. & Nemergut, D. R. 2016. Microbes as engines of ecosystem function: When does community structure enhance predictions of ecosystem processes? *Frontiers in Microbiology,* 7.

Guckland, A., Corre, M. D. & Flessa, H. 2010. Variability of soil N cycling and N_2O emission in a mixed deciduous forest with different abundance of beech. *Plant and Soil,* 336**,** 25-38.

Gulden, R. H. & Vessey, J. K. 1998. Low concentrations of ammonium inhibit specific nodulation (nodule number g^{-1} root DW) in soybean (*Glycine max* [L.] Merr). *Plant and Soil,* 198**,** 127-136.

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**,** 597-605.

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

Hallin, S., Welsh, A., Stenstrom, J., Hallet, S., Enwall, K., Bru, D. & Philippot, L. 2012. Soil functional operating range linked to microbial biodiversity and community composition using denitrifiers as model guild. *PLoS One,* 7.

Hamonts, K., Clough, T. J., Stewart, A., Clinton, P. W., Richardson, A. E., Wakelin, S. A., O'Callaghan, M. & Condron, L. M. 2013. Effect of nitrogen and waterlogging on denitrifier gene abundance, community structure and activity in the rhizosphere of wheat. *FEMS Microbiology Ecology,* 83**,** 568-584.

Hatzenpichler, R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. *Applied and Environmental Microbiology,* 78**,** 7501-7510.

Hauggaard-Nielsen, H., Ambus, P. & Jensen, E. S. 2001. Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Research,* 70**,** 101-109.

Helen, D., Kim, H., Tytgat, B. & Anne, W. 2016. Highly diverse *nirK* genes comprise two major clades that harbour ammonium-producing denitrifiers. *Bmc Genomics,* 17.

Henry, S., Baudoin, E., Lopez-Gutierrez, J. C., Martin-Laurent, F., Brauman, A. & Philippot, L. 2004. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. *Journal of Microbiological Methods,* 59**,** 327-335.

Henry, S., Bru, D., Stres, B., Hallet, S. & Philippot, L. 2006. Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in soils. *Applied and Environmental Microbiology,* 72**,** 5181-5189.

Henry, S., Texier, S., Hallet, S., Bru, D., Dambreville, C., Cheneby, D., Bizouard, F., Germon, J. C. & Philippot, L. 2008. Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: insight into the role of root exudates. *Environmental Microbiology,* 10**,** 3082-3092.

Herridge, D. F., Peoples, M. B. & Boddey, R. M. 2008. Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil,* 311**,** 1-18.

Herrmann, M., Saunders, A. M. & Schramm, A. 2008. Archaea dominate the ammonia-oxidizing community in the rhizosphere of the freshwater macrophyte Littorella uniflora. *Applied and Environmental Microbiology,* 74**,** 3279-3283.

Hirose, T. 2011. Nitrogen use efficiency revisited. *Oecologia,* 166**,** 863-867.

Holtan-Hartwig, L., Dorsch, P. & Bakken, L. R. 2000. Comparison of denitrifying communities in organic soils: kinetics of NO_3^- and N_2O reduction. *Soil Biology & Biochemistry,* 32**,** 833-843.

Hooker, T. D. & Stark, J. M. 2008. Soil C and N cycling in three semiarid vegetation types: Response to an in situ pulse of plant detritus. *Soil Biology & Biochemistry,* 40**,** 2678-2685.

Hu, B. L., Rush, D., van der Biezen, E., Zheng, P., van Mullekom, M., Schouten, S., Damste, J. S. S., Smolders, A. J. P., Jetten, M. S. M. & Kartal, B. 2011. New anaerobic, ammonium-oxidizing community enriched from peat soil. *Applied and Environmental Microbiology,* 77**,** 966-971.

Humbert, S., Tarnawski, S., Fromin, N., Mallet, M. P., Aragno, M. & Zopfi, J. 2010. Molecular detection of anammox bacteria in terrestrial ecosystems: distribution and diversity. *ISME Journal,* 4**,** 450-454.

Insam, H. & Domsch, K. H. 1988. Relationship between soil organic-carbon and microbial biomass on chronosequences of reclamation sites. *Microbial Ecology,* 15**,** 177-188.

IPCC 2013. Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK.

Jannoura, R., Joergensen, R. G. & Bruns, C. 2014. Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions. *European Journal of Agronomy,* 52**,** 259-270.

Jarvis, S., Hutchings, N., Brentrup, F., Olesen, J. E. & Van de Hoek, K. W. 2011. *Nitrogen flows in farming systems across Europe.* in: Sutton, Mark A., Howard, Clare M., Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., Grinsven, Hans van., Grizzetti, B. (Eds.) *The European Nitrogen Assessment* Cambridge university press. pp. 212.

Jin, X. B., Huang, J. Y. & Zhou, Y. K. 2012. Impact of coastal wetland cultivation on microbial biomass, ammonia-oxidizing bacteria, gross N transformation and N_2O and NO potential production. *Biology and Fertility of Soils,* 48**,** 363-369.

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**,** 417-426.

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**,** 801-805.

Jones, C. M., Stres, B., Rosenquist, M. & Hallin, S. 2008. Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Molecular Biology and Evolution,* 25**,** 1955-1966.

Kaiser, E. A., Kohrs, K., Kucke, M., Schnug, E., Heinemeyer, O. & Munch, J. C. 1998. Nitrous oxide release from arable soil: Importance of N-fertilization, crops and temporal variation. *Soil Biology & Biochemistry,* 30**,** 1553-1563.

Kelly-Gerreyn, B. A., Trimmer, M. & Hydes, D. J. 2001. A diagenetic model discriminating denitrification and dissimilatory nitrate reduction to ammonium in a temperate estuarine sediment. *Marine Ecology Progress Series,* 220**,** 33-46.

Kilian, S. & Werner, D. 1996. Enhanced denitrification in plots of N_2-fixing faba beans compared to plots of a non-fixing legume and non legumes. *Biology and Fertility of Soils,* 21**,** 77-83.

Kirk, G. J. D. & Kronzucker, H. J. 2005. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: A modelling study. *Annals of Botany,* 96**,** 639-646.

Knops, J. M. H., Bradley, K. L. & Wedin, D. A. 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters,* 5**,** 454-466.

Kobayashi, M., Matsuo, Y., Takimoto, A., Suzuki, S., Maruo, F. & Shoun, H. 1996. Denitrification, a novel type of respiratory metabolism in fungal mitochondrion. *Journal of Biological Chemistry,* 271**,** 16263-16267.

Kraft, B., Tegetmeyer, H. E., Sharma, R., Klotz, M. G., Ferdelman, T. G., Hettich, R. L., Geelhoed, J. S. & Strous, M. 2014. The environmental controls that govern the end product of bacterial nitrate respiration. *Science,* 345**,** 676-679.

Kuenen, J. G. 2008. Anammox bacteria: from discovery to application. *Nature Reviews Microbiology,* 6**,** 320-326.

Laan, P., Berrevoets, M. J., Lythe, S., Armstrong, W. & Blom, C. W. P. M. 1989. Root morphology and aerenchyma formation as indicators of the flood-tolerance of rumex species. *Journal of Ecology,* 77**,** 693-703.

Lam, P. & Kuypers, M. M. M. 2011. Microbial nitrogen cycling processes in oxygen minimum zones. *Annual Review of Marine Science, Vol 3,* 3**,** 317-345.

Larose, C., Dommergue, A. & Vogel, T. M. 2013. Microbial nitrogen cycling in Arctic snowpacks. *Environmental Research Letters,* 8.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., Prosser, J. I., Schuster, S. C. & Schleper, C. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature,* 442**,** 806-809.

Li, C. J., Li, Y. Y., Yu, C. B., Sun, J. H., Christie, P., An, M., Zhang, F. S. & Li, L. 2011. Crop nitrogen use and soil mineral nitrogen accumulation under different crop combinations and patterns of strip intercropping in northwest China. *Plant and Soil,* 342**,** 221-231.

Li, L., Tilman, D., Lambers, H. & Zhang, F. S. 2014. Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. *New Phytologist,* 203**,** 63-69.

Li, Y. F., Ran, W., Zhang, R. P., Sun, S. B. & Xu, G. H. 2009. Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. *Plant and Soil,* 315**,** 285-296.

Liu, B. B., Frostegard, A. & 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*. *Microbiology,* 5.

Logares, R., Haverkamp, T. H. A., Kumar, S., Lanzen, A., Nederbragt, A. J., Quince, C. & Kauserud, H. 2012. Environmental microbiology through the lens of high-throughput DNA sequencing: Synopsis of current platforms and bioinformatics approaches. *Journal of Microbiological Methods,* 91**,** 106-113.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., del Rio, T. G., Edgar, R. C., Eickhorst, T., Ley, R. E., Hugenholtz, P., Tringe, S. G. & Dangl, J. L. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature,* 488**,** 86-90.

Lyautey, E., Hallin, S., Teissier, S., Iribar, A., Compin, A., Philippot, L. & Garabetian, F. 2013. Abundance, activity and structure of denitrifier communities in phototrophic river biofilms (River Garonne, France). *Hydrobiologia,* 716**,** 177-187.

Maharjan, B., Venterea, R. T. & Rosen, C. 2014. Fertilizer and irrigation management effects on nitrous oxide emissions and nitrate leaching. *Agronomy Journal,* 106**,** 703-714.

Manevski, K., Borgesen, C. D., Andersen, M. N. & Kristensen, I. S. 2015. Reduced nitrogen leaching by intercropping maize with red fescue on sandy soils in North Europe: a combined field and modeling study. *Plant and Soil,* 388**,** 67-85.

Matzner, E. & Borken, W. 2008. Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science,* 59**,** 274-284.

Mckone, M. J. & Biesboer, D. D. 1986. Nitrogen fixation in association with the root systems of goldenrods (*Solidago L.*). *Soil Biology & Biochemistry,* 18**,** 543-545.

Melero, S., Perez-de-Mora, A., Murillo, J. M., Buegger, F., Kleinedam, K., Kublik, S., Vanderlinden, K., Moreno, F. & Schloter, M. 2011. Denitrification in a vertisol under long-term tillage and no-tillage management in dryland agricultural systems: Key genes and potential rates. *Applied Soil Ecology,* 47**,** 221-225.

Mergel, A., Kloos, K. & Bothe, H. 2001. Seasonal fluctuations in the population of denitrifying and N_2-fixing bacteria in an acid soil of a Norway spruce forest. *Plant and Soil,* 230**,** 145-160.

Mohan, S. B., Schmid, M., Jetten, M. & Cole, J. 2004. Detection and widespread distribution of the *nrfA* gene encoding nitrite reduction to ammonia, a short circuit in the biological nitrogen cycle that competes with denitrification. *FEMS Microbiology Ecology,* 49**,** 433-443.

Moorhead, K. K. & Reddy, K. R. 1988. Oxygen-transport through selected aquatic macrophytes. *Journal of Environmental Quality,* 17**,** 138-142.

Mueller, K. E., Hobbie, S. E., Tilman, D. & Reich, P. B. 2013. Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. *Global Change Biology,* 19**,** 1249-1261.

Mulder, A., Vandegraaf, A. A., Robertson, L. A. & Kuenen, J. G. 1995. Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor. *FEMS Microbiology Ecology,* 16**,** 177-183.

Mulvaney, R. L., Khan, S. A. & Mulvaney, C. S. 1997. Nitrogen fertilizers promote denitrification. *Biology and Fertility of Soils,* 24**,** 211-220.

Munro, P. E. 1966. Inhibition of nitrifiers by grass root extracts. *Journal of Applied Ecology,* 3**,** 231-238.

Müller, C., Kammann, C., Ottow, J. C. G. & Jager, H. J. 2003. Nitrous oxide emission from frozen grassland soil and during thawing periods. *Journal of Plant Nutrition and Soil Science,* 166**,** 46-53.

Niboyet, A., Barthes, L., Hungate, B. A., Le Roux, X., Bloor, J. M. G., Ambroise, A., Fontaine, S., Price, P. M. & Leadley, P. W. 2010. Responses of soil

nitrogen cycling to the interactive effects of elevated CO_2 and inorganic N supply. *Plant and Soil,* 327**,** 35-47.
Nicol, G. W. & Schleper, C. 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology,* 14**,** 207-212.
Nie, S. W., Eneji, A. E., Chen, Y. Q., Sui, P., Huang, J. X. & Huang, S. M. 2012. Nitrate leaching from mMaize intercropping systems with N fertilizer over-dose. *Journal of Integrative Agriculture,* 11**,** 1555-1565.
Nijburg, J. W. & Laanbroek, H. J. 1997. The influence of *Glyceria* maxima and nitrate input on the composition and nitrate metabolism of the dissimilatory nitrate-reducing bacterial community. *FEMS Microbiology Ecology,* 22**,** 57-63.
Niklaus, P. A., Wardle, D. A. & Tate, K. R. 2006. Effects of plant species diversity and composition on nitrogen cycling and the trace gas balance of soils. *Plant and Soil,* 282**,** 83-98.
Nogaro, G. & Burgin, A. J. 2014. Influence of bioturbation on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in freshwater sediments. *Biogeochemistry,* 120**,** 279-294.
Obia, A., Cornelissen, G., Mulder, J. & Dorsch, P. 2015. Effect of Soil pH Increase by Biochar on NO, N2O and N-2 Production during Denitrification in Acid Soils. *Plos One,* 10.
Odlare, M., Abubaker, J., Lindmark, J., Pell, M., Thorin, E. & Nehrenheim, E. 2012. Emissions of N_2O and CH_4 from agricultural soils amended with two types of biogas residues. *Biomass & Bioenergy,* 44**,** 112-116.
Odlare, M., Arthurson, V., Pell, M., Svensson, K., Nehrenheim, E. & Abubaker, J. 2011. Land application of organic waste - Effects on the soil ecosystem. *Applied Energy,* 88**,** 2210-2218.
Osanai, Y., Flittner, A., Janes, J. K., Theobald, P., Pendall, E., Newton, P. C. D. & Hovenden, M. J. 2012. Decomposition and nitrogen transformation rates in a temperate grassland vary among co-occurring plant species. *Plant and Soil,* 350**,** 365-378.
Oyaizumasuchi, Y. & Komagata, K. 1988. Isolation of free-living nitrogen-fixing bacteria from the rhizosphere of rice. *Journal of General and Applied Microbiology,* 34**,** 127-164.
Pappa, V. A., Rees, R. M., Walker, R. L., Baddeley, J. A. & Watson, C. A. 2011. Nitrous oxide emissions and nitrate leaching in an arable rotation resulting from the presence of an intercrop. *Agriculture Ecosystems & Environment,* 141**,** 153-161.
Patra, A. K., Abbadie, L., Clays-Josserand, A., Degrange, V., Grayston, S. J., Guillaumaud, N., Loiseau, P., Louault, F., Mahmood, S., Nazaret, S., Philippot, L., Poly, F., Prosser, J. I. & Le Roux, X. 2006. Effects of management regime and plant species on the enzyme activity and genetic structure of N-fixing, denitrifying and nitrifying bacterial communities in grassland soils. *Environmental Microbiology,* 8**,** 1005-1016.
Penton, C. R., Johnson, T. A., Quensen, J. F., Iwai, S., Cole, J. R. & Tiedje, J. M. 2013. Functional genes to assess nitrogen cycling and aromatic hydrocarbon degradation: primers and processing matter. *Frontiers in Microbiology,* 4.

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**,** 993-1008.

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**,** 1497-1504.

Philippot, L., Hallin, S., Borjesson, G. & Baggs, E. M. 2009. Biochemical cycling in the rhizosphere having an impact on global change. *Plant and Soil,* 321**,** 61-81.

Philippot, L., Hallin, S. & Schloter, M. 2007. Ecology of denitrifying prokaryotes in agricultural soil. *Advances in Agronomy, Vol 96,* 96**,** 249-305.

Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S. & Germon, J. C. 2002. Molecular analysis of the nitrate-reducing community from unplanted and maize-planted soils. *Applied and Environmental Microbiology,* 68**,** 6121-6128.

Philippot, L., Raaijmakers, J. M., Lemanceau, P. & van der Putten, W. H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology,* 11**,** 789-799.

Poly, F., Monrozier, L. J. & Bally, R. 2001. Improvement in the RFLP procedure for studying the diversity of nifH genes in communities of nitrogen fixers in soil. *Research in Microbiology,* 152**,** 95-103.

Porubsky, W. P., Weston, N. B. & Joye, S. B. 2009. Benthic metabolism and the fate of dissolved inorganic nitrogen in intertidal sediments. *Estuarine Coastal and Shelf Science,* 83**,** 392-402.

Prade, K. & Trolldenier, G. 1988. Effect of wheat roots on denitrification at varying soil air-filled porosity and organic-carbon content. *Biology and Fertility of Soils,* 7**,** 1-6.

Quispel, A. 1988. Bacteria-plant interactions in symbiotic nitrogen fixation. *Physiologia Plantarum,* 74**,** 783-790.

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

Reay, D. S., Davidson, E. A., Smith, K. A., Smith, P., Melillo, J. M., Dentener, F. & Crutzen, P. J. 2012. Global agriculture and nitrous oxide emissions. *Nature Climate Change,* 2**,** 410-416.

Reddy, K. R., Patrick, W. H. & Lindau, C. W. 1989. Nitrification-denitrification at the plant root - sediment interface in wetlands. *Limnology and Oceanography,* 34**,** 1004-1013.

Rice, W. A., Paul, E. A. & Wetter, L. R. 1967. The role of anaerobiosis in asymbiotic nitrogen fixation. *Can J Microbiol,* 13**,** 829-36.

Risch, S. J. 1983. Intercropping as Cultural Pest-Control - Prospects an Limitations. *Environmental Management,* 7**,** 9-14.

Romero, I. C., Jacobson, M., Fuhrman, J. A., Fogel, M. & Capone, D. G. 2012. Long-term nitrogen and phosphorus fertilization effects on N_2 fixation

rates and *nifH* gene community patterns in mangrove sediments. *Marine Ecology-an Evolutionary Perspective,* 33**,** 117-127.
Rotthauwe, J. H., Witzel, K. P. & Liesack, W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology,* 63**,** 4704-4712.
Rütting, T., Boeckx, P., Muller, C. & Klemedtsson, L. 2011. Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences,* 8**,** 1779-1791.
Sanchez-Martin, L., Sanz-Cobena, A., Meijide, A., Quemada, M. & Vallejo, A. 2010. The importance of the fallow period for N_2O and CH_4 fluxes and nitrate leaching in a Mediterranean irrigated agroecosystem. *European Journal of Soil Science,* 61**,** 710-720.
Sanford, R. A., Wagner, D. D., Wu, Q. Z., 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. & Loffler, 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**,** 19709-19714.
Scala, D. J. & Kerkhof, L. J. 1999. Diversity of nitrous oxide reductase (*nosZ*) genes in continental shelf sediments. *Applied and Environmental Microbiology,* 65**,** 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 & Biochemistry,* 43**,** 1607-1611.
Schroder, J. J., Assinck, F. B. T., Uenk, D. & Velthof, G. L. 2010. Nitrate leaching from cut grassland as affected by the substitution of slurry with nitrogen mineral fertilizer on two soil types. *Grass and Forage Science,* 65**,** 49-57.
Schultze, M. & Kondorosi, A. 1998. Regulation of symbiotic root nodule development. *Annual Review of Genetics,* 32**,** 33-57.
Shen, J. B., Li, C. J., Mi, G. H., Li, L., Yuan, L. X., Jiang, R. F. & Zhang, F. S. 2013. Maximizing root/rhizosphere efficiency to improve crop productivity and nutrient use efficiency in intensive agriculture of China. *Journal of Experimental Botany,* 64**,** 1181-1192.
Shoun, H. 1992. Fungal denitrification and cytochrome-P-450. *Nippon Nogeikagaku Kaishi-Journal of the Japan Society for Bioscience Biotechnology and Agrochemistry,* 66**,** 154-157.
Shu, D. T., He, Y. L., Yue, H. & Wang, Q. Y. 2016. Metagenomic and quantitative insights into microbial communities and functional genes of nitrogen and iron cycling in twelve wastewater treatment systems. *Chemical Engineering Journal,* 290**,** 21-30.
Silver, W. L., Herman, D. J. & Firestone, M. K. 2001. Dissimilatory nitrate reduction to ammonium in upland tropical forest soils. *Ecology,* 82**,** 2410-2416.
Silver, W. L., Thompson, A. W., Reich, A., Ewel, J. J. & Firestone, M. K. 2005. Nitrogen cycling in tropical plantation forests: Potential controls on nitrogen retention. *Ecological Applications,* 15**,** 1604-1614.

Simon, J. 2002. Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiology Reviews,* 26**,** 285-309.
Singh, B. K., Munro, S., Potts, J. M. & Millard, P. 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology,* 36**,** 147-155.
Sipila, T. P., Yrjala, K., Alakukku, L. & Palojarvi, A. 2012. Cross-site soil microbial communities under tillage regimes: fungistasis and microbial biomarkers. *Applied and Environmental Microbiology,* 78**,** 8191-8201.
Skiba, U., Smith, K.A. 2000. The control of nitrous oxide emissions from agricultural and natural soils. *Chemosphere-Glabal change science,* 2**,** 7.
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**,** 460.
Stern, W. R. 1993. Nitrogen fixation and transfer in intercrop systems. *Field Crops Research,* 34**,** 335-356.
Strous, M., Fuerst, J. A., Kramer, E. H. M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K. T., Webb, R., Kuenen, J. G. & Jetten, M. S. M. 1999. Missing lithotroph identified as new planctomycete. *Nature,* 400**,** 446-449.
Subbarao, G. V., Ishikawa, T., Ito, O., Nakahara, K., Wang, H. Y. & Berry, W. L. 2006. A biolumiuescence assay to detect nitrification inhibitors released from plant roots: a case study with *Brachiaria humidicola*. *Plant and Soil,* 288**,** 101-112.
Subbarao, G. V., Nakahara, K., Hurtado, M. P., Ono, H., Moreta, D. E., Salcedo, A. F., Yoshihashi, A. T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I. M., Lascano, C. E., Berry, W. L. & Ito, O. 2009. Evidence for biological nitrification inhibition in Brachiaria pastures. *Proceedings of the National Academy of Sciences of the United States of America,* 106**,** 17302-17307.
Subbarao, G. V., Sahrawat, K. L., Nakahara, K., Rao, I. M., Ishitani, M., Hash, C. T., Kishii, M., Bonnett, D. G., Berry, W. L. & Lata, J. C. 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). *Annals of Botany,* 112**,** 297-316.
Subbarao, G. V., Yoshihashi, T., Worthington, M., Nakahara, K., Ando, Y., Sahrawat, K. L., Rao, I. M., Lata, J. C., Kishii, M. & Braun, H. J. 2015. Suppression of soil nitrification by plants. *Plant Science,* 233**,** 155-164.
Sun, H. Y., Zhang, C. B., Song, C. B., Chang, S. X., Gu, B. J., Chen, Z. X., Peng, C. H., Chang, J. & Ge, Y. 2013. The effects of plant diversity on nitrous oxide emissions in hydroponic microcosms. *Atmospheric Environment,* 77**,** 544-547.
Sutton, M. A., Howard, C. M., Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., Grinsven, H. v. & Grizzetti, B. (Eds.) 2011. *The European nitrogen assessment : sources, effects, and policy perspectives,* Cambridge, UK ; New York, Cambridge University Press.
Sylvesterbradley, R., Mosquera, D. & Mendez, J. E. 1988. Inhibition of nitrate accumulation in tropical grassland soils - effect of nitrogen-fertilization and soil disturbance. *Journal of Soil Science,* 39**,** 407-416.

Thomas, T., Gilbert, J. & Meyer, F. 2012. Metagenomics - a guide from sampling to data analysis. *Microbial Informatics and Experimentation,* 2**,** 3.

Throbäck, I. N. 2004. Reassessing PCR primers targeting *nirS, nirK and nosZ* genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiology Ecology,* 49**,** 17.

Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E. & McDaniel, M. D. 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters,* 18**,** 761-771.

Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R. & Polasky, S. 2002. Agricultural sustainability and intensive production practices. *Nature,* 418**,** 671-677.

Tourna, M., Freitag, T. E., Nicol, G. W. & Prosser, J. I. 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. *Environmental Microbiology,* 10**,** 1357-1364.

Trias, R., Ruiz-Rueda, O., Garcia-Lledo, A., Vilar-Sanz, A., Lopez-Flores, R., Quintana, X. D., Hallin, S. & Baneras, L. 2012. Emergent macrophytes act selectively on ammonia-oxidizing bacteria and aArchaea. *Applied and Environmental Microbiology,* 78**,** 6352-6356.

Tsiknia, M., Tzanakakis, V. A. & Paranychianakis, N. V. 2013. Insights on the role of vegetation on nitrogen cycling in effluent irrigated lands. *Applied Soil Ecology,* 64**,** 104-111.

Walker, T. S., Bais, H. P., Grotewold, E. & Vivanco, J. M. 2003. Root exudation and rhizosphere biology. *Plant Physiology,* 132**,** 44-51.

Van den Heuvel, R. N., Bakker, S. E., Jetten, M. S. M. & Hefting, M. M. 2011. Decreased N_2O reduction by low soil pH causes high N_2O emissions in a riparian ecosystem. *Geobiology,* 9**,** 294-300.

van Kessel, M. A. H. J., Speth, D. R., Albertsen, M., Nielsen, P. H., Op den Camp, H. J. M., Kartal, B., Jetten, M. S. M. & Lucker, S. 2015. Complete nitrification by a single microorganism. *Nature,* 528**,** 555-559.

Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S. & Senoo, K. 2015. Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. *ISME Journal,* 9**,** 1954-65.

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

Venterea, R. T., Hyatt, C. R. & Rosen, C. J. 2011. Fertilizer management effects on nitrate leaching and indirect nitrous oxide emissions in irrigated potato production. *Journal of Environmental Quality,* 40**,** 1103-1112.

Wessen, E., Nyberg, K., Jansson, J. K. & Hallin, S. 2010. Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Applied Soil Ecology,* 45**,** 193-200.

Wessén, E., Soderstrom, M., Stenberg, M., Bru, D., Hellman, M., Welsh, A., Thomsen, F., Klemedtson, L., Philippot, L. & Hallin, S. 2011. Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. *ISME Journal,* 5**,** 1213-1225.

Whitmore, A. P. & Schroder, J. J. 2007. Intercropping reduces nitrate leaching from under field crops without loss of yield: A modelling study. *European Journal of Agronomy,* 27**,** 81-88.

Winogradsky, S. 1890. Recherches sur les organismes de la nitrification. *Ann. Inst.Pasteur (Paris),* 4**,** 18.

Vonberg, K. H. L. & Bothe, H. 1992. The distribution of denitrifying bacteria in soils monitored by DNA-probing. *FEMS Microbiology Ecology,* 86**,** 331-340.

Xi, D., Bai, R., Zhang, L. & Fang, Y. 2016. Contribution of anammox to nitrogen removal in two temperate forest soils. *Applied and Environmental Microbiology,* 82**,** 11.

Xue, K., Wu, L. Y., Deng, Y., He, Z. L., Van Nostrand, J., Robertson, P. G., Schmidt, T. M. & Zhou, J. Z. 2013. Functional Gene Differences in Soil Microbial Communities from Conventional, Low-Input, and Organic Farmlands. *Applied and Environmental Microbiology,* 79**,** 1284-1292.

Xu, H. J., Wang, X. H., Li, H., Yao, H. Y., Su, J. Q. & Zhu, Y. G. 2014. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. *Environmental Science & Technology,* 48**,** 9391-9399.

Yin, C., Fan, F. L., Song, A. L., Cui, P. Y., Li, T. Q. & Liang, Y. C. 2015. Denitrification potential under different fertilization regimes is closely coupled with changes in the denitrifying community in a black soil. *Applied Microbiology and Biotechnology,* 99**,** 5719-5729.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D. & Tilman, D. 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? *Ecology,* 84**,** 2042-2050.

Zechmeister-Boltenstern, S., Michel, K. & Pfeffer, M. 2011. Soil microbial community structure in European forests in relation to forest type and atmospheric nitrogen deposition. *Plant and Soil,* 343**,** 37-50.

Zhang, N. N., Sun, Y. M., Wang, E. T., Yang, J. S., Yuan, H. L. & Scow, K. M. 2015a. Effects of intercropping and Rhizobial inoculation on the ammonia-oxidizing microorganisms in rhizospheres of maize and faba bean plants. *Applied Soil Ecology,* 85**,** 76-85.

Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P. & Shen, Y. 2015b. Managing nitrogen for sustainable development. *Nature,* 528, 51-59.

Zhang, X. M., Liu, W., Schloter, M., Zhang, G. M., Chen, Q. S., Huang, J. H., Li, L. H., Elser, J. J. & Han, X. G. 2013. Response of the abundance of key soil microbial nitrogen-cycling genes to multi-factorial global changes. *PLoS One,* 8.

Zhu, G. B., Wang, S. Y., Feng, X. J., Fan, G. N., Jetten, M. S. M. & Yin, C. Q. 2011. Anammox bacterial abundance, biodiversity and activity in a constructed wetland. *Environmental Science & Technology,* 45**,** 9951-9958.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews,* 61**,** 533.

Zumft, W. G. & Korner, H. 1997. Enzyme diversity and mosaic gene organization in denitrification. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology,* 71**,** 43-58.
Zumft, W. G. & Kroneck, P. M. H. 2007. Respiratory transformation of nitrous oxide (N_2O) to dinitrogen by bacteria and archaea. *Advances in Microbial Physiology,* 52**,** 107-225.

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