PH.D. THESIS

Nitrous Oxide Production in Agricultural Soil

Linking Biogeochemical Pathways and Drivers

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One planet, one experiment.

E. O. Wilson

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Cover photo: Agricultural field experiment in Lanna, Sweden (Philipp Schleusner)

Abstract

Nitrous oxide (N_2O) is a long-lasting and potent greenhouse gas responsible for depletion of stratospheric ozone. As the atmospheric N_2O concentration reaches all-time highs, emission variability in space and time still leaves unresolved questions. The aim of this thesis is to improve our understanding of the origin of N_2O and its main drivers from the largest anthropogenic source: agricultural soil. Therefore, we investigated agricultural soil from long-term trial field sites in the laboratory and used ^{15}N-enriched tracers in two main approaches: partitioning of the sources of N_2O production and quantification of the gross rates of microbial processes competing for ammonium (NH_4^+) and nitrate (NO_3^-).

The varying relative contribution of NH_4^+, NO_3^- and organic nitrogen (N_{org}) to N_2O emission highlights the influence of site-specific factors apart from the field management. Without fertilizer, N_{org} was the dominant N_2O source related to high carbon (C) contents and C:N ratios. High N_2O emissions were caused by increasing contributions of nitrification and denitrification, which was drastically stimulated by mineral nitrogen (N) fertilizer. In addition, N fertilizer application more than doubled N_2O production from native non-fertilizer N compounds, which provides evidence for primed N_2O production. By using the *Ntrace* model, we quantified gross rates of N cycle processes that compete for substrates and regulate N_2O production. In the long term, cropping systems can shift the balance between denitrification and dissimilatory nitrate reduction to ammonium (DNRA), which determines the fate of NO_3^- in soil. A perennial cropping system that maintains high SOM contents and C/NO_3^- ratios has shaped the microbial community of dissimilatory nitrate reducers leading to higher N retention by DNRA and lower N_2O emissions. By applying selective inhibitors, we were able to quantify the specific activity of archaeal and bacterial nitrifiers competing for NH_4^+. While both can coexist and be equally active in agricultural soil with low N supply, bacteria outcompeted archaea with increasing NH_4^+ concentration, which can be responsible for higher N_2O emissions as well.

This thesis illustrates how human action drives N_2O emission from agricultural soil in a variety of ways since field management affects N cycle processes in the short- and long-term. While N fertilizer application strongly stimulates N_2O production from added- and native N sources, long-term field management can change the soil properties, which shifts the abundance of microbial communities and thereby alters the N cycle processes responsible for N_2O production.

Sammanfattning

Lustgas (N_2O) är en långvarig och stark växthusgas som är orsak till nedbrytning av stratosfäriskt ozon. Det finns fortfarande olösta problem med hänsyn till den höga variabiliteten i N_2O-utsläppen och den ökande N_2O-koncentrationen i atmosfären. Syftet med avhandlingen är att förbättra vår förståelse av N_2O:s ursprung och dess drivande faktorer från jordbruksmark, som är den största antropogena källan. Därför undersökte vi jord från långsiktiga fältförsök från jordbruksmark i laboratoriet och använde ^{15}N-berikat kväve (N) i två metoder: kvantifiering av källorna för N_2O-produktion och kvantifiering av bruttohastigheterna av mikrobiella processer som konkurrerar om ammonium (NH_4^+) och nitrat (NO_3^-).

Förutom markskötseln visar det varierande relativa bidraget av NH_4^+, NO_3^- och organiskt kväve (N_{org}) till N_2O-utsläpp på inflytandet av miljön. Oxidation av organiskt N dominerade N_2O-produktionen utan gödsling och var relaterad till höga kol(C)-innehåll och C:N-förhållanden. Nitrifikation och denitrifikation stimulerades drastiskt av mineralgödsling, vilket ledde till höga N_2O-utsläpp. I samband med detta fördubblade mineralgödslingen N_2O-produktionen från naturliga (icke-gödslade) N-föreningar, vilket ger bevis för *priming* av N_2O-produktion. Detta anses som en korttidseffekt. På lång sikt kan skördesystemet flytta balansen mellan denitrifikation och dissimilatorisk nitratreduktion till ammonium (DNRA), som bestämmer riktningen för NO_3^- i marken. En jordbruksmark som har högt SOM-innehåll och C/NO_3^- förhållande kan bilda ett mikrobiellt samhälle av nitratreducerare som leder till bevarande av N i marken och lägre N_2O-utsläpp. Dessutom kunde vi med hjälp av selektiva inhibitorer kvantifiera den specifika aktiviteten av nitrificerande arkéer och bakterier som konkurrerar om NH_4^+. Enligt tidigare studier producerar arkéer mindre lustgas än bakterier. Våra resultat visar att bägge organismgrupperna kan samexistera och vara lika aktiva i jordbruksmark med lågt N tillsättning, dock dominerar bakterier över arkéer när NH_4^+-koncentrationen blir högre.

Avhandlingen visar hur mänsklig aktivitet driver N_2O-utsläpp från jordbruksmark på olika sätt, eftersom markskötseln påverkar N-cykelprocesserna på kort och lång sikt. Medan N-gödselanvändningen stimulerar N_2O-produktion från tillförda och naturliga N-källor, så kan långsiktig markskötsel förändra jordens egenskaper och därmed deras mikrobiella samhällen. Detta i sin tur förändrar N-cykelprocesserna som är ansvariga för N_2O-produktionen.

Contents

List of papers

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

I. Schleusner P., Rütting T. Partitioning of nitrous oxide production pathways in agricultural soils. Under review in Geoderma.

II. Putz M.*, Schleusner P.*, Rütting T., Hallin S. Relative abundance of denitrifying and DNRA bacteria and their activity determine nitrogen retention or loss in agricultural soil (*equal contribution). Accepted with minor revision in Soil Biology and Biochemistry.

III. Schleusner P., Lammirato C., Tierling J., Lebender U., Rütting T. Primed N_2O emission from native soil nitrogen: a ^{15}N-tracing laboratory experiment. Accepted with minor revision in Journal of Plant Nutrition and Soil Science.

IV. Schleusner P., Rütting T. Substrate availability determines relative contribution of archaea and bacteria for gross ammonia oxidation. Manuscript.

Background and introduction

The nitrogen problem

Nitrogen (N) is an element essential for life on earth. Naturally, the bioavailable reactive N is supplied and cycled in soil, where it occurs as ammonium (NH_4^+), nitrate (NO_3^-), amino acids as a part of organic N (N_{org}) and in many other forms. Since the beginning of the 20th century, the introduction of artificial N fertilizers has facilitated intensive agriculture and greatly increased the availability of reactive N in agricultural soil due to industrial ammonia production via the Haber-Bosch process (Sutton *et al.*, 2011). In consequence, the increased food production has sustained a rapid growth of the human population in the 20th century.

However, the increasing concentration of reactive N in soil triggers a cascade, in which the same N atom has multiple effects in the Earth system with ultimately harmful consequences for the environment and human health (Galloway *et al.*, 2003). These include soil contamination, biodiversity loss of ecosystems (Aber *et al.*, 1995; Vitousek *et al.*, 1997), eutrophication and dead zones in aquatic environments (Howarth *et al.*, 2000) and atmospheric pollution (Cowling *et al.*, 1998). An adverse effect of global dimension is the emission of the greenhouse gas nitrous oxide (N_2O), which has also been described as the ‘forgotten’ greenhouse gas for receiving less attention than others (Thomson *et al.*, 2012). The harmful nature of N_2O is induced by a 265-fold global warming potential compared to carbon dioxide (CO_2) despite its lower atmospheric concentration (IPCC, 2013). In addition, N_2O is a long-lived greenhouse gas that stays in the atmosphere for ~114 years and eventually reaches the stratosphere as a main sink, where it is responsible for catalytic depletion of the ozone layer (Ravishankara *et al.*, 2009). The atmospheric N_2O concentration has increased since the beginning of the 20th century and continues to do so to this day (Fig. 1).

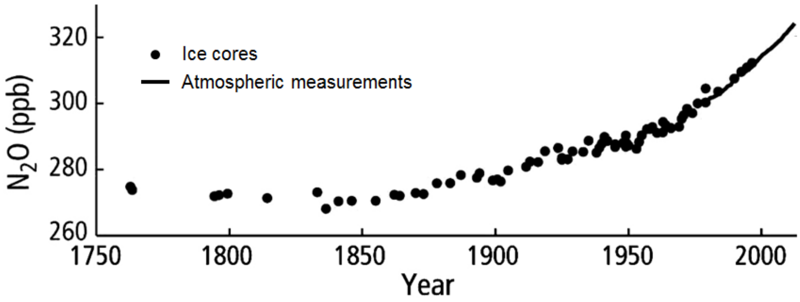

Fig. 1. Development of N_2O concentration in the earth atmosphere for the past 250 years (IPCC, 2013)

Microbial production and consumption of N_2O

Nitrous oxide emission from agricultural soil has been difficult to assess because of its high variability in space and time (Butterbach-Bahl *et al.*, 2013) and the different regulation of its underlying processes. Soil microorganisms are the main agents forming N_2O as an end- or byproduct when metabolizing reactive N compounds in a variety of red/ox reactions (Fig. 2). In general, the largest share of global N_2O production is ascribed to nitrification and denitrification (Butterbach-Bahl *et al.*, 2013), which can be further divided into different pathways.

Denitrification as a heterotrophic pathway is observed in bacteria, archaea (Philippot, 2002) and eukaryota, such as fungi (Shoun *et al.*, 1992) and benthic foraminifera (Risgaard-Petersen *et al.*, 2006), and comprises the stepwise reduction of NO_3^- to atmospheric dinitrogen (N_2) with N_2O as an important intermediate (Tiedje *et al.*, 1983). As a form of respiration, denitrifiers are capable of using oxidized N compounds as an electron acceptor under anaerobic conditions. It is a modular pathway, which means that many denitrifiers only perform certain parts of it (Graf *et al.*, 2014). Apart from being a major source, denitrification is also the only known microbial N_2O sink by reducing it to N_2 as the final step. Whether denitrification acts as a sink or source of N_2O varies because the responsible enzyme N_2O reductase (*nosZ*) can be regulated by acidity (Nömmik, 1956; Bergaust *et al.*, 2010), availability of the copper-containing coenzyme (Richardson *et al.*, 2009) and the composition of the denitrifying microbial community (Graf *et al.*, 2014). Co-denitrification is a process dominated by fungi that uses one of the two required nitric oxide molecules from another source such as from organic compounds (Laughlin and Stevens, 2002). This process has been identified as an important N_2O source (Spott and Stange, 2011) dominating over regular denitrification in grazed grasslands that regularly receive cattle manure (Selbie *et al.*, 2015). In direct competition to denitrification stands dissimilatory nitrate reduction to ammonium (DNRA), which may produce small amounts of N_2O (Rütting *et al.*, 2011).

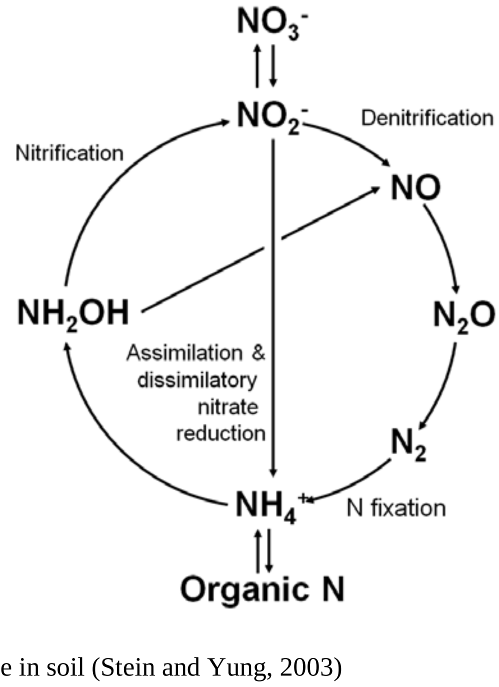

Fig. 2. The N cycle in soil (Stein and Yung, 2003)

Besides heterotrophic denitrification, autotrophic nitrification has been identified as a major N_2O production pathway (Stein and Yung, 2003). Nitrification is a two-step process comprising ammonia oxidation to nitrite and nitrite oxidation to NO_3^-. In the presence of oxygen and NH_4^+, ammonia oxidizers produce N_2O in different ways: hydroxylamine oxidation, nitrifier denitrification or a hybrid formation including both (Stieglmeier *et al.*, 2014). Nitrifier denitrification is performed by autotrophic nitrifiers under aerobic conditions possibly for detoxification (Wrage *et al.*, 2001). In addition, nitrite oxidation as the second part of nitrification provides the substrate (NO_3^-) for denitrification in different heterotrophic organisms. Evidence suggests that nitrification can be heterotrophic as well, which is performed by bacteria and fungi that use organic carbon as their primary source of energy (Papen *et al.*, 1989). Heterotrophic nitrifiers can dominate production of NO_3^- (Huygens *et al.*, 2008) and N_2O (Rütting *et al.*, 2010) and are frequently linked to acidic forest soils with high C/N ratios (Zhang *et al.*, 2015);

Table 1. Overview of microbial N_2O production processes in soil and important regulating factors

N cycle process	Microorganisms	Known environmental regulators
Heterotrophic denitrification*	Bacteria, archaea & eukaryota including fungi	Mineral N fertilizer Moisture (>60 % WFPS) (Stange *et al.*, 2013)
Dissimilatory nitrate reduction to ammonium (DNRA)	Bacteria	Natural N-limited soils (Rütting *et al.*, 2011) High C:NO_3^- ratio (van den Berg *et al.*, 2016)
Autotrophic nitrification (ammonia oxidation)	Bacteria & archaea	Mineral fertilizer/substrate availability Moisture (<65 % WFPS) (Stange *et al.*, 2013) Acidity inhibition of bacteria (Weber and Gainey, 1962)
Nitrifier-denitrification	Autotrophic nitrifiers	Aerobic conditions Nitrite accumulation under high pH (Tierling and Kuhlmann, 2018)
Co-denitrification	Bacteria & fungi	Organic matter (Spott and Stange, 2011) Manure application (Selbie *et al.*, 2015)
Heterotrophic nitrification	Bacteria & fungi	Acidity & forest soils (Zhang *et al.*, 2015)

* the N_2O sink capacity of denitrification depends on further factors as mentioned in the text.

Microbial competition for mineral N

Different microbial communities can utilize the same resources as substrate, which may be a key factor in regulating N_2O production. Therefore, it is crucial to understand what drives N cycling communities competing for the two major N_2O sources: NO_3^- and NH_4^+.

Dissimilatory processes competing for NO_3^-

Nitrate can be utilized by microorganisms as an electron acceptor in two different respiratory processes: denitrification and dissimilatory nitrate reduction to ammonium (DNRA). These two processes lead to contrasting consequences for the agroecosystem and the environment. While denitrification is the main pathway of N loss to the atmosphere and a major source of N_2O in agricultural soil, DNRA results in N retention by producing the less mobile cation NH_4^+ but only marginal amounts of N_2O (Rütting *et al.*, 2011; Stremińska *et al.*, 2012).

Denitrification, N_2O reduction and DNRA are performed by different microbial communities (Table 1), which may not necessarily be related to one another. Phylogenetic markers are therefore less reliable than the involved enzyme-encoding genes when estimating the abundance of the potentially active microorganisms (Braker *et al.*, 1998; Hallin and Lindgren, 1999). Denitrification is a modular pathway (Zumft, 1997) and typically studied genes are nitrite reductase (*nirK* & *nirS*) and nitrous oxide reductase (*nosZI* & *nosZII*), whereas the formate-dependent nitrite reductase (*nrfA*) characterizes DNRA bacteria. Evidence suggests that the relative abundance of these different genotypes in the soil community can affect the net N_2O production (Philippot *et al.*, 2011; Domeignoz Horta *et al.*, 2016).

Regarding the fate of NO_3^-, it has been suggested that the importance of DNRA increases relative to denitrification in soil with higher C/N ratios, or more specifically a high availability of electron donor relative to the electron acceptor NO_3^- (Tiedje, 1988). This has been confirmed for environments displaying such characteristics *e.g.* permanent grasslands (Rütting *et al.*, 2011), wetlands and aquatic sediments (Fernandes *et al.*, 2012; Song *et al.*, 2014). However, little is known about the environmental regulation of the competing dissimilatory NO_3^- reduction pathways in agricultural soils. In agroecosystems, long-term maintenance or increase of soil organic matter (SOM), as a mean to control the C/NO_3^- ratio, can be achieved by organic amendments as well as rotations including perennial crops (Post and Kwon, 2000; Deng *et al.*, 2014), with the latter systems typically also emitting less N_2O (Abalos *et al.*, 2016; Gelfand *et al.*, 2016).

Oxidation of NH_4^+ by bacteria and archaea

Oxidation of NH_4^+ (the protonated form of ammonia) to nitrite is a dissimilatory process performed by bacteria (AOB) and archaea (AOA). In a variety of soils, AOA are more abundant than AOB (Leininger *et al.*, 2006) and particularly environments with low NH_4^+ availability tend to favor AOA based on abundance data of marker genes (Hatzenpichler, 2012). However, microbial abundance does not necessarily mirror activity and evidence suggests that bacteria can dominate ammonia oxidation in agricultural soil despite being outnumbered by AOA (Jia and Conrad, 2009; Sterngren *et al.*, 2015). A precise distinction of AOA and AOB activity has become possible only recently after introducing the bacterial inhibitor 1-octyne (Taylor *et al.*, 2013), which confirmed significantly lower N_2O/NO_3^- product ratio of AOA compared to AOB (Hink *et al.*, 2016).

When it comes to quantification of gross rates of NH_4^+ oxidation, there have been widespread methodical limitations due to biased techniques. This includes net nitrification rates that only resemble a small fraction of the gross rate (Davidson *et al.*, 1992) and potential nitrification, which highly disturbs the soil environment (Prosser and Nicol, 2012). The existing findings suggest that substrate sources and availability allows for niche partitioning between AOA- and AOB activity by favoring AOB in fertilized soil and AOA when NH_4^+ is scarce *e.g.* when derived from mineralization of native organic N (N_{org}). This can be tested by conducting a laboratory incubation experiment that combines the quantification of gross NH_4^+ oxidation rates and selective inhibition of AOA and AOB.

Objectives

Thanks to research for decades, we have overcome important methodical limitations and gained a thorough understanding of the N cycle processes behind N_2O production. However, the high N_2O emission variability in space and time still leave unresolved questions as atmospheric concentration reaches all-time highs. This work is motivated to improve our understanding of the origin of N_2O and its main drivers from the largest anthropogenic source: agricultural soil.

The objectives are:

i. Quantifying the relative contribution of the three main N_2O sources in agricultural soil: NH_4^+, NO_3^- and N_{org} (paper I) in order to address their main environmental drivers and the influence of long-term field management; and to test the contribution of native soil N to N_2O emission after mineral N fertilizer application in soil with different organic matter content (paper III)

ii. Quantifying the gross rates of microbial N cycle processes competing for NH_4^+ and NO_3^- by comparing the abundance and activity between DNRA- and denitrifying bacteria in different agricultural systems (paper II) and to quantify the contribution of AOA and AOB to ammonia oxidation under different substrate availabilities (paper IV)

Field sites and methods

Sampling and soil properties

In order to study the sources of N_2O and its underlying pathways in agricultural soil, top-soils were sampled from long-term field trials (Fig. 3). The field samplings took place from 2013 to 2016 between late autumn and early spring (*i.e.* after harvest and before fertilizer application). The soils were sieved immediately after sampling to remove coarse particles and roots and stored until further processing at 4 °C in the dark or frozen at -20 °C when used for microbial analysis. Soil characteristics were analyzed in preparation for the experiments, including gravimetric water content (GWC), C, N, C:N ratio, pH and mineral N content.

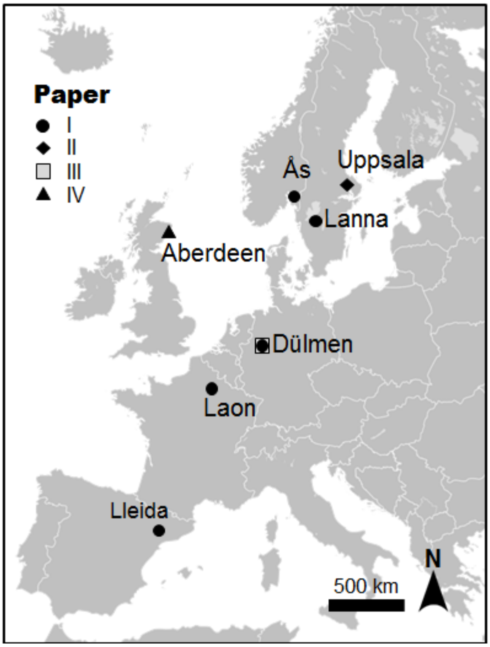

Fig. 3. Overview of the studied field sites in the respective papers

European transect (paper I)

The variability of three main source pools of N_2O production was studied on agricultural soil from five different European trial field sites in Ås (Norway), Lanna (Sweden), Dülmen (Germany), Laon (France) and Lleida (Spain), which constitutes a north-south transect. From each field site, we sampled conventionally N-fertilized crop soils (N+) and nearby unfertilized (N-) soils (cropped or permanent grassland, PG). At Laon, soil was additionally sampled from two bioenergy plant fields: *Panicum virgatum* (Swi) and *Miscanthus* (Mi).

Uppsala, Sweden (paper II)

In a set of agricultural soils, different substrate availability (SOM & NO_3^-) for denitrifiers and DNRA bacteria was met by sampling soil from two adjacent but differently managed long-term field experiments in Uppsala, Sweden (59°49'N 17°42'E) including an annual cereal rotation and a short-term ley rotation (Fig. 4). In addition, both crop managements were treated and sampled without and with mineral N application (120 kg N ha^{-1} $year^{-1}$ in the annual cereal and 150 kg N ha^{-1} $year^{-1}$ in the ley). The ley rotation is characterized by a four-year cycle starting with barley, under-sown grass and a red-clover mixture followed by three years of ley.

Dülmen, Germany (paper III)

We sampled a loamy sand soil from a 59-year old field experiment Yara, Germany (51°50’N, 7°15‘E). Similar to the Uppsala field site, the goal was to compare two soils with different SOM contents that are otherwise identical. Samples were collected from two field treatments with one receiving between 140-170 kg mineral N ha^{-1} $year^{-1}$ (LM-), whereas the other field treatment (LM+) received a similar amount of N supplied by both mineral N fertilizer and liquid manure.

Aberdeen (Craibstone), Scotland (paper IV)

We sampled soil from a long-term field experiment at Scotland’s Rural College (57°11’N, -2°13’W) in order to study the activity of AOA and AOB. The selected field treatment was a pH trial (pH ~6.5).

Fig. 4. The studied field sites in Uppsala, Sweden with conventional annual cereal (top) and perennial ley rotation (center) and bottles used for the soil incubations (bottom)

Incubations

We traced the N cycle fluxes in soil through ^{15}N-enriched NH_4^+ and NO_3^- under laboratory conditions. For this, glass flasks were filled with soil and adjusted to a water-filled pore space (WFPS) of 55% using deionized water. The samples were allowed gas exchange but, when gaseous inhibitors were applied, flasks were sealed air-tight and aerated every other day in order to avoid anaerobia. In darkness and under constant room temperature, samples were pre-incubated for at least two days at 55% WFPS in order to adapt the soil microbiota to the incubation conditions. After pre-incubation, ^{15}N-tracers in solution were sprayed evenly over the soil surface using syringes or pipettes. The ^{15}N tracer application resulted in 60% WFPS, which has been suggested as the approximate threshold where both aerobic- and anaerobic N_2O production (*i.e.* nitrification and denitrification) take place (Bateman and Baggs, 2005).

Analysis of mineral N and N_2O and their ^{15}N abundances

Analysis of mineral N and N_2O was performed immediately after N application and at pre-defined time-steps in increasing intervals. These time steps differed between the papers depending on the research question and the initial N application. When high amounts of N were applied while simulating fertilizer application (paper III and IV), sampling time steps were spread over a week in order to quantify the main mineral N turnover, whereas experiments with low N application (paper I and II) were sampled up to one day after since the substrate was quickly consumed.

For quantification of the N_2O concentration and its ^{15}N abundance, the incubation containers were sealed for one hour and headspace gas was sampled to be analyzed by isotope-ratio mass spectrometry (IRMS) at the Stable Isotope Facility at UC Davis.

For analysis of the mineral N species NH_4^+ and NO_3^-, destructive sampling was performed by extraction with a 1M KCl solution. For paper I, the ^{15}N

abundance of NH_4^+ was analyzed via the micro-diffusion technique (Brooks *et al.*, 1989), whereas NO_3^- was converted to N_2O using a Cd-reductor (Stevens and Laughlin, 1994). In the ensuing papers, the contents and ^{15}N enrichments of NH_4^+ and NO_3^- were analyzed automatically using the SPINMAS technique (Stange *et al.*, 2007). First, a sample preparation unit oxidizes NH_4^+ to N_2 in an alkaline medium or reduces NO_3^- to NO in an acidic medium. The produced N gases were then redirected through a helium carrier stream to a quadrupole mass spectrometer for quantification.

Nitrous oxide source partitioning (paper I & III)

^{15}N-tracers can directly link the produced N_2O to its sources and quantify their relative contributions. For that, we applied two different approaches:

In paper I, we quantified the relative contributions of the three main N_2O sources: NH_4^+, NO_3^- and N_{org} using the Inverse Abundance Approach (IAA), in which different N compounds are labelled with ^{15}N and traced into one common product (Stange *et al.*, 2009). While individual ^{15}N-labelling of NH_4^+ and NO_3^- traces their respective contribution to N_2O emission, N_{org} is taken into account through tracing of the non-labelled substrate into N_2O after simultaneous ^{15}N-labelling of NH_4^+ and NO_3^- (Fig. 5). A bias towards NH_4^+ and NO_3^- (and against N_{org}) following mineral N application was avoided by applying low amounts of mineral N (~25% of the native mineral N, ^{15}N-enriched by 99%). Thereby, we aimed to estimate the soil-inherent potential of different N_2O-production routes.

By contrast, the application of fertilizer N as a direct source of N_2O and its effect on N_2O production from non-fertilizer native soil N was quantified in paper III using a simple two-source isotope-mixing model where ^{15}N-enriched NH_4^+ and NO_3^- was considered as the first source (*i.e.* fertilizer N) and native soil N with ^{15}N at natural abundance was considered as the second source (Fry, 2006). The ^{15}N tracer was applied at a rate-equivalent of 100 kg N ha^{-1} $year^{-1}$ resembling typical field application of mineral fertilizer.

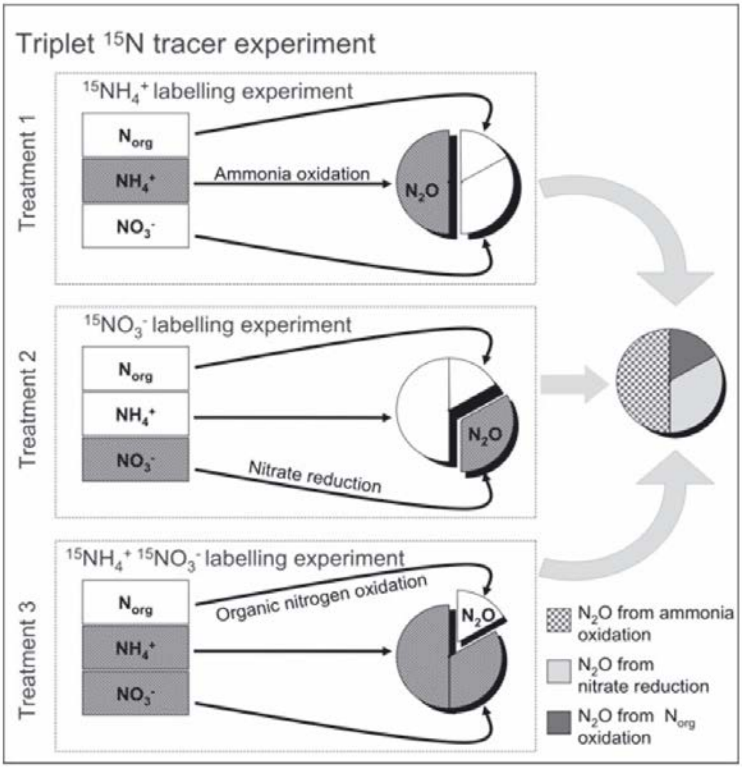

Fig. 5. Scheme of the Inverse Abundance Approach (IAA) with direct ^{15}N-tracing of NH_4^+ and NO_3^- into N_2O and indirect ^{15}N-tracing of the organic N (Stange *et al.*, 2009)

Quantification of gross denitrification and DNRA rates (paper II)

The gross N cycle rates were quantified by using a modified version of the *Ntrace* model (Müller *et al.*, 2007), which fits a conceptual N cycle model to the experimentally observed N contents and ^{15}N abundances (Fig. 6). As the model input, we used the mean values and standard deviations of the NH_4^+ and NO_3^- contents and their respective ^{15}N abundances. Besides NH_4^+ and NO_3^-, we considered an organic N (N_{org}) pool, for which we assumed constant pool size and initial ^{15}N concentration at natural abundance (0.366%).

The gross N cycle rates were then approximated with a Monte Carlo sampling through a random walk of 15 000 iterations aiming to minimize a misfit function between the modelled and observed values. The model accuracy was judged by comparing simulated with observed concentrations. The iterative approximation of the N cycle rates in the *Ntrace* simulation creates a normally-distributed probability density function, for which the mean values, which resemble a kinetic factor (*k*), and standard deviations of the means were calculated for each N flux (Müller *et al.*, 2007). The N fluxes followed zero- or first-order kinetics. We calculated the substrate-dependent gross N rates by multiplying the modelled parameters by the initial substrate concentration (*i.e.* first-order kinetics). Zero-order kinetics were applied to non-substrate dependent fluxes having N_{org} as substrate due to the static pool size (*i.e.* mineralization and oxidation to NO_3^-).

Quantification of bacterial- and archaeal ammonia oxidation rates (paper IV)

In order to quantify bacterial and archaeal ammonia oxidation, we set up two inhibition treatments including the bacterial *amoA* inhibitor 1-octyne and the AOA- and AOB inhibitor acetylene and one control treatment without inhibitor (ctrl, Table 2). 1-Octyne was prepared following Taylor *et al.* (2013) and injected with a syringe to reach a concentration of 0.03% *v/v*, whereas acetylene was applied at a concentration of 0.1 % *v/v*.

Table 2. Overview of the conducted inhibition treatments

Laboratory treatment	Inhibition	Ongoing processes
Positive control (Ctrl)	None	Complete nitrification
Acetylene (Ace)	AOA + AOB	Heterotrophic nitrification
1-Octyne (Oct)	AOB	Archaeal ammonia oxidation + heterotrophic nitrification

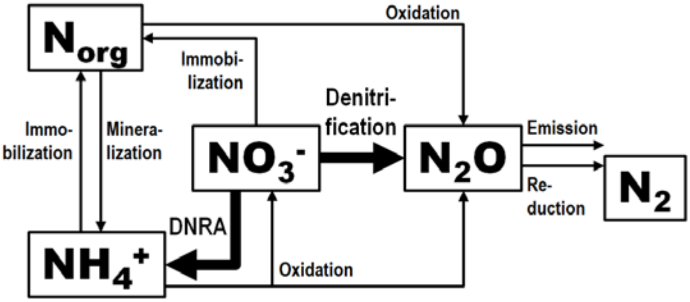

Fig. 6. Conceptual ^{15}N-tracing model as applied in the *Ntrace* simulation for comparison of the dissimilatory processes competing for nitrate: denitrification and DNRA.

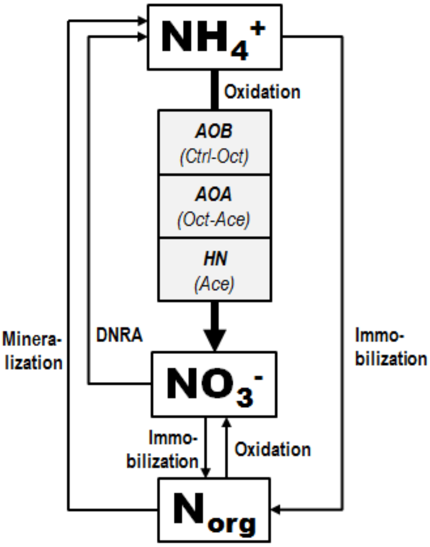

Fig. 7. Quantification of NH_4^+ oxidation with involvement of two different inhibition treatments (acetylene - ace, 1-octyne – oct, control - ctrl) in order to distinguish between ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB) and heterotrophic nitrification (HN).

These inhibition treatments were then included in a modified version of the above-described *Ntrace* model (Fig. 7). We considered the rate of archaeal ammonia oxidation as the difference between the 1-octyne and acetylene inhibition treatments ($k_{AOA} = k_{oct} - k_{ace}$), whereas the rate of bacterial ammonia oxidation was assumed to be the difference between the ctrl and the 1-octyne treatments ($k_{AOB} = k_{ctrl} - k_{oct}$).

To test the effect of substrate availability on AOA and AOB, we applied two different substrate applications rates with high NH_4^+ supply (HA, 50 µg N g^{-1}) and low NH_4^+ supply (LA, 5 µg N g^{-1}). While the former resembles fertilizer N application, the latter amount was estimated to be provided by mineralization of SOM after pre-incubation, which assumedly does not resemble fertilizer application.

Microbial community abundance (paper II)

The soil microorganisms performing denitrification and DNRA were quantified using the involved enzyme-encoding genes as proxies for functional abundance. Bulk DNA was extracted from frozen soil and quantitative PCR (qPCR) was performed for the nitrite reductase genes *nirK* and *nirS* in denitrification, the N_2O reductase genes *nosZ*I and *nosZ*II and the nitrite reductase gene *nrf*A in DNRA. In addition, the 16S rRNA gene and ITS2 region were quantified as proxies for the total bacterial and fungal communities.

Results and discussion

Contribution and variability of N_2O sources in agricultural soil (paper I and III)

The first aim was to study the environmental drivers that control the relative contribution of the three main N_2O sources apart from N fertilizer input and whether they are influenced by long-term field management, which was accomplished by adding low amounts of ^{15}N tracers in order to disturb the soil as little as possible. The relative contribution of the three sources to N_2O emission was calculated by the IAA (Stange *et al.*, 2009) as fractions: f_{NH4} (NH_4^+ oxidation), f_{NO3} (NO_3^- reduction) and f_{Norg} (oxidation of N_{org}). Our results show greater variability among the different field sites than between management practices within a field site (Fig. 8). Two exceptions are Ås (N-) and Laon (Mi) with considerably higher f_{Norg} compared to the conventionally N-amended plots. The f_{NH4} ranged between 5-58% and f_{NO3} ranged between 4-23%. The largest variation was found for f_{Norg} ranging between 25-90%.

This variability was correlated with different edaphic- and environmental factors (Table 3). The relative contribution of denitrification and NH_4^+ oxidation as sources for N_2O correlated positively with high soil moisture, high N_2O flux and low C:N ratios, whereas low pH correlated with increasing N_2O from denitrification. Oxidation of N_{org} contributed up to 90% during relatively low N_2O fluxes and has correlated with high organic C contents and C:N ratios. One exception was Laon (Mi) with f_{Norg} at ~70% even though N_2O fluxes were high.

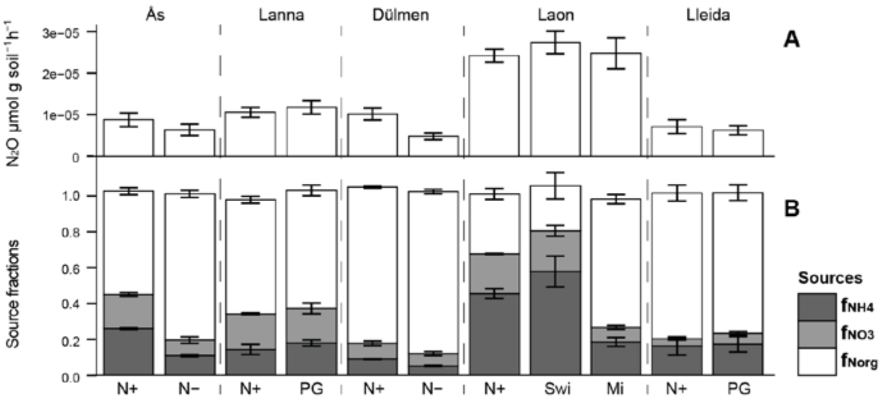

Fig. 8. (A) N_2O fluxes during the laboratory incubations. (B) Contribution of denitrification (f_{NO3}), NH_4^+ oxidation (f_{NH4}) and oxidation of organic N (f_{Norg}) to N_2O production calculated by the Inverse Abundance Approach (Stange *et al.*, 2009). The bars resemble means ± standard errors. Filed treatments include N fertilized (N+), not fertilized (N-), permanent grassland (PG), switchgrass (Swi) and *Miscanthus* (Mi).

Table 3. Pearson's correlation coefficient between the N_2O source fractions and soil properties. Bold numbers emphasize significant correlations ($p<0.05$).

	N_2O flux	C	ln C:N	pH	Field N application
f_{Norg}	**-0.69**	**0.56**	**0.38**	-0.05	-0.29
f_{NO3}	**0.41**	**-0.65**	-0.26	-0.33	0.17
f_{NH4}	**0.65**	**-0.39**	**-0.34**	0.25	0.26
ln f_{NH4}/f_{NO3}	0.21	0.11	-0.29	**0.74**	0.14
N_2O flux		**-0.52**	-0.17	0.06	**0.43**

In this study, the relative contribution of NH_4^+ oxidation, NO_3^- reduction and oxidation of N_{org} was more controlled by site specific factors than by different amounts of fertilizer application. Both mineral N sources: NO_3^- reduction and NH_4^+ oxidation increased N_2O emission rather than oxidation of N_{org} and lower soil pH promoted N_2O production from denitrification relative to NH_4^+ oxidation (f_{NH4}/f_{NO3}). Strong pH effects on denitrification and particularly on the product ratio (N_2O/N_2) are long known (Nömmik,

1956) and, indeed, increased N_2O emission with decreasing pH has been observed in field measurements on agricultural soils (Qu *et al.*, 2014) and forest soils (Weslien *et al.*, 2009). Apart from the inorganic N_2O sources, here we show that oxidation of N_{org} can be a dominating N_2O production source, which was positively correlated with high soil C content and C:N ratio. This was also observed for forest soils (Stange *et al.*, 2013) and on a sheep-grazed pasture (Rütting *et al.*, 2010). Even though we did not observe a significant long-term effect of fertilizer application on the contribution of the N_2O production sources, studies on the functional microbial communities suggest differently. Elevated nutrient supply reportedly shifts the N cycling communities in grassland soil (Leff *et al.*, 2015) and agricultural soil (Hallin *et al.*, 2009). The latter study, however, also demonstrated that soil bacteria and denitrifying communities were strongly controlled by soil properties. In agreement to this, the strong influence of soil properties in paper I suggests that field management may change N_2O production pathways when the agricultural practice changes the soil properties that control the N cycling microbiota.

Besides the above-mentioned environmental drivers, the amount of applied mineral N fertilizer is the most important factor explaining N_2O emission from agricultural soils (Rees *et al.*, 2013), which has been assigned to denitrification and autotrophic nitrification (Butterbach-Bahl *et al.*, 2011). If and how the application of N fertilizer can affect N_2O production from native N sources was tested in paper III. For that, we compared two field treatments with and without amendment of liquid manure (LM), which caused significantly higher SOM content and total C and N contents at LM+ compared to LM- ($p < 0.001$) but C:N ratios were still similar. The mineral N contents (NH_4^+ and NO_3^-) were below the detection limit; and since total N contents were significantly higher than the mineral N, we assume that most N was stored in organic compounds (*i.e.* SOM).

The total N_2O emissions after N fertilizer application were generally rising in the course of the week in both treatments but significantly higher emissions were observed for LM+ than from the LM- after one week of incubation (Fig. 9) considering all fractions: control N_2O emission (F_{ctrl}) from vessels that did not receive any mineral fertilizer, N_2O emission from fertilizer N (F_{fert}), N_2O emission from native soil N after fertilizer application (F_{nat}) and thereby also total N_2O emission (F_{tot}). In both field treatments, F_{nat} after N fertilizer application was significantly higher than F_{ctrl} without fertilizer application ($p < 0.001$) and more than doubled after one week of incubation. This increase in N_2O emission from native soil N contributed by 5-8% to the fertilizer-induced N_2O emission and indicates a positive priming effect on N_2O producing microorganisms, *i.e.* the stimulated microbial turn-over after substrate application (Dijkstra *et al.*, 2013).

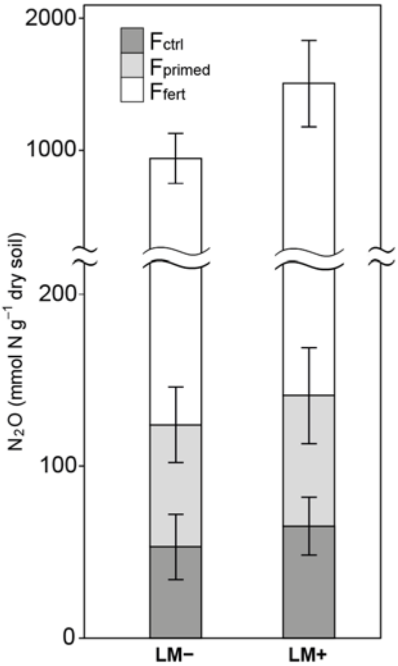

Fig. 9: Cumulative N_2O production (mean ± standard error, n = 3) from fertilizer N (F_{fert}) on the top, control N_2O emission from native soil N without fertilizer application (F_{ctrl}) on the bottom, and primed N_2O emission from native soil N after fertilizer application ($F_{primed} = F_{nat} - F_{ctrl}$) in the middle after one week of incubation for soil without (LM-) and with (LM+) application of liquid manure. Note the break within the y axis.

While we observed primed N_2O production after N fertilizer application in both field treatments, this effect was not significantly higher at the plots treated with liquid manure. Even though there is no immediate influence of the SOM content on the magnitude of priming-related N_2O production in the Dülmen field trial, it may well be observed in field trials with a larger range of soil C_{org} contents. The C contents in the studied soils were 1.09 to 1.44 %, which is considerably lower than in organic soils (van Beek *et al.*, 2010). Apart from the SOM and C contents, N_2O priming may be affected by the C:N ratio. Evidence suggests that N cycling processes, such as mineralization of SOM and nitrification, are controlled by the stoichiometric composition of C and N in the substrate (Klemedtsson *et al.*, 2005; Mooshammer *et al.*, 2012), which in our case was similar in both field treatments regardless of LM application and different SOM contents. The determination of the actual production pathways behind primed N_2O emission requires further research, *e.g.* in ^{15}N-tracing and -pool dilution experiments, in order to elucidate the influence of SOM mineralization and the actual production pathways behind primed N_2O emission.

Microorganisms competing for NH_4^+ and NO_3^-

Denitrification vs. DNRA (paper II)

The long-term field trial in Uppsala was selected for paper II in order to study agricultural soil with the same origin but with different long-term field management resulting in different substrate availability for denitrification and DNRA bacteria (*i.e.* SOM and mineral N). In fact, the perennial ley cropping systems resulted in higher SOM-, C- and N contents than the conventional annual crop rotation but no effects on soil properties were recorded through N fertilizer application. The difference in total C and N contents were mainly due to depletion in the cereal rotation, whereas the C and N levels were maintained in the ley when comparing to data from 1971, one year after establishing the experiments.

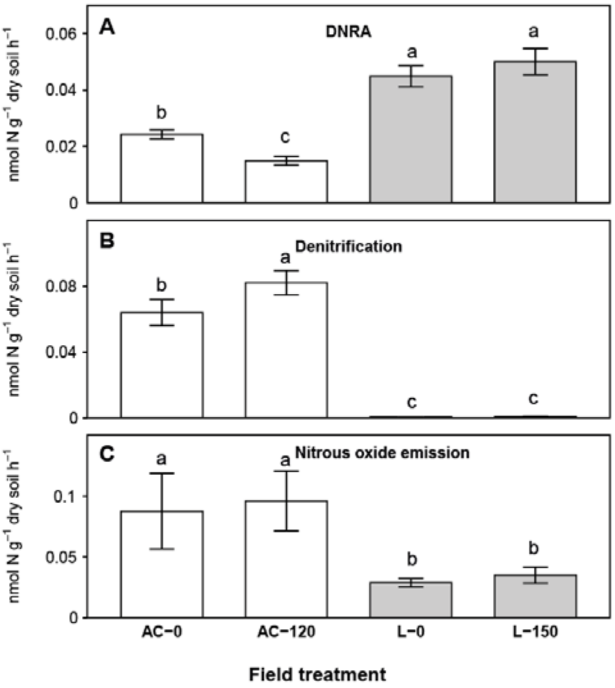

Fig. 10. Gross N cycle rates estimated by the *Ntrace* model (mean ± SD) with rates of (A) dissimilatory nitrate reduction to ammonium (DNRA), (B) reduction of nitrate (proxy for denitrification) and (C) emission of N_2O based on soils from an annual cereal rotation without and with 120 kg N ha^{-1} (AC-0, and AC-120) and a ley rotation without and with 150 kg N ha^{-1} added as fertilizer (L-0 and L-150) in Uppsala, Sweden. Significant differences are indicated with different letters ($p <$ 0.05, n=4).

The gross rates of denitrification and DNRA differed more between the cropping systems than between fertilizer application rates (Fig. 10). In the ley soil, DNRA dominated over denitrification and accounted for 99 % of dissimilatory reduction of NO_3^- and concurred with higher rates of mineralization and increased yields of barley kernels. By contrast, the annual cereal rotation had significantly higher rates of denitrification and N_2O emission. The long-term fertilizer application significantly increased denitrification and decreased DNRA in the annual cereal soil, but had no effect on these processes in the ley. While denitrification was the main N_2O source in the annual cereal soils with high emissions, N_{org} was the main N_2O

source in the ley were emissions were considerably lower. Altogether, our results show a positive relationship of the electron donor to electron acceptor (C/NO_3^-) ratio to the DNRA rates and a negative correlation with denitrification rates (Fig. 11). Moreover, the *nrfA*/*nir* gene ratio, reflecting the abundance of DNRA bacteria vs. denitrifiers, was positively correlated with the DNRA rates and negatively correlated with the denitrification rates, whereas N_2O emissions were negatively correlated with the abundance of *nosZ*.

Our results support the hypothesis that higher soil C/NO_3^- ratios promotes the abundance of DNRA bacteria relative to denitrifiers, resulting in increased DNRA rates and lower denitrification and N_2O production rates. The type of crop rotation had a stronger effect on the C/NO_3^- ratio than N fertilizer application and the ratios were driven by the differences in SOM content, with higher SOM content in the ley rotation. High DNRA activity has also been recorded in natural environments characterized by high input of organic C from plant litter (Huygens *et al.*, 2007; Fernandes *et al.*, 2012; Song *et al.*, 2014). The importance of organic C as a process-regulating factor in terrestrial systems has been controversial in previous studies and varied among different C sources (Rütting *et al.*, 2011). Instead, the capacity of DNRA bacteria to cope with low NO_3^- availability could be an important driver. During DNRA, bacteria gain more electrons per NO_3^- reduced compared to denitrification and if this results in a higher growth yield, this would be an advantage under low NO_3^- levels. Moreover, there are indications that DNRA bacteria have a higher affinity for NO_3^- (van den Berg *et al.*, 2016), although other studies suggest differently (Kraft *et al.*, 2014). The NO_3^- limitation hypothesis as a driver could explain the pattern of DNRA rates we observed. Even though there were no differences in NO_3^- content at the time of sampling, the NO_3^- content relative to C (C/NO_3^- ratio) differed among soils. Lowest DNRA was found in the fertilized cereal rotation, which had the lowest C/NO_3^- ratio. The fertilizer addition could not solely explain the low rate since DNRA rates in the fertilized ley were not different from those in the non-fertilized ley. This is potentially a consequence of the N input from N_2 fixation in the unfertilized ley (Börjesson et al., 2018). In any case, the soil C/NO_3^- ratio was a good explanatory variable for gross DNRA rates, in agreement with earlier studies (Silver et al., 2005).

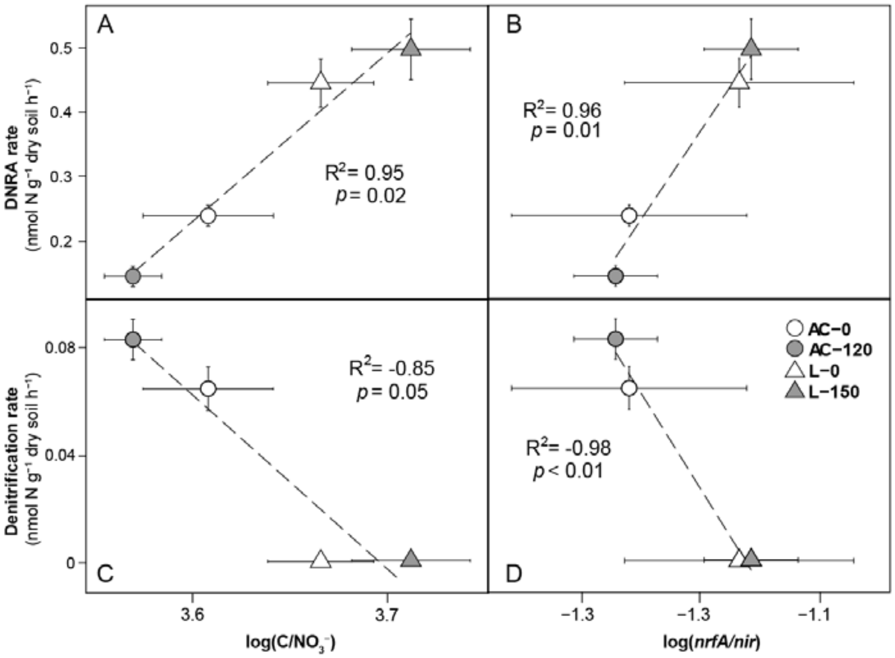

Fig. 11. Linear regressions for gross rates of dissimilatory nitrate reduction to ammonium (DNRA) and denitrification (NO_3^- reduction to N_2O) with (A, C) the C/NO_3^- ratio and (B, D) the ratio of *nrfA*/*nir*. The gene ratios and C/NO_3^- ratios were log10 transformed (mean ± SD, n=4)

AOA vs. AOB (paper IV)

The inhibitor treatments effectively separated the activity of AOA and AOB in the laboratory treatments with high (HA) and low (LA) application of NH_4^+, which is confirmed by impeded bacterial growth due to 1-octyne in a parallel experiment (Hink *et al.*, 2018). The higher NH_4^+ application in the HA treatment causes higher substrate-dependent gross rates (µmol N g^{-1} h^{-1}) compared to the LA treatment when the reaction kinetics are the same. Therefore, we compare not only the gross rates but also the kinetic factors (h^{-1}) estimated by the *Ntrace* model. The activity of AOA and AOB is reflected by the oxidation of NH_4^+ to NO_3^- (*i.e.* autotrophic nitrification). Bacterial ammonia oxidation of the HA treatment was found to have 60 % higher reaction kinetics than the AOB in the LA treatment and AOA in both treatments (Fig. 12a). Thereby, the relative contribution of AOB compared to AOA increased significantly with higher substrate application, whereas the AOA and AOB activity were similar in the LA treatment. Regarding the substrate-dependent gross rates (Fig. 12b), significantly higher ammonia oxidation was observed with higher NH_4^+ availability (*i.e.* HA > LA). In contrast to autotrophic nitrification that used NH_4^+ as substrate, nitrification from N_{org} was less important and contributed only marginally to nitrification. In addition, higher NH_4^+ application correlated with higher rates of mineralization at HA compared to LA, which may point towards a priming effect.

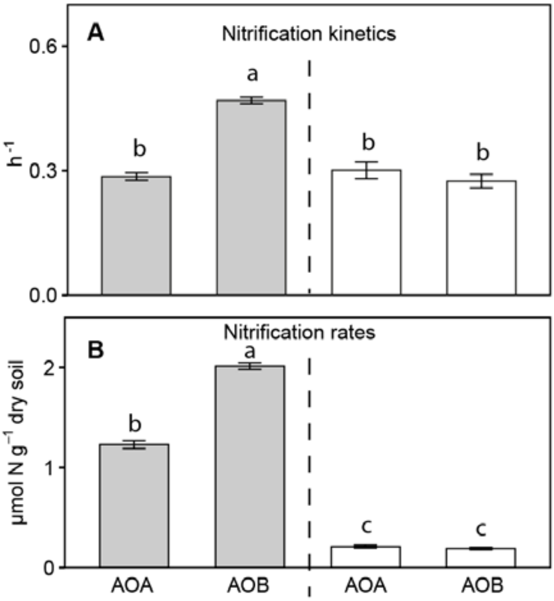

Fig. 12. Kinetic parameters (A) and gross rates (B, dependent on substrate availability) of ammonia oxidizing archaea (AOA) and ammonia oxidizing bacteria (AOB) for high (grey, left side) and low (white, right side) addition of ammonium (mean ± SD). Significant differences between treatments are indicated with different letters ($p < 0.05$, n=3)

Thus, a clear shift was recorded from a balanced contribution of AOA and AOB in the LA treatment to predominantly bacterial ammonia oxidation in the HA treatment. These findings confirm previous observations of a parallel increase in total gross nitrification rates and bacterial *amoA* gene abundance after NH_4^+ application (Sterngren *et al.*, 2015). Similar increases in bacterial relative to archaeal *amoA* gene abundances after NH_4^+ application have been observed repeatedly (Di *et al.*, 2010; Hink *et al.*, 2016). In turn, AOA were shown to outcompete AOB in abundance, growth and activity calculated by net nitrification when NH_4^+ is supplied at a continuously low rate (Hink *et al.*, 2018). While previous studies had to rely on correlations, we were able to differentiate AOA and AOB activity in soil and therefore provide evidence that increasing bacterial *amoA* abundance is directly linked to higher activity due to improved access to resources.

The equal contribution of AOA and AOB in the LA treatment supports previous studies suggesting that AOA and AOB coexist and can both be active in soil without NH_4^+ application (Di *et al.*, 2010; Sterngren *et al.*, 2015; Hink *et al.*, 2016). In addition, AOA are also reported to grow and be more active when easily available NH_4^+ is applied despite being outcompeted by AOB (Hink *et al.*, 2018). More recalcitrant N_{org}, on the other hand, can be substrate for mixotrophy and heterotrophy by both AOA and AOB (Jia and Conrad, 2009; Schmidt, 2009), which raises the question if AOA and AOB can also use N_{org} as substrate for autotrophic ammonia oxidation. This would be supported by previously observed increases in gross nitrification using N_{org} as substrate (without support from mineralization) parallel to increasing AOA- and AOB *amoA* gene abundances after organic amendments (Prommer *et al.*, 2014). Altogether these findings suggest that it is not the source but availability of substrate, which controls the contribution of AOA and AOB, and that usually mineral N fertilizer is more easily available than N_{org}.

The reason why AOA dominate in activity in unfertilized soil and when NH_4^+ is derived from N_{org} is still subject of research, but it may be a consequence of physical capacities. The smaller cell size but higher abundance of AOA allows them to cover a larger space in soil, which might explain their higher affinity for NH_4^+ at lower concentrations (Prosser and Nicol, 2012). In turn, the more than ten times larger cell size of AOB might explain their equally higher cell-specific activity, which gives them an advantage over AOA when substrate is highly concentrated in fertilized agricultural soil (Jia and Conrad, 2009) or in soil microsites with higher substrate availability such as the rhizosphere (Karlsson *et al.*, 2012).

Effects of N application on microbial N_2O production

The results of this work show partly controversial results in terms of N application and the role of soil organic matter (SOM) in the field, which will be discussed in the following two chapters.

In the short term, N fertilizer stimulates N_2O emission, which mostly occurs shortly after application (Müller *et al.*, 2014) and was also observed in paper III and IV. After a short lag- and log phase, high substrate availability activates microbial growth and activity (Zwietering *et al.*, 1990). This was deliberately prevented in paper I and II, as low amounts of ^{15}N-tracer were applied. However, mineral N is not the only substrate added to soil that drives N_2O emission. In earlier laboratory incubations, manure high in NH_4^+ and labile organic N compounds caused comparable N_2O emission relative to the N applied as mineral N application (Velthof *et al.*, 2003), which may explain higher N_2O emission from the liquid manure-treated soil in paper III. Even though we did not apply manure in the incubation experiment, the application of mineral fertilizer on soil from the LM+ field treatment caused the highest N_2O emission probably due to the higher soil C and N content as substrate for autotrophic- and heterotrophic N cycle processes. Similar to our findings, Yang *et al.* (2003) recorded increased N_2O emission due to manure application and a further increase after NO_3^- fertilizer was added to soil treated with liquid manure.

In the long-term, the link between N application in the field and N_2O emission is not as straight-forward. In paper II, the effect of long-term N fertilizer application on soil characteristics, microbial community composition and N cycle rates was less important compared to the different crop rotation practices for establishment of different SOM contents. Similarly, we observed no significant differences in N_2O emission and the contribution of the three sources between different N application levels in paper I, which suggests that N_2O production pathways are to a high degree regulated by the soil environment. This is supported by earlier recorded high spatial and temporal variability of N_2O emission within the same field site. Local patches of increased N_2O emission and N cycling microbial communities have been demonstrated independently of the agricultural practice (Ambus and Christensen, 1994; Wessen *et al.*, 2011). In our set of field sites, the geographic location rather than field management explains differences in the N_2O source fractions. Since the soil properties correlated with N_2O production pathways, land use may have an effect only if it permanently changes the soil properties.

However, N application in intensive agriculture reportedly does both, it changes the soil environment for instance by promoting soil acidification (Guo *et al.*, 2010) and by degrading soil organic matter (Matson *et al.*, 1997), and it changes the N cycling microbial communities (Hallin *et al.*, 2009). Such changes due to agricultural practice would assumedly alter the N_2O production pathways. Therefore, further ^{15}N-tracing studies on temporal changes in long-term field trials are needed in order to determine whether and how field management shifts the N_2O production pathways.

Effects of SOM on microbial N_2O production

The effect of SOM on N_2O production pathways was studied in different ways throughout this thesis. SOM can have an effect in two ways, by being a direct source of N_2O or by indirectly affecting N_2O-producing microbes that use mineral N as substrate. SOM is not only the largest terrestrial N pool in soil (Post *et al.*, 1985). It is also the main native (non-fertilizer) source for N cycling and N_2O production (Matson and Vitousek, 1987) and may even be a dominant N_2O source in agricultural soil (Zhu *et al.*, 2011; Müller *et al.*, 2014; Chen *et al.*, 2015; Zhang *et al.*, 2015). Under aerobic conditions, N_{org} oxidation reportedly dominates N_2O production in temperate forest soils accounting for 48-76% of N_2O emissions (Stange *et al.*, 2013) and on a sheep-grazed pasture, it contributed to 68.5–90.6% of N_2O production (Rütting *et al.*, 2010). Especially, the high amount of organic carbon supplemented by sheep feces favors heterotrophic soil processes. Microorganisms can use N_{org} as substrate in two known processes: heterotrophic nitrification and co-denitrification; both of which can be important N_2O sources. Heterotrophic nitrification may explain some N_2O emission in paper I and III but it has mainly been linked to acidic forest soils with high C/N ratios (Zhang *et al.*, 2015). Co-denitrification is dominated by fungi and was most importantly linked to grazed grassland (Selbie *et al.*, 2015). In contrast to grazed grassland, most soils of this work have been tilled regularly, which is known to destroy fungal hyphae (Laughlin *et al.*, 2009). Last but not least, mineralization of SOM is a source of NH_4^+, which is the substrate of nitrification and eventually denitrification (Fig. 2), which may be part of the reason why N_2O production from N_{org} has often been neglected.

In general, soils rich in organic C have been identified as hotspots for N_2O emission (Stehfest and Bouwman, 2006; Pärn *et al.*, 2018) with high emission factors after N application (van Beek *et al.*, 2010). When mineral N was applied at fertilizer concentration such as in paper III, we also recorded increasing N_2O emission correlating with higher SOM content. However, the N_2O derived directly from fertilizer N, as opposed to native N_{org}, was by far the largest source (>80 %) of N_2O emission across field treatments with different SOM contents, which suggests higher rates of nitrification and denitrification rather than direct N_2O production from N_{org} as substrate (Stehfest and Bouwman, 2006). In addition, organic carbon is the electron donor during heterotrophic metabolism, which therefore supports denitrification under high SOM contents.

In contrast to the above mentioned findings, low mineral N availability in soil can cause reducing effects of SOM on N_2O production. Previous studies found that oxidation of N_{org} contributed only little to total N_2O production (Stange and Döhling, 2005; Stange *et al.*, 2009) and may even have a suppressing effect on other N_2O production pathways. When comparing agricultural soils in paper I, the contribution of N_{org} to N_2O emission was, with exception of Laon (Mi), negatively correlated with the total N_2O flux during the laboratory incubation, whereas high N_2O emission correlated with nitrification and denitrification. An indirectly suppressing effect of SOM on N_2O production was observed in paper II, where the ley soil displayed significantly lower N_2O emission due to decreased denitrification and increased DNRA, which even reflected on the abundance of the functional microbial communities. A further explanation for decreased N_2O emission in soil high in organic matter may be based on ammonia oxidizing archaea, which are known to outcompete bacteria in natural environments high in SOM with a low but constant supply of mineral N such as during mineralization (Hink *et al.*, 2018). Recent studies provide evidence that AOA have significantly lower product ratios of N_2O/NO_3^- than AOB during autotrophic nitrification (Hink *et al.*, 2016). However, in paper IV we provide evidence that this effect disappears as soon as mineral N fertilizer is applied because AOB dominate under such conditions.

Conclusions

The results of this thesis highlight how human action drives N_2O emission from agricultural soil in a variety of ways. This can not only be explained by the direct conversion of fertilizer N. Field management affects N cycle processes in the short- and long-term. While N fertilizer application strongly stimulates N_2O production from added- and native N sources, long-term field management changes the soil characteristics, which shifts the abundance of microbial communities and thereby alters the N cycle processes responsible for N_2O production.

While the goal of ^{15}N-tracing is to identify and quantify the different sources of a common product, the laboratory experiments on a selection of European agricultural soils demonstrated that the site-specific factors control the N_2O production pathways apart from the field management (paper I). Here, we provide evidence for the contribution of parallel-occurring processes in soil and link them to their soil-inherent regulation by environmental factors. Ammonium oxidation and denitrification contributed dominantly to high N_2O emissions, whereas oxidation of N_{org} was the overall dominant N_2O source and especially when emissions were low and the C:N ratio and C content high. This changes drastically when mineral N fertilizer is applied to soil (paper III). As already known for decades, the highest absolute and relative N_2O emission is caused by nitrification and denitrification of the applied fertilizer N. Alongside this, however, N fertilizer application more than doubled N_2O production from native non-fertilizer N compounds, which clearly contributed to the enhanced N_2O emission and provides evidence for primed N_2O production due to stimulated microbial activity. Further research is needed to determine the underlying N- and C cycling pathways and whether or not N_{org} can be an important N_2O source in agricultural soils.

The application of the *Ntrace* model combines ^{15}N-tracing and ^{15}N pool dilution, which allowed us to quantify gross N cycle rates that compete for substrates and regulate N_2O production. As hypothesized, there is a strong influence by both short-term fertilizer application and long-term field management. This work demonstrates that cropping systems can shift the balance between the dissimilatory processes: denitrification and DNRA, which determines the fate of NO_3^- in soil and affects the N_2O emission rates (paper II). This suggests that DNRA can act as a mechanism to conserve N within the agroecosystems similar to what was observed in natural systems, which could potentially reflect on higher crop yields. Thus, the cropping systems controlled the soil properties (SOM content and C/NO_3^- ratio), which shaped the microbial community of dissimilatory nitrate reducers leading to changes in the biogeochemical cycling of N. Higher C/NO_3^- ratios were the main driver for DNRA, which highlights the importance of cropping systems maintaining SOM for soil N conservation. By combining the calculation of gross NH_4^+ oxidation rates and the application of selective inhibitors, we were able to quantify the specific activity of soil AOA and AOB competing for NH_4^+ (paper IV). While AOA and AOB can coexist and be equally active, AOB clearly outcompeted AOA with increasing NH_4^+ concentration. Therefore, it was not the substrate type that regulated AOA and AOB but its availability, which points towards AOB dominance in fertilized soil and microsites with high NH_4^+ concentration and AOA dominance under opposite conditions. We therefore provide evidence that activity is linked to previously presented variability in growth and abundance of AOA and AOB in agricultural soil.

Outlook

Since the establishment of soil microbiology as a subject in the beginning of the 20th century (Löhnis, 1913), we have gained a detailed understanding of N cycle processes in soil and their drivers due to technical advances including ^{15}N techniques (Kirkham and Bartholomew, 1954), inhibitors (Klemedtsson *et al.*, 1988) and molecular techniques (Philippot *et al.*, 2011). This work has connected some previously missing links by quantifying N_2O sources, separating microbial activity and testing hypotheses in terms of their environmental- and anthropogenic drivers in agricultural soil. However, the set of microbial communities and environmental drivers dealt-with in this thesis is by no means exhaustive as more factors influence N_2O emission in the field. In terms of the methodology, there are still challenges in quantifying gross rates of intermediate pathways such as nitrifier denitrification, which has been attempted to be solved using a dual ^{15}N + ^{18}O-tracing approach (Kool *et al.*, 2010) or by including nitrite into ^{15}N-tracing models (Müller *et al.*, 2014). Apart from N_2O production pathways, the potential for N_2O consumption is still widely unknown with regard to the gross activity, microbial basis and environmental regulation in agricultural soil (van Groenigen *et al.*, 2014). Also, new research questions may arise regarding the drivers of the high spatial and temporal variability of N_2O emission in the field. Answers may be given by *in-situ* ^{15}N-tracing, natural abundance techniques (*e.g.* isotopomers) (Köster *et al.*, 2011) and GIS-supported spatial analysis (Bengtson *et al.*, 2006). And eventually, a remaining challenge is to upscale our knowledge about individual N_2O production pathways *e.g.* by integration in atmospheric- and N cycle models (Butterbach-Bahl *et al.*, 2013).

Despite the knowledge we have gained about production pathways and drivers of N_2O, the atmospheric concentration is still increasing (IPCC, 2014). One important reason is, as mentioned above, the high variability in space and time due to the convoluted nature of N_2O production and -reduction with a plethora of drivers. There have been mitigation attempts in the field *e.g.* through biochar application (Nelissen *et al.*, 2015), pH regulation (Brumme and Beese, 1992), slow-release fertilizers or nitrification inhibitors (Akiyama *et al.*, 2010); often, however, with contradicting results, doubtful long-term success or potential side-effects for the environment. Another reason is the still increasing demand for food of an increasing global population (FAO, 2017). The problem about N_2O is that we are trying to resolve a global problem regulated on a micro-scale and the cure has not been found yet. Therefore, the simplest solution may be to heal the cause rather than the symptoms. N_2O emissions are foremost driven by excessive reactive N in intensive agriculture (Sutton *et al.*, 2011) and reducing the substrate may be the most important step for mitigation. Alternative cultivation methods such as organic farming, perennial cropping that recycle mineral N and organic carbon, or promotion of N_2 fixation by legumes can be promising ways to reduce the demand for fertilizer N (Maeder *et al.*, 2002) and reduce N_2O emissions (Abalos *et al.*, 2016; Gelfand *et al.*, 2016). Another important aspect is the need for reduction of animal agriculture because more than one third of the earths ice-free land area is occupied by livestock production, which requires large shares of fertilizer N and produced crops (Smith *et al.*, 2014) even though animal products are not necessary for human health (Craig and Mangels, 2009). Besides methane- and carbon dioxide, animal agriculture is estimated to contribute to global N_2O emission by 65 % (Steinfeld *et al.*, 2006). The high demand for cropping space is also the main cause for habitat destruction, deforestation, biodiversity loss and species extinction whereas nutrient excess is the main reason for ground water pollution, eutrophication and ocean dead zones (Tilman *et al.*, 2002; Diaz and Rosenberg, 2008; Machovina *et al.*, 2015). All these adverse effects seem to continue in the 21st century (Tilman *et al.*, 2002; Smith *et al.*, 2014).

Again, human land use fuels the global N cascade with adverse effects for the environment and human health. Emission of the greenhouse gas N_2O is only one part of it. Therefore, we are obliged to improve our understanding of the corresponding N cycle pathways and to critically address the underlying cause.

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