Gross nitrification in soils - Contribution of nitrification to N-gas emission from soils

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ABSTRACT

This work contributes to developing a better understanding of nitrification in soils as an important source of N gas emissions from soils. Therefore, the nitrification process as well as N gas produced by nitrification are considered. The work described the common methods and new developed approach for determining the gross nitrification rate. Both measuring and quantifying nitrification in soils have been shown to achieve the objective. One focus is to differentiate the sources of N gases and to quantify the contribution of nitrification to N gas emission from soils. The separation of N gas production into source-related pathways that simultaneously operate in soils requires comprehensive experiments with complex analyses. Therefore a new analytical approach and calculates the fractions of ammonia oxidation, N_{org} oxidation and denitrification for total soil NO and N_2O released from a soil probes at different oxygen states (2.5, 1.2 and 0 % O_2) is presented and tested for a five loamy Spanish forest soils. Whereas the relation between ammonia oxidation and denitrification as sources of soil N_2O gas release appear to be consistent, which is commonly accepted, the contribution of N_{org} oxidation was unexpectedly high (up to 76%). Also two model approaches to model the N-gas production in soils are parametrised on experimental data from laboratory studies. The findings are discussed in view of choosing the best approach to predict N_2O production during nitrification. and an approach to combine response functions in modelling is presented and tested on field data. The advantage against the conventional combining approaches (multiplicative or min/max approaches) is discussed. N_2O production data related to nitrification and nitrification rates were collected and multiple linear regression analysis between the soil properties and N_2O product ratios were applied to this dataset to identify functional relationships. Future works to support the development of sufficient model approaches are needed, and in particular, the nitrite and oxygen concentrations in soils are the most important factors for N_2O production.

ZUSAMMENFASSUNG

Diese Arbeit möchte zu einem besseren Verständnis über den Prozess der Nitrifikation als eine wichtige Quelle der N-Gasemission aus Böden beitragen. Daher werden einleitend die verschiedenen Prozesspfade der Nitrifikation und der Spurengasbildung beschrieben und bildlich dargestellt. Verfahren zur Messung der Nitrifikation und Versuche zu Bestimmung der Umsatzraten werden in der Arbeit vorgestellt. Dabei liegt der Fokus auf der Separation der verschiedenen Quellen von NO und N_2O und beschreibt die dafür notwendigen komplexen Versuche inclusive mathematischer Verfahren zu deren Analyse. Mit Hilfe dieser Tools werden die Anteile der Ammoniakoxidation (erster Schritt der autotrophen Nitrifikation), der direkten Oxidation von organischem Stickstoff und der Denitrifikation bei unterschiedlichen Sauerstoffpartialdrücken (2.5, 1.2 und 0 % O_2) bestimmt und in einem weiteren Schritt die Methoden auf fünf spanische Waldstandorte angewendet. Interessanterweise sind die Anteile der direkten Oxidation von organischem Stickstoff sehr hoch und auch relativ konstant bei verschiedenen Sauerstoffpartialdrücken. Zwei verschiedene Modellansätze zu Beschreibung der N-Spurengasproduktion in Böden werden vorgestellt und an Labordaten parametrisiert. Die beiden Ansätze und ihre Implikationen für die Bildungswege der N-Spurengasproduktion werden ausgiebig diskutiert. Zusätzlich wird für die Anwendung in Ökosystemmodellen ein auf dem harmonischen Mittel beruhenden Ansatz vorgeschlagen, um verschiedene Responsefunktionen (z.B. die für die Temperatur- und die für die Bodenfeuchteabhängigkeit) miteinander zu verbinden. Im letzten Abschnitt der Arbeit werden die Daten der zuvor beschriebenen Experimente sowie in der Literatur verfügbare Daten zur Bruttonitrifikation und der nitrifikatorischen N_2O-Produktion systematisch zusammengetragen, daraus das N_2O-Produktion/Nitrifikation-Verhältnis (N_2O product ratio) berechnet und dieses mittels multipler lineare Regression gegenüber den Bodeneigenschaften analysiert. Es deutet sich an, dass besonders der aktuelle Sauerstoffpartialdruck und die Nitritkonzentration starken Einfluss auf die N-Spurengasproduktion haben könnten, aber um kausale Zusammenhänge zu bestätigen, gibt es zu wenige insitu Messungen dieser beiden Faktoren in bisherigen Experimenten. Daher endet die Arbeit mit der Aufforderung zukünftig in N-Gasexperimenten immer auch Nitrit und Sauerstoff im Boden zu messen.

CONTENT

CHAPTER 1: AIM AND SCOPE

This work contributes to developing a better understanding of nitrification in soils as an important source of N gas emissions from soils. Therefore, the nitrification process as well as N gas produced by nitrification are considered. Unless expressly indicated, the term nitrification is used in the sense of gross nitrification instead of net nitrification, as usual. Both measuring and quantifying nitrification in soils have been shown to achieve the objective. One focus is to differentiate the sources of N gases and to quantify the contribution of nitrification to N gas emission from soils.

The isotopic pool dilution method is the common and the most used approach for determining the gross rate. When applied to nitrification, this method requires an adequate technique to determinate the ^{15}N abundance in nitrate. To overcome the difficulties of former approaches, an automated sample preparation unit for inorganic nitrogen (SPIN) species was developed and directly coupled to a quadruple Mass Spectrometer (MAS). Chapter 3, 'Automated and rapid online determination of ^{15}N abundance and the concentration of ammonium, nitrite or nitrate in aqueous samples by the SPINMAS technique' describes the set-up and performance of the system. The SPINMAS technique allows the most rapid determination of the ^{15}N abundance in inorganic N-species (e.g., NH_4^+, NO_3^-, and NO_2^-) at a very sensitive level. A more recent approach for determining the nitrification rate is the barometric process separation (BAPS, Ingwersen et al. 1998). In this work, the BAPS is used and tested extensively. Suggestions for improving BAPS analysis, which resulted from the test, are given in Chapter 4, 'Shortcomings in the commercialized barometric process separation measuring system'.

A separation of N gas production into source-related pathways that simultaneously operate in soils requires comprehensive experiments with complex analyses. The experimental data can be analysed by applying either a numerical or an analytical model. In Chapter 5, 'The ^{15}N tracing model SimKIM is used to analyse NO and N_2O production during autotrophic-, heterotrophic nitrification and denitrification in soils' the model is applied to an experiment with an agriculturally used silty soil. Chapter 6, 'An inverse abundance approach to optimize a separation of soil N pools and gaseous N fluxes into process-related fractions', introduces a new analytical approach and calculates the fractions of ammonia oxidation, Norg oxidation and denitrification for total soil N_2O released from a sandy Mollic Cambisol at different oxygen states (2.5, 1.2 and 0 % O_2). The approach is particularly suited to quantify the contribution of unlabelled Norg pools with less uncertainty than previous approaches.

The following four chapters describe the quantification of nitrification rates, and two of them also describe N gas production. Chapter 7, 'A novel approach to combine response functions in ecological process modeling', determines the influence of different temperature and moisture steps on the nitrification of beech litter. The results clearly note the interaction between the two environmental factors. Consequently, the combination of the temperature and moisture response functions is proposed based on the harmonic mean instead of the usual multiplicative combination. This concept is applied in Chapter 8, 'Measuring and modelling seasonal variation of gross nitrification rates in response to long-term fertilisation"', to the nitrification rates determined over one year in three different fertilized plots of a silty chernozem. The same soil, but with two different C_{org} contents, in which additional N gas production (N_2O and NO) is determinate, is investigated in Chapter 9, 'Analysis of the coexisting pathways for NO and N_2O formation in chernozem using the ^{15}N-tracer SimKIM-Advanced model'. The advanced version of the model presented in Chapter 5 is used to analyse the experimental data and to determine the influence of the oxygen concentration on nitrification and N gas production. Chapter 10, 'Use of the inverse abundance approach to identify the sources of NO and N_2O release from Spanish forest soils under oxic and hypoxic conditions', describes the influence of oxygen on nitrification and N gas production in five loamy forest soils. Whereas the relation between ammonia oxidation and denitrification as sources of

soil N_2O gas release appear to be consistent, which is commonly accepted, the contribution of N_{org} oxidation was unexpectedly high (up to 76%).

Chapter 11 describes the analysis of the actual state of knowledge about hybrid N_2O formation in soils. Although the name 'codenitrification' given by Tanimoto et al. (1992) and Shoun et al. (1992) points to a close link with denitrification, hybrid N_2O production is also possible during nitrification. Hydroxylamine and nitrite, the two substrates of hybrid N_2O production, are products in the pathway of microbial nitrification and therefore could react to hybrid N_2O. Inasmuch as the reaction mechanism for N_2O production during N_{org} oxidation has been undefined to date, hybrid N_2O formation provides a possible pathway, which should be tested in the future.

A comprehensive approach to modelling N_2O production by nitrification based on the synthesis of Chapters 2 to 11 is given in Chapter 12 'Synthesis'. N_2O production data related to nitrification and nitrification rates were collected across the chapters, and the literature and multiple linear regression analysis between the soil properties and N_2O product ratios were applied to this dataset to identify functional relationships. The findings are discussed in view of choosing the best approach to predict N_2O production during nitrification. Future works to support the development of sufficient model approaches are needed, and in particular, the nitrite and oxygen concentrations in soils are the most important factors for N_2O production; these factors must be measured in all future studies.

CHAPTER 2:
INTRODUCTION IN SOIL NITRIFICATION

Nitrification is a key process in the soil nitrogen cycle and is an important biological source of N_2O and NO emissions from soils. Nitrification promotes NO and N_2O formation, first, directly as a by-product of nitrate formation and second, indirectly as a producer for substrate for denitrification (Arth et al., 1998), and it links the reductive forms with the oxidative forms of nitrogen in the soil. Nitrification is the microbial oxidation of ammonium (NH_4^+) to nitrate (NO_3^-). While the cation ammonium is bound by electrostatic forces to negatively charged clay particles and functional groups of soil organic matter, the sorption of the nitrate anion to the clay surface is much weaker. Therefore, nitrification is known to promote nitrogen eluviation from the soils because the less mobile cation ammonium (NH_4^+) is oxidized by nitrifiers to the much more mobile anion nitrate (NO_3^-) (Abbasi and Adams, 1998).

Because soil forms many diverse microhabitats with a wide range of soil properties, including redox condition, water content, pH and substrate availability, the distribution of microorganisms and microbial activity is heterogeneous at a very fine scale (microscale) (e.g., Parkin, 1993; Strong et al., 1998; Nunan et al., 2003). For nitrification, different pathways for the different involved microorganism groups are proposed. Nitrification is divided into autotrophic nitrification and heterotrophic nitrification. Generally, autotrophic nitrification in soils is favoured by an increased availability of NH_3, a pH value close to neutral, and good aeration (Barnard et al., 2005). Commonly C_2H_2 is used to distinguish between the contribution of heterotrophic and autotrophic nitrifiers to nitrate production in soils. Ammonia mono-oxygenase, as the key enzyme of the autotrophic nitrification, is inhibited irreversibly by small quantities of acetylene and thereby provides a means for experimentally differentiating. In addition to bacteria, ammonia-oxidizing archaea (AOA) are also involved in the first step of autotrophic nitrification, and Zhalnina et al. (2012) proposed that the pathway for ammonium oxidation in AOA differs from the pathway outlined for bacteria (AOB). For detailed reviews about the organisms and processes responsible for nitrification in soils, see Schimel and Bennett (2004), Chapman et al. (2006), Prosser and Nicol (2008), Jackson et al. (2008), and Norton and Stark (2011). Comammox bacteria (e.g., *Nitrospira* species) comprise a previously overlooked fourth group of ammonia oxidizers (Daims et al., 2015; van Kessel, 2015). They appear to be environmentally widespread, but less is known about their physiological characterization and about the ecological niches in which comammox bacteria successfully compete with other nitrifiers.

CONTROL OF NITRIFICATION IN SOILS

Nitrification is influenced by a number of environmental and soil properties. The effects on the nitrification rates of environmental controls, including substrate availability (ammonia and oxygen), temperature, soil moisture and pH, are described. Most of these controls have been comprehensively investigated, and NH_3 availability is understood to be the most important. The response to the ammonia availability is mostly explained by a first-order kinetic (e.g., Müller et al., 2004). The control by substrate availability should be discussed, with particular attention to the factors affecting ammonia/ammonium availability, and includes the strong dependency of nitrification on the pH value caused by the NH_3/ NH_4^+ equilibrium ($NH_3+H^+ \leftrightarrow NH_4^+$; pKa = 9.25 at 25 °C).

Because nitrification is an obligate aerobic process, available O_2 is required. Bollmann and Conrad (1998) show that nitrification rates are more or less constant if the O_2 concentration ranges between 4 % and 20.9 %. At an O_2 concentration below 4 %, the nitrification rates strongly decreased (Bollmann and Conrad, 1998). Khalil et al. (2004) presented similar results, where the nitrification rates at 4.3 and 20.4 kPa were comparable, whereas at 1.5 kPa, the nitrification rate was nearly halved.

Wlodarczyk et al. (2004) reported that the ammonia oxidizer can use NO_2^- as an electron acceptor instead of O_2. In Chapter 9, nitrified N_2O production was also observed under an anaerobic condition.

Some of the more important factors are temperature and soil water content (Recous et al., 1998; Chapter 7 and 8). Soil properties such as temperature, soil moisture and microbial activity mineralization and consequently affect the

the temperature responses that the biochemical processes of NH_3 oxidation in AOA and AOB may differ from each other.

Low soil water content will stress the microbes, reduce microbial activity, and reduce the substrate supply by diffusion. Too much soil water will reduce the gas diffusion, consequently reducing the oxygen concentration of the soil and again resulting in a reduced nitrification rate. In Chapters 7 and 8, an

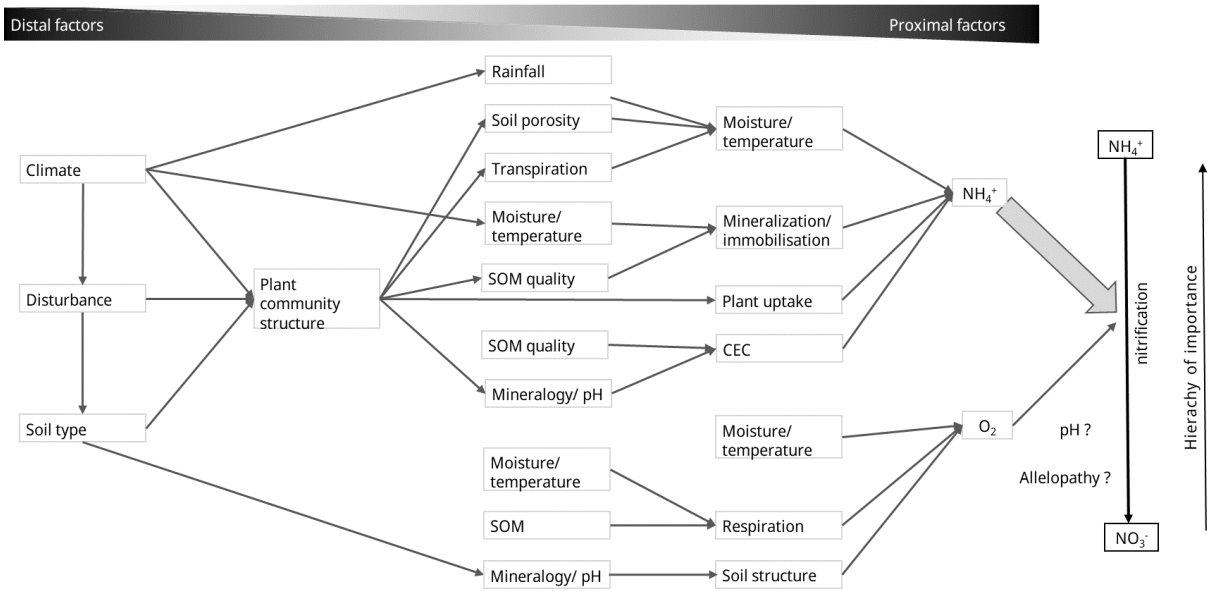

Figure 2.1: Factors that influence the nitrification rate in soils (adapted from Robertson and Groffman, 2007).

substrate availability. Optimal temperature of nitrification depends on the climatic condition of the specific site, so for temperate soils, microbes show mostly maximum activity between 25 °C and 37 °C (see Chapter 8), whereas on Mediterranean sites, Stark and Firestone (1996) found an optimum temperature in the forest of 32 °C and in open grassy interspaces of 36 °C. Below this maximum temperature, the rate of nitrification tends to decrease exponentially. Additionally, at a temperature higher than the maximum, the rate of nitrification reduced rapidly. Taylor et al. (2017) found significant differences in the response of the nitrification supported by AOA or AOB in eight soils from four different sites in Oregon, which contributed to the NPs, with AOA having a more than 12°C greater optimal temperature than AOB. They concluded from the significant differences in

optimum function is presented that describes the dependency of nitrification on the soil moisture. Optimal nitrification rates can be expected near field capacity (matric potential between -33 and -10 kPa, corresponding to a water-filled pore space (WFPS) from 60 to 90 %, depending on the soil type.

Sudden changes in environmental factors (e.g., frost or sudden wetting) can change substrate availability and microbial activity. As a result, dead microbial biomass can lead to higher nitrogen availability but also to lower microbial activity. Possible flushes of N mineralization are understood poorly and are implicated in the lack of ability to predict nitrification in the field (Campbell et al., 1988). Therefore, sudden changes in environmental conditions (e.g., in the soil water content) can have a profound influence on the rate of N mineralization and the single steps of nitrification and

consequently can affect the supply of substrate for nitrification and N_2O production (Liu et al., 2018).

Soil pH values strongly affect the nitrification rate, primarily through their effects on NH_3 availability. Low nitrification in acid soils (<pH 4) are thought to be due to substrate limitation and a low number of autotrophic nitrifiers (Klemedtsson et al., 1999). pH tolerance varies among the different species of ammonia-oxidizing microorganism (De Boer et al., 2001), and low pH has been suggested to favour the heterotrophic nitrification and the autotrophic nitrification by archaea compared to autotrophic nitrification by bacteria (e.g., Nicol et al., 2008; Jia and Conrad, 2009; Banning et al., 2015).

The fact that soil properties can be affected by other soil properties (variables) has led to hierarchical concepts, such as the distal/proximal concept from Robertson and Procter (1989) (Fig. 2.1) or the most/least fundamental concept from Strong et al. (1998). Nevertheless, the missing independencies of the soil properties complicate the analysis of causal relationships. Robertson and Groffman (2007) summarize the distal and proximal factors for nitrification (Fig. 2.1). A comprehensive literature review about the controls determining the nitrification in soils is given by Booth et al. (2005).

SMALL-SCALE VARIABILITY OF NITRIFICATION RATES IN SOIL

Soils are well known to vary spatially even over short distances. Spatial variability is understood to have an effect on diverse processes in soils, e.g., on transport processes, biomass turnover rates (Harden and Joergensen, 2000) and nutrient cycling processes (de Boer et al., 1996; Corre et al., 2003). In contrast to N_2O emission (Mathieu et al., 2006) and net nitrification (Strong et al., 1998 and 1999; Ollivier et al., 2011), to my knowledge, no study has addressed the spatial variability of gross nitrification rates at the field scale. Therefore, less is known about small-scale variability. In the 'Kreinitz Diversity Experiment' (for more information about the site see Chapter 6), gross nitrification rates under constant temperature (21 °C) and actual field moisture (between 10 and 15 % w/w) were determined at 172 locations inside a 50 m * 100 m area by the ^{15}N pool dilution technique.

The determined NRs vary in a wide range between 0.08 and 2.44 mg N kg^{-1} d^{-1}. Ollivier et al. (2011) have noted that net nitrification rates within temperate forest ecosystems can vary spatially by factors of 10 to 1000. The determined NRs in the investigation were distributed lognormally (Fig. 2.2), and the geometric mean NR for the sandy soil was 0.58 mg N kg^{-1} d^{-1} (median 0.60 mg N kg^{-1} d^{-1} and arithmetic mean 0.68 mg N kg^{-1} d^{-1}).

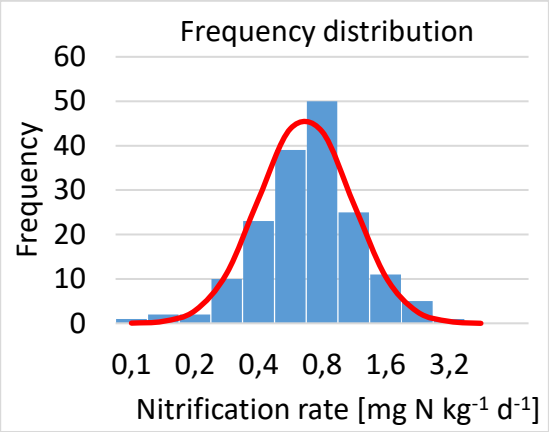

Figure 2.2: Frequency distribution of the observed nitrification rates at the 50 * 100 m plot. Please note the logarithmic scale of the x-axis. The line is the ideal lognormal distribution

The spatial variability of the nitrification rate at the plot scale was very high (coefficient of variation: 58 %), and the range of autocorrelation for nitrification was short (max. 10 cm) on this experimental plot. The observation of short autocorrelation for gross nitrification is in line with the previous observation for net nitrification. Strong et al. (1997) observed autocorrelation for net nitrification at a distance of 15 cm. Stark et al. (2004) examined the spatial structure of microbial C and N, as well as arginine ammonification, and did not observe any spatial structure at distances greater than 30 cm. Additionally, the results from Mendum (1999) suggest that the spatial distribution of bacterial colonies is critical in regulating nitrification rates in soils.

The results confirm former investigations (e.g., Chapter 8), where nitrification rates in soils were

observed as being lognormally distributed. Therefore, using the arithmetic mean is inappropriate. Instead, the geometric mean must be used to calculate mean nitrification rates in soils. The results of the experiment support the hypothesis that hotspots are mainly responsible for the turnover rates in soil including nitrification. Therefore, investigations should be performed on the volume and distribution density of nitrification hotspots. Representative soil sampling is a prerequisite for the quantification of the nitrification rate for a field site. Depending on the spatial variability and sampling volume, many replicates (approximately 20 in the "Kreinitz" soil) or composite samples are necessary for a representative measurement.

N_2O GAS PRODUCTION BY THE NITRIFICATION PATHWAYS

The contribution of nitrification to the N_2O emission of soils is significant, and in single ecosystems, nitrifiers dominate the N_2O production in soils (e.g., Siciliano et al., 2009; Ma et al., 2015). Gödde and Conrad (1999) have estimated, that ammonia oxidation contributes up to 80% of soil N_2O emissions, depending on particular soil ecosystem types and climatic regimes. However, controls for N_2O production by nitrification are not well established (Siciliano et al., 2009), and the quantification of relationships will be complicated due to the different pathways of N_2O production. Autotrophic ammonia-oxidizing bacteria (AOB) can produce N_2O directly due to an incomplete oxidation of hydroxylamine to nitrite (Firestone and Davidson, 1989) or as an intermediate in nitrifier denitrification (Poth and Focht, 1985). During the pathway of hydroxylamine oxidation, the intermediate nitroxyl (HNO) reacts with another NH_2OH molecule to form hyponitrous acid (HON=NOH), which is subsequently decomposed to N_2O and H_2O (Duan et al., 2017). However, two released HNO have been hypothesized to dimerize and dehydrate to the form N_2O. Caranto and Lancaster (2017) speculated that the product nitric oxide will attack NH_2OH and form N_2O. So, to date, the pathway of N_2O formation during NH_2OH oxidation is not fully understood.

Nitrifiers are able to reduce NO (or NO_2^-) to N_2O by a denitrification pathway: the so-called nitrifier denitrification (Poth and Focht, 1985; Wrage-Mülling et al. 2018). In particular, if diffusional constraints limit O_2 availability, the AOB will use nitric oxide or nitrite as an alternative electron acceptor and produce N_2O. Additionally, N_2O will be produced directly by heterotrophic nitrification and indirectly through the enhancement of nitrate availability for denitrifiers. Despite this important role of nitrification to the terrestrial nitrogen (N) cycle and N gas production, the contribution of autotrophic and heterotrophic nitrification to total gross nitrification and N_2O emission remains poorly understood (Wang et al. 2014).

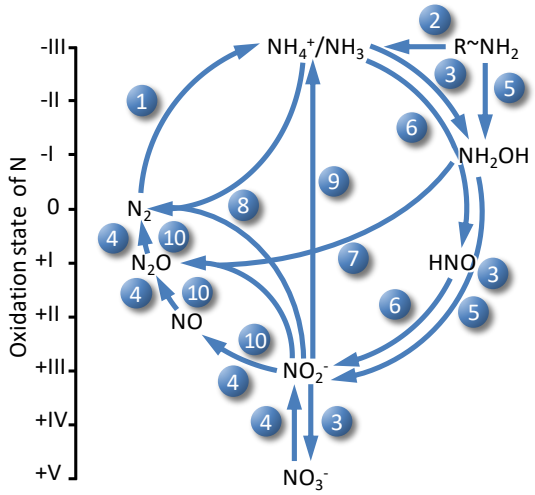

Figure 2.3: Oxidative and reductive processes in the microbial nitrogen cycle of soils. The oxidation state is given on the left site. The numbers indicate the different processes: 1. Dinitrogen fixation. 2. Dissimilatory ammonification 3. Aerobic oxidation of ammonia to nitrate by bacteria (autotrophic nitrification). 4. Classical denitrification. 5. Aerobic oxidation of organic N to nitrate by microorganism (different from heterotrophic nitrification). 6. Aerobic oxidation of ammonia to nitrite by archaea (autotrophic nitrification, the pathway is putative as based on genomic inference from Walker et al. (2010)). 7. Codenitrification. 8. Anoxic ammonia oxidation by bacteria (Anammox). 9. Dissimilatory nitrite reduction to ammonia (DNRA). 10. Aerobic nitrifier denitrification. Based on Cabello et al. (2004)

AUTOTROPHIC NITRIFICATION

Autotrophic nitrification is a two-step process, and for more than 100 years, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were believed to be the only microorganisms responsible for nitrification. Recent works have

shown that comammox bacteria (e.g., *Ca. Nitrospira inopinata*) are able to perform both ammonia and nitrite oxidation (Daims et al., 2015, van Kessel, 2015).

Ammonia-oxidizing bacteria (AOB) catalyse the oxidation of ammonia via hydroxylamine (NH_2OH) to nitrite. These slow-growing, autotrophic bacteria use this process as their sole source of energy. The first step of this reaction is mediated by the membrane-bound enzyme ammonia mono-oxygenase (AMO), which also can oxidize a variety of organic, nonpolar low-molecular-weight compounds, including, methanol and methane.

$NH_3 + 2H^+ + O_2 + 2e^- \rightarrow NH_2OH + H_2O$

The produced hydroxylamine is further oxidized to nitric oxide and chemical or biotic oxidized to nitrite according to the following reaction:

$NH_2OH + H_2O \rightarrow NO + H_2O + 3H^+ + 3e^- \rightarrow NO_2^- + 5H^+ + 4e^-$

Caranto and Lancaster (2017) proposed nitric oxide as an obligate intermediate in nitrification and as the end product of oxidation by the hydroxylamine oxidoreductase (HAO). They show that NO is the product of NH_2OH oxidation by HAO under anaerobic condition. Furthermore, they found evidence for NO as an obligate ammonia oxidation intermediate by using two different NO scavengers (catalase and ferrous-O_2 haemoglobin), also under aerobic condition. The produced NO will be fast-oxidized abiotically by O_2 to NO_2-, but the authors suggest that the ammonia oxidation requires an unidentified reagent that supported the NO to NO_2– oxidation to outcompete side reactions that produce NO_3– or N_2O. Such a reagent permits the full complement of the four electrons into the cellular electron transport chains (see Figure 2.4).

Because the first step of autotrophic nitrification, the oxidation of NH_3 to NH_2OH, requires two electrons, and 3 electrons result from the oxidation of NH_2OH to NO, overall, one electron generates the energy for microbial metabolism and cell growth. If the nitrite production is also involved and not abiotic, as supported by experiments with cells (Caranto and Lancaster, 2017), two electrons are available. Nitrite does not accumulate in soils, although it can be oxidized quickly to nitrate by nitrite-oxidizing bacteria according to the following reaction:

$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$

The majority of the energy gain (approx. 80%) through autotrophic nitrification (ammonia and nitrite oxidation) is required for the CO_2 fixation via the Calvin cycle (Prosser, 1990). Approximately 35 mol NH_3 or 100 mol NO_2^- must be oxidized to support the fixation of 1 mol CO_2 (Wood, 1986).

However, the common view of nitrification in soils has undergone a considerable change, especially in the last decade. Recently, the roles of ammonia-oxidizing archaea (AOA) and comammox bacteria have entered the conversation. Ammonia-oxidizing archaea (AOA) were discovered, bit by bit, in many ecosystems of varied environmental conditions and even found as the predominant nitrifying organisms in soils (Leininger et al 2006). In a few studies, the abundance of ammonia oxidizing archaea (AOA) genes exceeded the abundance of ammonia oxidizing bacterial (AOB) genes. A comprehensive review of Zhalnina et al. (2012) summarized the current knowledge on the environmental conditions related to the presence of AOA and discusses possible niches of AOA. Low availability of NH_3, reduced oxygen concentration and low pH have been suggested to favour the autotrophic nitrification by archaea compared to autotrophic nitrification by bacteria (Barnard et al. 2005; Jia and Conrad 2009). Furthermore, differences in substrate affinities allow AOA and AOB to inhabit distinct niches separated by substrate concentration and thereby reduce competition (Martens-Habbena & Stahl, 2009; Verhamme et al., 2011). Banning et al. (2015) observed a contrasting response of AOB and AOA; with increasing soil C content and pH value, the amoA gene copies of AOB increase, and the AOA-derived amoA gene copies decrease. These findings support the niche theory, which deducts possible niches based on pH, ammonia availability and nutrient levels (e.g., Nicol et al., 2008; Erguder et al., 2009; He et al., 2012).

Wang et al. (2016) found that, for alpine grasslands, the abundance of ammonia oxidizing archaea (AOA) genes exceeded the abundance of ammonia oxidizing bacterial (AOB) genes by approximately three orders of magnitude.

Nevertheless, compared to the AOA abundance, the AOB abundance had a stronger explanatory power for the variability of gross nitrification using all data. Alves et al. (2013) investigated 11 arctic soils and found only AOA in five of them, and AOA outnumbered the AOB in four of the remaining six soils. In addition to the low temperatures, this ecosystem is characterized by nitrogen limitation. The findings are in accordance with the hypotheses by Valentine (2007) and Schleper and Nicol (2010), who conclude that archaea are more stress tolerant than bacteria.

In contrast, high ammonia availability and ammonium fertilization benefit the AOB, whereas studies with high concentrations of ammonia indicate substrate inhibition of archaeal nitrification (Di et al., 2009; Tourna et al., 2010). For instance, in agricultural soils with continuous fertilization, ammonia oxidizing bacteria (AOB) dominate the microbial ammonia oxidation (Jia and Conrad, 2009; Xia et al., 2011). Generally, soil pH value is a strong separator for the different pathways, and nitrification in agricultural soils is mainly autotrophic (e.g., Cheng et al., 2015). Recently, evidence is growing that ammonia oxidizing archaea (AOA) are functionally dominant in acid soil (pH < 5.5) (He et al., 2012), potentially because AOA can use organic N instead of ammonia as a metabolic N source (Prosser and Nicol, 2012; Alves et al., 2013). However, AOA have since been demonstrated to numerically dominate AOB in agricultural soils and alpine grasslands soil (Wang et al. 2016), although their contribution to the nitrification rate is still unclear.

N_2O PRODUCTION BY AUTOTROPHIC AMMONIA OXIDIZING BACTERIA (AOB)

The production of N_2O results from the incomplete oxidation of ammonia and hydroxylamine by autotrophic ammonia oxidizing bacteria (AOB) such as *Nitrosomonas spp*. (Yoshida and Alexander, 1970), or it may result from nitrite reduction, which is referred to as nitrifier denitrification (Poth and Focht, 1985; Wrage et al. 2001). A net of two electrons are released in the reaction steps before the nitrifier is available for denitrification (Fig. 2.4) of the nitrite to N_2O. Nitrifier denitrification by autotrophic and heterotrophic bacteria has been acknowledged in pure cultures (Hooper et al., 1997; Ritchie and Nicholas, 1972). Kim et al. (2010) observed higher N_2O emission from an active sludge during undergoing NH_3 oxidation and a dropping emission rate as soon as the NH_4^+ was consumed. They observed only a slightly increase in the N_2O emission rate with increasing nitrite concentration. Additionally, experiments with NH_2OH as substrate and DCD and ATU as ammonia oxidase inhibitors show that ATU does not inhibit N_2O production, whereas DCD, which is also a NirK inhibitor, prevented N_2O emission. Therefore, the authors conclude that NirK from the ammonia-oxidizing bacteria plays an important role in N_2O production from active sludge. Kozlowski et al. (2014) compared the N_2O production of the wild-type of *Nitrosomonas europaea* and mutant strains deficient in the expression of NirK, NorB, and the proposed gene products. They found that NorB is the only nitric oxide reductase active in the nitrifier pathway and that NirK is not essential to the nitrifier denitrification pathway of *N. europaea*. From this, they conclude that an alternate nitrite reductase to NirK is active in the production of N_2O. According to the findings of Caranto and Lancaster (2017), nitric oxide is the end product of the hydroxylamine oxidation by HAO under anaerobic condition; therefore, no nitrite reductase is needed. Show et al. (2006) suggested that in beta-proteobacteria, ammonium oxidizer nitrifier denitrification could be a general trait. Kool et al. (2009) noted that nitrifier denitrification contributed significantly to N_2O emission from the soils. The relative importance of the two pathways in N_2O production is still under debate and has proven difficult to determine. Kozlowski et al. (2014) proposed that the copper-containing nitrite reductase (NirK) enzyme has a key function in controlling the two alternative pathways. They observed an enhanced N_2O production and a corresponding reduction in nitrite production in the mutant strains of *N. europaea* deficient in expression of NirK. Cantera and Stein (2007) suggest that slower NH_2OH oxidation in the NirK-deficient strain of *N. europaea* will be caused by interruption of electron flow from HAO to NirK through cytochrome *c* electron carriers.

Until now, two methods have been applied to

distinguish between incomplete oxidation of hydroxylamine and nitrifier denitrification: the dual labelling approach (Wrage et al., 2001) and more recently the Isotopomer approach (Sutka et al., 2006). Sutka et al. (2006) observed a site preference in the N_2O produced during NH_2OH oxidation by common autotrophic nitrifiers (*N. europaea* and *Nitrosospira multiformis*) also observed that the methane oxidizer *Methylosinus trichosporium* was between 32 and 36 ‰, whereas the site preference in the N_2O with nitrite as the substrate was near zero. This finding agrees with the site preference observed during denitrification by *Pseudomonas chlororaphis* and *Pseudomonas aureofaciens*. This approach will enable quantification of the contribution of the different pathways to the N_2O emission from soils and not only from pure culture.

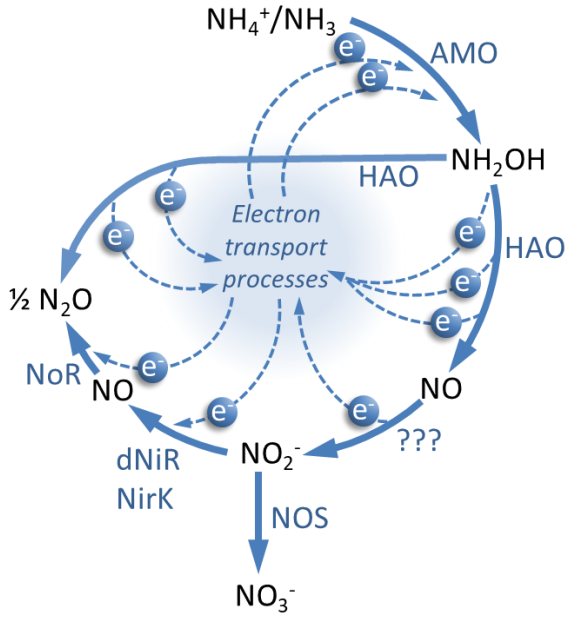

Figure 2.4: Pathways of N_2O production during autotrophic nitrification by bacteria with the enzymes responsible for the autotrophic N_2O production (direct by HAO, and nitrifier denitrification via nitrite), based on Kim et al. (2010), adapted to the findings of Caranto and Lancaster (2017).

bacteria. They discussed the question of whether ammonia (NH_3) or ammonium (NH_4^+) is the substrate for the archaeal AMO enzyme, which is particularly important for the observation that AOA is dominant in acid soils, where the equilibrium between ammonia and ammonium is far to the ammonium side (pKa 9.3). In addition, no evidence was observed that AOA possesses the HOA gene, a key gene for the N_2O production by nitrification. Walker et al. (2010) proposed nitroxyl as an intermediate for nitrite production by archaeal ammonia oxidizers (Fig. 2.3 pathway 6). Nitroxyl is also involved in the hybrid N_2O formation (Fig 11.3, Chapter 11). Stieglmeier et al. (2014) compared the ammonia-oxidizing bacterium *Nitrosospira multiformis* with the AOA *Nitrososphaera viennensis* and *Nitrosopumilus maritimus* and observed similar N_2O product ratios under comparable conditions. However, the ^{15}N experiments suggest that the N_2O production by the archaeal strains followed a hybrid formation pathway. Hybrid N_2O and hybrid N_2 formation is described in detail in Chapter 11.

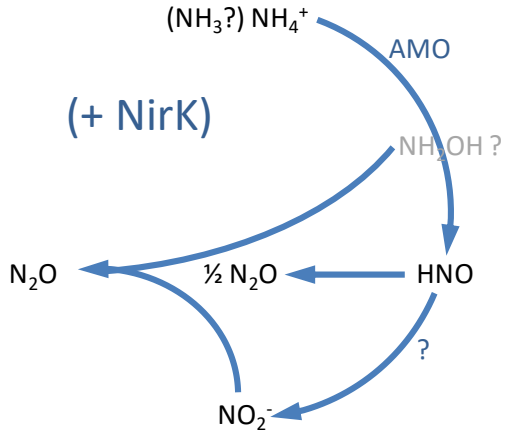

Figure 2.5: Proposed pathway of aerobic oxidation of ammonium to nitrite by archaea and two recently discovered pathways for N_2O production (direct via HNO, and hybrid N_2O formation). Additionally, the use of ammonium instead of ammonia is under discussion and would explain the acid tolerance of the archaeal nitrification.

N_2O PRODUCTION BY AMMONIA OXIDIZING ARCHAEA AOA

Zhalnina et al. (2012) suggest that the pathway for ammonium oxidation in ammonia oxidizing archaea (AOA) differs from the pathway outlined for

N_2O PRODUCTION BY HETEROTROPHIC NITRIFIER

Increasingly, evidence supports that heterotrophic nitrification is widespread in soils and important for N_2O production in acid soils.

Heterotrophic nitrification is not linked to energy gain for cellular growth, and N_{org} oxidation as well as ammonia oxidation by heterotrophic microorganisms was confirmed (Martens-Habbena et al., 2009). If sufficient C substrate exists to enable heterotrophic microbial growth, microbial heterotrophs have been suggested to be more competitive for NH_4^+ than AOB (Papen et al., 1989; Tietema and Wessel, 1992; Martens-Habbena et al., 2009). Soil pH value is accepted as a strong separator for the different pathways and for nitrification in acidic forest and acidic pasture soils, and for those with low C/N ratios, heterotrophic nitrification has been suggested to be the predominant NO_3^-– production pathway (De Boer and Kowalchuk, 2001; Daum et al., 1998; Huygens et al., 2008; Chapter 10; Islam et al., 2007; Killham, 1990; Schimel et al., 1984; Isobe, 2012, Zhang et al. 2015). On the other hand, in arable soils, the production of nitrate by heterotrophic microorganisms appears to be insignificant relative to that by autotrophic bacteria or archaea (e.g., Cheng et al., 2015). Heterotrophic nitrification is catalysed by a variety of microorganisms, including fungi, actinomycetes, and bacteria, presumably using a wide variety of metabolic pathways. In a recent overview article, Prosser et al. (2007) concluded that this great physiological diversity of heterotrophic nitrifiers was caused by an increase in the range of environments and environmental conditions in which nitrification is possible. Fungal activity was confirmed as a key driver in the soil nitrogen cycles (e.g. Laughlin and Stevens, 2002), and mounting evidence suggests that fungal activity contributes significantly to the soil emission of N_2O (e.g. Laughlin et al. 2008). N_2O production during heterotrophic nitrification has been confirmed for fungi, such as *Aspergillus flavus,* and for bacteria, such as *Thiosphaera pantothrapha* and *Alcaligenes fecalis*. A wide variety of heterotrophic nitrifiers can accomplish both nitrification and denitrification and also generate N_2O under aerobic condition (Robertson and Groffman, 2007). For example, McLain and Martens (2006) suggest that, in the investigated semiarid soil, heterotrophic oxidation of organic N is a major contributor to N_2O production. They observed that N_2O production is strongly correlated with the activity of fungi rather than with the activity of the bacteria (McLain and Martens, 2006). Zhang et al. (2018) observed high contributions of heterotrophic nitrification to the N_2O emission from forest soils (as observed in Chapter 10) and confirmed soil pH and C/N ratio as key factors to designate the N_2O production pathway. Zhang et al. (2015) reviewed recent investigations on the contributions of heterotrophic nitrification to the N_2O production in soils and found that the specific N_2O production per nitrified N by heterotrophic nitrification (heterotropic N_2O product ratio) is much higher than that in autotrophic nitrification. Notably, the determination of heterotrophic nitrification rate is challenging, and high uncertainty must be considered when interpreting these data.

Evidence exists for two different pathways for heterotrophic ammonia oxidation. The first is similar to that of autotrophic oxidation in that the nitrifying bacteria have a genome similar to that of the *amoA* gene of autotrophic ammonia oxidizers (Daum et al., 1998). The second heterotrophic pathway is organic and appears limited to fungi. This pathway involves the oxidation of amines or amides to a substituted hydroxylamine, followed by oxidation to a nitroso and then a nitro compound. The pathway for heterotrophic N_2O production is unclear, but great physiological diversity of heterotrophic nitrifiers can be concluded to cause a wide variety of N_2O formation pathways and the pathways described before are also concluded to be common for heterotrophic nitrification. The high heterotrophic N_2O product ratios reviewed by Zhang et al. (2015) suggest that the nitrifier denitrification pathway is important. However, especially for fungi, codenitrification was confirmed as hydride N_2O formation (see Chapter 11).

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Zhang, Y., Zhao, W., Cai, Z., Müller, C., Zhang, J., (2018). Heterotrophic nitrification is responsible for large rates of N_2O emission from subtropical acid forest soil in China. European Journal of Soil Science, 69(4): p. 646-654.

CHAPTER 3:

AUTOMATED AND RAPID ONLINE DETERMINATION OF ^{15}N ABUNDANCE AND CONCENTRATION OF AMMONIUM, NITRITE OR NITRATE IN AQUEOUS SAMPLES BY THE SPINMAS TECHNIQUE

Stange CF, Spott O, Apelt, B, Russow R.. Isotopes in Environmental and Health Studies 43 (3) 227-236: 2007.

http://www.tandfonline.com/doi/abs/10.1080/10256010701550658

ABSTRACT

On the basis of the principle of Reaction Continuous-Flow Quadrupole Mass Spectrometry an automated Sample Preparation unit for Inorganic Nitrogen (SPIN) species was developed and coupled to a quadrupole Mass Spectrometer (MAS). The SPINMAS technique was designed for an automated, sensitive, and rapid determination of ^{15}N-abundance and concentration of a wide variety of N-species involved in nitrogen cycling (e.g. NH_4^+, NO_3^-, NH_2OH etc.). In this paper the SPINMAS technique is evaluated with regard to the determination of ^{15}N-abundance and concentration of the most fundamental inorganic nitrogen compounds in ecosystems such as NH_4^+, NO_2^-, and NO_3^-. The presented paper described the newly developed system in detail and demonstrated the general applicability of the system. For a precise determination of ^{15}N-abundance and concentration a minimum total N-amount of 10 µg NH_4^+-N, 0.03 µg NO_2^--N, or 0.3 µg NO_3^--N have to be supplied. Currently, the SPINMAS technique represents the most rapid and only fully automated all-round method for a simultaneous determination of ^{15}N abundance and total N-amount of NH_4^+, NO_2^-, or NO_3^- in aqueous samples.

CHAPTER 4:
SHORTCOMINGS IN THE COMMERCIALIZED BAROMETRIC PROCESS SEPARATION MEASURING SYSTEM

Ingwersen J, Schwarz U, **Stange CF**, Xiaotang J, Streck T.. SSSAJ 72 (1): 135-142, 2008.

https://acsess.onlinelibrary.wiley.com/doi/pdf/10.2136/sssaj2007.0092

ABSTRACT

In a growing number of studies the Barometric Process Separation (BaPS) has been applied for measuring gross nitrification rates in soil. In 2000, the company Umweltanalytische Mess-Systeme (UMS) Ltd. (Munich, Germany) presented the sole commercially available automatic BaPS measuring system. In an ongoing project we have used the UMS-BaPS system for measuring gross nitrification rates in an alkaline soil. During data evaluation we came across some shortcomings in the calculations implemented in the UMS data evaluation software. We identified three problems: (1) an unit error in the calculation of the carbonate equilibrium, (2) an erroneous calculation in case of a respiration quotient unequal to unity, and (3) an inappropriate procedure for handling negative ΔNxOy values. Particularly the flaw in the calculation of the carbonate equilibrium causes a tremendous overestimation of the gross nitrification rate at pH values above six. In a literature review we identified three studies that applied the UMS-BaPS system for measuring gross nitrification in soils with a pH value higher than six. A re-evaluation of the data would be necessary to clarify whether the results were affected by the shortcomings in the UMS-BaPS system. Moreover, the literature review showed that the BaPS method works well in acidic to weakly acidic soils. For soils with higher pH values at present only one study tested the BaPS against the ^{15}N pool dilution technique. The results of this study indicate that in weakly neutral to alkaline soils the BaPS method is less accurate due to uncertainties in the computation of the carbonate equilibrium in soil solution. More research is needed to test the applicability of the BaPS method in neutral and alkaline soils and to find new methods to quantify accurately the transfer of gaseous carbon dioxide to soil solution during the incubation period.

CHAPTER 5:

^{15}N TRACING MODEL SIMKIM TO ANALYSE THE NO AND N_2O PRODUCTION DURING AUTOTROPHIC, HETEROTROPHIC NITRIFICATION, AND DENITRIFICATION IN SOILS

Stange F, Döhling F. Isotopes in Environmental and Health Studies, 41(3): 261-274, 2005.

https://www.tandfonline.com/doi/abs/10.1080/10256010500230205

ABSTRACT

An adjusted model was developed to analyse measured data of nitrous oxide and nitric oxide fluxes from an arable black earth soil. The existing models for kinetic isotope studies ignore N-trace gas fluxes. The novel model includes both N-gas production by heterotrophic and autotrophic nitrification and N-gas production and consumption by denitrification. Nitrous oxide and nitric oxide production through nitrification was simulated following the 'hole-in-the-pipe' model ([4]: M.K. Firestone et al. Microbiological basis of NO and N_2O production and consumption in soil), N-gas production by denitrification was described with first-order kinetics.

The model has been evaluated in a triplicate laboratory experiment, which involved three treatments (glycine, NH_4^+ , or NO_3^- -pool labeled) to distinguish the different sources of N_2O and NO.

Heterotrophic nitrification was negligible, whereas autotrophic nitrification and denitrification occur simultaneously in soils. Nitrification was the main source of NO and N_2O in the black earth soil by field capacity (water content: 0.22 g H_2O g^{-1} soil). The NO release was higher than the N_2O release, the N_2O/NO ratio was 0.05 in this soil.

CHAPTER 6:
AN INVERSE ABUNDANCE APPROACH TO OPTIMIZE A SEPARATION OF SOIL N POOLS AND GASEOUS N FLUXES INTO PROCESS-RELATED FRACTIONS

Stange CF, Spott O, Müller C. European Journal of Soil Science, 60: 907-915, 2009.

https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2389.2009.01188.x

SUMMARY

The soil nitrogen cycle exhibits a variety of complex biochemical reactions in which nitrogen species such as NO_2^-, NO, and N_2O are produced and consumed by coexisting processes which respond differently to the local environmental conditions. Key to understanding the soil nitrogen cycle in its full complexity is the development and application of methods that allow a quantification of individual pathways and processes that are responsible for the build up and/or emission of N compounds. Triplet ^{15}N tracer experiments (TTE) have been developed and applied to allow a source-related quantification of nitrogen species (e.g. NO_2^-, N_2O) by different biochemical pathways (e.g. ammonia oxidation, nitrate reduction) that are related to multiple nitrogen sources (NH_4^+, NO_3^-, N_{org}). An analysis of a TTE requires the application of either a numerical or analytical model. Due to the ease of application it is desirable to use analytical models. However, available analytical solutions suffer from serious drawbacks concerning the quantification of nitrogen fluxes related to soil organic nitrogen. In this paper we describe the development and application of a new inverse abundance approach (IAA) to analyse a TTE. A theoretical as well as experimental data set of soil N_2O release was analysed by the new method. The IAA was also applied to a data set by Müller et al. (2006) to identify fractions of the soil nitrite pool related to NH_4^+, NO_3^-, and Norg. We show that the IAA provides a reliable and comprehensive data evaluation of a TTE.

CHAPTER 7:
A NOVEL APPROACH TO COMBINE RESPONSE FUNCTIONS IN ECOLOGICAL PROCESS MODELING

Stange CF.. Ecological Modelling, 204 (3-4): 547-552, 2007.

https://www.sciencedirect.com/science/article/abs/pii/S0304380007000233

ABSTRACT

A novel approach to combining response functions, e.g. temperature and soil moisture dependency, is presented. This approach is in analogy of resistances connected in parallel and mathematically to the inverse function of the sum of reciprocal response functions. The approach presented is applicable for a wide range of response functions, and demonstrate better performance as the multiplicative approach if the limiting factor dominates the process rate more than the other factors. It was applied to a gross nitrification data set acquired from beech litter samples in the laboratory using the Barometric Process Separation (BaPS) method. Compared with the minimum and the multiplicative approaches, the best fit was achieved with the novel approach, using the Residual Sum of Squares and r^2 values as indicators. Additionally, two examples from the literature were presented to demonstrate the potential and benefits of the approach, which is a good alternative combining two or more response functions.

CHAPTER 8:
MEASURING AND MODELLING SEASONAL VARIATION OF GROSS NITRIFICATION RATES IN RESPONSE TO LONG-TERM FERTILISATION

Stange CF, Neue HU. Biogeosciences, 6: 2181-2192, 2009.

http://www.biogeosciences.net/6/2181/2009/bg-6-2181-2009.pdf

ABSTRACT

The formation of nitrate (nitrification) in soils is an important process that influences N availability for plant uptake and potential N losses as well. Gross nitrification is an effective measure by which to test mechanistic ecosystem models for predictability because gross rates can widely differ between sites, even if net production is similar between these sites.

A field experiment was designed to (i) determine gross nitrification rates in response to fertilisation and (ii) to verify the idea that seasonal variations of gross rates in soils can be readily predicted by soil moisture and soil temperature.

Gross nitrification rates were measured by a Barometric Process Separation (BaPS). The BaPS measurements were validated with the commonly used ^{15}N pool dilution technique measurements at six times. In general, the rates determined from both measurement approaches were in the same order of magnitude and showed a good correlation.

The effects of 100 years of fertilisation (mineral fertiliser, manure and control) on gross nitrification rates were investigated. During 2004 soil samples from the long-term "static fertilisation experiment" at Bad Lauchstädt were sampled weekly and were measured in the laboratory under field conditions and subsequently under standardised conditions (16 °C soil temperature and -30 kPa matrix potential) with the BaPS system. Gross nitrification rates determined under standardised conditions did not show any seasonal trend but did, however, reveal a high temporal variability. Gross nitrification rates determined by the BaPS-method under field conditions showed also a high temporal variability and ranged from 5 to 77 µg N h^{-1} kg^{-1} dry mass, 2 to 74 µg N h^{-1} kg^{-1} dry mass and 0 to 49 µg N h^{-1} kg^{-1} dry mass with respect to manure, mineral fertiliser, and control. The annual average was 0.34, 0.27 and 0.19 g N a^{-1} kg^{-1} dry mass for the manure site, mineral fertiliser site and control site, respectively. On all sites gross nitrification revealed a strong seasonal dynamic. Three different models were applied for reproducing the measured results. Test models could explain 75 % to 78 % of variability at the manure site, 66 % to 77 % of variability at the mineral fertiliser site, and 39 % to 63 % of variability at the control site. The model parameterisation shows that the temperature sensitivity of gross nitrification differs between the three neighbouring sites. Hence, a temperature response function in an ecosystem model has to consider the site specificity in order to adequately predict the effects of future climate change on the soil N cycle.

CHAPTER 9:
ANALYSIS OF THE COEXISTING PATHWAYS FOR NO AND N_2O FORMATION IN CHERNOZEM USING THE ^{15}N-TRACER SIMKIM-ADVANCED MODEL

Stange CF, Spott O, Russow R.. Isotopes in Environmental and Health Studies, 49 (4) 503-519, 2013.

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ABSTRACT

The nitrogen cycle consists of a variety of microbial processes. These processes often occur simultaneously in soils, but do respond differently to local environmental conditions due to process-specific biochemical restrictions (e.g., oxigen levels). Hence, soil N cycling (e.g., soil N gas production through nitrification and denitrification) is individually affected through these processes, resulting in the complex and highly dynamic behavior of total soil N turnover. The development and application of methods that facilitate the quantification of individual contributions of coexisting processes is a fundamental prerequisite for (i) understanding the dynamics of soil N turnover and (ii) implementing these processes in ecosystem models.

To explain the unexpected results of the triplet tracer experiment (TTE) of Russow et al. [1], the existing SimKIM model was extended to SimKIM-Advanced model through the addition of three separate nitrite sub pools associated with ammonia oxidation, organic N (Norg) oxidation, and denitrification, respectively. For the TTE, individual treatments with ^{15}N-ammonium, ^{15}N-nitrate, and ^{15}N-nitrite were conducted under oxic, hypoxic, and anoxic conditions, respectively, to clarify the role of nitric oxide as a denitrification intermediate during N_2O formation. Using a split nitrite pool, this analysis model explains the observed differences in the ^{15}N enrichments in nitric oxide (NO) and nitrous oxide (N_2O), which occurred in dependence on different oxygen concentrations. The change from oxic over hypoxic to anoxic conditions only marginally increased the NO and N_2O release rates (1.3-fold). The analysis using the model revealed that, under oxic and hypoxic conditions, Norg-based N_2O production was the dominant pathway, contributing to 90 and 50% of the total soil N_2O release. Under anoxic conditions, denitrification was the dominant process for soil N_2O release. The relative contribution of Norg to the total soil NO release was small. Ammonia oxidation served as the major pathway of soil NO release under oxic and hypoxic conditions, while denitrification was dominant under anoxic conditions. The model parameters for soil with moderate soil organic matter (SOM) content were not scalable to an additional data set for soil with higher SOM content, indicating a strong influence of SOM content on microbial N-turnover. Thus, parameter estimation had to be re-calculated for these conditions, highlighting the necessity of individual soil-dependent parameter estimations.

CHAPTER 10:

USE OF THE INVERSE ABUNDANCE APPROACH TO IDENTIFY THE SOURCES OF NO AND N_2O RELEASE FROM SPANISH FOREST SOILS UNDER OXIC AND HYPOXIC CONDITIONS

Stange, CF, Spott, O, Arriaga, H, Menéndez, S, Estavillo, JM, Merino, P, 2013. Soil Biology and Biochemistry 57, 451-458, 2013.

http://dx.doi.org/10.1016/j.soilbio.2012.10.006

ABSTRACT

Forest soils exhibit a variety of complex biochemical nitrogen (N) reactions in which nitric oxide (NO) and nitrous oxide (N_2O) can be produced by coexisting processes that respond differently to the same environmental conditions. In general, two biochemical processes, (i) the oxidation of ammonia (nitrification) and (ii) the reduction of nitrate (denitrification), are known as the major sources of nitrogen oxides. Few reports indicated that a direct oxidation of soil organic N compounds (Norg) to NO and N_2O may also be significant in soils.

A ^{15}N triplet tracer experiment (TTE) combined with an inverse abundance approach (IAA) was applied to quantify NO and N_2O formation in soil related to different but simultaneously utilised soil N sources (ammonium, nitrate, and Norg). In addition, the impact of oxic and hypoxic conditions (21 and 2 % v/v O2, respectively) on total soil NO/N_2O release and source composition was studied. Experiments were conducted with soil samples from 5 different Basque forest stands (mature beech, young beech, mature pine, young pine, and new pine plantation). The release rates of NO and N_2O were higher in the soil samples from beech stands than in the samples from pine stands. The change from oxic to hypoxic conditions increased the NO release rate 2- to 14-fold and the N_2O release rate 3.6- to 25-fold. The study suggests that, under oxic conditions, N_2O formation based on N_{org} appears to be the dominant pathway of soil N_2O production (48 to 76 % to total N_2O release). Under hypoxic conditions, the relative contribution of N_{org} significantly decreased, whereas its absolute contribution increased concomitantly. Denitrification was the dominant process of soil N_2O release under hypoxic conditions and served as the major pathway of soil NO release under both oxic and hypoxic conditions (40 and 60 % of total soil NO release, respectively).

We conclude that the individual contribution of different soil N pools to the total soil N gas release and the impact of environmental parameters (e.g., O_2 availability) are site-specific. Nonetheless, further research is required to elucidate the impact of forest stands on soil NO and N_2O production, particularly N_2O formation directly based on N_{org} transformation.

CHAPTER 11:

FORMATION OF HYBRID N_2O AND HYBRID N_2 DUE TO CODENITRIFICATION: FIRST REVIEW OF A BARELY CONSIDERED PROCESS OF MICROBIALLY MEDIATED N-NITROSATION.

Spott, O, Russow R, **Stange, CF**, 2011. Soil Biology and Biochemistry 43, (10): 1995-2011.

doi: 10.1016/j.soilbio.2011.06.014

ABSTRACT

Already at the end of the 19th century an experimental study reported N gas production during microbial nitrate reduction, which significantly exceeded the amount of nitrate N supplied to the microorganism. The observed excess gas production was suggested to be caused by a reaction of nitrous acid (produced during microbial nitrate reduction) with amino acids contained in the nutrient solution. Since the 80ies of the former century a number of ^{15}N tracer experiments revealed that this biotic excess gas production is based on a formation of hybrid N_2O and/or hybrid N_2. It was shown that the N-N linkage is formed due to a microbially mediated N-nitrosation reaction by which one N atom of nitrite or nitric oxide combines via a nitrosyl intermediate with one N atom of another N species (e.g. amino compound). Because of its cooccurrence with conventional denitrification this process was later on termed "codenitrification". Although the phenomenon of N_2O and N_2 formation by codenitrification is known since more than a century its impact on global N cycling is still unclear today. Nonetheless, the present literature review reveals codenitrification as a potentially important process of biospheric N cycling since (i) most codenitrifying species are already known as typical denitrifiers (e.g. *Pseudomonas spec.*, *Fusarium spec.* etc.) and (ii) codenitrification was already reported to occur within the three domains *archaea*, *bacteria*, and *eukarya* (kingdom *fungi*). Furthermore, the present literature suggests that codenitrification does not only act as an additional source of N gas formation due to a mobilisation of organic N by N-nitrosation, but also acts as an N immobilising process due to a bonding of inorganic N (e.g. from NO_3^- or NO_2^-) onto organic compounds due to e.g. N- or even C-nitrosation reactions. From this it can be concluded that N gas formation by codenitrification represents a sub-phenomenon of a variety of possible biotic nitrosation reactions. Moreover, the review reveals that biotic nitrosation also occurs among nitrifying species, even under aerobic conditions. Furthermore, recent studies support the assumption that even anaerobic ammonium oxidation (anammox) appears to be based on biotically mediated N-nitrosation. Therefore, we propose to introduce the term BioNitrosation, which includes all biotically mediated nitrosation reactions resulting either in N gas production or in N immobilisation, independently from the acting microbial species or the environmental conditions.

CHAPTER 12: SYNTHESIS

This chapter should not be considered a summary (that was provided in Chapter 1. Aim and Scope); instead, it is a synthesis that brings the elements of the individual chapters together in an entirely new way to form a new proposition. In general, consensus exists that nitrification is a key process in the soil nitrogen cycle, and intensification of agriculture and the subsequent increased fertilization regimes result in higher nitrification rates. Increasingly, studies have found that nitrification is the dominant N_2O source in aerated soils (e.g., Bollmann and Conrad, 1998; Morkved et al., 2007; Cheng et al., 2012; Chapter 9 and 10). Morse and Bernhard (2013) conclude from the results of a stable isotope tracer experiment that nitrification contributes to an important and underappreciated role in the N_2O emission from wetlands with acid-organic soils. Zhu et al. (2013) showed nitrifier denitrification is the dominant pathway of N_2O production at O_2 concentrations ≥0.5 %. For example, in a high-arctic lowland ecosystem, Ma et al. (2007) found that at 50 to 55 % WFPS, nitrification by ammonia oxidizing bacteria dominates the N_2O production in the soil to more than 80%. Cheng et al. 2015 observed in a review that pH is a critical factor regulating the contributions of nitrification and denitrification to the total N_2O emission and postulate that, below pH 4.8, nitrification was the dominant process contributing to the N_2O production in soils.

Despite these observations, to date, nitrification as source for N_2O is considered insufficient in biogeochemical models to predict soil nitrogen cycle and N gas fluxes (e.g., Hu et al., 2015; Chen et al., 2008). Most of the models calculate only net nitrification, and only a few approaches for more complex modelling of nitrification-derived N_2O have been published, for example, Li et al. (2001) and Rubol et al. (2013). The need exists to implement the current knowledge about abundance and community structure of microbes and to develop new model structures and evaluate more hypotheses for N_2O production by nitrification, especially by linking nitrification and its N_2O production with other processes in the soil. For example, Zhang et al. (2015) proposed transfer of the HIP-Model from Firestone and Davidson (1998) to heterotrophic nitrification and calculated the heterotrophic N_2O product ratio from the available data. Those authors found that the specific N_2O production per nitrified N by heterotrophic nitrification depends on the soil organic C content, but the available data are limited.

While the contribution of nitrification to the N_2O emission from soils is large, its regulation is poorly understood. To date, two approaches are commonly used to model N_2O production due to nitrification. First, the N_2O production depends on the substrate pool (see model Chapter 9), and in the second approach, the N_2O production depends on the turnover rate (see model Chapter 5). This approach is usually known as the 'hole-in–the-pipe' (HIP) model offered by Firestone and Davidson (1989). Ni et al. (2013) used four different metabolic computer models to elucidate the mechanisms of aerobic N_2O production by nitrification, but the widespread diversity of the microorganisms and individual enzymes involved caused a multitude of possible pathways, with individual regulation and specific responses to environmental conditions complicating a simple prediction. Until now, the contribution of different nitrification pathways and involved microorganisms have been difficult to separate (Zhang et al. 2015) and still are under debate (e.g., de Boer and Kowalchuk, 2001; Banning

et al., 2015). Detailed information is given in Chapters 2, 6, and 11.

Nevertheless, to date, no comprehensive analysis modelling the N_2O product ratio according to the most important factors has been presented to my knowledge. Therefore, a single chapter on the current state of knowledge about the dependence on environmental factors is provided as a review, and a synopsis of the available data is presented. The proposed approach includes data presented in the previous chapters, includes additional literature, and does not make distinctions among the contributions of the different microorganisms and pathways.

ENVIRONMENTAL FACTORS AFFECTING SOIL N_2O PRODUCT RATIO OF NITRIFICATION

Based on the 'hole-in–the-pipe' (HIP) model offered by Firestone and Davidson (1989), an extended model should be derived from the present results and from additional data from the literature. In the HIP-model, the flow-through-the-pipe rate of N turnover (e.g., nitrification) and the leak-out-of-the-holes rate are analogous to the N gas production. The sizes of the holes will be determined by environmental conditions, and Firestone and Davidson have suggested that the size is determined primarily by the soil moisture. N_2O product ratios of nitrification, equivalent to the size of the holes, were defined as the amount of N emitted as N_2O during nitrification as per mill of the nitrified N by nitrification, calculated using Eq. 12.1:

$$R = N_2O\_N / NO_3^-\_N * 1000 \qquad \text{(Eq. 12.1)}$$

where R is the N_2O product ratio (‰).

The N_2O product ratios of nitrification observed in this work and in the literature are wide ranging. Morkved et al. (2007) demonstrated the limitation of the N_2O product ratio concept for specific soils, in particular for soils with very low pH values. Therefore, the aim of this work was to find the important factors for inclusion in an enhanced N_2O product ratio model.

SOIL MOISTURE AND O_2 CONCENTRATION

Contrary to the observations from single experiments (Stevens et al., 1997; Maag and Vinther, 1996; Goodroad and Keeney, 1984; Bollmann and Conrad, 1998; Klemedtsson et al., 1988; Mathieu et al., 2006; Liu et al., 2017), overall, the N_2O product ratios showed no significant correlation to indicate that they were determined by the soil water content (expressed in WFPS; water field pore space) or WHC (water holding capacity). Notably, problems with methodology may influence the results. Due to the different methods used to describe soil moisture in the studies (vol%, weight%, WFPS and % WHC), scaling factors had to be used to calculate %WHC or WFPS from the other units. The scaling factors used were estimated from soil type and bulk density, if that information was available. Consequently, agreement on a single standard method for soil moisture or a published report on site-specific scaling factors among the different methods is urgently required for better comparability. In contrast to the observation cited before, Stange and Neue (2009) observed that the determined N_2O product ratios can be weakly explained by soil moisture alone. No clear trend over the investigated range of moisture was observed by Khalil and Baags (2005) in their study. In contrast to most studies, Cheng et al. (2012) observed decreasing N_2O product ratios with increasing soil moisture (30 to 90 % WHC) in the two soils, which was caused by the stronger increase of the nitrification rate with the increasing soil moisture compared to the N_2O production rates. Overall, I hypothesized that soil moisture is more important as an indirect factor that drives the soil O_2 concentration, than as a direct factor. This hypothesis may also explain the high variability of the observed response in the different soils.

However, differentiating between the direct influence of soil moisture and the indirect influence (e.g., by determining the oxygen concentration of soils) is challenging (Drury et al., 1992). Soil moisture influences most processes involved in the O_2 household of soils, and therefore, it is the most important predictor. Due to the complex interaction between the involved factors, no simple correlation between O_2 concentration and soil moisture could be expected (Smith, 1980; Tiedje et al., 1984).

Studies dealing with both the direct influence of O_2 and the complex influence of soil moisture (e.g., Bollmann and Conrad, 1998) suggest that the O_2 influence outcompetes the influence of soil moisture.

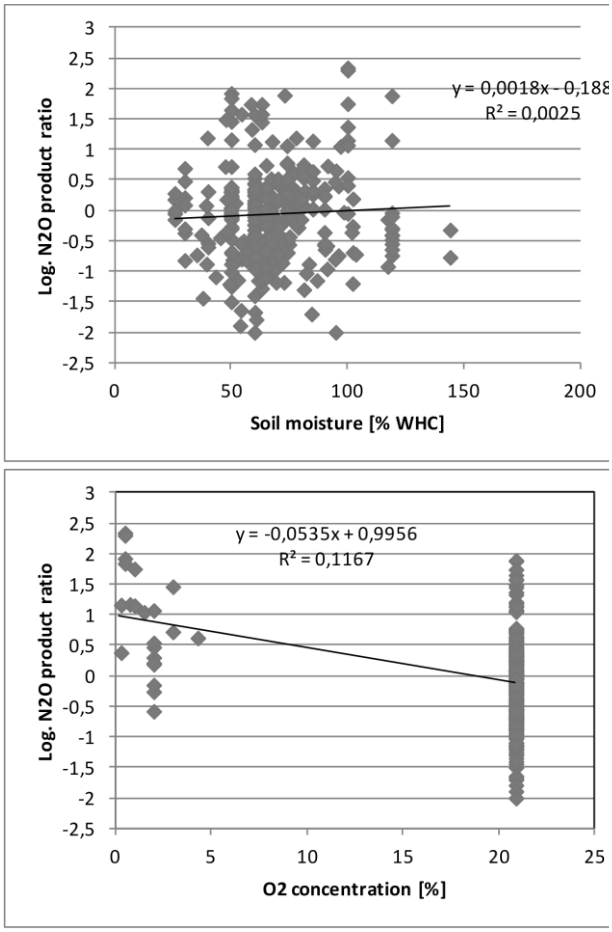

Fig 12.1: Relationship between soil moisture (given in % water holding capacity (WHC) (top) or the O_2 concentration (bottom) and the logarithmic value of the N_2O product ratios of nitrification.

Zhu et al. (2013) demonstrated how O_2 concentration regulates the magnitude and pathways of N_2O production by working with batch reactions with nitrifiers supporting the pre-eminence of the O_2 available for nitrification and particularly for the N_2O product ratio from nitrification (e.g., Peng et al., 2014). Based on these results and the previous chapter, I hypothesized that the change in nitrification rates in soils and N_2O product ratios is caused by changing the O_2 availability at high soil moisture and changing the substrate availability at low soil moisture rather than by the soil water itself. Soil moisture and oxygen concentration were considered when modelling the N_2O production by nitrification in process-oriented ecosystem models (e.g., Stange, 2001; Rubol et al., 2013). Whereas Stange (2001) suggested the N_2O product ratio depends on the soil temperature and soil moisture, based on the results of Maag and Vinther (1996) and Ingwersen et al. (1998), Rubol et al. (2013) modelled the N_2O product ratio as dependent on the oxygen concentration.

Khalil et al. (2004) found that nitrification was the main source of N_2O under oxic and hypoxic condition (≥0.76 kPa), and the N_2O product ratio of nitrification was dependent on the O_2 concentration. The N_2O product ratio of nitrification increased from 1.6 to 14.8‰ when the O_2 decreased from 20.4 to 0.76 kPa. In addition, Zhu et al. (2013) observed N_2O product ratios of nitrification for soils under ambient O_2 concentration (0.8 to 1.1‰), whereas the N_2O product ratios of nitrification were two orders of magnitude higher when the O_2 concentration was lowered to 0.3 kPa. Other studies with pure autotrophic nitrifier cultures had shown a strong influence of the O_2 concentration on the N_2O product ratio of nitrification for marine bacteria. The observed N_2O product ratios by Goreau et al. (1980) can be calculated as being approximately $r=40/O_2$, if the oxygen concentration O_2 is given in %.

An analysis of all available data has showed a significant correlation between the logarithmic value of the N_2O product ratio and the O_2 concentration. The efficacy of this model is low (r^2=0.117), and results are marginally better with a logarithmic regression model (r^2=0.132). One reason for the low predictability may be the assumed O_2 concentration of 20.9 % for experiments deducted under normal atmosphere. In most investigations, the real O_2 concentration is unknown, which is a drawback, and therefore, the O_2 concentration must be assumed for this analysis. Because O_2 concentration is an influential factor, more care must take in the determination of the real O_2 condition in the experiments, including the high spatial variability of the O_2 concentration in the soil samples and soil aggregates. Therefore, the assumed O_2 concentration of 20.9 % for the experiments under normal atmosphere is a very rough estimation (upper bound), and the real O_2 concentration varies positively with the soil

moisture and O_2 consumption in the soil sample (for more information about O_2 concentration in real soils, see e.g., Smith, 1980). Consequently, the assumption of constant 20.9 % is too high in most cases, and I suggest that the determination of real O_2 concentration in future studies would lead to a better predictability of the N_2O product ratio by nitrification. Despite the strong influence of oxygen on N_2O product ratios in soil samples, Stieglmeier et al. (2014) observed no dependency of the N_2O product ratio using a pure culture of AOA. This lack of dependency may be caused by the AOA being incapable of generating N_2O via nitrifier denitrification. The different N_2O production pathways in the different microorganism groups and the divergent response to O_2 change must be considered when simulating N_2O production during nitrification in ecosystems, particularly if nitrification is dominated by AOA.

SOIL TEMPERATURE

Maag and Vinther (1996) investigated the relative change of the N_2O product ratio with temperature and soil moisture for the sandy loam. The ratio decreased to a third with increasing temperature in the interval of 5 to 20°C. Li et al. (2001) implemented this moisture and temperature dependency in the PNET-N-DNDC model to predict N_2O production by nitrification. Maag and Vinther (1996) hypothesized that higher nitrite accumulation at lower temperature might be causing the increase in the N_2O product ratio of nitrification with decreasing temperature. In contrast (Goodroad & Keeney, 1984) reported an increasing N_2O product ratio with temperature (interval 10 to 30 °C), but these ratios were calculated without distinguishing between nitrification and denitrification. Additionally, Lang et al. (2011) investigated the influence of temperature to the N_2O emission and nitrification and observed no consistent trend in all four soils over the incubation time of 15 days. In all soils, the N_2O product ratio of nitrification was higher in the treatment with higher temperature (15°C versus 10°C) at day 1, but during the incubation in the same soils, the temperature effect was marginal or contrasted with the effect at the beginning.

In addition to determining the nitrification rate (Chapter 8), Stange and Neue (2009) determined rates of the N_2O released from these soil cores from differently treated plots of the long-term static fertilization experiment 'Bad Lauchstädt'. The N_2O emissions show very high temporal variability and ranged from 0 to 192 $ngNh^{-1}\ kg^{-1}$ dry matter (DM), 0 to 372 $ngNh^{-1}\ kg^{-1}$ DM, and 0 to 16 $ngNh^{-1}\ kg^{-1}$ DM with respect to the manure fertilizer site, the mineral fertilizer site, and the control site. The N_2O product ratios of nitrification were calculated for the times where the BAPS system witnessed only nitrification. N_2O product ratios of nitrification ranged from 0.02 to 3.8‰ (Fig. 12.2).

No clear trend was observed from Stange and Neue (2009), where the correlation between the temperature and the N_2O product ratio of nitrification was low, and the general trends (positive or negative) differed between the different fertilizer types. The N_2O product ratios of nitrification were positively correlated with temperature only at the mineral fertilizer site. The N_2O product ratios of nitrification determined in the long-term field experiment during the year 2004 are given in Figure 12.2.

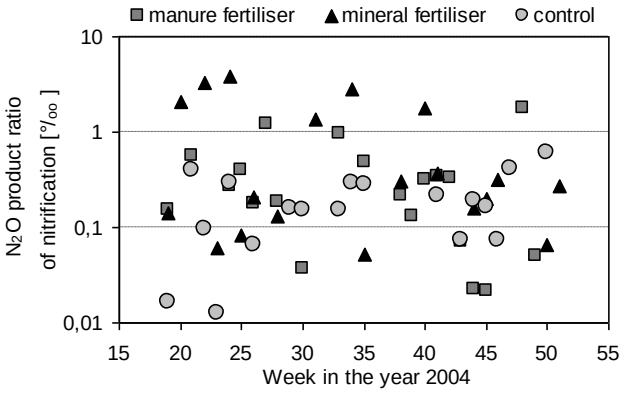

Fig 12.2: N_2O product ratios of nitrification in response to long-term fertilization (manure, mineral or no fertilizer). Please note the logarithmic scale of the y axis (Data from Stange & Neue 2009).

A significant relationship was observed between temperature and the logarithmic value of the N_2O product ratio (Fig. 12.3), but the r^2 was very low due to the high variability at a given temperature.

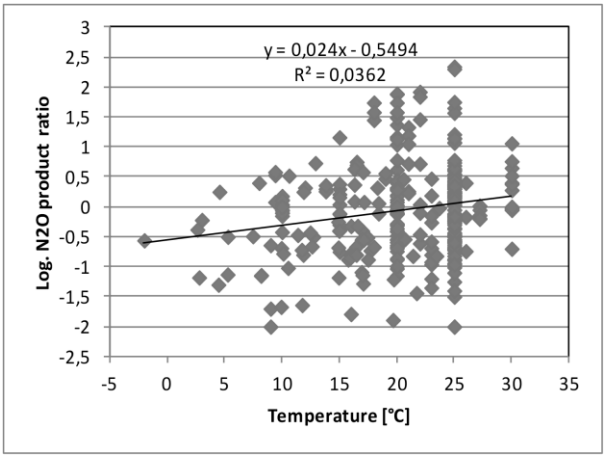

Fig 12.3: Positive relationship between soil temperature and the logarithmic value of the N_2O product ratios of nitrification.

Whether the observed shifts were a direct effect of the temperature or were related instead to changes of processes, for example, changing substrate availability, in response to the increased temperature is unclear. The shifts included a possible shift in the microbial community. Taylor et al. (2017) concluded that the different response of AOA and AOB to temperature could possibly explain the difficulties involved in modelling the response of nitrification to temperature changes in the soils. The observation of Lang et al. (2011) may indicate a changing temperature effect over time, due to changes in the conditions in the soil during the experiment.

FERTILIZATION

Li and Lang (2014) quantified N_2O emission and gross nitrogen transformation rates in a laboratory study with uncultivated and cultivated black soils. They found that the average N_2O emission rate in cultivated soil (21.6 ng N_2O-N kg^{-1} h^{-1}) was significantly higher than that in the uncultivated soil (11.6 ng N_2O-N kg^{-1} h^{-1}). Not only the nitrification rates but also the N_2O product ratios of nitrification were significantly higher in the cultivated soil than in the uncultivated soil. Zhu et al. (2013) cannot explain the enhanced N_2O gas production after N fertilizer applications with the size of the pools (NH_4^+ or NO_3^-) alone and concluded that the NH_3 oxidation pathways contributed a significant portion to total N_2O production under low O_2 availability. These findings are in agreement with the observations in cultivated and uncultivated wetlands in central Saskatchewan (Bedard-Haughn et al., 2006). In a general sense, the nitrification rate was higher, the N_2O product ratio was higher, and the N_2O emission by nitrification was higher in the cultivated soil than in the uncultivated soil. Normally, the N_2O emission by denitrification was also higher in the cultivated soil, but in July, the N_2O emission by denitrification was lower in the cultivated soil, possibly due to the lower WFPS as consequence of the higher water demand by the crops.

The mean N_2O product ratios of nitrification observed by Stange and Neue (2009) for the mineral-fertilized plot (0.96‰), the manure-fertilized plot (0.38‰), and the control sites (0.20‰) support the other observation that fertilization not only had an impact on nitrification rates but also on the N_2O product ratio. The interaction between the fertilizer and the pH values must be considered. Lebender et al. (2014) observed a higher N_2O production due to nitrification if a nitrogen fertilizer, such as urea, which leads to an alkaline condition, is used, than when a fertilizer such, as an ammonium fertilizer, which produces acidic conditions, was used. Additionally, the results of Zhu et al. (2013) support the influence of fertilizer to N_2O product ratio by nitrification.

PH

The soil pH value plays a central role for controlling N_2O emissions due to nitrification (e.g., Cheng et al. 2015), partly by affecting the nitrification rate by itself and the N_2O product ratio of nitrification. The soil pH value has been considered a master factor of N transformation (Morkved et al., 2007), and its strong influence on nitrification and the population of ammonia oxidizers was demonstrated *in situ* and in culture (e.g., Morkved et al., 2007; Nicol et al., 2008, Baggs et al., 2010; Stieglmeiser et al., 2014). Morkved et al. (2007) observed low N_2O product ratios of nitrification for soils with pH ≥5 (0.2 to 0.9‰),

whereas for the soils with pH 4.1 and 4.2, the N_2O product ratios of nitrification was two orders of magnitude higher. They underlined the important role of nitrite for regulating the nitrifier-derived N_2O emission and hypothesized chemo-denitrification of NO_2^- as possible processes for the higher N_2O production at low pH-values. Chemical nitrite conversion to N_2O is generally accepted to rapidly increase with decreasing pH value. Venterea and Rolston (2000) noted that, in addition to the nitrite concentration itself, the protonated form of nitrogen (nitrous acid, HNO_2) is the substrate. This result would explain the pH dependency and the strong change in the range of the pKa value of nitrous acid (pKa 3.3 at 25°C). Cheng et al. (2013) confirmed the observation of the higher N_2O product ratio in the soil with lower pH values and also used pure bacteria cultures to demonstrate that the N_2O product ratio of nitrification increases with decreasing pH values (e.g., Jiang and Bakken, 1999).

REGRESSION ANALYSIS

The determined N_2O product ratios of nitrification presented in Fig. 12.4 were systematically amended by the N_2O product ratios in the available literature. A dataset of 398 N_2O product ratios of nitrification was established for the analysis. If available, comprehensive soil parameters, such as NH_4^+ and NO_3^- content, C_{org} and N_{tot} content, pH value, soil texture, temperature, and soil moisture were included in the dataset during the measurement. Ammonia concentration (NH_3) computed by NH3=0.944*NH4/(10**((2728.8/(temperature+273.15))+0.0925-pH)+1) was tested. Land use was classified in agricultural (A), forest (F), grassland (G) and uncultivated land (u). Observed N_2O product ratios of nitrification were classified in four subsets, depending on the type of determinate nitrification rate (gross/net) and N_2O production (N_2O production by nitrification/total N_2O production). The Indexes 1 to 4 were allocated as follows: 1 includes the ratios from N_2O production by nitrification and gross nitrification, 2 includes the total N_2O production and gross nitrification, 3 includes the N_2O production by nitrification and net nitrification, and 4 includes the total N_2O production and net nitrification if the N_2O production by denitrification might be of minor importance. Statistical tests were performed using SPSS version 20, and if the parameter was log normal distributed, the values were logarithmized. Logarithmic values of the product ratio of nitrification and the NH_4^+, NO_3^-, C_{org} and N_{tot} contents were used in the statistical analysis of the dependency of the N_2O product ratio of nitrification from the soil parameters.

Observed N_2O product ratios were log normal distributed and ranged from 0.006‰ to 220‰, with a geometric mean of 0.99‰ (0.91‰ - 1.08‰) and a median of 1.05‰ (arithmetic mean 5.11‰). The geometric means of the subsets were 0.90‰ (n=138), 0.64‰ (n=127) 2.14‰ (n=56) and 1.34‰ (n=67) for Indexes 1, 2, 3 and 4, respectively (Fig. 12.4).

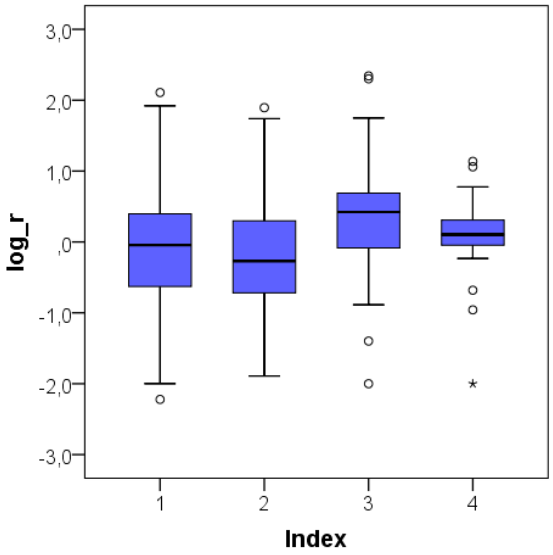

Fig 12.4 Boxplot analysis of the logarithmic values of the product ratio of nitrification depending on the determinate nitrification rate (gross/net) and the N2O origin (nitrification only/total)

The statistical test showed that groups 2 and 4 do not differ significantly from group 1, but the N_2O product ratios from group 3 (N_2O production by nitrification and net nitrification) were significantly higher compared to the other 3 groups. Although the net rates are always lower than or equal to the gross rates and consequently the N_2O product ratios are systematically higher, these data were retained in the analysis. The most obvious cause for the higher value is the relatively high measurements with low O_2 concentration in group 3.

Methodical differences as the acetylene inhibition versus the isotope approach to distinguish between N_2O production by nitrification and denitrification may have caused additional differences in the observations. Acetylene inhibition only suppresses the autotrophic nitrification, and possibly, heterotrophic nitrification was attributed to denitrification during the N_2O production. Additionally, acetylene inhibition stops the substrate production for denitrification and consequently underestimated the N_2O production by denitrification. Due to the small number of samples used in the investigation with the acetylene inhibition method, the impact of using both methods as comparable could not be investigated systematically.

A multiple linear regression analysis was applied to the whole dataset, including the parameter N_{tot}, C/N ratio, temperature, soil moisture [WFPS], log(O_2 concentration), pH value, clay content, sand content, log(NO_3), and log(NH_4). The model only explained 20 % of the observed variability in the N_2O product ratio. Separate analyses of the subset differed by the methods found r^2 values of 0.543, 0.582 and 0.722 for Indexes 1, 2, and 4, respectively.

With the exception of Index 4, where the NH_4^+ concentration was the dominate factor, no dominant factor could be detected. With exception of the pH value, all tested parameters are used in at least one of the three models. This lack of a dominate factor demonstrates that predicting the N_2O product ratio by nitrification is challenging due to the great number of influential soil parameters.

The missing consensus between the multiple regression analysis in this work and previously observed influence of soil factors in the single experiments indicate the insufficient consideration of interactions between factors. To analyse and parameterize these interactions and to implement this knowledge into biogeochemical models will be the most challenging work in the future. Chapter 7 gives an example for modelling the interaction between two or more factors. As another example, O_2 diffusion is mostly influenced by WFPS, but O_2 consumption is strongly dependent on the microbial activity and consequently on the temperature. Therefore, for O_2 concentration in the soils, diffusion limitation by high WFPS is more important than high temperature (e.g., Tiedje et al., 1984).

Despite the great number of soil parameters included in the multiple regression analyses, the general model explained only 20 % of the observed variability in the N_2O product ratio. Additionally, in the separate analysis of the subset, 28% to 46% of the variability in the N_2O product ratio of nitrification remained unexplained. Futhermore the three models (index 1, 2 and 4) varied tremendously from each other. This fact and the observed strong dependency of the output on the input data, indicate that the applicability of the 'hole in the pipe' for site specific N_2O predictions by nitrification is questionable. Generally, different pathways are accepted to produce N_2O during nitrification, and the rates of the nitrification processes likely regulate the N_2O production (Zhang et al. 2016). Based on this, Zhang et al. (2015) proposed a separate parameterization of the HIP model for the different pathways (e.g., heterotrophic and autotrophic nitrification) to advance the accuracy of this approach. Perhaps if the techniques are available to separate the different pathways of N_2O production by nitrification (including the different involved microorganisms, such as fungi, actinomycetes, bacteria, and archaea) and extensive data are available, it will be possible to consider both (the different pathways and the environmental factors) by the development of advanced models. In the present situation, I conclude that the widespread diversity of the microorganisms and the many processes involved may interact in combination with the processes and soil parameters (e.g., Chapter 7), which is too complex for the enhanced HIP approach. Even if the implementation will reduce the error of omission, the increasing number of parameters (and associated uncertainties) tend to increase the uncertainty of the model. Therefore, a simpler approach should be used in the near future, when more knowledge is available about the O_2 and NO_2^- variability in the soils. Land use affects all important soil parameters, and therefore, the influence of land use on the N_2O product ratios of nitrification were investigated as a simplified model. The results are provided in the box plot diagram in Fig. 12.5.

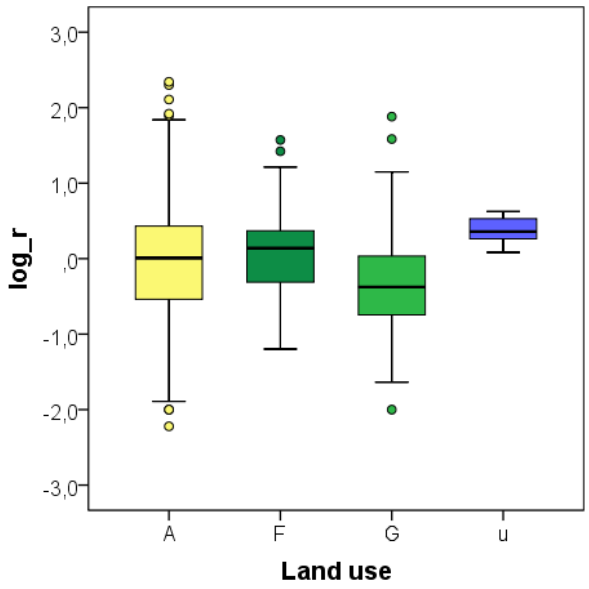

Fig 12.5 Boxplot analysis of the logarithmic values of the product ratio of nitrification depending on the land use

The mean values according to land use are 1.01 ‰ (n=269), 1.11 ‰ (n=68) and 0.52 ‰ (n=42) for agricultural, forest, and grassland soils, respectively. The mean N_2O product ratios of nitrification for uncultivated land (u) was 2.26‰ and differed strongly from the three other land use forms, but only a few measurements (n=9) were available for the analysis.

IMPROVING THE PERFORMANCE OF BIOGEOCHEMICAL N_2O MODELS

In view of the uncertainties in the estimation of N_2O emissions at the field and regional scales, future efforts in ecosystem modelling should attempt to represent the different pathways and associated microbial populations, such as AOA, AOB, heterotrophic nitrifier and comammox. Upscaling the microbial communities and processes to consistent ecosystem models requires close collaborations among soil scientists, microbial ecologists, biogeochemists, and modellers and more robust field measurements, including process separation. New method development, such as the online determination of the N_2O isotopomer (isotopoloque) (e.g., Mohn et al., 2012), stimulate hope for obtaining the needed data in the future.

However, in addition to this upscaling approach, simple modelling approaches, which generalize among the different processes of N_2O production and consumption, need to be developed. In contrast to the 'hole in the pipe' philosophy (N_2O production as a by-product during the first nitrification step; Figure 2.4, direct by HAO) recent works (e.g., Wrage et al., 2001, Wrage-Mönnig et al., 2018) have noted the importance of nitrifier denitrification for the N_2O production by nitrification (Figure 2.4, nitrifier denitrification via nitrite). Ni et al. (2013) analysed experimental results by using four different metabolic computer models to elucidate the mechanisms of aerobic N_2O production by nitrification. Neither approach can explain all experiments successfully, and the authors conclude that the two pathways are of different importance in different soils. Ni et al. (2013) assume that the concentration of the free nitrous acid regulates the pathways. In accordance, sludge N_2O production by nitrifier denitrification was more important if the NO_2- concentration was higher than 10 mg N/l at all O_2 concentrations (Peng et al., 2014).

The occurrence of nitrite decomposition in association with N gas production is widely accepted, e.g., the abiotic production of N gases from nitrite (Chalk and Smith, 1983). Increasingly, evidence shows that the nitrite concentration or rather the concentration of its protonated form, free nitrous acid (HNO_2), also determines the biotic N_2O production in soils (e.g., Russow et al., 2000; Maharjan and Venterea, 2013; Venterea et al., 2015, Ma et al., 2015). Nitrite occurs as an intermediate of a few microbial processes in soils (e.g., nitrification and denitrification). Ma et al. (2015) suggested that N_2O emission peaks observed after ammonium or urea application are caused by nitrite accumulation due to inhibition of the last step of nitrification (NO_2^- oxidation) and N_2O production via nitrifier denitrification. Additionally, Liu et al. 2018 observed that nitrite was the most relevant factor to explain N_2O emission after rewetting. Inhibitions of nitrite oxidation due to ammonia (NH_3) and substrate inhibition of ammonia oxidation were described by Smith et al. (1997) and Venterea et al. (2015). Maag and Vinther (1996) proposed nitrite accumulation as the reason for the higher N_2O product ratio of nitrification. The nitrous acid as substrate for N_2O formation would explain the strong pH dependency and may be a possible explanation for the uncertain mechanisms responsible for the apparently high N_2O product

ratios of nitrification in acid soils (Morkved et al., 2007). All these works support the theory of nitrifier denitrification as an important pathway for N_2O formation by nitrification. Consequently, divergent approaches to the HIP model should be developed to model N_2O production by nitrification. Examples of this new generation of models are given by Müller et al. (2015) or by the model presented in Chapter 9. In contrast to the previous modelling approach based on the HIP model (Chapter 5), the advanced approach rejects the HIP model philosophy (N_2O production depending from the turnover rate) and calculated the N gas production depending of the different nitrite pools (NO_2^--concentration in the soil). This approach also considers that chemical reduction, aerobic denitrification, codenitrification, or active exoenzymes can contribute to the N_2O production from nitrite, which would support a N_2O production model based on the nitrite (free nitrous acid) and oxygen concentration in soils. To date, nitrite is neglected in the majority of ecosystem models, and measurements of nitrite in the field to validate these models are scarce. Usually nitrite turnover occurs rapidly in soils (e.g., Russow et al., 2000), and therefore, its concentration normally is low in soil solutions (e.g., Davidson et al., 1991, Van Cleemput and Samater, 1995, Venterea et al., 2003). However, under certain conditions, nitrite consumption is less than nitrite production, and nitrite can accumulate in soil solutions (Van Cleemput and Samater, 1995; Burns et al., 1996). Consequently, nitrite concentration can vary highly in time and space (Gelfand and Yakir, 2008). More experimental results are necessary for the successful implementation of existing approaches to describe nitrite turnover in ecosystem models. Difficulties in the determination of nitrite must be considered (e.g., Homyak et al. 2015) if concepts to monitor the temporal and spatial variability of nitrite in soils are to be developed. To predict the nitrite concentration in the field sufficiently, comprehensive knowledge of the producing and consuming processes and spatial variability are urgently needed. In particular the right balance between producing and consuming processes must be modelled accurately for the prediction of nitrite concentration, which is challenging. To include these processes with high spatial and temporal variability in ecosystem models, novel approaches should be developed.

CONCLUSION

The presented work will contribute to developing a better understanding of nitrification in soils as an important source for N gas emissions and provide the basis to include nitrification as a crucial process in the next generation of N cycle models in greater detail. The analysis clearly indicates the importance of oxygen for determining the N_2O product ratio by nitrification. Therefore, measurement of the O_2 concentration in soil during experiments on N_2O production by nitrification is strongly recommended. Additional nitrite contents and nitrite dynamics in soils regulate N gas production. Consequently, nitrite measurements and monitoring in field studies are essential to develop a better understanding of the N cycles and gas production. Both O_2 and nitrite measurements would be helpful for enhancing the modelling approaches. Because the N_2O product ratio of nitrification depends on several soil parameters (e.g., soil moisture, O_2-concentration, and temperature), a general model can only explain the observation to a small degree. The proposed approaches, such as the tested enhanced HIP approach, are inappropriate for predicting N_2O gas production by nitrification at specific sites with high accuracy. New modelling concepts, such as neuronal networks and self-learning systems, must be tested to predict the emissions of N_2O at the site and regional scale. I conclude that the widespread diversity of the microorganisms involved and the multitude of processes may interact in combination in the processes and that the soil parameters (e.g., Chapter 7) are too complex for the presented approach. These large uncertainties allow me to suggest that the mean N_2O product ratios that are differentiated by land use are the most sufficient approach for ecosystem modelling to date.

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