Impact of induced summer drought and nitrogen fertilizer application method on net exchange of nitrous oxide and methane in arable soils

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Summary

In wide parts of Europe, a higher frequency of summer drought is expected with global climate change from the increase in greenhouse gases. Formation and uptake of N_2O and CH_4 are regulated by soil climatic conditions (e.g. water content, temperature) and affected by soil texture, plant cover and fertilizer management. In this context, agriculture faces multiple challenges: It needs to feed a growing world population, cope with climatic extremes while being committed to reduce its contribution to greenhouse gas emissions.

To address the impact of enhanced summer drought, a field experiment was conducted at a sandy soil. N_2O and CH_4 fluxes were measured during 18 months, including two summer periods. Dry treatment plots were covered with transparent shields during rain events and compared to well-watered wet control treatments. To assess the effect of different crops, maize and sorghum cropping was compared. While the effect of drought treatment on N_2O emissions was small, with only insignificantly lower annual N_2O emissions at dry treatments, CH_4 uptake was significantly enhanced with drought. There was no significant impact of plant type on annual N_2O or CH_4 fluxes, but yield-scaled N_2O emissions were higher from sorghum than maize due to lower biomass yields.

In a second 2-year field experiment, the impact of CULTAN (Controlled Uptake Long Term Ammonium Nutrition) fertilization on N_2O emissions from two sites cropped with winter wheat (loam and sandy loam soils) was assessed. Lower N_2O emissions compared to broadcast surface application were expected to result from the injection of ammonium sulfate solution (130 kg N ha^{-1}) in CULTAN treatments due to reduced nitrification rates at high NH_4^+ salt concentration in fertilizer depots. However, no substantial stabilization of NH_4^+ fertilizer could be detected. N_2O emissions were higher at the loam than the sandy loam site, and the difference was most pronounced in the CULTAN treatment. N_2O emission factors were low (< 0.6% of applied fertilizer N) and did not depend on treatments. Fertilizer-derived emission measured from ^{15}N tracing at a CULTAN plot revealed the importance of soil N for N_2O emissions at the sandy loam site, as only 1% - 17% of annual N_2O emission were directly derived from the fertilizer.

A laboratory study was conducted to gain further insight into nitrification and N_2O emission at high NH_4^+ concentrations as occurring in fertilizer depots. Since inhibition of nitrification was expected to increase with N content, N_2O emission was measured at five N levels from 0 to 5000 µg NH_4^+-N g^{-1} soil, in sandy loam soil at 50% water filled pore space. Acetylene inhibition was used to determine the contribution of autotrophic nitrification and ^{15}N tracing to distinguish between nitrate and NH_4^+ derived N_2O, both showing the dominance (≥ 70% of total N_2O emission) of nitrification. With an isotopomer approach, nitrifier denitrification was estimated to contribute 10% - 40% of total N_2O emission at these conditions. Nitrification was increasingly inhibited with increasing N content in soil, but there was no evidence for increasing contribution of denitrification. Results from ^{15}N tracing revealed that the ^{15}N-labeling was highly heterogeneous, indicating that nitrification and denitrification were spatially separated, which might affect source-partitioning results if neglected.

It was shown that investigated climate and fertilizing effects have potential impact on N_2O, but due to spatial heterogeneity as well as low site and year-specific fluxes, effects were not significant. This indicates a need for long-term measurements at more sites. The small direct impacts of drought and fertilizer injection on area-based greenhouse gas fluxes and the clear impact on biomass and grain yields indicate that greenhouse gas mitigation strategies in agriculture should be yield rather than area based.

Keywords: N_2O emission, CULTAN, nitrification

Zusammenfassung

Für weite Teile Europas werden im Zuge des Klimawandels häufiger sommerliche Dürreperioden zu erwarten sein. Die Freisetzung und Aufnahme der Treibhausgase Lachgas (N_2O) und Methan (CH_4) wird von bodenklimatischen Bedingungen gesteuert (z.B. Wassergehalt, Temperatur) und von Bodentextur, Pflanzenbedeckung und Düngermanagement beeinflusst. Die Landwirtschaft steht dabei vor der komplexen Herausforderung, eine wachsende Weltbevölkerung ernähren und gleichzeitig die Freisetzung von Treibhausgasemissionen reduzieren zu müssen.

Der Einfluss von durch Regenausschluss induzierter Sommertrockenheit auf N_2O- und CH_4-Flüsse aus einem lehmigen Sandboden wurde in einem 18-monatigen Feldexperiment untersucht. Die annuellen N_2O-Emissionen wurden durch die verstärkte Sommertrockenheit nur geringfügig reduziert, wobei diese Reduktion vor allem auf geringeren Winteremissionen beruht. Die CH_4-Aufnahme hingegen war im Vergleich zur Kontrollvariante signifikant erhöht. Emissionsunterschiede zwischen Mais und Hirse, einer dürreresistenteren Frucht, konnten nur bei ertragsbezogener Berechnung festgestellt werden und sind vor allem auf die niedrigeren Biomasseerträge der Hirsepflanzen zurückzuführen.

In einem weiteren zweijährigen Feldexperiment wurde untersucht, ob mit der CULTAN-Düngung (Controlled Uptake Long-Term Ammonium Nutrition) von Weizen die N_2O-Emission reduziert werden kann. Dazu wurden auf 2 Standorten (Lehm und sandiger Lehm) N_2O-Flüsse nach Punktinjektion (CULTAN) im Vergleich zur oberflächlichen Düngerapplikation (Ammoniumsulfatlösung, 130 kg N ha^{-1}) gemessen. Erwartet wurden geringere N_2O-Emissionen nach CULTAN-Düngung durch die nitrifikationshemmende Wirkung von hohen Ammonium (NH_4^+)-Konzentrationen in den Düngerdepots. Eine deutliche Stabilisierung des NH_4^+ in den Depots konnte allerdings nicht beobachtet werden. Die N_2O-Emissionen waren am Lehm- höher als am sandigen Standort, vor allem durch (wenn auch nicht signifikant) höhere Emissionen der CULTAN-Variante. Die N_2O-Emissionsfaktoren waren generell niedrig (< 0.6% des ausgebrachten Stickstoffs), und die Art der Düngerapplikation hatte keinen signifikanten Einfluss. Ein ^{15}N-Tracerversuch in der CULTAN-Variante am Standort mit sandigem Lehmboden zeigte nur einen geringen direkten Anteil des Düngerstickstoff (1% - 17%) an der annuellen N_2O-Emission.

Um den Einfluss hoher NH_4^+-Konzentrationen bei der CULTAN-Düngung auf die Nitrifikation und N_2O-Emissionen besser zu verstehen, wurde außerdem ein Laborversuch durchgeführt. Sandiger Lehmboden wurde mit NH_4^+ in Konzentrationen von 0 bis 5000 µg NH_4^+-N g^{-1} inkubiert. Mit der Acetylen-Inhibitionsmethode wurde der Anteil der Nitrifikation, und mit ^{15}N-Markierung der Anteil von nitratbürtigem N_2O gemessen. Es zeigte sich eine deutliche Hemmung der Nitrifikation und der N_2O Freisetzung mit steigender NH_4^+-Konzentration; der Anteil der Nitrifikation, der einen Großteil (≥70%) der N_2O Bildung ausmachte, war aber kaum beeinflusst. Ein Isotopomeransatz zeigte, dass die Nitrifizierer-Denitrifikation zwischen 10% und 40% zur Gesamt-N_2O-Bildung beitrug. Aus den Ergebnissen des ^{15}N-Traceransatzes kann abgeleitet werden, dass die ^{15}N-Markierung im Boden inhomogen verteilt, und Nitrifikation und Denitrifikation räumlich getrennt waren. Durch Nichtbeachten dieses Effekts kann die Quellenzuordnung von N_2O-Emissionen deutlich beeinflusst werden.

Es wurde gezeigt, dass die untersuchten Klima- und Düngereffekte die N_2O-Emission beeinflussen könnten, die Unterschiede allerdings aufgrund hoher räumlicher Heterogenität sowie niedriger standort- und jahresspezifischer Flüsse nicht signifikant waren. Dies verdeutlicht den Bedarf an Langzeituntersuchungen. Die geringen direkten Effekte von Sommertrockenheit und Düngerinjektion auf flächenbasierte Flüsse und der deutlichere Effekt auf Biomasse- und Kornerträge deuten an, dass Emissionsminderungsstrategien eher auf ertrags- als auf flächenbasierte Emissionen abzielen sollten.

Schlagworte: N_2O Emission, CULTAN, Nitrifikation

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Authorship Declaration

The three main chapters of this thesis (chapters 4 to 6) comprise individual studies published or intended to be published as research papers. Chapter 5 has already been published in a revised version in Agriculture, Ecosystems & Environment.

I am the first, but not the only, author of these three articles. To declare my contribution to the individual phases of the three studies, the following scale is used:

A: I contributed to the work (0-33%)
B: I made a substantial contribution (34-66%)
C: I did the majority of the work independently (67-100%)

It is applied to four categories:

- **Concept:** Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/ investigations
- **Planning:** Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered
- **Execution:** Involvement in the analysis or the concrete experiments/ investigation
- **Manuscript preparation:** Presentation, interpretation and discussion of the results obtained in article form

	Concept	Planning	Execution	Manuscript
Chapter 4 - Summer drought study	A	B	C	C
Chapter 5 - CULTAN field study	A	B	C	C
Chapter 6 - Laboratory study	B	B	C	C

1. General Introduction

1.1. The N cycle and its changes under human influence

The vast majority of nitrogen (N) on earth is unreactive, gaseous, molecular dinitrogen (N_2). High energy input is needed to break the stable bond between the two N atoms. All life depends on processes that convert this N_2 into reactive N species (N_r). In the natural N cycle, nitrogen fixing prokaryotes (bacteria and archaea) containing the enzyme nitrogenase, that converts N_2 to ammonia (NH_3), are the main source of N_r, and the only one apart from lightning that produces NO_x (Fowler et al. 2013).

N is comprised in all living cells: in proteins, enzymes, DNA, and many others. Plant growth depends on sufficient supply with N_r, mostly in the form of inorganic ammonium (NH_4^+) and nitrate (NO_3^-). With the domestication of plants and animals, humans began to interfere with the natural N cycle, and did so deliberately at least since the time they realized that returning dung/feces and food residues and growing leguminous plants helped to maintain or increase the soils fertility (Galloway et al. 2013). The transition from a hunters and gatherers society to husbandry was the prelude of a comprehensive reshaping of the land surface.

In the 19th century, nitrogen was recognized as a crucial compound of fertilizer to increase crop production (Galloway et al. 2013). With the invention of the Haber-Bosch process in the early 20th century, large-scale production of anthropogenic N_r started, not exclusively but with growing contribution for synthetic fertilizers (Galloway et al. 2008). Compared to pre-industrial times, the amount of N_r that circulated through soils, waters and the atmosphere increased drastically, and feeding a growing world population of 7.16 billion people in the year 2013 (UN 2015) would not have been possible without the supply with cheap synthetic fertilizer (Erisman et al. 2008). Industrialization, the usage of fossil fuels, and application of synthetic (N) fertilizers all supply N_r to the environment. Consequently, the global N cycle changed enormously, and nowadays the anthropogenic (from agricultural symbiotic N_2 fixation and synthetic N_r from the Haber-Bosch process) equals the biological N fixation (Fowler et al. 2013).

Such an extensive intervention in the N cycle causes complex side effects that are still difficult to predict, including eutrophication of rivers, lakes and ocean water, acidification of surface water bodies, a decline in species richness in formerly low-N ecosystems and photochemical processes in the atmosphere leading to high ozone levels in the troposphere while destroying the ozone layer in the stratosphere (Erisman et al. 2013; Robertson & Vitousek 2009).

The increase of reactive carbon and nitrogen compounds in the atmosphere causes furthermore a change in the greenhouse effect that is inherently a prerequisite for life on earth. The most important contributors to the natural greenhouse effect are water vapor, carbon dioxide (CO_2) and ozone (O_3); minor contribution comes from methane (CH_4) and nitrogen oxides (NO_x), including nitrous oxide (N_2O). With fossil fuel burning and intensification of agriculture since the industrial revolution, the contribution of anthropogenic emissions of these gases increased. The concentration of N_2O in the atmosphere rose from preindustrial 270 ppb (i.e. in 1750) to 326 ppb in 2013, at a growth rate of 0.82 ppb yr^{-1} within the last decade (WMO 2014). Direct and indirect emissions from agriculture have a share of 79% on this anthropogenic increase (Ciais et al. 2013). In the stratosphere, N_2O furthermore participates in photochemical reactions leading to the transformation of ozone (O_3) to O_2. Since emission of chlorofluorocarbons drastically declined after

their restriction by the Montreal Protocol, N_2O is the dominant O_3 depleting substance (Ravishankara et al. 2009).

Methane (CH_4) has an even greater share on the radiative forcing in the stratosphere, and about 60% of its emission to the atmosphere comes from anthropogenic sources as fossil fuel exploitation, biomass burning, rice cultivation and ruminants. Aerobic soils, however, are CH_4 sinks compensating roughly 5% of total CH_4 emissions to the atmosphere (Ciais et al. 2013).

The increase in greenhouse gases leads to increased trapping of solar energy in the atmosphere, increasing global mean temperatures. Likely consequences are the increase in extreme weather events, as flooding, storms or droughts (Seneviratne et al. 2012). Globally, the area prone to drought periods is expected to increase, and drought periods to extend (Burke et al. 2006).

Summer precipitation and soil moisture are expected to decrease in large parts of Southern and Central Europe (Bindi & Olesen 2011; Calanca et al. 2006), with increasing risk of extreme events as summer drought and heavy rain (Christensen et al. 2013; Feyen & Dankers 2009; Seneviratne et al. 2012). These changes will and already do affect agriculture. The growing season lengthens, and cereal and seed crop cultivation could become possible further north (Bindi & Olesen 2011; Gornall et al. 2010). Crop growth, however, will be negatively affected by the resulting drought stress (Gornall et al. 2010).

With the increased need for food for a growing global population (UN 2015) and for bioenergy crops (e.g., due to the compliant binding target of the European Union to increase the renewable-based share of total gross final energy consumption to 20% by 2020 (Directive 2009/28/EC)), there is a need to increase the knowledge of how to cope with extreme events, as summer drought. Furthermore, feedback mechanisms between changes in the environmental parameters (e.g., soil moisture, plants, temperature, fertilization) and greenhouse gas fluxes are not conclusively understood. Therefore, more insight into the underlying processes is needed.

The international community acknowledges its responsibility for the global climate change, and 192 parties ratified the Kyoto Protocol to decrease the emission of CO_2 and other greenhouse gases (UNFCCC 2014). Whether we will be able to counteract the ongoing increase in N_2O concentration in the atmosphere will depend on reduction of N_r input to the environment and the implementation of strategies to mitigate N_2O formation in anthropogenic systems as agricultural soils (Schreiber et al. 2012).

Much is already known about how N_2O production is regulated in soil, and a short summary of the processes and some major control parameters is given in the next chapter. Interactions between these control parameters, climatic and weather conditions and the anthropogenic intervention from field management add further complexity. For conceiving and implementing management strategies for the mitigation of N_2O emissions and adaptation to changing climate conditions, further insight is needed.

1.2. N_2O production in soil

In soil, the majority of N_2O is produced during enzymatically mediated processes. Firestone and Davidson (1989) described the production of N_2O in soil with their 'hole-in-the-pipe' theory. N_2O is thereby no target product for the organisms producing it, but it is emitted as a side- or intermediate product and leaks out (Figure 1-1). Although the model is a simplification, reducing N_2O producing processes to nitrification and denitrification, it is vivid. Microorganisms capable of producing N_2O are found in various microbial groups, e.g. ammonia oxidizing bacteria (AOB) and archaea (AOA), denitrifying bacteria and fungi (Braker & Conrad 2011). While denitrification is the main source of N_2O under anaerobic conditions, nitrification and, with an often indefinite fraction, nitrifier denitrification are considered the main N_2O producing processes in aerobic compartments (Bouwman et al. 2010; Butterbach-Bahl et al. 2013).

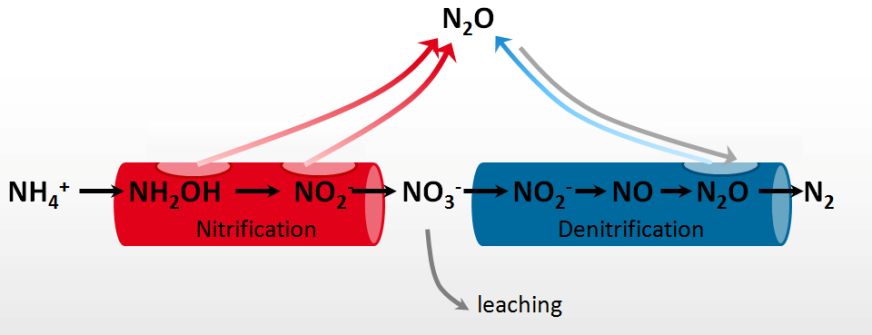

Figure 1-1: Conceptual 'hole-in-the-pipe' model, adapted after Davidson et al. (2000). N_2O is leaked from 'holes' in the processes of nitrification and denitrification, with the size of holes determined by controlling factors as the water content. NO emissions may occur from the same processes as N_2O emissions and are omitted for clarity.

Denitrification is a form of heterotrophic respiration, an anaerobic process where NO_3^- is used as alternative electron acceptor by heterotrophic organisms in the absence of oxygen (Knowles 1982), which can also occur in anaerobic micro-sites in aerobic soils (Parkin 1987). During denitrification, NO_3^- is stepwise reduced, the intermediates and products being nitrite (NO_2^-), nitric oxide (NO), N_2O, and N_2. Each of these reaction steps is catalyzed by a specific enzyme. A broad range of microorganisms are capable of denitrification, including fungi and archaea. Their relative contribution has only seldom been studied. Due to high fungal biomass in soil, and the lack of N_2O reductase, this contribution may be large (Braker & Conrad 2011).

Also many AOB are capable to reduce NO_2- to NO and N_2O, with the pathway and related enzymes resembling those in denitrifiers. This nitrifier denitrification (Wrage et al. 2001) may also be a means of detoxification, when NO_2- accumulates (Beaumont et al. 2004; Beaumont et al. 2002; Schreiber et al. 2012).

Nitrification, in the proper sense, is the oxidative production of nitrate from reduced N species. Autotrophic nitrification is a two-step process, generating the energy for CO_2 fixation: Ammonia is first reduced via hydroxylamine (NH_2OH) to NO_2^- by AOB or AOA. The second step, reduction of NO_2^- to NO_3^- is mediated by a separate group, the nitrite oxidizing bacteria (NOB). In both steps, O_2 is the terminal electron acceptor. The oxidation of NH_3 to NH_2OH is catalyzed by ammonia monooxygenase (AMO), a membrane-bound enzyme. The further oxidation of NH_2OH to NO_2^- is

catalyzed by the periplasmic hydroxylamine oxidoreductase (HAO). The nitrite oxidoreductase in NOB is also a membrane-bound enzyme; 2 electrons are released by the oxidation, which are again used to reduce O_2 in a terminal oxidase to induce a proton gradient for ATP production. N_2O is no primary intermediate in these reactions but a side-product from chemical decomposition of intermediates as NH_2OH or NO_2^- (Schreiber et al. 2012; Wrage et al. 2001).

While nitrifier denitrification describes NH_4^+ oxidation and NO_2^- reduction within the same microbes, in coupled nitrification denitrification these processes proceed in separate microbial groups. Coupled nitrification denitrification can occur in micro environments where conditions are suboptimal for both nitrification and denitrification, or nitrifying and denitrifying microsites are in close proximity. The NO_2^- or NO_3^- produced during nitrification can then be used by other organisms to be denitrified (Wrage et al. 2001). In Figure 1-2, a depiction of the main N_2O producing pathways is given.

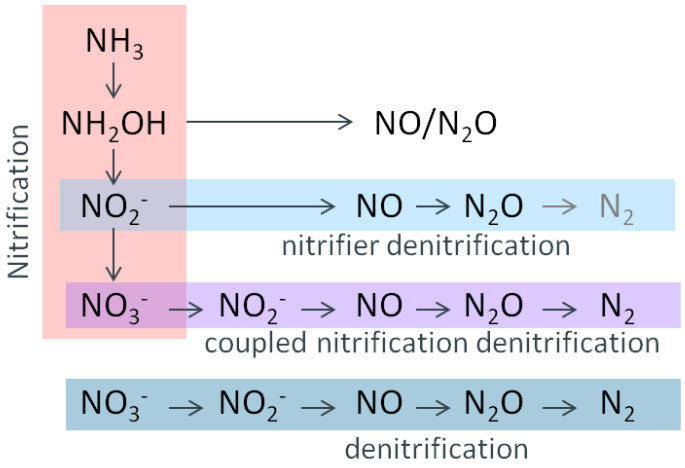

Figure 1-2: Depiction of major pathways of N_2O production. (Adapted from:Kool et al. 2011; Wrage et al. 2001; Zhu et al. 2013)

While nitrification (including nitrifier denitrification) and denitrification (including coupled nitrification and denitrification as well as fertilizer denitrification) are generally considered the major N_2O generating processes in soil (Butterbach-Bahl et al. 2013), others do certainly exist. Under certain circumstances (e.g. low pH), N_2O can be formed by chemical reactions between intermediates of NH_4^+ oxidation to NO_2^-, or between NO_2^- and organic or inorganic substances. This chemodenitrification can hardly be differentiated from nitrification, as they are closely linked (Schreiber et al. 2012; Van Cleemput & Baert 1984; Wrage et al. 2001).

Heterotrophic nitrification is similar to autotrophic nitrification in that it also oxidizes NH_4^+ to NO_2^- and NO_3^-, with the same intermediates, but it can also oxidize organic N compounds. The enzymes (AMO and HAO), though catalyzing the same reaction, are different. Heterotrophic nitrifiers use organic compounds for their energy gain and are often capable of denitrification, even under aerobic conditions. Heterotrophic nitrification is more common among fungi than bacteria. Heterotrophic nitrification is generally thought to be of minor importance for N_2O production, but it may become important under low pH, high O_2 and high organic C conditions (Guo et al. 2013; Wrage et al. 2001). From the results of a ^{15}N tracing model, Müller et al. (2014) concluded that

organic nitrogen compounds might have contributed as much as 50% to total N_2O emission from grassland soil.

Furthermore, other processes as dissimilatory nitrate reduction to ammonium (DNRA) or co-denitrification of NO or N_2O with another N compound can build N_2O in soil (Butterbach-Bahl et al. 2013), but their contribution to N_2O production under normal conditions in soil is thought to be low.

1.3. Methane production and oxidation in terrestrial soil

CH_4 is produced by archaea during methanogenesis from fermentation products. There are mainly two pathways; either is acetic acid converted to CH_4 and CO_2 or CO_2 is reduced to CH_4 with H_2. The production of CH_4 in soil is thermodynamically limited to anaerobic conditions, and CH_4 emission from mineral soils is mostly confined to waterlogged conditions or the presence of anaerobic microsites at high C contents (Conrad 1996). Under aerobic conditions CH_4 fluxes are dominated by CH_4 uptake and oxidation (Smith et al. 2000). CH_4 oxidation depends on availability of O_2 and CH_4, and is thus controlled by diffusive transport of these gases into the soil. Soil texture and soil water content affect CH_4 uptake rates, with increasing CH_4 uptake at drier conditions. Extreme drought, however, may limit methanotrophic activity (Dobbie & Smith 1996). CH_4 oxidizers are autotrophs, using CH_4 as their sole energy and carbon source, and they are structurally very similar to NH_4^+ oxidizers. Although they depend on N for growth, CH_4 oxidation may be competitively inhibited by NH_4^+ in soil (Kravchenko et al. 2002; Nyerges & Stein 2009). This effect is, however, apparently of minor importance in agricultural soil with a history of N fertilization (Dobbie & Smith 1996; Hartmann et al. 2011)

1.4. Ecological and environmental factors affecting N_2O production

Both process rates of nitrification and denitrification and their relative importance for N_2O production in soil are affected by a range of environmental conditions, including soil temperature, soil humidity, the availability of O_2, organic carbon and the respective N substrates, pH and the availability of other nutrients.

Furthermore, the diverse processes may be differently regulated by the same parameter. Relationships between the physiological and environmental factors can furthermore not be examined separately, as they are often interrelated. Still, some relevant environmental controls are presented here.

1.4.1. Water content

Water is essential for all organisms living in soil, being the main component of cell plasma and intercellular fluids. At very low water contents in soil, microorganisms as well as plants suffer drought stress (Bennett et al. 1989; Manzoni et al. 2011). The importance for N_2O and CH_4 production and consumption in soil is furthermore based on the influence of water content on gas and solute transport in the soil matrix. Both NO_3^- and NH_4^+ are mobile in soil only in the water phase; transport to the sites of microbial activity and thus availability for microorganisms depends on water films or water filled pores, and on the soil texture defining the length of transport paths. While mobility of NO_3^- and NH_4^+ increases with increasing water content, O_2 diffusion is drastically reduced, as the diffusion in water is by a factor of 10^4 lower in water than in the gas phase (Lerman

1988). Via its control on O_2 availability, the water content affects which processes prevail in soil and the N_2O product ratio of the processes. With increasing water content, or decreasing O_2 availability, N_2O production from NH_4^+ oxidation increases (Goreau et al. 1980; Khalil et al. 2004; Maag & Vinther 1996; Zhu et al. 2013). Increasing aerobicity, on the other hand, increases the N_2O/N_2 product ratio during denitrification (Betlach & Tiedje 1981; Knowles 1982). However, emission rates of N_2O generally increase with increasing soil moisture (Bateman & Baggs 2005; Dobbie et al. 1999; Maag & Vinther 1996). A maximum has often been found around 70% - 90% water filled pore space (WFPS) or higher (del Prado et al. 2006; Skiba & Smith 2000). Decreasing N_2O emission above this maximum can be explained by reduced diffusion and thus less outgassing of N_2O, which is instead more completely reduced to N_2 (Butterbach-Bahl et al. 2013; Smith et al. 1998).

1.4.2. pH

Per NH_4^+ that is oxidized, $4H^+$ are released, leading to acidification in soil by nitrification. Conversely, the soil pH is also affecting N turnover processes in soil. Nitrification is affected by pH via the substrate availability: since NH_3 rather than NH_4^+ is the substrate of the AMO, at low pH (~pH 4) nitrifiers may starve from NH_4^+ limitation (Mørkved et al. 2007; Subbarao et al. 2006). The $N_2O/(NO_2^-+NO_3^-)$ product ratio of nitrification has been reported to be higher in soils with pH 4 than pH > 5, possibly due to chemodenitrification of NO_2^- under acid conditions (Mørkved et al. 2007). The $N_2O/(N_2O+N_2)$ product ratio of denitrification is also negatively correlated with pH (in a range of pH 5-8), due to a higher sensitivity of N_2O reductase compared to other denitrification enzymes or the inhibition of N_2O reductase formation at low pH (Baggs et al. 2010; Bakken et al. 2012). While the $N_2O/(N_2O+N_2)$ ratio decreases with increasing pH, total denitrification rates are usually highest at high pH (Focht & Verstraete 1977; Müller & Clough 2013). Acidification of soil with a pH of 7 to pH 5.6 and 4.3, however, has led to a decrease in N_2O emission and a shift in the predominant N_2O source from denitrification to nitrification (Baggs et al. 2010).

1.4.3. Temperature

The temperature is an important factor for biological processes. Chemical reactions are faster with increasing temperature and enzymatically mediated processes generally have an optimum curve (e.g. 25-35°C as optimum temperature for nitrification; Focht and Verstraete (1977)). Respiration, nitrification and denitrification rates increase with increasing temperature and often there is a positive correlation between soil temperature and N_2O emission (Smith et al. 1998). Higher respiration at warmer temperature furthermore accelerates O_2 consumption, thus leading to more anaerobic conditions that promote denitrification (Linn & Doran 1984; Mathieu et al. 2006). Some nitrification and denitrification is, however, found under temperatures as low as 0°C, and N_2O emissions peaks during frost/thaw cycles or during the winter period (Flessa et al. 1995; Kaiser et al. 1998) may contribute substantially to annual N_2O emissions, thus averting a linear correlation between N_2O emission and temperature.

1.4.4. Nitrogen substrate availability

N_2O emissions generally increase with increasing N input, be it from atmospheric deposition or from direct fertilization (e.g. Bouwman 1996; Breitenbeck & Bremner 1986; Stehfest & Bouwman 2006). In contrast to natural systems, substrate availability in agricultural systems is both in the amount and the chemical form controlled by fertilizer application. Application of NO_3^- stimulates

denitrification rates, and both N_2O production and the N_2O/N_2 ratio (Blackmer & Bremner 1978) increase with increasing NO_3^- content in soil. Application of NH_4^+ fertilizer promotes nitrification rates, and the produced NO_2^- and NO_3^- may subsequently be denitrified. Some studies indicate higher emission from ammonium-based than nitrate-based fertilizers, and still higher emissions from urea (Bouwman et al. 2002; Snyder et al. 2009). Organic fertilizers supply not only N but also easily available carbon to the soil and thus promote mineralization and N immobilization and the formation of anaerobic microsites. While initial N_2O formation may be low after organic fertilizer addition as a result of NO_3^- limitation and consequently low N_2O/N_2 ratios from denitrification (Senbayram et al. 2009), N_2O emissions were often higher after organic than mineral fertilization, especially in soils with low carbon content (Kaiser & Ruser 2000; Köster et al. 2011; Pelster et al. 2012)

1.4.5. Soil texture

The soil texture affects nitrification and denitrification and resulting N_2O production in several ways. The pore distribution is directly affected by the soil texture, and thus is the connectivity of pores, the aeration and the diffusive transport of gases and solutes. The clay content furthermore determines the abundance of binding sites for NH_4^+ cations, and by adsorption of NH_4^+ at the cation exchange sites of clay minerals nitrification is supported as this is also the place where microorganisms are located in soils (Powell & Prosser 1991; Subbarao et al. 2006). Higher N_2O emission has been found in heavy than light textured soils (Bouwman 1996; Subbarao et al. 2006), and higher water filled pore spaces (WFPS) and often higher organic carbon contents were proposed as the reason for higher N_2O emission from loamy than sandy soil (Pelster et al. 2012)

1.4.6. Plants

Plants affect N_2O production in soil in several ways. They extract mineral nitrogen compounds from the soil to meet their N demand, thus lowering the amount of N available for microbial turnover and N_2O producing processes. Their demand for water affects soil water content, and the crop type with its corresponding rooting depth influences the distribution of water within the soil (Singh & Singh 1995; Zegada-Lizarazu et al. 2012). Dense plant stands (dense foliage) affect the microclimate at the soil surface. Furthermore, plants supply organic material to the soil, as root exudates and plant litter during the growing season or as crop residues after harvest. The easily mineralizable carbon can serve as electron donor for denitrification. At sufficient NO_3^- availability, denitrification rates increase in the rhizosphere (Philippot et al. 2009); the N_2O/N_2 ratio of denitrification decreases with the availability of organic carbon and increasing C/N ratios (Knowles 1982).

Although the influence of the mentioned control parameters on N_2O emission have been studied in numerous experiments, we still do not completely understand N_2O turnover and the corresponding production processes at the field scale, where all the parameters vary concurrently, especially under transient conditions (Butterbach-Bahl et al. 2013).

1.5. Fertilization-effects on N_2O production

Nitrogen fertilization, including the production of nitrogen fertilizer, is responsible for a great part of the increase in N_2O emission. It is thus straightforward to look at nitrogen fertilization when trying to mitigate anthropogenic N_2O emission. As maintenance of crop yields depends on sufficient

nitrogen supply, complete abandonment of fertilization is no option. Optimum fertilization strategies thus aim at increasing nitrogen use efficiency and yields, while simultaneously avoiding N losses (Dinnes et al. 2002; Robertson & Vitousek 2009).

1.5.1. Fertilizer application method

Placement of fertilizer within the soil, in bands, nests or as granules, may improve N efficiency and crop yields (Hou & Tsuruta 2003; Ladha et al. 2005; Malhi & Nyborg 1985; Stecker et al. 1993; Yadvinder et al. 1994) by reduction of N losses, e.g. from NH_3 volatilization, nitrification, denitrification and NO_3^- leaching. Passioura and Wetselaar (1972) suggested ammonium fertilizer placement to avoid NO_3^- leaching.

As N_2O fluxes are concerned, results from fertilizer placement studies are contradictory, though. Fertilizer placement often led to higher N_2O emissions as compared to broadcast application when urea ammonium nitrate (Smith et al. 2012), urea (Cheng et al. 2002; Chu et al. 2007; Engel et al. 2010), or ammonium nitrate sulfate (one year in Pfab et al. (2012)) were applied. Deep (≥10 cm) injection or banding of fertilizer, however, has also caused a reduction in N_2O emission in some studies (Liu et al. 2006; van Kessel et al. 2013). High ammonium concentrations have been found to inhibit nitrification (Harada & Kai 1968), decelerating or preventing the accumulation of NO_3^- after fertilizer banding (Petersen et al. 2004; Wetselaar et al. 1972). With highly concentrated NH_4^+ nests in soil, N_2O production from nitrification should thus be limited, as well as N_2O production from denitrification without NO_3^- accumulation.

Nitrification rates have been shown to be effectively reduced at NH_4^+ contents as high as 2000 ppm, although it is not completely clear, whether this toxic effect is a result of ammonium specific toxicity or mainly due to high osmotic pressure due to the high salt content (Harada & Kai 1968; Wetselaar et al. 1972).

1.5.2. CULTAN

The CULTAN fertilization technique (an abbreviation of **C**ontrolled-**u**ptake **l**ong-**t**erm **a**mmonium **n**utrition) is a form of fertilizer management with ammonium-rich, mainly nitrate-free fertilizers, aiming at improving N nutrition of plants by supplying them with NH_4^+ as the dominant N form (Sommer 2005). Uptake of N in the form of NH_4^+ is less energy consuming for the plant, as it is directly incorporated into organic compounds in the root tissue. This incorporation depends on the proper supply of carbohydrates within roots, and thus on the photosynthesis and transport via the phloem. Nitrate, by contrast, can be transported via the transpirational flow to the upper plant parts (leafs, stems) and then stored in vacuoles or be reduced to NH_4^+ before incorporation into organic substances. This difference alters the sink-source relationships and the phyto-hormone balance within the plant. Ammonium nutrition leads to less N being transported and stored in older leaves and to better supply of young plant tissue and roots with N. Sommer (2005) refers to root-dominant growth under NH_4^+ nutrition and shoot-dominated growth under urea or NO_3^- nutrition.

Pure ammonium nutrition has been shown to have several negative effects on plants, when grown in uniformly fertilized soil or hydrocultures (Bloom 1997; Gerendás et al. 1997). Furthermore, plants compete with microorganisms in soil for the NH_4^+ from fertilizer (Inselsbacher et al. 2010), and nitrification could lead to N losses via NO_3^- leaching and thus counteract the strategy of ammonium nutrition with CULTAN. Thus, the N fertilizer is not supplied via broadcast surface application but in depots of high NH_4^+ concentration. Fertilizer injection with spoke wheels is

common, creating depots of some cm diameter within the soil. The fertilizer depots thus comprise only a small portion of the complete soil (usually < 5 - 10%). Negative effects on plants, e.g. from NH_4^+ antagonisms with potassium (K^+) or acidification, are thus avoided. Passioura and Wetselaar (1972) observed higher root density around ammonium sulfate bands, and lower NO_3^- contents as compared to urea banding.

Some studies showed positive yield effects of CULTAN fertilization (Richter 2010; Weber et al. 2008), and only seldom did CULTAN treatments lead to lower crop yields. The majority of studies showed only small effects (Flisch et al. 2013; Kozlovsky et al. 2010).

The high ammonium concentration in the fertilizer depots is thought to have similar effects on nitrification as chemical nitrification inhibitors. Nitrate leaching could be reduced with CULTAN fertilization in some field studies in Germany (Maier et al. 2011). Analogously, inhibition of nitrification should also lead to lower N_2O emission from nitrification. Studies on N_2O emission after fertilizer application according or similar to the CULTAN strategy often used different fertilizer types for broadcast surface application and CULTAN. While this helps to distinguish between management systems, it is not suitable to decide on whether the method of application is effective in preventing N_2O emission.

2. Research questions and hypotheses

Summer drought field experiment (Chapter 4)

Facing possible changes in summer climate in Europe and the growing demand for bioenergy crops, further knowledge is needed about the reaction of greenhouse gas fluxes to more extreme weather conditions during the growing period and about possible feedbacks with the crop type. Specifically, we measured N_2O fluxes from a sandy loam soil under maize and sorghum cultivation facing increased summer drought that was induced by rain exclusion.

The questions were:

- *Are soil mineral N dynamics significantly changed by the induced drought?*

Mineralization and nitrification are reduced at very dry soil conditions, but also plant N uptake could be reduced due to drought stress. We thus hypothesized higher mineral nitrogen content in soil under dry conditions.

- *Does rain exclusion in summer significantly affect N_2O and CH_4 fluxes from soil?*

Due to reduced soil moisture, less anaerobic microsites in soil are available where CH_4 could be produced. Additionally, diffusion of CH_4 into the soil is eased. Therefore, higher CH_4 uptake rates in the dry treatments were expected.

With the exclusion of rain events, the probability for conditions suitable for denitrification is lower in the dry treatments. As also N_2O emissions from NH_4^+ oxidation decrease with decreasing soil moisture, N_2O emission are expected to be lower during the period of rain exclusion in the dry treatments.

- *Do effects during the rain exclusion period transfer into changes in annual fluxes?*

If differences in the growing period are strong enough, they will have an effect on annual fluxes. However, if nitrogen contents in soil shows distinctively higher values after the growing period in dry treatments, higher N_2O emissions from denitrification of the surplus N could be expected, counterbalancing low fluxes from the growing season. Last, if drier conditions in the soil persist during fall, lower N_2O emission will occur.

- *Does sorghum, that is better adapted to dry conditions, affect total and yield-related N_2O / CH_4 fluxes from the soil compared to maize?*

Sorghum has been shown to be able to withdraw water from deeper soil depths than maize, and it is more resistant to drought conditions. It may thus affect both the soil water content and the mineral nitrogen content in soil. If sorghum yields are less affected by drought than maize yields, yield related fluxes may decrease in comparison to maize.

An additional focus lay on the control parameters of N_2O emission and CH_4 oxidation and their interaction, to gain further insight into dependencies at the prevailing conditions.

CULTAN field experiment (Chapter 5)

At a similar site, a CULTAN experiment had been established in 2007. Here, measurements of N_2O fluxes were performed for a period of two full years to study the impact of pure ammonium injection fertilization in contrast to broadcast application of the same fertilizer (ammonium sulfate). As soil texture is an important parameter for both nitrification and N_2O emissions, the experiment was also newly established at a second site, with higher clay content.

With a ^{15}N tracer experiment, the contribution of the applied fertilizer-N to total N_2O fluxes was measured. Thereby, more insight into the relative importance of fertilizer and soil N for N_2O fluxes was sought.

The questions addressed with this experiment were:

- *Is nitrification of the applied ammonium fertilizer inhibited by fertilizer injection in depots of high ammonium concentration?*

Fertilizer nitrogen is expected to remain in the ammonium form for longer when applied by point-injection compared to broadcast spraying. Correspondingly, lower nitrate contents in soil are expected at the CULTAN plots during the growing period.

- *Is N_2O emission lower from CULTAN than surface application?*

As inhibition of nitrification is expected, and thus also less nitrate for denitrification is available in the CULTAN plots, lower N_2O emission are expected during the growing period. If nitrogen uptake is equal or even higher at CULTAN plots, lower emission can also be expected on annual base.

- *How large is the contribution of fertilizer-N to N_2O emission?*

As the fertilizer is confined to a small part of the soil only, and nitrification of NH_4^+ in the CULTAN depots is expected to be inhibited, the contribution of fertilizer to N_2O emission may be low.

- *Is there a difference between sites regarding total fluxes and the impact of fertilizer application method on N_2O emissions?*

Soil moisture and N_2O emission is expected to be higher at the loamy than the sandy site. Therefore, we also assume a higher mitigation potential at this site by inhibition of nitrification.

Laboratory experiment (Chapter 6)

To analyze the effect of high NH_4^+ concentrations as they may occur in CULTAN depots, an incubation study was performed. Here, the sandy loam soil of the field experiment was used and different concentrations of NH_4^+ were installed. The water content was installed at 50% WFPS, which was comparable to field conditions after fertilization. Different methods were used to distinguish between sources of N_2O production, and the product ratio of nitrification (N_2O/NO_3^-) was determined.

The following questions were addressed:

- *Are concentrations of NH_4^+ after CULTAN injection of $(NH_4)_2SO_4$ fertilizer appropriate to inhibit nitrification?*

Increasing inhibition is expected with increasing NH_4^+ contents. Highest concentrations used (5000 µg/g) represent conditions in the depot centers and are well above the concentrations that had been found to inhibit nitrification in earlier studies. Complete inhibition of nitrification is thus expected.

- *Is the N_2O yield of nitrification dependent on the NH_4^+ concentration in soil?*

Increases in N_2O yield of nitrification were reported under suboptimal conditions. As high NH_4^+ concentration likely affects oxidation of NO_2^- and thus may cause accumulation of hydroxylamine or NO_2^-, an increase in N_2O yield is expected with increasing NH_4^+ content.

- *Which process dominates the N_2O production under high NH_4^+ conditions?*

In general, NH_4^+ oxidation is expected to dominate N_2O production, as the water content is too low for intense denitrification. With increasing initial NH_4^+ content, the contribution of NO_3^- derived N_2O is expected to increase. With increasing NO_3^- content from nitrification under low or moderate initial NH_4^+ content, the contribution of NO_3^- is also expected to rise.

3. Flux measurements with the closed chamber technique

In both field studies included in this thesis, N_2O fluxes (and CH_4 in the summer drought study) were measured with static chambers. As methodological considerations are not addressed in the individual research papers, an overview and some considerations regarding the method are given here.

The principle behind static chamber measurements is to trap the gas that diffuses across the soil surface within a certain time, to measure the increase in its concentration in the chamber, and to calculate a flux rate from the increase in concentration over time, taking into account the air temperature and pressure. Closed chamber methods are the most widely used measurement technique for the quantification of N_2O fluxes from field experiments (Butterbach-Bahl et al. 2013). They are relatively cheap and easy to build, and allow measurement of fluxes at a small spatial scale without interference from neighboring plots (Hensen et al. 2013).

The static closed chamber method has several severe shortcomings, though. One source of several different errors is that chamber measurements interfere with the processes they shall measure, thus affecting the flux while measuring it.

Firstly, N_2O and CH_4 fluxes in soil are mainly due to diffusion, and according to Fick's first law, the gas flux is dependent on the concentration gradient, in this case between the soil air and the overlying atmosphere. In static chambers, the concentration in the chamber must change during the measurement period (increase in the case of efflux, decrease in the case of net flux into the soil), and thereby the concentration gradient between the soil and chamber atmosphere is lowered. It has been shown that also in the soil atmosphere below the chamber the concentration may increase during measurements, further affecting the flux and leading to underestimation in the case of linear flux calculation (Conen & Smith 2000; Davidson et al. 2002). Especially at high fluxes, the assumption of linear fluxes was estimated to cause 20% - 40% underestimation of CO_2 fluxes (Kutzbach et al. 2007). There is still a debate about whether linear or non-linear calculation of fluxes is more appropriate for CH_4 and N_2O measurements, with a clear trend towards non-linear in the last years (Kroon et al. 2008; Pedersen et al. 2010), although especially at low flux conditions, non-linear calculation has a higher uncertainty (Pihlatie et al. 2013). For calculation of CH_4 and N_2O fluxes in the field studies comprised here, a mixed approach was thus used (Leiber-Sauheitl et al. 2014).

Secondly, chamber installation on the chamber bases/collar induces pressure differences that could affect the concentration in the chamber and thus the calculated fluxes (Davidson et al. 2002; Pihlatie et al. 2013). To avoid high pressure during chamber closure (and thus pushing air into the soil and altering the flux), a vent tube was installed (in both chambers for the summer drought and the CULTAN experiment) and in the chambers used in the CULTAN experiment sampling valves were left open during chamber closure. The vent tubes were installed at each chamber to avoid pressure differences between inside and outside atmosphere during the chamber closure, as reduced pressure in the chamber (e.g. due to sampling), or overpressure due to temperature differences, would lead to over- or underestimation of fluxes, respectively (Davidson et al. 2002). To avoid a possible Venturi effect that would suck air out of the chamber and induce reduced pressure (Conen & Smith 1998), the vent outlets were placed near the soil surface.

Thirdly, due to the covering of the soil surface with the chamber, microclimatic conditions in the chamber atmosphere are affected. Exclusion of turbulence can alter the concentration (gradient) in the chamber, especially at large chamber volumes, and Christiansen et al. (2011) report

underestimation of 36% for CH_4 fluxes without a fan (in an unvented chamber, however). To account for this issue, fans were placed in one corner of the large chambers used in the CULTAN study, also because of high plants in the chambers during the growing season that were assumed to prevent proper mixing by diffusion.

Besides the effects during flux measurement itself, other problems may derive from installation of the chamber bases: To avoid effects of root cutting by insertion of chamber bases (collars) on gas fluxes, they remained in the soil as long as management was possible without removing. For soil tillage, seeding, fertilizer injection and the first surface application (to avoid "spray shadows") in the CULTAN experiment, as well as for planting, fertilizing and tillage in the summer drought study, however, chamber bases had to be removed. After tillage, the soil matrix was disturbed anyway, so that disturbance by collar insertion was regarded negligible.

Due to the relatively small dimensions of the chambers, spatial heterogeneity of the study area may not be covered. Soil processes, also denitrification and N_2O emission, are highly heterogeneous on both spatial and temporal scales, characterized by hotspots (Mathieu et al. 2006). Mathieu et al. (2006) found N_2O emission to be highly variable, but spatially independent, at a grid of 3m x 3m. They suggest heterogeneity to occur at the microscale level. Spatial heterogeneity of fluxes is often driven by heterogeneity in underlying environmental parameters, as soil moisture, or nutrient availability (Butterbach-Bahl et al. 2013). To account for the spatial heterogeneity in the CULTAN experiment, chamber bases were in their dimension (including 8 injection spots each, and thus covering the area above and between injections spots in representative ratio) and in their placement (e.g. avoiding tire tracks) adjusted to the experimental setup.

The high temporal variability of N_2O fluxes is also not covered if manual sampling is performed only weekly (plus additional measurements at certain events) and therefore peak emissions may be missed despite proper planning and adjustment of measurement days/periods according to management and weather conditions. The low temporal resolution of weekly measurement has caused annual fluxes to differ by up to 50% from near-continuous measurements (Flessa et al. 2002; Kroon et al. 2008), and Thornton et al. (1996) reported N_2O fluxes to differ by a factor of 2.5 if calculated from weekly instead of daily measurements after anhydrous ammonia injection to a loess soil. The deviation of annual emissions calculated from weekly measurements could, however, be substantially reduced by extending the measurement scheme to additional measurements after fertilization and strong rain events (Flessa et al. 2002). To account for fertilization peaks, in addition to weekly measurement we sampled in higher frequency after fertilization and irrigation.

Alternatives for measuring gas fluxes in field experiments with multiple treatments are scarce. Micrometeorological methods like eddy covariance supply much higher time resolution but are far more expensive and not applicable at stable atmospheric conditions. They furthermore require large homogeneous surfaces, thus not allowing simultaneous measurement of different treatments within a crop field. For research questions with several different treatments, aiming to improve the process understanding, chamber flux measurement are thus still the method of choice (Hensen et al. 2013).

It has to be mentioned, that although annual fluxes are measured and discussed in the studies presented, the main focus is the comparison of different treatments.

4. Small effects of reduced summer precipitation on net exchange of CH_4 and N_2O fluxes on a sandy soil under maize and sorghum[1]

4.1. Abstract

For most of Central Europe climate change is expected to lead to higher frequencies of extreme weather events with hotter and drier summers. The resulting lower soil water content directly affects turnover rates of nitrogen and carbon and, consequently, production rates and fluxes of the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) from soil. Type and mass of plant coverage can modify the degree of desiccation. Over a time period of 18 months, we measured the net exchange of these greenhouse gases and nitrogen dynamics on an experimental field site on a sandy loam soil in Northern Germany, which was planted with sorghum and maize. The measurement period included two periods of experimental drought: During spring and summer, plants on ambient wet control plots were irrigated to keep water content above 50% water filled pore space (WFPS), whereas on dry plots rain was excluded by transparent rain shelters. Soil water content and nitrogen dynamics were measured from soil samples, and fluxes of N_2O and CH_4 were measured between plant rows using static chambers.

N_2O emission was generally low, with a mean annual emission over all treatments of 1.8 ± 0.5 kg N ha^{-1} yr^{-1}. There was a trend to higher emissions (20% – 25% lower on annual base, driven by winter emission) from wet than dry plots, but the difference was not significant. Uptake of atmospheric CH_4 averaged 1.9 ± 0.3 kg C ha^{-1} yr^{-1}, and was significantly higher (by 46%) at dry than wet maize plots when cumulated over the whole experiment (18 months) and during one of the drought periods at both maize and sorghum plots. Linear mixed effect models showed correlation between N_2O fluxes and WFPS and nitrate content in the upper 10 cm of soil and soil temperature; CH_4 fluxes were correlated with WFPS and nitrate content in soil, and their interaction. There was no consistent plant impact on greenhouse gas fluxes, but due to higher maize than sorghum aboveground biomass yields, yield-scaled emissions were approx. 35% higher from sorghum than maize plots. Yield-scaled N_2O emission was similar for dry and wet conditions because both yields and N_2O emission were lower at dry plots.

Summer drought thus affected yield-scaled N_2O emission by changing both emission dynamics and crop yield. The results suggest that reduced precipitation during summer months will have only minor effects on N_2O emission at this site but will increase the uptake of atmospheric CH_4.

[1] This chapter is in preparation for submission with the following authors: Marianna Deppe, Reinhard Well, Remigius Manderscheid Roland Fuß, Hans-Joachim Weigel, Heinz Flessa

4.2. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas contributing 6% of the radiative forcing to the anthropogenic greenhouse effect (Myhre et al. 2013) and its decomposition currently is the main process depleting ozone in the stratosphere (Ravishankara et al. 2009). The concentration of N_2O in the atmosphere increased since industrialization from 270 ppb in 1750 to 325 ppb in 2012 (WMO 2013). Agriculture is one of the most important anthropogenic sources of N_2O, emitting approx. 2.8 (1.7 - 4.8) Tg N yr^{-1} (Denman et al. 2007). Besides carbon dioxide (CO_2) and N_2O, methane (CH_4) is one of the earth's most important greenhouse gases, and CH_4 concentration in the atmosphere more than doubled from pre-industrial values (WMO 2013).

Biogeochemical processes involved in both N_2O production and CH_4 uptake in soils are controlled, among other factors, by the soil water content (e.g. Ruser et al. 2006; Smith et al. 1998). N_2O is mainly produced biologically in soils as intermediate or by-product during nitrification and denitrification. N_2O production is often dependent on soil water filled pore space (WFPS), and peak emission of N_2O are expected when WFPS increases to > 60% (Linn & Doran 1984; Skiba & Smith 2000), and after disturbances as fertilization or rewetting of dry soil (Ruser et al. 2006). CH_4 is produced in soil under anaerobic conditions and it is oxidized under aerobic conditions. Aerobic terrestrial soils have a sink function for atmospheric CH_4 of ~ 30 Tg yr^{-1}, counterbalancing roughly 5% of total CH_4 emissions (Ciais et al. 2013).

Changes in precipitation and temperature, as they are likely to occur in the next decades, will affect biogeochemical processes in soils. For Europe, the Intergovernmental Panel on Climate Change (IPCC) predicted warmer summers and for some regions decreasing precipitation, with increasing risk of extreme events as summer drought and heavy rain events (Christensen et al. 2013; Seneviratne et al. 2012). Nitrogen availability in soil is another factor controlling production of N_2O (Conrad 1996; Mosier 1994), and its dynamics in soil might change with changing climatic conditions. Drier summers may affect soil nitrogen dynamics by several processes such as reduced plant N uptake, slower nitrate leaching to deeper soil layers, and lower N mineralization rates (Bimüller et al. 2014; Borken & Matzner 2009; Larsen et al. 2011; Rimski-Korsakov et al. 2009).

One of the most important crops produced worldwide is maize and its production is increasing to meet global need for food and, still more, feed and energy plants for the growing global population (Alexandratos & Bruinsma 2012; FAO 2008; Ray et al. 2013; USDA 2013). Sorghum is more drought resistant than maize due to a higher ability of extracting water from deeper soil layers (e.g. Singh & Singh 1995; Zegada-Lizarazu et al. 2012) thus sustaining biomass production under drier conditions, and might have a higher potential for energy production on marginal-yield sites (Farré & Faci 2006; Yuan et al. 2008). In view of the risk of more frequent summer drought and increasing temperatures, sorghum might become an advantageous alternative to growing maize in Central Europe.

There have been several experiments regarding the impact and feedbacks of changing climate on N_2O and CH_4 fluxes. Simulation of future climate by rain exclusion experiments were performed e.g. in peatlands, pastures, forests and shrubland, with inhomogeneous results: Carter et al. (2012) report reduced N_2O efflux caused by induced drought at several European shrubland sites and Carter et al. (2011) no effect of experimentally increased summer drought as a single factor on N_2O and CH_4 emissions from a heathland. For forest floor, Borken et al. (2000) and Borken et al. (2006) showed small to important increases in CH_4 oxidation, Goldberg et al. (2010) a reduction in N_2O emission and Muhr et al. (2010) reduced nitrogen mineralization with increased summer drought. Hartmann and Niklaus (2012) found a large reduction of N_2O emission from fertilized but not from

unfertilized alpine pastures. We are not aware of studies regarding the effect of experimentally reduced summer precipitation in sandy cropland soils on N_2O and CH_4 exchange.

Taking into account earlier drought studies and controlling environmental parameters, we hypothesize that (1) drier soil conditions due to rain exclusion during summer lead to an increase in CH_4 uptake; and (2) N_2O emission during the drought period is reduced because of low denitrification activity at low soil moisture. At the annual scale, drought may either reduce N_2O emission due to lowering of denitrification with decreasing soil moisture, or N_2O emission may be increased because of higher nitrogen (N) content resulting from reduced plant N-uptake during drought periods. Furthermore, (3) the impact of crop type on yield-scaled N_2O emission is supposed to be controlled by better adaptation to dry conditions of sorghum compared to maize.

To test these hypotheses, a field experiment was conducted on a sandy soil planted with maize and sorghum. Rain exclusion was used to intensify summer drought during the growing season. Measuring CH_4 and N_2O fluxes and mineral in soil, the reaction of greenhouse gas fluxes and N turnover to enhanced drought conditions was studied.

4.3. Materials/Methods

4.3.1. Research Site

The experimental field site was located at the Johann Heinrich von Thünen-Institute in Braunschweig, Germany (52°18' N, 10°26' E, 79 m a.s.l.). The soil is a luvisol with sandy loam texture (69% sand, 24% silt, 7% clay), a pH of 6.5 and an organic carbon content of 1%, total N content of 0.09% (C/N = 10.7). Bulk density was 1.54 ± 0.10 g cm^{-3} in uncompacted soil and 1.63 ± 0.07 g cm^{-3} in plant rows after harvest. Mean annual temperature at the site is 8.8 °C, annual precipitation is 618 mm. A more detailed site description is given in Manderscheid et al. (2014).

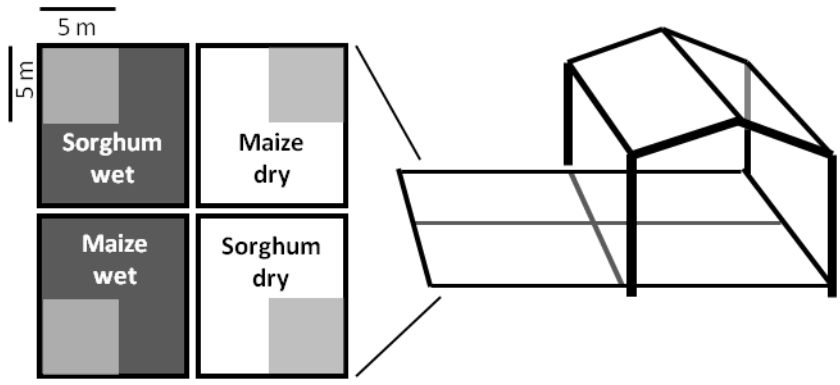

Figure 4-1: Scheme showing one of three replicate plots with subplots. Shaded areas mark subplots used for gas and soil measurements in this study

4.3.2. Treatments

This study took place as part of a more complex experiment on the impact of climate change on maize and different sorghum species (Manderscheid et al. 2012). Sowing of plants was done timely

in 2011 (18th of May) but was delayed in 2010 (10th of June) due to cool weather conditions in May. Measurements were performed on 3 replicate plots (5 x 5 m, within larger plots of the main experiment, see Figure 4-1) in both years. The experiment was two-factorial with different crops (maize and sorghum) and experimental drought (dry) vs. control (wet). Two out of 4 subplots per plot were planted with maize (*Zea mays*, cultivar Simao, 8 plants m^{-2}, 0.75 m row distance) and sorghum (*Sorghum bicolor*, cultivar Bulldozer, 20 plants m^{-2}, 0.75 m row distance), respectively. The preceding crop at the site was ryegrass that was mulched and incorporated. One subplot for each crop type was under a tent that was manually covered with transparent shields at days with > 10 mm day^{-1} precipitation forecast (dry treatments) during the period with rain exclusion (7/21 – 9/9/2010 and 7/22 - 10/4/2011). The other subplots, designated as well-watered control plots (wet), were drip irrigated to keep water content above 50% water filled pore space during the growing season (see Erbs et al. 2012 for detailed description of field installations). Fertilizer was applied according to local fertilization practices (N fertilization: calcium ammonium nitrate, 150 kg N ha^{-1}, in May 2010 and 2011) and weed control was performed manually in experimental plots in June 2010 and 2011, and chemically with bromoxynil in May 2011. Maize and sorghum plants were harvested at the end of October (10/4/10 and 10/4/11 for biomass and N yield samples, 10/25/2010 and 10/28/2011 total fields) and the field was ploughed before seedbed preparation. After ploughing in October 2011, winter wheat was sown.

4.3.3. Measurement of plant biomass yield and nitrogen uptake

Aboveground biomass of maize and sorghum plants was harvested at the beginning of October (4th - 5th October) from a ground area of 3 m^2 in each of the 12 subplots. After drying (105°C) to constant weight, total dry weight was determined. Total N concentration was measured in ground sample material of the total aboveground biomass using an element analyzer (TruSpec CNS, Leco). Total N in aboveground biomass was calculated from biomass yield data and N concentration of the biomass.

4.3.4. Flux measurements

Fluxes of N_2O and CH_4 at the soil surface were measured between plant rows of maize and sorghum with closed chambers at weekly intervals, with some larger intervals due to field management. As bases for the chambers, PVC rings (30 cm diameter, 15 cm height) were permanently installed approx. 10 cm deep into the soil. PVC chambers (30 cm diameter, 20 cm height) were placed on these rings and sealed with rubber collars at the start of each flux measurement. Vent tubes permitted equilibration of air pressure. Four samples of chamber atmosphere were taken after chamber closure in intervals of 12 to 30 min in 50 ml evacuated glass bottles equipped with teflon stop-cocks. Concentrations of N_2O and CH_4 in gas samples were analyzed with a gas chromatograph (GC 2014, Shimadzu, Duisburg, Germany) equipped with an automated rack and an ^{63}Ni electron-capture detector for N_2O and an FID for CH_4 (Loftfield et al. 1997). Gas fluxes were calculated from measured concentrations, air pressure and temperature inside the chamber using either linear regression, robust linear regression (Huber, 1981) or the Hutchinson-Mosier non-linear regression (HMR, Pedersen et al. 2010). The method used for further analysis was chosen as described by Leiber-Sauheitl et al. (2013). In short, we applied the following criteria: robust linear regression was used as default. If only three data points for a flux measurement were available, linear regression was used. HMR was used if HMR flux could be fitted, had a smaller value of Akaike's information criterion (AIC) and a lower p-value than the linear flux and its absolute value was not more than 4 times that calculated using robust regression. This reproducible method avoids severe

overestimation of fluxes (Leiber-Sauheitl et al. 2013) and potential bias due to personal decision-making when analyzing concentration trends. Reported flux rates represent net emission to the atmosphere when values are positive and net uptake into soil when negative. Cumulated fluxes per period and per year were calculated by linear interpolation between measurement dates.

4.3.5. Climatic condition and water content

The two growing seasons 2010 and 2011 were considerably different regarding precipitation (Figure 4-2). 2010 was a rather wet year with 750 mm annual precipitation and highest precipitation of 195 mm in August (see also Erbs et al. 2012). 2011 was a relatively dry year (488 mm annual precipitation) and both precipitation and irrigation were more evenly distributed in summer. During the periods between start of rain exclusion and harvest (subsequently referred to as drought periods) in 2010/2011, precipitation was 334/170 mm, of which 176/53 mm were excluded by rain shelters on dry plots, and irrigation on wet plots was 20 mm in 2010 and 108 mm (sorghum)/118 mm (maize) in 2011 (DWD, Erbs et al. 2012, Figure 2). This results in total water inputs during the drought period of 158 (2010) and 117 mm (2011) on dry plots, 354 mm on wet plots 2010 and 288 (sorghum)/ 298 mm (maize) on wet plots in 2011.

Soil moisture was measured on composite soil samples taken on five spots halfway between plant rows in 0 – 10 cm and 10 – 30 cm depth. Gravimetric water content was obtained by weighing before and after drying at 105°C; WFPS was calculated from gravimetric soil water content and soil bulk density.

4.3.6. Mineral nitrogen in soil

Content of ammonium (NH_4^+) and nitrate (NO_3^-) in 0 – 10 cm and 10 – 30 cm soil depth were determined weekly unless soil was completely frozen. Field-fresh soil samples for N analyses were stored at 5 °C for up to 24 h until extraction. Contents of NH_4^+-N and NO_3^--N were analyzed photometrically (SA 5000, Skalar Analytical B.V., Netherlands) in filtrates from 40 g field fresh soil with 200 ml 0.01 M $CaCl_2$ solution shaken for 1 h (according to ISO 14255; MN614 ¼ filters, Macherey & Nagel, Düren, Germany). Extracts were stored frozen until analyses.

4.3.7. Statistics

Data analyses were performed with the software R (version 3.0.2, R Core Team 2013). To test for treatment and environmental parameter impact on measured N_2O and CH_4 flux rates, we conducted regression analysis with linear mixed effects models using the *nlme* package in R (Pinheiro et al. 2013). This was necessary, as flux measurements were repeatedly performed at the same positions and the resulting time series thus violate the assumption of independence needed for ordinary least square regression. The recommendations in *Zuur et al. (2009)* were followed to develop appropriate model structures. Data transformation was performed when necessary. The dataset was divided into 6 periods (Figure 4-3) to see whether the expected effects occurred over the whole year or differed between periods. Logit transformation was applied to WFPS (log(WFPS/(1-WFPS)); Warton & Hui 2010) when used as dependent variable but not when used as an independent variable in models describing N_2O fluxes. N_2O fluxes were log-transformed before further analyses because residual plots showed strong deviation from normal distribution when untransformed flux rates were used. An offset of 10 µg N $m^{-2}h^{-1}$ was used to keep most of the negative fluxes in the dataset, as they most likely represent variation around zero resulting from

measurement uncertainty. However, the 12 lowest measurements of N_2O fluxes were excluded from statistical analyses, as they might represent real N_2O uptake that we did not want to address with the models applied. A nested random intercept was included in all models to account for the experimental setup with one subplot per treatment in three different plots. Standard error of flux measurements was used as variance covariate; this allows stronger deviation of less precisely measured fluxes from the model and improved the homogeneity of residual variance. Autocorrelation of measured fluxes was considered by applying first or second order autoregressive correlation structures.

Different plant type, water regime and period were included as fixed effects with interactions in the models for WFPS, N_2O and CH_4; and models were fitted based on maximum likelihood. AIC was used to identify the best model fit. Interactions and fixed effects without significant influence ($p > 0.05$) were then step-wise excluded from the model to find the optimal model structure, which was then fitted using restricted maximum likelihood (REML) to get the final estimates. Mean fluxes were additionally tested for significant differences between periods within treatments and between treatments within periods using the *glht* function from the *multcomp* package in R (Hothorn et al. 2008) and the *fdr* correction (Benjamini & Hochberg 1995) for multiple comparisons. To test for correlation between gas fluxes and environmental parameters, additional mixed effects models were fitted. To account for substrates of both nitrification and denitrification, NO_3^--N and NH_4^+-N content in soil (sum of 0 – 10 cm and 10 – 30 cm), each of them with interaction with WFPS, soil temperature and plant type (for its possible impact on carbon availability for denitrification), were chosen as relevant parameters and thus used as fixed effects with interactions (starting with ((NH_4 + NO_3) * WFPS * soil temperature * plant type) in the full model). For CH_4 fluxes, NO_3^--N and NH_4^+-N content were included again, both in interaction with WFPS and soil temperature (WFPS * soil temperature * (NH_4 + NO_3) as full model fixed effects), as ammonium oxidation may compete with methane oxidation in soil (Bédard & Knowles 1989) and thus N content can affect CH_4 uptake rates in soil (e.g. Acton & Baggs 2011; Tlustos et al. 1998). Here again, step-wise exclusion of insignificant ($p > 0.05$) interactions and parameters led to final models.

Cumulated fluxes per year were additionally tested for significant differences between treatments using analysis of variance (ANOVA) and Tukey's HSD test for pair-wise comparisons.

4.4. Results

4.4.1. Treatment effect on soil water content

Drought treatment led to significant lowering of WFPS ($p < 0.001$) in dry plots during both drought periods in 0 – 10 cm and 10 – 30 cm depth. The magnitude of desiccation during drought was slightly greater in 2011 than 2010, which corresponds to the fact that water input during the drought period was 16%/19% (wet sorghum/maize treatment) and 26% (dry treatment) lower in 2011 than 2010. Summing the effect of rain exclusion at dry and additional irrigation at wet plots resulted in 180 mm total difference in precipitation between treatments until early September 2010, and 171 mm in early October 2011, when lower WFPS occurred at dry plots. A quarter of this difference (~40 mm) was visible in the soil at the end of the drought period as a difference in soil moisture.

At the beginning of the drought period 2010, WFPS was between 20% and 45% in all treatments. While precipitation and irrigation led to an increase in WFPS to > 60% in wet plots, rain exclusion on dry plots resulted in drying to < 20% WFPS until September 2010 (Figure 4-2). Increasing WFPS on dry plots at the end of September 2010 resulted from lateral inflow (amount not quantifiable) from outside the plots due to an extreme precipitation event of 38 mm (9/27/10). Highest water contents were measured in winter 2010/2011. As the soil is well-drained, water-saturation occurred only when soil was frozen in December 2010 (Figure 4-2). In 2011, desiccation in summer was faster on plots under maize than under sorghum, but at the end of the drought period there was no significant effect of plant type on WFPS. Lowest water contents reached during the drought periods were 13 – 14% in 10-30 cm and 10% in 0–10 cm on dry plots. While WFPS in the first winter season was almost equal in both treatments, values trended 9% and 10% lower in 'dry' treatments at 0-10 cm and 10-30 cm soil depth, respectively. Mean values and Tukey's test results of significant differences of logit-transformed WFPS between wet and dry treatments are shown in the appendix (Table A 1 and A 2). Differences between the treatments were significant (no overlap of confidence intervals ($p > 0.95$), see Appendix, Figure A 1) at several dates in November and December of the second winter season.

4.4.2. Plant growth and nitrogen uptake

Biomass yields were higher in 2011 than in 2010, and total maize biomass yields were generally higher than sorghum biomass yields (Figure 4-4). However, yield reduction in dry plots relative to wet plots was stronger for maize (33%) than for sorghum (24%) in 2011.

Crop N content in harvested aboveground biomass (N yield; shown in Figure 4-4) was between 177 and 258 kg N ha^{-1}, and lower at dry than wet plots in both years. Reduction was stronger on sorghum plots in 2010 (18%, 6% on maize plots) and on maize plots in 2011 (12%, 5% on sorghum plots).

4.4.3. Dynamics of mineral soil N and N_2O emission

Measured **nitrate** (NO_3^-) content in the upper 10 cm of soil was high at the beginning of measurements, with concentrations of up to 420 µg NO_3^--N cm^{-3} and total mineral N in the plough horizon reaching 400 - 500 kg N ha^{-1} in late July/early August (fertilization was 150 kg NO_3^--N ha^{-1} in May, Figure 4-5). It sharply decreased in wet maize and sorghum at the end of July/ early August

2010, with a subsequent increase in the 10 - 30 cm depth increment. Up to 148 ± 91 µg NO_3^- N cm^{-3} were measured on wet maize plots in the second half of August 2010 in 10 – 30 cm depth; on dry plots, NO_3^- content increased more slowly, with highest amounts reached in September (Figure 4-5). These dynamics are attributed to transport with the seepage water resulting from high amounts of precipitation (Figure 4-2). In 2011, when sowing was successful in May, N content after fertilization was lower than 2010, despite equal amounts of fertilizer added. NO_3^- content decreased to < 9 µg NO_3^--N cm^{-3} before the drought treatment began. An increase in NO_3^- content in 10 – 30 cm occurred in all treatments in early August 2011 when the soil became drier (WFPS decreasing to < 40%). At dry maize plots, NO_3^- content remained relatively high during the following drought period (WFPS < 30% for 9 weeks; Figure 4-2 and 4-5), and NO_3^- content was significantly higher in dry than wet plots during drought 2011.

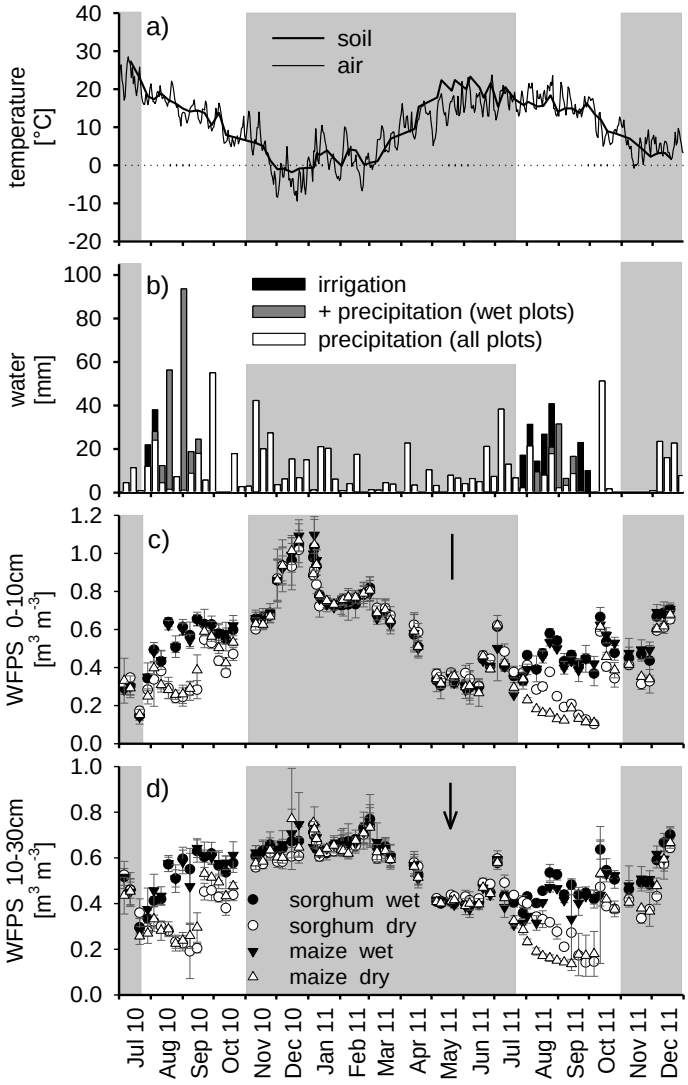

Figure 4-2: (a) Soil temperature in 10 cm depth (mean of all plots) and air temperature in 2 m; (b) Weekly precipitation/irrigation over time. White bars show water supplied to dry plots (precipitation – rain exclusion) per week, grey plots show precipitation excluded on dry plots and thus only available on wet plots and black bars represent irrigation on wet plots. (c) Water filled pore space (WFPS) in soil at 0–10 cm and (d) 10–30 cm depth in all treatments. Symbols show means of 3 replicates; error bars represent standard deviation. White fields highlight drought periods from beginning of rain exclusion/irrigation until harvest.

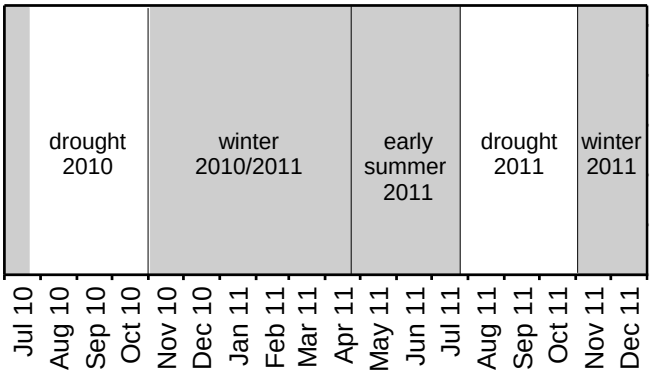

Figure 4-3: Scheme of periods used for statistical analyses and calculation of cumulative fluxes of N_2O and CH_4.

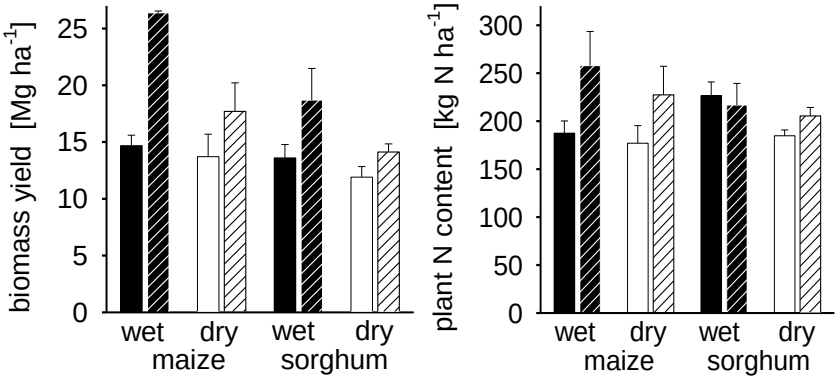

Figure 4-4: Biomass yield (dry matter, left) and nitrogen (N) content of aboveground biomass (right) of maize and sorghum with (white bars) and without (black bars) rain exclusion in 2010 (plain bars) and 2011 (shaded bars). Means (n=3); error bars represent standard deviation of replicate plots.

Ammonium (NH_4^+) concentration on all plots was mostly low (< 10 kg NH_4^+-N ha^{-1}), except for some weeks after fertilization with calcium ammonium nitrate (Figure 4-5). At the beginning of rain exclusion, approx. 10 – 30 µg NH_4^+-N cm^{-3} (10 - 30 kg N ha^{-1}) were available in 0-10 cm depth in 2010 and only < 0.4 µg NH_4^+-N cm^{-3} in 2011 (Figure 4-5). NH_4^+ content in 10-30 cm soil depth was always < 10 µg NH_4^+-N cm^{-3} and did not show any distinct dynamics (data not shown).

Calculated **N_2O** fluxes ranged from -41 to 920 µg N m^{-2} h^{-1}, with a mean flux over all treatments of 20 ± 11 µg N m^{-2} h^{-1} (median: 8.99 µg N m^{-2} h^{-1}). The automatic decision scheme of the used flux calculation led to 12% of fluxes being calculated with the HMR procedure, 86% were calculated with robust linear regression and 2% by linear regression due to only 3 concentration measurements per flux. Relatively high N_2O fluxes occurred:

(1) in summer 2010, before and shortly after beginning of the drought treatment (up to 150 µg N m^{-2} h^{-1});

(2) after harvest and precipitation in November 2010, when WFPS reached > 50% also in dry treatments (up to 250 µg N m^{-2} h^{-1});

(3) mostly on wet plots in winter 2010/2011, when temperatures increased while or shortly after soil was frozen (up to 920 µg N m^{-2} h^{-1}); and

(4) on sorghum plots in June 2011 when soil moisture increased to > 60% WFPS at high mineral N content some weeks after fertilization (up to 112 µg N m^{-2} h^{-1}, Figure 4-6).

Rates declined in both dry and wet treatments during drought 2010 to 8 ± 12 µg N m^{-2} h^{-1} before harvest and were continuously low (< 20 µg N m^{-2} h^{-1}) during drought 2011 in all plots (Figure 4-6). Net uptake of N_2O into the soil was measured at some dates, especially in September 2010 when WFPS increased to > 55% after the drier summer in both wet and dry plots.

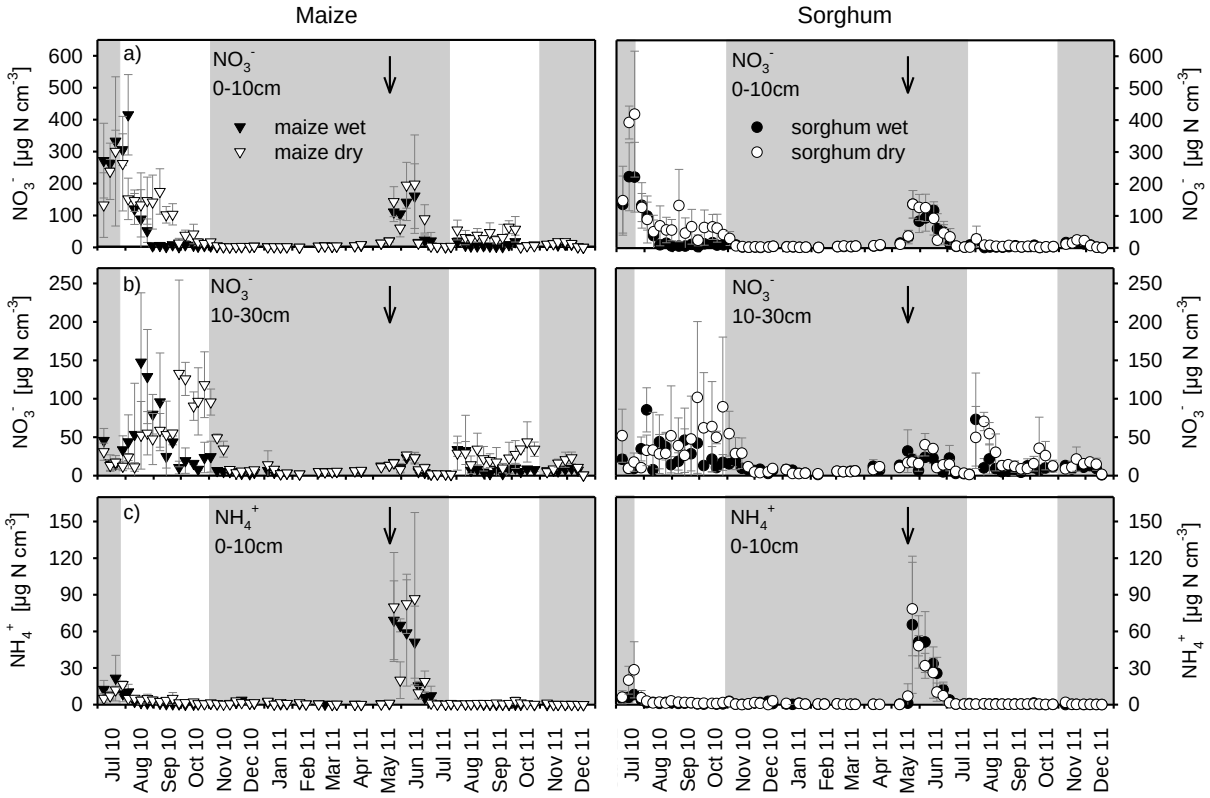

Figure 4-5: Mineral nitrogen content in soil under maize (left) and sorghum (right column). a) Nitrate (NO_3^-) content in 0-10 cm and b) 10-30 cm depth and c) ammonium (NH_4^+) content in 0-10 cm depth. Symbols show means of 3 replicates (open: dry treatment, filled: wet treatment); error bars represent standard deviation. White fields mark drought periods from beginning of rain exclusion/irrigation until harvest; the arrows mark the time of fertilization in 2011; fertilization in 2010 was conducted in May.

Linear mixed effect models of treatment impact show significant impact of the interaction between water treatment and period, as well as between plant type and period. The model explained 26% of the variance in log-transformed N_2O fluxes. Total annual N_2O emission exhibited a trend to be lower by 20% under sorghum and 25% under maize in drought treated plots; however, differences between wet and dry plots were not significant, neither over the whole year, nor when mean fluxes of all periods were compared (see Table 4-1). During the early summer 2011 period before the rain exclusion was started, mean N_2O fluxes from soil under dry sorghum were significantly higher than from dry maize plots. Neither in the other periods nor on an annual scale, did the plant effect translate into significantly different cumulated fluxes between maize and sorghum.

Regarding linear mixed effect models with driving parameters of N_2O fluxes, log-transformed N_2O fluxes were significantly correlated with WFPS and NO_3^- content in soil, and soil temperature

($p < 0.005$). Although these effects are highly significant, the total linear mixed effects model explains only 13% of the variance in log-transformed fluxes.

N_2O emission from harvest 2010 to harvest 2011 accounted for between 0.9% (dry maize) and 1.6% (wet sorghum) of N applied as fertilizer per year. However, real emission factors for N fertilization cannot be derived, as the study design did not include unfertilized plots. Due to higher yields and slightly lower N_2O emission, yield-scaled emissions were 0.08 and 0.13 kg N_2O-N t^{-1} d.w. maize and sorghum in the wet treatments and 0.07 and 0.11 kg N_2O-N t^{-1} d.w. in the dry treatments, respectively. Yield-scaled N_2O emissions in dry and wet treatments were thus by 36 and 35% (but insignificantly) lower, respectively, in the maize compared to the sorghum plots due to lower sorghum yields. Based on N content in aboveground biomass, N yield-scaled emissions amounted to 8 ± 3 g N_2O-N kg^{-1} N in plants, without correlation or visible pattern to plant or water treatment.

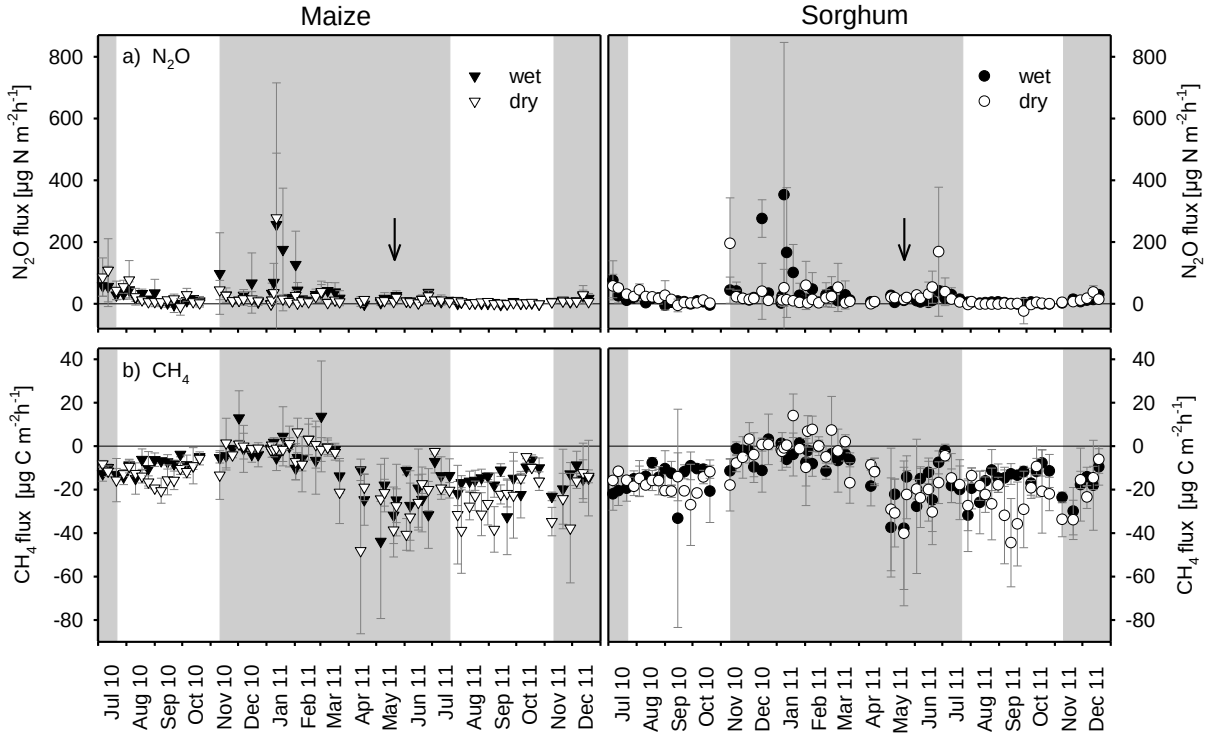

Figure 4-6: Fluxes of nitrous oxide (N_2O, a) and methane (CH_4, b) between plant rows of maize (left) and sorghum (right column). Symbols show means of 3 replicates (open: dry treatment, filled: wet treatment); error bars represent standard deviation. Negative flux rates represent uptake into the soil. White fields mark drought periods from beginning of rain exclusion/irrigation until harvest; the arrow marks time of fertilization; fertilization in 2010 was conducted in May.

4.4.4. Dynamics and total amounts of atmospheric CH_4 consumption in soil

Measured CH_4 fluxes ranged from –91 to +32 µg C m^{-2} h^{-1}, with a mean rate of -14 ± 3 µg C m^{-2} h^{-1} over all treatments and periods. At the beginning of measurements in July 2010, mean CH_4 flux was -15 ± 5 µg C m^{-2} h^{-1}. With increasing WFPS on wet plots, less CH_4 was consumed in wet than in dry treatments. In winter 2010/2011, when soil was wettest and partly saturated (Figure 4-2), CH_4 emission occurred at some days, with highest emission of 20 – 30 µg C m^{-2} h^{-1} from wet maize and dry sorghum plots (Figure 4-6).

Fluxes were highly variable in early summer and during drought 2011, and uptake increased during April/May 2011, when WFPS decreased to < 60%. Uptake decreased again with increasing WFPS after fertilization in June 2011, and was higher under dry than wet maize in August and under dry than wet sorghum in September 2011, when WFPS decreased to < 30% in the respective dry plots (Figure 4-2 and 4-6).

Linear mixed effect models of CH_4 fluxes show significant impact of drought treatment, plant type and period, and interaction between both plant type and drought treatment with period. During drought 2011, CH_4 uptake was significantly higher in dry than wet plots under both maize and sorghum; and in the following winter period, uptake in wet maize still differed from that in dry maize and sorghum. The difference was smaller during drought 2010 and significant only between wet maize and dry sorghum. Over the whole experiment, significant effects of treatments on cumulated fluxes were detectable between wet and dry maize plots ($p<0.05$, see Table 4-1).

Linear mixed effect models of CH_4 fluxes in dependence of control parameters additionally showed that CH_4 uptake significantly correlates with WFPS and NO_3^--N content in soil, with higher uptake rates at low WFPS and NO_3^--N content. The interaction of WFPS and NO_3^--N modifies the impact of these parameters, with lower CH_4 uptake with increasing NO_3^--N content below 60.1% WFPS, and higher CH_4 uptake with increasing NO_3^--N content at higher water content. This model explains 34% of total flux variance. Although the impact of NO_3^--N and its interaction with WFPS on CH_4 uptake is highly significant ($p < 0.001$ for NO_3^- and $p = 0.012$ for the interaction), only a small part of CH_4 fluxes is explained with NO_3^--N. Excluding NO_3^--N and its interaction with WFPS leads to still 33% of variation in fluxes being explained by the model.

Table 4-1: Mean fluxes of N_2O and CH_4 for different periods, cumulated fluxes over the experiment and per year and calculated emission factors (EF) for yield based and fertilizer based emissions. Error terms are standard deviations (n=3). Results of posthoc pairwise comparisons of mean fluxes between treatments (within periods) are given in capital letters, and between periods (within treatments) in lower case letters. *fdr* correction (Benjamini & Hochberg 1995) was used to correct for multiple comparisons. Mean fluxes differ significantly ($p<0.05$) when they do not share the same letter. For fluxes cumulated over the whole experiment (sum), over a complete year (harvest 2010 - harvest 2011) and for yield-scaled fluxes, separate tests were performed.

crop	treatment	Pre-drought 2010	drought 2010	winter 2010/ 2011	early summer 2011	drought 2011	winter 2011	Sum	harvest 2010 – harvest 2011	fertilizer based emissions	Yield-scaled emissions
		6.7.-21.7.2010	22.7.- 1.11.2010	2.11.2010- 27.4.2011	28.4.- 24.7.2011	25.7.- 2.11.2011	3.11.- 31.12.2011			N_2O-N/ fertilizer N	g N_2O-N/ t d.w. biomass
		16d	102d	177d	88d	101d	59d	543d	365d	365d	365d
N_2O emission gN m^{-2} d^{-1}								**N_2O emission kgN ha^{-1}**			
sorghum	**wet**	7.5 ± 5.8 A ac	2.2 ± 0.8 A bc	11.4 ± 3.8 A a	3.8 ± 1.2 AB cd	1.2 ± 0.3 A b	2.8 ± 0.7 A bcd	2.94 ± 0.70 A	2.43 ± 0.69 A	1.6%	131 ± 47 A
	dry	11.1 ± 3.3 A ab	3.5 ± 0.6 A ac	4.8 ± 1.2 A c	9.1 ± 7.7 A a	0.8 ± 0.5 A b	3.6 ± 1 A c	2.35 ± 0.72 A	1.61 ± 0.71 A	1.1%	113 ± 56 AB
maize	**wet**	11.6 ± 10.1 A a	3.9 ± 0.6 A b	10.3 ± 4.3 A a	3.5 ± 1.4 AB b	1.2 ± 0.1 A c	3 ± 0.7 A b	2.95 ± 0.80 A	2.19 ± 0.78 A	1.5%	78 ± 29 AB
	dry	20.3 ± 17 A a	4.2 ± 2.5 A b	5.4 ± 3 A b	2.8 ± 0.6 B b	1.3 ± 0.9 A c	2.7 ± 1.8 A bc	2.20 ± 0.66 A	1.29 ± 0.54 A	0.9%	75 ± 38 B
CH_4 uptake gC m^{-2} d^{-1}								**CH_4 uptake kgC ha^{-1}**			g CH_4-C/ t d.w. biomass
sorghum	**wet**	-4.9 ± 0.5 A a	-3.4 ± 0.8 AB a	-1.3 ± 0.4 Ab	-4.5 ± 2.0 A a	-3.6 ± 0.5 A a	-4.9 ± 1.0 AB a	-1.70 ± 0.22 AB	-0.99 ± 0.19 A	-	-58 ± 12 A
	dry	-3.3 ± 0.3 AB a	-4.4 ± 0.7 A ab	-0.6 ± 0.5 A c	-5.2 ± 1.1 A b	-5.8 ± 0.5 B b	-5.6 ± 0.5 A b	-1.99 ± 0.16 AB	-1.16 ± 0.11 A	-	-85 ± 9 B
maize	**wet**	-2.7 ± 0.4 B ab	-2.0 ± 0.6 B bc	-0.9 ± 0.2 A c	-5.0 ± 0.8 A d	-3.8 ± 0.9 A ad	-3.8 ± 1.8 B ad	-1.45 ± 0.17 **B**	-0.98 ± 0.15 A	-	-41 ± 3 A
	dry	-2.9 ± 0.5 B a	-3.3 ± 0.5 AB a	-1.7 ± 0.4 A a	-5.8 ± 1.0 A b	-5.6 ± 1.0 B b	-5.8 ± 2.2 A b	-2.11 ± 0.20 **A**	-1.39 ± 0.18 A	-	-81 ± 11 B

4.5. Discussion

4.5.1. Calculation of gas fluxes – linear vs. non-linear

Calculation of N_2O and CH_4 fluxes using the routine to select the most suitable regression approach (see 4.3) yielded higher fluxes compared to default linear calculation (Appendix, Table A 3 and Figure A 2). The sequence of treatments, when ordered according to their total fluxes per period or year, remained the same, though. Peak N_2O emissions were approx. 25% and annual fluxes thereby 10–20% lower when calculated purely linearly than with the protocol including HMR. Moreover, results of statistical analyses differ slightly. N_2O emission would be reduced by 27% in sorghum and 42% in maize due to drought treatment (dry) compared to non-drought treatment (wet), respectively. For CH_4, there were significant differences between wet and dry maize during drought 2010 but not during drought 2011 with linear fluxes.

Due to relatively low flux rates in our experiment, HMR was less often used as in datasets analyzed by Pedersen et al. (2010), where 47% of fluxes were calculated non-linearly and gave approx. 50% higher fluxes. Systematic underestimation of CH_4 fluxes when calculated with linear regression were also shown in a chamber comparison campaign by Pihlatie et al. (2013). Difference between partly HMR and purely linear calculation of fluxes led to discrepancies comparable to ours in a study by Schelde et al. (2012). Different methods of flux calculation result in different annual fluxes, and this adds to the impreciseness of upscaled emission rates. However, in our case, discrepancy is not extraordinarily high.

4.5.2. Range of gas fluxes

Annual **N_2O emission** in this study was between 1.29 and 2.4 kg N ha^{-1} yr^{-1} and thus within the range of N_2O fluxes from cropland with mineral fertilization in Germany (Kaiser & Ruser 2000). As often reported elsewhere, high fluxes were measured after typical events as fertilization, harvest, heavy rainfall and during frost-thaw-cycles (Hellebrand et al. 2003; Kavdir et al. 2008; Sehy et al. 2003). That a great part of the N_2O emission occurred during winter (53-83% in our study for winter 2010/2011) is a common phenomenon under temperate climate with frost periods during winter (Flessa et al. 1995; Kaiser et al. 1998; Teepe et al. 2000).

With approx. 0.9–1.6% of applied N, measured fertilizer-based emissions were in the lower range of values given in the literature for arable soils in Germany (Jungkunst et al. 2006; Kaiser & Ruser 2000; Skiba & Smith 2000). While it is common to calculate fertilizer-based emissions by dividing total emissions by total N input from fertilizer (Dobbie et al. 1999; Kaiser & Ruser 2000), this procedure neglects background emissions. Subtracting these, fertilizer-induced emission might be substantially smaller (Jungkunst et al. 2006).

Measured annual **methane fluxes** of -1 to -1.4 kg C ha^{-1} yr^{-1} were also in the range given for northern European arable soils (Dobbie et al. 1996; Smith et al. 2000). Annual uptake was slightly higher than in a study by Hellebrand et al. (2003) on loamy sand soil under energy plants, where annual precipitation was comparable to our dry treatments. Flux dynamics showed a typical seasonal pattern, with highest uptake rates of methane during summer and considerably lower uptake activity and single events of net CH_4 emission during and after winter, when soil was wettest.

4.5.3. Effect of drought and other environmental parameters

Our hypothesis of higher N content under drought due to lower crop N uptake was supported by the observation of **nitrate** content being higher in dry than wet treatments in 2011. Nitrate content in soil increased during drought 2011, and N content in plant biomass was indeed higher at wet than dry plots (by 5-18%).

In 2010, possible drought effects were interfered with extremely high nitrate contents, probably attributable to high mineralization rates in soil caused by high amounts of organic material from the incorporated ryegrass. Also the appearance of relevant amounts of NH_4^+ in July 2010 points to high mineralization rates. Additionally, late sowing (3 weeks delay) might have caused lower root density between plant rows and reduced N uptake in plants during the first weeks of measurements compared to 2011. Time courses show depth translocation of NO_3^- in soil during drought 2010 that started earlier in wet than dry plots. If the NO_3^- was not completely taken up by plant roots in deeper depths, this implies considerable nitrate leaching. In the dry plots, more than 200 kg NO_3^--N were still available at the end of rain exclusion (9/9/2010) and more than 100 kg N after harvest 2010 in the plough layer. In the wet plots, the amount of available NO_3^- in the plough layer was much smaller at that time (20 - 30 kg N); the NO_3^- that was presumably translocated might, however, still have been present and available for N_2O production in deeper depths.

We furthermore hypothesized that increased summer drought reduces **N_2O fluxes** during the drought phase. Although the drought effect on water content itself was strong, and there was a positive correlation between WFPS and N_2O flux, this did not result in differences in N_2O fluxes between treatments due to low water content even in well-watered wet plots. Fluxes during summer drought periods (101 days = 28% of the year) itself were low and contributed only 2% – 26% to annual fluxes. There was a weak trend towards higher fluxes from wet than dry treated plots in winter 2010/2011 with a significant difference between wet sorghum and dry maize only when purely linearly calculated fluxes are regarded (Appendix, Table A 3).

Analyzing the conditions associated with observed peak fluxes of N_2O might identify situations which would generally lead to a substantial increase of annual N_2O losses. No distinct N_2O peak fluxes occurred with initial **rewetting** after rain exclusion, but emission rates > 200 µg N $m^{-2}h^{-1}$ occurred in the winter 2010/2011 period directly after harvest in November and when WFPS was > 80% after the soil had been frozen, with higher peaks at wet than dry treated plots. Peak emission during frost-thaw cycles may be attributed to release of physically stored N_2O produced in deeper soil layers during frost and/or conditions favorable for denitrification after thawing; i.e. high water content and high carbon availability due to breaking down of plant material and microbial residues into microbiologically usable forms (Goodroad & Keeney 1984a; Mørkved et al. 2006; Risk et al. 2013). N_2O emission during frost/thaw cycles was found to be affected by incorporation of crop residues (Pelster et al. 2013). In particular, residues with high N contents can increase N_2O emission after harvest and over winter (Kaiser et al. 1998). High soil moisture and frost intensity are further factors that affect N_2O losses over winter (Koponen & Martikainen 2004; Öquist et al. 2004; Risk et al. 2013). In our study, the water content in 0 – 10 cm depth did not differ visibly between wet and dry treatments throughout the winter 2010/2011 period. In 10 – 30 cm depth, however, desiccation in dry plots lasted somewhat longer, with small differences visible until December (Figure 4-2 and Figure A 1 in the Appendix). Higher N_2O peaks from wet than dry plots during winter 2010/2011 might thus be accredited to N_2O produced in or below 10 – 30 cm soil depth, with higher water content before freezing and higher organic matter input from plant residues and belowground biomass.

Another peak flux > 200 µg N m^{-2} h^{-1} was detected following heavy precipitation at high WFPS after fertilization 2011 in dry sorghum. It is well-known that N_2O emission depends on the water content (Dobbie et al. 1999; Ruser et al. 2006), and the threshold for high N_2O emission is generally given at somewhere between 60% – 80% WFPS (Laville et al. 2011; Linn & Doran 1984; Ruser et al. 2006). Several studies (e.g. Dobbie et al. 1999; Sehy et al. 2003; Smith et al. 1998) showed a significant effect of WFPS only when N substrates were not limiting. Statistical analysis of our data with mixed linear effect models partly supports this finding, with a significant effect of both WFPS and NO_3^- on N_2O fluxes. Distinct peaks in N_2O emission occurred when soil nitrate content was high after fertilization in both 2010 and 2011, and WFPS above 40%. Overall, N_2O flux of peak events (> 200 µg N $m^{-2}h^{-1}$) contributed approx. 30% to total cumulated fluxes.

Regarding **CH_4 fluxes**, the assumed increase in methane uptake under experimental drought occurred, with approx. 20% – 40% higher annual CH_4 uptake with increased summer drought. This is in accordance with results from forest and alpine grassland sites that showed increased annual CH_4 uptake under drought conditions (Borken et al. 2000; Borken et al. 2006; Hartmann et al. 2011). CH_4 uptake is controlled by diffusivity of CH_4 and O_2 into the soil, which depends on soil physical parameters such as bulk density and WFPS (e.g. Le Mer & Roger 2001; Smith et al. 2000). Thus expectedly, mixed linear effect models showed a correlation between WFPS and CH_4 emission, with higher uptake at lower water content. This impact is well-known (e.g. Carter et al. 2011; Smith et al. 2000); however, it was not clear in how far it would translate into significantly different seasonal or annual emissions between treatments.

There was also a correlation between CH_4 fluxes and **NO_3^- content** in soil and its interaction with WFPS. This interaction implies lower net CH_4 uptake with increasing NO_3^- content at WFPS < 60% and with decreasing NO_3^- content at WFPS > 60%. It has to be kept in mind that net CH_4 uptake into the soil is the consequence of uptake of CH_4 from the atmosphere into the soil as a result of CH_4 oxidation and emission of CH_4 from soil after methanogenesis in anaerobic soil compartments. Several studies showed a negative correlation between N content or NH_4^+ oxidation and CH_4 oxidation in soil (e.g. Acton & Baggs 2011; Dobbie & Smith 1996; Flessa et al. 1996); and for thermodynamical reasons, methanogenesis generally takes place only after other electron acceptors (e.g. O_2, nitrate or sulfate) have been largely depleted. Ammonium contents in our plots were low except for some weeks after fertilization, but nitrate as the product of nitrification might be another proxy for ammonium oxidation. CH_4 uptake in the experiment reported here was higher during drought 2011 than 2010 while NO_3^- contents in soil were lower (Figure 4-5 and 4-6). However, the effect of N content on CH_4 fluxes was small and might be driven by parameters not included in the model or even result from coincidence of low NO_3^--N content with CH_4 emission (positive fluxes) occurring during winter, when high WFPS and low temperature limited CH_4 oxidation.

To summarize the effect of summer drought treatment on N_2O and CH_4 fluxes, CO_2-equivalents can be calculated. As the GWP (global warming potential) of CH_4 (34) is much smaller than that of N_2O (298; Myhre et al. 2013), the reduction in annual N_2O emission (0.8/0.9 kg N_2O-N; or 1.3/1.4 kg N_2O for sorghum and maize) has a much higher share on total greenhouse gas potential reduction than the increase in annual CH_4 uptake (0.17/0.41 kg CH_4-C; or 0.23/0.55 kg CH_4). Together, they amount to approx. 0.4 t CO_2-equivalents ha^{-1} yr^{-1}. The reduction in N_2O emission due to summer drought is in a range comparable to reduced N_2O emission after a 15% reduction of N input or with the use of nitrification inhibitors (Eagle et al. 2012).

4.5.4. Plant impact on N_2O and CH_4 fluxes

As the effect of plants on WFPS and NO_3^- content was not statistically significant, great impact on gas exchange rates could not be expected. Consequently, there were only faint impacts of plant types in interaction with periods on both CH_4 and N_2O fluxes – CH_4 uptake trended to be higher under sorghum in summer 2010, and N_2O emission was higher from dry sorghum than maize in early summer 2011 - but cumulated fluxes per year or over the experiment did not differ significantly between plant types.

Regarding the contribution of agriculture to greenhouse gas exchange and the global need for food production, not only area-based emissions, as mostly reported, but also emissions scaled to dry weight or N yield should be given (Van Groenigen et al. 2010). A range of 0.13 - 0.48 kg N_2O - N t^{-1} d.w. maize yield-scaled N_2O emissions can be calculated from N_2O emissions and biomass yields reported in studies on loamy soils (Liu et al. 2013; Sehy et al. 2003; Zebarth et al. 2008). The 0.07 - 0.08 kg N_2O - N t^{-1} d.w. maize biomass yield measured here are slightly below this range, presumably because of drier conditions in our sandy soil. As both yields and N_2O emissions were higher on wet than on dry plots, yield-scaled emissions did not change with drought treatment. Higher yield-scaled N_2O emissions with sorghum than maize result from lower biomass yields. N yield-scaled emissions based on aboveground plant N uptake were shown to be relatively constant with fertilizer addition in the range of 0 to 190 kg N ha^{-1} and increased at higher fertilization rates in a review that summarized datasets where both N_2O and N yields were reported (Van Groenigen et al. 2010). For total N_2O emissions, Bouwman et al. (2002) also report increasing N_2O emission above a threshold of 100 kg N $ha^{-1}yr^{-1}$ N fertilization. Our finding of increased yield–scaled emissions due to lower yields in sorghum illustrates the general need to optimize the adaptation of fertilization to expected yield in order to keep yield-scaled N_2O emission as low as possible.

4.6. Conclusions

Sandy soils and climatic conditions as present during this study represent a wide range of northern Germany and Central Europe. Although conditions on the sandy soil were relatively dry even in the wet control treatment during treatment phases, small effects of increased summer drought were detectable. We showed that increased summer drought led to higher uptake rates of atmospheric methane during summer. A reduction of N_2O emission in the drought treatment during the period of rain exclusion did not occur. In both treatments, highest N_2O emission peaks occurred during the winter. While water contents of ambient wet control plots were higher than in the dry plots during early winter, there was only a weak trend towards higher cumulated N_2O emission during the entire winter season. Taken together, this indicates that effects of summer climate changes on greenhouse gas fluxes in crop production have to be evaluated on the basis of long-term measurements covering at least a whole year. Increased CH_4 uptake and the small difference in N_2O emission under increased summer drought together reduced the area related greenhouse gas balance by approx. 0.4 t CO_2-equiv. ha^{-1} yr^{-1} compared to ambient wet control plots. However, N_2O fluxes of this sandy soil were relatively low and the generally good drainage rarely leads to strong inhibition of diffusive CH_4 uptake even under wet conditions. Drought effects on the greenhouse gas balance might thus be more pronounced in soils with lower sand content where changes in soil moisture during drought can be much larger.

Regarding sorghum as an alternative to maize for energy plant production, there was no impact on greenhouse gas exchange detectable in our study on an annual base. As yields were lower and biomass yield-scaled emissions higher from sorghum than from maize plots, sorghum does not

seem to be a worthwhile substitute for energy plant production at the selected site under the present conditions. With changing climatic conditions, strategies to limit yield-scaled greenhouse gas emissions must thus take specific crop responses into account.

4.7. Acknowledgements

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5. Impact of CULTAN fertilization with ammonium sulfate on field emissions of nitrous oxide[2]

5.1. Abstract

Agricultural soils have a great share on global nitrous oxide (N_2O) emissions. The method of nitrogen fertilization is a manageable control parameter of N_2O production in soil. Controlled uptake long-term ammonium nutrition (CULTAN) intends to aliment field growing crops mainly with ammonium instead of nitrate, aiming at a better N use efficiency and less N leaching by placing ammonium-based N fertilizer in highly concentrated depots in the soil. In this two years field study, we analyzed N_2O flux rates and dynamics of mineral N in soils after injection of ammonium sulfate solution (CULTAN) and conventional surface application of the same fertilizer type (ammonium sulfate at a rate of 130 kg N ha^{-1}) to winter wheat at two sites with different soil texture. Using ^{15}N-NH_4^+ as a tracer, we additionally measured fertilizer-derived emissions and fertilizer N uptake at one CULTAN plot.

Grain yields were higher after CULTAN fertilization than after surface application of N fertilizer; significantly so in one year at each site. Neither N uptake nor N use efficiency were consistently different between fertilization methods. Nitrate accumulation in CULTAN treated plots occurred after fertilizer injection, showing that the concentrated NH_4^+ depots did not sufficiently inhibit nitrification. Total annual N_2O emission ranged from 0.29 to 1.9 kg N ha^{-1} yr^{-1}, with higher emissions from fertilized than unfertilized plots, but no significant difference between fertilizer application methods. N_2O emission was higher at the loam than the sandy loam site, with twice as high annual emission at the loam site (1.2 ± 0.5 kg N ha^{-1} yr^{-1}) compared to the sandy loam site (0.6 ± 0.2 kg N ha^{-1} yr^{-1}) after CULTAN fertilization. Temporal N_2O emission dynamics were influenced by weather conditions (i.e. thawing of soil) and irrigation and could partly be explained by changes in soil moisture and soil mineral N. With only 1% - 17% of total annual fluxes at the ^{15}N CULTAN plot, fertilizer-derived emissions were small, highlighting the dominance of soil N for N_2O emission.

In terms of N_2O emission, CULTAN fertilization did thus not proof beneficial over surface application of the same fertilizer. Without effective inhibition of nitrification, and with the high concentration of fertilizer N in small zones within soil, there is even the possibility of increased N_2O emission from CULTAN fertilization at fine textured soils.

[2] This chapter is a modified form of an article, which was in the reviewing process of the journal Agriculture, Ecosystems & Environment when the thesis was submitted. After submission of the thesis the article was published in a revised version (DOI: 10.1016/j.agee.2015.12.015).
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5.2. Introduction

Nitrogen (N) management is an integral part of agriculture. It offers the potential to maintain and increase crop yields necessary for feeding a growing world population, but carries the burden of responsibility that comes along with intervening in biogeochemical cycles. Negative effects of increased N input to ecosystems include nitrate leaching, changes in biodiversity, and the increase in nitrous oxide (N_2O) emissions (Erisman et al. 2013; Vitousek et al. 1997). In fact, agriculture is an important source of N_2O, contributing 79% (59% from direct and another 20% from indirect emissions) to global anthropogenic emissions (Ciais et al. 2013); thereby it contributes substantially to global climate change and the destruction of the stratospheric ozone layer (Myhre et al. 2013; Ravishankara et al. 2009). Besides the amount of N applied, the chemical speciation of N fertilizer, and timing and method of N application are key parameters in N management (Cameron et al. 2013).

The application method determines the fertilizer distribution within soil and it also influences the contact between fertilizer and plant roots. After surface application, N is leached to the rooting zone with precipitation or irrigation and is thus broadly dispersed in soil. In contrast, banding within the soil or point-injection of fertilizer supplies N directly to the rooting zone. These application methods were developed to improve N efficiency and reduce N leaching (Dinnes et al. 2002; Janzen et al. 1990; Petersen et al. 2004).

The CULTAN (Controlled Uptake Long-Term Ammonium Nutrition) fertilization strategy according to (Sommer 2005) combines ammonium-rich/nitrate free nitrogen fertilizers with fertilizer placement techniques such as point injection or banding. Point injection of concentrated fertilizer solution by spoke-wheels is common, creating fertilizer depots of small volume and high NH_4^+ concentration in the soil. The CULTAN method primarily aims at a more beneficial nutrition of plants using NH_4^+-N as the dominant nitrogen form (Sommer 2005). The fertilization strategy is assumed to result in a more efficient N assimilation within the plant: Whereas NH_4^+ assimilation occurs mostly in the roots directly after uptake, NO_3^- needs to be reduced prior to assimilation, which is requiring more energy than NH_4^+ assimilation. Negative effects of pure ammonium nutrition, such as potassium antagonism, are assumed to be negligible, since fertilizer depots are confined to a small part of the soil, leaving the remaining space for uptake of other nutrients (Sommer 2005).

Higher grain yields and higher N uptake have indeed been observed after NH_4^+ injection compared to broadcast NO_3^- fertilization in pot experiments with barley (Schittenhelm & Menge-Hartmann 2006), however, similar yields on winter wheat, barley and oilseed rape fields have been reported comparing CULTAN with conventional fertilization by surface application (Flisch et al. 2013; Kozlovsky et al. 2010; Peklova et al. 2012; Sedlář et al. 2011). Higher yields from point-injected urea ammonium sulfate than from surface applied fertilizers have been observed on winter wheat fields in a study by Weber et al. (2008).

The long-term nutrition of plants with ammonium in CULTAN treatments is assumed to be ensured by high concentrations of ammonium that inhibit nitrification (Harada & Kai 1968; Petersen et al. 2004; Wetselaar et al. 1972). Due to inhibition of nitrification and thus slower build-up of nitrate and due to the high concentration of root-tips in the proximity to nitrate formation at the margins of NH_4^+ depots, it has been suggested that nitrate leaching could be reduced by ammonium fertilizer placement (Passioura & Wetselaar 1972; Petersen et al. 2004). Analogously, CULTAN fertilization might help to reduce both nitrification- and denitrification- derived N_2O emission.

Earlier studies that report N_2O emissions after fertilizer injection have often used urea or nitrate containing fertilizer or even organic fertilizers. Band injection of ammonium sulfate nitrate resulted in reduced nitrification rates but not in lower N_2O emission on an annual scale in vegetable production (Pfab et al. 2012), and both urea-ammonium nitrate and poultry litter banding increased N_2O emissions in corn fields (Smith et al. 2012). Subsurface-banding of urea has often increased N_2O emission in the growing period as compared to broadcast application (e.g. Cheng et al. 2002; Engel et al. 2010; Maharjan & Venterea 2013), likely because of higher NO_2^- accumulation (Maharjan & Venterea 2013). Organic fertilizers (e.g. animal manure) do not contain suitable concentrations of ammonium to build highly concentrated depots, and they add a substantial C source that might promote denitrification and thus N_2O emission (Kaiser & Ruser 2000). We are not aware of studies that report on N_2O fluxes measured over whole years after point injection of pure ammonium fertilizer according to the CULTAN method.

To investigate the effect of CULTAN fertilization with ammonium sulfate on N_2O fluxes, we conducted field experiments at two sites differing in soil texture: a loam and a sandy loam site, both planted with winter wheat. N_2O fluxes from broadcast surface application and point-injected ammonium sulfate (CULTAN) fertilized plots were measured over a two years period. As after fertilizer placement in depots only a small portion of the soil is in contact with the fertilizer N, we used ^{15}N tracing to distinguish between N_2O originating directly from the turnover of ^{15}N labeled fertilizer-N from CULTAN depots and N_2O from turnover of soil N.

We hypothesized that inhibition of nitrification in fertilizer depots of CULTAN treated plots would lead to a) lower built-up of NO_3^- in soil, b) lower total and fertilization-induced N_2O emission, and c) lower yield related N_2O emission compared to broadcast surface application at both sites. We further hypothesized that soil moisture and N_2O emission activity would be higher in the loamy than in the sandy soil and that the expected N_2O mitigating effect of point NH_4^+ injection (CULTAN) would be larger in the loamy soil.

5.3. Materials and methods

5.3.1. Field sites and management

Both field sites are located near Braunschweig in Lower Saxony, Germany (loam site: 52°12'N 10°36'E, sandy loam site: 52°18'N 10°26'E), and were managed according to local farm practice except for fertilization. The main soil properties of the two sites are summarized in Table 5-1. The mean annual temperature is 9.1°C, with an annual precipitation of 617 mm (German climate service, nearby weather station *Braunschweig*). Whenever possible and reasonable, management was performed at the same or subsequent days at both sites. Winter wheat was sawn in fall 2010 and 2011 at both sites and at the sandy loam site in 2012; at the loam soil, barley was sawn in fall 2012. Plants were harvested the last week of July or the first week of August in both years. The sandy loam site was ploughed (to a depth of 30cm) after harvest and a field cultivator was used approx. 5 and 10 weeks later; tillage at the loam site was performed only once in late September. The sandy loam site is commonly irrigated during the growing season and irrigation was performed in both years (2011 and 2012). In May/June 2011 a total of 45 mm irrigation water was applied via a sprinkler irrigation gun (split in two applications), higher amounts of water were supplied in May 2012 (three applications of 30 mm each) to achieve wetter conditions after fertilization and to increase N uptake rates.

Table 5-1: Soil properties in 0- 30cm soil depth (means ± standard deviation) of the two experimental sites

site	soil type	clay %	silt %	sand %	bulk density	pH ($CaCl_2$)	C_{tot} g kg^{-1}	N_{tot} g kg^{-1}
sandy loam	haplic Luvisol	9*	23*	68*	1.51 ± 0.07	5.9 ± 0.3	11 ± 1	1.0 ± 0.1
loam	stagnic Luvisol	26 ± 2	41 ± 1	33 ± 2	1.49 ± 0.11	7.3 ± 0.0	13 ± 1	1.2 ± 0.1

*source of soil texture for the sandy loam site: (Sauerbeck 2005)

5.3.2. Fertilization treatments

The following fertilization treatments were established with three replicates in spring 2011 at both experimental sites: point-injection of NH_4^+-N (CULTAN), surface application of NH_4^+-N, and no fertilizer application (control). Ammonium sulfate ($(NH_4)_2SO_4$) solution (100 g NH_4^+-N L^{-1}) was used as nitrogen fertilizer. Fertilizer was either applied by injection (CULTAN method: single application, 130 kg N ha^{-1}) or as split application by spraying (60, 30, 40 kg N ha^{-1}) at intervals of 6 and 4 weeks in 2011 and 3 weeks in 2012, respectively. Unfertilized plots served as control. As at the sandy loam site a long-term experiment has been established in 2004, unfertilized plots had not received any N fertilizer for 7 years in 2011. The injection was conducted using a 3 m spoke wheel fluid fertilizer injector (distance between depots was 17 cm in line and 25 cm perpendicular to crop rows, and approx. 5.5 ml were applied per depot). For spraying, a commercial field sprayer was used. At the sandy loam site, one plot of the CULTAN and the surface application treatment each was fertilized with ^{15}N-enriched $(NH_4)_2SO_4$ (target enrichment: 5 at%^{15}N) to enable the determination of fertilizer-derived N_2O fluxes. The ^{15}N-fertilizer-solution was prepared from $(^{15}NH_4)_2SO_4$ and deionized water to a concentration of 100 g N L^{-1}, equivalent to the concentration in the commercial N fertilizer. Fertilization at ^{15}N plots was performed at the same day as on the other plots; tanks and hoses of the fertilizer application devices were rinsed with water before filling with the ^{15}N labeled fertilizer solution.

Samples of ^{15}N labeled fertilizer solution were taken from the tank of the fertilizer distributors after fertilizer application to check N concentration and ^{15}N label. The analyses revealed that there were severe problems with dilution of the ^{15}N tracer solution by residual water and nitrogen which was not completely removed from the tubing system of the application devices by our rinsing procedure. In consequence, the ^{15}N treated plot of each treatment unfortunately received N amounts differing from their unlabeled equivalents and results cannot be compared. This shortcoming was taken into account when analyzing our data. At the sandy loam site, only the two non-labeled replicated plots per fertilized treatment received an identical amount of fertilizer and could be used for statistical analysis. Consequently, we could not analyze the impact of application methods on total fluxes per year and per site with ANOVA of cumulated fluxes. Instead, we combined both sites and analyzed the time series of fluxes with regression analysis.

Emission of N_2O from the fertilizer N pool was calculated from labeled fertilizer at the CULTAN treated plots of the sandy loam site. Here, we had three chambers on the labeled plot that received the target fertilization rate of 130 kg N ha^{-1} in 2011, whereas the rate was only 90 kg N ha^{-1} in 2012. Actual ^{15}N abundance of applied fertilizer was 2.88 at%^{15}N in 2011 and 4.25 at%^{15}N in 2012. Results from the plot with surface application of ^{15}N labeled fertilizer were excluded.

5.3.3. Mineral soil N

Soil samples (0-10 cm and 10-30 cm soil depth) were taken in weekly to biweekly intervals from March 2011 to March 2013 to determine dynamics of mineral nitrogen. Soil mineral nitrogen (N_{min} = NO_3-N + NH_4-N) was measured photometrically (continuous flow autoanalyzer SKALAR; DIN ISO 14255) in soil sample extracts (200 ml 1 M KCl, 50 g field fresh soil, shaken for 1 h, MN614 ¼ filters, Macherey & Nagel, Düren, Germany). At CULTAN plots, soil was sampled separately at injection spots (3 depth segments of 5 cm with a radius of 3.5 cm and an outer segment with a radius of 8 cm and 15 cm depths around the injection channel) and between injection spots to calculate area based N contents. Total sample material was used for N_{min} extraction of the 3 inner depot samples (approx. 500 g wet soil and 600 ml KCl solution).

5.3.4. Gas fluxes

Fluxes of N_2O and CO_2 at the soil surface were measured with closed chambers, generally at weekly intervals, but with higher frequency after fertilization and with some larger intervals due to field management especially in fall/winter 2012. Both chambers and chamber bases consisted of white PVC. Chambers were 30 cm high and covered 64 x 48 cm of the soil surface. These dimensions were chosen to include 8 injection spots each at the CULTAN plots, so that the ratio of injection spots to unfertilized soil within the area covered by the chamber was identical to the total plot. Chamber bases were permanently installed approx. 10 cm deep into the soil and only removed for fertilization, harvest and tillage. The chambers were ventilated with small fans to ensure complete mixing of the gas phase even with plants in the chamber. Vent tubes permitted equilibration of air pressure. At times when wheat plants were too high to be enclosed in the 30 cm high chamber, extensions of the same dimensions as the chambers were installed between chamber bases and chambers. Four air samples of chamber atmosphere were taken after chamber closure over a time period of 60 minutes, which was extended to 120 minutes during low-flux conditions, in 50 ml evacuated glass bottles equipped with Teflon stop-cocks. With each flux measurement, soil and chamber temperature were measured. Additionally, at the sandy loam site, gas samples were taken in 100 ml crimp vials with butyl septa for ^{15}N analyses in N_2O.

For analysis of CO_2 and N_2O concentration in gas samples, a gas chromatograph (GC 2014, Shimadzu, Duisburg, Germany) equipped with an automated rack and an ^{63}Ni electron-capture detector was used (Loftfield et al. 1997). The GC was calibrated for each sample run with 4 standards ranging from 1 to 10 times ambient concentration. The performance of the GC system was checked weekly by measuring a standard of ambient concentration 10 times consecutively. The peak area's coefficient of variation was always better than 3%.

Gas fluxes were calculated in R (version 3.0.2, R Core Team 2013) with an automated procedure using either linear regression, robust linear regression with a Huber-M estimator or the Hutchinson-Mosier non-linear function as implemented in the HMR package (HMR, Pedersen et al. 2010). The flux calculation used for further calculation and modeling was chosen as described by Leiber-Sauheitl et al. (2014) according to the following criteria: robust linear regression was used as default; HMR was only used if it could be fitted, had a smaller Akaike's Information Criterion (AIC, Burnham & Anderson 2004) and a lower *p*-value than that calculated for the linear flux and was not more than 4 times the robust regression flux. If only three data points for a flux measurement were available, linear regression was used. CO_2 concentrations were used to control flux measurements – N_2O fluxes were removed from the dataset when no increase in CO_2 was measureable, unless soil was snow-covered. The median of the resulting N_2O fluxes' standard

errors was 2.16 µg N m^{-2} h^{-1} and 95% of the fluxes had a standard error smaller than 7.35 µg N m^{-2} h^{-1}. Cumulated fluxes per year were calculated based on linear interpolation between measurement dates.

5.3.5. Isotope analyses

Gas samples taken at the end of flux measurements were analyzed for ^{15}N content of N_2O by isotope ratio mass spectrometry (IRMS) as described previously (Brand 1995; Lewicka-Szczebak et al. 2014). Briefly, a pre-concentrator and gas chromatograph (PreCon+ Trace GC Isolink, ThermoFinnigan, Bremen, Germany) were connected to a Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) where N_2O was pre-concentrated, separated, purified and analyzed for m/z 44, 45 and 46 of intact N_2O molecules. Analytical precision, determined as the standard deviation of internal standards, was typically $3.7 \cdot 10^{-5}$ at%^{15}N.

Fertilizer-derived emissions were then calculated with Equation 5-1.

$$n_{fertilizer} = \frac{n_{mix} \cdot \left(at\%^{15}N_{mix} - at\%^{15}N_{soil}\right) - n_{air} \cdot \left(at\%^{15}N_{air} - at\%^{15}N_{soil}\right)}{\left(at\%^{15}N_{fertilizer} - at\%^{15}N_{soil}\right)} \qquad \textbf{Equation 5-1}$$

with:

$n_{fertilizer}$ = amount of fertilizer-derived N_2O,
n_{mix} = amount of N_2O in the chamber at the end of a flux measurement,
n_{air} = amount of air N_2O at the start of the flux measurement,
$at\%^{15}N_{mix}$ = ^{15}N content in chamber atmosphere at the end of the measurement,
$at\%^{15}N_{air}$ = ^{15}N content in air measured above the field site.
$at\%^{15}N_{soil}$ = ^{15}N content in soil-derived mineral N (0.3627 at%^{15}N), calculated as the mean of values derived from flux measurement at unfertilized plots.

The ^{15}N content in plant biomass was measured after grinding of dried samples. Samples were transferred to zinc capsules and then analyzed for at%^{15}N using an elemental analyzer coupled to a Delta Plus IRMS (ThermoFinnigan, Bremen, Germany).

5.3.6. Statistical analyses

Statistical analyses were performed with R (version 3.0.2, R Core Team 2013). Both single and cumulative fluxes of N_2O were log-transformed before further analysis, as residual plots showed strong deviation from normal distribution when untransformed flux rates were used. An offset of 20 µg N m^{-2} h^{-1} was added to N_2O fluxes for transformation. Thus, negative fluxes were kept in the dataset, except for the 2 most negative fluxes measured, assuming they mainly represent fluctuation around zero due to measurement uncertainty. To test for significant treatment and site effects on cumulated fluxes, WFPS and N_{min}, linear mixed effects models were analyzed using the *nlme* (Pinheiro et al. 2013) package in R. The recommendations in Zuur et al. (2009) were followed to develop appropriate model structures. A random intercept grouped by chamber was included in all models to account for the experimental setup. Pairwise comparisons of cumulated fluxes were performed with post-hoc tests using the *multcomp* package (Hothorn et al. 2008) in R.

To test for the impact of measured environmental parameters on N_2O fluxes, a generalized additive mixed model (*gamm*) was applied on log-transformed N_2O fluxes using the *mgcv* (Wood 2006) package. The model relates N_2O fluxes to a linear combination of predictor variables, which are estimates from parametric or smoother functions of explaining parameters. The degree of smoothing is estimated during fitting with a penalized maximum likelihood approach. Parameters used were NO_3^- and NH_4^+ content in 0- 30 cm soil depth, the WFPS in 10-30 cm soil depth and the CO_2 flux as a proxy for microbial activity. As N_{min} samples were not always taken at the days of flux measurements, values were linearly interpolated between measurement dates for statistical analyses. Missing values (7 of 675 at the loam site) were filled with the average of the 2 available replicates of the respective treatment and date. Including WFPS in 0-10 cm soil depth or soil temperature did not improve the model fit. Treatment and site were included to analyze whether the impact of environmental parameters differed according to fertilizer application or soil types.

5.4. Results

5.4.1. Precipitation, WFPS and soil temperature

Rainfall pattern was similar at both sites (Figure 5-1 and 5-2), with relatively dry conditions in spring and early summer in both years. Precipitation was 518 mm from the first fertilization in 2011 to March 2012 and 549 mm between March 2012 and March 2013 at the sandy loam site. 640 mm were precipitated at the loam site between March 2011 and March 2012 and 616 mm from March 2012 until March 2013.

Water content in soil followed a seasonal pattern, with lowest water contents in May, June and September, and highest in winter (Figure 5-1 and 5-2). Mean WFPS at the loam site ranged from 29% to 100% (mean = 58%) in 0-10 cm depth and from 29% to 87% (mean: 68%) in 10-30 cm depth. At the sandy loam site, mean WFPS was in the range of 10% to 86% in the upper 10 cm (mean: 50% WFPS) and between 13% and 69% in 10-30 cm depth (mean: 48% WFPS). In 10-30 cm depth, there was a trend to lower WFPS in the fertilized than unfertilized plots, most distinctively so in May 2011 and July 2012 at the sandy loam site. There was no significant difference of soil WFPS between treatments with point injection (CULTAN) and surface application of fertilizer.

Soil temperature at both sites showed a typical seasonal pattern with highest values of 25 to 30 °C in summer 2011 at the sandy loam site and about 23°C at the loam site (Figure 5-1 and 5-2). Soil frost occurred at several dates in winter; in December 2011 and in February/March of both 2012 and 2013. Differences between treatments are small and fall in the range of standard deviations; however, during summer, temperatures at unfertilized control plots trended to be slightly higher at the sandy loam site.

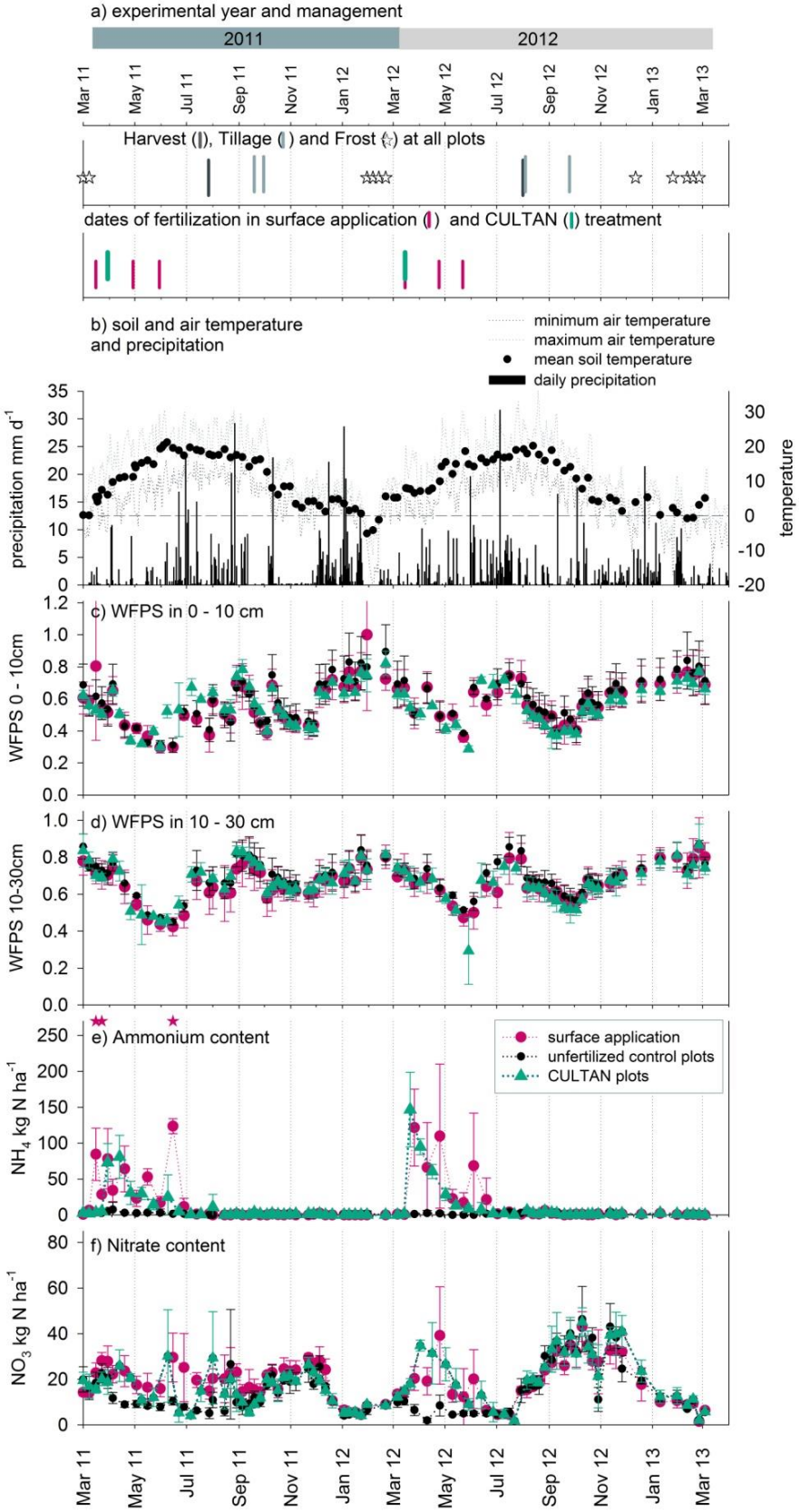

Figure 5-1: Dates of management (a), temperatures and precipitation (b), water filled pore space (WFPS) (c and d) and N_{min} content (e and f, 0-30 cm) at the loam site during the experiment. Management dates were the same at all plots. Fertilization dates are marked with pink (surface application) and green (CULTAN) lines. Pink stars at the ammonium plot denote dates when single samples were removed as outliers.

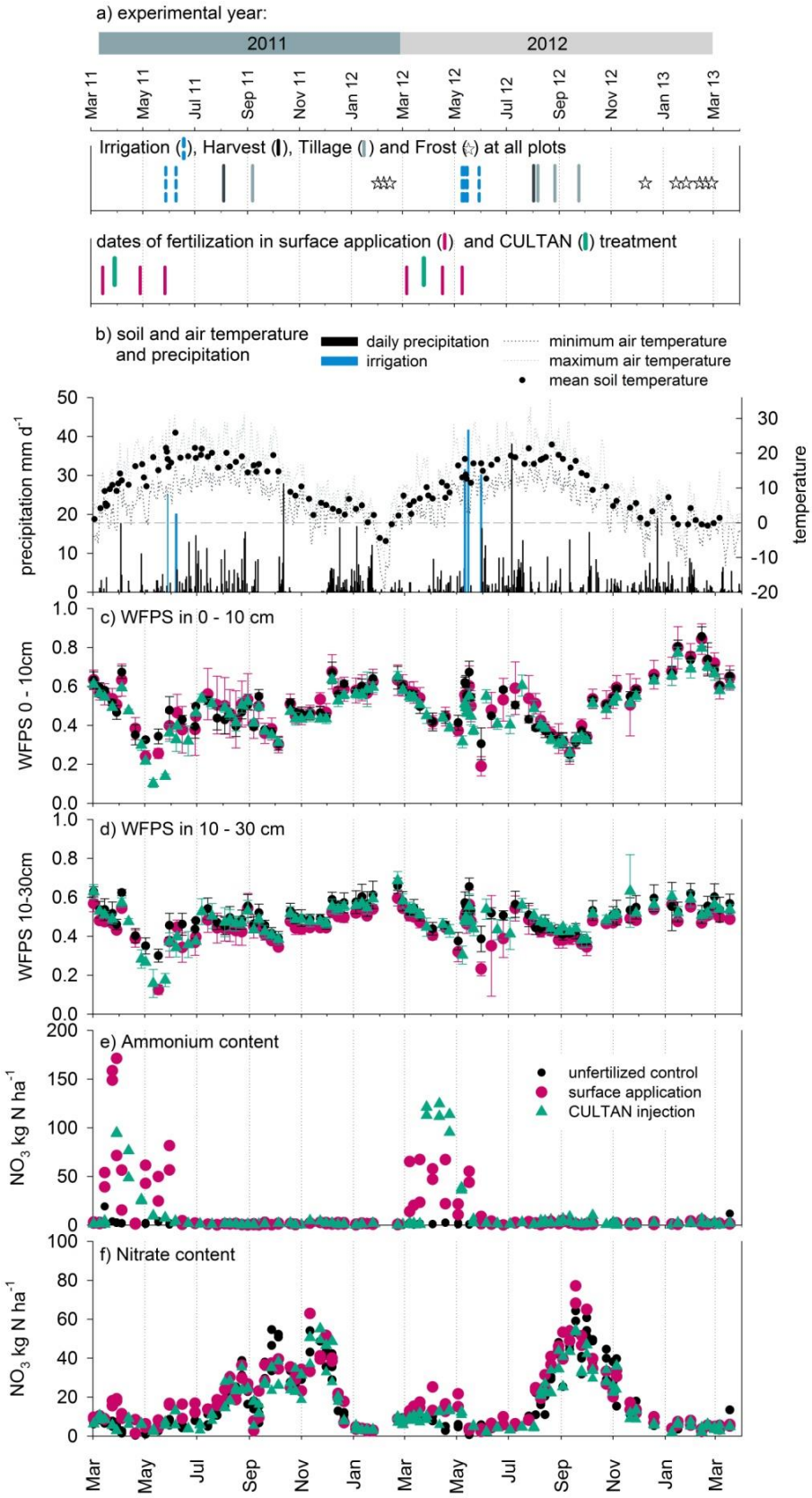

Figure 5-2: Dates of management (a), temperatures and precipitation/irrigation (b), water filled pore space (WFPS) (c and d) and N_{min} content (e and f, 0-30 cm) at the sandy loam site during the experiment. Management dates were the same for all plots. Fertilization dates and soil properties are shown for the three fertilization treatments. Values of the unfertilized control represent means with standard deviation (n=3). For the fertilized treatments in results of the 2 non-labeled replicates per treatment were shown.

5.4.2. Ammonium and nitrate dynamics

Within two weeks after fertilizer injection at the CULTAN treatment, 73 ± 27 kg NH_4^+-N ha^{-1} (2011) and 147 ± 52 kg NH_4^+-N ha^{-1} (2012) were measured at the loam site, and 76-130 kg NH_4^+-N ha^{-1} (2011) and 112-125 kg N ha^{-1} (2012) at the sandy loam site, respectively (Figure 5-1 and 5-2). The NH_4^+ content strongly decreased within one month, and depots were completely depleted in NH_4^+ at the end of May in both years at the sandy loam, and in 2012 at the loam site. The decline was slower at the loam site in 2011, with complete depletion of depots at the end of June. At the surface application treatment, NH_4^+ content was more variable during the growing season, which can be attributed to split application with three fertilization dates. After fertilization events, mean values of up to 120 kg N ha^{-1} (loam) and 170 kg N ha^{-1} (sandy loam) in 2011 and 240 kg N ha^{-1} (both sites) in 2012 were measured. These unexpectedly high NH_4^+ contents are attributed to sampling artifacts and inhomogeneous distribution of fertilizer applied on top of the plant stand. From June (sandy loam) and July (loam) until fertilization in the following spring, NH_4^+ contents remained below 10 kg N ha^{-1}. Soil ammonium contents were generally low (< 10 kg NH_4^+-N ha^{-1} in 98% of the sampling dates) in the unfertilized control treatments. Despite higher temporal variability at the surface application plots, dynamics of mean soil NH_4^+ did not differ strongly from CULTAN plots. After fertilizer injection at CULTAN treatments, however, NH_4^+ content was much higher within the 8.5 cm radius sampled separately, than between depots (data not shown). While after surface application the NH_4^+ concentration in soil was at most 180 µg NH_4^+-N g^{-1} soil, the highest NH_4^+ concentration in samples from CULTAN depots was 2000 µg NH_4^+ g^{-1} soil. The spatial distribution of fertilizer within soil was thus different between treatments.

Temporal dynamics of soil NO_3^- content showed a similar pattern in all treatments (Figure 5-1 and 5-2). Periods of NO_3^- accumulation occurred in early spring until mid-March in both years, followed by low NO_3^- contents during the growing period. After harvest, soil nitrate contents increased again and highest values were reached in autumn 2012 in all treatments. Nitrate contents in 0-30 cm decreased during periods of precipitation events in September and November 2011 and they generally declined over winter. Fertilized plots showed a similar seasonal pattern as unfertilized plots, with the exception of NO_3^- accumulation after fertilization. Accumulation of soil NO_3^- in the CULTAN treatments occurred already in the first two weeks after NH_4^+ injection. Since fertilizer injection at CULTAN plots lagged two weeks behind the first surface application at the sandy loam site (both years) and in 2011 at the loam site, the accumulation of NO_3^- started later at CULTAN plots. Peaks in NO_3^- content occurred 2-6 weeks after fertilizer injection, corresponding to the decrease in NH_4^+ contents.

At CULTAN plots of the sandy loam site, mean NO_3^- content was smaller than at the loam site, with 8.5 ± 2.5 kg NO_3^--N ha^{-1} measured in April 2011 and 13 kg NO_3^--N ha^{-1} in March and May 2012. At the loam site, the variability was high during the growing season 2011, with values of 26 ± 7 kg N ha^{-1} two weeks after injection without a clear decrease in the following weeks. In 2012, by contrast, NO_3^- content steadily declined from 35 ± 2 kg N ha^{-1} two weeks after fertilization to 2.1 ± 1.8 kg N ha^{-1} in late July. Nitrate dynamics differed between sites insofar as NO_3^- accumulation at the sandy loam site was lower during the growing season but higher in fall compared to the loam site (Figure 5-1 and 5-2). Mean NO_3^- contents were not significantly lower after point injection (CULTAN treatment) than surface application, except for the 2 weeks that lay between the first surface application and CULTAN fertilization. However, as for NH_4^+ contents, soil NO_3^- was very heterogeneously distributed in the CULTAN treatment. While NO_3^- content was low between depots, NO_3^- concentrations of up to 180 µg NO_3^- N g^{-1} soil were measured in depots, compared to at most 30 µg NO_3^- N g^{-1} soil in single samples after surface application.

5.4.3. N_2O emission rates

Mean emission rates of N_2O from the analyzed treatments were 12.2 ± 23.0 (median 5.0) µg N m^{-2} h^{-1}, 10.2 ± 15.8 (median 5.6) µg N m^{-2} h^{-1} and 5.7 ± 9.8 (median 3.1) µg N m^{-2} h^{-1} in the CULTAN, surface application and unfertilized treatments, respectively. The majority of fluxes were calculated by robust linear regression, and only 6% (sandy loam) and 15% (loam) were calculated with the HMR procedure. Most fluxes measured (90% at the sandy loam site and 95% at loam site) were below 30 µg N m^{-2} h^{-1}. Higher fluxes occurred mostly during freeze-thawing events (stronger in February/March 2012 than in 2011), after fertilization (stronger in the wetter season 2012 than in 2011) and after tillage in fall. The 5-10% of all fluxes that were >30 µg N m^{-2} h^{-1} accounted for > 40% (sandy loam) and > 60% (loam) of total annual emissions.

At the sandy loam site, highest N_2O emission rates occurred in all treatments in February/March 2012 after thawing (Figure 5-3). Besides this peak, emission from unfertilized plots was low throughout the experiment. At fertilized plots, elevated fluxes were measured after the first (60 kg N) and third (40 kg N) fertilizer application of surface application plots in both years, directly after injection at CULTAN plots in 2011, and following harvest and tillage at both fertilid treatments. These peaks were small (<40 µg N m^{-2} h^{-1}), however, in comparison to the thaw peak in 2012. Emission peaks at the sandy loam site were higher in 2012 than in 2011 at both CULTAN and surface application plots. N_2O emission was increased at the surface application plots from the first application in 2012, with a further increase after irrigation in May. On the contrary, CULTAN plots exhibited no increase directly after injection in 2012, but a sharp peak after the first irrigation, which occurred 7 weeks after fertilization. Emissions reverted to background level at the CULTAN plots shortly after this peak.

At the loam site, N_2O emission exhibited much higher variability, especially at CULTAN plots. Emissions attributable to freeze-thawing events were much smaller compared to the sandy loam site (Figure 5-3 and 5-4). Peak fluxes occurred at CULTAN and surface application plots after the respective fertilization in both years, and in fall 2011 after tillage. Higher fluxes from CULTAN than surface application plots were measured especially in spring 2012 within 5 weeks after fertilization.

Cumulated emission rates per plot and site (Figure 5-5) were between 0.26 kg N ha^{-1} yr^{-1} (at control plots 2012) and 1.9 kg N ha^{-1} yr^{-1} (at CULTAN plots 2011). There was no significant effect of experimental year, and after considering the AIC, it was removed from the statistical model. Annual emissions of both years were then treated as replicates in the model, thus allowing pair-wise comparisons within treatments and sites. Both treatment and site significantly affected annual N_2O emission, with higher emissions from fertilized than unfertilized plots and from the loam than the sandy loam site. Pair-wise comparisons revealed significant differences between CULTAN and control plots at the loam site ($p<0.01$) and between surface application and control plots at the sandy loam site ($p < 0.05$) (Table 5-2). The difference between sites within the CULTAN treatment was not significant ($p=0.055$), despite twice as high mean emissions from CULTAN at the loam site. On both sites, there was no effect of the fertilizer application technique (surface application versus injection) on the annual emission of N_2O.

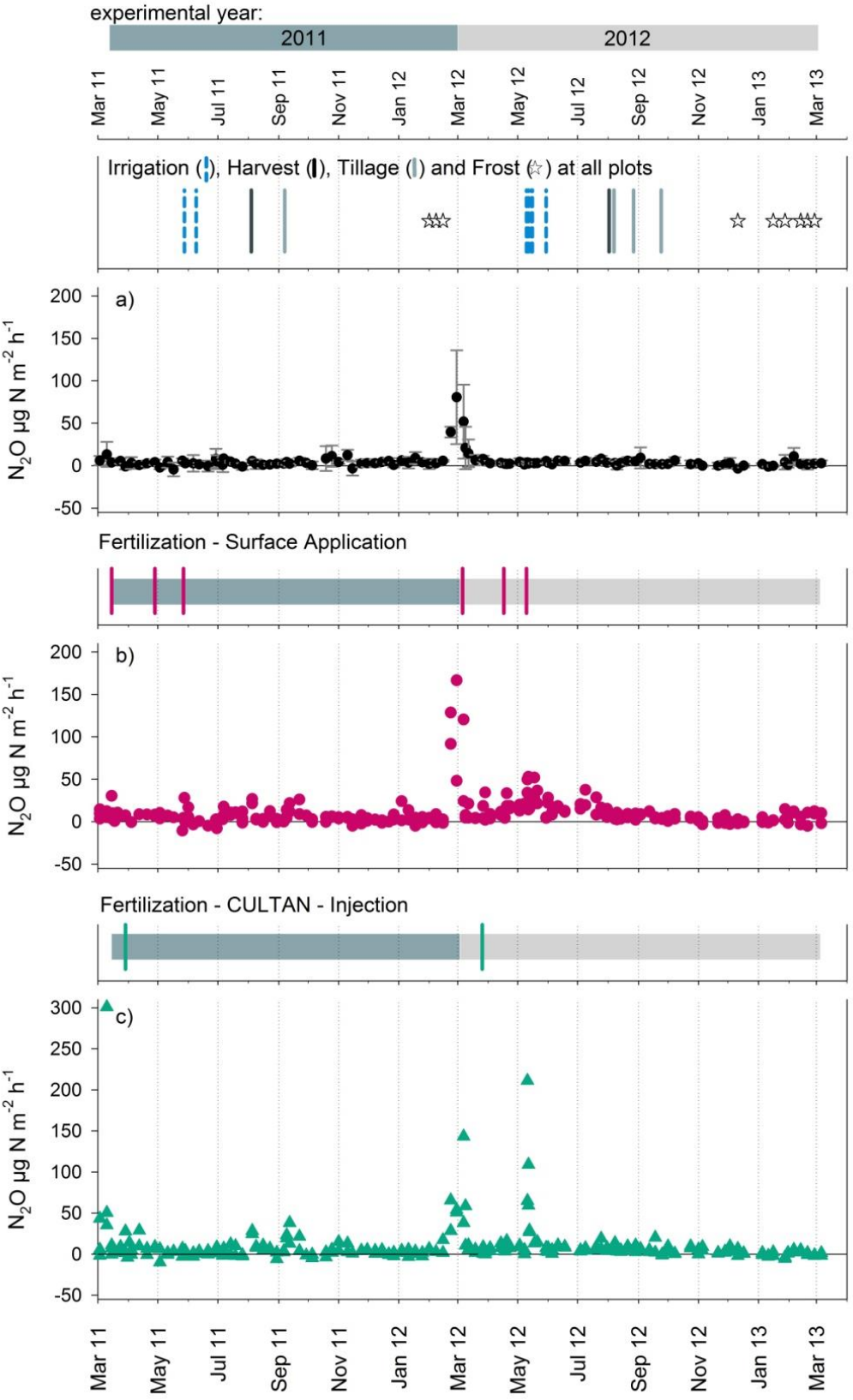

Figure 5-3: N_2O emission rates at the sandy loam site over time at a) unfertilized control plots, b) surface application of NH_4^+ plots, and c) NH_4^+ injection (CULTAN) plots. Values are mean fluxes with standard deviation (n=3) for the unfertilized plots in a. For the fertilized treatments in b) and c) single flux measurements of the 2 non-labeled replicates per treatment were shown. Management dates, as marked above a) with blue (irrigation), black (harvest) and grey lines (tillage), and days with soil temperature below zero (5 – 10 cm depth, stars), were the same at all plots. Fertilization dates are marked with colored lines above b) and c).

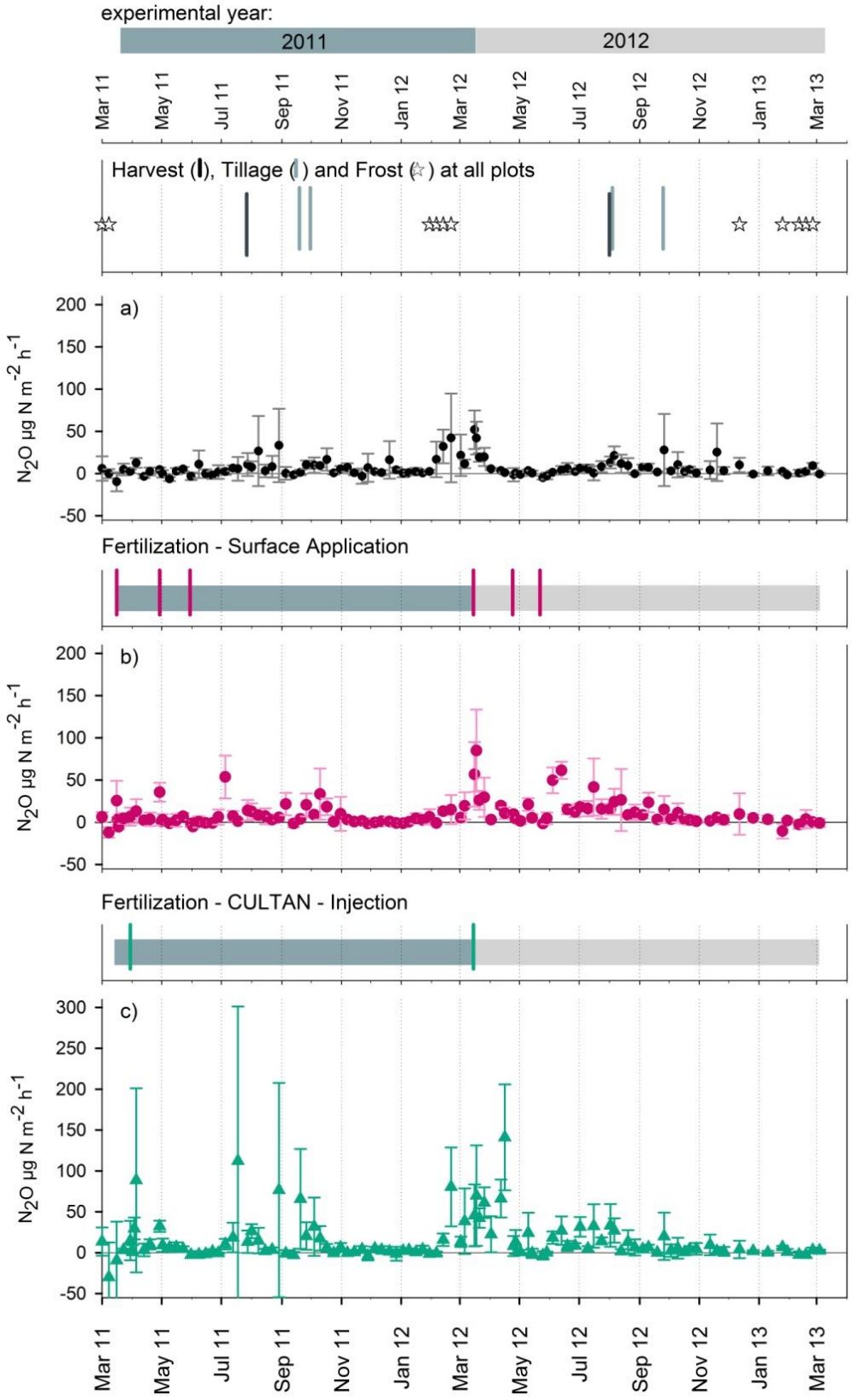

Figure 5-4: N_2O flux rates at the loam site at a) unfertilized control plots, b) surface application of NH_4^+ plots, and c) NH_4^+ injection (CULTAN) plots. Values and error bars show mean fluxes (n=3) with standard deviation. Management dates, as marked above a) with black (harvest) and grey lines (tillage), and days with soil temperature below zero (measured in 5-10cm depth, stars), were the same at all plots. Fertilization dates are marked with colored lines above b) and c).

Table 5-2: Mean annual fluxes of fertilization treatments at the loam and the sandy loam site. Groups sharing the same letter are not significantly different. Only plots without ^{15}N labeling were included (n=4 for surface application and CULTAN at the sandy loam site, n=6 for all other treatments). As fluxed did not differ significantly between years, both years were pooled

treatment		kg N_2O-N ha^{-1}	
		Loam	Sandy loam
		A	B
Control	A	0.55 ± 0.28 ab	0.36 ± 0.09 a
Surface application of NH_4^+	B	0.82 ± 0.31 bc	0.77 ± 0.07 bc
Injection of NH_4^+ (CULTAN)	B	1.22 ± 0.47 c	0.56 ± 0.02 ac

The contribution of **fertilizer-induced** emission to total annual emission was calculated from the increase in N_2O flux at the fertilized in comparison to the unfertilized treatment (($N_2O_{fertilized}$ − $N_2O_{unfertilized}$)/$N_2O_{fertilized}$). At the sandy loam site, 25% and 46% of total annual emission of the CULTAN treatment and 45% and 61% of the surface application treatment were attributed to fertilization in 2011 and 2012, respectively. At the loam site, fertilizer-induced N_2O emissions amounted to 49% ± 26% and 59% ± 12% at CULTAN and 7% ± 22% and 51% ± 11% at surface application treatment in 2011 and 2012, respectively.

Emission factors of fertilization were calculated by relating these emissions to the applied amount of N fertilizer (($N_2O_{fertilized}$ − $N_2O_{unfertilized}$)/$N_{applied}$; Bouwman (1996)). They amount to between 0.03 and 0.54% of the applied fertilizer N (Table 5-3). Without subtraction of background emissions from unfertilized plots, as calculated by Jungkunst et al. (2006) for N_2O emission studies in Germany, emission factor would range from 0.3% to 1.4% of applied fertilizer.

5.4.4. Dependence of N_2O fluxes on explaining variables

The applied *gamm* (model output see supplementary material) explains 23% of the variance in log-scaled N_2O fluxes. It shows highly significant effects of treatment ($p < 10^{-4}$) and site ($p < 10^{-14}$), with higher fluxes from fertilized than unfertilized plots. The model did not identify significant difference between fertilizer application methods. If differences between treatments existed, they were explained by the predictor variables included in the model. The interaction between WFPS and NH_4^+ content in soil affects N_2O fluxes ($p < 10^{-9}$) in all treatments, with highest emission rates at WFPS of approx. 80% and NH_4^+ content of 100 kg N ha^{-1} and relatively lower fluxes at drier conditions and lower NH_4^+ content. At unfertilized plots the interaction between WFPS and NH_4^+ represents mainly an increase in N_2O fluxes with increasing WFPS at low NH_4^+ contents. High NH_4^+ contents at these plots were rare and resulted in mean (log-scaled) N_2O fluxes regardless of moisture conditions. The relationship of log-scaled N_2O fluxes with NO_3^- is weaker than the impact of WFPS and NH_4^+ but still highly significant ($p < 10^{-7}$). CO_2 was included in the model as a proxy of plant and microbial activity and the model indicates an increase in N_2O fluxes with increasing CO_2 at low values that levels off at increasing CO_2 fluxes.

5.4.5. Grain Yields, N use efficiency and N content in aboveground biomass

Grain yields at the fertilized plots were 28% - 300% (surface application) and 28% - 440% (CULTAN) higher compared to unfertilized plots. Yields were higher at the loam than the sandy loam site. Highest yields were found at the CULTAN plots and the difference between CULTAN and surface application was significant at the loam soil in 2011 and at the sandy loam soil in 2012, respectively (Table 5-3). The amount of N in the aboveground biomass measured at harvest was approx. 30 kg N ha^{-1} (sandy loam) and 55 kg N ha^{-1} (loam) in unfertilized treatments, and amounted to 100–120 kg N ha^{-1} (sandy loam) and 90-170 kg N ha^{-1} (loam) in fertilized treatments. The N use efficiency (calculated as the difference in aboveground N content in plants at harvest between fertilized and unfertilized plots, divided by the amount of fertilizer N applied) showed a range of 26% - 89% after surface application and 51% - 75% after CULTAN (Table 5-3), without consistent pattern.

Grain yield-scaled emissions calculated as the ratio of annual N_2O-N emission to dry weight grain yield accounted to between 8.8 and 29.6 g N_2O-N dt^{-1} $d.w.^{-1}$ biomass, with a higher variation at the sandy loam site (Table 5-3). Grain yield-scaled N_2O emissions did not differ significantly between fertilizer application methods, but there was a tendency towards higher grain yield scaled N_2O emission from unfertilized than fertilized plots at the sandy loam site where also yields were much higher with than without fertilization.

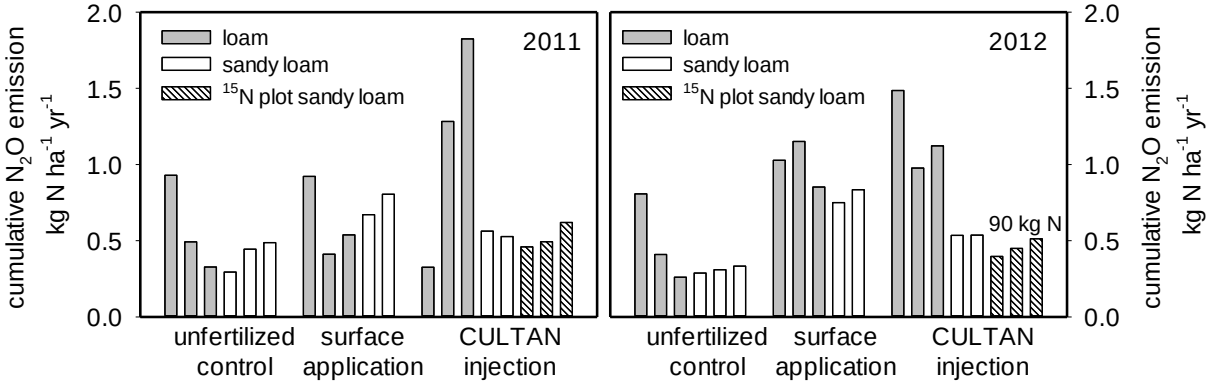

Figure 5-5: Cumulative N_2O emission at the loam (grey) and sandy loam sites (black/white) in 2011 (left) and 2012 (right). Each bar represents cumulative fluxes of one plot. Cumulative emissions from the ^{15}N labeled CULTAN plots are represented by hatched bars. The N application rate was 130 kg N ha^{-1} yr^{-1} except for the ^{15}N CULTAN plots in 2012 where 90 kg N ha^{-1} yr^{-1} were applied.

Table 5-3: N_2O emission factors and yield scaled emissions. Emission factors are calculated as the difference in N_2O emission between fertilized and unfertilized plots, divided by the amount of fertilizer N applied. N uptake is the amount of N in aboveground plant biomass (grains and straw) at harvest. Nitrogen use efficiency (NUE) was calculated as the difference in N uptake between fertilized and non-fertilized plots in relation to the total amount of fertilizer N applied. Values given are means ± 1 standard deviation. Values marked with *were derived from fluxes of two chambers only instead of three; n=3 for all other values.

treatment	emission factor		grain yield		grain yield scaled emission		N uptake		NUE	
	%		dt d.w. ha^{-1}		g N_2O-N dt^{-1}		kg N ha^{-1}		%	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Loam site										
unfertilized			34.7 ± 3.4 bc	42.1 ± 5.5 bc	16.8 ± 3.0	11.7 ± 2.3	54.1 ± 5.3 ab	55.3 ± 6.5 ab		
Surface application	0.03 ± 0.20	0.40 ± 0.12	44.3 ± 10.6 bc	70.6 ± 4.9 de	14.1 ± 2.3	14.3 ± 0.8	87.3 ± 23.1 bc	170.5 ± 22.7 e	26 ± 18	89 ± 18
CULTAN	0.43 ± 0.58	0.54 ± 0.20	61.2 ± 8.3 d	73.2 ± 2.5 e	18.7 ± 4.2	16.3 ± 1.2	120.9 ± 26.6 cd	145.7 ± 30.2 de	51 ± 21	70 ± 23
Sandy loam site										
unfertilized			13.8 ± 6.3 a	11.7 ± 1.1 a	29.6 ± 5.2	26.4 ± 1.0	31.3 ± 6.2 a	24.8 ± 2.8 a		
Surface application	0.22 *	0.37 *	33.9 ± 6.4 b	48.6 ± 5.6 c	22.4 *	15.7 *	110.6 ± 17.8 cd	98.3 ± 8.5 bc	61 ± 14	57 ± 7
CULTAN	0.10 *	0.18 *	42.0 ± 3.8 bc	63.4 ± 3.4 de	12.4 *	8.8 *	116.9 ± 15.4 cd	122.5 ± 8.5 cd	70 ± 7	75 ± 7

5.4.6. ^{15}N fertilizer-derived N in N_2O fluxes and plant biomass

With the ^{15}N tracer technique, we were able to calculate the percentage of N_2O fluxes originating directly from the applied fertilizer N. The share of these fertilizer-derived fluxes to total N_2O fluxes varied over time (Figure 5-6). During the first weeks after fertilization, 3% - 36% of the N_2O emitted from CULTAN plots were derived from fertilizer N in 2011; values were lower afterwards, with a maximum of fertilizer N on total fluxes of 3% in August. Despite lower fertilizer N addition to the ^{15}N labeled plot in 2012 than in 2011 (i.e. 130 kg N ha^{-1} in 2011 and 90 kg N ha^{-1} in 2012), fertilizer-derived fluxes were higher in 2012; 12% - 60% of total N_2O flux was derived from fertilizer after fertilization until May 2012. Again, the share of fertilizer N on total N_2O fluxes was lower after harvest, with 1% - 10% of fluxes at single dates from August 2012 to February 2013.

On an annual base, the fertilizer-derived fluxes (i.e. calculated from the ^{15}N signature of added NH_4^+ and emitted N_2O) accounted for only 1.16% ± 0.57% of total cumulated N_2O fluxes at the CULTAN plot in 2011. This percentage is equal to a fertilizer-derived emission of 0.006 kg N_2O-N ha^{-1} yr^{-1} and represents 0.005% of the total amount of fertilizer N added. In 2012, 0.5% of the N_2O emissions from this plot were still derived from fertilizer applied in 2011. The CULTAN plot that was fertilized with ^{15}N labeled fertilizer in 2012 received only 90 kg N ha^{-1} and results can thus not directly be compared. In 2012 17.0% ± 2.5% of the total N_2O emission of 0.47 kg N_2O-N ha^{-1} emitted from the CULTAN plot originated directly from the labeled fertilizer N. This percentage is equal to a fertilizer-derived emission of 0.09 kg N_2O-N ha^{-1} yr^{-1} and represents 0.1% of the total amount of fertilizer N added.

The fertilizer-induced N_2O emission calculated from the difference in annual emission between the ^{15}N CULTAN plot and unfertilized plots was much higher, with 21% ± 14% of the total annual emission in 2011 and 43% ± 10% in 2012.

The contribution of fertilizer N to N uptake into aboveground crop biomass was calculated from the ^{15}N abundance in straw and grain nitrogen at harvest. The ^{15}N abundance in grains was 1.0 ± 0.03 at%^{15}N in 2011 and 2.2 ± 0.03 at%^{15}N in 2012. In straw the ^{15}N abundance was slightly lower (0.6 ± 0.01 at%^{15}N in 2011 and 2.1 ± 0.03 at%^{15}N in 2012, respectively). Considering the ^{15}N enrichment of the applied fertilizer (2.88 at%^{15}N in 2011 and 4.25 at%^{15}N in 2012), the contribution of fertilizer N to total aboveground crop N was 35% (i.e. 40 kg N of the total biomass N of 115 kg N ha^{-1}) in 2011 and 52% (i.e. 46 kg N of the total biomass N of 89 kg N ha^{-1}) in 2012. Recovery of fertilizer N in aboveground plant biomass was 31% and 51% of the applied ^{15}N fertilizer, respectively.

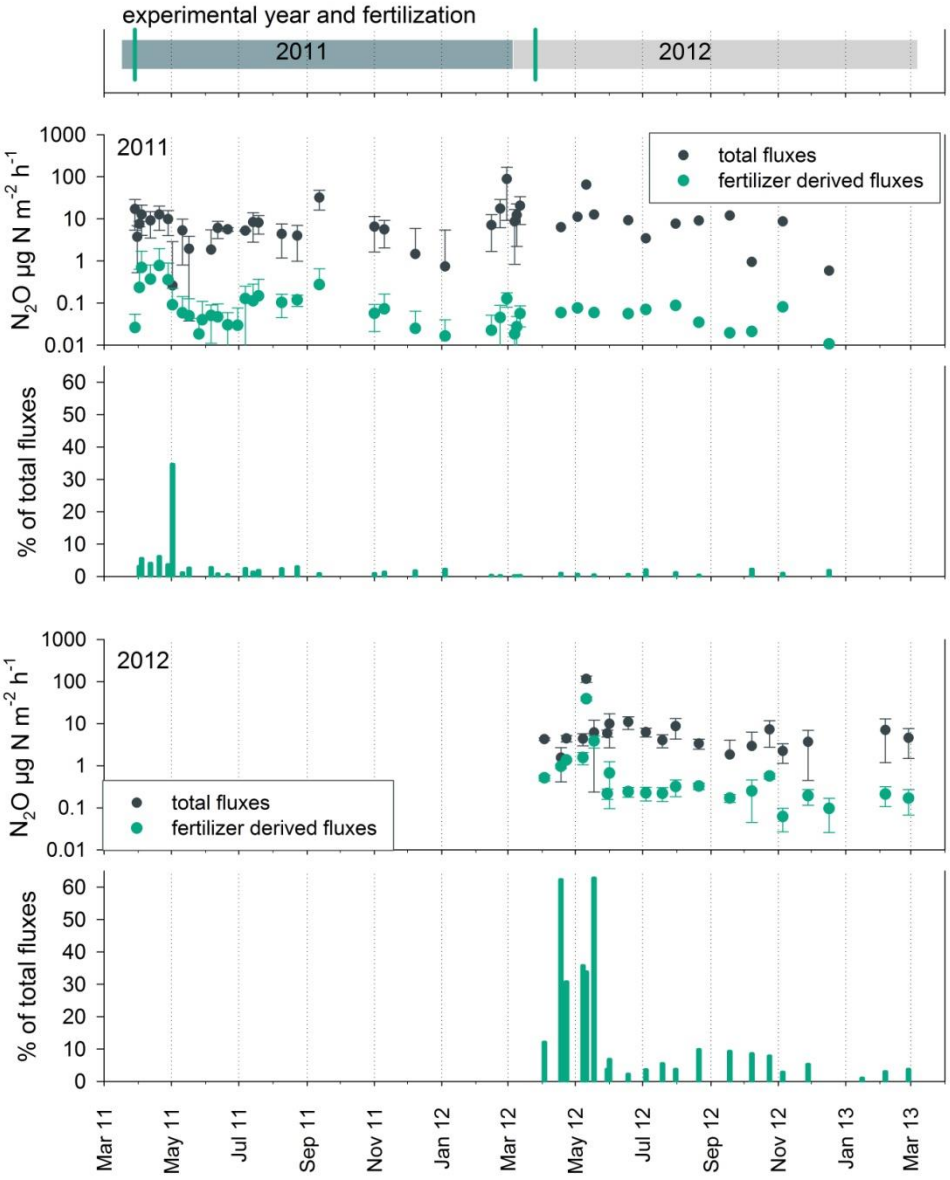

Figure 5-6: Total and fertilizer-derived N_2O emission rates after NH_4^+ injection (CULTAN) at the sandy loam site (upper figures of each year) and percentage of fertilizer-derived fluxes on total N_2O fluxes (lower figure of each year). Fertilizer-derived fluxes were calculated from at%^{15}N in gas samples of flux measurements and the ^{15}N signature of the applied NH_4^+-N. Note the logarithmic scale of N_2O fluxes.

5.5. Discussion

This study presents the first year-round measurements of N_2O fluxes after real CULTAN fertilization (NH_4^+ point injection) in comparison to broadcast application of the same fertilizer (ammonium sulfate). Earlier studies showed the inhibiting effect of ammonium sulfate banding on nitrification (Petersen et al. 2004) without measured N_2O fluxes, or the impact of fertilizer injection or banding on N_2O emission, but with fertilizers containing nitrate or urea (Maharjan & Venterea 2013; Pfab et al. 2012; Smith et al. 2012).

Emissions from the two unfertilized treatments were relatively, but not particularly, low when compared to background emissions from cropland soils (1.1 ± 1.6 kg N_2O-N ha^{-1} yr^{-1}, median: 0.6 kg N ha^{-1} yr^{-1}) summarized by Kim et al. (2013). Emissions from fertilized plots and emission factors are also in the lower range of emissions summarized by Jungkunst et al. (2006) for cropland soils in Germany, even compared to measurements at similar clay, C or N contents, and well in the range of N_2O fluxes measured worldwide (supporting information in Shcherbak et al. 2014).

5.5.1. Environmental controls of N_2O emission

N_2O emission from soils to the atmosphere depends mainly on the availability of substrates of N_2O forming processes and environmental conditions controlling their transformation rates. Under aerobic conditions, nitrification is the main source of N_2O, depending on NH_4^+ as substrate. When O_2 availability decreases because of increasing O_2 consumption and/or decreasing gas diffusion rates, other processes come into effect. Some nitrifiers can use the nitrite (NO_2^-) produced from ammonium oxidation as an electron acceptor and reduce it to NO and N_2O. This nitrifier denitrification can contribute significantly to N_2O production when conditions are suboptimal for both nitrification and denitrifier denitrification (Kool et al. 2011; Wrage et al. 2001). Peak fluxes of N_2O, however, often occur under denitrifying conditions at high soil water content, i.e. after rain events, further supported by high nitrate contents after fertilization and/or high organic matter contents after harvest (Drury et al. 2006; Pelster et al. 2012; Sehy et al. 2003). The aeration status, which is affected by climatic conditions as well as soil texture and microbial O_2 consumption, thus exercises control over predominant N_2O producing processes and the amount of N_2O emitted. Both nitrification and denitrification, however, depend also and particularly on the availability of NH_4^+ and NO_3^- or nitrite (NO_2^-), respectively. The relationship between N input to and N_2O emission from ecosystems has often been described (e.g. Liu & Greaver 2009; Stehfest & Bouwman 2006). For agricultural systems, an increase in N_2O flux rates with increasing N fertilization has often (e.g. Acton & Baggs 2011; Breitenbeck & Bremner 1986; Kaiser et al. 1998; Mulvaney et al. 1997) although not always (high yielding areas in Sehy et al. 2003; Zebarth et al. 2008) been observed in both field and laboratory studies.

The generalized additive mixed model applied to our data confirms the importance of WFPS, NH_4^+ and NO_3^-, as well as the microbial activity (represented by CO_2 emission) for N_2O emission. The interaction of NH_4^+ with WFPS shows that high mineral nitrogen content alone was not sufficient to cause peak events of N_2O emission. Besides after frost, peaks in N_2O fluxes at both sites particularly occurred when nitrogen content as well as WFPS were relatively high. These flux dynamics are in accordance with the literature (Cannavo et al. 2004; Fuß et al. 2011; Hellebrand et al. 2003; Laville et al. 2011; Sehy et al. 2003). Substantial increase in N_2O fluxes may be expected at WFPS above 60 to 70% (Linn & Doran 1984; Ruser et al. 2006; Sehy et al. 2003) and the applied *gamm* (see Appendix A2) showed a similar trend. At the sandy loam site, such high WFPS values

were only reached in winter. Substantial peak fluxes after irrigation in 2012, however, show the potential for higher annual fluxes under wetter conditions after fertilization.

5.5.2. Impact of fertilizer application technique

Soil mineral N dynamics did not show distinctly different behavior between fertilized treatments. Accumulation of NO_3^- in or around CULTAN depots started within 2 weeks, and the soil NO_3^- content was not significantly lower than on surface application plots, except shortly after CULTAN injection. Hence, nitrification was not effectively inhibited. This is in agreement with earlier studies that reported mixed N nutrition after CULTAN fertilization with diammonium phosphate in pot experiments (Menge-Hartmann & Schittenhelm 2008; Schittenhelm & Menge-Hartmann 2006) or showing that nitrification was not inhibited after ammonium sulfate banding in 10 cm depth (Pfab 2011). On an annual scale, soil NO_3^- dynamics were largely dominated by turnover of the native soil N pool, as shown by similar NO_3^- contents and dynamics at fertilized and unfertilized plots. In accordance with the small differences in N_{min} contents between fertilizer treatments, there was no consistent effect of fertilizer application technique on N uptake and N use efficiency.

Despite the relatively low rate of N fertilization, annual N_2O emission roughly doubled with fertilization as compared to unfertilized plots. Fertilizer application method, however, did not have a significant effect on annual N_2O emission. There was only a weak trend to lower emission from CULTAN at the sandy loam and from surface application at the loam site. Analogously, N_2O emission factors and grain-yield based emissions did not differ significantly. Our results thus resemble earlier findings of no change or even enhanced N_2O emission after subsurface fertilizer banding of nitrate-containing fertilizers compared to broadcast application (Pfab et al. 2012; Smith et al. 2012). Given the observed accumulation of NO_3^-, differences between pure ammonium-fertilizer and nitrate containing fertilizer might be negligible, at least as N_2O production is concerned. Significantly higher grain yields, however, on CULTAN than surface application plots (significantly so in 2011 at the loam site and in 2012 at the sandy loam site) indicate an advantage for plant cultivation. However, available studies on specific yield effects of CULTAN fertilization provide no consistent results. While Weber et al. (2008) and Richter (2010) reported higher yield of various cereals from CULTAN compared to broadcast application of different fertilizers in Germany, other studies showed no significant yield effects (Flisch et al. 2013; Kozlovsky et al. 2010).

The temporal pattern of N_2O fluxes was primarily influenced by weather-dependent peaks. N_2O emission from fertilized plots was higher than from unfertilized plots after irrigation or abundant rain during the vegetation period, when also NO_3^- content was higher. Despite similar NO_3^- content and WFPS, higher peak fluxes occurred on CULTAN than on surface application plots after irrigation at the sandy loam site in 2012, and N_2O fluxes were more variable at CULTAN plots at the loam site. The N_{min} data shown do not provide information about spatial distribution of NO_3^- in soil. However, nitrification in or at the margins of depots might have formed patches with high NO_3^- contents. Together with high WFPS and possibly high organic carbon contents from exudates at the margins of depots, where high root density is assumed (Passioura & Wetselaar 1972), this may have led to conditions favorable for denitrification. Peaks of N_2O emission were broader, with lower amplitude, after surface application. Different spatial distribution of N_2O production zones may be the reason, with more homogeneous distribution in surface application plots and more heterogeneous conditions with spots of temporal mineral N surplus at CULTAN plots, allowing the formation of N_2O hotspots.

5.5.3. Site effect

Higher nitrification rates and N_2O emissions have been observed from loamy than from sandy soils in incubation studies (Maag & Vinther 1996). Pelster et al. (2012) proposed that higher N_2O rates from silty clay than sandy loam soil resulted from higher WFPS, greater organic content and finer texture (and thus higher restrictions to O_2 diffusion and more anaerobic microsites). Our hypothesis of higher emissions from the loam than the sandy loam site has been confirmed, but mainly for the CULTAN treatment. That the difference between sites was not significant after surface application and without fertilizer may be the result of dry conditions during summer and the low emission level at both sites. While WFPS was indeed higher at the loam site during most of the year, it was below 60% at both sites after fertilization when highest fluxes were expected. N_{min} dynamics were also similar at both sites, except for the higher NO_3^- accumulation at the sandy loam site during winter. Differences in dynamics of environmental factors controlling N_2O production were thus less severe than expected. Although pairwise comparisons revealed no significant difference between sites within treatments, annual N_2O emission at the loam was twice as high as at the sandy loam site after fertilizer injection (CULTAN). Especially after CULTAN fertilization, the loam site showed also much higher temporal and spatial variability of N_2O flux rates then the sandy loam soil which might be a result of higher nitrification and denitrification activity in the fine-grained soil. Adsorption of added NH_4^+ to clay minerals can affect nitrification by its influence on the contact of NH_4^+ with nitrifying microorganisms present on clay surfaces (Powell & Prosser 1991; Subbarao et al. 2006). In addition, soil texture also affects the inhibitory effect of high NH_4^+ concentration on nitrification (Abbès et al. 1994) and may thus lead to faster nitrification after point injection at the loam site. NO_3^- contents at the loam site were indeed twice as high as at the sandy loam site in the first weeks after fertilization. The formation of O_2 depleted microniches, where (nitrifier) denitrification may occur, is more likely in the finer textured loam soil (Pelster et al. 2012). We found no evidence of our hypothesis that NH_4^+ point injection (CULTAN) is able to reduce N_2O emission, particularly in a fine-grained soil. In contrast, the CULTAN induced accumulation of mineral N and roots in small areas within the soil might even promote local N surplus and N_2O emission by coupled nitrification denitrification, in particular at higher soil moisture in fine-grained soils.

5.5.4. Fertilizer-derived N_2O emissions and crop N uptake

For a more detailed analysis of fertilization effects on direct N_2O emission it is helpful to differentiate between N_2O emission that originates directly from the added fertilizer N (here called fertilizer-derived N_2O emission) and a more comprehensive fertilization effect which is determined from the increase in total N_2O emission compared with an unfertilized treatment (here called fertilizer-induced N_2O emission). Unfortunately, it was not possible to compare fertilizer-derived emissions from application methods. However, the ^{15}N signature of N_2O emitted from the CULTAN treatment of the sandy soil indicates that only a small fraction of the added fertilizer N (0.005% in 2011 and 0.10% in 2012) was lost as direct annual N_2O-N emission within 12 months after application. Most of the N_2O emitted thus originated from transformation of soil N. Large contributions from added fertilizer N (up to 65% of the measured N_2O flux) were only measured in the first weeks after fertilization and support the above mentioned assumption that CULTAN N depots represent at least transient hotspots of N_2O production. Annual fertilizer-induced N_2O-N emission, calculated from difference in total N_2O emission between the ^{15}N fertilized CULTAN plots and the unfertilized treatments (i.e. emission factor, 0.09% - 0.26% of the total amount of fertilizer N added), was considerably higher than annual fertilizer-derived N_2O emission. A similar

phenomenon has earlier been described for N turnover and was attributed to N pool substitution through immobilization or isotopic displacement (Jenkinson et al. 1985). Other possible reasons for this obvious difference are effects of fertilization on N_2O production from the soil N pool and effects of fertilization on soil carbon availability and microbial activity (i.e. larger plant biomass at fertilized plots) that can stimulate N_2O emission. That N_2O fluxes depend on microbial activity was affirmed by our statistical model.

The relative contribution of ^{15}N fertilizer to N content in the aboveground plant biomass was much higher than its relative contribution to total N_2O emission. With 35% and 50% of total N in aboveground plant biomass at harvest in 2011 and 2012, respectively, the results reflect the efficiency of CULTAN N in crop nutrition. Higher N use efficiency of point injection of ammonium based fertilizer compared to surface application has been reported earlier (Janzen et al. 1990; Richter 2010) and was also described as a benefit of subsurface urea banding (Yadvinder et al. 1994). Analogously to the calculation of fertilizer-derived N_2O emission we calculated the fertilizer-derived nitrogen use efficiency from the amount of fertilizer N in the aboveground crop biomass at harvest and the amount of fertilizer N applied. The fertilizer-derived NUE corresponds to 31% and 52% of the applied fertilizer N in 2011 and 2012, while NUE calculated from the difference in N content of aboveground crop biomass between fertilized and unfertilized plots was 64% to 72%. The ^{15}N fertilizer recovery was rather high in comparison to earlier studies (Carranca et al. 1999; Tran & Tremblay 2000). Petersen (2001), however, found higher ^{15}N fertilizer recovery in spring wheat (55% - 59%), without a significant effect of subsurface banding of ammonium sulfate compared to surface application.

The ^{15}N results show the great importance of mineralization of organic soil N for crop N uptake and in particular for soil N_2O emission. Common methods to determine fertilization effects on NUE and N_2O emission factors include transformation processes of soil organic N and reflect only partly direct transformation of fertilizer N.

5.6. Conclusions

Inhibition of nitrification in fertilizer depots after CULTAN fertilization was not strong enough to prevent nitrate accumulation in both the loam and the sandy loam soil. In terms of N_2O emission, this first study on CULTAN fertilization provides no evidence that this fertilization technique has the potential to reduce direct N_2O emission from fertilizer application. Higher yields after CULTAN than surface application, however, indicate the potential of higher crop yields at equal N fertilization rates at our experimental sites.

Higher N_2O emissions from the loam than the sandy loam site especially at CULTAN plots were attributed to the higher soil moisture and the propensity to the formation of denitrifying microsites at the finer textured soil. In combination with the patchy distribution of N_{min} which probably results in transient local N_{min} surplus and possibly also root biomass in soil after CULTAN, this may cause N_2O emission hotspots. This explanation is further supported by a higher variability of N_2O fluxes after CULTAN fertilization compared to surface application. It also remains to be tested, whether results would differ under wetter conditions, on high emission sites, or at higher N application rates. We'd like to point out that CULTAN fertilization may also change nitrate leaching and thus affect indirect N_2O emission. This potential effect on nitrogen leaching was not included in our study.

According to point injection of ^{15}N-labeled NH_4^+, added CULTAN N had only a small direct effect on annual N_2O emission, with less than 20% of annual N_2O emission originating from the applied fertilizer. The results indicate that the presence and mineralization of active organic N pools were decisive for annual N_2O emission. The dominance of native soil N for N turnover processes was also apparent from N_{min} dynamics and high crop N uptake in unfertilized treatments. The results stress the importance to optimize long-term N management in cropping systems in order to reduce N_2O emission from agricultural soils. In addition, they indicate that measurements of N_2O emissions with the goal to derive emission factors for different fertilizers or fertilizer application techniques should cover several years to include short-term and also medium term effects of specific N management on N_2O emission.

5.7. Acknowledgements

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6. Soil N_2O fluxes and processes in laboratory incubations simulating ammonium fertilizer depots[3]

6.1. Abstract

High concentrations of ammonium in soil have been shown to inhibit nitrification, and fertilizer injection as conducted during CULTAN management might thus have the potential to reduce N_2O emission from arable soil. We conducted an incubation experiment with different NH_4^+ concentrations in soil that resembled concentrations as expected at and around injection spots (5000, 2250, 1000, 450, 0 µg NH_4^+-N g^{-1} soil) directly after fertilization and after dilution due to plant uptake or precipitation. N_2O emission was measured in dynamic soil mesocosms over a period of 21 days. Acetylene inhibition and ^{15}N tracer approaches were used to calculate the relative contribution of nitrification and denitrification to N_2O emission. An isotopomer approach was applied to gain further insight into N_2O producing processes. We expected lower contribution of nitrification-derived N_2O to total N_2O emission and a higher N_2O/NO_3^- ratio from nitrification with increasing N levels. Nitrification indeed declined with increasing N level, and no nitrification occurred in the 5000 µg NH_4^+-N g^{-1} soil treatment. A pool dilution approach showed that gross nitrification in 450 µg NH_4^+-N g^{-1} soil (nitrification rate: 4.96 mg NO_3^--N kg soil d^{-1}) was by a factor of 2.6 and 6 higher than in 1000 and 2250 µg NH_4^+-N g^{-1} soil treatments. In the 5000 µg NH_4^+-N g^{-1} soil treatment, gross nitrification occurred at very small rates (0.1 mg NO_3^--N kg soil d^{-1}). Similarly, N_2O emission declined with increasing N level. The N_2O yield of nitrification was between 0.07% and 0.15% of NO_3^- production, but was not affected by increasing N level. Nitrification was the dominant source of N_2O throughout the incubation at all N levels, and there was no significant change in the relative contribution of nitrification and denitrification with N level or time. We thus conclude that denitrification derived N_2O emissions were similarly reduced at high N levels. Applying the non-equilibrium technique to our ^{15}N tracer data revealed heterogeneous distribution of denitrification in soil, with at least two distinct NO_3^- pools and spatial separation of NO_3^- formation and consumption. The isotopomer approach provided reasonable results in comparison with the acetylene inhibition and ^{15}N tracer approaches and indicated substantial contribution of nitrifier denitrification (10% - 40%) to total N_2O production.

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6.2. Introduction

The CULTAN (Controlled uptake long-term ammonium nutrition) fertilization strategy as described by Sommer (2005) uses fertilizer placement techniques (point injection, banding) of ammonium-rich/nitrate free nitrogen fertilizer, aiming at a more beneficial nutrition of plants with ammonium (NH_4^+) as the dominant nitrogen form. Point injection of concentrated fertilizer solution by spoke-wheels is common, creating fertilizer depots of high NH_4^+ concentration within the soil. It has been shown that both grain yields and N uptake can equal or exceed those from conventional surface application of fertilizer (chapter 5; Flisch et al. 2013; Kozlovsky et al. 2010; Peklova et al. 2012; Schittenhelm & Menge-Hartmann 2006; Sedlář et al. 2011; Weber et al. 2008). The relative stability of nests with high concentration of NH_4^+ (Wang et al. 1998) is a main aspect why N fertilizer injection during CULTAN management, at one dose to the root zone, may be a convenient method of mineral fertilizer application. This anticipated stability comes from the relative immobility of NH_4^+ in soil as compared to nitrate (Olesen et al. 1999), preventing broadening of fertilizer, and the toxicity of high concentrations of NH_4^+ for microbial nitrification (Harada & Kai 1968; Wetselaar et al. 1972). This toxicity effect is crucial for the mitigation potential of CULTAN fertilization; hence dynamics of nitrification and coupled denitrification processes under this treatment must be more thoroughly investigated.

Nitrification is the microbially mediated oxidation of NH_4^+ or, more specifically, ammonia (NH_3) to nitrate (NO_3^-). The first step of nitrification is performed by ammonia oxidizing bacteria (AOB) or archaea (AOA): The enzyme ammonia monooxygenase (AMO) catalyzes the oxidation of NH_3 with O_2 to hydroxylamine (NH_2OH), which is further oxidized to nitrite (NO_2^-) by hydroxylamine oxidoreductase (HAO). Thereby, a certain fraction of the NH_2OH is chemically transformed to nitrous oxide (N_2O) as a side product (Butterbach-Bahl et al. 2013; Heil et al. 2014). The second step of nitrification, the oxidation of NO_2^- with O_2 to NO_3^-, is catalyzed by nitrite oxidoreductase in nitrite oxidizing bacteria. Various AOB are furthermore capable to reduce the NO_2^- from ammonia oxidation to NO and N_2O via the nitrifier denitrification pathway (Kool et al. 2011; Wrage et al. 2001). The N_2O, which is built as a side- or intermediate product during these processes, is a potent greenhouse gas, and international efforts are made to diminish the N_2O emission from anthropogenic sources (to which agricultural sources contribute roughly 79%, including indirect emission (Ciais et al. 2013; UNFCCC 2014)).

While nitrification is regarded as the most important N_2O producing process under strictly aerobic conditions, several other N_2O source processes exist (Butterbach-Bahl et al. 2013; Kool et al. 2011). Under suboxic conditions, nitrifier denitrification can substantially add to N_2O production (Kool et al. 2011; Zhu et al. 2013). Under anaerobic conditions, N_2O is built as an intermediate in denitrification, the stepwise reduction of NO_3 to NO_2-, NO-, and finally N_2O and N_2 (Knowles 1982). Besides the aforementioned processes, which are assumed to be responsible for the majority of N_2O from terrestrial soils, other N_2O producing processes exist (e.g. dissimilatory nitrate reduction to ammonia, heterotrophic nitrification, co-denitrification; Butterbach-Bahl et al. (2013)).

In general, there is a positive correlation between NH_4^+ as a substrate and nitrification rates. In incubation studies, nitrification and N_2O flux from nitrification have been shown to increase with NH_4^+ contents from 0 to 400 mg NH_4^+-N kg^{-1} soil (Avrahami et al. 2002; Huang et al. 2014; Vermoesen et al. 1996; Well et al. 2008). After band application or point injection of NH_4^+ fertilizer, however, NH_4^+ content in soil can be much higher, easily exceeding 3000 mg NH_4^+-N kg^{-1} (e.g. Menge-Hartmann & Schittenhelm 2008; Pfab et al. 2012). Such high concentrations of NH_4^+ in cells are toxic to both plant tissue (Gerendás et al. 1997) and microorganisms (Müller et al. 2006).

Contents of 2000 to 20,000 mg N kg^{-1} as ammonium sulfate ($(NH_4)_2SO_4$) have been shown to completely inhibit nitrification in soil (Nishio & Fujimoto 1990; Shaviv 1988; Wetselaar et al. 1972) for a time span of 3 – 4 weeks.

If NH_4^+ contents in soil after CULTAN injection are high enough to successfully inhibit nitrification, for a time span sufficient to stabilize the depot until most of the N is taken up by plants, they should have similar effects as the use of industrial nitrification inhibitors, which also retard NH_4^+ oxidation (Prasad & Power 1995). Studies on the effect of nitrification inhibitors were summarized by Akiyama et al. (2010) and showed a reduction in N_2O emission of 26% - 43% (95% confidence interval). If depot fertilization during CULTAN management reduces N_2O emission, it will thus have the potential to be not only beneficial to plant nutrition but also a climate friendly method of mineral fertilization.

Most studies on the dissolution of fertilizer depots or inhibition of nitrification at high NH_4^+ content concentrated on NO_3^- or nitrite (NO_2^-) accumulation in soil as the parameter of interest (Menge-Hartmann & Schittenhelm 2008; Petersen et al. 2004; Shaviv 1988; Wang et al. 1998; Wetselaar et al. 1972). In an incubation experiment with increasing NH_4NO_3 content, net nitrification and N_2O production from nitrification decreased by one and two thirds, respectively, at an increase from 355 to 710 mg NH_4^+-N kg^{-1} (Acton & Baggs 2011). Whether the inhibition of nitrification at high NH_4^+ content is due to NH_4^+ specific properties or mainly because of osmotic pressure due to high salt concentration has not been conclusively determined for all processes or organisms. Different NH_4^+ salts exhibited different strength in decreasing nitrification rates (Darrah et al. 1985), and often the effect is attributed to the increasing osmotic pressure (Darrah et al. 1986; Müller et al. 2006).

At conditions unfavorable for microbial turnover in one way or another, product ratios of nitrification (N_2O_{nit}/NO_3^-) or denitrification (N_2O_{denit}/N_2) have been shown to change due to varying sensibility of enzymes responsible for specific process steps. For instance, increasing salinity has an inhibiting effect on denitrification, but may concurrently increase N_2O emission. This is a result of shifts in the N_2O/N_2 product ratio due to the fact that N_2O reductase is the enzyme most vulnerable to inhibition, and hence N_2O reduction to N_2 is the enzymatic reaction to be mostly inhibited (Menyailo et al. 1998; Menyailo et al. 1997). The product ratio of N_2O/N_2 during denitrification also increases with decreasing pH values (Baggs et al. 2010; Čuhel et al. 2010; Knowles 1982) and increasing partial pressure of O_2 (Betlach & Tiedje 1981). Lower O_2 conditions, to the contrary, lead to higher N_2O production from nitrification (Mørkved et al. 2006; Zhu et al. 2013), supposedly due to increasing importance of nitrifier denitrification. Increasing salinity also slowed down gross nitrification (Low et al. 1997), as long as low NH_4^+ content in soil was not limiting. However, N_2O production increased with increasing salinity, and decoupling of processes with NO_2^- accumulation were suggested to be the reason (Low et al. 1997), since NO_2^- oxidation may be more effectively inhibited than NH_4^+ oxidation (Harada & Kai 1968). Also acid conditions may lead to increased N_2O/NO_2^- ratio of nitrification (Jiang & Bakken 1999). On the other hand, acidification during nitrification could be a reason for short-term decreases in N_2O production, as growth of nitrifiers and NH_4^+ oxidation is affected by changes in the NH_4^+/NH_3 equilibrium with pH (Baggs et al. 2010).

The CH_4 molecule is structurally similar to NH_4^+, and the methane monooxygenase (MMO) of methanotrophs is very similar to the AMO in ammonia oxidizers (Bédard & Knowles 1989). The addition of NH_4^+ to soil has the potential to inhibit CH_4 oxidation, although mechanisms are complex and comprise a pH effect from nitrification, competitive inhibition of MMO by NH_3/NH_4^+ and

inhibition by the nitrification intermediates NH_2OH and NO_2^- (Hütsch 1998; King & Schnell 1994; Nyerges & Stein 2009). Both AMO and MMO are furthermore susceptible to acetylene (C_2H_2) as an inhibitor (Bédard & Knowles 1989; Hyman & Wood 1985; Prior & Dalton 1985). Due to the close resemblance between the monooxygenases of methane and ammonia oxidation, factors affecting nitrification may also have impacts on rates of methane consumption in soil.

Little is known about the relative importance of different processes (i.e. mainly denitrification vs. nitrification) under high NH_4^+ levels. Several methods exist to quantify their relative contributions to measured N_2O fluxes, all having their advantages and difficulties (Baggs 2008; Decock & Six 2013). The inhibition of NH_4^+ oxidation at 0.01vol% (C_2H_2) in the gas phase (Bollmann & Conrad 1997; Hyman & Wood 1985; Klemedtsson et al. 1988) can be used to quantify the contribution of autotrophic nitrification, if N_2O production is compared to a control without inhibition. This method may suffer from the consequences of its own mode of action, because NO_3^- formation is inhibited and the contribution of denitrification may thus be underestimated. After addition of an N substrate enriched in the heavy isotope ^{15}N, its fate can be traced in the different products, thus allowing to distinguish between NO_3^- derived and NH_4^+ derived N_2O, but not between single processes. Bateman and Baggs (2005) used the combination of acetylene inhibition and the ^{15}N tracer approach to distinguish between denitrification, autotrophic and heterotrophic nitrification; and Well et al. (2008) showed good correspondence between acetylene inhibition and the ^{15}N tracer approach in source partitioning N_2O production under nitrifying conditions. The natural abundance of the heavy isotopes ^{15}N and ^{18}O in N_2O without tracer addition comprise information about the substrates and production processes of N_2O (Baggs 2008). Moreover, the analysis of the intramolecular distribution of ^{15}N within the N_2O molecule, i.e. the ^{15}N-site preference (SP, defined as difference in the abundance of isotopomers $^{14}N^{15}NO$ and $^{15}N^{14}NO$ relative to $^{14}N^{14}NO$) is a tool to investigate N_2O source processes at natural abundance (Decock & Six 2013; Ostrom & Ostrom 2012). For different processes (e.g. NH_2OH oxidation, NO_3- or NO_2- reduction), the SP has been measured in pure culture studies of bacteria and fungi (Rohe et al. 2014; Sutka et al. 2008; Sutka et al. 2006; Sutka et al. 2003; Sutka et al. 2004; Toyoda et al. 2005) and soil incubations (Köster et al. 2015; Köster et al. 2013; Lewicka-Szczebak et al. 2015; Lewicka-Szczebak et al. 2014; Perez et al. 2006; Well & Flessa 2009). Based on the finding that SP is independent of the ^{15}N abundance in precursors (Toyoda et al. 2002) but depends only on the producing process or enzymes, it can be used to estimate the relative contribution of different processes to N_2O production. However, SP alone has not been specific enough to quantify the relative contribution of nitrification and denitrification to N_2O production, as fractionation during the reduction of N_2O to N_2 during bacterial denitrification changes the SP, moving values closer to those of nitrification (Ostrom et al. 2007; Well & Flessa 2009). As not only the SP but also the $\delta^{18}O$ of remaining N_2O increase during N_2O reduction (Ostrom et al. 2007), taking into account also the oxygen atom of the N_2O molecule may help to improve the estimation of source processes (Snider et al. 2013). Köster et al. (2015) used the SP and $\delta^{18}O$ of N_2O to distinguish between denitrification and autotrophic nitrification under denitrifying conditions (in a helium-oxygen atmosphere) in laboratory soil incubations, taking into account the reduction of N_2O by measuring N_2 production.

The present laboratory study was conducted to examine the impact of concentrated NH_4^+ solution as after fertilizer injection on nitrification and associated N_2O fluxes. Concentrations of NH_4^+ were chosen according to concentrations in CULTAN depots of a parallel field experiment (chapter 5) and earlier studies (Menge-Hartmann & Schittenhelm 2008; Pfab et al. 2012). Using N levels from 0 to 5000 mg kg^{-1} soil, we aimed at a range from no to complete inhibition of nitrification. Three methods were used to measure nitrification rates and source partitioning of N_2O fluxes in

laboratory incubation experiments with sandy loam soil. With the acetylene inhibition approach, we determined the contribution of autotrophic nitrification. Using the ^{15}N tracer approach, we calculated the fraction of N_2O that originated from NO_3^-. The pool dilution approach (Davidson et al. 1991) was used to calculate gross nitrification rates, which were then used to calculate the N_2O/NO_3^- yield of nitrification. An isotopomer approach was additionally used to estimate the relative contributions of denitrification and nitrification to the emitted N_2O.

The experiment aimed at testing whether concentrations of NH_4^+ after CULTAN injection of $(NH_4)_2SO_4$ fertilizer are appropriate to successfully inhibit nitrification and associated N_2O fluxes. Our hypotheses were that 1) Concentrations as they occur at the field sites are high enough to limit nitrification after fertilization; 2) N_2O yield of nitrification increases with increasing initial NH_4^+ content in soil; 3) The fraction of N_2O from NH_4^+ oxidation predominates N_2O production, with increasing contribution of NO_3^--derived N_2O with time and NO_3^- accumulation.

6.3. Materials and methods

6.3.1. Soil properties

The soil used for the incubation experiment was taken from the upper 20 cm at a temperate arable field site at the Thünen Institute in Braunschweig, Germany (52°18'01"N, 10°26'50"E) in September 2012. The soil type is a Haplic Luvisol derived from glaciofluviatile sediments with sand, silt and clay contents of 68%, 23% and 9%, respectively. Carbon and nitrogen contents were 11 g C kg^{-1} and 1 g N kg^{-1}, respectively, pH($CaCl_2$) was 5.9, and bulk density 1.5 g cm^{-3}. The soil was rather dry when sampled (28% WFPS, September 2012). It was manually sieved to 4 mm, stored at 5°C, and preincubated at room temperature 24h prior to addition of fertilizer solutions. After preincubation, the soil contained 0.2 mg NH_4^+-N kg^{-1}, 13 mg NO_3^--N kg^{-1} and 8.1 g water kg^{-1} d.w. soil.

6.3.2. Experimental design

Simulating a range of NH_4^+ concentrations as expectable after CULTAN fertilization, different amounts (N levels) of $(NH_4)_2SO_4$ were applied to the soil. Treatments were named after the NH_4^+ content added, i.e. 0N (no $(NH_4)_2SO_4$), 450N, 1000N, 2250N and 5000N for 450 µg NH_4^+-N (g dry soil)$^{-1}$, 1000 µg NH_4^+-N (g dry soil)$^{-1}$, 2250 µg NH_4^+-N (g dry soil)$^{-1}$ and 5000 NH_4^+-N µg (g dry soil)$^{-1}$, respectively. All treatments received the same amount of KNO_3 (13 mg NO_3^--N kg^{-1} d.w. soil).

To achieve the respective NH_4^+ concentrations for different N levels, varying amounts of $(NH_4)_2SO_4$ were mixed with distilled water and potassium nitrate (KNO_3) to achieve a water content of 145 g kg^{-1} in soil, which later resulted in an initial water filled pore space (WFPS) of 50%. For each N level, 30 kg of air-dry soil were thoroughly mixed with the respective $(NH_4)_2SO_4/KNO_3$ solution. 3217 g of wet soil of the respective N level were then filled to a height of 12.5 cm in cylindrical incubation vessels with 14.4 cm inner diameter and 18 cm height. A bulk density of 1.5 g cm^{-3} was chosen according to field conditions.

Three different methods were used to measure the fraction of nitrification-derived N_2O to total N_2O (f_N) emissions from soil: With the ^{15}N tracer method (Stevens et al. 1997) in combination with the ^{15}N pool dilution approach (Davidson et al. 1991), N_2O fluxes from NO_3^- turnover were determined after adding ^{15}N labeled KNO_3 to the soil (12.5 at%^{15}N; 15N batch). Similar batches with unlabeled

KNO_3 were used for, the isotopomer approach (Decock & Six 2013; Ostrom & Ostrom 2012) of emitted N_2O (using $\delta^{18}O$, average $\delta^{15}N$ and ^{15}N site preference; ^{14}N batch), and the C_2H_2 inhibition approach (Hyman & Wood 1985; Klemedtsson et al. 1988), i.e. comparison of N_2O production in batches 14N and 15N with C_2H_2-amended treatments (C2H2 batch). Soil for the 14N and C2H2 batches was prepared in one go, as these batches differed only in the headspace flow applied (compressed air for 14N and 15N, compressed air with 0.01vol% acetylene added for the C2H2 batch). Details of these methods for source partitioning are given in section 6.3.5.2.

Preparation and installation of the 15N batch was started and finished approx. 24h later than 14N and C2H2 batches, respectively. Four soil cores were used per N level and batch. Four additional replications of each N level of the 15N batch were installed and destructively sampled for mineral N content (see Section 6.3.4.1) at day 10. These cores were otherwise treated identically to those used for flux measurements. Duration of incubation for all other soil cores was 21 days.

After filling with the fertilized soil, incubation vessels were sealed airtight and connected to an automated incubation system as described by Hantschel et al. (1994) in a climate chamber (16°C) in darkness. The headspace of vessels was continuously flushed with compressed air at a rate of approx. 4ml min^{-1}. For incubation vessels of the C2H2 batch, 100 ppm acetylene (Linde, solvent free) were mixed to the compressed air with a gas mixer (HovaGAS digital G8-vTI, IAS GmbH, Frankfurt, Germany). Due to mismatched pipes, N levels 0N and 450N of the C2H2 batch did not receive acetylene during the incubation period. These N levels were therefore repeated immediately after the end of the first incubation period with soil from the same sampling date which had been equally prepared and stored at 5°C.

6.3.3. Gas sampling and analytical procedures

Glass vials for N_2O and CH_4 concentration (20ml crimp vials with butyl rubber septa) and isotope analysis of N_2O (120ml crimp vials with butyl rubber septa; and 12ml septum capped glass vials, Labco™, High Wycombe) were connected in line to the headspace outlet of the incubation vessels as described in Well et al. (2008) and sampled on each of days 1, 2, 3, 6, 10, 14, 18, 21. Before and after sampling, gas flow rates were additionally measured using a high precision digital flow meter (Alltech Associates Inc., Deerfield, IL, USA).

6.3.3.1. Determination of N_2O and CH_4 concentrations and fluxes

Concentrations of N_2O and CH_4 were measured with a gas chromatograph (GC 2014, Shimadzu, Duisburg, Germany) equipped with an ECD detector (N_2O, CO_2) and FID (CH_4) and an automated rack (P 65, Loftfields Analytical Solutions, Neu Eichenberg, Germany). Precision was checked weekly by repeated determination of standard gases (1810 ppb CH_4, 320 ppb N_2O) and was consistently < 3%. Gas fluxes were calculated from change in concentration in the gas stream between headspace inlet and outlet of the incubation vessel, the flow rate of the respective headspace gas, and the amount of dry soil per incubation vessel. To calculate cumulative emissions, flux rates were linearly interpolated between measurement dates.

6.3.3.2. Determination of isotopic signatures of N_2O

Isotopologue values of N_2O were obtained by analyzing m/z 44, 45 and 46 of intact N_2O molecules as well as m/z 30, 31 of NO^+ fragments by isotope ratio mass spectrometry as described previously (Lewicka-Szczebak et al. 2014) using a DeltaV IRMS (ThermoFisher Scientific, Bremen, Germany) allowing simultaneous detection of m/z 30, 31, 44, 45 and 46. The IRMS was connected to a

modified pre-concentrator (Precon Finnigan MAT, Bremen, Germany) equipped with an autosampler (model Combi-PAL CTC-Analytics, Zwingen, Switzerland) as described by Casciotti et al. (2002). The scrambling factor reflecting the N-exchange between N_2O^+ and NO^+ in the ion source of the mass spectrometer was determined as described by Röckmann et al. (2003) and was 0.08. The isotopologue ratios of $^{15}R^{bulk}$, ^{18}R, ^{30}R and $^{15}R^{\alpha}$ were determined and $^{15}R^{\beta}$ was obtained by the relationship of $^{15}R^{bulk} = (^{15}R^{\alpha} + {}^{15}R^{\beta})/2$, where $^{15}R^{\alpha} = [^{14}N^{15}N^{16}O]/[^{14}N^{14}N^{16}O]$, $^{15}R^{\beta} = [^{15}N^{14}N^{16}O]/[^{14}N^{14}N^{16}O]$, $^{18}R = [^{14}N^{14}N^{18}O]/[^{14}N^{14}N^{16}O]$ and $^{31}R = [^{15}N^{16}O]/[^{14}N^{16}O]$ (Toyoda and Yoshida, 1999) . Isotopologue ratios of a sample (R_{sample}) were expressed as ‰ deviation from $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of the standard materials (R_{std}), atmospheric N_2 and standard mean ocean water (SMOW), respectively: $\delta X = (R_{sample}/R_{std} - 1)\times 1000$, where X = $^{15}N^{bulk}$, $^{15}N^{\alpha}$, $^{15}N^{\beta}$, or ^{18}O. Typical analytical precision was 0.12, 0.33, and 0.3 ‰ for $\delta^{15}N^{bulk}$, SP, and $\delta^{18}O$, respectively. The detection limit for N_2O-N was 1.5 nM. The difference between the isotopomer ratios of N ($\delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$) is referred to as ^{15}N-site preference (SP, in ‰). Pure N_2O (Westfalengas, Münster, Germany; purity > 99.995) was used as reference gas in concentrations corresponding to the expected N_2O amounts in samples. The pure N_2O was analyzed for isotopologue values by Toyoda and Yoshida in the laboratory of the Tokyo Institute of Technology (Toyoda & Yoshida 1999). This reference signature was used to correct the raw $\delta^{15}N^{\alpha}$ determined by our instrumentation.

6.3.4. Soil analyses

6.3.4.1. Determination of mineral soil N contents

To measure soil mineral nitrogen ($N_{min} = NO_3^- + NH_4^+$) content, subsamples from soil prepared for each N level of both 14N and 15N batches were collected at day 0 (n=5 per batch). At the end of the incubation (day 21) soil cores were sampled individually (n=3), as were the additional soil cores of the 15N batch sampled at day 10. Soil samples were mixed with $CaCl_2$ solution (0.01M; soil to solution ratio of 1:10 (v/v) for control soil and N levels 0N to 1000N and 1:30 for 2250N and 5000N levels). After filtration (MN614 ¼ filters, Macherey & Nagel, Düren, Germany) the extracts were stored at -20°C until analysis of NH_4^+-N and (NO_3^- + NO_2^-)-N concentrations with a continuous flow analyzer (SA 5000, Skalar Analytical B.V., Netherlands). Water content in soil was determined gravimetrically by drying overnight at 105°C.

6.3.4.2. Determination of ^{15}N enrichment of mineral N

To determine the isotope ratios of N_{min} in soil extracts from the 15N batch, a diffusion technique adapted after Goerges and Dittert (1998) was used. Aliquots of extracts containing NH_4^+ and NO_3^- were alkalized with magnesium oxide, so that NH_4^+ formed NH_3 gas that was trapped on fibre glass filters (Macherey-Nagel, MN85/90BF) which had been acidified with $KHSO_4$. In a second step, NO_3^- was reduced to NH_3 by addition of Devarda's alloy (containing Al, Cu, Zn) to the same sample, and trapped on a second fiber glass filter. The filters were then analyzed for at%^{15}N using an elemental analyzer coupled to a Delta Plus IRMS (ThermoFinnigan, Bremen, Germany).

A blank correction was applied to at%^{15}N values of NH_4^+ samples according to Equation 6-1 and 6-2.

$$at\%_{NH4} = \frac{at\%_{mix} * N_{mix} - at\%_{blank} * N_{blank} * \frac{ml_{sample}}{ml_{blank}} - at\%_{KCl} * N_{KCl} * \frac{ml_{KClsample}}{ml_{KCL}}}{N_{NH4}} \qquad \textbf{Equation 6-1}$$

$$N_{NH4} = N_{mix} - N_{blank} * \frac{ml_{sample}}{ml_{blank}} - N_{KCl} * \frac{ml_{KClsample}}{ml_{KCL}}$$ **Equation 6-2**

Incomplete outgassing of NH_4^+ in the first reaction step resulted from high NH_4^+ concentrations in the samples relative to NO_3^- concentrations. Additional calculations were performed to correct the measured at%^{15}N in NO_3^- samples for this surplus N. Therefore, standard samples containing either labeled NH_4^+ and unlabeled NO_3^-, or vice versa, were used. The amount of N in the measured sample stemming from the NH_4^+ standard was calculated from mass balance and mixing equations. The correlation between this N surplus and the N input was used as a correction function for the samples (Equation 6-3).

$$N_{NH4} = \frac{N_{mix} * (at\%_{mix} - at\%_{NO3}) - N_{blank} * (at\%_{blank} - at\%_{NO3})}{(at\%_{NH4} - at\%_{NO3})}$$ **Equation 6-3**

Many of the N_{min} samples from 2250N and 5000N levels contained much higher amounts of NH_4^+ than NO_3^-. As the carryover of NH_4^+ into the NO_3^- sample was too high to determine the at%^{15}N of the NO_3^- correctly, even with the abovementioned correction, ^{15}N abundance of NO_3^- in these samples was measured according to the procedure described in Stange et al. (2007): NO_3^- was reduced to NO by vanadium chloride ($V(III)Cl_3$), and NO was used as measurement gas. Measurements were performed with a quadrupole mass spectrometer (GAM 200, InProcessInstruments, Bremen, Germany). According to the measured standard solutions, precision was 0.05 at%^{15}N.

6.3.5. Quantification of N-transformation processes

6.3.5.1. Determination of nitrification rates

Net nitrification was calculated as the change in NO_3^- content over time (difference between initial and final sampling). **Gross nitrification** (n_g) rates were determined with the ^{15}N pool dilution approach and thus calculated from at%^{15}N in NO_3^- of soil samples taken before filling of soil cores (day 0), from the additional soil cores sampled at day 10 and at the end of measurements (day 21) using Equation 6-4 (after Davidson et al. 1991).

$$n_g = \frac{c_1 - c_2}{t_2 - t_1} \cdot \frac{\ln\left(\frac{at\%_1}{at\%_2}\right)}{\ln\left(\frac{c_1}{c_2}\right)}$$ **Equation 6-4**

With *c*=concentration, *t*=time of sampling, and numbers indicating initial (1) and final (2) values. Assumption for the applicability of this equation are 1) constant process rates during the incubation, 2) negligible isotopic discrimination, 3) uniform distribution of ^{15}N label within the soil and the NO_3^- pool, and 4) no remineralization of the assimilated ^{15}N (Davidson et al. 1991; Murphy et al. 2003). Values of at%^{15}N of NO_3^- for N_2O sampling dates without soil sampling were calculated from the same equation solved for at%$_2$ to allow calculation of NO_3^- derived N_2O for each gas sampling event, applying it to sampling times and NO_3^- contents in soil as derived from net nitrification rates.

6.3.5.2. Identification of N_2O source processes

Different approaches were used to calculate the contribution of nitrification (f_N) and denitrification (f_D = 1 - f_N) to total N_2O production. All eight measurement dates were used for calculation of

cumulated fluxes with the acetylene inhibition technique (0). For isotope-based methods (6.3.5.4 - 6.3.5.6), only four dates were used and f_N for the whole incubation period was then calculated as the weighted average over these sampling dates. Table 6-1 gives an overview of the methods used and the processes they address.

Table 6-1: Overview of methods used for source partitioning of N_2O and the targeted processes or N_2O sources they can distinguish from other processes.

	Method	Distinguished process		Remarks
		Target process	Other processes	
(a)	C_2H_2 inhibition	N_2O from autotrophic nitrification (and, resulting from inhibited NO_2^-/ NO_3^- formation: nitrifier denitrification and coupled nitrification denitrification)	Other N_2O production, including denitrification and heterotrophic nitrification.	This apportionment was used also in (Zhu et al. 2013)
(b)	^{15}N tracing with NO_3^- labeling based on extracted bulk $^{15}NO_3^-$	N_2O from labeled NO_3^- pool in case bulk NO_3^- pool is identical to active denitrifying pool ; includes denitrification coupled to nitrification	NH_2OH oxidation, nitrifier denitrification; heterotrophic nitrification	-
(c)	^{15}N tracing with NO_3^- labeling based on the non-equilibrium approach	N_2O from labeled NO_3^- pool instantaneously undergoing denitrification, includes denitrification coupled to nitrification only under ideal homogeneity of pools and processes	NH_2OH oxidation, nitrifier denitrification; heterotrophic nitrification	-
(d)	isotopomers	N_2O from NH_2OH oxidation (enzymatic and abiotic)/ fungal denitrification	Nitrifier denitrification, bacterial denitrification, including denitrification coupled to nitrification(in case N_2O reduction is low and has thus negligible impact on isotopomers)	Unknown apportionment of heterotrophic nitrification
(e)	Difference approach	Fertilizer induced fluxes	Background flux (e.g. nitrification of initial or mineralization-derived NH_4^+, denitrification of initial and added tracer NO_3^-)	

6.3.5.3. Acetylene inhibition approach to determine the fraction of N_2O from autotrophic nitrification (a)

With C_2H_2 inhibition, f_N (contribution of autotrophic nitrification) of N_2O production was calculated from the difference in N_2O production between soil cores of the 15N and 14N batches (no inhibition) and the C2H2 batch (where the AMO was inhibited by C_2H_2 addition to the headspace).

6.3.5.4. ^{15}N tracing approach using at%^{15}N of N_2O and of extracted NH_4^+ and NO_3^- (b)

To calculate f_N and f_D with the ^{15}N tracer approach of Stevens et al. (1997), we assumed that the N pools of these processes were NH_4^+ and NO_3^-, respectively. Therefore, the soil NO_3 -pool labeled with ^{15}N (KNO_3) and the isotopic abundance of ^{15}N in soil emitted N_2O (aN_2O_{soil}) were compared to the ^{15}N abundance in soil NH_4^+ (aNH_4^+) and NO_3^- (aNO_3^-). If soil derived N_2O was emitted into an enclosure initially free of N_2O, f_N and f_D could be calculated from the mass balance using Equation 6-5.

$$f_N = 1 - f_D = \frac{aN_2O_{soil} - aNO_3}{aNH_4 - aNO_3} \qquad \textbf{Equation 6-5}$$

As our gas flux contained background N_2O, the ^{15}N abundance in the soil derived N_2O (aN_2O_{soil}) had to be corrected for background N_2O using with Equation 6-6.

$$aN_2O_{soil} = \frac{aN_2O_{sample} \cdot cN_2O_{sample} - aN_2O_{bgd} \cdot cN_2O_{bgd}}{cN_2O_{sample} - cN_2O_{bgd}} \qquad \textbf{Equation 6-6}$$

With the application of Equation 6-5, one assumes that the measured ^{15}N abundance in the extracted NO_3^- represents the ^{15}N abundance in the N pool undergoing denitrification.

The impact of isotope fractionation during N_2O formation on the estimation of f_N and f_D is assumed to be negligible, since the NO_3^- pool was always highly enriched compared to background N (2–5 at%^{15}N measured in NO_3^-). Initial and final ^{15}N abundance in NH_4^+ was always at natural abundance (Appendix, Table A 8) and recycling of immobilized NO_3^- thus negligible (Mathieu et al. 2007).

6.3.5.5. ^{15}N tracing approach based on non-equilibrium distribution of N_2O isotopologues

In addition to Equation 6-5, we used the non-equilibrium approach to calculate the ^{15}N enrichment of the N_2O producing NO_3^- pool (a_2) as well as the fraction of pool-derived N_2O (Bergsma et al. 2001; Spott et al. 2006). This procedure is based on the assumption that within N_2O from a single source of a given ^{15}N abundance, the N_2O isotopologues of distinct number of ^{15}N substitutions ($^{14}N^{14}NO$, [$^{14}N^{15}NO$ + $^{15}N^{14}NO$] and $^{15}N^{15}NO$) follow a binomial distribution. When N_2O from different pools with different ^{15}N abundance is mixed, the distribution deviates from the binomial. Given the ^{15}N abundance in one of the pools (here background, a_1) and in the resulting mixture (a_m), the ^{15}N abundance in the second pool (a_2) and the contribution of N_2O originating from both pools (f_D and f_N) can be calculated. In our experiment, the ^{15}N abundance in the background air and the N_2O derived from NH_4^+ were assumed identical (i.e., with negligible deviation from natural abundance) and they were thus treated as one pool. Hence, the ^{15}N abundance in the NO_3^- from which N_2O was produced could be calculated (Spott et al. 2006) using Equation 6-7 and Equation 6-8.

$$a_2 = \frac{\alpha_m - a_1 * a_m}{a_m - a_1}$$ **Equation 6-7**

With

$$\alpha_m = \frac{^{30}R}{^{28}R + ^{29}R + ^{30}R}$$ **Equation 6-8**

To use the previous equation for N_2O isotopologues differing in the number of ^{15}N substitution ($^{14}N^{14}NO$, $^{14}N^{15}NO$+$^{15}N^{14}NO$; $^{15}N^{15}NO$) isotope ratios representing intact N_2O molecules (^{45}R = ($^{14}N^{15}N^{16}O$+$^{15}N^{14}N^{16}O$+$^{14}N^{14}N^{17}O$)/$^{14}N^{14}N^{16}O$; ^{46}R = ($^{15}N^{15}N^{16}O$+$^{14}N^{14}N^{18}O$)/$^{14}N^{14}N^{16}O$)) must be converted to respective ratios excluding the oxygen of N_2O using Equation 6-9 and Equation 6-10, with the assumptions that ^{18}R = 0.0020052 and R^{17} = 0.0073 (Bergsma et al., 2001):

$$^{30}R = {}^{46}R - {}^{17}R \cdot {}^{29}R - {}^{18}R$$ **Equation 6-9**

$$^{29}R = {}^{45}R - {}^{17}R$$ **Equation 6-10**

The fraction of NO_3^--derived N_2O to total N_2O (f^*_{NO3}) in a sample was then calculated with

$$f^*_{NO3} = \frac{a_m - a_1}{a_2 - a_1}$$ **Equation 6-11**

and the fraction of NO_3^--pool derived N_2O (i.e. denitrification derived) to soil-derived N_2O with

$$f_D = \frac{f^*_{NO3}}{f_{soil}}$$ **Equation 6-12**

where f_{soil} is calculated from the difference in N_2O concentration between sample and background air

$$f_{soil} = \frac{c_{sample} - c_{background}}{c_{sample}}$$ **Equation 6-13**

In contrast to the conventional ^{15}N tracing approach (b) that neglects the non-random distribution of N_2O isotopologues (3.6.3), the non-equilibrium approach (c) directly determines the ^{15}N enrichment of the labeled N pool that is instantaneously undergoing denitrification. Both approaches must yield identical results in case of perfect pool homogeneity.

6.3.5.6. Isotopomer approach using ^{15}N site preference and $\delta^{18}O$ of N_2O

To estimate the fraction of N_2O derived from the NH_2OH-N_2O pathway of nitrification (f_{NH2OH}), we analyzed SP and $\delta^{18}O$ values of gas samples and used an isotopomer mixing approach similar to, e.g., Zou et al. (2014) but with $\delta^{18}O$ instead of $\delta^{15}N$ as suggested earlier (Well et al. 2012). Input data, i.e. $\delta^{18}O$ and SP in soil-derived N_2O, were calculated analogously to ^{15}N abundance with Equation 6-6. The isotopomer map of SP vs. $\delta^{18}O$ (Figure 6-1) shows the calculation of f_{NH2OH} and f_D with this approach. Endmember areas are given for bacterial denitrification and nitrification, and mixing lines represent values for N_2O which would result from varying contributions of the two processes. The mixing lines were calculated from ranges reported for SP and $\delta^{18}O$ of bacterial denitrification (including nitrifier denitrification) and nitrification (hydroxylamine oxidation),

respectively. The values characteristic for soil incubation not influenced by N_2O reduction were selected (bacterial denitrification: SP_D -10 to 0 (Sutka et al. 2006; Toyoda et al. 2005); $\delta^{18}O_D$: +10 to +20 (Lewicka-Szczebak et al. 2014; Snider et al. 2013); bacterial nitrification: SP_N: +33 to +37, $\delta^{18}O_N$ +40 to +50 (Heil et al. 2014; Sutka et al. 2006)). Additionally, a mixing line from average endmember values was calculated (mean mixing line). For $\delta^{18}O$ endmember values we used the range suggested by Köster et al. (2015), which excluded extreme values from pure cultures that are not considered to be representative for soil emitted N_2O, as they showed more variable and lower O-exchange with water compared to soil incubations (Köster et al. 2015).

The maximum difference in f_N calculated for individual sample resulted from using minimum and maximum endmember values, respectively (mixing lines shown in Figure 6-1). To account for N_2O reduction to N_2, a reduction line was calculated, using the average of reported reduction slopes (0.35; (Jinuntuya-Nortman et al. 2008; Lewicka-Szczebak et al. 2015; Ostrom et al. 2007; Well & Flessa 2009) and SP and $\delta^{18}O$ values of N_2O of each sample as origin of the reduction line. The point of interception between the sample-specific reduction line and the mixing line gave the estimated initial isotope values (SP^*, $\delta^{18}O^*$) of produced N_2O before reduction. If SP* was higher than the measured SP value of the sample, the measured value was used, since N_2O reduction was assumed to be negligible. The fraction of nitrification-derived N_2O to total N_2O produced (f_{NH2OH} in this case) was then calculated from SP values (or SP*) and SP values of nitrification and denitrification as endmembers (Equation 6-14). This calculation was done for maximum, minimum and mean mixing lines, respectively.

$$f_{NH2OH} = 1 - f_D = \frac{SP - SP_D}{SP_N - SP_D} \qquad \textbf{Equation 6-14}$$

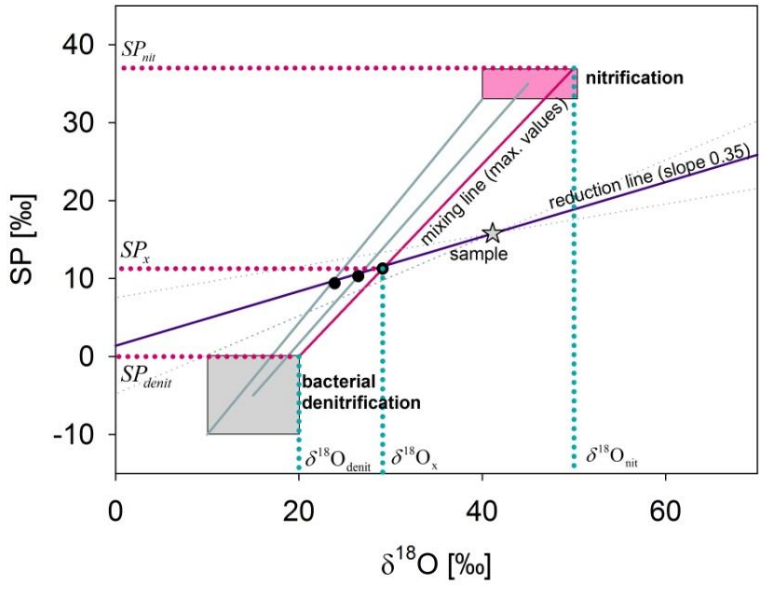

Figure 6-1: Isotopomer map showing the estimation of f_N from SP and $\delta^{18}O$ in N_2O. Top and bottom boxes indicate the expected ranges for bacterial denitrification (values and references see text in section 6.3.5.6) and nitrification. Mixing lines were drawn between minimum and maximum values for both SP and $\delta^{18}O$ of the respective processes, and the reduction line was then placed through a (in this scheme fictional) sample value.

6.3.5.7. Fertilization induced fluxes

As all treatments received the same amount of NO_3^-, difference between unfertilized and NH_4^+ - fertilized treatments is another method to calculate the amount of N_2O produced from added NH_4^+. Besides N_2O directly produced during nitrification, also coupled nitrification-denitrification is included in the amount attributed to NH_4^+ additions. The approach is based on the assumption of increasing N_2O emission with NH_4^+ additions, which does not hold true when N_2O production is inhibited at increasing NH_4^+ content in soil and has to be kept in mind.

6.3.6. N_2O yield from nitrification

The N_2O yield of nitrification was calculated from the total N_2O flux, the ratio of nitrification-derived N_2O determined by the C_2H_2 inhibition approach and the gross nitrification rate (n_g) of the respective N level. For other methods of source partitioning, values were not available for all dates. As f_N was measureable with these approaches mainly at days with high N_2O emission but the gross nitrification is an average over the incubation period, N_2O yields would be biased. However, as nitrification rates are the same irrespective to the source partitioning approach, the N_2O yield would differ only according to differences in f_N.

$$N_2O\ yield = \frac{f_N \cdot N_2Oflux}{n_g}$$ **Equation 6-15**

6.3.7. Statistics

Statistical analyses were performed with the software R (version 3.0.2, R Core Team 2013). To tests for differences in concentrations and (cumulative) emissions between treatments, analysis of variance (ANOVA) was performed, followed by pairwise comparisons between groups (t-tests) with adjustments correcting for multiple testing. Therefore, the *fdr* method was used (Benjamini & Hochberg 1995; Benjamini & Yekutieli 2001). Effects were considered significant if $p < 0.05$. Uncertainty values given represent one standard deviation for measured parameters, and standard errors calculated using Gauss's error propagation for calculated values.

6.4. Results

6.4.1. Nitrification

Soil NO_3^- content was similar at all N levels and batches before incubation. In the C2H2 batch, NO_3^- content did not increase during the incubation at 0N, 450N and 5000N levels, and was only slightly increased in soil cores of 1000N and 2250N (Table 6-2).

In both 15N and 14N batches, NO_3^- content increased at all but the 5000N levels during the incubation. Highest net nitrification occurred at the 450N level, followed by the 1000N level. At 2250N and 0N levels, the increase in NO_3^- content was small (Table 6-2). Soil NO_3^- contents in the additional cores of the 15N batch sampled at day 10 showed that nitrification was faster in the first half of the experiment (Table 6-2 and Table 6-3).

Gross nitrification was in the same range as net nitrification and highest in the 450N level of 14N and 15N batches as well. With increasing initial NH_4^+ content, gross nitrification also decreased, and nitrification was faster in the first than the last 10 days of incubation (Table 6-3).

Table 6-2: NO_3^- concentrations in soil before and after incubation. The given values represent mean and standard deviation per treatment. For day 10, separate soil cores in the 15N batch were used for soil analysis and gas sampling. Concentrations were tested for significant differences between batches and N levels within sampling sampling days. Treatments with the same letters within time of sampling are not significantly different (i.e. $p > 0.05$). Stars indicate significant increase in NO_3^- content compared to the preceding sampling, the star in brackets (*) denotes statistical difference between the terminal (day 21) and initial (day 0) sampling only. (* $p<0.05$;***: $p<0.001$, p-values adjusted for multiple comparison). The single value for 14N batch, day 0 is the expected value calculated from initial NO_3^- content of soil plus added NO_3^- from KNO_3, as samples were mixed up during preparation.

N level	NO_3^- content in soil						
	mg NO_3-N (kg soil)$^{-1}$ 15N batch			mg NO_3-N (kg soil)$^{-1}$ 14N batch		mg NO_3-N (kg soil)$^{-1}$ C2H2 batch	
	day 0	day 10	day 21	day 0	day 21	day 0	day 21
0N	27.6 ± 1.4 bc	34 ± 3 ab	36 ± 3 bcd	26+	32 ± 1 ace	27.9 ± 0.8 bc	27 ± 1 ac
450N	24 ± 2 b	83 ± 15 d***	134 ± 14 g ***	28 ± 7 bc	151 ± 5 h ***	32 ± 1 c	31 ± 2 acd
1000N	22 ± 2 ab	54 ± 9 c ***	76 ± 13 f ***	26 ± 6 bc	77 ± 1 f ***	= 14N	37 ± 6 cd
2250N	23 ± 4 ab	38 ± 2 b	40 ± 7 de (*)	28 ± 1 bc	44 ± 5 d *	= 14N	36 ± 12 acd
5000N	23 ± 2 ab	26 ± 1 a	23 ± 1 ab	27 ± 3 bc	23 ± 2 a	= 14N	27 ± 2 ac

Table 6-3: Nitrification rates in different batches. For 14N and C2H2 batches, only net nitrification rates could be calculated. For the 15N batch, gross nitrification is given for the whole incubation period (days 0-21) and for the periods between days 0 and 10, and days 10 and 21, respectively. Values are mean ± standard error of gross rates (n=5 for day 0 and n=4*3 for days 10 and 21); for the period between day 0 and 10, errors were calculated from Gauss' error propagation.

N level	net nitrification			gross nitrification		
	mg N (kg soil)$^{-1}$ d^{-1}			mg N (kg soil)$^{-1}$ d^{-1}		
	15N batch	14N batch	C2H2 batch	15N batch		
		days 0-21		days 0-21	days 0-10	days 10-21
0N	0.39 ± 0.11	0.18 ± 0.03	- 0.05 ± 0.02	0.29 ± 0.04	0.37 ± 0.06	0.24 ± 0.07
450N	5.12 ± 0.36	5.74 ± 0.24	- 0.06 ± 0.07	4.96 ± 0.51	4.87 ± 0.48	5.99 ± 1.29
1000N	2.51 ± 0.33	2.36 ± 0.04	0.52 ± 0.27	1.96 ± 0.13	2.30 ± 0.26	1.85 ± 0.14
2250N	0.80 ± 0.19	0.75 ± 0.07	0.38 ± 0.45	0.82 ± 0.06	1.15 ± 0.18	0.64 ± 0.08
5000N	- 0.01 ± 0.00	- 0.21 ± 0.03	- 0.03 ± 0.04	0.10 ± 0.01	0.17 ± 0.08	0.06 ± 0.02

6.4.2. pH ($CaCl_2$)

Values of pH measured in $CaCl_2$ solution are given in Table 6-4. They were determined from soil samples of each N level before filling of incubation vessels and from each core at day 21. Acidification of soil was significant in 450N and 1000N levels of both 15N and 14N batches. A slight increase in pH was measured at the 5000N level. No change in pH occurred in the C2H2 batch.

Table 6-4: Values of pH, mean and standard deviation (n=4 per N level and batch).

N level	pH		pH		
	day 0	day 21	day 0	day 21	
	15N batch		14N+C2H2 batches	14N batch	C2H2 batch
0N	6.4 ± 0.0	6.2 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.1
450N	6.2 ± 0.0	5.3 ± 0.1***	6.2 ± 0.0	5.2 ± 0.1***	6.2 ± 0.0
1000N	6.3 ± 0.0	5.9 ± 0.0***	6.3 ± 0.0	6.0 ± 0.1***	6.4 ± 0.0
2250N	6.4 ± 0.0	6.5 ± 0.4***	6.5 ± 0.2	6.4 ± 0.0***	6.5 ± 0.0
5000N	6.4 ± 0.0	6.7 ± 0.2***	6.4 ± 0.1	6.8 ± 0.3***	6.6 ± 0.0

6.4.3. N_2O fluxes

6.4.3.1. Flux dynamics

Fluxes of N_2O from soil cores of 0N were always below 40 ng N_2O-N kg^{-1} h^{-1} during the first two weeks and increased to 111 and 163 ng N_2O-N kg^{-1} h^{-1} in single soil cores of non-acetylene addition batches (14N and 15N) in the third week. Lowest fluxes occurred in the 5000N level in all batches; fluxes in 2250N were slightly (but insignificantly when cumulated) higher. Temporal dynamics were also similar in 14N and 15N batches, with high fluxes at days 2 and 3, lower fluxes at days 6-14 and higher emission again in the third week of incubations (Figure 6-2). The initial peak was missing in most soil cores of the C2H2 batch and single soil cores of 0N or 5000N levels of 14N and 15N batches.

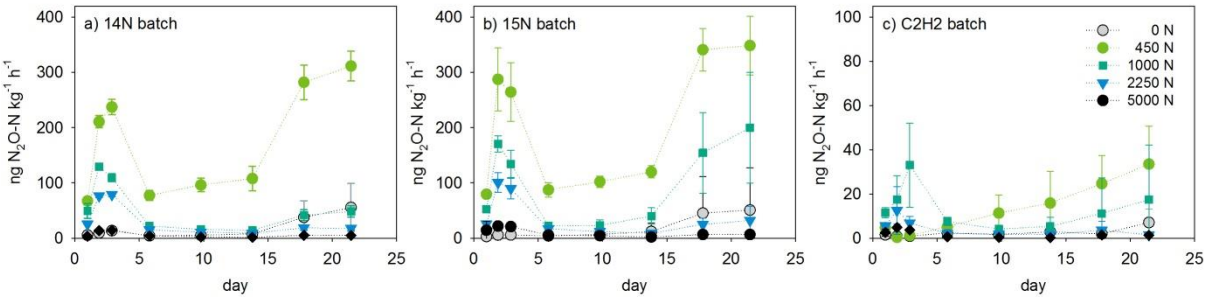

Figure 6-2: N_2O fluxes at different sampling dates in a) 14N batch with addition of unlabeled NO_3^-, b) 15N batch with addition of 12.5at%^{15}N labeled NO_3^-, and c) C2H2 batch with unlabeled NO_3^- addition and 0.01vol% acetylene in the headspace gas. Colors and symbols denote different N levels (see legend in c). Error bars show one standard deviation (n=4). Please note the different scale of the y-axis in c).

6.4.3.2. Total cumulative fluxes

Cumulated fluxes of N_2O are shown in Table 6-5. Highest N_2O fluxes were measured in the 450N level of both the unlabeled (14N) and ^{15}N-labeled (15N) batches. N_2O fluxes were low and not statistically different between N levels in the C2H2 batch. Cumulated fluxes within N levels of the 14N and 15N batches were comparable except for the 1000N level where fluxes of the 14N batch were significantly lower than those of the 15N batch. This difference was mainly due to higher fluxes in 15N soil cores in the third week of incubation (see also Figure 6-2).

Table 6-5: Cumulated N_2O fluxes from soil cores over the 21 days incubation period. Values given are means ± standard deviation **(n=4)**. N_2O fluxes are not significantly different (p>0.05) if they share the same letter.

N level	µg N_2O-N kg^{-1}		
	15N batch	14N batch	C2H2 batch
0N	9.0 ± 12.3 ab	8.6 ± 5.6 ab	1.3 ± 1.0 a
450N	98.1 ± 12.8 d	83.9 ± 9.1 d	7.2 ± 4.1 ab
1000N	43.1 ± 13.2 c	20.4 ± 2.4 b	5.7 ± 3.7 ab
2250N	14.6 ± 2.6 ab	12.0 ± 1.2 ab	1.7 ± 1.1 a
5000N	4.0 ± 1.0 ab	2.5 ± 0.1 a	0.7 ± 0.1 a

6.4.3.3. CH_4 uptake and emission

Whereas in soil cores of the 0N level without C_2H_2 addition 8 µg CH_4-C kg^{-1} (15N) and 22 µg CH_4-C kg^{-1} (14N) were oxidized throughout the incubation, all other treatments showed either low CH_4 uptake (at most –3 µg CH_4-C kg^{-1}) or low emission (up to 0.8 µg CH_4-C kg^{-1}). The inhibiting effect of both C_2H_2 and high NH_4^+ concentration on oxygenases (AMO and MMO) can also be shown by CH_4 fluxes (Figure 6-3).

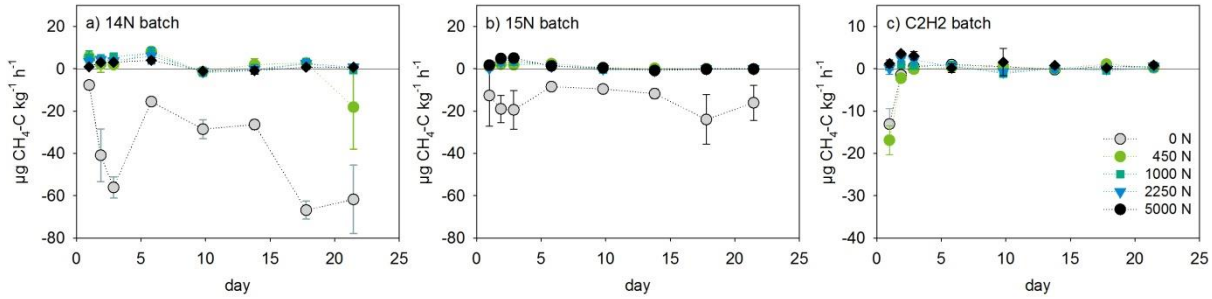

Figure 6-3: CH_4 fluxes at different sampling dates in a) 14N batch with additions of unlabeled NO_3^-, b) 15N batch with addition of 12.5 at%^{15}N labeled NO_3^-, and c) C2H2 batch with unlabeled NO_3^- addition and 0.01vol% acetylene in the headspace gas. Colors and symbols denote different N levels (see legend in c). Error bars show one standard deviation (n=4). Please note the different scale of the y-axis in c).

6.4.4. Source partitioning

The fractions of N_2O attributed to nitrification with the different approaches applied are shown in Table 6-6. In the appendix, results for f_N are given for each single measurement day (Table A 9). N_2O production in the C2H2 batch was less than 30% of N_2O from other batches; the C_2H_2 inhibition approach (a) thus implies that the majority of the produced N_2O originated from autotrophic nitrification (more than 70% from all treatments).

The ^{15}N tracer approach using at%^{15}N in extracted bulk NO_3^- (b) indicated between 44% (1000N) and 79% (0N) of N_2O fluxes to originate from the NO_3^- pool and thus only a small contribution of NH_4^+ oxidation in 0N and approx. 32% - 56% at N levels with NH_4^+ addition (Table 6-6).

The fraction of soil-derived N_2O in samples (f_{soil}) was between 0.03 and 0.89. For calculations with the non-equilibrium approach, values with $f_{soil} < 0.55$ were discarded. This was necessary because by calculation of ^{30}R from Equation 6-8 constant $\delta^{18}O$ values are assumed. Since the major part of

^{46}R was from $^{14}N^{14}N^{18}O$ in most samples, and a minor only from $^{15}N^{15}N^{16}$, uncertainty in a_2 resulted from variability in $\delta^{18}O$ of the produced N_2O. As ^{18}R calculated from soil derived N_2O ranged from 0.002025 to 0.002126 in samples with $f_{soil} > 0.55$, the assumption of constant $\delta^{18}O$ could have lead to an overestimation of up to 0.6 at%^{15}N for a_2. The inaccuracy in a_2 increased at lower f_{soil} and reached up to 2 at%^{15}N when $f_{soil} < 0.1$. The calculated ^{15}N abundance of the NO_3^- pool producing N_2O (a_2) was between 6.6 and 15 at%^{15}N, with most values below the ^{15}N abundance of added NO_3^- (12.5 at%^{15}N). The few higher values are attributed to the uncertainty in $\delta^{18}O$ of N_2O described above. Application of the non-equilibrium approach resulted in f_N of 0.90±0.17 in 450N over the whole incubation period, with decreasing contribution of NO_3^- over time (from 0.33% ± 0.08% at day 2 to 5% ± 11% at day 21; Table A 9 in the appendix). For 1000N and 2250N, f_{soil} was high enough for calculation of f_N only at days 2, 3, and 21 (1000N only), and f_N did not substantially differ from values at 450N if compared for the same day of measurements.

Values of $\delta^{18}O$ and SP in soil derived N_2O are shown in Figure 6-4. Values of $\delta^{18}O$ were highest for samples of the 0N level. The shift to substantially higher $\delta^{18}O$ in 0N samples cannot be explained with mixing of nitrification and denitrification derived N_2O, and is considered to be indication of N_2O reduction. Such a shift has only been observed in samples of the 0N level, where no NH_4^+ was added. Depending on the endmember signatures used for calculations, f_{NH2OH} varied between 44 and 96%. Highest SP values were measured in samples of the 450N level, but all samples (except from 0N) were close together (Figure 6-4). Using mean values for SP and $\delta^{18}O$ of nitrification and denitrifier denitrification, 77% ± 15% of N_2O were produced from NH_2OH oxidation at the 450N level. At day 21, when samples of 0N and 1000N could be compared to 450N, f_{NH2OH} was 0.51 ± 0.14 in 0N, 0.77 ± 0.06 in 450N and 0.60 ± 0.10 in 1000N, respectively, indicating slightly lower contribution of nitrification (NH_2OH) to N_2O production in the 0N and 1000N levels (Appendix, Table A 9).

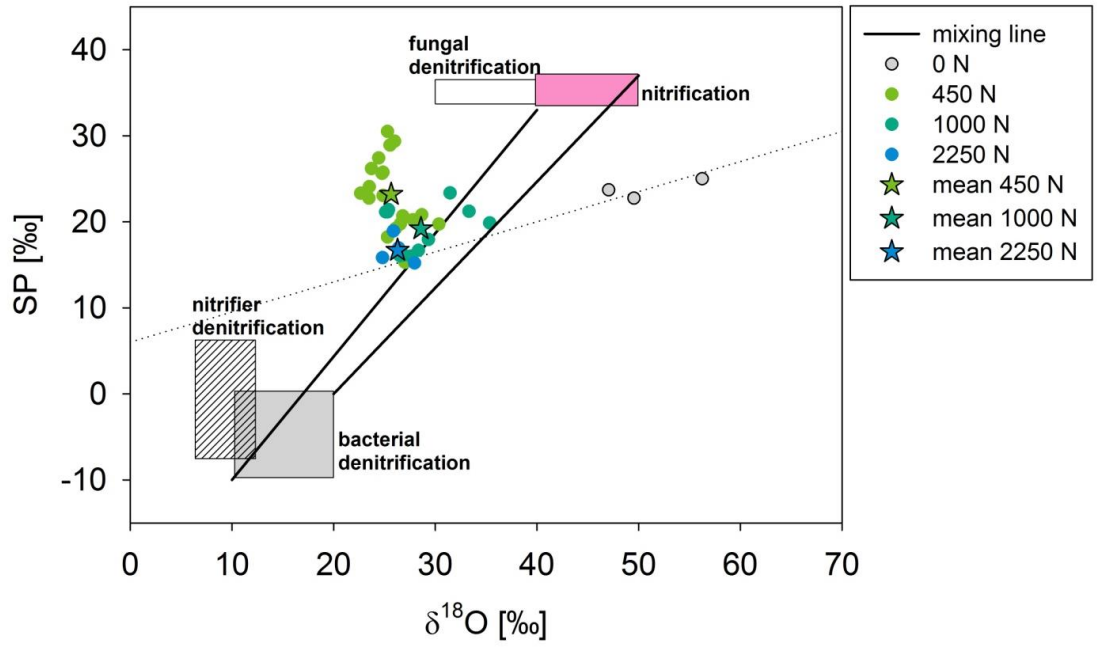

Figure 6-4: Site preference (SP) and $\delta^{18}O$ in N_2O produced at different N levels. Stars denote average values per N level. Concentration of N_2O was too low to derive $\delta^{18}O$ and SP in samples from the 5000N level.

6.4.5. N_2O yield of nitrification

The ratio of N_2O from nitrification (calculated from C_2H_2 inhibition) and NO_3^- production from nitrification (N_2O yield = $N_2O_{nit}/NO_3^-{}_{nit}$) was between 0.07% and 0.15%. In 0N and 5000N levels, N_2O yields were highly uncertain, as both N_2O production and nitrification were low. They were slightly higher compared to the other N levels, but the difference was small. No trend in N_2O yield was observed between 450N and 2250N (Table 6-7).

Table 6-6: Contribution of nitrification to N_2O fluxes (f_N) derived from the different methods applied. Concentration was too small in samples from 5000N to measure isotopomers of N_2O with sufficient precision. The number of individual samples (n) used to calculate means and standard errors, and the days they were taken are given above the respective values. SPmean, SPmin and SPmax denote the respective mixing lines in Figure 6-1 that were used for calculation. The target processes of the respective methods are given in brackets below the method (NN=autotrophic nitrification, ND=nitrifier denitrification, CND=coupled nitrification denitrification)

Method		fraction of nitrification derived N_2O(f_N)				
		0N	**450N**	**1000N**	**2250N**	**5000N**
(a)	**Acetylene inhibition** (NN, ND, CND)	n=32 all days	n=32 all days	n=32 all days	n=32 all days	n=32 all days
	14N batch	0.86 ± 0.90	0.93 ± 0.09	0.87 ± 0.21	0.88 ± 0.13	0.83 ± 0.16
	15N batch	0.85 ± 0.44	0.91 ± 0.08	0.72 ± 0.12	0.86 ± 0.08	0.73 ± 0.04
(b)	**^{15}N tracer approach based on extracted bulk NO_3^-** (NN, ND)	n=3 day 21	n=23 days 2,3,14,18,21	n=18 days 2,3,6,14,21	n=18 days 2,3,6,14,21	n=8 days 2,3
	15N batch	0.21 ± 0.20	0.53 ± 0.10	0.32 ± 0.08	0.56 ± 0.15	0.54 ± 0.10
(c)	**^{15}N tracer non-equilibrium approach** (NN+ND(+CND))	n=3 days 21	n=23 days 2,3,6,14,18,21	n=12 days 2,3,21	n=7 days 3	none
	15N batch	0.71 ± 0.72	0.90 ± 0.17	0.82 ± 0.17	0.70 ± 0.14	-
(d)	**Isotopomer approach** (NN)	n=3 day 21	n=19 days 2,3,14,18,21	n=11 days 2,3,21	n=4 day 3	none
	SP_{mean} 14N batch	0.71 ± 0.19	0.77 ± 0.15	0.58 ± 0.12	0.54 ± 0.02	-
	SP_{min} 14N batch	0.79 ± 0.21	0.81 ± 0.17	0.65 ± 0.12	0.63 ± 0.03	-
	SP_{max} 14N batch	0.63 ± 0.17	0.70 ± 0.15	0.50 ± 0.11	0.45 ± 0.02	
(e)	**Difference approach** (NH_4^+ induced N_2O)	not applicable	n=32 all days	n=32 all days	n=32 all days	n=32 all days
	14N batch	-	0	0.90	0.58	0.29
	15N batch	-	0	0.90	0.79	0.38
	C2H2 batch	-	0	0.82	0.77	0.25

Table 6-7: N_2O yield from nitrification calculated from f_N of the C_2H_2 inhibition approach.

	N_2O yield of nitrification $gN_2O\ (NO_3^-\text{-}N)^{-1}$ *100				
	0N	**450N**	**1000N**	**2250N**	**5000N**
15N batch	0.12 ± 0.10	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.15 ± 0.02

6.5. Discussion

6.5.1. Inhibition of nitrification

6.5.1.1. Did inhibition of nitrification take place?

Decreasing net and gross nitrification rates with increasing N level clearly show inhibition of nitrification at NH_4^+ contents higher than 450 mg N kg^{-1} d.w. soil, with concurrent inhibition of N_2O production (Table 6-3 and Figure 6-2). At the highest (5000N) level, the NO_3^- content even decreased during the incubation, and gross nitrification was negligible. This pattern is in accordance with earlier studies on inhibition of nitrification at high NH_4^+ concentrations (Harada & Kai 1968; Wetselaar et al. 1972). However, some N_2O was still emitted at the 5000 N level. As N_2O production was further reduced with the addition of C_2H_2, the majority of this small production was attributed to nitrification (Table 6-5 and Table 6-6). Besides incomplete inhibition from high salt levels, one possible explanation for these small fluxes might be the existence of microsites within the soil matrix that had not been fully reached by the added NH_4^+, possibly due to low diffusivity of NH_4^+ in the relatively dry soil.

The inhibiting effect of high NH_4^+ concentration is also reflected in the change of pH values in soil, with acidification in the treatments with substantial nitrification rates but only small changes in 0N, 2250N and 5000N levels. The inhibition of CH_4 oxidation in all treatments that received NH_4^+ fertilizer showed the even higher sensitivity of CH_4 oxidation to factors inhibiting nitrification (either high NH_4^+ content specifically, or via salinity). Inhibition of CH_4 oxidation at the 0N level in the C2H2 batch furthermore emphasizes the proper functioning of inhibition by C_2H_2 addition.

6.5.1.2. Gross nitrification rates

The measured gross nitrification rates were well in the range of rates summarized by Stange and Neue (2009) for agricultural soils. Gross rates determined by isotope pool dilution were very similar to measured net nitrification rates (from change in NO_3^- content in soil) indicating negligible NO_3^- assimilation and denitrification losses. The difference between the first and the second half of incubation (day 0-10 and day 10-21, respectively), with higher rates in the first phase in all but the 450N treatment, shows that rates weren't constant with time. Time courses of N_2O emission support variable nitrification rates, with high initial emission, a subsequent low emission phase and increasing emission again in the third week of incubation (Figure 6-2). Constant rates are, however, a prerequisite for applicability of the equation used, and underestimation of rates is to be expected when this condition is not met (Nason & Myrold 1991). Gross rates were in fact slightly (but insignificantly) lower than net rates in most cases in this experiment. Still, underestimation of gross nitrification would imply that large amounts of NO_3^- were consumed by denitrification or NO_3^- assimilation. Denitrification rates were low in this experiment, and in the absence of plants and the presence of high NH_4^+ concentrations, large NO_3^- assimilation rates seem also implausible (McCarty & Bremner 1992; Rice & Tiedje 1989). At low NH_4^+ contents, however, Burger and Jackson (2003) measured NO_3^- immobilization rates as high as one third of nitrification, which is in the range of error at our 0N level.

6.5.2. Sources and processes of N_2O production

There was a large discrepancy of 40% (450N, 2250N) to > 60% (0N) in the fraction of nitrification derived N_2O between the ^{15}N tracer method using extracted bulk NO_3^- (b) and the C_2H_2 inhibition approach in this study. Estimation of f_{NH2OH} with the isotopomer approach indicated that a lower share of N_2O was derived from nitrification (in this case NH_2OH oxidation) than f_N measured by the C_2H_2 inhibition approach (that includes nitrifier denitrification), but a higher share than the NH_4^+ derived flux calculated with the ^{15}N tracer approach using extracted bulk NO_3^-.

In contrast, a study by Well et al. (2008), which was also conducted under nitrifying conditions, showed good agreement between the C_2H_2 inhibition and a ^{15}N tracer approach. However, they calculated f_N for the second day of incubation only, and at this time the difference between approaches was also small in our incubations. To dissolve the inconsistency, it hast to be kept in mind that f_N from different approaches comprises different sources and processes (Table 6-1). If autotrophic nitrification (NH_2OH - N_2O pathway) and denitrification of homogeneously mixed (initial + fertilizer) NO_3^- were the only processes contributing to N_2O production, all three approaches should have given consistent results. That this is a very strong simplification of actual processes in soil, became clear in numerous studies over the last decade (van Groenigen et al. 2015).

While the two methods using ^{15}N tracer (b and c) should yield similar results if preconditions of the methods are fulfilled, deviations of the C_2H_2 inhibition and the isotopomer approach (a and d) from (b) and (c) are an indication of coexistence of different pathways of N_2O production from NH_4^+. In the following we will first address the difference between the two tracer approaches used, as they showed the highest inconsistencies (6.5.3). Then, different pathways of N_2O production from NH_4^+ will be discussed (6.5.4). Finally, our findings will be converged with respect to the hypotheses of decreasing f_N with time and N level.

6.5.3. NO_3-derived fluxes

The conventional ^{15}N tracer approach (b) using measured ^{15}N abundance in extracted (bulk) NO_3^- (+NO_2^-), indicates a much higher contribution of NO_3^- derived N_2O than the C_2H_2 inhibition approach (a). Results from the ^{15}N non-equilibrium approach (c), in contrast, gave f_N very similar to the C_2H_2 inhibition approach. This comes from the large discrepancy between ^{15}N abundance in NO_3^- from soil extracts (Appendix, Table A 8) and the active N_2O producing NO_3^- pool (a_2) as calculated with the non-equilibrium approach (Bergsma et al. 2001; Spott et al. 2006) (Appendix, Table A 7). The discrepancy even increased during the experiment – while ^{15}N in the extracted bulk NO_3^- pool was diluted by nitrification, a_2 increased over time. At day 2, a_2 was only slightly higher than the ^{15}N abundance in the bulk NO_3^- pool (6.6 ± 0.5 at%^{15}N vs. 5.3 at%^{15}N in 450N level); at day 21 this difference was much higher (10.6 ± 0.9 at%^{15}N vs. 1.3 ± 0.1 at%^{15}N). Consequently, f_N also differed when it was calculated from the non-equilibrium approach.

These results imply two different NO_3^- pools in soil, governed by different processes. The first pool is the added NO_3^- (12.5 at%^{15}N enriched KNO_3) plus at least initially the old NO_3^- contained in soil (natural abundance), the second was built up from nitrification of the unlabeled NH_4^+. There are two, presumably concurrent, conceivable reasons for separate NO_3^- pools in this experiment. First (1), inhomogeneities from tracer application cannot be completely ruled out, despite fine spraying of fertilizer solution (on approx. 1 cm soil layers) and thorough mixing of soil, which was repeated several times per soil until all fertilizer solution was applied. Due to the dryness of the soil before

mixing, the initial distribution of the fertilizer in soil may not have been well mixed, causing an initially low dilution of ^{15}N from the tracer with soil NO_3^- (with inhomogeneity at a small, i.e. mm, scale). At the same time (2), denitrification was favored in non-diluted (fertilizer-NO_3^--rich) domains due to higher water content, while nitrification caused dilution in aerobic domains but not in anaerobic microsites. Initially, i.e. two days after application of the tracer, a_2 was very close to the ^{15}N abundance of NO_3^- expected from mixing of initial and fertilizer NO_3^- (6 at%^{15}N, as unlabeled and 12.5 at%^{15}N labeled NO_3^- mixed in approx. equal amounts). Later, a_2 was more close to the ^{15}N label of the applied tracer NO_3^-. These observations may be explained by the following scenario: Towards the end of incubation, denitrification was restricted to wetter parts with more or less undiluted NO_3^- from fertilizer. In the initial phase, in contrast, there was some denitrification also in drier parts, since labile carbon was possibly mobilized during wetting and favored denitrification due to enhanced O_2 consumption and availability of electron donors (Bergstermann et al. 2011; Ruser et al. 2006). Dilution of added plus soil NO_3^- by NO_3^- from nitrification of the unlabeled NH_4^+, which is shown by the ^{15}N abundance in extracted NO_3^- at the end of incubations, on the other hand, occurred only in aerobic domains, as otherwise it should have mixed with the denitrifying pool.

Both causes of inhomogeneity (1 and 2) lead to failure of the ^{15}N tracing based on extracted bulk $^{15}NO_3^-$ to quantify NO_3^- -derived N_2O. Hence, only the estimates of NO_3^- -derived N_2O based on non-equilibrium approach (c) are considered valid. While the values for f_N derived from this approach seem plausible, they were measurable only for a confined number of samples, as precision was low at a low ratio of soil-derived to background N_2O fluxes. Furthermore it has to be mentioned that the non-equilibrium approach leads to overestimation of a_2 and underestimation of f_D if different labeled NO_3^- pools contribute to N_2O production (Boast et al. 1988). However, in the case of a homogenous background, the underestimation due to multiple N_2O sources should not exceed 25% of the f_D value (Arah 1992), and thus not substantially affect our results of very low contribution of denitrification to total N_2O production.

Distinct pools of NO_2^-, separated into an added NO_2^- pool and pools produced from NH_4^+ and NO_3^-, respectively, have been shown by Russow et al. (2009). They furthermore concluded that this rendered the application of the pool dilution method for determination of gross production rates problematic. Besides constant rates, which were addressed in section 6.5.1.2, another assumption for the calculation of gross nitrification is homogeneous distribution and equal turnover of tracer and background NO_3^-, i.e. one homogenous NO_3^- pool (Davidson et al. 1991; Herrmann et al. 2007; Murphy et al. 2003). From the calculation of ^{15}N abundance in the denitrifying pool with the non-equilibrium approach, we deduced that at least parts of the labeled pool underwent a different process than the non-labeled native pool, and thus this assumption is not met. Preferential use of the non-labeled/newly produced NO_3^- pool for nitrification, and preferential consumption of the old/labeled pool, would result in overestimation of gross nitrification. For application of the pool dilution method (using ^{15}N labeled NH_4^+) to calculate mineralization rates, Davidson et al. (1991) estimated errors of approx. 10% if ^{15}N tracer was supplied to less than 70% of the mineralization micro-sites, with errors further increasing if the bias in ^{15}N distribution corresponded with a gradient in N transformation rates. The error in gross nitrification should have been analogous. As gross nitrification determined in this study was lower than net nitrification, this overestimation can hardly be large. Furthermore, anaerobic microsites, where undiluted fertilizer solution was dominant, were probably small and comprised only a tiny fraction of the total soil volume (Parkin 1987). Therefore, the inhomogeneity shown by the comparison between tracer methods is not supposed to significantly bias the pool dilution approach used to estimates gross nitrification.

6.5.4. Processes of NH_4-derived N_2O

Irrespective of the method used, the majority of N_2O emission stemmed from NH_4^+ oxidation processes in all our treatments. Given the high uncertainties of the isotopomer approach, significant distinction of f_{NH2OH} estimates from f_N of the other approaches is not possible. Still, the results indicate a much higher contribution of denitrification than the ^{15}N tracer (non-equilibrium) and the C_2H_2 inhibition approach. An explanation for this difference may be the contribution of nitrifier denitrification to N_2O production. The addition of C_2H_2 inhibits the formation of NH_2OH, NO_2^- and NO_3^- concurrently to inhibition of N_2O production and the NO_2^- reduced during nitrifier denitrification is derived from unlabeled NH_4^+ but not from labeled NO_3^-. The N_2O produced during nitrifier denitrification is thus attributed to nitrification (f_N) in both the C_2H_2 and the ^{15}N tracer approaches.

When calculating f_{NH2OH} from the isotopomer approach using SP and $\delta^{18}O$ in the produced N_2O, we also assumed nitrification (NH_2OH - N_2O pathway) and denitrifier denitrification to be the only relevant processes. Both SP and $\delta^{18}O$ of other N_2O yielding processes, however, showed complete or partial overlap with the used ranges. As enzymes and reactions of nitrifier denitrification in NH_4^+ oxidizers are similar or identical to those of denitrifier denitrification, the produced N_2O results in similar SP. Therefore, nitrifier denitrification is comprised in f_D with the isotopomer approach.

Neglecting the high uncertainty of f_N values from the isotopomer approach (d), the contribution of nitrifier denitrification (f_{ND}) could be derived from the difference between f_{NH2OH} and f_N of the C_2H_2 approach ($f_{ND} = f_N - f_{NH2OH}$) and would amount to 10% - 40% at single days and treatments, and to approx. 14% of total N_2O in the 450N, that was measurable at most dates. This proportion would include also coupled nitrification denitrification, as this would also be inhibited due to a lack of NO_3^- production under C_2H_2 inhibition.

The values of $\delta^{18}O$ used for nitrification were partly derived from abiotic N_2O production from NH_2OH, and were higher than in N_2O produced during NH_2OH oxidation in pure culture studies (Heil et al. 2014; Sutka et al. 2006). There is only limited information for $\delta^{18}O$ values specific for N_2O produced from NO_2^- in nitrifiers (i.e. nitrifier denitrification), but these reported values are also at the lower end of values assumed for denitrification (8.8 ± 1.4 ‰ and 10.8 ± 1.4 ‰, (Sutka et al. 2006; Sutka et al. 2004)). Besides the low f_{NH2OH} from the isotopomer approach (SP- $\delta^{18}O$) compared to the C_2H_2 inhibition approach, substantial contribution of nitrifier denitrification could thus also explain the observation of $\delta^{18}O$ values lower than expected from mixing lines between nitrification and denitrification in our samples. These suppositions cannot be validated, though, as our methods did not specifically target nitrifier denitrification independently. The fraction of nitrifier denitrification to total N_2O production calculated is consistent to the literature. It has been shown that nitrifier denitrification can contribute as much as 37-57% to total N_2O production, or 46-71% to NH_4^+-derived N_2O at 50% WFPS in sandy soil incubations (Kool et al. 2011). Under O_2 deficiency (0.5% and 3% O_2), even the majority of NH_4^+-derived N_2O was produced from nitrifier denitrification (Zhu et al. 2013).

The contribution of other processes than autotrophic nitrification, nitrifier denitrification, and bacterial heterotrophic nitrification to N_2O production cannot be excluded. Their occurrence could have affected SP and $\delta^{18}O$ and thus the value of f_{NH2OH} from the isotopomer approach. Fungal denitrification showed similar SP and lower $\delta^{18}O$ values than nitrification (SP_f: +34 to +37, $\delta^{18}O_f$: +30 to +40; (Rohe et al. 2014; Sutka et al. 2008)), and would thus be included in f_{NH2OH} with this approach. In both the tracer approaches (b+c) and the C_2H_2 inhibition approach, fungal denitrification is included in f_D, which was very low. We thus assume fungal denitrification to be

negligible in this experiment. Heterotrophic nitrification is not inhibited by C_2H_2 addition, and the N_2O is also built from unlabeled NH_4^+ or organic N compounds. It is, however, assumed to be negligible under the present conditions, since f_N by ^{15}N tracing would be higher compared to f_N by C_2H_2 inhibition if heterotrophic nitrification was significant (Well et al. 2008).

6.5.5. Impact of N level on N_2O source processes

Our results support the hypothesis of nitrification as the main N_2O source insofar as NH_4^+ derived N_2O dominates total N_2O fluxes throughout the incubation, although the underlying process is not exclusively autotrophic nitrification/NH_2OH oxidation. With respect to the expected changes in source processes with increasing N level, there is no clear result. Comparing different N levels, the C_2H_2 inhibition, the ^{15}N tracer (non-equilibrium) and the isotopomer approaches show slightly higher nitrification-derived proportion of N_2O in the 450N level compared to the 0N and the 1000-5000N levels, although not significantly if averaged over time and not at all sampling times (Appendix, Table A 9). This may partly be caused by the limited applicability of the ^{15}N tracer approach at low N_2O production rates at very low (0N) and high NH_4^+ content, and the observation of heterogeneous distribution and rates of denitrification. The isotopomer approach is less affected by inhomogeneities as the tracer approaches. With decreasing total N_2O fluxes but without concurrent increase in the fraction of denitrification derived N_2O, we presume that also N_2O production during denitrification must be inhibited at the high NH_4^+ content. This assumption is furthermore supported by the differences in N_2O emission between N levels in the C2H2 batch, which indicate other N_2O producing processes besides NH_4^+ oxidation to be inhibited by the high rates of NH_4^+ addition. As NO_3^- was added to all treatments and declined slightly only in the 5000N treatment, NO_3^- limitation could not have caused low denitrification-derived N_2O. High salt levels, which we induced here by adding high rates of NH_4^+-salt, have been shown to affect denitrification (Menyailo et al. 1998; Menyailo et al. 1997), although N_2O reductase was the enzyme that was most effectively inhibited.

6.5.6. Temporal dynamics

Background fluxes in the 0N level without NH_4^+ additions are mainly produced from nitrification, at least at the beginning of incubations. High SP and $\delta^{18}O$ values (above the mixing lines) at the end of incubations, that indicate N_2O reduction, show that denitrification-derived N_2O substantially added to N_2O emission in 0N. The background flux (from 0N) contributed less than 5% at the beginning of incubations but 15% and 18% at day 21 at the 450N level of 15N and 14N batches (Appendix, Table A 10). Increasing N_2O emission with time in the 0N level show that mineralization fueled nitrification, and thus possibly also denitrification. While the initial peak in N_2O emission may be explained by increased mineralization and nitrification (Borken & Matzner 2009; Davidson 1992), as well as denitrification in microsites after wetting (Bergstermann et al. 2011), the increase in the last week of the incubation may be the result of adaptation or growth of nitrifiers.

However, we did not find evidence for increasing contribution of denitrification to total N_2O emission with time. With the C_2H_2 inhibition approach, this question cannot be appropriately addressed, as NO_3^- accumulation, and thus the base for increased contribution of denitrification, was inhibited. The ^{15}N tracer approach (non-equilibrium, c) indicated that newly produced NO_3^- did not contribute to the denitrifying pool, at least did not homogeneously mix with it. The apparent problems with the conventional tracer method (b) also prevent proper conclusion. The isotopomer approach was less affected by inhomogeneities than the tracer approaches. Since the SP and $\delta^{18}O$

values do not distinctly change during the incubation, there is no indication for changing N_2O sources with time (Appendix, Table A 9).

6.5.7. N_2O yield of nitrification

The N_2O/NO_3^- product ratio from nitrification was relatively low compared to the literature, that gives a range of 0.01% –1.8% (Flessa et al. 1996; Goodroad & Keeney 1984b; Well et al. 2008), with higher values (up to 7%) under unfavorable conditions as low O_2 concentrations (Mørkved et al. 2006; Zhu et al. 2013), or low pH (Jiang & Bakken 1999; Mørkved et al. 2007). Accumulation of NO_2^- was proposed as the reason for reduced nitrification and N_2O production under acidic conditions (Subbarao et al. 2006). As high salt contents are correspondingly supposed to inhibit the nitrite oxidase (Harada & Kai 1968; Low et al. 1997), similarly higher N_2O yield from nitrification was expected following increasing NH_4^+-content in soil. Slightly higher N_2O yield in the 5000N level occurred, but was based on very low N_2O emission and highly uncertain despite low variability. As N_2O yield was calculated from gross nitrification rates, the observed problems due to inhomogeneity of tracer distribution affect also these values. As gross rates, if they deviated from out calculated values, would have been underestimated, N_2O yield only could have been overestimated. High N_2O yield due to increasing NH_4^+ concentrations can thus be excluded. The inhibiting effect of NH_4^+ thus seems not to affect one enzymatic process specifically but to act more generally. An NH_4^+ ion specific toxicity as the reason for nitrification inhibition after NH_4^+ addition has earlier been challenged, and osmotic pressure or ionic strength was instead proposed as a reason for inhibition (Darrah et al. 1986; Müller et al. 2006).

6.5.8. Potential for nitrification inhibition by CULTAN

Recalculating N_2O production in this laboratory study to area based emissions is difficult, as the fertilizer depots comprise only small portions of the surface soil. However, the mean N_2O flux at the 0N level was 17ng kg^{-1} h^{-1}; with a surface area of 0.016m^2 and 2.8 kg soil per soil core this would correspond to a flux of 3 µg m^{-2} h^{-1}, which is pretty similar to fluxes from unfertilized plots of the site the soil was taken from (chapter 5.4.3, median = 3.1 µg m^{-2} h^{-1}). Measured values of gross nitrification of 0N and 450N levels in this study are also comparable to other incubation studies from arable land and we thus regard them to meet typical conditions and related N_2O production.

The strength of reduction in N_2O production rates shows that no substantial N_2O emission should be expected from depot centers during the first weeks after fertilizer placement at these high NH_4^+ concentrations, as was expected from results of earlier studies (Wetselaar et al. 1972). The relatively low N_2O yield also at increasing NH_4^+ concentrations further supports the assumption that low rates of N_2O emission are expectable from nitrification of concentrated fertilizer depots. However, the time frame of this experiment was short as compared to field conditions, where plants need to take up nitrogen over a longer time period. In relation to the methods used, on the other hand, the experiment was already quite long, which is shown by inconstant gross nitrification rates. Plant uptake was furthermore not included in this study, but strongly affects N dynamics in or at the margins of fertilizer depots at the field. Due to mass flow in unsaturated soil, with stones and roots affecting the flow path of fertilizer after application, the NH_4^+ applied may be less concentrated, and resemble the lower 2250N or 1000N level even initially, where nitrification is not completely inhibited and NO_3^- accumulation occurred. Our results suggest that emission peaks of N_2O after CULTAN injection are thus likely to be dominated by nitrification under comparably dry soil conditions. However, in view of incomplete inhibition of nitrification, a shift towards

denitrification under wetter conditions can be expected due to the nitrified fertilizer at the margins of depots or after fertilizer dilution.

6.6. Conclusions

The inhibiting effect of high NH_4^+ levels on nitrification and N_2O emission from soil has been confirmed, at N_2O emission rates and nitrification rates comparable to field conditions. The N_2O yield of nitrification was not affected by high NH_4^+ level, which further adds to the expectation of low N_2O emission after fertilizer point injection. However, there was no evidence for a decreasing contribution of nitrification to total N_2O emission with increasing N level or time. If inhibition of nitrification at NH_4^+ level was indeed mainly due to an osmotic effect, as supposed by Darrah et al. (1986) and supported by our results that show also denitrification to be retarded, the inhibition may be weaker under field conditions, where heterogeneous soil conditions affect initial fertilizer distribution in soil, and plant uptake and precipitation may dilute the fertilizer depots.

This incubation study showed that inhomogeneities of ^{15}N tracer distribution in soil due to incomplete initial mixing or heterogeneity of N processes may considerably affect the results of ^{15}N pool-derived fluxes. It was also shown that non-homogeneity can be identified by applying different calculation procedures. But to which extend such an approach may be suitable for the quantification of non-homogeneity and the bias resulting therefrom needs to be further investigated.

7. Synthesis and General Discussion

7.1. The impact of water content on N_2O and CH_4 fluxes, and how annual emissions are affected by summer drought and temporal dynamics of irrigation and precipitation

The water content was a key controlling factor in the summer drought experiment (Chapter 4). Emission of N_2O from soils generally increases with increasing soil moisture (Bateman & Baggs 2005; Dobbie et al. 1999; Maag & Vinther 1996) and a maximum has often been found around 70-90% WFPS or even higher (del Prado et al. 2006; Skiba & Smith 2000). While denitrification proceeds at higher water content, N_2O reduction to N_2 is strongly enhanced due to limited gas diffusivity (Drury et al. 1992). In field studies in different ecosystems, the water content often explained a great part of the variability in N_2O emissions (correlations of 0.27-0.54 between the water content and N_2O emissions, e.g., in temperate deciduous forest (Berger et al. 2013), maize fields (Adviento-Borbe et al. 2007), grassland (Dobbie et al. 1999), and semi-arid wheat fields (Barton et al. 2008)). The correlation between water content and N_2O was less strong in the field experiments on summer drought or CULTAN fertilization presented here (Chapter 5). There were indeed highly significant effects of WFPS on N_2O emissions, but less than 25% of the variance in N_2O fluxes could be explained with the statistical models. Even taking N_{min} (in form of NH_4^+ or NO_3^-) and temperature or microbial activity (in terms of CO_2 fluxes) into account, only 13% (summer drought) or 23% (CULTAN) of N_2O fluxes were explained.

While the lower water content induced by rain exclusion during the growing seasons significantly increased the annual CH_4 uptake, the effect was not correspondingly clear with respect to N_2O emissions. In the first drought period, N_2O emission even tended to be higher from dry than wet treatments. The direct effect of the increased summer drought was negligible in the second drought period, and also on annual base the drought treatment effect on N_2O emission was not significant. Thus we had to conclude that, while the water content significantly affected the temporal dynamics of N_2O emission of both the summer drought and the CULTAN experiments, increased summer drought had only a negligible effect on annual N_2O emissions.

That drought had no effect on N_2O emission has earlier been reported: neither alone nor in combination with artificially increased CO_2 concentration in the atmosphere and/or increased soil temperature did enhanced drought change annual or even seasonal N_2O emissions in extensively managed grassland (Cantarel et al. 2011) or heathland (Carter et al. 2011). CH_4 oxidation, in contrast, showed a clear reaction to increased summer drought in our study, which is in accordance with the effect of drought in forest ecosystems (Borken et al. 2000; Borken et al. 2006), and consistent with the observation of higher CH_4 oxidation rates with decreasing soil moisture (Flessa et al. 1995; Smith et al. 2000).

Considering the generally strong dependence of N_2O emission on the water content in soil and the observation that temporal dynamics of N_2O emission in both field experiments were mainly driven by climatic factors as precipitation (or irrigation) and thawing, the question remained why the summer drought treatment did not lead to more distinct reactions. Lower N_2O fluxes from dry than wet plots in the summer drought study were expected to result from a lower probability for the existence of denitrifying microsites as compared to well-watered plots. Very low water contents in the both wet and dry plots at the beginning of the first drought period (< 20% in 0-10cm in July/August) and low water content while NO_3^- content was high in the second drought period

indicate a predominance of nitrifying conditions in both treatments. At 50% WFPS, nitrification was responsible for the largest proportion of N_2O emission in several studies (Bateman & Baggs 2005; Well et al. 2008), and the high fraction of nitrification-derived to total N_2O emission was supported by the results of the laboratory experiment (Chapter 6), where 90% of N_2O emission were derived from nitrification even though no NH_4^+ but relatively high amounts of NO_3^- were added. In the dry treatments, and in the second drought period also in the wet treatment of the summer drought study, 50% WFPS were seldom exceeded. There was a weak trend to even higher N_2O emission from the dry than well-watered wet treatments in the first drought period, when enhanced N_{min} contents persisted longer at dry than at wet plots where apparently leaching of N to deeper soil layers occurred with precipitation (Figure 4-5). This might be due to relatively dry soil conditions and low N_2O emissions in our well-drained soils. Leaching of NO_3^- with rain events has been proposed as a reason for even negative correlation between water content and N_2O emission (Hellebrand et al. 2008; Kavdir et al. 2008). However, a correlation of 44% - 55% between WFPS and N_2O fluxes was reported by for a field cropped with wheat in semi-arid Western Australia that showed very low annual N_2O emission (0.11 kg N ha^{-1} yr^{-1}).

In winter, on the other hand, high N_2O fluxes occurred mainly from the wet treatments. We attributed this to higher organic matter input due to higher biomass production at wet plots, and to still (although insignificantly) higher water contents in 10-30cm depth, and thus higher propensity to denitrifying conditions at least in the winter period. High organic matter content was also suggested as the reason for higher N_2O peak fluxes at the CULTAN than the surface application plots in the CULTAN experiment. It has been reported that roots formed dense nets around fertilizer depots or bands when urea or ammonium were placed in high concentrations (Passioura & Wetselaar 1972; Sommer 2005). In this way, confined spaces with high N content and high organic matter density may have formed surrounding the CULTAN depots. Denitrification was thus regarded as the process responsible for peak emission from CULTAN plots.

With respect to N_2O dynamics, water content may obviously not be regarded independently. It has earlier been shown that the relationship between N_2O emissions and the soil water content is affected by the availability of N substrates (Kavdir et al. 2008). Laville et al. (2011) report N_2O emission of > 20µg m^{-2} h^{-1} only when WFPS was > 50% and N_{min} > 20 mg kg^{-1}, and also Sehy et al. (2003) and Smith et al. (1998) found a correlation between WFPS and N_2O only if neither NO_3^- content nor temperature were limiting. Consistently, also N_2O emission peaks in the CULTAN experiment occurred mainly when both water and N_{min} were enhanced. This became obvious e.g. with the irrigation peaks in 2012 at surface application and CULTAN plots while no considerable emission occurred from the unfertilized treatment (Figure 5-3) and was also supported by the statistical model applied to the data from the CULTAN field experiment, that showed a strong impact of the interaction between WFPS and NH_4^+ (Chapter 5) on N_2O fluxes.

7.2. How N_2O fluxes are affected by fertilizer injection and the impact of fertilization on annual N_2O emission

There is a well-known and often analyzed relationship between fertilization, which contributes substantially to N input in agricultural systems, and N_2O emission (e.g. Acton & Baggs 2011; Liu & Greaver 2009; Stehfest & Bouwman 2006). The type of fertilizer may have an important impact on N_2O emission. N_2O emission has been shown to be lower from NH_4^+- and NO_3^--based fertilizers than from urea-based or organic fertilizers by Bouwman et al. (2002) in their review summarizing > 800 N_2O emission measurements. However, the relationship is not always straightforward and in individual studies the relative N_2O emission may differ, e.g. according to soil texture or climatic conditions, with higher emission from NO_3^--based fertilizers under wet and from NH_4^+-based fertilizers under dry soil conditions (Lebender et al. 2014; Liu & Greaver 2009). Furthermore, it has been shown that urea, urea-ammonium nitrate and organic fertilizers like poultry litter may lead to higher N_2O emission when they are banded below the surface compared to broadcast surface application or incorporation (Cheng et al. 2002; Engel et al. 2010; e.g. Maharjan & Venterea 2013; Smith et al. 2012). The increase in N_2O emission with urea banding may be explained with alkalization and NO_2^- accumulation (Wetselaar et al. 1972).

Banding of ammonium sulfate or ammonium chloride, on the other hand, has been shown to slow down nitrification (Petersen et al. 2004), and point-injection of fertilizer should lead to even higher concentration of fertilizer in a smaller volume than banding, thereby entailing a still higher potential for inhibition of nitrification. Using NH_4^+ fertilizer instead of urea might have prevented the accumulation of high NO_2^- content, since instead of alkalization from urea hydrolysis NH_4^+ oxidation leads to acidification. Lower accumulation of NO_2^- at low pH might be the reason for the low N_2O/NO_3^- ratio from nitrification. The pH in fertilizer depots of the CULTAN field experiment was indeed by one unit lower than between fertilizer depots and in bulk soil of the surface application treatment (loam: pH 6.5 in depots, pH 7.3 on unfertilized and surface application plots; sandy loam: pH 4.5 in depots and pH 5.5 and 6.0 on surface application plots and unfertilized plots, respectively; data not shown). A similar acidification has also been found in earlier studies (Menge-Hartmann & Schittenhelm 2008; Wetselaar et al. 1972) and in the laboratory experiment, where pH decreased from 6.2 to 5.2 within 3 weeks (Table 6-4).

The laboratory experiment showed clearly that nitrification in soil decreased with increasing NH_4^+ content, confirming earlier reports (Harada & Kai 1968; Wetselaar et al. 1972). N_2O emission was also correspondingly inhibited, and we did not find an increase in N_2O/NO_3^- ratio (N_2O yield of nitrification) as well. Besides N_2O production from nitrification, also denitrification-derived N_2O obviously decreased with the high NH_4^+ salt addition, as could be concluded from the decrease in N_2O emission in the acetylene amended treatments with increasing N level. Denitrification has been shown to be inhibited by high salinity, although generally an increase in the N_2O/N_2 ratio of denitrification occurred (Menyailo et al. 1998; Menyailo et al. 1997). From the results of the laboratory experiment and earlier studies, we expect that at NH_4^+ concentrations as high as 5000 $\mu g\ g^{-1}$, negligible N_2O formation should occur. This concentration was calculated to be present in the depot center directly after fertilization at the sandy loam site.

However, under field conditions nitrification obviously occurred already within the first two weeks after fertilizer application. At the margins of depots there is always a diffusion zone of decreasing NH_4^+ concentration with distance from the depot center (Wang et al. 1998). In this diffusion zone, nitrification may occur and thus formation of both N_2O and NO_3^-. In case of a non-spherical depot, the surface of the depot itself, and consequently the volume of soil with non-inhibiting NH_4^+

concentration around the depot, increases. A test with Brilliant Blue colored water machine-injected with the spoke wheel injector at equal rate and pressure as the fertilizer solution visualizes the depot geometry at the sandy loam site. The photographs in Figure 7-1 show that the fertilizer-depot must not be considered as a perfect sphere. Additionally, dilution of the depot occurs with N uptake by plants which have been shown to be the better competitors for N_{min} in soil compared to microbes (Inselsbacher et al. 2010). Plant uptake, however, reduces the N available for nitrification and denitrification and thus N_2O formation.

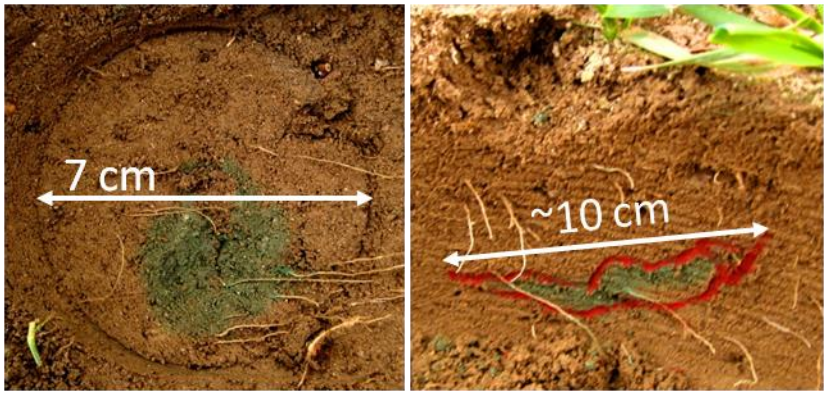

Figure 7-1: Simulated fertilizer depots from injection of Brilliant Blue colored water with the spoke wheel injector, directly after injection. Left: top view on a depot cross section, the circle denotes the dimension of the core sampler used for N_{min} sampling of depot centers. Right: vertical section of a fertilizer depot, the red line showing the shape of the fertilizer depot.

In consequence of the incomplete inhibition of nitrification with fertilizer injection, N_2O may be produced. The N_2O emission from the 0N treatment in the lab experiment resembled the background fluxes at the field site very well. The highest fluxes from the 450N treatment of incubations would correspond to 75 µg m^{-2} h^{-1} if upscaled, which is in the range of higher N_2O field fluxes. However, due to the concentration of NH_4^+ to a confined volume, more than 90% of the soil volume doesn't receive any fertilizer N. Even if it exhibited high nitrification rates, this small volume would not drastically increase area based N_2O emission (90% · 3 µg m^{-2} h^{-1} + 10% · 75 µg m^{-2} h^{-1} = 10 µg m^{-2} h^{-1}). This was also reflected by the high contribution of soil-derived to total N_2O emission in the CULTAN field experiment, where fertilizer-derived N_2O emissions contributed only 1% - 17% of total N_2O emissions. Peak emissions as they occurred at the CULTAN field site are thus not expected to be derived from nitrification of the depot fertilizer. This was also concluded from the temporal pattern of N_2O emissions at field experiments, where N_2O peak emissions occurred with irrigation some weeks after fertilization, and peak emission especially at the CULTAN treatments were attributed to denitrifying conditions. Higher propensity for denitrifying conditions is also regarded as the reason for higher N_2O emission from the loam than the sandy loam site, especially after CULTAN treatment (Chapter 5 and in correspondence to the literature (Bouwman et al. 2002; Pelster et al. 2013)) and indicates a potential of increased N_2O emissions from CULTAN management.

At well drained sandy sites and under predominantly nitrifying conditions, on the contrary, there might be the potential for lower N_2O emissions from CULTAN fertilization. Banding of N fertilizer has furthermore been shown to increase N uptake and yields under early season drought conditions as compared to broadcast incorporation (Hartman & Nyborg 1989), presumably by easing the accessibility of fertilizer to the roots by supplying it in the root zone thereby alleviating

the dependence of N uptake on precipitation for transport. This is of importance mainly in dry regions, and without irrigation. CULTAN fertilization may thus be of advantage in relatively dry and light soils. There was indeed a trend towards lower emission from the CULTAN than the surface application treatment (not statistically significant, though) at our sandy loam site (Table 5-2).

The temporal dynamics in both field experiments and the statistical evaluation revealed the relationship between N_2O emissions and the amount of N in soil. The N input by fertilization is a parameter that is relatively easy to control - and to assess. Calculation of large-scale (i.e. national) greenhouse gas inventories thus utilizes the amount of fertilizer N applied to estimate annual N_2O emissions. The *Tier-1* approach of the IPCC (2006) uses 1% of the applied fertilizer as an estimate of annual N_2O emission, regardless of the crop type, soil type or fertilizer. The model of Stehfest and Bouwman (2006), which was derived from empirical data, considers classes for fertilizer type (organic vs. mineral), soil (organic C content, pH and texture), climate and vegetation/crop type, for the estimation of N_2O emission with an exponential function of the amount of fertilizer input. Although none of the studies in this thesis aimed at showing the effect of increased N fertilization on annual N_2O emissions, emission rates can be compared to the predictions of the Stehfest and Bouwman (2006) model. Due to unforeseen problems with the ^{15}N tracer application method, different amounts of fertilizer N were applied to the respective plots at the sandy loam site of the CULTAN experiment. Although not presented in Chapter 5, these data show an exponential increase in N_2O emission with increasing fertilizer N amount at the sandy loam site (Figure 7-2, R^2=0.70). Figure 7-2 shows the annual N_2O emission of the summer drought and the CULTAN experiment. Each data point represents the cumulative annual emission of one plot. The N amount applied at the surface application plot receiving ^{15}N labeled fertilizer at the sandy loam site of the CULTAN experiment was roughly estimated to be 190-200 kg N from the concentration and ^{15}N fraction of NH_4^+ in the fertilizer solution that was in the tank after application.

According to the Stehfest and Bouwman (2006) model, much higher annual fluxes would be expectable for cereals grown at the sites and fertilized with N amounts applied during the CULTAN experiment, especially at the sandy loam soil. Well-watered control plots of the summer drought experiment show very high variation but are close to values as expected from the Stehfest and Bouwman model. Soil conditions were pretty similar between the summer drought experimental site and the sandy loam site of the CULTAN experiment, as were climatic conditions at the sites. The two sites of the CULTAN experiment were approx. 15 km apart, and the distance between the summer drought site and the sandy loam site of the CULTAN experiment was only 1 km. Measurements overlapped by one growing season (as the summer drought experiment ran from July 2010 to December 2011, and the CULTAN experiment from March 2011 to March 2013). Slightly higher N_2O emission at the 120 kg N level were measured at the loam site in the CULTAN experiment than at the sandy loam site, which was explained by the finer texture. Higher annual N_2O emission from the summer drought study may be explainable by the: 1) different fertilizers (CAN-prills broadcasted in the summer drought, $(NH_4)_2SO_4$ solution in CULTAN experiment), 2) high additional N mineralization from the preceding crop at the summer drought site, or 3) surficial soil compaction between plant rows in the summer drought experiment, decreasing the gas diffusivity and thus increasing the propensity for denitrifying conditions (Sitaula et al. 2000).

Besides the measured N loss as N_2O, N_2 fluxes from denitrification and nitric oxide (NO) fluxes from denitrification and nitrification add to total gaseous loss of N_r (Butterbach-Bahl et al. 2013; Cameron et al. 2013). The share of N_2 production from denitrification depends on various factors, e.g. pH, soil NO_3^- and organic carbon contents, and the aerobicity, that may affect denitrification rates and the product ratio of denitrification, i.e. the $N_2O/(N_2O+N_2)$ ratio. A range of 1-55 for the

N_2/N_2O ratio from agricultural soils has been given by Butterbach-Bahl et al. (2013). N_2 and NO emissions were not measured in the studies presented here; still some thought should be given to their importance for total gaseous N loss. While N_2 is unreactive, and its production the only permanent N_r sink, NO is highly reactive and plays a vital role in photochemistry by contributing to stratospheric O_3 formation. As N_2O and NO are produced during the same processes, they are generally regulated by the same environmental control factors, although due to directly acting as a greenhouse gas more attention had been given to N_2O (Pilegaard 2013). The ratio of NO/N_2O emission is mainly affected by the soil water content (Figure 7-3). During denitrification (including nitrifier denitrification), NO is produced before being further reduced to N_2O; the NO/N_2O ratio thus declines with increasing anaerobicity.

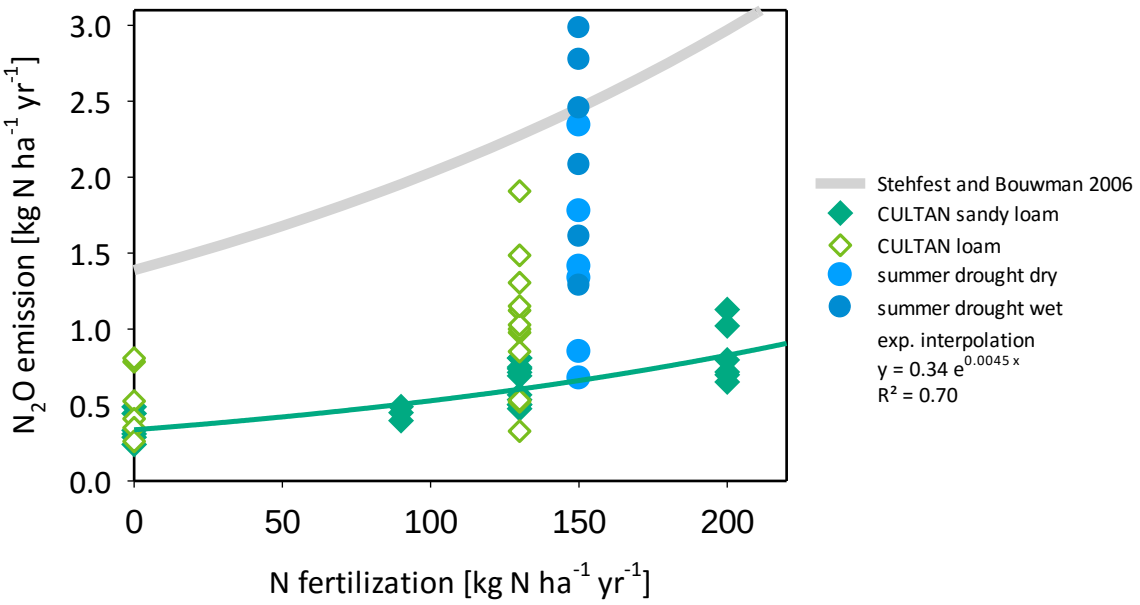

Figure 7-2: Annual N_2O emission determined in summer drought and CULTAN field experiment in relation to the amount of fertilizer applied. For comparison, the model according to (Stehfest & Bouwman 2006) is shown, adapted with the respective mean effect values for climatic, soil, and fertilization classes of this thesis' study sites. The exponential interpolation was derived from N_2O emissions of the CULTAN sandy loam site.

The relative contribution of denitrification and nitrification was assessed in the laboratory experiment, taking into consideration N_2O production during nitrification (hydroxylamine oxidation and nitrifier denitrification) and heterotrophic denitrification. Heterotrophic nitrification and fungal denitrification apparently had no great share on N_2O emission under the well-aerated (50% WFPS) conditions and at high NH_4^+ concentrations, although they were not specifically targeted with the methods applied. While background N_2O emissions in the field experiments were assumed to result from nitrification, high flux events under wetter conditions were attributed to denitrification.

The exact pathway of NO production during ammonia oxidation is not known, but the sequence may be $NH_3 \rightarrow NH_2OH \rightarrow HNO \rightarrow \mathbf{NO} \rightarrow NO_2^-$ (Firestone & Davidson 1989; Pilegaard 2013). Under dry well-aerated conditions, NO may leak out of soil before being further oxidized, and the NO/N_2O ratio is thus also higher at drier conditions. At low pH (<5) and when NO_2^- accumulates,

chemodenitrification of NO_2^- may be a source for NO emission (Medinets et al. 2015). Pilegaard (2013) summarized impact factors of NO production, showing that NO emission increases linearly with N input or availability, exponentially with temperature, has a maximum at intermediate soil water content and may both increase at low (due to chemodenitrification) and high (due to nitrification) pH values. Due to the low water content during most of the year in both field experiments, NO emissions might well have been much higher than N_2O emissions.

N_2 production from denitrification, on the other hand, was probably low. Dense root systems around CULTAN depots in the field experiment could have provided easily available organic carbon compounds and might have locally enhanced denitrification rates. High amounts of organic carbon would result in more complete reduction during denitrification and would thus have lowered the N_2O/N_2 product ratio (Focht & Verstraete 1977). High NO_3^- concentrations around the CULTAN depots, on the other hand, would enhance the N_2O/N_2 ratio, as would high salt concentrations (Menyailo et al. 1998) and the low pH of CULTAN depots (Bakken et al. 2012). Acid conditions, however, also reduce total denitrification rates (Focht & Verstraete 1977) which adds to the assumption that N_2 losses from denitrification were probably not very high.

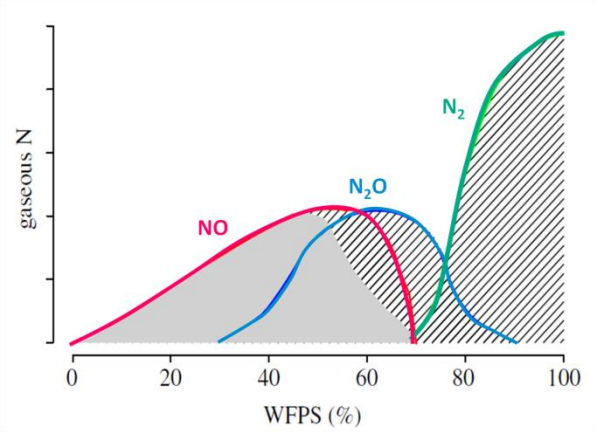

Figure 7-3: Proposed relative contributions of nitrification (solid grey shading) and denitrification (hatched shading) to gaseous N losses as a function of WFPS. Adapted from Davidson et al. (2000) as in Pilegaard (2013).

7.3. Heterogeneity at different scales and its impact on fluxes and flux determination

The field experiments as well as the laboratory experiment showed the high impact of spatial as well as temporal heterogeneity on the determination of N_2O emissions and underlying processes.

High spatial variability, especially at the loam site, led to large differences between individual chambers and thus high standard errors of mean fluxes per measurement day. The coefficient of variation (CV) was ≤ 200% for fluxes higher than 10 µg N m^{-2} h^{-1} at single measurement days, ≤ 300% for fluxes between 3 and 10 µg N m^{-2} h^{-1}, and partly higher at fluxes close to 0. For annual fluxes, the CV was higher at the loam than the sandy loam site (38% - 51% and 4% - 25%, respectively). This high spatial variability would have prevented the detection of small differences (smaller than the within treatment variation) between treatments. N_2O fluxes as measured with closed static chambers are often much higher or at least much more variable (Jones et al. 2011; Schäfer et al. 2012) than fluxes measured with methods integrating over a larger surface area, as

e.g. eddy covariance measurements or large chamber or closed tunnels (Jones et al. 2011; Schäfer et al. 2012). The probable explanation are hotspots (and coldspots) of N_2O production in soil that may be caused by patchy distribution of organic matter (root systems, litter), fertilizer, water content or differences in soil structure (Groffman et al. 2009; Kim et al. 2012). The spatial heterogeneity causing environmental conditions to differ between individual chambers might be lower in arable compared to grassland or forest ecosystems because of tillage and uniform application of fertilizer and management. The distribution of N substrate in soil of arable fields is mainly controlled by fertilization, and thus better known than in forest or grassland ecosystems. In the CULTAN field experiment, the distribution of fertilizer in CULTAN depots was addressed by the chamber geometry. However, CVs in the CULTAN experiment were relatively high but still comparable to other field studies (Flessa et al. 1995; Laville et al. 2011; Mathieu et al. 2006).

Temporal heterogeneity is another challenge: The measurement scheme – with weekly measurement plus additional measurements after fertilization, thawing, and precipitation after long dry periods – should have reduced the missing of peak events as compared to strictly weekly measurements and thus provide a reasonable estimate for the annual flux (Flessa et al. 2002; Pfab 2011). However, high flux events can be very short, and while missing very short emission events might not even cause extreme underestimation of annual fluxes, their inclusion and extrapolation to a complete week could have led to substantial overestimation of annual emission (Flessa et al. 2002). Peak fluxes (> 200 $\mu g\ m^{-2}\ h^{-1}$) contributed 30% to total annual fluxes in the summer drought experiment, and 40% (sandy loam) and 60% (loam) of annual fluxes were attributable to the 5-10% of measurement dates with high fluxes (> 30$\mu g\ m^{-2}\ h^{-1}$) in the CULTAN experiment. Peak fluxes were furthermore higher in the summer drought experiment than in the CULTAN experiment, which also translated into higher annual emission. That the temporal and spatial variability complicates the detection of small differences between treatments is a well-known problem that to date cannot be easily solved. It could possibly be avoided by higher temporal and/or spatial resolution, which might, depending on the measurement technique of choice, result in higher investment costs, more labor and/or a much higher number of samples to be taken and measured, causing additional costs. Model-based gap filling could be another possibility, but would depend on the predictability of N_2O fluxes from measurable parameters (Luo et al. 2011). Application of methods integrating over larger spatial scales would necessitate larger experimental plots, with the potential drawback of reduced comparability of the soil parameters underlying treatments.

Heterogeneity at a much smaller scale was shown to be important in the laboratory experiment (Chapter 6). Despite thorough homogenization of fertilizer solution and soil, the formation of microsites with spatial separation of nitrification and denitrification became evident from the comparison of a standard ^{15}N tracer approach (using ^{15}N abundance in bulk extracted NO_3^-, (Stevens et al. 1997)) and the non-equilibrium approach (Bergsma et al. 2001; Spott et al. 2006) used to calculate the fraction of NO_3^- derived N_2O. While the impact of heterogeneity in NO_3^- pools on the calculation of nitrification rates by pool dilution was not too strong, the results of source partitioning were substantially affected. Without the application of the non-equilibrium approach, the high impact of this small-scale heterogeneity would not have been disclosed. The data show (maybe for the first time) evidence for a large discrepancy between bulk and actively denitrifying pools and the resulting consequences for source partitioning. Neglecting that, the contribution of denitrification to N_2O production may be massively overestimated when the standard method is applied. In future studies, this discrepancy should be further addressed, as obviously homogeneous distribution and especially turnover cannot easily be stated even in relatively "homogeneous" systems as sieved and repacked sandy soil cores.

8. Conclusions and Implications

Methane oxidation was significantly enhanced with induced increased summer drought in this study, while the effect on N_2O emission was weak. This led to the conclusion that changes in N_2O emission with increasing summer drought frequency might not be severe, which is in accordance to earlier studies in other ecosystems. While the increased frequency of summer drought is a severe problem especially for the Mediterranean and other semi-arid or arid regions, it is not the only change in climate we will be faced with in the next decades. While precipitation in Southern and Central Europe tends to decrease in summer, it is likely to increase in the winter months. Regarding the high contribution of winter emissions to total N_2O emissions in these and other studies, more severe effects may be expected from higher water contents.

The direct effect of CULTAN fertilization on N_2O emission was also small and no substantial N_2O mitigation potential could be identified. A greenhouse gas balance has not been calculated here, but since single fertilizer injection necessitates fewer field operations compared to broadcast split application, the CULTAN strategy could help reduce both the manpower and the fuel consumption needed for fertilization. Furthermore, although dynamics of NO_3^- content in the CULTAN study did not show substantial inhibition of nitrification, there have been indications of reduced NO_3^- leaching with CULTAN fertilization in other studies. Since higher yields were achieved with CULTAN in comparison to broadcast surface application, its application may be worthwhile. To further improve the inhibition of nitrification and to avoid N_2O peaks under wet conditions, the combination with nitrification inhibitors may be promising.

In the summer drought as well as in the CULTAN study, biomass yields showed a stronger reaction to treatment than N_2O emissions. With the target being to increase crop and biomass yields while minimizing the negative environmental and climate impacts of agriculture, the minimization of greenhouse gases may not be the main focus at these sites. As yield-related N_2O emissions were mainly affected by plant yields at similar total N_2O emission, such strategies would be reasonable also from the greenhouse gas emission point of view.

While there was a significant relationship between N content in soil and N_2O emission, soil N derived N_2O caused the bigger part of N_2O emission, as was concluded from the low ratio of ^{15}N-labeled fertilizer-derived emissions in the CULTAN field experiment and more generally by the low fertilizer N emission factors. While N mineralization and nitrification were not directly addressed in the summer drought study, N_{min} dynamics were affected by the treatments, with higher NO_3^- contents persisting during drought. Considering that differences in N_2O emissions occurred mainly during the winter period, longer-term effects may be assumed to result from changes in precipitation pattern. Taking the possibility that the relatively high N_2O emission in the summer drought experiment resulted from high mineralization of the incorporated preceding crop into account, this points to the demand of long-term measurements or monitoring of N dynamics. At least the consideration of fertilization and cropping history when relationships between greenhouse gas emissions and fertilizer input shall be derived seems to be advisable.

Both spatial and temporal heterogeneity were high in the field studies and complicated the identification of treatment effects. Due to the small effects, it might be debatable whether a much higher effort to identify small changes would be worthwhile. However, even a reduction of N_2O emissions by 10% (which would not have been significant at the high variability in the field experiments) would sum up over large scales when simple management adjustments, like using another method of fertilizer application, were sufficient to cause this reduction.

The small scale heterogeneity, which has been detected in the laboratory experiment with the application of the non-equilibrium approach, has often been neglected in earlier ^{15}N tracer studies. In cases where the magnitude of the inhomogeneity is as high as in this study, it will have severe impacts on the results of N_2O source-partitioning in ^{15}N tracer studies. This should be considered in future studies and the small-scale heterogeneity should be further addressed in field and laboratory studies.

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A Appendix

A1. Supplementary data - summer drought study

A1.1. Linear mixed effect models applied to fluxes of N_2O and CH_4 calculated with the automated decision scheme

In this section, the R output of the final models applied to log transformed N_2O fluxes and CH4 fluxes is given.

A1.1.1 Linear mixed effect model of N_2O fluxes – Impact of treatment and period

```
> summary(fittreat)
Linear mixed-effects model fit by REML
 Data: data
       AIC      BIC    logLik
  1736.273 1844.727 -845.1367

Random effects:
 Formula: ~1 | Field
        (Intercept)
StdDev:   0.1046794

 Formula: ~1 | Plot %in% Field
         (Intercept)  Residual
StdDev: 3.197762e-05 0.5741102

Correlation Structure: ARMA(1,0)
 Formula: ~numdate | Field/Plot
 Parameter estimate(s):
     Phi1
0.7019846
Variance function:
 Structure: Exponential of variance covariate
 Formula: ~SE
 Parameter estimates:
     expon
0.02759175
Fixed effects: logn2o ~ treat_w + period + treat_p + treat_w:period + period:treat_p
                            Value  Std.Error  DF  t-value p-value
(Intercept)             3.0701809 0.08134886 816 37.74092  0.0000
treat_wdry              0.0536392 0.06201374   7  0.86496  0.4157
period.L               -0.4048288 0.16729608 816 -2.41983  0.0157
period.Q                0.1344373 0.15550273 816  0.86453  0.3875
period.C                0.0594079 0.12823040 816  0.46329  0.6433
period^4                0.7689338 0.10518707 816  7.31016  0.0000
period^5                0.0557389 0.09276522 816  0.60086  0.5481
treat_pMaize            0.0633118 0.06192204   7  1.02244  0.3406
treat_wdry:period.L    -0.2825416 0.19000120 816 -1.48705  0.1374
treat_wdry:period.Q     0.2600630 0.17644912 816  1.47387  0.1409
treat_wdry:period.C    -0.1497618 0.14642968 816 -1.02276  0.3067
treat_wdry:period^4     0.0008974 0.12077233 816  0.00743  0.9941
treat_wdry:period^5     0.2577939 0.10605444 816  2.43077  0.0153
period.L:treat_pMaize -0.3419686 0.18963279 816 -1.80332  0.0717
period.Q:treat_pMaize  0.1852457 0.17610183 816  1.05192  0.2931
period.C:treat_pMaize  0.0688767 0.14624102 816  0.47098  0.6378
period^4:treat_pMaize -0.3196402 0.12071942 816 -2.64779  0.0083
period^5:treat_pMaize -0.1939479 0.10601132 816 -1.82950  0.0677

> cor(fitted(fittreat), fittreat$data$logn2o)^2
[1] 0.2627685
> cor((predict(fittreat, level=0)), fittreat$data$logn2o)^2
[1] 0.2418775

> anova(fittreat)
               numDF denDF   F-value p-value
(Intercept)        1   816 2154.7885  <.0001
treat_w            1     7    0.3559  0.5696
period             5   816   39.2195  <.0001
treat_p            1     7    0.6021  0.4632
treat_w:period     5   816    2.4170  0.0345
period:treat_p     5   816    3.4087  0.0047
```

A1.1.2. Linear mixed effect model of N_2O fluxes – Impact of WFPS, NO_3^- and soil temperature

```
> fit_fin <- lme(logn2o ~ NO3 + WFPS_O+soil_temp,random=~1|Field/Plot,weights=varExp(form=~SE),
+                cor=corARMA(c(0.5,0.25),form=~numdate|Field/Plot,p=2,q=0),
+                data=data[order(data$Field,data$Plot, data$numdate),])
>
>
> summary(fit_fin)
Linear mixed-effects model fit by REML
 Data: data[order(data$Field, data$Plot, data$numdate), ]
       AIC      BIC    logLik
  1786.487 1833.809 -883.2436

Random effects:
 Formula: ~1 | Field
        (Intercept)
StdDev:  0.08299311

 Formula: ~1 | Plot %in% Field
         (Intercept)  Residual
StdDev: 3.069248e-05 0.6347878

Correlation Structure: ARMA(2,0)
 Formula: ~numdate | Field/Plot
 Parameter estimate(s):
     Phi1      Phi2
0.3828825 0.3962768
Variance function:
 Structure: Exponential of variance covariate
 Formula: ~SE
 Parameter estimates:
     expon
0.02427524
Fixed effects: logn2o ~ NO3 + WFPS_O + soil_temp
                Value  Std.Error  DF   t-value p-value
(Intercept) 2.1491305 0.15522318 828 13.845423  0.0000
NO3         0.0001284 0.00003094 828  4.150649  0.0000
WFPS_O      1.1429818 0.18477755 828  6.185718  0.0000
soil_temp   0.0164614 0.00563860 828  2.919408  0.0036
 Correlation:
            (Intr) NO3    WFPS_O
NO3         -0.015
WFPS_O      -0.888  0.006
soil_temp   -0.758 -0.324  0.645

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-3.50728372 -0.56355489 -0.04809658  0.52324362  5.44463457

Number of Observations: 843
Number of Groups:
          Field Plot %in% Field
              3              12
>
>
> anova(fit_fin)
            numDF denDF   F-value p-value
(Intercept)     1   828 2773.9805  <.0001
NO3             1   828   15.0866  0.0001
WFPS_O          1   828   31.7139  <.0001
soil_temp       1   828    8.5229  0.0036
>
> cor(fitted(fit_fin), fit_fin$data$logn2o)^2
[1] 0.1348275
> cor((predict(fit_fin, level=0)), fit_fin$data$logn2o)^2
[1] 0.1179196
```

A1.1.3. Linear mixed effect model of CH_4 fluxes – Impact of treatment and period

```
> summary(fittreatCH4)
Linear mixed-effects model fit by REML
 Data: data
       AIC       BIC   logLik
  -5119.319 -5010.533 2582.66

Random effects:
 Formula: ~1 | Field
         (Intercept)
StdDev: 0.0002172846

 Formula: ~1 | Plot %in% Field
         (Intercept)    Residual
StdDev: 6.106501e-08 0.008629856

Correlation Structure: ARMA(1,0)
 Formula: ~numdate | Field/Plot
 Parameter estimate(s):
     Phi1
0.7644571
Variance function:
 Structure: Exponential of variance covariate
 Formula: ~SE.2
 Parameter estimates:
   expon
87.00438
Fixed effects: ch4flux ~ treat_w + period + treat_p + treat_w:period + period:treat_p
                              Value    Std.Error  DF    t-value p-value
(Intercept)             -0.014730587 0.0008587806 828 -17.152911  0.0000
treat_wtrocken          -0.002832860 0.0009751111   7  -2.905166  0.0228
period.L                -0.002797657 0.0025186710 828  -1.110767  0.2670
period.Q                -0.007774533 0.0023874349 828  -3.256437  0.0012
period.C                 0.004700208 0.0019746504 828   2.380274  0.0175
period^4                 0.001596824 0.0016737088 828   0.954063  0.3403
period^5                -0.006589590 0.0016079412 828  -4.098154  0.0000
treat_pMais              0.001502526 0.0009748368   7   1.541310  0.1671
treat_wtrocken:period.L -0.005143119 0.0028785221 828  -1.786722  0.0743
treat_wtrocken:period.Q  0.001687938 0.0027309741 828   0.618072  0.5367
treat_wtrocken:period.C  0.000162181 0.0022693826 828   0.071465  0.9430
treat_wtrocken:period^4  0.004791182 0.0019341892 828   2.477101  0.0134
treat_wtrocken:period^5 -0.001238248 0.0018614012 828  -0.665224  0.5061
period.L:treat_pMais    -0.003437415 0.0028773293 828  -1.194655  0.2326
period.Q:treat_pMais     0.005229881 0.0027299284 828   1.915757  0.0557
period.C:treat_pMais     0.001988982 0.0022688170 828   0.876660  0.3809
period^4:treat_pMais    -0.002984275 0.0019340061 828  -1.543053  0.1232
period^5:treat_pMais    -0.001043077 0.0018613331 828  -0.560393  0.5754

> anova(fittreatCH4)
               numDF denDF   F-value p-value
(Intercept)        1   828 1031.7507  <.0001
treat_w            1     7   15.3533  0.0058
period             5   828   43.0568  <.0001
treat_p            1     7    1.5742  0.2499
treat_w:period     5   828    2.1820  0.0543
period:treat_p     5   828    2.1789  0.0546
> cor(fitted(fittreatCH4), fittreatCH4$data$ch4flux)^2
[1] 0.3103728
> cor(predict(fittreatCH4, level=0), fittreatCH4$data$ch4flux)^2
[1] 0.3100168
```

A1.1.4. Linear mixed effect model of CH_4 fluxes – Impact of WFPS and NO_3^-

```
>
> fit_fin<-lme(ch4flux~ NO3 + WFPS_O + NO3:WFPS_O ,
+              random=~1|Field/Plot,
+              weights=varExp(form=~SE.2),
+              cor=corARMA(c(0.7),form=~numdate|Field/Plot,p=1,q=0),
+              data=data[order(data$Field,data$Plot, data$numdate),],
+              method="REML")
>
> summary(fit_fin)
Linear mixed-effects model fit by REML
 Data: data[order(data$Field, data$Plot, data$numdate), ]
       AIC       BIC   logLik
  -5301.261 -5258.543 2659.63

Random effects:
 Formula: ~1 | Field
         (Intercept)
StdDev: 0.0002899314

 Formula: ~1 | Plot %in% Field
         (Intercept)    Residual
StdDev: 1.331702e-06 0.008309337

Correlation Structure: ARMA(1,0)
 Formula: ~numdate | Field/Plot
 Parameter estimate(s):
    Phi1
0.740254
Variance function:
 Structure: Exponential of variance covariate
 Formula: ~SE.2
 Parameter estimates:
  expon
89.4451
Fixed effects: ch4flux ~ NO3 + WFPS_O + NO3:WFPS_O
                   Value    Std.Error  DF    t-value p-value
(Intercept) -0.03327244 0.0013132388 840 -25.336166  0.0000
NO3          0.00000350 0.0000010476 840   3.341546  0.0009
WFPS_O       0.03581696 0.0021862664 840  16.382705  0.0000
NO3:WFPS_O  -0.00000577 0.0000023015 840  -2.507740  0.0123
 Correlation:
            (Intr) NO3    WFPS_O
NO3         -0.560
WFPS_O      -0.923  0.556
NO3:WFPS_O  0.435 -0.942 -0.495

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-8.7472569 -0.4557990  0.2005358  0.6101907  2.3828436

Number of Observations: 855
Number of Groups:
          Field Plot %in% Field
              3              12
>
> anova(fit_fin)
            numDF denDF   F-value p-value
(Intercept)     1   840 1071.5126  <.0001
NO3             1   840    6.6559  0.0101
WFPS_O          1   840  303.6586  <.0001
NO3:WFPS_O      1   840    6.2888  0.0123
```

```
> cor(fitted(fit_fin), fit_fin$data$ch4flux)^2
[1] 0.3341724
> cor(predict(fit_fin, level=0), fit_fin$data$ch4flux)^2
[1] 0.3334404
```

A1.2. Results of linear mixed effect models of water filled pore space

Table A 1: Means and standard deviation of water filled pore space (WFPS) in soil under wet and dry plots in different periods in 0 – 10 cm soil depth. Capital letters below WFPS values give results of Tukey's test on logit transformed WFPS performed with R; groups differ significantly in their WFPS when they do not share the same letter. Significant difference between wet and dry treatment thus occurred during both drought phases 2010 and 2011. During winter and before drought treatment, WFPS did not differ significantly.

	Predrought 2010	Drought 2010	Winter 2010/2011	Early summer 2011	Drought 2011	Winter 2011
Wet plots	0.40 ± 0.13 CD	**0.56 ± 0.09 D**	0.77 ± 0.15 D	0.36 ± 0.09 AB	**0.47 ± 0.09 CD**	0.58 ± 0.12 ABD
Dry plots	0.44 ± 0.15 D	**0.37 ± 0.12 BC**	0.77 ± 0.14 D	0.38 ± 0.10 B	**0.27 ± 0.14 A**	0.49 ± 0.14 ABD

Table A 2: Means and standard deviation of water filled pore space (WFPS) in soil under wet and dry plots in different periods in 10 - 30 cm soil depth. Capital letters below WFPS values give results of Tukey's test on logit transformed WFPS performed with R; groups differ significantly in their WFPS when they do not share the same letter. Significant difference between wet and dry treatment thus occurred during both drought phases 2010 and 2011. During winter and before drought treatment, WFPS did not differ significantly.

	Predrought 2010	Drought 2010	Winter 2010/2011	Early summer 2011	Drought 2011	Winter 2011
Wet plots	0.42 ± 0.11 C	**0.54 ± 0.10 D**	0.65 ± 0.07 D	0.42 ± 0.06 C	**0.44 ± 0.09 C**	0.57 ± 0.09 CD
Dry plots	0.42 ± 0.10 C	**0.34 ± 0.11 B**	0.63 ± 0.07 D	0.44 ± 0.06 C	**0.27 ± 0.12 A**	0.47 ± 0.12 BCD

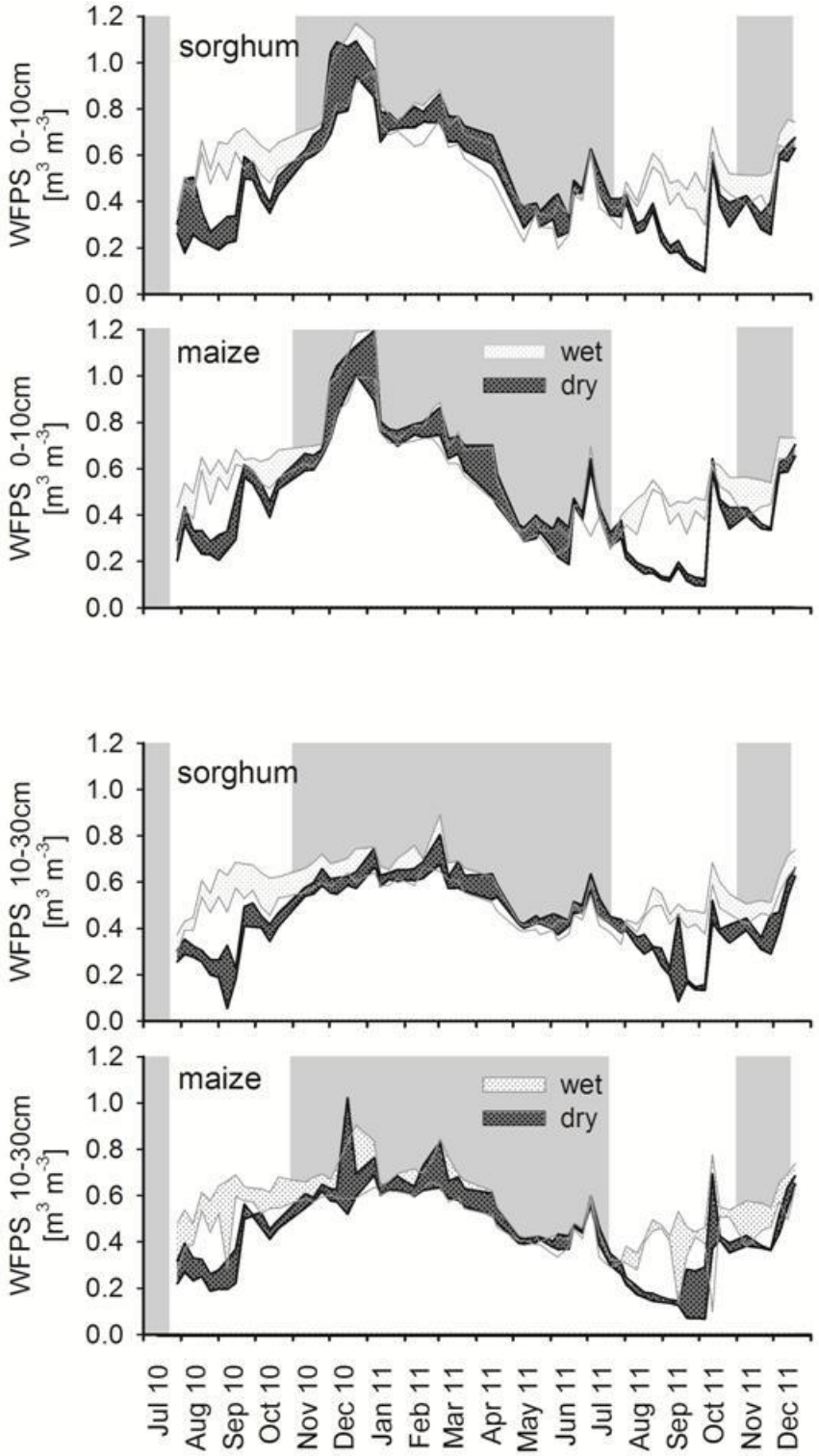

Figure A 1: Confidence intervals of water filled pore space (WFPS) in 0 – 10 cm and 10 – 30 cm soil depth. Light gray areas stand for WFPS in ambient wet plots of the respective plant, dark gray areas for WFPS in dry plots.

A1.3. Results from linear calculation of flux rates

Fluxes of CH_4 and N_2O were additionally calculated linearly. Resulting fluxes rates, mean fluxes per period and annual fluxes are presented in this section.

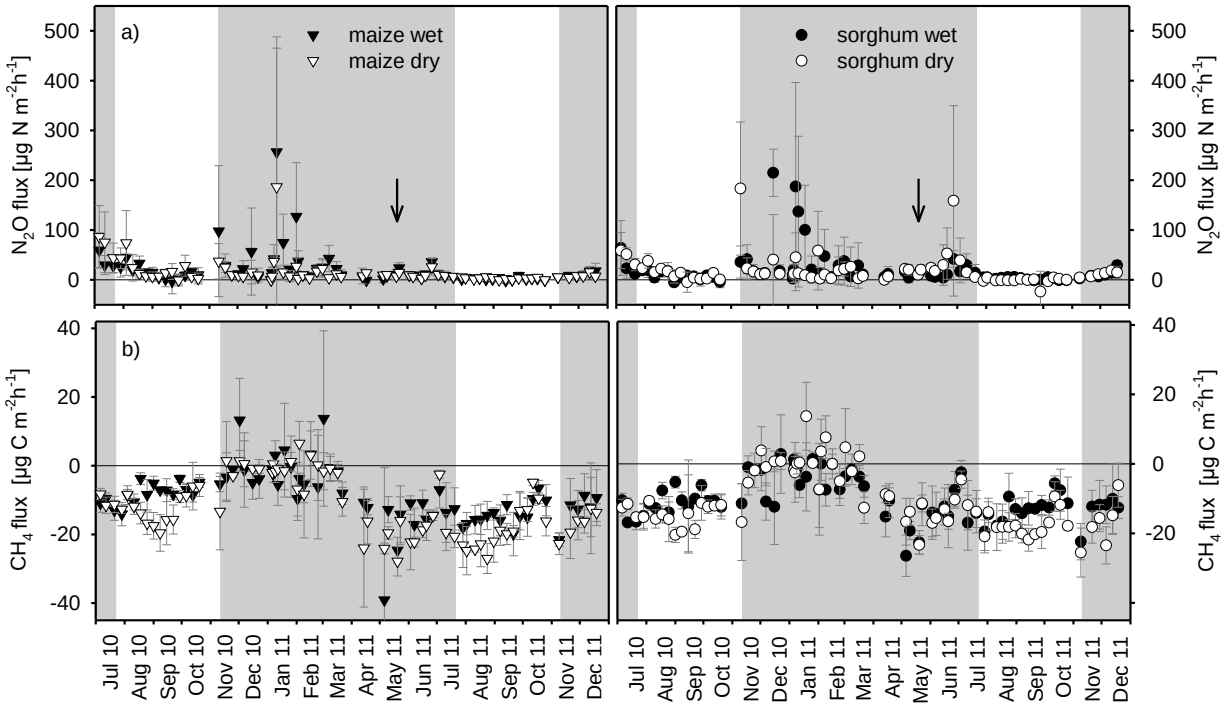

Figure A 2: Fluxes of nitrous oxide (N_2O, a) and methane (CH_4, b) calculated with linear regression of concentration over time. Error bars represent standard deviation (n=3).

Table A 3: Results from purely linearly calculated fluxes: Mean fluxes of N_2O and CH_4 for different periods, cumulated fluxes over the experiment and per year and calculated emission factors (EF) for yield based and fertilizer based emissions. Error terms are standard deviations (n=3). Results of posthoc pairwise comparisons of mean fluxes between treatments within periods are given in capital letters, and between periods (within treatments) in lower case letters. *fdr* correction

crop	treatment	Pre-drought 2010	drought 2010	winter 2010/2011	early summer 2011	drought 2011	winter 2011	Sum	harvest 2010 – harvest 2011	fertilizer scaled emissions	Yield-scaled emissions
		6.7.-21.7.2010	22.7.-1.11.2010	2.11.2010-27.4.2011	28.4.-24.7.2011	25.7.-2.11.2011	3.11.-31.12.2011			N_2O-N/ fertilizer N	g N_2O-N/ t d.w. biomass
		16d	102d	177d	88d	101d	59d	543d	365d	365d	365d
N_2O emission kgN ha^{-1}											
sorghum	**wet**	6.7 ± 4.7 A ce	1.9 ± 0.7 A b	9.2 ± 4.1 A e	3.7 ± 1.2 AB cd	0.6 ± 0.1 A a	2.3 ± 0.3 A bd	2.46 ± 0.74 A	2.02 ± 0.73 A	1.3%	110 ± 53 A
	dry	11.2 ± 3.2 B d	2.8 ± 0.3 A b	4.3 ± 1.1 AB bc	8.6 ± 7.4 A cd	-0.4 ± 0.8 A a	2.6 ± 0.6 A b	2.09 ± 0.69 A	1.47 ± 0.69 A	1.0%	97 ± 49 A
maize	**wet**	8.4 ± 4.7 AB c	3.4 ± 0.9 A b	8.1 ± 2.4 AB c	3 ± 1.1 B b	0.6 ± 0.4 A a	2.4 ± 0.6 A b	2.37 ± 0.45 A	1.75 ± 0.44 A	1.2%	62 ± 17 A
	dry	16.4 ± 11.4 B d	4.3 ± 2.5 A bc	4.2 ± 2.1 B c	2.5 ± 0.8 B bc	0.7 ± 0.5 A a	1.9 ± 0.5 A b	1.83 ± 0.49 A	1.02 ± 0.37 A	0.7%	60 ± 30 A
CH_4 uptake kgC ha^{-1}											g CH_4-C/ t d.w. biomass
sorghum	**wet**	-3.7 ± 0.1 A a	-2.5 ± 0.2 AB b	-1.2 ± 0.3 A c	-3.3 ± 1.1 AB ab	-3 ± 0.7 A ab	-3.2 ± 1.2 A ab	-1.31 ± 0.15 A	-0.81 ± 0.14 A	-	-46 ± 7 AB
	dry	-3.1 ± 0.3 AB a	-3.6 ± 0.4 A ac	-0.5 ± 0.4 A b	-3.3 ± 0.3 A ad	-4.2 ± 0.5 B cd	-4.4 ± 0.7 B c	-1.47 ± 0.10 A	-0.79 ± 0.07 A	-	-58 ± 4 A
maize	**wet**	-2.6 ± 0.5 B ac	-1.7 ± 0.4 B a	-0.7 ± 0.3 A b	-3.5 ± 0.6 AB c	-3.4 ± 0.7 AB c	-3 ± 1.1 A c	-1.17 ± 0.13 A	-0.77 ± 0.11 A	-	-32 ± 4 B
	dry	-2.6 ± 0.3 B a	-3 ± 0.7 A a	-1 ± 0.2 A b	-4.4 ± 0.4 B c	-4.4 ± 0.7 B c	-4.1 ± 1 AB c	-1.60 ± 0.13 A	-1.00 ± 0.10 A	-	-61 ± 12 A

(Benjamini and Hochberg, 1995) was used to correct for multiple comparisons. Mean fluxes differ significantly ($p < 0.05$) when they do not share the same letter. For fluxes cumulated over the whole experiment (sum) and over a complete year (harvest 2010 - harvest 2011), separate tests were performed.

A2. Supplementary material – CULTAN field study

A2.1. Results of the *gamm* applied to log-scaled N_2O fluxes and soil parameters of the CULTAN field study

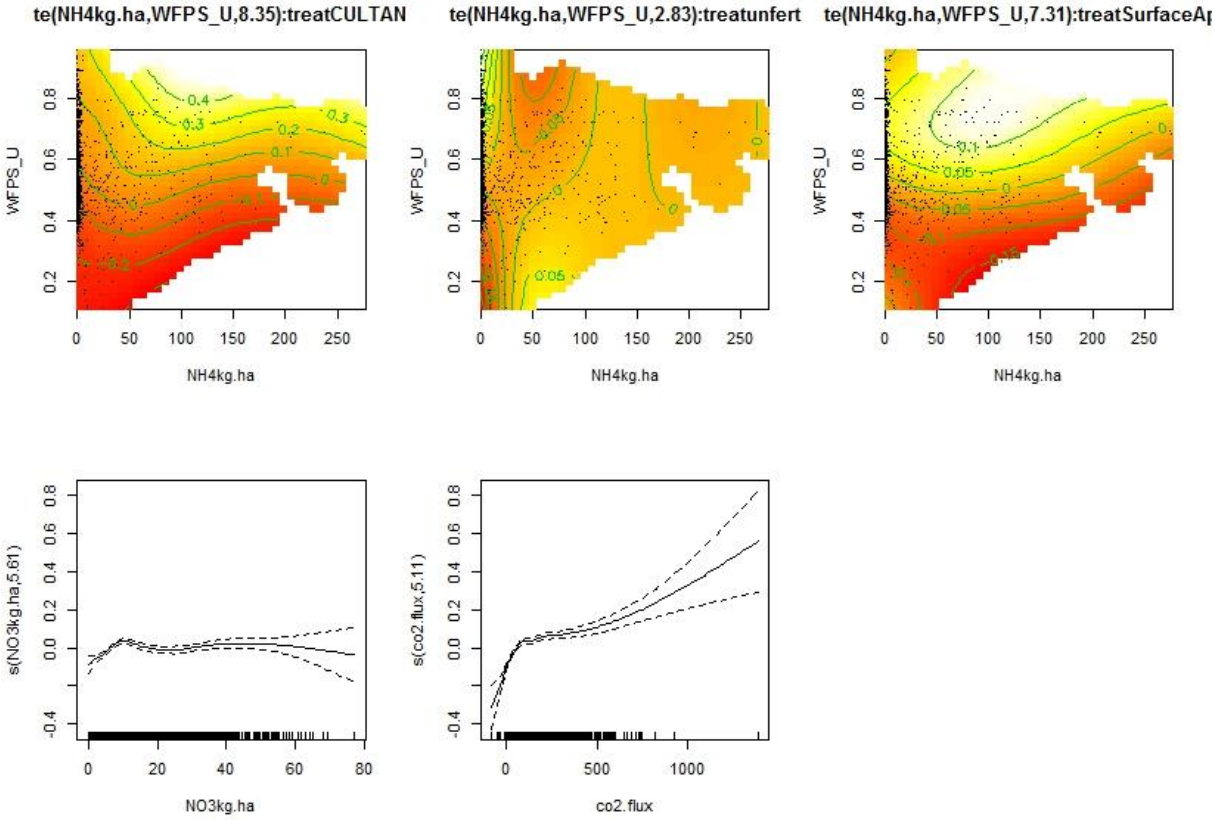

Figure A 3: Results of the *gamm* applied to log-scaled N_2O fluxes and soil parameters. Shown are the smoothers of WFPS (y axis) and NH_4^+ content (x axis) at the upper figures, for CULTAN injection (upper left), unfertilized control (upper middle) and broadcast surface application (upper right figure). The figures in the lower row show the smoothers for NO_3^- and CO_2. The values given at the y axis in lower figures and in color or at lines in the upper figures give the values that are inserted in the equation to calculate the modeled N_2O flux at the respective value-combinations of NH_4^+, WFPS, NO_3^- and CO_2.

```
> summary(fit$gam)

Family: gaussian
Link function: identity

Formula:
logN2O ~ te(NH4kg.ha, WFPS_U, by = treat, bs = "ts") + s(NO3kg.ha,
    bs = "cs") + s(co2.flux, bs = "cs") + Field + treat

Parametric coefficients:
                 Estimate Std. Error t value Pr(>|t|)
(Intercept)      1.475446   0.008697 169.658  < 2e-16 ***
FieldS          -0.101718   0.012789  -7.954 3.15e-15 ***
treatunfert     -0.043188   0.011912  -3.626 0.000296 ***
treatSurfaceApp  0.009916   0.010351   0.958 0.338199
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
                                    edf Ref.df      F  p-value
te(NH4kg.ha,WFPS_U):treatCULTAN     8.352 24.000  8.443  < 2e-16 ***
te(NH4kg.ha,WFPS_U):treatunfert     2.834 19.000  4.401  < 2e-16 ***
te(NH4kg.ha,WFPS_U):treatSurfaceApp 7.308 24.000  2.237 2.55e-10 ***
s(NO3kg.ha)                         5.610  5.610  8.586 1.30e-08 ***
s(co2.flux)                         5.111  5.111 46.062  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.213  Scale est. = 0.024715  n = 1862
```

Figure A 4: R output of the applied *gamm,* showing the significance of linear terms (field and treatment) and smooth terms. (FieldS= loam, treatunfert = control, SurfaceApp=surface application, CULTAN injection and the sandy loam site are represented by the intercept due to treatment contrasts)

A3. Supplementary data - Laboratory experiment

Table A 4: Concentration of N_2O measured in the headspace of incubation vessels at different days. Values given represent mean ± standard deviation (n=4). Background values were derived from incubation vessels without soil.

batch	N level	N_2O concentration [ppm]							
		Day 1	2	3	6	10	14	18	21
14N	0N	353 ± 42	409 ± 60	435 ± 60	332 ± 13	358 ± 40	393 ± 60	668 ± 283	840 ± 405
	450N	984 ± 46	2348 ± 134	2748 ± 178	1082 ± 102	1267 ± 123	1426 ± 191	3212 ± 262	3502 ± 371
	1000N	820 ± 56	1544 ± 50	1429 ± 44	513 ± 60	465 ± 40	475 ± 48	718 ± 86	801 ± 90
	2250N	553 ± 20	1056 ± 47	1106 ± 75	459 ± 24	420 ± 38	408 ± 26	488 ± 47	493 ± 40
	5000N	344 ± 11	431 ± 8	456 ± 6	331 ± 15	333 ± 11	332 ± 3	352 ± 5	363 ± 6
15N	0N	334 ± 6	351 ± 31	366 ± 42	329 ± 22	374 ± 96	436 ± 158	752 ± 646	830 ± 775
	450N	1166 ± 105	3434 ± 709	3146 ± 637	1212 ± 171	1434 ± 154	1647 ± 188	3879 ± 622	4543 ± 815
	1000N	852 ± 50	2065 ± 138	1708 ± 255	515 ± 6	564 ± 65	741 ± 134	1879 ± 669	2441 ± 964
	2250N	557 ± 36	1307 ± 174	1193 ± 164	455 ± 11	429 ± 25	408 ± 8	544 ± 110	626 ± 254
	5000N	445 ± 161	504 ± 43	506 ± 37	339 ± 14	351 ± 10	335 ± 12	362 ± 14	364 ± 10
C2H2	0N	317 ± 6	314 ± 3	316 ± 7	327 ± 25	324 ± 18	343 ± 28	364 ± 39	388 ± 63
	450N	354 ± 11	309 ± 12	319 ± 11	356 ± 31	440 ± 81	534 ± 112	577 ± 131	656 ± 167
	1000N	441 ± 31	489 ± 148	674 ± 224	375 ± 26	356 ± 7	373 ± 43	419 ± 169	502 ± 268
	2250N	360 ± 8	416 ± 112	376 ± 26	311 ± 16	336 ± 27	333 ± 14	336 ± 38	326 ± 9
	5000N	325 ± 17	328 ± 5	341 ± 7	293 ± 4	310 ± 6	318 ± 7	311 ± 3	323 ± 7
background		304 ± 7	286 ± 3	306 ± 4	290 ± 9	309 ± 10	316 ± 4	299 ± 4	311 ± 2

Table A 5: Fraction of soil derived N_2O to total N_2O (f_{soil}) in samples

batch	N level	f_{soil}																					
		Day 1			2			3			6			10			14			18			21		
14N	0N	0.13	±	0.10	0.29	±	0.10	0.29	±	0.09	0.13	±	0.03	0.13	±	0.09	0.18	±	0.13	0.48	±	0.25	0.54	±	0.27
	450N	0.69	±	0.01	0.88	±	0.01	0.89	±	0.01	0.73	±	0.03	0.75	±	0.03	0.78	±	0.03	0.91	±	0.01	0.91	±	0.01
	1000N	0.63	±	0.03	0.81	±	0.01	0.79	±	0.01	0.43	±	0.06	0.33	±	0.06	0.33	±	0.06	0.58	±	0.05	0.61	±	0.04
	2250N	0.45	±	0.02	0.73	±	0.01	0.72	±	0.02	0.37	±	0.03	0.26	±	0.07	0.22	±	0.05	0.38	±	0.05	0.37	±	0.05
	5000N	0.12	±	0.03	0.33	±	0.01	0.33	±	0.01	0.12	±	0.04	0.07	±	0.03	0.05	±	0.01	0.15	±	0.01	0.14	±	0.01
15N	0N	0.09	±	0.01	0.17	±	0.10	0.15	±	0.09	0.12	±	0.07	0.15	±	0.18	0.22	±	0.22	0.41	±	0.32	0.42	±	0.33
	450N	0.74	±	0.02	0.91	±	0.02	0.90	±	0.02	0.76	±	0.03	0.78	±	0.02	0.81	±	0.02	0.92	±	0.01	0.93	±	0.01
	1000N	0.64	±	0.02	0.86	±	0.01	0.82	±	0.02	0.45	±	0.01	0.45	±	0.07	0.57	±	0.08	0.82	±	0.07	0.86	±	0.06
	2250N	0.45	±	0.03	0.78	±	0.03	0.74	±	0.03	0.37	±	0.01	0.28	±	0.04	0.23	±	0.01	0.44	±	0.10	0.46	±	0.16
	5000N	0.29	±	0.23	0.43	±	0.06	0.39	±	0.06	0.16	±	0.04	0.13	±	0.01	0.07	±	0.03	0.18	±	0.03	0.16	±	0.02
C2H2	0N	0.06	±	0.02	0.03	±	0.01	0.03	±	0.02	0.07	±	0.07	0.05	±	0.05	0.09	±	0.08	0.13	±	0.09	0.17	±	0.13
	450N	0.15	±	0.03	0.01	±	0.04	0.04	±	0.03	0.15	±	0.08	0.29	±	0.14	0.39	±	0.14	0.43	±	0.15	0.49	±	0.16
	1000N	0.31	±	0.05	0.38	±	0.15	0.50	±	0.17	0.22	±	0.05	0.13	±	0.02	0.14	±	0.09	0.22	±	0.23	0.28	±	0.25
	2250N	0.16	±	0.02	0.28	±	0.15	0.18	±	0.06	0.07	±	0.05	0.08	±	0.07	0.05	±	0.04	0.10	±	0.09	0.05	±	0.03
	5000N	0.08	±	0.05	0.16	±	0.01	0.12	±	0.02	0.02	±	0.01	0.01	±	0.02	0.01	±	0.02	0.05	±	0.01	0.04	±	0.02

Table A 6: ^{15}N abundance in soil derived N_2O (aN_2O_{soil}), calculated from N_2O concentrations and ^{15}N abundance in sample and background N_2O

batch	N level	^{15}N abundance in soil derived N_2O [at%^{15}N]						
		Day 1	2	3	6	10	14	18	21
15N	0N						6.03 n=1		3.74 ± 0.32
	450N		1.66 ± 0.47	1.68 ± 0.41	1.75 ± 0.57		0.94 ± 0.11	0.84 ± 0.08	0.82 ± 0.06
	1000N		1.84 ± 0.24	2.01 ± 0.41			2.80 ± 0.16		1.98 ± 0.21
	2250N		2.10 ± 0.02	2.06 ± 0.02			3.87 ± 0.81		2.12 ± 0.60
	5000N		2.71 ± 0.41	2.89 ± 0.28					(2.73 ± 0.23)

Table A 7: ^{15}N abundance in the labeled N_2O producing pool (a_2) calculated from the non-equilibrium approach after Spott *et al.* (2006) and Bergsma *et al.* (2001).

batch	N level	a_2 [at%^{15}N]							
		Day 1	2	3	6	10	14	18	21
15N	0N	-	-	-	-	-	-	-	15.4 ± 0.9
	450N	-	6.6 ± 0.5	8.6 ± 0.8	9.7 ± 2.5	-	11.1 ± 1.4	10.9 ± 1.1	10.6 ± 0.9
	1000N	-	7.6 ± 1.8	9.6 ± 1.7	10.3 / 12.6	-	-	-	13.0 ± 0.9
	2250N	-	6.1 ± 0.1	6.5 ± 0.1	-	-	-	-	-
	5000N	-	-	-	-	-	-	-	-

Table A 8: ^{15}N abundance in bulk extracted NH_4^+ and NO_3^- at different days of incubation. The initial samples were taken from soil before filling of incubation vessels (n=5 per N level), at days 10 and 21, individual soil cores were sampled (n=4 per N level)

batch	N level	^{15}N abuncance in bulk N_{min} [at%^{15}N]				
		day 0	day 10		day 21	
		NO_3^-	NO_3^-	NH_4^+	NO_3^-	NH_4^+
15N	0N	5.30 ± 0.01	2.2 n=1	n.d.	4.42 ± 0.09	0.369 ± 0.000
	450N	5.27 ± 0.02	4.8 n=1	0.373 ± 0.002	1.31 ± 0.11	0.368 ± 0.001
	1000N	5.30 ± 0.01	2.98 ± 0.02	0.370 ± 0.001	2.24 ± 0.02	0.377 ± 0.003
	2250N	5.50 ± 0.05	3.88 ± 0.03	0.370 ± 0.001	3.26 ± 0.04	0.377 ± 0.004
	5000N	5.72 ± 0.04	5.36 ± 0.06	0.372 ± 0.001	5.23 ± 0.04	0.369 ± 0.000

Table A 9: Fraction of nitrification derived N_2O to total N_2O (f_N) from different methods over time, and weighted mean over the whole experiment (n=3 or 4 for mean or standard error of values). NN: (autotrophic) nitrifier nitrification; ND: nitrifier denitrification; CND: coupled nitrification denitrification

batch	N level	Approach	Process	f_N								f_N
				Day 1	2	3	6	10	14	18	21	Ø
14N	0N	C_2H_2 inhibition (a)	NN+ND+CND	0.71 ± 0.53	0.92 ± 0.14	0.93 ± 0.32	0.46 ± 0.31	0.68 ± 0.51	0.64 ± 0.48	0.95 ± 0.55	0.87 ± 0.53	**0.85 ± 0.44**
15N	0N	C_2H_2 inhibition (a)	NN+ND+CND	0.50 ± 0.12	0.86 ± 0.39	0.84 ± 0.46	0.43 ± 0.46	0.75 ± 0.90	0.76 ± 0.82	0.96 ± 1.01	0.86 ± 0.99	**0.86 ± 0.90**
15N	0N	^{15}N tracer non-equil (c)	NN+ND(+CND)	-	-	-	-	-	-	-	0.71 ± 0.72	**0.71 ± 0.72**
15N	0N	^{15}N tracer measured (b)	NN+ND	-	-	-	-	-	-	-	0.21 ± 0.20	**0.21 ± 0.20**
14N	0N	Isotopomer SP_{mean} (d)	NN (f_{NH2OH})	-	-	-	-	-	-	-	0.51 ± 0.14	**0.51 ± 0.14**
14N	0N	Isotopomer SP_{min} (d)	NN (f_{NH2OH})	-	-	-	-	-	-	-	0.57 ± 0.15	**0.57 ± 0.15**
14N	0N	Isotopomer SP_{max} (d)	NN (f_{NH2OH})	-	-	-	-	-	-	-	0.46 ± 0.12	**0.46 ± 0.12**
14N	450N	C_2H_2 inhibition (a)	NN+ND+CND	0.93 ± 0.08	1.00 ± 0.04	0.99 ± 0.04	0.93 ± 0.08	0.88 ± 0.10	0.85 ± 0.15	0.91 ± 0.08	0.89 ± 0.06	**0.91 ± 0.08**
15N	450N	C_2H_2 inhibition (a)	NN+ND+CND	0.94 ± 0.07	1.00 ± 0.14	1.00 ± 0.14	0.94 ± 0.10	0.89 ± 0.08	0.87 ± 0.09	0.93 ± 0.08	0.90 ± 0.11	**0.93 ± 0.09**
15N	450N	^{15}N tracer non-equil (c)	NN+ND(+CND)	-	0.77 ± 0.08	0.82 ± 0.10	0.83 ± 0.09	-	0.94 ± 0.06	0.95 ± 0.07	0.95 ± 0.11	**0.90 ± 0.17**
15N	450N	^{15}N tracer measured (b)	NN+ND	-	0.62 ± 0.06	0.57 ± 0.06	0.36 ± 0.11	-	0.55 ± 0.04	0.55 ± 0.05	0.50 ± 0.06	**0.53 ± 0.10**
14N	450N	Isotopomer SP_{mean} (d)	NN (f_{NH2OH})	-	0.61 ± 0.02	0.60 ± 0.03			0.72 ± 0.10	0.90 ± 0.11	0.77 ± 0.06	**0.77 ± 0.15**
14N	450N	Isotopomer SP_{min} (d)	NN (f_{NH2OH})	-	0.69 ± 0.02	0.68 ± 0.03	-	-	0.79 ± 0.14	0.96 ± 0.11	0.84 ± 0.11	**0.81 ± 0.17**
14N	450N	Isotopomer SP_{max} (d)	NN (f_{NH2OH})	-	0.53 ± 0.02	0.51 ± 0.03	-	-	0.65 ± 0.17	0.83 ± 0.10	0.70 ± 0.06	**0.70 ± 0.15**
14N	1000N	C_2H_2 inhibition (a)	NN+ND+CND	0.77 ± 0.17	0.87 ± 0.05	0.70 ± 0.10	0.66 ± 0.19	0.74 ± 0.18	0.63 ± 0.16	0.73 ± 0.24	0.64 ± 0.29	**0.72 ± 0.12**
15N	1000N	C_2H_2 inhibition (a)	NN+ND+CND	0.78 ± 0.07	0.90 ± 0.07	0.75 ± 0.14	0.66 ± 0.05	0.82 ± 0.27	0.87 ± 0.25	0.93 ± 0.33	0.91 ± 0.34	**0.87 ± 0.21**
15N	1000N	^{15}N tracer non-equil (c)	NN+ND(+CND)	-	0.78 ± 0.05	0.82 ± 0.10	-	-	-	-	0.85 ± 0.30	**0.82 ± 0.17**
15N	1000N	^{15}N tracer measured (b)	NN+ND	-	0.65 ± 0.03	0.60 ± 0.06	0.46 ± 0.03	-	-	-	0.10 ± 0.04	**0.32 ± 0.08**
14N	1000N	Isotopomer SP_{mean} (d)	NN (f_{NH2OH})	-	0.53 ± 0.02	0.67 ± 0.04	-	-	-	-	0.60 ± 0.10	**0.58 ± 0.12**
14N	1000N	Isotopomer SP_{min} (d)	NN (f_{NH2OH})	-	0.62 ± 0.02	0.75 ± 0.04	-	-	-	-	0.67 ± 0.15	**0.65 ± 0.12**
14N	1000N	Isotopomer SP_{max} (d)	NN (f_{NH2OH})	-	0.44 ± 0.02	0.59 ± 0.04	-	-	-	-	0.52 ± 0.09	**0.50 ± 0.13**

Table A 9: continued

batch	N level	Approach	Process	f_N								f_N
				Day 1	2	3	6	10	14	18	21	Ø
14N	2250N	C_2H_2 inhibition (a)	NN+ND+CND	0.78 ± 0.03	0.84 ± 0.07	0.91 ± 0.04	0.86 ± 0.18	0.84 ± 0.20	0.82 ± 0.18	0.80 ± 0.18	0.91 ± 0.13	**0.85 ± 0.44**
15N	2250N	C_2H_2 inhibition (a)	NN+ND+CND	0.78 ± 0.14	0.88 ± 0.13	0.92 ± 0.14	0.87 ± 0.07	0.85 ± 0.13	0.82 ± 0.11	0.85 ± 0.32	0.95 ± 0.54	**0.86 ± 0.90**
15N	2250N	^{15}N tracer non-equil (c)	NN+ND(+CND)	-	0.68 ± 0.08	0.73 ± 0.14	-	-	-	-	-	**0.70 ± 0.14**
15N	2250N	^{15}N tracer measured (b)	NN+ND	-	0.64 ± 0.08	0.66 ± 0.10	0.36 (n=1)	-	-	-	0.28 ± 0.11	**0.56 ± 0.15**
14N	2250N	Isotopomer SP_{mean} (d)	NN (f_{NH2OH})	-	-	0.54 ± 0.02	-	-	-	-	-	**0.54 ± 0.02**
14N	2250N	Isotopomer SP_{min} (d)	NN (f_{NH2OH})	-	-	0.63 ± 0.03	-	-	-	-	-	**0.63 ± 0.03**
14N	2250N	Isotopomer SP_{max} (d)	NN (f_{NH2OH})	-	-	0.45 ± 0.02	-	-	-	-	-	**0.45 ± 0.02**
14N	5000N	C_2H_2 inhibition (a)	NN+ND+CND	0.28 ± 0.28	0.64 ± 0.07	0.74 ± 0.02	0.85 ± 0.27	0.84 ± 0.31	0.83 ± 0.23	0.72 ± 0.07	0.73 ± 0.07	**0.73 ± 0.04**
15N	5000N	C_2H_2 inhibition (a)	NN+ND+CND	0.82 ± 0.73	0.78 ± 0.13	0.82 ± 0.16	0.89 ± 0.18	0.92 ± 0.16	0.88 ± 0.41	0.78 ± 0.16	0.79 ± 0.14	**0.83 ± 0.16**
15N	5000N	^{15}N tracer non-equil (c)	NN+ND(+CND)	-	-	-	-	-	-	-	-	**-**
15N	5000N	^{15}N tracer measured (b)	NN+ND	-	0.56 ± 0.10	0.53 ± 0.09	-	-	-	-	-	**0.54 ± 0.10**
14N	5000N	Isotopomer SP_{mean} (d)	NN (f_{NH2OH})	-	-	-	-	-	-	-	-	**-**
14N	5000N	Isotopomer SP_{min} (d)	NN (f_{NH2OH})	-	-	-	-	-	-	-	-	**-**
14N	5000N	Isotopomer SP_{max} (d)	NN (f_{NH2OH})	-	-	-	-	-	-	-	-	**-**

Table A 10: Ratio of fertilizer-induced fluxes, calculated from the difference in fluxes between the respective N level and the 0N level, in relation to the total N_2O emission from the respective N level.

batch	N level	Fertilizer- induced N_2O emission (% of total N_2O flux)							
		Day 1	2	3	6	10	14	18	21
14N	0N								
14N	450N	0.92 ± 0.17	0.95 ± 0.07	0.94 ± 0.09	0.94 ± 0.16	0.95 ± 0.18	0.93 ± 0.28	0.87 ± 0.18	0.82 ± 0.18
14N	1000N	0.89 ± 0.38	0.93 ± 0.06	0.88 ± 0.10	0.80 ± 0.39	0.67 ± 0.44	0.44 ± 0.45	0.10 ± 0.76	-0.15 ± -0.93
14N	2250N	0.79 ± 0.19	0.88 ± 0.05	0.83 ± 0.10	0.71 ± 0.33	0.52 ± 0.51	0.13 ± 0.72	-1.02 ± -1.66	-2.11 ± -2.49
14N	5000N	-0.49 ± -1.33	0.29 ± 0.19	0.06 ± 0.45	-0.06 ± -0.50	-1.38 ± -2.06	-4.25 ± -4.18	-6.10 ± -5.75	-9.97 ± -8.69
15N	0N								
15N	450N	0.96 ± 0.14	0.98 ± 0.28	0.98 ± 0.28	0.95 ± 0.21	0.93 ± 0.16	0.90 ± 0.18	0.87 ± 0.24	0.85 ± 0.30
15N	1000N	0.94 ± 0.14	0.97 ± 0.12	0.96 ± 0.26	0.82 ± 0.14	0.71 ± 0.66	0.70 ± 0.60	0.71 ± 0.72	0.75 ± 0.73
15N	2250N	0.88 ± 0.30	0.94 ± 0.24	0.93 ± 0.29	0.76 ± 0.18	0.44 ± 0.82	-0.34 ± -1.75	-0.82 ± -2.74	-0.58 ± -2.55
15N	5000N	0.79 ± 1.44	0.74 ± 0.30	0.72 ± 0.35	0.27 ± 0.56	-0.48 ± -2.12	-4.66 ± -7.86	-5.69 ± -9.90	-6.78 ± -11.8

Curriculum Vitae

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Publications

Marianna Deppe, Reinhard Well, Martin Kücke, Roland Fuß, Anette Giesemann, Heinz Flessa: *Impact of CULTAN fertilization with ammonium sulfate on field emissions of nitrous oxide.* Agriculture, Ecosystems & Environment 2016

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A short summary