NITROUS OXIDE EMISSIONS FROM ARABLE SOILS

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Effect of long-term tillage and identification of production and consumption processes using stable isotope approaches

Dissertation

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vorgelegt von

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Ich werde nie vergessen, wie ich zu Beginn meines wissenschaftlichen Tuns auf einem Acker stehend große Mengen Boden zur Untersuchung von Stickstoffumsetzungsprozessen entnahm und einige Bauern vorbei kamen.

Sie waren auf der Suche nach ein paar ausgebrochen Rindviehchern und fragten mich nach meinen Beweggründen für den Bodenaushub.

Auf meine Antwort, ich würde Proben für die Untersuchung der Lachgasfreisetzung aus Ackerböden nehmen, reagierten sie nicht nur amüsiert, ich bin mir sicher, dass sie wirklich nicht wussten, welchen bedeutenden Anteil landwirtschaftlich genutzte Böden am Klimawandel haben.

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C	carbon	N_2O	nitrous oxide
CH_4	methane	NH_3	ammonia
CO_2	carbon dioxide	NH_4^+	ammonium
CO_{2Eq}	CO_2-equivalent	N_{mic}	microbial nitrogen
C_{mic}	microbial carbon	N_{min}	mineral nitrogen
C_{org}	organic carbon	NO	nitric oxide
CT	conventional tillage	NO_2^-	nitrite
CV	coefficient of variation	NO_3^-	nitrate
DENIS	denitrification incubation system	NT	no-tillage
DNDC	denitrification-decomposition (model)	N_t	total soil nitrogen
DOC	dissolved organic carbon	O_2	oxygen
G	Garte Süd	r^2	correlation coefficient
GHG	greenhouse gas	RT	reduced tillage
H	Hohes Feld	SOC	soil organic carbon
He	helium	SOM	soil organic matter
MT	minimum tillage	SP	site preference
N	nitrogen	WPFS	water-filled pore space
N_2	dinitrogen		

Abstract

One of the main anthropic sources of nitrous oxide (N_2O) emissions, being an important greenhouse gas (GHG), is arable soil. With respect to the increasing world population an enhanced agricultural production with large-scale impacts on the nitrogen (N) cycle is most likely. Anyway, not all N flows and transformations in soils are yet fully understood, in particular denitrification as one of the key processes. Denitrification transforms nitrate (NO_3^-) via nitrite and nitric oxide to N_2O and finally into dinitrogen (N_2) and both production and consumption of N_2O take place simultaneously. The policy is engaged in developing mitigation strategies especially with respect to the agricultural sector to reduce GHG. To predict those emissions process-based models were used and field studies help to evaluated and improve them. Furthermore, for instance isotopomer measure-ments contribute to a better understanding of N_2O processes in soils.

This thesis presents results with respect to N_2O emissions from arable soils and provides information which contribute to fill the gap of knowledge with respect to pathways and influencing factors of N_2O emissions from arable soils.

Firstly, the long-term effect of different tillage (conventional vs. reduced) systems on the stocks and the distribution of soil organic carbon and total nitrogen and on the annual N_2O emission and the methane (CH_4) uptake are described and discussed, particularly with regard to spatial and seasonal variation of N_2O and CH_4 flux rates and the factors that control the spatial and temporal variability of the flux rates.

Additionally, those N_2O emissions and crop yields were modeled using the denitrification-decomposition (DNDC) model, in order to test the usefulness of the model in describing and predicting crop growth and N_2O emissions of differently managed soils.

Secondly, two laboratory experiments using stable isotope approaches are presented dealing with the production and consumption processes of N_2O during denitrification in arable soils. The first laboratory study aimed to simultaneously measure production and consumption of N_2O during denitrification in order to determine whether the N_2O isotopologue signatures of emitted N_2O under the condition of non-homogenous distribution of NO_3^- and denitrification in soil could be used to better define the processes involved.

The second laboratory experiment intended to determine the impact of antecedent soil moisture on N_2 and N_2O fluxes, to evaluate how N_2 fluxes and the N_2O/N_2 ratio are reflected by the isotopic signatures of emitted N_2O and of NO_3^- in soil and thus to test isotopologue signatures of N_2O as a tool to study denitrification in soil.

With respect to the effect of different tillage systems two long-term experimental sites Garte Süd (G) and Hohes Feld (H), both located near Göttingen, Germany, were selected. The loess derived Haplic Luvisols have been managed under conventional (CT) and reduced tillage (RT) for about 40 years with maximum tillage depths of 25 - 28 cm and 5 - 8 cm, respectively. N_2O and CH_4 fluxes (closed chamber method), physical and chemical properties (e.g. water content, mineral N content) were measured weekly and climate data were collected on a daily basis for two subsequent years. Additionally, at the beginning of the investigation a soil inventory was accomplished. Crop yields were determined separately for sites, tillage systems and years.

For the modeling a test was performed based on a model parameterization to best describe the case G-CT. This parameterization was then applied to the other cases as a retrospective simulation.

Laboratory experiments were conducted at the Institute of Grassland and Environmental research, North Wyke, UK. Twelve replicate cylinders filled with arable soil were placed in a specialized denitrification incubation system (DENIS), where they were sealed inside chambers to avoid the influx of N_2. Atmospheric N_2 was removed by flushing the headspace and cylinders with a helium-oxygen mixture and glucose (400 kg C ha^{-1}) and potassium nitrate (75 kg N ha^{-1}) were applied to the soil surface via a secondary vessel fitted to the center of each lid leading to a final water-filled pore space (WFPS) of 85%. After 7.5 days oxygen (O_2) was shut off in order to achieve totally anaerobic denitrifying conditions. Gas fluxes (N_2O, N_2 and carbon dioxide) and isotope signatures ($\delta^{18}O$-N_2O, $\delta^{15}N^{bulk}$-N_2O, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and ^{15}N site preference) of emitted N_2O were determined over a period of 13 days.

For the second laboratory experiment one batch of soil samples was kept dry (20% WFPS) and another was incubated under wet conditions (75% WPFS) for a period of 4 weeks. Then both batches were adjusted to the same high water content of 85% WFPS, placed in the DENIS and incubated for 10 days under a helium-oxygen atmosphere. When atmospheric N_2 was removed by flushing the system, potassium nitrate (75 kg N ha^{-1}) and glucose (400 kg C ha^{-1}) were added leading to a final WFPS of 90% in each soil core. Gas fluxes and isotope signatures of emitted N_2O were determined over a period of 13 days whereas after 6 days O_2 was shut off.

Results of the first study indicates that the annual N_2O fluxes and CH_4 uptakes of the investigated arable soils were influenced rather by soil properties as well as climate and short-term management effects than by tillage systems. Winter emissions accounted for up to 50% of annual N_2O fluxes and cumulative annual N_2O fluxes were reflected by precipitation level. Moreover the two long-term tillage systems resulted in a different soil carbon distribution whereas total carbon stocks did not differ between tillage systems but due to different clay contents between sites.

Site specific calibration within the second study has shown to be an essential requirement for the modeling of N_2O emissions and crop yields. Altogether the results indicates that calibration with experimental data and available literature data may result in approximate agreement between modeled and measured crop yields and annual N_2O emissions. Anyway, modeled and measured annual distributions of N_2O emissions were not accurate. Thus, the pedotransfer functions and the denitrification sub-model of the used DNDC model may need further improvement.

The third study shows, that the N_2O isotopologue values reflected the temporal patterns observed in N_2O and N_2 fluxes and gain helpful process information even if due to the occurrence of several factors the evaluation of identifying source processes is hampered and could thus not be fully explained. Anyway, the simultaneous increase in SP and $\delta^{18}O$-N_2O was found to be indicative of N_2O reduction to N_2.

The fourth study demonstrates the important effect of rewetting soil on N_2O emissions. The approach of combining measurement of N_2 and N_2O fluxes and isotopic signatures of NO_3^- and N_2O with isotope fractionation modeling gives insight into the spatial distribution of N species and microbial activity in soils.

Summarizing the results, the long-term effect of conventional and reduced tillage on the net exchange of N_2O was low and the modeling of N_2O emissions from arable soils with differing tillage quite good. Using stable isotope approaches improved the understanding of N_2O production and consumption processes and antecedent soil moisture conditions effected emissions and isotopologue distribution of N_2O during denitrification in an arable soil.

Kurzfassung

Eine Hauptquelle des vom Menschen verursachten klimaschädlichen Distickstoffoxids (N_2O), das auch Lachgas genannt wird, sind landwirtschaftliche Böden. Im Hinblick auf die ansteigende Weltbevölkerung ist mit einer Erhöhung der landwirtschaftlichen Produktion zu rechnen - mit weitreichenden Auswirkungen auf den Stickstoffkreislauf. Allerdings sind noch immer nicht alle Stickstoffflüsse und Umbauprozesse in Böden bis ins Detail verstanden, im Speziellen die Denitrifikation als einer der Schlüsselprozesse. Bei der Denitrifikation wird Nitrat (NO_3^-) über Nitrit (NO_2^-) und Stickstoffmonoxid (NO) zu N_2O und schließlich zu Di-Stickstoff (N_2) umgesetzt, wobei N_2O parallel entstehen und verbraucht werden kann. Die Politik befasst sich angesichts des Klimawandels und dessen Folgen mit Maßnahmen zur Reduzierung der Treibhausgase gerade im Agrarbereich. Um die Emissionen von Klimagasen vorhersagen zu können, werden prozessbasierte Modelle verwendet, die mit Hilfe von Feldstudien eingeschätzt und verbessert werden sollen. Weiterhin können beispielsweise Isotopomermessungen dazu beitragen, die N_2O-Prozesse im Boden besser zu verstehen.

Diese Arbeit beinhaltet verschiedene Untersuchungsergebnisse zum Thema „N_2O-Emissionen landwirtschaftlicher Böden“ und liefert hilfreiche Informationen, die dazu beitragen, die Wissenslücke bezüglich der N_2O-Prozesse und deren Einflussfaktoren zu füllen.

In einer ersten Teilstudie wird der Langzeiteffekt unterschiedlicher Bodenbearbeitung (pflugbasiert vs. pfluglos) einerseits auf die Vorräte und die Verteilung organischen Kohlenstoffs und des Gesamtstickstoffs und andererseits auf die Jahresemission von N_2O und die jährliche Methanaufnahme beschrieben und diskutiert. Dabei sollte insbesondere untersucht werden, wie sich die Bearbeitung auch auf die Variation der Gasflüsse und auf die Faktoren, die die zeitliche und räumliche Variabilität bedingen, auswirkt.

Zusätzlich wurden mit dem „Denitrification-Decomposition“-Modell (DNDC) die bei den Feldversuchen erfassten N_2O-Emissionen und Ernteerträge der zwei Bearbeitungsvarianten modelliert. Damit sollte die Eignung des Modells im Hinblick auf die Beschreibung und Vorhersagbarkeit der Emissionen und Erträge der unterschiedlich bewirtschafteten Böden getestet werden.

Des Weiteren werden zwei Laborexperimente zur Identifizierung von Produktions- und Reduktionsprozessen des N_2O während der Denitrifikation in Ackerböden mit Hilfe stabiler Isotope präsentiert. Der erste Versuch zielte durch die zeitgleiche Erfassung der N_2O-Produktion und -Reduktion darauf ab herauszufinden, ob die Isotopensignaturen des emittierten N_2O unter der nicht-homogenen NO_3^-- und Denitrifikationsverteilung im Boden geeignet sind, die involvierten Prozesse besser zu beschreiben.

Der zweite Versuch sollte neben dem Einfluss der initialen Bodenfeuchte auf die N_2- und N_2O-Flüsse auch dazu dienen festzustellen, inwieweit die Isotopensignaturen des emittierten N_2O und des NO_3^- im Boden die N_2-Flüsse und das Verhältnis von N_2O/N_2 widerspiegeln und ob die Isotopensignaturen des N_2O als Werkzeug zur Untersuchung der Denitrifikation im Boden geeignet sind.

Für die Untersuchung des Einflusses der Bodenbearbeitung wurden die Versuchsstandorte Garte Süd und Hohes Feld bei Göttingen ausgewählt. Die lössbasierten Parabraunerden unterliegen seit über 40 Jahren der konventionellen (pflugbasierten) und der reduzierten (pfluglosen) Bodenbearbeitung, mit den jeweiligen Bearbeitungstiefen von 25 bis 28 und 5 bis 8 Zentimetern. Über einen Zeitraum von zwei Jahren wurden die N_2O- und Methan-Flussraten mittels Haubenmethode sowie einige Bodenparameter (Wassergehalt und mineralischer Stickstoffgehalt) wöchentlich gemessen und Wetterdaten (Temperatur und Niederschlag) täglich erfasst. Zusätzlich wurde zu Beginn der Untersuchung eine Bodeninventur durchgeführt. Ernteerträge wurden getrennt für die Flächen, Jahre und Bodenbearbeitungsvarianten bestimmt.

Für die Modellierung wurde ein Testmodel, basierend auf der Parametrisierung einer Variante der ersten Teilstudie (Garte Süd, pflugbasiert) generiert, welches die erfassten Daten (N_2O-Emissionen, Erträge, Bodenwasserdynamik) am besten beschrieben hat. Diese Parametrisierung wurde dann an den anderen Varianten als zurückblickende Simulation angewendet.

Die beiden Laborversuche fanden in England am Institute of Grassland and Environmental Research, North Wyke, statt. Mit Hilfe eines speziellen Denitrifikations-Inkubationssystems unter Ausschluss des N_2 wurden zwölf mit Ackerboden gefüllte Zylinder eingebaut und nach Über- und Durchströmen mit einem Helium/Sauerstoff Gemisch wurde Glukose (400 kg C ha^{-1}) und Kaliumnitrat (75 kg N ha^{-1}) bei einem wassergefüllten Porenvolumen von 85% über ein mittig angebrachtes zweites Gefäß von oben zugegeben. Nach 7,5 Tagen wurde statt des Helium/Sauerstoff Gemisches reines Helium verwendet, um eine vollständige Denitrifikation zu gewährleisten. Die Gasflüsse (N_2O, N_2 und Kohlenstoffdioxid) und Isotopensignaturen ($\delta^{18}O$-N_2O, $\delta^{15}N^{bulk}$-N_2O, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ und die ^{15}N Positionspräferenz) des emittierten N_2O wurden über einen Zeitraum von 13 Tagen erfasst.

Bei dem zweiten Laborversuch wurde ein Teil der Bodenproben bei trockenen (20% wassergefülltes Porenvolumen), der andere Teil bei deutlich feuchteren Bedingungen (75% wassergefüllter Porenvolumen) über einen Zeitraum von vier Wochen vorinkubiert. Anschließend wurden alle Proben auf denselben hohen Wassergehalt (85% wassergefülltes Porenvolumen) eingestellt, in die Versuchsanlage eingebaut, unter Helium/Sauerstoff Atmosphäre gesetzt. Nach Zugabe von Glukose (400 kg C ha^{-1}) und Kaliumnitrat (75 kg N ha^{-1}) (90% wassergefülltes Porenvolumen) wurden die Gasflüsse und Isotopensignaturen analog zum ersten Versuch zehn Tage lang untersucht. In diesem Versuch wurde nach sechs Tagen die Sauerstoffzufuhr gestoppt.

Die Ergebnisse der ersten Studie ergeben, dass die jährlichen N_2O-Flüsse und Methan-Aufnahmen der untersuchten Ackerböden mehr von den Bodeneigenschaften, dem Klima und der Bewirtschaftung abhingen als vom Bearbeitungssystem. Winteremissionen machten bis zu 50 Prozent der jährlichen N_2O-Emissionen aus und die Jahresemissionen spiegeln die Unterschiede der Jahresniederschläge wieder. Außerdem hat sich das jahrzehntelange Pflügen auf die Verteilung des organischen Kohlenstoffs im Bodenprofil ausgewirkt, jedoch nicht auf den Gesamtkohlenstoffvorrat der gepflügten und minimal bearbeiteten Flächen. Unterschiede der Gesamtkohlenstoffvorräte zwischen den Flächen lassen sich auf den unterschiedlichen Tongehalt zurückführen.

Die standortspezifische Kalibration hat sich als essenzielle Voraussetzung für die Modellierung der N_2O-Flüsse und Ernteerträge herausgestellt. Insgesamt zeigen die Ergebnisse, dass die Kalibration mit experimentellen Daten und verfügbaren Literaturangaben zu annähernder Übereinstimmung zwischen modellierten und gemessenen Erträgen und den jährlichen N_2O-Emissionen geführt hat. Es wurden jedoch große Abweichungen bezüglich der modellierten und gemessenen N_2O-Emissionen im Jahresverlauf festgestellt. Die Pedotransferfunktionen das Denitrifikationsteilmodell des verwendeten DNDC Modells bedürfen daher weiterer Verbesserungen.

Die dritte Studie legt dar, dass die N_2O-Isotopologen den zeitlichen Verlauf der beobachteten N_2O- und N_2-Flüsse widerspiegelten und hilfreiche Prozessinformationen lieferten. Die eindeutige Identifizierung der Quellprozesse wurde durch das Auftreten mehrerer Faktoren behindert und konnte abschließend nicht aufgeklärt werden. Dennoch wies der zeitgleiche Anstieg der ^{15}N-Positionspräferenz und der $d^{18}O$-N_2O-Signaturen auf die N_2O-Reduktion zum N_2 hin.

Der bedeutende Einfluss der Wiederbefeuchtung eines Bodens auf die N_2O-Emissionen belegt die vierte Studie. Der Versuchsansatz zeigt, dass das zeitgleiche Erfassen von N_2- und N_2O-Flüssen und der Isotopensignaturen von NO_3^- und N_2O zusammen mit der Modellierung der Isotopenfraktionierung Einblicke in die räumliche Verteilung von N Spezies und der mikrobiellen Aktivität im Boden erlaubt.

Insgesamt bleibt festzuhalten, dass sich kein genereller Einfluss der betrachteten Bodenbearbeitungssysteme auf den Nettoaustausch des N_2O gezeigt hat und dass die Modellierung der N_2O-Gesamtemissionen der zwei Bodenbearbeitungs-systeme mit den gemessenen Werten übereinstimmte. Die Nutzung stabiler Isotope hat das Verständnis der N_2O-Produktions- und -Verbrauchsprozesse verbessert und die initialen Feuchtebedingungen haben die Emissionen und die Isotopensignaturen während der Denitrifikation in einem Ackerboden beeinflusst.

Preface and Outline

This thesis was part of the framework of the research training group 1397 „Regulation of soil organic matter and nutrient turnover in organic agriculture“ at the Büsgen-Institute, Soil Science of Temperate Ecosystems, University of Göttingen. Most of this research was funded by the German Research Foundation (DFG). Research activities for the laboratory experiments took place at the North Wyke Research Institute and were funded by the Biotechnology and Biological Sciences Research Council (BBSRC) within the Cross-Institute Program for Sustainable Soil Function (SoilCIP).

This thesis contains 6 chapters. First, the key question of nitrous oxide (N_2O) emissions from arable soils is introduced in a preliminary section (chapter 1) that imparts knowledge about agriculture as driver for climate change and about agricultural soil as a sink and source of greenhouse gases and their processes and regulating factors. Furthermore chapter 1 includes aspects of modeling N_2O emissions from soils and gives an insight into isotopomer signatures being a beneficial tool for investigating production and consumption processes of N_2O. Finally an overview about the study sites and experimental setup is given. Chapter 1 ends with the objectives of this thesis. Chapter 2 presents results of field experiments, in which the long-term effects of conventional and reduced tillage on the stocks and distribution of soil organic carbon and total nitrogen and the net exchange of N_2O and methane (CH_4) were investigated. In chapter 3 data of the experiment described in chapter 2 are used to model crop yields and N_2O emissions and the results of the modeling are discussed. Chapter 4 describes the setup and results of a laboratory experiment, focusing on N_2O production and consumption during denitrification in an arable soil. Results of a second experiment, which examines the effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification, are discussed in chapter 5. Finally the synthesis and general conclusions and future research perspectives are presented in chapter 6.

In the framework of this thesis, the following articles (chapters 2 to 5) were written or published in peer-reviewed journals:

Chapter 2 Sielhorst, A., Well, R., Ludwig, B., Rauber, R., Flessa, H.: Long-term effects of conventional and reduced tillage on soil organic carbon stocks and net exchange of N_2O and CH_4 (in progress).

Chapter 3 Ludwig, B., Bergstermann, A., Priesack, E., Flessa, H. (2011): Modelling of crop yields and N_2O emissions from silty arable soils with differing tillage in two long-term experiments. Soil & Tillage Research, Vol. 112, Issue 2, pages 114-121.

Chapter 4 Meijide, A., Cardenas, L. M., Bol, R., Bergstermann, A., Goulding, K., Well, R., Vallejo, A., Scholefield, D. (2010): Dual isotope and isotopomer measurements for the understanding of N_2O production and consumption during denitrification in an arable soil. European Journal of Soil Science, Vol. 61, Issue 3, pages 364-374.

Chapter 5 Bergstermann, A., Bol, R., Cárdenas, L., Gilliam, L., Goulding, K., Meijide, A., Scholefield, S., Vallejo, A., Well, R. (2011): Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification. Soil Biology & Biochemistry, Vol. 43, Issue 2, pages 240-250.

I am first author of the first article (chapter 2). I took the soil samples, measured gas fluxes, did the laboratory work with additional help of the technical staff, analyzed the data, evaluated them statistically, produced all tables and figures, interpreted and compared the results and wrote the text.

To the second article (chapter 3) I contributed by providing data material. I calculated N_2O flux rates and total N_2O fluxes for the respective time periods, generated data of soil characteristics (pH, bulk density, organic carbon, total nitrogen and texture), water-filled pore spaces and gravimetrically water contents, yields and climate data (precipitation, temperature), and supplied soil

management information (dates, tillage operations, time and amount of fertilization). Furthermore, I verified all data gained with reference to previous studies and checked the manuscript for compliance.

The third article (chapter 4) is a result of collaboration between the first author and me. I was involved in the development of the research idea and traveled to Great Britain to carry out the experiment. I prepared the soil (sieving, fertilization), and the experimental system (calibration of gas chromatographs, tightness, flow control), set up the experiment (soil core preparation, inserting cores, sealed cores, applied fertilizer) and conducted the experiment (regular calibration, soil core sampling, checking the system for leaks, flow control, gas sampling, soil analysis). After the experiment I calculated isotopomer data, flux rates (N_2O and N_2) and parameter of soil properties (water content, mineral nitrogen, microbial N, dissolved organic carbon). I contributed to the overall interpretation, especially to the isotopomer part, where I calculated a pool-model which helped with regard to interpretation but which was finally not incorporated in the article. Furthermore I checked drafts and assisted with reviewer demands.

I am first author of the forth article (chapter 5). This experiment was also carried out in Great Britain and my contribution was similar to that mentioned in the section before. In addition I evaluated the data statistically, produced tables and figures, interpreted and compared the results, calculated a pool model, and wrote the text. Moreover I incorporated and implemented the reviewers' comments.

Furthermore I contributed to and co-authored the following articles:

Mueller, E., Rottmann, N., Bergstermann, A., Wildhagen, H. & Joergensen, R.G. (2011): Soil CO_2 evolution rates in the field - a comparison of three methods. Archives of Agronomy and Soil Science, Vol. 57, No.6, pages 597-608.

Jacobs, A., Ludwig, B., Schmidt J.-H., Bergstermann, A., Rauber, R., Joergensen, R.G. (2011): Influence of tillage on degradation kinetics using the litter-bag method. European Journal of Soil Biology, Vol. 47, Issue 3, pages 198-204.

1 General introduction

1.1 Agriculture as driver of climate change

The world population is rapidly increasing and is expected to reach circa 9 billion in the middle of the 21st century with projected associated effects on all terrestrial ecosystems (BARNOSKY ET AL., 2012). Agricultural soils are the basis for food production, not only for humans but also for cattle breeding. Deforestation is a common way to get more land, being able to build up farms for industrial livestock farming. Burning woodland releases tones of climate-damaging carbon dioxide (CO_2). Fermentative digestion by ruminant livestock produces the even more detrimental methane (CH_4), as well as rice grown under flooded conditions and stored manure (MOSIER ET AL., 1998). Furthermore, a parallel increase in nitrogen (N) consumption, predominantly originating from industrial N fixation, has been observed to ensure the food production by fertilization. This N may be lost via leaching as nitrate (NO_3^-), or as gaseous product (ammonia (NH_3), nitric oxide (NO), nitrous oxide (N_2O), dinitrogen (N_2)) if it is not applied in an appropriate way. The atmospheric concentrations of N_2O, CO_2 and CH_4 are related to climate change. But which processes contribute to which proportion to global warming and climate change?

1.1.1 Global warming and greenhouse gases

N_2 and oxygen (O_2) basically form the Earth's atmosphere by proportions of 78% and 21%, respectively. Two thirds of the solar radiation passes through the atmosphere and reaches the Earth's surface, adsorbing the radiation and emitting back longwave radiation in form of infrared rays (Figure 1.1). Some of this heat is transmitted to the lower atmosphere by conduction and convection. Some of that heat is reflected to space, but some is re-emitted and the escape is prevented by the presence of "greenhouse gases" (GHG) in the atmosphere. These GHG adsorb this heat and re-emit it as infrared radiation, increasing the temperature of the atmosphere, which is called "global warming".

Negative effects of the presence of GHG in the atmosphere besides global warming are climate change (extreme climate events), ozone depletion, changes of snow cover and land ice expansion, sea level rise, water shortage and adverse effects on biodiversity.

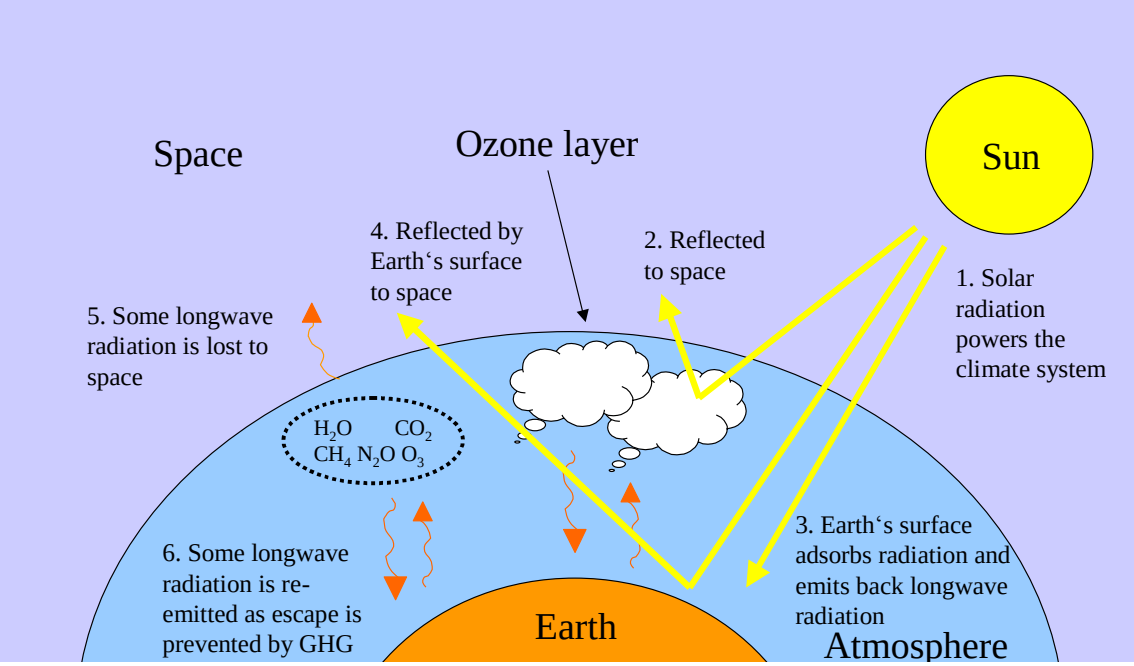

Figure 1.1: The natural greenhouse effect.

Important GHG due to human activities are CO_2, CH_4 and N_2O, which have grown since pre-industrial times (Figure 1.2a). The word wide increase of CO_2 concentration is mainly caused by the consumption of fossil fuels, deforestation, decay and peat (Figure 1.2b). Annual CH_4 emissions of anthropogenic sources have increased (Figure 1.2a) and emissions result from fossil fuel combustion, biomass burning, paddy fields, landfill and cattle breeding (DENMAN ET AL., 2007; FORSTER ET AL., 2007). CH_4 has a life time of 12 years and a global warming potential 25 times greater than that of CO_2 (FORSTER ET AL., 2007). The main sources of N_2O are the application of N-fertilizers to soil, fossil fuel consumption and some natural mechanisms that occur in terrestrial and aquatic ecosystems and the annual increase rate varies from 0.2 - 0.3% (SIGNOR AND CERRI, 2013). It has to be highlighted that N_2O has a 298 times greater global warming potential than that of CO_2 and a life time of 114 years (FORSTER ET AL., 2007).

Following the sectorial breakdown adopted in the Intergovernmental Panel on Climate Change Report (IPCC, 2007), in 2004 about 13.5% of total anthropogenic GHG emissions were derived from agriculture (Figure 1.2c). Even if this proportion seems to be low in 2005, agriculture accounts for about 50% of global anthropogenic CH_4 and about 60% of global anthropogenic N_2O emissions (IPCC, 2007). Agricultural soils in turn share 15.3% of the total amount of N_2O emissions, or 41.8% of anthropic emissions (DENMAN ET AL., 2007).

The impact of arable soils as driver of climate change emphasizes the need to understand the responsible processes for N2O production and consumption and how those processes are influenced.

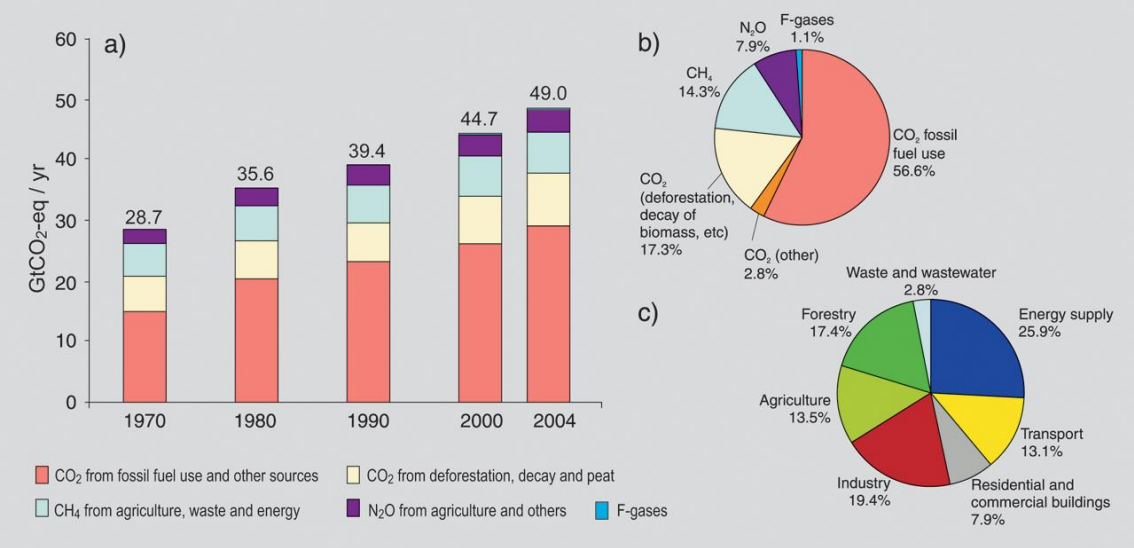

Figure 1.2: (a) Global annual emissions of anthropogenic GHG from 1970 to 2004. (b) Share of different anthropogenic GHG in total emissions in 2004 in terms of CO_2-eq. (c) Share of different sectors in total anthropogenic GHG emissions in 2004 in terms of CO_2-eq. (Forestry includes deforestation.) [IPCC, 2007].

1.1.2 Agricultural soils as sink and source of GHG - processes and regulating factors

The net balance between fixed CO_2 through photosynthesis and storage in soil as organic matter and the amount of soil C oxidized to CO_2 determines the net temporal status of soils as either sink or source. CO_2 is mostly produced by heterotrophic organisms and plant root respiration and is emitted from the soil surface to the atmosphere. It is the largest component of soil derived GHG fluxes and it nearly counterbalances the plant carbon fixation. Organic matter, which might be incorporated by intense tillage management or remain on the soil surface, consists of a variety of compounds with different residence time and easily or hardly compostable material. Labile compounds are composed by microbial organisms to CO_2 which is emitted to the atmosphere, whereas some soil organic carbon (C_{org}) is converted to for example organic-mineral complexes and may retain in the soil for centuries. The CO_2 flow from soils is thus highly variable and depends amongst others on root activity, microbial processes which are in turn influenced by climatic variables, crop residue and litter content, and soil properties.

In flooded conditions, such as wetland environments and paddy rice production, a significant fraction of the decomposing dead organic matter and soil organic matter is returned to the atmosphere as CH_4 (IPCC, 2006). In temperate oxic soils that are continuously exposed to atmospheric concentrations of CH_4 methanotrophs use CH_4 as carbon (C) and energy source and O_2 availability is the main factor limiting their activity (LE MER AND ROGER, 2001). Chemolithotropic ammonium oxidizing bacteria are also able to oxidize CH_4 (KNOWLES, 1993). Cultural practices mostly affect the potential of arable soil to oxidize atmospheric CH_4 both by destroying micro-acrophilic niches of CH_4 oxidizers and compaction by agricultural equipment which may also reduces atmospheric CH_4 oxidation (LE MER AND ROGER, 2001).

1.1.2.1 Production and consumption processes of N_2O in soil

In general, N_2O is released from arable soil surfaces to the atmosphere and is the result of production and consumption processes at different soil depths. N_2O is predominantly produced through the microbial processes of nitrification and denitrification (Figure 1.3). Nitrification is an autotrophic aerobic process by ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. The first step is called nitritation when ammonium (NH_4^+) or NH_3 is oxidized to nitrite (NO_2^-). Following the first step, nitratation is the oxidation of NO_2^- to NO_3^-. When the O_2 availability during the oxidation of NH_4^+ decreases and the composed NO_2^- is used as electron acceptor N_2O and N_2 are formed. This process is called nitrifier-denitrification (POTH AND FOCHT, 1985; WRAGE ET AL., 2001).

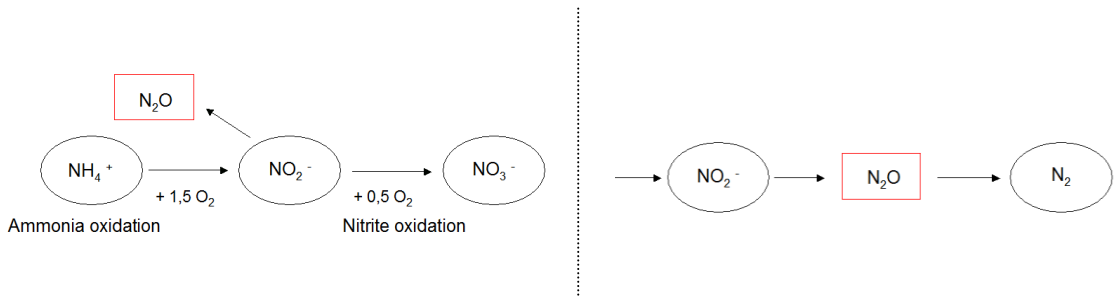

Figure 1.3: N_2O production during two-step nitrification and as intermediate during denitrification.

Denitrification occurs under anaerobic conditions by denitrifying bacteria and NO_3^- or NO_2^- are reduced to the gaseous products NO, N_2O and N_2 (FIRESTONE AND DAVIDSON, 1989) (Figure 1.3). N_2O is an intermediate of this reaction and may therefore be produced and consumed under certain conditions simultaneously. Furthermore denitrification and nitrification can take place at the same time within different microsites of the same soil. According to this, attributing N_2O production to different processes is a challenge.

Besides these two main N_2O production processes in soil further microorganisms and non-biological processes are able to contribute to N_2O formation. Some fungi can produce N_2 and N_2O by denitrification and codenitrification, and archeae

mediate nitrification in marine ecosystems and are capable of promoting denitrification in soils (HAYATSU ET AL., 2008). Chemodenitrification and hydroxylamine oxidation belong to non-biological processes. The amount of N_2O produced by decomposition of NO_2^- is almost negligible (BREMNER ET AL., 1980; BREMNER, 1997). The hydroxylamine oxidation can produce much more N_2O than the chemodenitrification process (BREMNER, 1997) and hydroxylamine is formed when NH_4^+ is oxidized to NO_3^-.

1.1.2.2 Factors influencing soil derived N_2O emissions

The microbial processes nitrification and denitrification are the most important ones with respect to N_2O formation in soils and are highly influenced by complex interactions among several factors.

The processes of nitrification and denitrification are influenced by variables at the microbial level, called "proximal" variables (BEAUCHAMP, 1997). At higher scales (microsite, field, landscape), these "proximal factors" are in turn affected by various physical, chemical and biological factors ("distal factors") (GROFFMAN ET AL., 1988). Ultimately climate, soil characteristics, cropping practices, and their interactions affect the nitrification and denitrification processes and hence the production and emission of N_2O (BEAUCHAMP, 1997).

The main factors influencing emissions of N_2O from nitrification are temperature and soil density (DAVIDSON AND SWANK, 1986). Moreover nitrification is controlled by the availability of O_2 and NH_4^+. NH_4^+ is normally the limiting factor for nitrification in cultivated soils, whereas low pH values, low water potentials and extreme temperatures reduce the nitrification rates (HAYNES, 1986b). As nitrification is a more or less spatial and temporal constant process the background emissions of arable soils are constant and low whereas high emission events are generally correlated with denitrification (FIRESTONE AND DAVIDSON, 1989). The amount of water-filled pores space (WFPS) is the main factor for N_2O formation during denitrification. Furthermore the presence of denitrifying bacteria and appropriate reducing agents (e.g. C_{org}) and reducible N-oxides under inhibited O_2 availability are required for the denitrification process.

Temperature

Temperature determines the activity of microorganisms and enzymes and influences not only the N_2O production but also its diffusion to the atmosphere. The close relationship between seasonal variation of N_2O flux and soil and air temperatures were documented in several studies (WOLF AND BRUMME, 2002; ZANG AND HAN, 2008). Moreover high temperatures stimulate soil respiration, which increases the formation of anaerobic sites, enhancing denitrification and therefore N_2O production. High N_2O emissions are often described in combination with freeze-thaw cycles, which might account for about 50% of annual losses of arable soils (FLESSA ET AL., 1995; KAISER ET AL., 1998). The importance of this period for the assessment of total N_2O losses from arable cops in the temperate climate zone can be explained in two different ways: a) release of trapped N_2O by melting of the ice barrier (BURTON AND BEAUCHAMP, 1994; TIETEMA ET AL., 1991); b) increased denitrification activity due to the release of organic matter available for denitrification by killing soil organisms and disintegrating aggregates (CHRISTENSEN AND CHRISTENSEN, 1991; CHRISTENSEN AND TIEDJE, 1990).

Soil properties

Water content is another factor that determines the activity of microorganisms and influences the diffusion capability, affecting the synthesis and release of N_2O to the atmosphere. High soil moisture is connected with high N_2O emissions (BAGGS ET AL., 2000; GIACOMINI ET AL., 2006) due to decreased aeration resulting from a smaller number of soil pores filled with air and therefore enhancing N_2O production by denitrification. Contrariwise, in total anaerobic soils most part of the N_2O is reduced to N_2, before being released to the atmosphere (DAVIDSON ET AL., 2000). Soil type and texture influence N_2O emissions due to a higher amount of anaerobic microsites in fine textures soils and thus increasing N_2O emissions. A high soil pH value stimulates the N_2O production during nitrification, but if denitrification is the main process, higher pH values decrease soil emitted N_2O (SIGNOR AND CERRI, 2013).

Interactions

As mentioned before, lots of factors regulate both nitrification and denitrification which might occur at the same time (aerobe and anaerobe conditions within the same aggregate). The interactions of these factors determine the amounts and rates of soil derived N_2O.

The interaction of production, consumption and disposal of N_2O during nitrification and denitrification is described by DAVIDSON (1991) as the "hole-in-the-pipe"-model (Figure 1.4). Therefore, three different variables control the N_2O flux: a) the N-transformation rate, b) the proportion of N_2O to other reaction products, and c) the magnitude of diffusion and consumption of N_2O before its escape to the atmosphere (DAVIDSON, 1991). The latter factor is affected by the location of the N_2O production within the soil profile, the texture and the soil water content.

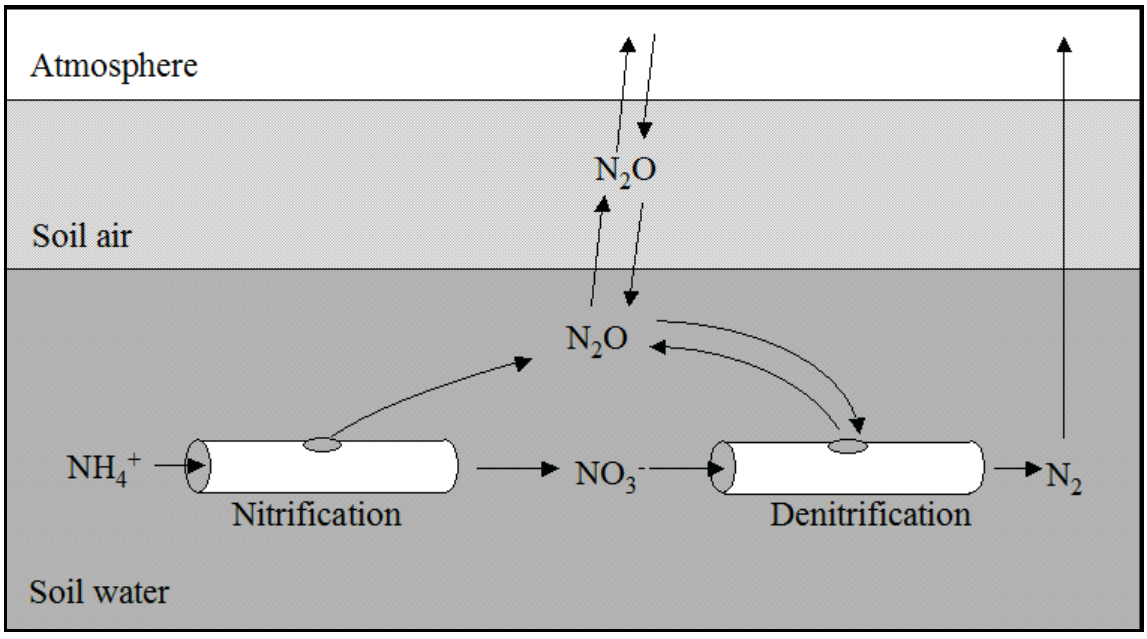

Figure 1.4: Turnover of N_2O during nitrification and denitrification ("hole-in-the-pipe-model"), according to Davidson (1991).

1.1.2.3 Agricultural management effects on N_2O emissions from soil

Besides the high number of influencing factors and their interactions, management factors may also alter N_2O formation of arable soils.

Soil tillage, recycling N from crop residues and the application of N-fertilizers (mineral or organic) are of great importance (SIGNOR AND CERRI, 2013) and may influence the factors previously described, too.

Three main tillage systems can be distinguished, conventional tillage (CT) which is characterized by a relatively deep tillage that either disrupts (chisel plough, cultivator) or inverts (moldboard plough) the arable top soil; conservation or reduced tillage (RT) which represents any form of non-inversion tillage with low application frequency and tillage depth (rotary harrow, rotavator) retaining a soil protecting mulch cover on the soil surface and no-tillage (NT) where the only soil disturbance is caused by planting.

The effect of soil tillage practices on N_2O emissions results from changes in soil structure, soil aeration, microbial activity, rate of residue decomposition and rate of N mineralization, as well as soil temperature and moisture (SIGNOR AND CERRI, 2013). Tillage and compaction are known to influence soil properties and there is little consensus, whether RT leads to increased or decreased N_2O emissions and what are the most important factors regulating the magnitude of these emissions (VENTEREA ET AL., 2005).

RT, where soil disturbance is small may increase the occurrence and stability of macroaggregates (JACOBS ET AL., 2009; KUSHWAHA ET AL., 2001; PAUSTIAN ET AL., 2000; SIX ET AL., 2000a, 2000b). Such stable macroaggregates may reduce the physical impact of machinery. Furthermore, these aggregates, especially when particulate organic matter is occluded, may form hot spots for denitrification due to anaerobic conditions inside those aggregates (SIX ET AL., 2002). The surface mulching of residues under RT has been shown to create conditions conductive to denitrification under the residues by increasing the soil water content, supplying available C as indicated by high measured microbial activity, and it has been supposed that this favored the creation of anaerobic microsites (BAGGS ET AL., 2003).

The higher soil water contents of RT soil results from the barrier against evaporation which the crop residues form at the surface. Furthermore, retained crop residues isolate the soil from heating up and reflect solar radiation (SHINNERS ET AL., 1993). Incorporating the crop residue and arranging them in deeper layers by ploughing may cause contrary effects. Temperature in turn affects microbial activity, respiration, O_2 content, and diffusion and finally N_2O emissions.

Available C influences nitrification and denitrification reactions (BREMNER, 1997), because it can stimulate microbial growth and activity and C_{org} is needed by soil denitrifiers (CAMERON ET AL., 2013). The concentrations of NO_3^- and NH_4^+ are another important factor influencing N_2O emissions as the amount influences the reaction rates and for the denitrification additionally the N_2O/N_2 ratio. Furthermore, the increase of microbial activity enhances O_2 consumption and creates anaerobic conditions, favoring denitrification. When moisture and N availability are not the limiting factors for N_2O production, greater fluxes are determined in soils with high available C content (RUSER ET AL., 2006). The biochemical composition of plant residues added to the soil is responsible for differences in N_2O emissions (GOMES ET AL., 2009). The balance between immobilization and mineralization depends on the C/N ratio and soils with a small ratio tend to have higher mineralization rates. Therefore plants are able to adsorb the released N or it is used in microbial processes like nitrification and denitrification and higher N_2O emissions may be the result. But not only the distribution of nutrients within the soil profile alter the soil conditions, the quantity and quality of plant residues influences the N mobilization and immobilization and therefore the N availability in the soil. This in turn affects the nitrification and denitrification processes and N_2O production.

CT soils increases the availability of soil organic matter by soil aggregate disruption, enhancing C and N mineralization (VERACHTERT ET AL., 2009). KANDELER ET AL. (1999) reported that after a 4-year period, N mineralization in the CT treatment was significantly higher than in MT and RT plots due to buried organic materials. The increased crop residue-soil contact under CT creating a

more oxidative soil environment which results in a more rapid decomposition of soil C_{org} relative to MT was confirmed by HALVORSON ET AL. (2002) for a silt loam soil.

Besides the differences between tillage systems in C_{org} and total N distribution, it is widely believed that RT has a beneficial effect on total C_{org} and N stocks.

The use of N-fertilizers directly influences the amount of NH_4^+ or NO_3^- available in the soil (SIGNOR AND CERRI, 2013). NH_4^+ based fertilizers may favor the nitrification process and the greater the amount of N-NH_4^+, the greater will be the nitrification process (MOSIER, 2001; KHALIL ET AL., 2004; LIU ET AL., 2005). When NO_3^- availability decreases, N_2O emissions will also decrease, because denitrification is reduced (HELLEBRAND ET AL., 2008). The type of fertilizer also plays an important role. Ammoniacal fertilizers increase N_2O emissions slower than nitric fertilizers, because nitric sources can be denitrified immediately, while ammonia sources still have to be nitrified before denitrification can occur (SIGNOR AND CERRI, 2013). Interactions of N-fertilizers should also be highlighted, as fertilizer application only induces high N_2O emissions in combination with moist conditions e.g. precipitation events, while fertilizer applications during dry weather result in small N_2O emissions.

The question arises if long-term reduced tillage is beneficial with respect to C storage ability and the accomplishment of mitigation GHG emissions. There are political and economic reasons to be able to estimate GHG and GHG mitigation strategies not only on a regional scale and process-based model are commonly used.

1.2 Simulation approaches of N_2O emissions

The high spatially and temporally variability of soil derived N_2O results from highly complex interaction of various factors, presented previously. To provide quantifications of N_2O emissions with respect to developing mitigation strategies for policy, simulation models are required with the ability to integrate all of these variables.

CHEN ET AL., (2008) summarized the simulation approaches generally used for N_2O emissions from agricultural lands. They abstracted that each model has its own philosophy in constructing simulation components as well as performance strengths and they range from those that attempt to comprehensively simulate all soil processes to more empirical approaches requiring minimal input data. According to them, process-based field-scale N_2O simulation models, which simulate whole agroecosystems, can be used to develop N_2O mitigation measures and are widely used, compared to those on a laboratory or regional/global level.

DAYCENT, CANDY, ExpertN or the Denitrification-Decomposition (DNDC) model are used for the task of understanding or even predicting the effects of different tillage systems on crop yields and greenhouse gas emissions. The DNDC model is able to simulate C and N biogeochemistry in agroecosystem and is used for prediction crop growth, soil temperature and moisture regimes, soil carbon dynamics, nitrogen leaching and GHG emissions (N_2O, NO, N_2, NH_3, CH_4 and CO_2).

Whereas simulation models may help to develop mitigation strategies, these models are only as good as their underlying functions and input data. This emphasizes the need of field data and laboratory experiments to get a better understanding of the processes involved. For instance natural abundance stable isotopic signatures have increasingly been used to identify N_2O source processes in the soil.

1.3 Isotopomers and isotopologue signatures of N_2O

One way to identify sink and source processes of N_2O in terrestrial systems is the use of its stable isotopic signature (STEIN AND YUNG, 2003).

Due to the linear molecule structure, there is a peripheral (β) and a central (α) N position within the N_2O molecule (Figure 1.5). Produced N_2O via nitrification and/or denitrification yields isotopically lighter N_2O in relation to its precursors because of kinetic isotope effects, whereas reduction during denitrification in soils results in an enrichment of ^{15}N and ^{18}O in the residual N_2O (BARFORD ET AL., 1999; MANDERNACK ET AL., 2000). N_2O derived from denitrification in soil was found to be higher in $\delta^{15}N$ and $\delta^{18}O$ signatures than derived from nitrification (BAGGS, 2008).

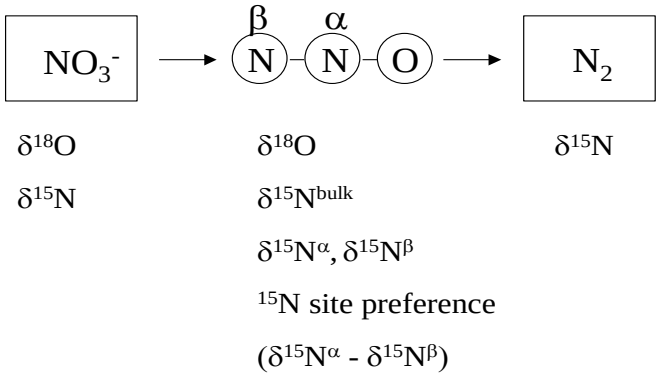

Figure 1.5: Isotopic signatures of N-species of the denitrification pathway.

In contrast to average ^{15}N, the difference between α and β ^{15}N enrichment, so called site preference (SP) is considered to be independent of the isotopic signature of the precursor (POPP ET AL., 2002; TOYODA ET AL., 2002). The intramolecular ^{15}N SP can be used to identify production processes at the scale of bacteria species or enzymes involved (TOYODA ET AL., 2005; BAGGS, 2008) and can differentiate between fungal and bacterial denitrification (SUTKA ET AL., 2008). Even if isotopic signatures of additional N species are missing, it is possible to supply clear process information with the help of SP values. However, reduction of N_2O during denitrification may increase the α-site enrichment in the residual N_2O, and hence also the SP index and thus bias the evaluation of its origin (BOL ET AL., 2003a; OSTROM ET AL., 2007).

1.4 Study sites and experimental setup

1.4.1 Study sites

The study described in chapter 2 was carried out on two long-term experimental sites near Göttingen, southern Lower Saxony / Germany. Both sites were established by the University of Göttingen about 40 year ago to determine the influence of different tillage systems on soil chemical, physical and biological parameters, crop yields, weed control and straw management.

Tillage management strategies for both sites were conventional tillage (CT) by plowing up to 25 cm depth followed by a seedbed preparation with a rotary harrow. The other tillage operation is reduced tillage (RT) with shallow cultivation down to 5 - 8 cm depth with a rotary harrow. More detailed descriptions of the study sites are provided in the *material and methods* sections of chapters 2 and 3.

Garte Süd (G) is a plot of the Reinshof Experimental Farm for crop science research located in the south of Göttingen. Hohes Feld (H) belongs to the Marienstein Experimental Farm in the north of Göttingen (Figure 1.6).

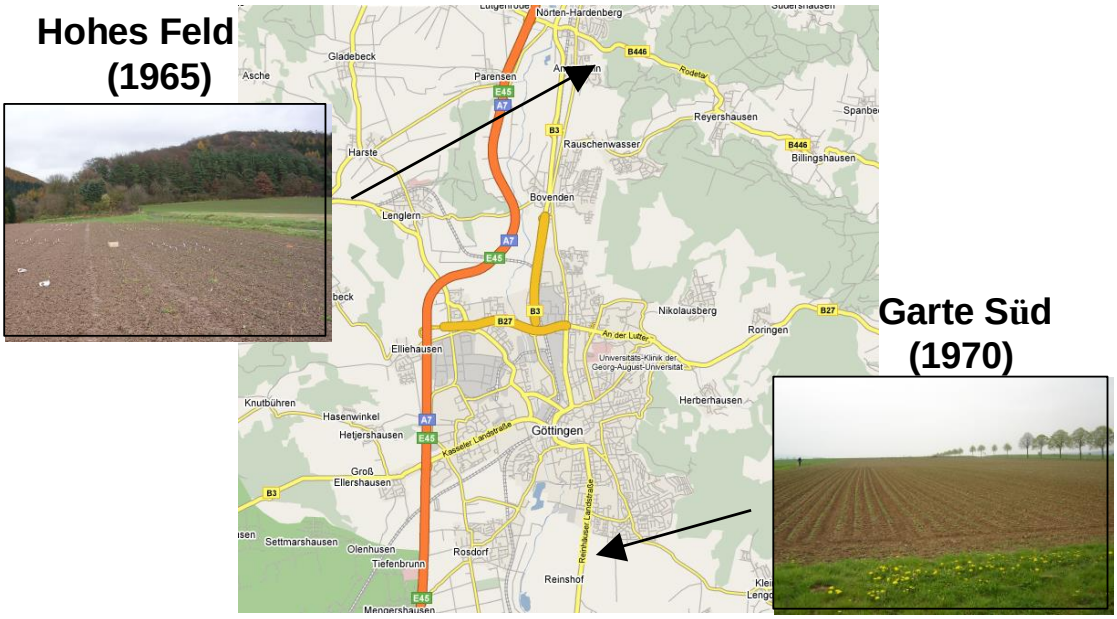

Figure 1.6: Location of the two long-term experimental sites Garte Süd and Hohes Feld.

G is a randomized complete block design with eight replicate plots (20 m x 40 m), but for our purpose only 4 replicates were used (Figure 1.7). Due to a smaller dimension of H a split plot design with three replicate plots (12.75 m x 36 m) was established (Figure 1.7).

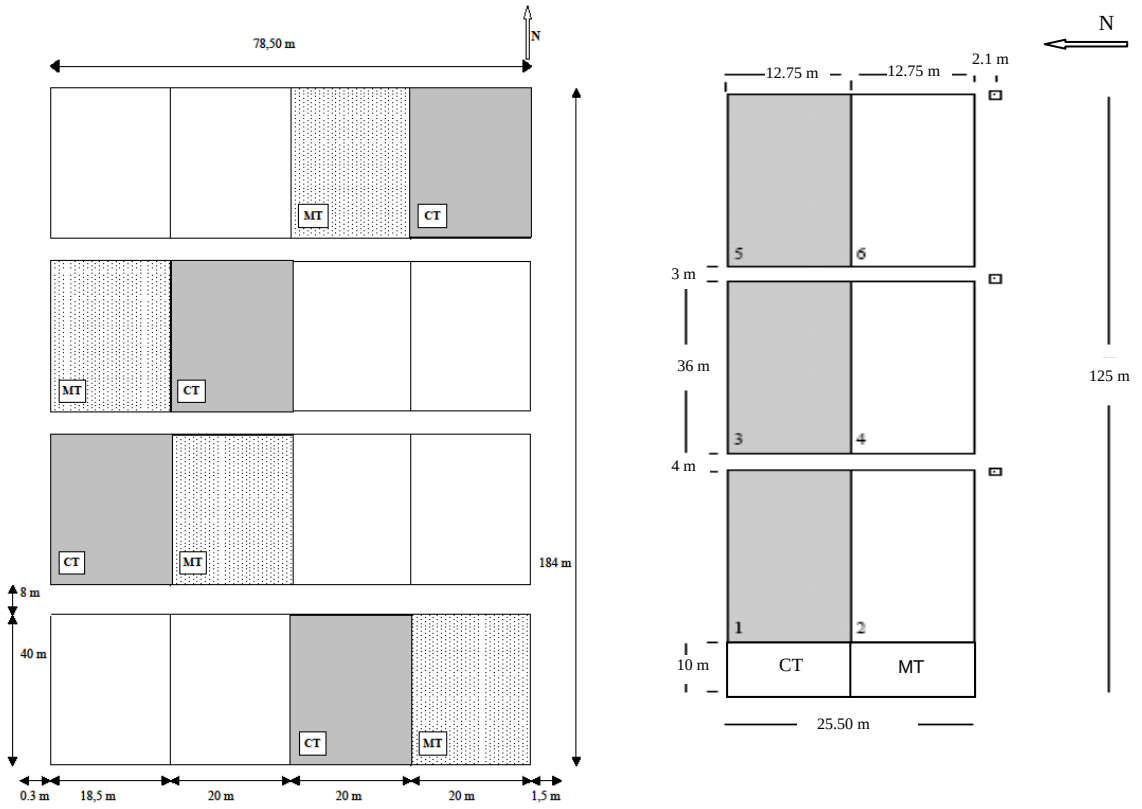

Figure 1.7: Experimental designs of the two sites Garte Süd (left) and Hohes Feld (right) with four (Garte Süd) and three (Hohes Feld) replications of conventional tillage (CT) and reduced tillage (MT), respectively.

1.4.2 Experimental design

The laboratory experiments of chapter 4 and 5 were conducted at the North Wyke research station in Devon, United Kingdom. A specialized incubation system called DENIS (denitrification incubation System) (CÁRDENAS ET AL., 2003) was used for both experiments. The system allows continuous measurements of N_2O and N_2 emissions from soil cores utilizing a helium-oxygen atmosphere allowing direct quantification of N_2 emissions. More detailed

descriptions of the experimental design are provided in the *material and methods* sections of chapters 4 and 5.

For N_2O and N_2 fluxes gas chromatographs with an electron capture detector for N_2O and a helium ionization detector for N_2 (CÁRDENAS ET AL., 2003) were used. Samples for isotopic analysis of emitted N_2O and for measurements of CO_2 fluxes were collected in duplicated 115 ml septum-capped serum bottles and 20 ml glass vials, respectively. Replicate sampling bottles were connected in line via needles (Figure 1.8). The outlet of each vessel was then connected to one sample bottle creating a flow-through sampling system.

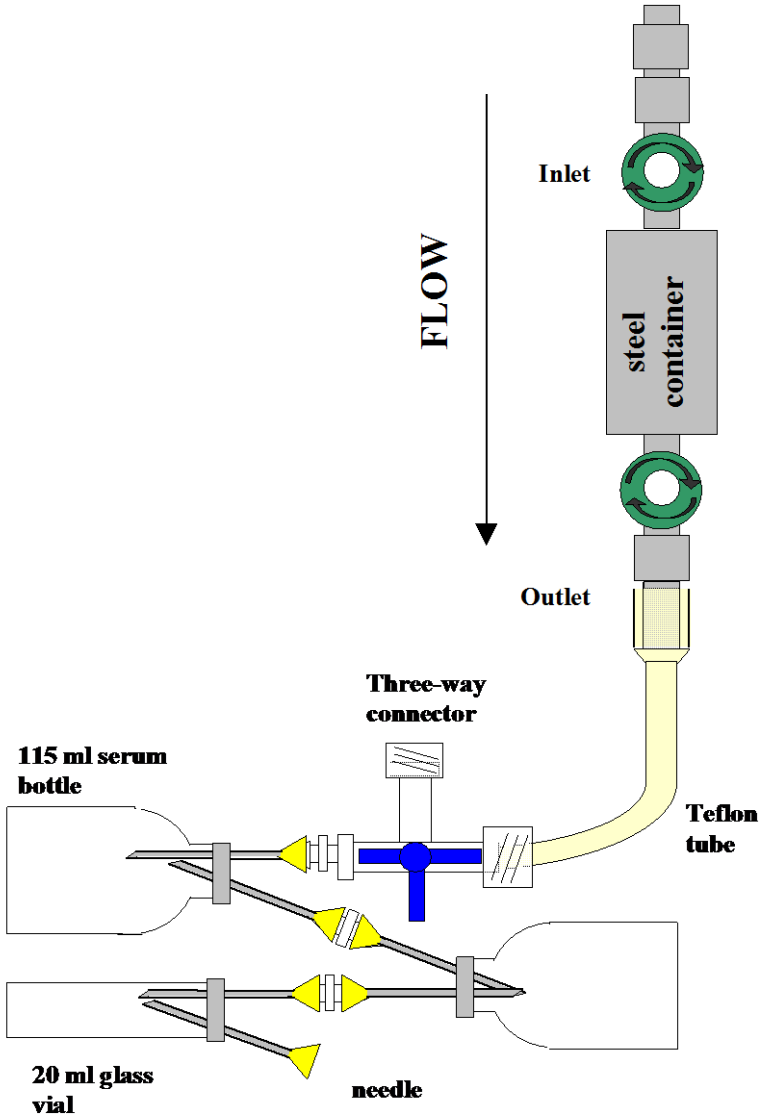

Figure 1.8: Illustration of the sampling design.

1.5 Objectives of this thesis

Agricultural soil management with all influencing factors and interactions shows potential to reduce greenhouse gas emissions. Arable soils are pointed out as a relevant source of climate-damaging N_2O and several studies have shown different effects of the tillage strategy on the emissions of greenhouse gases. Modeling these fluxes may help to better understand the interactions of the processes involved. Source partitioning of N_2O is considerably important to develop mitigation strategies and the isotopic fingerprint of N_2O may help to better understand controls of the different processes. This thesis aims at providing information which contribute to fill the gap of knowledge with respect to pathways and influencing factors of N_2O emissions from arable soils

The objectives of the first study (chapter 2) were to determine the long-term effects of conventional tillage (CT) and reduced tillage (RT) of two arable loess soils in Germany on i) the stocks and distribution of soil organic carbon and total nitrogen ii) the annual N_2O emission and the CH_4 uptake, iii) the spatial and seasonal variation of the N_2O and CH_4 flux rates and iv) the factors that control the spatial and temporal variability of the flux rates.

In the second study (chapter 3) those results were used to apply a proposed calibration and validation scheme for applications of the process-based dinitrification-decomposition (DNDC) model for field experiments with differing tillage in order to test the usefulness of the DNDC model in describing and predicting crop growth and N_2O emissions.

To get a better understanding of N_2O production and consumption during denitrification in arable soils, a laboratory experiment (chapter 4) was conducted in which the N_2O and N_2 fluxes and the isotopic fingerprint of N_2O were measured simultaneously. The aim of this third study was to determine whether the N_2O isotopologue signatures of emitted N_2O under the condition of non-homogenous distribution of nitrate and denitrification in soil could be used to better define the processes involved.

In the fourth study (chapter 5) the effect of antecedent soil moisture conditions in a laboratory experiment under denitrifying conditions was tested. One objective was to determine the impact of antecedent soil moisture on N_2 and N_2O fluxes. Furthermore, it should be evaluated how N_2 fluxes and the N_2O/N_2 ratio are reflected by the isotopic signatures of emitted N_2O and of NO_3^- in soil and to test isotopologue signatures of N_2O as a tool studying denitrification in soil.

2 Long-term effects of conventional and reduced tillage on soil organic carbon stocks and net exchange of N_2O and CH_4*

* note the information in the preface and outline section (XVIII)

2.1 Introduction

Agricultural activities contribute to over 20% of global anthropogenic greenhouse gas (GHG) emissions and cultivated soils have been identified as one of the main GHG sources within the agricultural sector (LOKUPITIYA AND PAUSTIAN, 2006). The most important biological GHGs are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O).

N_2O in arable soils is mainly produced by the microbial processes of nitrification and denitrification (BEAUCHAMP, 1997; SKIBA AND SMITH, 2000). Soil oxygen (O_2), mineral nitrogen (N_{min}), temperature, pH and the abundance of organic carbon or other electron donors directly affect nitrifiers and denitrifiers ("proximal factors") (GROFFMAN ET AL., 1988). At higher scales (microsite, field, landscape), "proximal factors" are in turn affected by various physical, chemical and biological factors ("distal factors") such as climate, soil characteristics, cropping practices and their interactions.

Soil management practices such as soil tillage, recycling N from crop residues and the application of N-fertilizer (mineral or organic) are of great importance, being significantly involved in the N_2O production (SIGNOR AND CERRI, 2013). Changes in soil structure by tillage strongly influence the production, consumption and transport of N_2O (MÜLLER AND CLOUGH, 2013). Reduced tillage (RT) concentrates organic carbon and nitrogen at the upper centimeters, as crop residues are left at the soil surface. Such mulch layer influences the temperature and water budget of the soil which in turn affects microbial processes. Conventional tillage (CT) incorporates crop residues within the maximum ploughing depth. The conversion and decomposition, the content and distribution of residues and finally the storage of soil organic matter (SOM) can affect both nitrification and denitrification (FARQUHARSON AND BALDOCK, 2008).

There is little consensus, whether RT leads to increased or decreased N_2O emissions and what are the most important factors regulating the magnitude of these emissions (VENTEREA ET AL., 2005). RT can enhance gas diffusivity in

soils due to more stable soil structure and higher connectivity of pores (BALL ET AL., 1997A; KESSAVALOU ET AL., 1998; ROBERTSON ET AL., 2000). Inhibited diffusivity may favor anaerobic conditions and net N_2O production by denitrification, but also N_2O reduction to dinitrogen (N_2) (SMITH ET AL., 2003).

The gas exchange between soil and the atmosphere exhibits a dual impact on GHG emissions since it is a prerequisite for the influx of atmospheric O_2 and CH_4. CH_4 is oxidized in soils by methanotrophic bacteria and also chemolithotrophic ammonium oxidizing bacteria are able to oxidize CH_4 (KNOWLES, 1993). Little information is available concerning the influence of different management practices on CH_4 uptake in arable soils. Reduced diffusivity by tillage management can reduce CH_4 uptake rates in soils (BRONSON AND MOSIER, 1993; HANSEN ET AL., 1993).

The objectives of our study were to determine the long-term effects of conventional tillage (CT) and reduced tillage (RT) of two arable loess soils in Germany on i) stocks and the distribution of soil organic carbon and total nitrogen ii) the annual N_2O emission and the CH_4 uptake, iii) the spatial and temporal variation of the N_2O and CH_4 flux rates and iv) the factors that control the spatial and temporal variability of the flux rates.

2.2 Materials and methods

2.2.1 Study sites

The study was conducted on the long-term experimental sites Garte Süd (51°29´15.50´´N, 9°56´9.17´´E) and Hohes Feld (51°37´14.18``N, 9°56`30.98``E) located near Göttingen in southern Lower Saxony, Germany. Mean annual temperatures in the years 2007 and 2008 were 10.0 and 9.8°C for Garte Süd (G) and 10.1 and 9.8°C for Hohes Feld (H). The annual precipitation levels in 2007 and 2008 were 842 and 544 mm for G and 1015 and 564 mm for H, respectively. The soil type of both sites is a Haplic Luvisol (WRB, 2006) derived from loess (EHLERS ET AL., 2000; REITER ET AL., 2002). Soil properties at the site G are shown in Table 2.1 and for H in Table 2.2. The long-term tillage

experiments were established in 1970 at G and in 1967 at H consisting of 8 and 3 replicates whereas we investigated only four replicates at G and all at H. The sizes of single field plots were 20 m x 40 m and 13 m x 36 m at the sites G and H, respectively. At both sites long-term conventional tilled (CT) plots and reduced tilled (RT) plots had been formed, hereinafter referred to as tillage systems. CT plots were tilled with a regular moldboard plough to a depth of 25 - 30 cm followed by a rotary harrow with a tillage depth of 5 - 8 cm. For the RT plots, a shallow cultivation down to 5 - 8 cm with a rotary harrow for seedbed preparation was used.

At G-CT, ploughing occurred on the 27th of March 2007 and the 1st of November 2007. Seedbed preparations with a rotary harrow at G-CT and G-RT were carried out one day later. At H-CT, management was the same as for G-CT, except that harvest of bean and moldboard ploughing were on the 28th of March 2007 and the second moldboard ploughing was on the 31st of October 2007. Seedbed preparation at H-CT and H-RT was carried out on the 29th of March and 1st of November 2007. The rotary harrow was used again after harvest on the 15th of August 2008 at both sites (G and H). Before soils were tilled at the beginning of November 2007 they were mulched with a chopper (26th of September 2007) and milled (31st of October 2007). In addition the G-RT treatments were hoed on the 3rd of May 2007.

The main crop in both experiments was field bean (*vicia faba* L., Fuego) in 2007 and winter wheat (*triticum aestivum* L., Hermann) in 2008. Cropping periods were between the 29th of March 2007 and the 30th of August 2007 (field bean) and between the 1st of November 2007 and the 31st of July 2008 (winter wheat).

After harvest, all crop residues remained on the field and were incorporated by the respective tillage operation. Fertilizer was applied as calcium ammonium nitrate on the 31st of March 2008 (54 kg N ha^{-1}) and 29th of April 2008 (48.6 kg N ha^{-1}) at all four treatments. On the third fertilization date at the 28th of May 2008 G got less calcium ammonium nitrate (43.2 kg N ha^{-1}) than H (67.5 kg N ha^{-1}) but was fertilized a fourth time on the 6th of June 2008 (37.8 kg N ha^{-1}).

Table 2.1: Soil properties of Garte Süd (G) for conventional (CT) and reduced tillage (RT) systems; mean values and standard errors (n = 4). Different letters indicate significant ($p < 0.05$) differences between tillage systems (CT vs. RT comparing the same depth). * indicates a trend of differences ($p < 0.1$) between tillage systems (CT vs. MT comparing the same depth).

	Depth	Sand	Silt	Clay	Bulk density	pH (H_2O)	C_{org}	C_{org}	Total N	Total N
	cm	%	%	%	g cm^{-3}		g kg^{-1}	t ha^{-1}	g kg^{-1}	t ha^{-1}
CT	0-5	11.1 ±0.5	74.6 ±1.0	14.3 ±0.8	1.36 ±0.09	7.0 ±0.1	8.26 ±0.82	5.53 ±0.20	0.90* ±0.08	0.60 ±0.02
	5-10	11.3 ±0.4	74.7 ±1.6	14.0 ±1.2	1.47 ±0.05	7.0 ±0.1	9.20[a] ±0.39	6.73[a] ±0.22	0.99[a] ±0.03	0.72[a] ±0.01
	10-15	11.2 ±0.5	74.1 ±1.7	14.7 ±1.5	1.50 ±0.03	7.0 ±0.1	8.36 ±0.36	6.26[a] ±0.18	0.87 ±0.04	0.65 ±0.02
	15-20	11.5 ±0.7	75.2 ±1.2	13.4 ±0.8	1.48 ±0.02	7.0 ±0.1	8.61* ±0.33	6.36 ±0.27	0.89[a] ±0.03	0.66* ±0.02
	20-25	11.1 ±0.3	75.4 ±1.5	13.5 ±1.3	1.45 ±0.03	7.0* ±0.1	8.88[a] ±0.33	6.43[a] ±0.29	0.92[a] ±0.03	0.67[a] ±0.02
	25-30	11.1 ±0.3	74.8 ±1.7	14.1 ±1.4	1.51 ±0.06	7.1 ±0.1	7.62[a] ±0.31	5.77* ±0.41	0.81[a] ±0.02	0.61 ±0.03
	30-40	10.7 ±0.5	75.1 ±1.6	14.3 ±1.1	1.61[a] ±0.04	7.2 ±0.2	5.80 ±0.80	9.41 ±1.47	0.67 ±0.05	1.08 ±0.10
	40-50	10.1 ±1.0	75.7 ±2.1	14.3 ±1.2	1.50 ±0.07	7.1 ±0.2	4.16 ±0.12	6.28 ±0.48	0.51 ±0.02	0.77 ±0.06
	∑ 0-50							52.77 ±1.68		5.76 ±0.13
MT[1)]	0-5	10.9 ±0.3	77.3 ±0.7	11.8 ±0.4	1.25 ±0.05	6.9 ±0.1	10.49 ±0.94	6.62 ±0.81	1.15* ±0.08	0.72 ±0.07
	5-10	10.7 ±0.1	76.9 ±1.1	12.4 ±1.0	1.41 ±0.02	7.0 ±0.1	11.43[b] ±0.25	8.06[b] ±0.23	1.12[b] ±0.03	0.79[b] ±0.02
	10-15	11.1 ±0.5	77.1 ±1.2	11.9 ±0.8	1.54 ±0.02	7.1 ±0.1	8.92 ±0.17	6.86[b] ±0.08	0.90 ±0.3	0.69 ±0.02
	15-20	11.4 ±0.4	77.1 ±0.8	11.5 ±0.5	1.53 ±0.02	7.2 ±0.2	7.16* ±0.53	5.50 ±0.49	0.76[b] ±0.03	0.58* ±0.03
	20-25	11.0 ±0.4	77.3 ±0.8	11.7 ±0.4	1.52 ±0.01	7.3* ±0.1	6.32[b] ±0.29	4.81[b] ±0.21	0.72[b] ±0.02	0.55[b] ±0.02
	25-30	11.6 ±0.2	77.9 ±1.8	10.6 ±1.5	1.52 ±0.01	7.4 ±0.1	6.30[b] ±0.21	4.77* ±0.12	0.73[b] ±0.02	0.55 ±0.01
	30-40	10.5 ±1.1	76.3 ±1.2	13.2 ±0.0	1.46[b] ±0.03	7.3 ±0.1	5.41 ±0.32	7.86 ±0.33	0.63 ±0.30	0.91 ±0.03
	40-50	9.0 ±0.4	76.0 ±1.3	14.9 ±0.9	1.47 ±0.02	7.2 ±0.1	4.10 ±0.44	6.03 ±0.65	0.51 ±0.04	0.74 ±0.06
	∑ 0-50							50.52 ±1.24		5.54 ±0.12

[1)] n = 2 for texture analysis

Table 2.2: Soil properties of Hohes Feld (H) for conventional (CT) and reduced tillage (RT) systems; mean values and standard errors (n = 3). Different letters indicate significant ($p < 0.05$) differences between tillage systems (CT vs. RT comparing the same depth). * indicates a trend of differences ($p < 0.1$) between tillage systems (CT vs. MT comparing the same depth).

	Depth	Sand	Silt	Clay	Bulk density	pH (H_2O)	C_{org}	C_{org}	Total N	Total N
	cm	%	%	%	g cm^{-3}		g kg^{-1}	t ha^{-1}	g kg^{-1}	t ha^{-1}
CT	0-5	16.4 ±0.9	65.7 ±1.4	17.9 ±0.6	1.32 ±0.08	7.0 ±0.1	9.54[a] ±0.37	6.31[a] ±0.50	0.98[a] ±0.03	0.65[a] ±0.04
	5-10	15.8 ±0.5	65.8 ±1.4	18.4 ±1.2	1.53 ±0.03	7.0 ±0.1	10.37 ±0.97	7.95 ±0.87	1.06 ±0.07	0.81 ±0.07
	10-15	16.0 ±0.6	66.0 ±0.8	18.0 ±0.4	1.48 ±0.04	6.9 ±0.1	11.74[a] ±1.56	8.75 ±1.41	1.10 ±0.07	0.81 ±0.07
	15-20	16.9 ±0.6	65.4 ±0.5	17.7 ±0.4	1.33[a] ±0.06	7.1 ±0.1	9.20 ±0.13	6.11 ±0.38	0.95* ±0.02	0.63 ±0.04
	20-25	19.1[a] ±3.0	64.1 ±2.9	16.9 ±1.0	1.54 ±0.07	7.1 ±0.1	7.59 ±1.32	5.83 ±0.98	0.81 ±0.11	0.62 ±0.09
	25-30	12.8 ±1.6	69.4 ±0.5	17.8 ±1.5	1.60 ±0.04	7.1 ±0.1	4.91 ±0.21	3.94 ±0.23	0.61 ±0.03	0.49 ±0.03
	30-40	11.3 ±2.4	70.5 ±0.3	18.2 ±2.2	1.53 ±0.07	7.1 ±0.2	4.52 ±0.36	6.96 ±0.87	0.57 ±0.02	0.87 ±0.07
	40-50	9.5 ±1.5	71.4 ±1.1	19.1 ±2.6	1.42 ±0.11	7.1 ±0.1	4.03 ±0.55	5.79 ±1.09	0.51 ±0.03	0.73 ±0.09
	∑ 0-50							51.65 ±2.46		5.62 ±0.19
MT	0-5	14.9 ±0.7	67.0 ±0.4	18.2 ±1.1	1.40 ±0.04	6.7 ±0.1	15.82[b] ±0.99	11.05[b] ±0.66	1.52[b] ±0.06	1.06[b] ±0.05
	5-10	16.0 ±0.2	66.1 ±0.7	17.9 ±0.7	1.57 ±0.00	6.9 ±0.1	10.26 ±0.09	8.04 ±0.06	1.09 ±0.30	0.85 ±0.02
	10-15	16.6 ±0.2	65.9 ±0.4	17.5 ±0.4	1.56 ±0.01	7.0 ±0.0	8.53[b] ±0.27	6.64 ±0.17	0.94 ±0.04	0.73 ±0.03
	15-20	14.9 ±1.1	66.4 ±0.1	18.7 ±1.2	1.55[b] ±0.03	7.2 ±0.0	8.30 ±0.44	6.45 ±0.38	0.89* ±0.02	0.69 ±0.02
	20-25	15.4[b] ±0.2	66.8 ±0.9	17.8 ±0.9	1.53 ±0.04	7.1 ±0.0	7.56 ±0.36	5.82 ±0.40	0.83 ±0.04	0.64 ±0.04
	25-30	15.0 ±0.7	68.5 ±0.7	16.6 ±0.7	1.52 ±0.02	7.1 ±0.0	5.44 ±0.57	4.15 ±0.46	0.65 ±0.05	0.49 ±0.04
	30-40	13.1 ±0.6	70.4 ±0.4	16.5 ±0.8	1.43 ±0.07	7.1 ±0.1	5.41 ±0.91	7.72 ±1.41	0.65 ±0.08	0.92 ±0.13
	40-50	10.7 ±1.1	73.2 ±0.7	16.2 ±0.4	1.45 ±0.01	7.1 ±0.1	5.04 ±0.58	7.30 ±0.76	0.59 ±0.05	0.86 ±0.06
	∑ 0-50							57.16 ±1.88		6.26 ±0.17

2.2.2 Measurements of field N_2O and CH_4 fluxes

Weekly gas flux measurements started in March 2007 using static closed chambers (HUTCHINSON AND MOSIER, 1981) over a period of two years. The chamber system consisted of polypropylene chambers which were 28 cm high and 29.5 cm in diameter. These chambers were placed on top of frames of the same diameter and 20 cm height which were randomly inserted into the soil to a depth of 12 cm. The frames remained in place for the entire period, except when removal and reinsertion were required to allow for tillage and harvesting events. Three chambers per plot were used to capture plot variability. The total number of chambers was 24 at G and 18 at H (2 tillage systems, 3 chambers per plot, 4 or 3 replicates, respectively). Chambers were placed between bean plants in 2007, while winter wheat was allowed to grow within the chambers in 2008.

Four gas samples were taken at regular intervals, normally 0, 20, 40 and 60 minutes after closing a chamber. The closing time was extended up to 80 minutes in dry periods with low N_2O flux rates and when the chamber volume was enlarged by extension rings (30 cm height) to include the growing winter wheat in 2008 (11^{th} of June to 24^{th} of July 2008).

Gas samples were collected in 100 ml glass bottles fitted with Teflon locks which were evacuated to a pressure of < 1 mbar. After purging the connection between lock and chamber three times with chamber air, a gas sample was collected by opening the evacuated bottle. Samples were analyzed within one week using an automated gas chromatograph described previously (LOFTFIELD ET AL., 1997).

Two approaches were used and compared to account for very low flux rates which generally show low correlation coefficients (r^2) of the concentration slope during chamber closure. In a first approach, flux rates with $r^2 \geq 0.75$ for N_2O and $r^2 \geq 0.85$ for CH_4 were accepted for further analysis. Fluxes with a r^2 smaller than these limits, or when the difference between repeated standard gas measurement (1050 ppb) were higher than concentration difference of the four subsequent samples, were rated to be insignificant and were assumed to be equal to zero. This approach might have led to an underestimation of small positive fluxes or negligence of N_2O uptake events. The second approach assumed that small

positive N_2O fluxes were existent at the dates when our criteria (r^2 and concentration difference) were not met and minimum detectable N_2O flux rates were thus used. Detection limits for N_2O fluxes were 1.9 µg N_2O-N m^{-2} h^{-1} and for CH_4 fluxes 1.0 µg CH_4-C m^{-2} h^{-1}. Cumulative N_2O and CH_4 fluxes were calculated assuming constant flux rates between two consecutive measurement dates.

2.2.3 Climate data, soil analyses and yields

Precipitation and temperatures for G were acquired from the Deutscher Wetterdienst station that was located 1 km near the site. At H temperature and rainfall were measured directly at the experimental site. At each gas flux measurement day, soil samples were taken from 0 - 10 cm depth on each plot as a mixed sample for determination of soil mineral N and gravimetric water content (mass loss during drying at 105°C for 24 h). Mineral N was determined after extracting 50 g fresh soil with 100 ml of 0.01 mol calcium chloride ($CaCl_2$) for 1 h at 20°C on a horizontal shaker. Extracts were filtered and stored frozen until photometric analysis using a continuous flow analyzer (SA 20/40 Skalar Analytical, Erkelenz, Germany).

At the beginning of the experiment, soil samples were taken in 5 cm steps down to 30 cm and in 10 cm steps from 30 - 50 cm from one soil profile at each site, tillage system and replication. Samples were analyzed for pH in a 0.01 mol $CaCl_2$ solution (5 g soil, 15 ml solution). Organic carbon (C_{org}) and total nitrogen (N_t) were analyzed with an elemental analyzer (Heraeus Vario EL, Hanau, Germany) after pre-treatment with hydrochloric acid (HCl) to destroy carbonates. Soil texture was determined using the pipette method (SCHLICHTING ET AL., 1995). Bulk density was determined gravimetrically on undisturbed soil cores (250 cm^3). Total stocks of C_{org} and N_t were calculated using the bulk densities and C_{org} or N_t contents within the corresponding depths, respectively. Water-filled pore space (WFPS) were calculated by WFPS = (soil gravimetric water content x bulk density) x (1- (bulk density / particle density))$^{-1}$ (LINN AND DORAN, 1984) by

using a particle density of 2.65 g cm^{-3}. Crop yields were quantified after harvest separately for fields, tillage systems and replications.

2.2.4 Statistics

For statistical calculations the software Statistica version 10 (Statsoft inc., USA) was used. Mean values were expressed as arithmetic means with standard errors. Data were checked for normality using the Shapiro-Wilk test ($p \leq 0.05$). When normality was given differences between treatments (CT vs. RT separately for both fields and G vs. H separately for both tillage systems) and partly between the years within treatments were checked using a parametric test (t-test). If the data were not normally distributed and no successful transformation was achieved (in the cases of texture, bulk density, C_{org} content; C_{org}- and N_t-stocks of the soil profile, flux rates, soil properties and crop yields), a nonparametric (Kruscal-Wallis) test was used. Differences were considered significant at a probability value (α) ≤ 0.05. A trend was assumed for $\alpha \leq 0.1$. Correlation analyses were performed using Spearman rank correlation tests.

2.3 Results

2.3.1 Crop yields

Field bean yields neither differed between tillage systems nor between study sites. Mean yields across G-CT and G-RT were 45.7 ± 1.2 and 45.8 ± 2.0 dt ha^{-1} and across H-CT and H-RT 49.1 ± 1.7 and 49.2 ± 0.9 dt ha^{-1}, respectively. The yields of winter wheat did not differ between tillage systems if two of three subplots of the H-CT treatment, which were damaged by wild boars, were excluded. Mean yields across G-CT and G-RT were 103.4 ± 2.1 and 103.8 ± 2.3 dt ha^{-1} and significant higher compared to H with yields of 75.4 dt ha^{-1} (52.7 dt ha^{-1} and 55.5 dt ha^{-1} excluded) and 88.2 ± 1.0 dt ha^{-1}, respectively.

2.3.2 Soil properties

Soil texture was generally dominated by high silt content and soil of all plots was within the texture class silt loam. Differences between tillage systems were not found (Table 2.1 and Table 2.2), whereas the comparison of the two sites exhibited distinct differences. Soil at G contains on average (0 - 30 cm) a lower proportion of sand (11.2 ± 0.3%) and clay (13.2 ± 0.9%) but more silt (75.6 ± 1.0%) compared to the site H (15.8 ± 0.9%, 17.8 ± 0.6%, 66.4 ± 0.9% for sand, clay and silt, respectively). Due to failure of one sample batch during texture analysis of the G-RT treatment, results of soil texture were available for only two replicate plots instead of four.

Soil bulk density (0 - 10 cm) ranged from 1.25 g cm^{-3} to 1.57 g cm^{-3} and no effects of tillage or experimental site were found (Table 2.1and Table 2.2). There were only significant differences in 30 - 40 cm depth at G-CT showing a higher bulk density compared to G-RT and in 15 - 20 cm depth at H, with a significant higher bulk density at H-RT compared to H-CT. For the G-RT treatment, bulk density significantly increased with depth.

The pH values varied between 6.7 and 7.4 independent of tillage system and site. For RT, pH significantly increased with depth down to 30 cm.

Differences in soil moisture (expressed as WFPS) between tillage systems were only found at H with significant higher mean values for H-RT than H-CT within both years. Differences in soil moisture between years were significant (except for G-CT) with higher mean WFPS in the first year than in the second year (Table 2.3). Overall, the time courses of WFPS were similar at both sites (Figure 2.1 and Figure 2.2) with significant lower values during summer (June to August) and higher values during winter (December to February) (data not shown). Differences in WFPS between sites comparing the same tillage system were only significant for RT with higher mean WFPS at H-RT compared to G-RT in both years.

Total stocks of C_{org} and N_t down to a depth of 50 cm did not differ between tillage systems (Table 2.1 and Table 2.2). However, there was a site effect with higher total C_{org} and N_t stocks (0 - 50 cm) at H-RT than at G-RT.

At both sites, tillage systems influenced the distribution of C_{org} and N_t contents (per mass of dry soil) within the soil profiles (Table 2.1 and Table 2.2). While the C_{org} and N_t contents (0 - 25 cm) at the CT systems did not exhibit a significant change, the respective values for the RT systems showed significant decreasing contents with increasing depth. Similar results were found for the stocks per depth (G-RT: $r^2 = 0.45$ and $r^2 = 0.53$; H-RT: $r^2 = 0.80$ and $r^2 = 0.84$ for C_{org} and N_t, respectively).

Mean annual soil NO_3^- contents significantly differed between tillage systems (G: both years; H first year) with higher contents for RT than CT at both sites and years (Table 2.3). With respect to the summer period mean NO_3^- contents were significant higher (> 12.8 mg kg^{-1}) in the second year (main crop winter wheat, N fertilization 170 kg N ha^{-1} as calcium ammonium nitrate) compared to the first year (< 5 mg kg^{-1}) (main crop field bean, no N fertilization). Also mean NO_3^- contents in the winter period were significant higher in the second year, compared to the first year (except H-RT). There were no differences in mean soil NO_3^- contents between sites comparing the same tillage system.

Mean NH_4^+ contents did neither differ between tillage systems nor between sites. Also mean NH_4^+ contents of the summer and winter periods did not differ. Differences of mean NH_4^+ contents between years were significant for the summer periods with higher values in the second year compared to the first for both RT systems.

2.3.3 N_2O fluxes

2.3.3.1 Spatial variability of N_2O fluxes

The results showed a large spatial variation in daily N_2O fluxes. Mean coefficients of variation (CV) within single plots ranging between 85% and 130% and within treatments between 77 and 132%. In general, mean CV within single plots and within treatments were higher for the CT system (106%, 97%) compared to RT system (97%, 89%) and higher for the second year (116%, 109%) compared to the first (92%, 81%), respectively.

The variation of cumulative N_2O fluxes showed even smaller mean CV varying from 18% to 60%. Differences in mean CV between tillage systems were low with 38% for CT and 32% for RT. Except for G-RT, mean CV of cumulative N_2O fluxes were clearly higher for the second year (43%), compared to the first (28%).

Table 2.3:Weather conditions (mean air temperature and total precipitation), mean soil moisture (water-filled pore space), mean soil mineral N content (nitrate and ammonium) and cumulative N_2O-N and CH_4-C fluxes for conventional (CT) and reduced tillage (RT) systems at the two experimental sites Garte Süd (G) and Hohes Feld (H) during the first and the second experimental year; mean values and standard errors (n = 4 for G, n = 3 for H). Different lowercase letters within a column indicate significant ($p < 0.05$) differences between tillage systems (CT vs. RT comparing the same site) and different capital letters indicate significant differences between the sites (G vs. H comparing the same tillage system). # indicates a significant ($p < 0.05$) difference between years (comparing the same treatment).

Treatment	Temp.	Precip.	WFPS		NO_3^-		NH_4^+		N_2O-N		CH_4-C	
	°C	mm	%		mg/kg		mg/kg		g ha^{-1}		g ha^{-1}	
First experimental year (19th March 2007 to 18th March 2008)												
G-CT	9.4#	±0.5	805	65.7	±1.2	2.9a	±0.2	0.04	±0.01	1716#	±380	-1018A ± 20
G-RT				66.3A#	±1.1	3.6b	±0.2	0.04	±0.02	2075#	±331	-1012 ± 53
H-CT	9.5	±0.6	889	69.7a#	±1.8	2.8a#	±0.3	0.08	±0.04	2199#	±147	-544aB ± 62
H-RT				80.4bB#	±1.7	3.9b	±0.3	0.02#	±0.01	1936	±171	-863b# ± 26
Second experimental year (19th March 2008 to 18th March 2009)												
G-CT	7.7#	±0.5	514	61.9	±1.7	5.8a	±0.9	0.67	±0.28	581a#	± 93	-1142A ±109
G-RT				58.7A#	±1.4	7.1b	±1.1	0.69	±0.34	940b#	± 88	-1014A ± 56
H-CT	8.0	±0.6	495	63.2a#	±1.8	6.5#	±1.0	0.67	±0.26	951#	±151	-580B ± 7
H-RT				71.8bB#	±2.1	6.2	±0.9	0.69#	±0.27	1945	±471	-643B# ± 72

2.3.3.2 Temporal flux pattern and tillage effect at Garte Süd

No significant differences between tillage systems were found relating to mean N_2O emission rates at G.

The mean emission rates were significantly higher in the first year (22.9 ± 3.3 and 29.4 ± 4.7 µg N_2O-N m^{-2} h^{-1} for G-CT and G-RT) than in the second year (6.9 ± 1.1 and 12.8 ± 1.9 µg N_2O-N m^{-2} h^{-1} for G-CT and G-RT).

N_2O emissions exhibited pronounced temporal variability in both tillage systems (Figure 2.1). N_2O fluxes were mostly relatively small (< 50 µg N_2O-N m^{-2} h^{-1}), but short periods of high emission rates were observed after hoeing and mulching, attended by elevated WFPS and NO_3^- contents. After a heavy rainfall in May 2007 with 25.5 and 10.7 mm N_2O emissions increased while WFPS became about 70%. During the fertilization period in the second year, only the first fertilizer application resulted in elevated N_2O emissions, coincident with high WFPS, but further fertilization events showing nearly no increase in N_2O emission when WFPS were small.

Maximum emissions occurred during the first winter period with emission rates of 165.1 ± 74.6 and 266.6 ± 109.6 µg N_2O-N m^{-2} h^{-1} for the G-CT and G-RT treatments, respectively. Highest winter emissions of the second year were much lower with maximum values of 25.5 ± 12.4 and 47.4 ± 17.2 µg N_2O-N m^{-2} h^{-1} for G-CT and G-RT, respectively. The portion of winter emissions on total fluxes is presented in section 2.3.3.4.

Within the entire experimental phase, a linear correlation between N_2O emissions and WFPS were found, but with relatively low coefficients of determination (G: $r^2 = 0.08$; G-CT: $r^2 = 0.06$; G-RT: $r^2 = 0.12$). N_2O emissions and NO_3^- contents showed a significant dependency for G-RT, but with a low coefficient of determination ($r^2 = 0.02$). Higher correlations of N_2O fluxes with WFPS were found when NO_3^- contents were elevated (> 5 mg kg^{-1}) and WFPS were higher than 20% (G: $r^2 = 0.24$; G-CT: $r^2 = 0.15$; G-RT: $r^2 = 0.33$).

2.3.3.3 Temporal flux pattern and tillage effect at Hohes Feld

Within the first year, mean N_2O emission rates were not affected by the two different tillage systems (24.2 ± 4.8 and 26.1 ± 5.1 µg N_2O-N m^{-2} h^{-1} for H-CT and H-RT, respectively), but for the second year mean emission rates were about two times higher for H-RT than for H-CT (25.0 ± 3.5 and 10.9 ± 1.7 µg N_2O-N m^{-2} h^{-1}). Comparing mean fluxes of G and H within the same tillage system, significant differences were found for the second year with 1.6-fold and 2-fold higher flux rates under H-CT and H-RT compared to G-CT and G-RT, respectively (data not shown).

H showed a similar temporal pattern of N_2O fluxes compared to G (Figure 2.2). Elevated N_2O fluxes after fertilization occurred solely after the first fertilization event.

The highest N_2O fluxes occurred during the first winter period with maximum emission rates of 316.4 ± 36.9 and 296.7 ± 106.6 µg N_2O-N m^{-2} h^{-1} for H-CT and H-RT, respectively. Maximum winter emissions of the second year were much lower with maximum values of 68.0 ± 16.3 and 73.1 ± 15.2 µg N_2O-N m^{-2} h^{-1} for H-CT and H-RT, respectively. The portion of winter emissions on total fluxes is presented in section 2.3.3.4.

Within the entire experimental phase, a linear correlation of N_2O emissions and WFPS were found, but with relatively low coefficient of determination (H: r^2 = 0.07; H-CT: r^2 = 0.05; H-RT: r^2 = 0.05). N_2O emissions and NO_3^- contents showed a significant dependency for H-RT, but with a low coefficient of determination (r^2 = 0.04). WFPS (> 20%) were found to be positively correlated with N_2O fluxes when NO_3^- contents were elevated (> 5 mg kg^{-1}) (H: r^2 = 0.33; H-CT: r^2 = 0.13; H-RT: r^2 = 0.42).

2.3.3.4 Annual N_2O emission

The annual N_2O emissions ranged from 0.6 to 2.2 kg N_2O-N ha^{-1} yr^{-1} (Table 2.3). At both sites, no differences between tillage systems were found in the first year. In the second year, G-RT showed significantly higher annual N_2O emission than G-CT. Annual N_2O fluxes were 3.0, 2.2 and 2.4 times and thus significantly higher in the first year compared to the second year at G-CT, G-RT, H-CT, respectively. Only H-RT did not show a significant difference between years. However, no significant differences were found in annual N_2O emissions between sites comparing the same tillage system.

Relevant fractions of annual N_2O fluxes originated from the highest N_2O rates during winter (28th of December to 10th of January). These fractions accounted for 20.5%, 30.7%, 27.1% and 23.3% of annual N_2O emissions in the first year and for 19.3%, 30.5%, 23.7% and 21.8% in the second year (G-CT, G-RT, H-CT and H-RT, respectively). In the first year, the three winter months (December to February) accounted for 50.3%, 51.5%, 33.8% and 44.8% of annual N_2O emission at G-CT, G-RT, H-CT and H-RT respectively. In the second year proportions were lower (32.6%, 38.0%, 30.5% and 37.4%), especially for G.

Yield-scaled fluxes derived as the ratio of cumulated N_2O fluxes and dry matter yields were expressed as CO_2-equivalents ($CO_{2\ Eq}$), considering a global warming potential of 298 for N_2O (IPCC, 2007). For G yield based N_2O emissions were 11.4 ± 2.9 and 13.7 ± 2.1 kg $CO_{2\ Eq}$ dt^{-1} for G-CT and G-RT within the first year, respectively. For the second year yield based emissions were significant higher for G-RT (2.7 ± 0.2 kg $CO_{2\ Eq}$ dt^{-1}) than for G-CT (1.7 ± 0.3 kg $CO_{2\ Eq}$ dt^{-1}). No differences between tillage systems were found at the site H with values of 13.4 ± 1.2 and 4.4 ± 0.2 kg $CO_{2\ Eq}$ dt^{-1} for H-CT, and 11.8 ± 1.1 and 6.5 ± 1.3 kg $CO_{2\ Eq}$ dt^{-1} for H-RT within the first and second year, respectively.

2.3.4 CH_4 fluxes

2.3.4.1 Spatial variability of CH_4 fluxes

The results showed a large spatial variation in daily CH_4 fluxes within single plots with coefficients of variation (CV) ranging between 65 and 93% across all treatments. In general, CV were similar for the CT systems (76% and 62%) compared to RT systems (72% and 51%) and also for the second year (79% and 57%) compared to the first (70% and 56%) within single plots and within treatments, respectively.

The variation of cumulative CH_4 fluxes was clearly smaller with CV varying from 7% to 29%. No difference in CV between tillage systems was found (18% for CT and RT). Except for H-CT, CV of cumulative CH_4 fluxes were clearly higher for the second year, compared to the first (G-CT 10% and 25%, G-RT 14% and 29% and H-RT 7% and 26% for the first and second year, respectively). CV of H-CT was 23% and 14% for the first and second year.

2.3.4.2 Temporal flux pattern and tillage effect at Garte Süd

Fluxes were always negative and thus indicating soil uptake of atmospheric CH_4 (Figure 2.1). Mean CH_4 flux rates did not differ between tillage systems and were similar (-11.6 ± 0.6 and -11.8 ± 0.6 µg CH_4-C m^{-2} h^{-1} and -12.5 ± 0.7 and -12.4 ± 0.7 µg CH_4-C m^{-2} h^{-1}) for G-CT and G-RT for the first and second year, respectively.

The pattern of the CH_4 uptake followed a seasonal trend with higher uptake in warm and dry periods. Comparing the CH_4 flux rates of the summer (June to August) and winter (December to February) periods, significant differences were found for all treatments. The highest CH_4 uptake was observed during a dry period with flux rates of -30.3 ± 1.7 µg CH_4-C m^{-2} h^{-1} for G-CT and -28.1 ± 2.3 µg CH_4-C m^{-2} h^{-1} for G-RT.

Significant positive linear regression were found between CH_4 uptake and temperature (G: $r^2 = 0.14$; G-CT: $r^2 = 0.22$; G-RT: $r^2 = 0.08$).

Moreover the WFPS was negatively correlated with the CH_4 uptake. Correlations were generally higher in the second year (r^2 = 0.26) than in the first year (r^2 = 0.14). Within the first year, correlations between CH_4 uptake and WFPS were similar for both tillage systems (r^2 = 0.14 and r^2 = 0.15 for G-CT and G-RT, respectively). In the second year the correlation at G-CT was higher (r^2 = 0.35) than at G-RT (r^2 = 0.16).

2.3.4.3 Temporal flux pattern and tillage effect at Hohes Feld

Mean CH_4 uptake rates of the first year were significantly different between tillage systems (-6.0 ± 0.6 and -9.2 ± 0.6 µg CH_4-C m^{-2} h^{-1} for H-CT and H-RT, respectively) whereas the rates of the second year did not differ between tillage systems (-6.4 ± 0.7 and -6.6 ± 0.7 µg CH_4-C m^{-2} h^{-1} for H-CT and H-RT, respectively). In general, CH_4 uptake was lower at the H site compared to G.

The temporal pattern of CH_4 uptake observed at G was found at H as well (Figure 2.2). Uptake rates varied seasonally with higher consumption in dry and warm seasons and lower or even no uptake during wet and cold periods. Comparing the CH_4 flux rates of the summer (June to August) and winter (December to February) periods, significant differences were found for all treatments (except H-CT for the second year).

Correlations at H were found between CH_4 uptake and temperature with uptake increasing with temperature in the first year (H-CT: r^2 = 0.30; H-RT: r^2 = 0.24). No such correlation was found for H-CT in the second year and only a weak correlation at H-RT (r^2 = 0.09).

Furthermore negative correlations between CH_4 uptake and WFPS were found in both years, but only for the H-RT treatment (first year: r^2 = 0.23; second year: r^2 = 0.11, respectively).

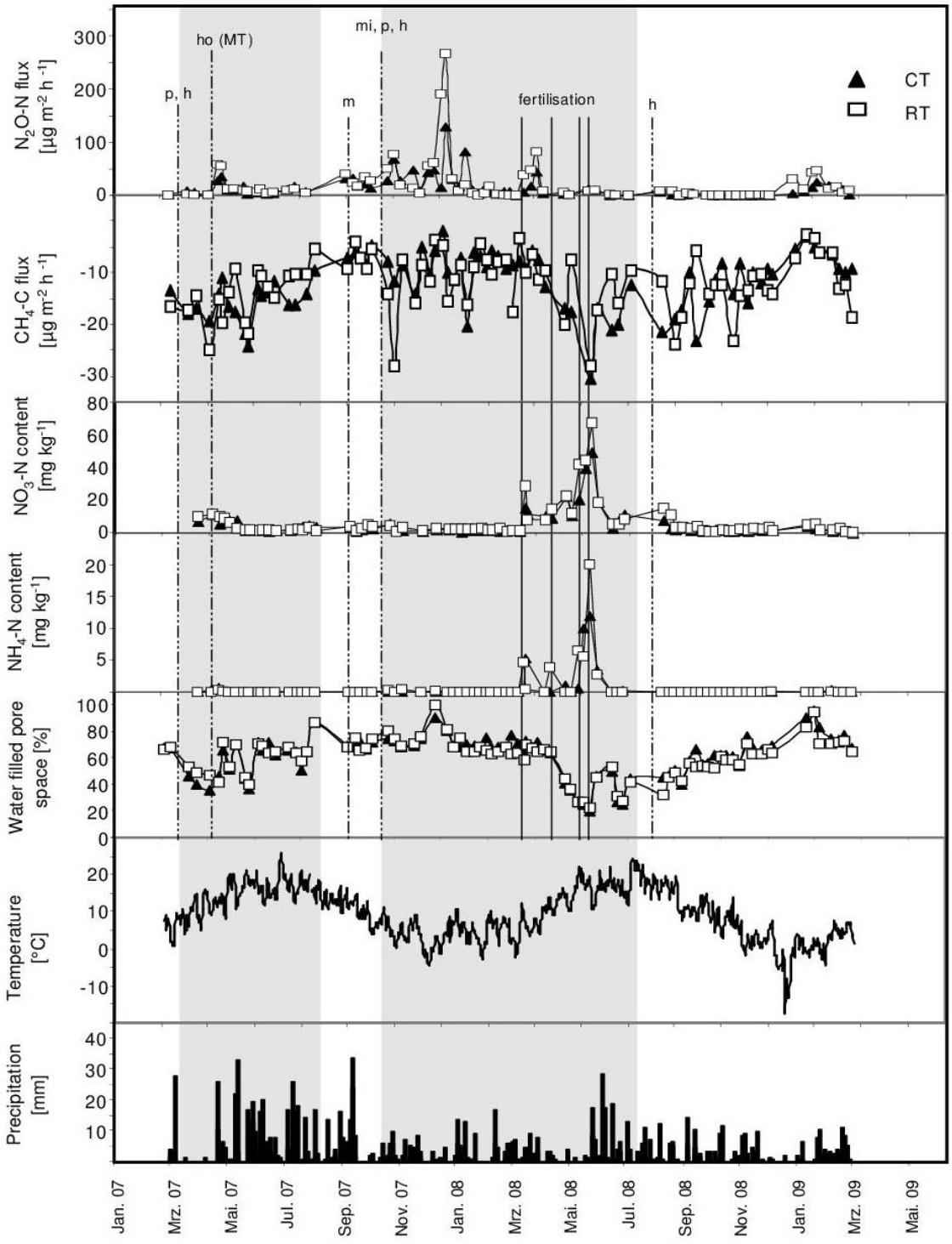

Figure 2.1: Time courses of N_2O and CH_4 flux rates, soil nitrate and ammonium content (0 - 10 cm), soil water-filled pore space (0 - 10 cm), air temperature and weekly precipitation for the conventional tilled (CT) and reduced tilled (RT) plots of the site Garte Süd. Dates of soil management are indicated by dashed vertical lines (p=ploughing, h=harrowing, ho=hoeing, m=mulching, mi=milling), dates of fertilizer application are marked by solid vertical lines (54, 49, 43, 38 kg N ha^{-1} as calcium ammonium nitrate). Light gray shaded areas represent vegetation periods (main crop in 2007 field beans and in 2008 winter wheat).

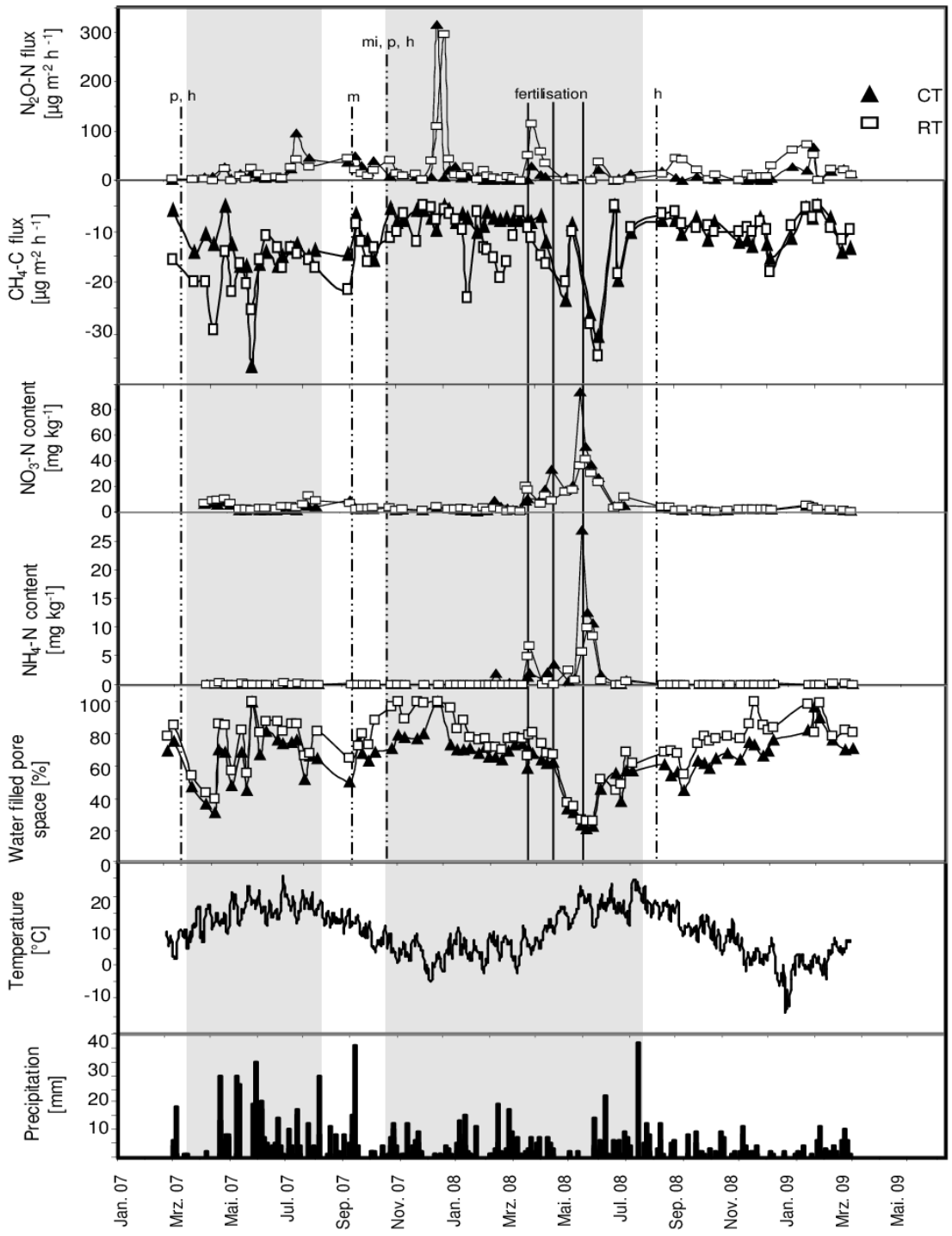

Figure 2.2: Time courses of N_2O and CH_4 flux rates, soil nitrate and ammonium content (0 - 10 cm), soil water-filled pore space (0 - 10 cm), air temperature and weekly precipitation for the conventional tilled (CT) and reduced tilled (RT) plots of the site Hohes Feld. Dates of soil management are indicated by dashed vertical lines (p=ploughing, h=harrowing, m=mulching, mi=milling), dates of fertilizer application are marked by solid vertical lines (54, 49, 67.5 kg N ha^{-1} as calcium ammonium nitrate). Light gray shaded areas represent vegetation periods (main crop in 2007 field beans and in 2008 winter wheat).

2.3.4.4 Annual CH_4 fluxes

Annual CH_4 uptakes at G were about 1 kg CH_4-C ha^{-1} yr^{-1} with no significant effect of tillage system (Table 2.3). At H, a significant difference between tillage systems was found within the first year with a higher uptake of H-RT than H-CT. Significant differences between years comparing the same treatment were only found for H-RT with a higher CH_4 uptake within the first year. CH_4 uptake at H was significantly lower (0.5 to 0.9 kg CH_4-C ha^{-1} yr^{-1}) than at G comparing the same tillage system for both years (except RT first year).

2.4 Discussion

2.4.1 Long-term effects of tillage on N_2O emissions

2.4.1.1 Annual N_2O emissions

The total annual N_2O losses measured at the loess derived fields in Germany varied between 0.6 and 2.2 kg N_2O-N ha^{-1} yr^{-1} and fell within the range of other investigations under comparable conditions. JUNGKUNST ET AL. (2006) summarized all available data from Germany on annual N_2O emission rates derived from field experiments of at least an entire year and reported rates from 0.04 to 17.1 kg N_2O-N ha^{-1} yr^{-1}. ERNST (1997), KAISER ET AL. (1998) and RÖVER ET AL. (1998) published N_2O emission rates resulting from the same soil type located in Lower Saxony and with similar clay, C_{org} and N_t contents under similar climate conditions compared to our sites. Emissions reported in these studies ranged between 1.8 - 2.5, 1.7 - 3.2 and 1.8 - 3.5 kg N_2O-N ha^{-1} yr^{-1}, respectively.

In our study, no significant differences in annual N_2O emissions between tillage systems were found (except for G within the second year). ABDALLA ET AL. (2010) reported for Irish sandy loams under temperate oceanic climate that reduced tillage had no significant effect on N_2O fluxes from soils in a 2-year field experiment cropped with barley. USSIRI ET AL. (2009) observed no differences in N_2O emissions between CT and chisel till systems on a silt loam for continuous

corn under humid continental climate, too. Contrariwise BEHEYDT ET AL. (2008) reported for minimum tillage (MT) and CT systems under temperate, humid marine climate on silty soils that N_2O losses from MT maize fields were significantly higher than those from a CT maize field (period of 1 year) comparable to the G site in the second year.

Due to the cropping of a legume in 2007 the two to three times higher annual N_2O emissions in the first year were not expected. Also because of the application of mineral N fertilizer in 2008 higher N_2O emissions were expected but the increase of N_2O emissions was probably counterbalanced by the considerable drier conditions in 2008 and the higher frost-induced effect in 2007 (see 4.1.3).

With respect to the yield-scaled N_2O emissions, smaller values are attributed to highly productive and environmentally sustainable cropping systems. High crop yields and high nitrogen use efficiency contribute to low yield-scaled N_2O emission. Our investigation only covered two years but differences of yield scaled N_2O fluxes between tillage systems were negligible although the ranges of crop yields are above the average crop yields for field beans and winter wheat in Germany. Crop yields of field bean were about 10 to 15 dt dry matter (dm) ha^{-1} higher compared to the average crop yields of 35.3 dt dry matter (dm) ha^{-1} for field beans in Germany in 2007 (STATISTISCHES BUNDESAMT DEUTSCHLAND, 2009). For winter wheat, yields were about 10 and 20 dt dm ha^{-1} higher than the lowest and highest averaged crop yield range (65.5 to 82.1 dt dm ha^{-1} in the years 2003 to 2007, STATISTISCHES BUNDESAMT DEUTSCHLAND, 2009). Anyway, crop yields were almost identical for both tillage systems whereas the yields of winter wheat were higher at the G site.

N_2O emissions in agricultural soils are estimated from the total nitrogen input via fertilizer and crop residues assuming that on average 1% of this nitrogen input is emitted as N_2O-N (IPCC, 2006). At our experimental sites a very low fraction of applied fertilizer (0.003, 0.006, 0.05 and 0.011 kg N_2O-N kg^{-1} of applied N) was lost as N_2O for G-CT, G-RT, H-CT and H-RT in the second year, respectively.

In general, long-term effects of CT and RT system on annual N_2O emissions were not found. Anyway, our results are not transferable to no-till soils, where effects of tillage on N_2O emissions were often more pronounced as found amongst others by SMITH AND CONEN (2004) and ROCHETTE (2008). Nonetheless, annual fluxes are in the range of previously published studies and the considerably differences between years are discussed in the following two sections.

2.4.1.2 Spatial and seasonal variation of N_2O emissions

The high spatial variation in daily N_2O fluxes found in our study reflected the natural soil heterogeneity, and were determined by comparable studies as well (CARRAN ET AL., 1995; CHOUDHARY ET AL., 2002). Cumulative CV of treatments were between 18 to 60%, whereas CHOUDHARY ET AL. (2002) observed CV ranging between 39 and 140% and LEHMKE ET AL. (1998) reported up to 92% due to differences in soil clay content in that experimental site.

In several studies (BRONSON AND MOSIER, 1993; CATES AND KEENEY, 1987) similar temporal patterns of N_2O fluxes from arable soils with short peaks of very high emission rates which emphasizes the need for long-term studies to calculate annual emission rates (FLESSA ET AL., 1995). To accurately estimate annual net greenhouse gas fluxes for an agroecosystem, gas flux determinations must also be made at critical times near tillage and wetting events (KESSAVALOU ET AL., 1998). Our results underline this need as we found significant differences between years, large variation in time and increased N_2O emissions appearing with tillage, rewetting, fertilization and thawing events.

According to the two approaches described in section 2.2.2, substitution of zeroized values (approach one) with minimum detectable N_2O fluxes (approach two) did not change the differences between systems, sites or years. But daily mean fluxes calculated with approach two were 1.2% to 1.7% higher for the first and 2.8% to 14.3% higher for the second year. Cumulative N_2O fluxes calculated with approach two showed the same significant differences between systems, sites or years compared to the calculated fluxes of approach one, but showed

1.6% to 8.5% higher values. The comparison show that the two approaches to account for low and uncertain flux rates led to similar results. We like to point out that it is important not to omit but to include these close to zero fluxes in the data evaluation as they are an important part of the emission characteristics of many sites.

The high spatial and temporal variations of N_2O emissions follow from the complexity of interactions between various factors, even if those factors are not always strongly correlated with N_2O fluxes (CHOUDHARY ET AL., 2002). In the following, we discuss to which extent the mentioned high spatial and temporal variability within our study are explainable by various parameters and their interactions.

2.4.1.3 Factors that control the variability of N_2O emissions

2.4.1.3.1 Soil properties

Under CT management plant residues and soil are mixed up to the maximum tillage depth, causing soil organic matter being evenly distributed within the tilled layer. This explains why C_{org} and N_t contents only decrease with depth (0 – 25 cm) under RT in our study whereas STOCKFISCH ET AL. (1999) made similar observations. Several studies have reported higher soil C_{org} concentrations under conservation tillage practices than ploughed soils due to disruption of soil aggregates and increased soil respiration as a result of the release of protected soil C_{org} in the latter (CAMPBELL ET AL., 1996; SIX ET AL., 2000a; LEWIS ET AL., 2011; KAHLON ET AL., 2013). We indeed found higher C_{org} contents in the topsoil of the RT treatments but total C_{org} and N_t stocks (0 - 50 cm) did not show differences between tillage systems.

Investigations where changes in C retention have been estimated through soil sampling of different tillage experiments had led to the widely belief that considerable soil organic carbon (SOC) sequestration can be achieved by adoption of conservation tillage. However, sampling protocol may have biased the results as BAKER ET AL. (2006) figured out. They revealed that in studies

which found C sequestration under RT, soil was only sampled to a depth of 30 cm or less. When sampling extended deeper than 30 cm, CT has shown no consistent accrual of SOC (BAKER ET AL., 2006).

Even if total stocks did not differ between tillage systems, the differences of C_{org} and N_t stocks between the RT treatments of the two sites might be attributed to a higher proportion of clay-humus complexes derived at the site containing more clay.

In our study, increased fluxes after fertilization (calcium ammonium nitrate) were only found in combination with high WFPS (> 60%) and high NO_3^- contents at the beginning of the fertilization period. The mineral N content is known as a main factor influencing N_2O emissions (BALL ET AL., 1997b; CASTALDI AND SMITH, 1998; SENEVIRATNE AND VAN HOLM, 1998) but correlation between the N_2O emissions and the NO_3^- contents were small.

The missing precipitation and warm temperatures during further fertilizer applications resulted in relatively dry soil. Therefore, conditions were not favorable for denitrification. We conclude further that there were no elevated fluxes from nitrification, possibly because of intensive N-uptake of the vegetation and because the N_2O yield of nitrification is relatively small at all, i.e. mostly < 1% (WELL ET AL., 2008). The peak emissions at H three weeks after the first fertilizer application occurred in combination with raised water content after a heavy rainfall and high NO_3^- contents, suggesting denitrification as source process.

We confirm that long-term tillage influences the C_{org} and N_t distribution but overall stocks did not differ between CT and RT, and that soil texture influences the C_{org} and N_t storage ability. Anyway, the differences of C_{org} and N_t distribution between tillage systems did not affect annual N_2O emissions. As substrate for N_2O production mineral N is essential but not a key factor controlling temporal variability of N_2O emission at our sites.

2.4.1.3.2 Climate

Most of the time the flux rates during winter were small due to low temperatures and therefore low microbial activity. However, highest flux peaks were found during the winter periods confirmed by studies of FLESSA ET AL. (1995) and KAISER ET AL. (1998).

The extraordinarily high N_2O release during thawing of the frozen soil can be explained in two different ways: a) release of trapped N_2O by melting of the ice barrier (BURTON AND BEAUCHAMP, 1994; TIETEMA ET AL., 1991); b) increased denitrification activity due to the release of organic matter available for denitrification by killing soil organisms and disintegrating aggregates (CHRISTENSEN AND CHRISTENSEN, 1991; CHRISTENSEN AND TIEDJE, 1990). The N_2O peak of the first winter was found after a 7 day period with soil freezing in December 2007, followed by temperatures about 0°C the day before measurement. The day the samples were taken, soil started to thaw with free water on the topsoil and cracks were created in soil. Freezing soil for less than one day had negligible effects, but freezing for longer periods caused concomitant increases in emissions (TEEPE ET AL., 2004). It is likely that the observed high N_2O fluxes in December 2007 originated from N_2O production of deeper soil layers, which escaped along the frost-induced cracks. This incident has also been observed by KAISER AND HEINEMEYER (1996) and KAISER ET AL. (1998) in soils of the same type. The daily freezing and thawing cycles resulting in high N_2O emissions were observed during spring thaw by CHRISTENSEN AND TIEDJE (1990) and FLESSA ET AL. (1995), too.

The magnitude of cumulative N_2O loss appeared to be related to the annual precipitation, as two to three times higher N_2O losses were found in the first year when precipitation was marginal 200 mm higher than the long-term average, compared to the second year, which was about 100 mm less than the long-term average. If winter derived N_2O emissions were subtracted, differences between years became even more obvious with two to five times higher emissions in the wetter year. MALHI AND LEMKE (2007) observed a similar relationship between precipitation level and N_2O emissions in a 4 year rotation cycle in Canada.

Differences in WFPS between sites were only evident for the RT system and might be attributed to the higher clay and C_{org} content at H and the water retention effect of RT in general. The water retention effect of RT results from the crop residues left at the soil surface which forms a barrier against evaporation and those retained crop residues isolate the soil from heating up and reflect solar radiation (SHINNERS ET AL., 1993).

Higher precipitation levels result in higher mean annual WFPS values. The WFPS in turn differs between tillage systems at the site H in contrast to the amount of rain which is equal for the tillage treatments.

Even if we found significant higher WFPS at H-RT compared to H-CT in both years, these differences were not reflected in annual N_2O emissions. This might be come from some kind of threshold for N_2O released during denitrification at our sites. N_2O fluxes from denitrification typically start around 55% and increase until 70% WFPS after which the N_2O emission often declines in favor of N_2 production (BEHEYDT ET AL., 2008). Our results showed that important field peak N_2O fluxes coincided with WFPS values higher than 60%. However, not all high WFPS values resulted in enhanced N_2O emissions. In the first wetter year annual N_2O emissions reached about 2 kg N_2O-N ha^{-1} at mean WFPS of 66% to 80% and mean NO_3^- contents of 3 to 4 mg kg^{-1}. Although differences in WFPS and NO_3^- contents between tillage systems were almost significant and higher values might have favor denitrification and thus higher N_2O emissions, our investigations did not capture N_2O increase for the RT treatment. Probably this might result from N_2O reduction to N_2. This would explain why H-RT in the second year, with a mean WFPS of 72% and a mean NO_3^- content of 6 mg kg^{-1} showed the same amount of cumulative N_2O emission compared to the other treatments in the first year. Contrary, G-CT, G-RT and H-CT showed mean WFPS < 63% and consequently lower N_2O emissions in the second year compared to the first year.

At both sites, heavy rainfall events after dry periods had been observed with increased N_2O fluxes. Released C from die off of microbial biomass in dry periods can become available during rewetting, (MARIOTTI ET AL., 1981;

KALBITZ ET AL., 2000). Furthermore a reactivation of the soil microbial communities (CABRERA, 1993; DAVIDSON ET AL., 1993) and a release of available C by the disruption of soil aggregates (LUNDQUIST ET AL., 1999) might have resulted in N_2O pulses as the increased C turnover is associated with an enhanced O_2 consumption which stimulates denitrification (FLESSA AND BEESE, 1995).

We conclude that up to 50% of annual N_2O emissions were caused by freeze-thaw cycles and the freezing effect was besides other factors (e.g. precipitation level) responsible for the significantly higher annual N_2O losses of the first experimental year. The coincidence of high WFPS and NO_3^- reveals denitrification events with peak fluxes in some cases. The lack of elevated N_2O fluxes at certain dates under conditions favorable for denitrification might be due to increased N_2O reduction. But this hypothesis can only be validated using further field studies including N_2O reduction, e.g. using stable isotopes.

2.4.2 Long-term effects of tillage on CH_4 uptake

2.4.2.1 Annual CH_4 uptake

Although soils can act as both a sink and a source for CH_4, none of our investigated soils exhibited a net CH_4 production, possible due to adequate soil aeration and no impact of groundwater down to 5 m depth was observed at our sites.

The annual uptakes of all treatments (0.5 to 1.1 kg CH_4-C ha^{-1} yr^{-1}) were lower than values published by SMITH ET AL. (2000) who found an annual CH_4 uptake of 1.6 kg CH_4-C ha^{-1} yr^{-1} in Northern European soils. FLESSA ET AL. (1995) found 0.4 kg CH_4-C ha^{-1} yr^{-1} in a similar textured soil compared to the H site.

In our study neither significant differences in mean CH_4 oxidation rates between tillage systems nor between years were found (except at H, first year). SMITH ET AL. (2000) summarized CH_4 oxidation rates of arable soils in Denmark, Norway and the UK of 7 to 16, 1 to 10 and -3 to 46 µg CH_4-C m^{-2} h^{-1}. BOECKX ET AL. (1997) observed oxidation rates of 11.4 to 14.5 µg CH_4-C m^{-2} h^{-1} for intact soil

cores of arable land differing in texture. While rates observed for G were within the range, CH_4 oxidation rates found at H were lower (6.0 to 9.2 µg CH_4-C m^{-2} h^{-1}). Anyway, FLESSA ET AL. (1995) found flux rate of 4.7 µg CH_4-C m^{-2} h^{-1} in southern Germany, which was in the same order of magnitude as uptake rates in arable soils reported by BRONSON AND MOSIER (1993) and HANSEN ET AL. (1993).

With respect to the effect of long-term tillage on CH_4 uptake rates, other studies found higher CH_4 uptake rates under RT (COCHRAN ET AL., 1997; HÜTSCH, 1998); KESSAVALOU ET AL., 1998; ROBERTSON ET AL., 2000; VENTEREA ET AL., 2005) as CH_4 diffusion into oxidizing zones is facilitated due to more stable and porous soil structure (BALL ET AL., 1997a). CT alters therefore soil structure such that porosity decreases and CH_4 diffusion into the soil is thus reduced (DEL GROSSO ET AL., 2000). Furthermore the higher microbial activity, the higher C_{org} content and the higher water holding capacity at RT soils favor CH_4 oxidation.

The fact that the first year was wetter compared to the second year might be an explanation for the observed differences in CH_4 uptake between tillage systems at the site containing more clay. Although WFPS at H-RT was significant higher compared to H-CT the CH_4 uptake was higher. Under general higher WFPS in combination with the proposed improved pore continuity the positive effect of enhanced diffusion of atmospheric CH_4 into soil under RT might be more important than at drier conditions. That would imply a negligible inhibitory effect of the elevated moisture. Moreover, the difference in aggregate stability and diffusion conditions between RT and CT might be more important for H with higher clay and surface C_{org} content compared to G. More information is needed to confirm our hypothesis. The vertical distribution of CH_4 concentrations profiles in combination with soil properties might be helpful.

The differences in CH_4 uptake observed for the two experimental sites are related to textural discrepancy. The mean annual CH_4 uptake amounted to 1.1 kg ha^{-1} yr^{-1} at G independent of tillage system and year. CH_4 uptake of H was significantly lower (about half as high) varying between 0.5 and 0.6 kg CH_4-C ha^{-1} yr^{-1}, except for H-RT within the first year (0.9 kg CH_4-C ha^{-1} yr^{-1}). It has

been reported, that coarse textured soils showed a higher CH_4 uptake rate than fine textured soils (BOECKX ET AL., 1997) and were linked to an increasing soil tortuosity, reducing the CH_4 oxidation rate in fine textured soils.

Overall, we did not find differences between different tillage systems on the CH_4 uptake in general, but texture apparently affected the total CH_4 consumption at our sites.

2.4.2.2 Spatial and seasonal variation of CH_4 uptake and factors that control those variability

Spatial variation of daily CH_4 uptake rates within single plots and within treatments as well as for cumulative CH_4 uptake were lower than variations of N_2O rates. CV of total annual CH_4 fluxes range from 7 to 29% and were in the range of CV published by FLESSA ET AL. (1995) (6 to 23%). They found slightly lower CV than those for N_2O fluxes as well. That CV for the RT treatments were smaller than for the CT treatments possibly resulted from the more undisturbed soil structure and therefore supported methanogenic bacteria. Tillage disrupts soil structure, probably perturbing biological, chemical and physical parameters that define ecological niche for methanotrophic bacteria (HÜTSCH, 1998). Studies have shown that vertical distributions of aerobic and anaerobic microbial populations and potential denitrifying activity tend to vary in ploughed versus untilled soil profiles (GROFFMAN, 1985; LINN AND DORAN, 1984b; VENTEREA ET AL., 2005). In the present study, inhibitions of CH_4 uptake directly after tillage events were found after several management operations.

The greater CH_4 uptake observed in the summer month was likely due to enhanced diffusion of atmospheric CH_4 into the soil under drier conditions. Similar relations between soil factors and CH_4 flux rates were found by FLESSA ET AL. (1995) and PETERJOHN ET AL. (1994).

Heavy rainfall events resulted in clear reduction of CH_4 uptake on several occasions, attributed to retarded gas diffusion as reported by CRILL (1991) and GUCKLAND ET AL. (2009). This is particularly true for fine-textured soils where diffusive gas exchange is slow (BORN ET AL., 1990; CRILL, 1991; KOSCHORRECK

AND CONRAD, 1993; SMITH ET AL., 2000), which might explain the lower CH_4 uptake at H.

We conclude that the significantly high and positive correlations between WFPS and CH_4 uptake for all treatments demonstrate that moisture was a main control of CH_4 uptake on a daily basis. Temperature accounted for up to 30% of variation and played also an important role. No clear effect of tillage on the CH_4 uptake was found and differences between years despite distinct differences in moisture were insignificant. The differences of CH_4 uptake between sites were attributed to different soil structure.

2.5 Interim conclusions

No significant tillage effect on N_2O emissions was found and the heights of N_2O emissions (0.6 to 2.2 kg N_2O-N ha^{-1} yr^{-1}) were found in other studies with comparable characteristics. We found significant differences in the C_{org} and N_t distribution in the soil profile between tillage systems which did not affect annual N_2O emissions. Overall C_{org} and N_t stocks did not differ between tillage systems, but the site containing more clay showed higher stocks. The combination of high NO_3^- availability and WFPS resulted in elevated N_2O fluxes, whereas those peaks were attributed to denitrification as source process. Freezing/thawing cycles were identified as hot moments of N_2O fluxes accounting for about 23% to 27% of the annual fluxes and winter emissions (December to February) represented 34% to 52% of total annual N_2O fluxes. The freezing effect was in addition to the precipitation level responsible for the higher N_2O loss of the first year.

Soils of all treatments act as a CH_4 sink and the tillage system had no general effect on annual CH_4 uptakes. Differences in CH_4 consumption between years were sparsely observed and inconsistent with respect to precipitation level. General differences between sites could be attributed to textural effects with smaller uptake rates of atmospheric CH_4 at the fine textured soil. The time patterns reflected temperature and moisture effects.

Overall our data indicated that the annual N_2O fluxes and CH_4 uptakes of the investigated arable soils were influenced rather by soil properties as well as climate and short-term management effects than by tillage systems.

2.6 Summary of the chapter

Soil management effects on greenhouse gas fluxes are of growing importance due to the high contribution of agriculture to global warming. The objectives of our study were to determine the effects of long-term conventional tillage versus long-term reduced tillage of two arable loess soils in Germany on i) stocks and the distribution of soil organic carbon and total nitrogen ii) the annual N_2O emission and the CH_4 uptake, iii) the spatial and temporal variation of the N_2O and CH_4 flux rates and iv) the factors that control the spatial and temporal variability of the flux rates. N_2O and CH_4 fluxes were measured weekly over a period of two years by using the closed chamber technique. The main crops in both experiments were field bean (*vicia faba* L., Fuego) and winter wheat (*triticum aestivum* L., Hermann) in the first and the second experimental year, respectively. Annual N_2O emission varied between 0.6 and 2.2 kg N_2O-N ha^{-1} yr^{-1}. We found no significant differences in N_2O fluxes between tillage systems (except at one site within the first year), but differences between years could be attributed to differences in precipitation level and a higher contribution of freeze-thaw cycles of the first winter. During the vegetation period, N_2O fluxes were highest after fertilization in combination with high soil water contents. During winter, highest emissions were found when freeze/thaw cycles induced high N_2O losses accounting for up to 27% of the annual N_2O emission. Winter emissions (December to February) ranged from 34% to 52% of annual N_2O loss. Net uptake of atmospheric CH_4 varied between 0.5 and 1.1 kg CH_4-C ha^{-1} yr^{-1}. The tillage systems did not affect annual CH_4 uptake in general whereas differences were significant between sites which was attributed to textural discrepancy. Lower CH_4 uptake was found at the site with the higher clay content. At this site the RT system showed a higher CH_4 uptake compared to CT although the mean WFPS

was higher, which was attributed to an improved pore continuity having a stronger impact on CH_4 exchange than the inhibitory effect of elevated moisture. Soil temperature was positively and water content was negatively correlated with CH_4 uptake. At our investigated German loess soils the net N_2O and CH_4 exchange were not affected by long-term adaptation of conventional versus minimum tillage in general. But, soil properties as well as climate and management effects could be attributed to spatial and temporal variation of flux rates.

3 Modelling of crop yields and N_2O emissions from silty arable soils with differing tillage in two long-term experiments*

* note the information in the preface and outline section (XVIII)

3.1 Introduction

Tillage systems affect crop yields and nitrous oxide (N_2O) emissions. DECKER ET AL. (2009) studied the economics of five wheat (*Triticum aestivum* L.) production systems with no-till (NT) and conventional tillage (CT) in the Southern Plains of the United States and reported that average wheat grain yields were greater with CT, whereas average fall wheat forage yields were significantly greater on the NT plots. ALMARAZ ET AL. (2009) reported for clay loam Alfisols in Quebec in a corn cropping system that the CT and NT systems had either no significant or only small differences in grain yields, but significantly higher forage yields under NT. The tillage systems generally had similar cumulative carbon dioxide (CO_2) emissions but NT had higher cumulative N_2O emissions (3.7 to 5.5 kg N_2O-N ha^{-1}) than CT (2.3 to 4.1 kg N_2O-N ha^{-1}) in fertilized (180 kg N ha^{-1}) and unfertilized treatments. Furthermore, ROCHETTE (2008) summarized 25 field experiments on a number of soil types with a wide range of textures by concluding that the impact of NT on N_2O emissions depended markedly on the aeration of the soils: on average, N_2O emissions under NT were 0.1 kg N ha^{-1} lower, 0.1 kg N ha^{-1} higher and 2.0 kg N ha^{-1} higher than from tilled soils with good, medium and poor aeration, respectively.

Less information is available for fields with reduced (RT) or minimum tillage (MT). BEHEYDT ET AL. (2008) reported for MT and CT systems on silty soils that N_2O losses from MT maize (*Zea mays* L.) and MT oats (*Avena sativa* L.) fields (5.3 and 3.6 kg N_2O-N ha^{-1}, respectively) were significantly higher than those from a CT maize field (0.3 kg N_2O-N ha^{-1}) over a period of 1 year. ABDALLA ET AL. (2010) observed for Irish sandy loams that RT had no significant effect on N_2O fluxes from soils and barley (*Hordeum vulgare* L.) grain yield in a 2-year field experiment compared to CT. Similarly, USSIRI ET AL. (2009) reported no differences in N_2O emissions for CT and MT systems on a silt loam for continuous corn. Overall, different tillage systems may affect crop yields and

emissions of greenhouse gases, with soil aeration being one of the factors affecting the magnitude of the emissions.

Process-based models help us to understand or even predict the effects of different tillage systems on crop yields and greenhouse gas emissions. Several process-oriented models exist for this task (e.g. DAYCENT, CANDY, ExpertN or the Denitrification-Decomposition (DNDC) model (PARTON ET AL., 2001; FRANKO ET AL., 2007; KAHARABATA ET AL., 2003; LI, 2009; for a review see CHEN ET AL., 2008)). Together with long-term experiments, these computer models may improve our understanding of the interactions between agricultural management, soil quality and the global environment.

The DNDC model has been successfully applied to sites with different tillage systems in several studies. FARAHBAKHSHAZAD ET AL. (2008) applied the DNDC model to a row-crop field in Iowa, USA and carried out sensitivity tests. Model results indicated that NT significantly increased storage of soil organic carbon (SOC) and reduced nitrate-N leaching rate, but slightly decreased crop yield and increased N_2O emissions. LI ET AL. (2010) applied a similar sensitivity study to a winter wheat maize rotation system in China and reported that temperature, initial SOC content, tillage, and quantity and quality of the organic matter added to the soil had significant effects on simulated greenhouse gas emissions.

In contrast, ABDALLA ET AL. (2009) used the DNDC model less successfully for Irish agriculture. They reported that the simulations using DNDC underestimated measured N_2O fluxes from RT plots up to 55%. The main problems were an overestimation of the water filled pore space (WFPS) and the effect of SOC on the flux. BEHEYDT ET AL. (2008) reported for field experiments in Belgium a marked overestimation of N_2O emissions with DNDC (CT, maize), a good agreement (MT, oats) and an underestimation (MT, maize).

The differences in performance of the DNDC model might be due to different soil and climatic conditions and may depend to a large extent on the model parameterization. Unfortunately, not in all studies cited above, model parameterization is sufficiently documented.

Therefore, the objectives of our study were to apply a proposed calibration and validation scheme for field experiments with CT and RT on silty soils near Göttingen (Germany) in order to test the usefulness of the DNDC model in describing and predicting crop growth and N_2O emissions.

3.2 Materials and methods

3.2.1 Study sites

We tested the DNDC model with data from two long-term trials near Göttingen, Germany (JACOBS ET AL., 2009; BERGSTERMANN AND FLESSA, 2009). Briefly, the experimental trials were carried out at Garte (51°29'15.50"N, 9°56'9.17"E) and Hohes Feld (51°37'14.18"N, 9°56'30.98"E). The mean temperature in the years 2007 and 2008 were 10.0 and 9.8°C for Garte and 10.1 and 9.8°C for Hohes Feld. The annual precipitation in 2007 and 2008 was 842 and 544 mm for Garte and 1015 and 564 mm for Hohes Feld. The soil type at both sites is a Haplic Luvisol (WRB, 2006) derived from loess (EHLERS ET AL., 2000; REITER ET AL., 2002). For soil properties see Table 3.1.

Table 3.1: Site characteristics for the soil (0 - 5 cm) of the conventional (CT) and reduced tillage (RT) treatments at Garte[a] (G-CT, G-RT) and Hohes Feld[b] (H-CT, H-RT) (means and standard errors, n = 4 for Garte and n = 3 for Hohes Feld).

	pH	Bulk density ($g\ cm^{-3}$)	SOC ($mg\ g^{-1}$)	N_t ($mg\ g^{-1}$)	Sand (%)	Silt (%)	Clay (%)
G-CT	7.0 (0.1)	1.36 (0.09)	8.3 (0.8)	0.9 (0.1)	11.1 (0.5)	74.6 (1.0)	14.3 (0.8)
G-RT	6.9 (0.1)	1.25 (0.05)	10.5 (0.9)	1.2 (0.1)	11.8 (0.6)	78.2 (0.7)	10.1 (1.0)
H-CT	7.0 (0.1)	1.32 (0.08)	9.5 (0.4)	1.0 (0.0)	16.4 (0.9)	65.7 (1.4)	17.9 (0.6)
H-RT	6.8 (0.1)	1.40 (0.04)	15.8 (1.0)	1.5 (0.1)	14.9 (0.7)	67.0 (0.4)	18.2 (1.1)

[a] Garte is located at 51°29'15.50" latitude north and 9°56'9.17" longitude east. The soil type is Haplic Luvisol.

[b] Hohes Feld is located at 51°37'14.18" latitude north and 9°56'30.98" longitude east. The soil type is Haplic Luvisol.

A field experiment was established in 1970 at Garte and in 1967 at Hohes Feld consisting of 4 and 3 field-replicates, respectively. The treatments were CT at Garte (G-CT) and Hohes Feld (H-CT) with a regular moldboard plough to 25 cm depth followed by seedbed preparation with a rotary harrow and RT (G-RT and H-RT) with shallow cultivation down to 5 - 8 cm depth with a rotary harrow for seedbed preparation. Before the start of the experiment the soil had been moldboard ploughed. At Garte, the experimental design was a randomised complete block design with four replicate plots (20 m × 40 m). At Hohes Feld, a split plot design with three replicate plots (12.8 m × 36 m) was established due to a smaller dimension of the field. The crops grown were the same at both sites and the crop rotation was cereal based since 1970 (REITER ET AL., 2002). Crops in the period 2006 to 2008 were forage maize in 2006, field bean (*Vicia faba* L.) in 2007 and winter wheat in 2008. All residues were incorporated by the respective tillage operations. Timing and amount of N fertilization, timing of tillage and number of herbicide applications at G-CT are given in Table 3.2.

Table 3.2: Summary of selected input data of the denitrification-decomposition (DNDC) model for the conventional tillage (CT) treatment at Garte (G-CT).

Data	Value	Data	Value
Climate data		*Crop data*	
Latitude (degree)	51.488	1. crop: bean[c] (dates)	29.03.07 - 29.08.07
Annual mean temperature in 2007, 2008 (°C)	9.97, 9.82	2. crop: winter wheat[d] (dates)	02.11.07 - 31.07.08
Annual precipitation in 2007 and 2008 (mm)	842, 544		
Atmospheric CO_2 concentration (ppm)	350	*Tillage*	
N concentration in rainfall (ppm)	2.7	Ploughing[e] down to 20 cm (dates)	27.03.07 01.11.07
		Ploughing with disk or chisel[f] (date)	28.03.07 02.11.07
Soil data[b]			
Soil texture	silt loam		
bulk density (0 - 10 cm) (g cm^{-3})	1.42	*Irrigation and weeding*	
Fertilization		Irrigation	none
Calcium ammonium nitrate applied on 31.03.0 29.04.08, 28.05.08, 06.06.08 (kg N ha^{-1})	54.0, 48.6, 43.3, 37.8	Application of herbicides[g]	on eight dates

[a] Garte is located at 9°56'9.17" longitude east. The soil type is Haplic Luvisol.
[b] pH and C_{org} are given in Table 3.1.
[c] field bean (*Vicia faba* L.) was cultivated, the crop bean was used in DNDC.
[d] Triticum L.
[e] ploughing depth was 25 cm (25 cm depth is not included in DNDC).
[f] soil was tilled using a rotary harrow (not included in DNDC, thus ploughing with disk or chisel was used in the model).
[g] not included in the model, since weed problems on the field were minimal.

For G-RT, management was the same as for G-CT, except that no moldboard ploughing was carried out. Seedbed preparation with a rotary harrow was carried out on the 28th of March and 2nd of November 2007.

For H-CT, management was also the same as for G-CT, except that harvest of bean was one day later and moldboard ploughing was carried out on the 28th of March and 31st of October 2007. Seedbed preparation was carried out on the 29th of March and 1st of November 2007. In contrast to G-CT, no N fertilizer was applied on the 6th of June, instead, the application on the 28th of May 2008 was increased to 67.5 kg N ha^{-1}. Thus, N fertilization of H-CT in 2008 was 170.1 kg N ha^{-1}, slightly less (13.6 kg N ha^{-1}) than at G-CT.

Management for H-RT was the same as for H-CT, except that no moldboard ploughing was carried out. Seedbed preparation was carried out on the 29th of March and 1st of November 2007.

3.2.2 Field N_2O fluxes and water content of soil

Trace gas fluxes of N_2O were measured approximately once a week from April 2007 to December 2008 (Figure 3.1). This temporal resolution allowed the estimation of the seasonal pattern of the fluxes. No specific attention was given to heavy rainfall events. However, the variation of water filled pore space (determined on the same day as the flux measurements, Figure 3.2) indicated that a large range of soil water contents was covered by the measurement scheme.

The N_2O fluxes were measured using the closed chamber method with three chambers on each of the four (Garte) or three (Hohes Feld) plots per treatment (thus the number of field replicates was n = 4 (Garte) or n = 3 (Hohes Feld) for each treatment). The method is described in detail by RUSER ET AL. (2001). The circular chambers were made of dark PVC with an inner diameter of 30 cm and an initial height of 15 cm. By using extensions of the same material the height of the chamber could be adjusted to plant growth. For each gas measurement these chambers were placed on permanently installed PVC-soil collars with the same diameter and sealed with a lid. The closed chamber method was chosen because of its well known advantages - small fluxes can be measured, chambers are cheap

and the disturbance of the site is limited (FAO, 2001). However, a major disadvantage is that the area covered by the chambers is small, which results in uncertainties of the emission estimates due to the spatial variability in the field (LAVILLE ET AL., 1999).

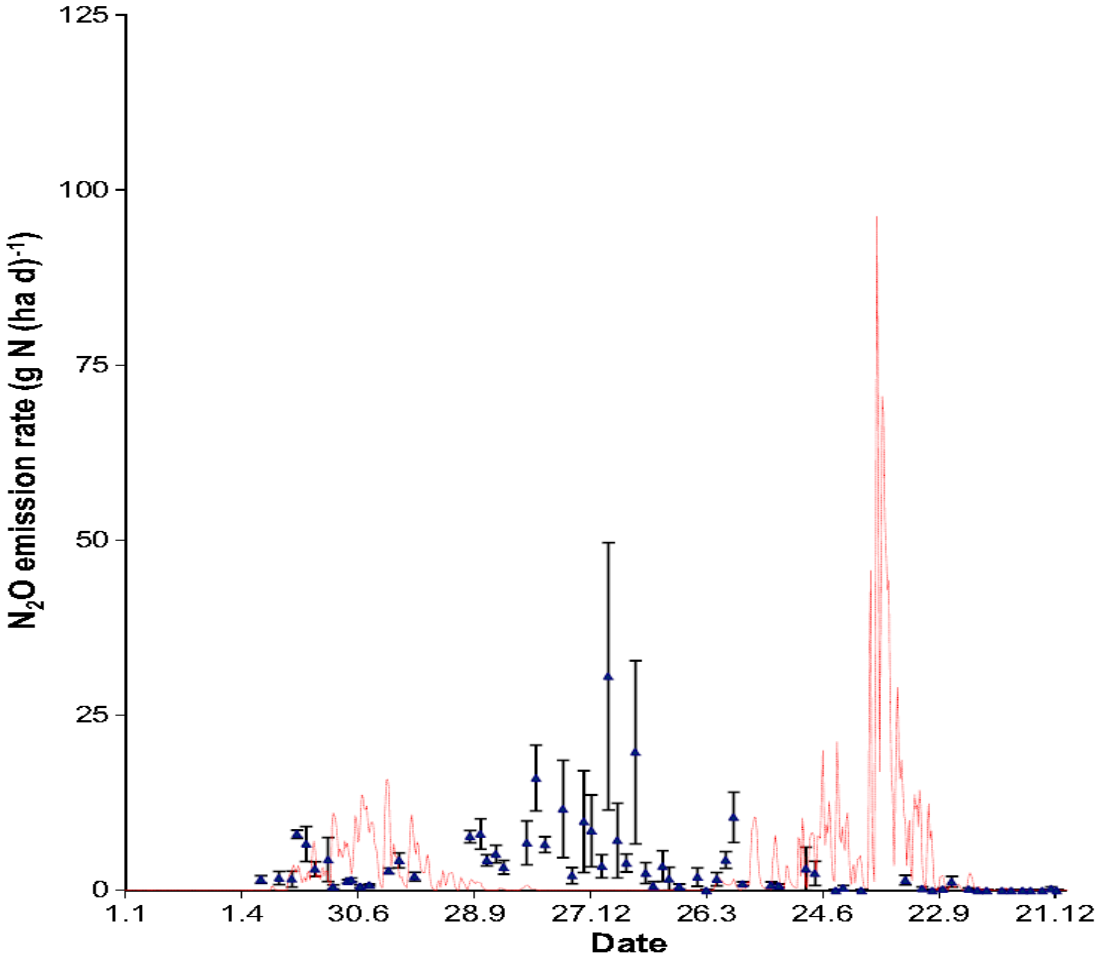

Figure 3.1: Modelled (lines, model variant v4) and measured (symbols, means and standard errors) N_2O emissions from soils of the conventional tillage treatment at Garte (G-CT, located at 51°29'15.50" latitude north and 9°56'9.17" longitude east. The soil type is Haplic Luvisol).

On the same day as the flux measurements, water content of soil was determined with four replicates for each treatment. Briefly, soil samples were taken at a depth of 0 - 10 cm. Samples were weighed, oven dried to constant mass at 105°C, and reweighed. The dry weight and differences between fresh and dry weight were used to calculate the gravimetric water content. Water filled pore

space is calculated by WFPS = (soil gravimetric water content x bulk density) x (1- (bulk density / particle density))$^{-1}$ (LINN AND DORAN, 1984) and by using a particle density of 2.65 g cm^{-3}.

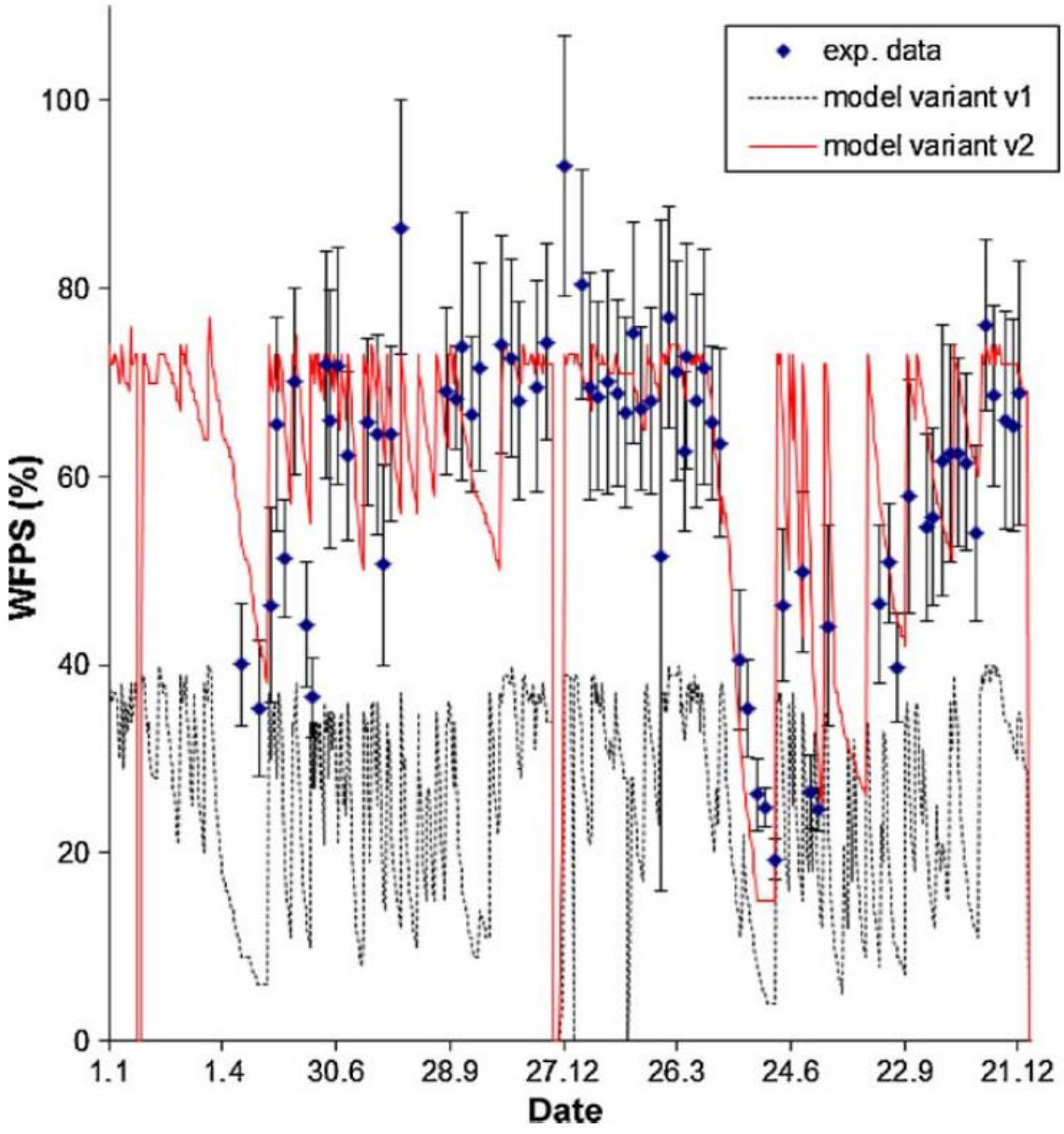

Figure 3.2: Modelled (at 5 cm depth, lines, model variants v1 and v2) and measured (in the 0 - 10 cm depth range, symbols, means and standard errors) water filled pore space in soils of the conventional tillage treatment at Garte (G-CT, located at 51°29'15.50" latitude north and 9°56'9.17" longitude east. The soil type is Haplic Luvisol).

3.2.3 DNDC model

We used the DNDC model (model version version 9.3; http://www.dndc.sr.unh.edu) to describe and predict water dynamics, crop growth and N_2O emissions on a field scale. The DNDC model consists of soil climate, crop growth and decomposition sub-models and calculates soil temperature, water content, pH, redox potential (Eh), and substrate concentration profiles driven by ecological drivers (e.g., climate, soil, vegetation and anthropogenic activity). Additionally, nitrification, denitrification, and fermentation sub-models calculate emissions of CO_2, methane (CH_4), ammonia (NH_3), nitric oxide (NO), N_2O and dinitrogen (N_2) from the plant-soil systems (LI, 2009). In the model, denitrification is calculated by assuming the presence of an "anaerobic balloon". Overall, the model relies on the assumption of universal microbial parameters for the kinetics of their growth and N_2O production (LI, 2009). The following model variants were considered:

3.2.3.1 Model variant v1 – no calibration, sole use of the measured initial data and default values

No calibration was carried out. For a prediction of soil water dynamics, crop growth, and N_2O emission in the G-CT treatment, the model used the measured input for the SOC content and pH (Table 3.1), bulk density (0 - 10 cm) and the crop management (Table 3.2). The hydraulic soil characteristics were estimated by the model using the implemented pedotransfer function for a silt loam. For crop growth of field bean and winter wheat, the default values for "bean" and "winter wheat" provided by DNDC were used. Weeding, as carried out at the field sites, could not be considered by the model, since the options were only "no weed problem" or "moderate weed problems".

3.2.3.2 Model variant v2 – adjustment of soil water dynamics using curve fitting

The model variant v2 was the same as model variant v1 except that we now used modified soil hydrology parameters in order to get an optimum agreement between measured and calibrated WFPS. Optimum agreement was achieved by setting the WFPS at field capacity to 72%, the WFPS at wilting point to 15% and the hydro-conductivity to 0.0045 m hr^{-1}.

3.2.3.3 Model variant v3 – calibration to observed yields using literature data of crop properties

Crop growth is affected by a number of factors (site specific soil and climate conditions, crop variety used, method of pest control, use of growth promoters) and calibration of crop growth may be achieved by optimizing several combinations of parameters. In the DNDC manual (LI, 2009), it is suggested that for a calibration of crop growth, the crop heat/water/N demands, growth curve, biomass partitioning, or yield may be adjusted.

Model variant v3 was based on model variant v2. An additional calibration was carried out based on the yield and crop growth data of the experimental treatment G-CT.

For field bean, the maximum grain yield was increased from 600 kg C ha^{-1} (default value in DNDC, 16.2 decitonnes (dt) dry matter (dm) ha^{-1}) to 1800 kg C ha^{-1} (49 dt dm ha^{-1}) in order to match the large yield in our field experiment. The N fixation index was increased from 1.2 (default) to 4 in order to reach an N_2 assimilation in the range of 80 to 90 kg N ha^{-1} (SCHILLING, 2000).

For winter wheat, maximum grain yield was increased from 2500 kg C ha^{-1} (default value in DNDC, 67.6 dt dm (including 14% water) ha^{-1}) to 4500 kg C ha^{-1} (122 dt dm ha^{-1}). Additionally, the biomass fractions for winter wheat were modified: the biomass fraction for grain was changed from 0.3 (default value) to 0.42 and the biomass fraction for leaf plus stem was changed from 0.53 (default value) to 0.41 since the grain:straw ratio of winter wheat is approximately 1 (KTBL, 2005). The biomass fraction for root was thus 0.17 (same as the default

value), since the sum of the three fractions equals 1. The thermal degree days (with daily averages > 10.0°C) were decreased from 2500 (default value) to 1600°C (the minimum value of the range reported by ENTRUP AND OEHMICHEN, 2000), the C/N ratio of grains was increased from 30 (default value) to 40 and the transpiration coefficient was reduced from 280 (default value) to 169 kg water $(kg\ dm)^{-1}$ (the minimum value of the range reported by EHLERS, 1996).

3.2.3.4 Model variant v4 – calibration to observed yields and N_2O emissions using additional parameter fitting

Model variant v4 was based on model variant v3 with the following additional adaptations of parameters: The decomposition rates of the three soil organic C pools were reduced by a factor of 0.3 and the initial SOC content was reduced from 8.3 mg g^{-1} to 2.7 mg g^{-1} to better match the cumulative N_2O emission of the G-CT treatment in 2007 (April until December) and 2008 (January until December).

3.2.3.5 Retrospective predictions

Retrospective predictions were carried out for the remaining treatments and sites G-RT, H-CT and H-RT based on the parameters obtained by the calibration in variant v4. The models were the same as described above with the following exceptions:

G-RT: RT was used in the model instead of CT.

H-CT and H-RT: the different latitude and climate data were used. The slightly different N fertilization scheme (application of 170.1 instead of 183.7 kg N ha^{-1}) and the minimal changes in harvest and tillage dates described above were considered. Again, the model considered either CT (H-CT) or RT (H-RT).

3.2.4 Statistics

The performance of the model calibration and retrospective prediction of the soil water dynamics was evaluated by calculation of the root mean square error (RMSE), model efficiency (EF), and relative error (E) as defined in SMITH ET AL. (1997):

$$\mathrm{RMSE} = \frac{100}{\overline{O}} \sqrt{\sum_{i=1}^{n} (P_i - O_i)^2 / n} \quad (1)$$

$$\mathrm{EF} = \frac{\sum_{i=1}^{n} (O_i - \overline{O})^2 - \sum_{i=1}^{n} (P_i - O_i)^2}{\sum_{i=1}^{n} (O_i - \overline{O})^2} \quad (2)$$

$$E = \frac{100}{n} \sum_{i=1}^{n} (O_i - P_i) / O_i \quad (3)$$

where O_i are the observed (measured) values, P_i are the predicted values, $\overline{O}$ is the mean of the observed data and n is the number of paired values. RMSE ranges from 0 to ∞, EF from $-\infty$ to 1 and E from $-\infty$ to ∞. For an ideal fit, RMSE and E equal zero and EF equals 1.

3.3 Results and discussion

3.3.1 Yields and N_2O emissions

Grain yields of field beans were almost identical for both tillage treatments and also similar between the two sites (Table 3.3 and Table 3.4). The range of 45.7 to 49.2 dt dm ha^{-1} in our study is above the average grain yield of 35.3 dt dm ha^{-1} for field beans in Germany in 2007 (STATISTISCHES BUNDESAMT DEUTSCHLAND, 2009) and indicates a high soil fertility for both sites. For winter wheat, grain yields at Garte were high without differences between the tillage treatments, whereas for Hohes Feld, grain yield was slightly smaller (Table 3.3 and Table 3.4). Again, yields were above the average yields in Germany (65.5 to 82.1 dt dm ha^{-1} in the years 2003 to 2007, STATISTISCHES BUNDESAMT DEUTSCHLAND, 2009).

Cumulative N_2O emissions for the silty soils (Haplic Luvisols) ranged from 1.2 to 1.8 kg N_2O-N in 2007 (01.04. until 31.12.) and from 0.8 to 2.5 kg N_2O-N ha^{-1} in 2008 (01.01. until 31.12., Table 3.3 and Table 3.4) and were thus in the lower range reported by JUNGKUNST ET AL. (2006) who summarized that annual N_2O emissions from fertilized German arable soils (seven Anthrosols, one Arenosol, eight Cambisols, one Chernosem, two Fluvisols, two Gleysols, ten Luvisols, and one Planosol) ranged from 0.1 to 17.1 kg N_2O-N ha^{-1}. In our study, a marked increase of N_2O emissions in 2008 due to the cropping of a legume in 2007 was expected (e.g. WAGNER-RIDDLE ET AL., 1997; FLESSA ET AL., 2002), but was probably counterbalanced by the considerably drier conditions in 2008.

Table 3.3:Measured (means and standard errors, n = 4) and modelled yields of bean and wheat and cumulative N_2O emissions in the conventional tillage (CT) treatment at Garte[a] (G-CT). Statistics on the measured and modelled soil water dynamics are also given. Modelled data refer to a retrospective prediction (model variant v1) and calibration results (model variants v2 - v4).

	Measured	v1	v2	v3	v4
Yields					
Field bean[b] grain[c] (dt dm[d] ha^{-1})	45.7 (1.2)	9.6	10.4	39.3	39.3
Winter wheat[e] grain[c] (dt dm ha^{-1})	103.4 (2.1)	5.2	8.4	76.8	76.8
N_2O emissions[f]					
Cumulative N_2O emissions (g N_2O-N ha^{-1})					
(2007)	1160 (140)	1470	800	6610	640
(2008)	1050 (430)	10210	10530	51210	1570
Sum	2210	11680	11330	57820	2210
Soil water					
Mean WFPS[g] (%) for all sampling dates	60.1	26.2	63.1	62.2	62.2
RMSE (WFPS)	-	59.4	17.0	16.3	16.3
EF (WFPS)	-	-4.5	0.5	0.6	0.6
E (WFPS)	-	167	-2.4	-0.9	-0.9

RMSE: root mean square error, EF: model efficiency, and *E*: relative error.

[a] Garte is located at 51°29'15.50" latitude north and 9°56'9.17" longitude east. The soil type is Haplic Luvisol.

[b] *Vicia faba L.*

[c] Amounts include 14% water content for grain. Modelled yields were recalculated using a C content of 43% (grains, straw) and considering a water content of 14% (grains).

[d] Decitonnes dry matter.

[d] Decitonnes dry matter.

[e] *Triticum aestivum L.*

[f] Measured and modelled emissions refer to the periods from April to December (2007) and from January to December (2008).

[g] Water filled pore space.

Table 3.4:Measured (means and standard errors, n = 4 for the yields at Garte or n = 3 for the yields at Hohes Feld) and predicted grain yields of bean and wheat and cumulative N_2O emissions in the reduced tillage treatment (RT) at Garte[a] (G-RT) and in the RT and conventional tillage (CT) treatments at Hohes Feld[b] (H-RT, H-CT). Statistics on the measured and modelled soil water dynamics are also given.

	G-RT		H-CT		H-RT	
	Measured	v4	Measured	v4	Measured	v3
Yields						
Field bean[c] grain[d] (dt dm[e] ha^{-1})	45.8 (2.0)	39.3	49.1 (1.7)	41.8	49.2 (0.9)	41.8
Winter wheat[f] grain[d] (dt dm ha^{-1})	103.8 (2.3)	77.1	75.4[g]	72.8	88.2 (1.0)	73.0
N_2O emissions[h]						
Cumulative N_2O emissions						
(2007) (g N_2O-N ha^{-1})	1360 (100)	660	1770 (110)	710	1260 (20)	740
(2008) (g N_2O-N ha^{-1})	1640 (290)	2750	770 (20)	1510	2460 (490)	1850
Sum	3000	3410	2540	2220	3720	2590
Soil water						
Mean WFPS[i] for all sampling dates	60.0	62.3	63.8	60.4	73.9	60.3
EF (WFPS)	-	0.6	-	0.4	-	0.0

EF: model efficiency.

[a] Garte is located at 51°29'15.50" latitude north and 9°56'9.17" longitude east. The soil type is Haplic Luvisol.

[b] Hohes Feld is located at 51°37'14.18" latitude north and 9°56'30.98" longitude east. The soil type is Haplic Luvisol.

[c] *Vicia faba L.*

[d] Amounts include 14% water content for grain. Modelled yields were recalculated using a C content of 43% (grains, straw) and considering a water content of 14% (grains).

[e] Decitonnes dry matter.

[f] *Triticum aestivum L.*

[g] Data for one subplot, yields on the other subplots were 52.7 and 55.5 dt dm ha^{-1} due to damages caused by wild boars.

[h] Measured and modelled emissions refer to the periods from April to December (2007) and from January to December (2008).

[i] Water filled pore space.

Differences between the tillage treatments were not marked in 2007. In 2008, however, the emission of 2.5 kg N_2O-N ha^{-1} at H-RT was 2 to 3 times greater than emissions from the other three plots. Seasonal dynamics of N_2O emissions were pronounced, especially in 2007 where the N_2O emissions were largest in autumn and winter 2007. Emissions of N_2O are generally associated with soil management and cropping systems, variable rainfall, especially drying/rewetting, freeze/thawing events, and amount and type of fertilizer N (KAISER ET AL., 1996; KAISER AND RUSER, 2000; JUNGKUNST ET AL., 2006). In our study, increased N_2O emissions coincided with the cropping of winter wheat after harvest of field

bean and tillage in November 2007. Spatial variability of the emissions, however, was strong. Fertilization in 2008 had only small effects on N_2O emissions.

3.3.2 Performance of model variant v1 – no calibration, sole use of the measured initial data and default values

Model variant v1 was applied to provide an independent simulation without any parameter optimization. The performance was poor (Table 3.3), indicating that the default values given by DNDC may not always be useful. The pedotransfer function used for this site (silt loam) resulted in large deviations between modelled and measured WFPS data (Figure 3.2, Table 3.3). Mean WFPS on the 75 sampling dates differed between 60 (measured) and 26% (modelled). The root mean square error RMSE and the relative error *E* were the largest of all four model variants tested (Table 3.3). The negative efficiency value EF of -4.5 indicated that the model variant described the WFPS data less well than the mean of the data. Deviations between measured and simulated WFPS were also reported in some other studies. ABDALLA ET AL. (2009) applied the DNDC model for an Irish grassland soil (texture: sandy loam) and reported that WFPS was considerably overestimated by DNDC (version 9.2). Similar to our results, SMITH ET AL. (2008) reported for Canadian sites (textures: loam and clay loams) that DNDC underestimated soil water content particularly during the growing season.

Since soil water dynamics was not simulated correctly in model variant v1, it is not surprising that modelled crop growth was exceptional low and modelled N_2O emissions in 2008 were high (Table 3.3). The main reason is the strongly underestimated N uptake by winter wheat (49 kg N ha^{-1}), which left large amounts of N available for losses through denitrification and nitrification.

3.3.3 Performance of model variant v2 – adjustment of soil water dynamics using curve fitting

The satisfactory agreement between modelled and measured WFPS data (Figure 3.2) indicated that the calibration of soil hydraulic characteristics using optimized WFPS data for field capacity (72%) and wilting point (15%) and an optimized hydro-conductivity (0.0045 m hr^{-1}) was successful: RMSE decreased to 17.0, *E* decreased markedly to -2.4, and EF increased to 0.5 (Table 3.3). Similarly, TONITTO ET AL. (2007) reported that an accurate simulation of NO_3-N leaching and drainage dynamics using DNDC required significant changes to key soil physical and chemical parameters relative to their default values for arable soils in the USA.

The calibration in our study may have been required because of experimental uncertainties. For instance, additional water sources such as groundwater or aquifers in the subsoils may largely affect soil water dynamics and crop growth. However, no impact of groundwater down to 5 m depth was observed at this site. Calibration may have also been required because of uncertainties in the DNDC algorithms. For instance, inaccuracies in the estimation of the potential evapotranspiration cannot be ruled out: In DNDC, the Thornthwaite approach is implemented. However, this approach is assumed to be less accurate than the Penman Monteith equation (GUO ET AL., 2007; MÜLLER, 1982). Additionally, inaccuracies in the calculation of the flow of soil water are likely: KRÖBEL ET AL. (2010) tested the DNDC model for a soil (Calcaric Cambisol with a silty-loamy texture) in the North China Plain and concluded that the cascade model approach used by the DNDC model appeared to be unsuitable to simulate soil water dynamics at their site.

In our study, despite the improved description of soil hydrology in model variant v2, there was no overall improved performance of modelled crop growth and N_2O emissions, which was still poor (Table 3.3).

3.3.4 Performance of model variant v3 – calibration to observed yields using literature data of crop properties

The need for a calibration of crop properties for different sites is evident, due to differences in growth performance because of site specific soil and climate conditions and also due to ongoing improvements of crop growth by having better varieties, improved pest control and growth promoters. The adjustments made in model variant v3 – a threefold (field bean) and a 1.8-fold (winter wheat) increase of maximum grain yields, an increase of the N fixation index to 4 (bean), a change of the biomass fraction for grain and leaf plus stem, a decrease of the transpiration coefficient and thermal degree days – are backed up by the literature (SCHILLING, 2000; ENTRUP AND OEHMICHEN, 2000; EHLERS, 1996) and resulted in large reductions of water and nitrogen stress: simulated grain yields increased from 10.4 to 39.3 dt dm ha^{-1} (86% of the measured data) for field bean and from 8.4 to 76.8 dt dm ha^{-1} (74% of the measured data) for winter wheat (Table 3.3). Overall, one has to keep in mind that several crop parameters, especially the transpiration coefficient and the thermal degree days are highly site specific. We used the ranges summarized by EHLERS (1996) and ENTRUP AND OEHMICHEN (2000). However, the available literature merely gives overviews of ranges observed and does not give distinct minimum and maximum data for all sites and crop varieties.

Despite an improved description of crop yields, overall performance of model variant v3 was still unsatisfactory: simulated cumulative N_2O emission in the 2-year period was 26-fold larger than the measured emission (Table 3.3), indicating that this model variant is not usable for predictive purposes for the other three treatments. According to the model, much more nitrate is present in the soil at the beginning of 2008 (63 kg N ha^{-1} compared to 3 kg N ha^{-1} in model variant v2), which is used for denitrification and this leads to the calculated large N_2O emission in 2008.

3.3.5 Performance of model variant v4 – calibration to N_2O emissions using additional parameter fitting

Model variant v4 included as additional adjustments a reduction of the C content in the surface soil and of the mineralization rate of the C pools. These adjustments resulted in a matched cumulative N_2O emission (the modelled sum and the measured sum amounted to 2210 g N_2O-N ha^{-1}, Table 3.3) for the entire period (April 2007 until December 2008). The sensitivity of the N_2O emissions to changes of the initial SOC content in the surface soil is well known (ABDALLA ET AL., 2009). The main reasons for the markedly smaller N_2O emissions in model variant v4 compared to v3, especially in 2008, are that less nitrate was present in the soil at the beginning of 2008 (44 instead of 63 kg N ha^{-1}), that less N was mineralized in 2008 (3.9 instead of 19.4 kg N ha^{-1}) and that more N was present as nitrate in the soil at the end of 2008 (95 instead of 49 kg N ha^{-1}).

Considering its overall performance for a description of crop yields and N_2O emissions in treatment G-CT, variant v4 was satisfactory and markedly more accurate than variants v1 to v3. This overall satisfactory performance indicates the need for site-specific calibration of the DNDC model to describe the complex plant-soil-atmosphere interactions and emphasizes the relationship between model and site (BEVEN, 2002; PRIESACK ET AL., 2006).

However, besides the need for parameter fitting discussed above, the main shortcomings of model variant v4 were the underestimation (in 2007) and overestimation (in 2008) of N_2O emissions and a lack of agreement between simulated and observed annual distributions of the emissions (Table 3.3, Figure 3.1). Thus, the assumption of universal microbial parameters for the kinetics of their growth and N_2O production (LI, 2009) may be challenged and/or the denitrification sub-model of DNDC may need further improvements.

Soil water dynamics were similar for all four treatments, with slightly wetter conditions at H-RT (Table 3.4). The calibration done for the G-CT had a similar performance for a prediction of soil hydrology for G-RT and H-CT (EF = 0.6 and 0.4, respectively, Table 3.4) and was slightly less useful for H-RT (EF = 0.0, Table 3.4).

Prediction accuracy for the grain yields of field bean was similar for all three treatments and overall satisfactory, whereas the smaller grain yields of winter wheat at Hohes Feld were closer to the modelled ones than those at Garte (Table 3.4).

For the cumulative N_2O emissions, modelled results for G-RT, H-CT and H-RT were similar to the experimental ones (Table 3.4). Moreover, the DNDC predicted correctly that the RT treatments had slightly higher N_2O emissions than the CT treatments. However, for all three cases, DNDC slightly underestimated (H-CT, H-RT) or overestimated (G-RT) the cumulative N_2O emissions (Table 3.4) and the annual distribution of emissions was not reproduced correctly (data not shown).

3.4 Interim conclusions

The DNDC model is a very user-friendly model, requiring inputs that are generally easy to obtain. Our results for German silty arable sites indicate that site-specific calibration is essential and that calibration with experimental data and literature data available may result in approximate agreement between modelled and measured crop yields but large deviations between modelled and measured N_2O emissions. If a better agreement is required then a parameter fitting procedure (as in our model variant v4) may be necessary. Thus, the pedotransfer functions and the denitrification sub-model of DNDC may need further improvement.

3.5 Summary of the chapter

The choice of tillage system affects crop growth and soil nitrogen dynamics. Models help us to better understand these systems and the interaction of the processes involved. Objectives were to test a calibration and validation scheme for applications of the Denitrification-Decomposition (DNDC) model to describe a long-term field experiment with conventional tillage (CT) and reduced tillage (RT) at two sites (G and H, silty Haplic Luvisols) near Göttingen, Germany (G-CT, G-RT, H-CT, H-RT). Crop growth of field bean (*Vicia faba L.*) and winter wheat (*Triticum aestivum L.*) as well as soil water dynamics and nitrous oxide (N_2O) emissions were determined for two subsequent years. A model test was performed based on a model parameterization to best describe the case G-CT. This parameterization was then applied to the other cases as a retrospective simulation. Results of model variant v1 (no parameter optimization) indicated that soil water contents were not accurately simulated using the DNDC default values for a silt loam. After successful calibration of the soil water flow model using modified water-filled pore spaces at field capacity and wilting point and a modified hydro-conductivity that led to a good fit of the measured water content data, grain yields were markedly underestimated and modelled N_2O emissions were too large (v2). An optimization of the crop properties (maximum grain yield, N fixation index, thermal degree days, transpiration coefficient) was essential for a better match of measured yields (v3). Further adjustments in the model (v4) were required to better match cumulative N_2O emissions: reducing the initial soil organic carbon content and mineralization rates. Predictions of crop yields and annual cumulative N_2O emissions using model variant v4 were fairly accurate for the reduced tillage system G-RT and also for the second field experiment H-CT and H-RT, but annual distributions of N_2O emissions were not. Overall our results indicate that site specific calibration was an essential requirement for the silty German sites, and that the pedotransfer functions and denitrification sub-model of DNDC may need further improvement.

4 Dual isotope and isotopomer measurements for the understanding of N_2O production and consumption during denitrification in an arable soil *

* note the information in the preface and outline section (XIX)

4.1 Introduction

Nitrous oxide (N_2O) is an important greenhouse gas that also contributes to the depletion of the ozone layer (PRATHER ET AL., 2003). Nitrogen fertilizers applied to agricultural land are the major source of N_2O from terrestrial ecosystems (NEVISON & HOLLAND, 1997; MOSIER & KROEZE, 1998). Nitrous oxide is produced predominantly by microbial processes, as by-products of nitrification and products of denitrification (FIRESTONE & DAVIDSON, 1989). Under denitrifying conditions, which mainly occur under low oxygen (O_2) concentrations in soil, the associated increases in N_2O tend to be short-lived, lasting from a few days to several weeks (SCHOLEFIELD ET AL., 1997a). During denitrification, both production and consumption of N_2O can take place simultaneously and significant amounts of N may also be lost to the atmosphere as dinitrogen (N_2).

Natural abundance stable isotopic signatures, such as $\delta^{15}N$ and $\delta^{18}O$ of N_2O, have increasingly been used to identify N_2O source processes in the soil (BAGGS, 2008; WELL ET AL., 2008b), both in the field (PÉREZ ET AL., 2001; YAMULKI ET AL., 2001) and in laboratory-based studies (BOL ET AL., 2003; CARDENAS ET AL., 2007). Generally, N_2O emitted from denitrification in soils has higher $\delta^{15}N$ and $\delta^{18}O$ signatures than when derived from nitrification (BAGGS, 2008). Recent studies have also provided information on isotope fractionation during N_2O production (PÉREZ ET AL., 2006; WELL & FLESSA, 2008, 2009a) and N_2O reduction (MENYAILO & HUNGATE, 2006a; OSTROM ET AL., 2007; JINUNTUYA-NORTMAN ET AL., 2008). Few, however, have studied the isotopic signature in systems where both production and consumption take place simultaneously and where process rates have been measured simultaneously (WELL ET AL., 2006; CARDENAS ET AL., 2007).

There are several isotopologues differing in isotopic substitution of oxygen and/or the two N atoms within the N_2O molecule. Techniques have recently been developed that allow the determination of all isotopologues, including the isotopomers of N_2O, which are those differing in the peripheral (β) and central

N-positions (α) of the linear molecule (BRENNINKMEIJER & RÖCKMANN, 1999; TOYODA & YOSHIDA, 1999). The intra-molecular ^{15}N site preference (SP; the difference between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$) can be used to identify production processes at the scale of bacterial species or enzymes involved (TOYODA ET AL., 2005; BAGGS, 2008). Site preference is generally independent of the isotopic signature of the precursor (POPP ET AL., 2002); however, OSTROM ET AL. (2007) suggested that SP may be altered during reduction of N_2O and thus bias the evaluation of its origins.

The isotopic signature of N_2O may be affected by fertilizer N application and/or endogenous N present in the soil. Most isotopic studies, examining the changes in N_2O isotopologues associated with N_2O production and reduction in soils during denitrification, have used acetylene inhibition (PÉREZ ET AL., 2006) or ^{15}N tracer techniques (WELL ET AL., 2006) to estimate total N loss because of the difficulty in measuring N_2, the final product of denitrification, directly. In both cases, process rates, and thus the associated isotope effect, are determined indirectly. However, incubation under a N_2-free atmosphere (in which air is replaced by a He/O_2 mixture), as developed by SCHOLEFIELD ET AL. (1997a,b), allows direct estimation of N_2O and N_2 production, and isotopologue changes, during N_2O production and reduction under denitrifying conditions, and thereby reduces the potential for artefacts yielding more accurate results.

The aim of the present study was to simultaneously measure production and consumption of N_2O during denitrification, with the objective of determining whether the N_2O isotopologue signatures of emitted N_2O under the condition of non-homogenous distribution of nitrate and denitrification in soil could be used to better define the processes involved.

4.2 Materials and methods

4.2.1 Experimental system

Soil was collected in October 2006 from the Highfield long-term experiment at Rothamsted Research, Harpenden, UK. The soil was under arable management and had been sown with winter wheat in September 2005 and harvested in August 2006. After collection the soil was transported to the laboratory where worms and large stones were removed.

The soil was a well-structured and free-draining flinty, silty, clay-loam (FAO Chromic Luvisol) known locally as the Batcombe Series (GOULDING ET AL., 1993). Soil properties (0 - 10 cm) were: pH in water, 6.3; total C, 1.28%; and total N, 0.15%. Soil was sieved to 5 mm, dried to 20% water-filled pore space (WFPS) and re-packed into 14 stainless steel cylinders (143 mm diameter, 120 mm height) to a bulk density of 1.22 g cm^{-3} (1.55 kg dry soil to each cylinder). The base of each cylinder was covered with a nylon mesh (1.0 mm) to retain the soil. The cylinders were placed in individual trays for rewetting, and aliquots of water were applied, allowing the soil to take up water from the base of the cylinder; more water was applied to the top of the cylinder so that the soil was evenly wetted to field capacity (equivalent to 77% water-filled pore space – WFPS). Together with the water applied to the top of the cylinder, the equivalent of 5 kg N ha^{-1} in 100 ml water was applied as nitrate (KNO_3) to the soil (priming solution) in order to prime the denitrifying microbial community. Forty-eight hours after rewetting, 12 of the soil-packed cylinders were incubated, as described below, with the remaining two kept for subsequent soil analysis.

The 12 cylinders were placed in a specialized incubation system (CARDENAS ET AL., 2003), where they were sealed inside chambers to avoid the influx of atmospheric N_2. The N_2 from the headspace of the cylinders and soil atmosphere was flushed out with a 90% He, 10% O_2 mixture (this is subsequently referred to as the aerobic period) at 30 ml per vessel per minute from the bottom to the top of the vessel (flow-through mode). The vessels had three-way valves (Swagelok®, Bristol Valve and fittings, Bristol, UK) on the bottom to allow

switching the flow between flow-through to flow-over (across the surface of the soil core). The flows were controlled by means of mass flow controllers (Tylan FC-2900 series®, Millipore, Billerica, MA, USA) within the ranges 0 - 250 ml $minute^{-1}$ for He and 0 - 100 ml $minute^{-1}$ for O_2. After 36 hours, the flow was reduced to 10 ml per vessel per minute and the flow changed to flow over which is more typical of field conditions (flow-over mode). The concentration of N_2 in the headspace was monitored (see later) and when > 99.999% of atmospheric background level had been removed, a second application of KNO_3 was made to the soil surface via a secondary vessel fitted to the centre of each lid (CARDENAS ET AL., 2003) consisting of 50 ml solution (amendment) containing the equivalent of 75 kg N ha^{-1} as KNO_3 and 400 kg C ha^{-1} as glucose. This solution had been previously flushed with He to remove N_2 and other dissolved gases. The final WFPS after all the amendments were made was 85%; the incubation temperature was maintained at 20°C. Headspace gases were subsequently ducted to waste or to gas chromatographs (GC) with an ECD detector for N_2O and an HID detector for N_2 (CARDENAS ET AL., 2003), with detection limits of the equivalent of 2.3 and 9.6 g N ha^{-1} day^{-1}, respectively.

When N_2O fluxes were at their maxima, the gas flow through the headspace was reduced to 5 ml per vessel per minute in order to increase the sensitivity of the N_2 analysis. After 12 days incubation, the O_2 concentration was reduced to zero (anaerobic period), in order to try to achieve totally anaerobic denitrifying conditions. The gas flow was maintained at 5 ml per vessel per minute by replacing the O_2 with additional He.

Cylinders were removed from the incubation system for soil analysis in groups of three at different stages of the experiment: (i) when N_2O emissions were increasing (day 1), (ii) when N_2O emissions were decreasing (day 3), and (iii) at the N_2 peak (day 4). When a cylinder was removed, a length of tubing was put in place to connect what was the inlet of the vessel to the outlet. This allowed us to keep the flow going through the line (with no vessel) rather than closing the line, which would cause an increase in flow rate in the other vessels. This also avoided the need for changing the sampling programme (to skip one sample) as

measurements could still be carried out without the vessel, with the He/O_2 gas mixture only. After the N_2 emissions peaked (day 4), the three remaining cylinders were left in place until the end of the experiment (day 9).

Changes in the N_2O and N_2 emissions were used to define periods during the incubation (Figure 4.1a,b): phase 0 was immediately after the application of the amendment, and before the increase in N_2O emission (day 0); phase I covered the period with increasing N_2O, but before the start of N_2 production (days 0 - 2); phase II covered the period from the beginning of N_2 production until the maximum $N_2O + N_2$ emission (days 2 - 3.5); and phase III covered the period from the maximum $N_2O + N_2$ emission until N_2O emissions were near baseline levels (days 3.5 - 5). In phase IV, all N_2O produced was consumed and consequently only N_2 was emitted (before the removal of O_2) (days 5 until 7.5). The last phase (V) corresponded to the period when the O_2 supply was removed, which provides information about total denitrification (days 7.5 - 9, when nitrification was absent and N_2O was fully reduced to N_2). Broadly speaking, denitrification can be expected to be the dominant process in phases I-IV, whereas in phase V it is total denitrification (removal of O_2 or total anaerobicity).

4.2.2 Measurement of N_2O isotopic signatures and CO_2 emissions

Gas samples for isotopologue analysis were collected in 115-ml serum bottles sealed with grey butyl crimp-cap septa (Part No 611012, Altmann, Holzkirchen, Germany). To facilitate collection, serum bottles were connected by a Teflon tube to the end of the cylinder vents. These serum bottles were, in turn, connected with needles to 20-ml glass vials in series. The 115-ml serum bottles were used for isotopic N_2O analysis and the 20-ml vials for CO_2 analysis. The last vial of a series was vented to the atmosphere through another needle, to maintain flow through the experimental system. Glass vials were replaced three times each day.

Dual isotope and isotopomer signatures of N_2O, i.e. $\delta^{18}O$ of N_2O ($\delta^{18}O$-N_2O), average $\delta^{15}N$ ($\delta^{15}N^{bulk}$) and $\delta^{15}N$ from the central N-position ($\delta^{15}N^{\alpha}$) were analysed, after cryo-focussing, by isotope ratio mass spectrometry using a Delta

XP IRMS (Thermo-Finnigan, Bremen, Germany), allowing the simultaneous detection of m/z 30, 31, 44, 45 and 46 (WELL ET AL., 2008B). The IRMS was connected to a modified Precon (Thermo-Finnigan) equipped with an autosampler (model Combi-PAL, CTC-Analytics, Zwingen, Switzerland) (WELL ET AL., 2008B). ^{15}N site preference (SP) was obtained as SP = 2 x ($\delta^{15}N^{\alpha}$ - $\delta^{15}N^{bulk}$). Dual isotope and isotopomer ratios of a sample (R_{sample}) were expressed as ‰ deviation from $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of the reference standard materials (R_{std}), atmospheric N_2 and standard mean ocean water (SMOW), respectively:

$$\delta X = (R_{sample}/R_{std} - 1) \times 1000, \quad (1)$$

where X = $^{15}N^{bulk}$, $^{15}N^{\alpha}$, $^{15}N^{\beta}$, or ^{18}O.

CO_2 was measured with a GC equipped with flame ionisation detector (FID) connected to a methaniser. A Perkin Elmer Clarus 500 GC (Perkin Elmer Instruments, Beaconsfield, UK) with a TurboMatrix 110 Headspace autosampler was used with a megabore Elite Plot Q column kept at 35ºC.

4.2.3 Soil analysis

The soils kept for analysis at the start of the incubation and those removed from the incubation system were assessed for changes in nitrate (NO_3^-), ammonium (NH_4^+), total C and total N. Mineral-N was determined using 2 m KCl soil extracts and automated colorimetry (SEARLE, 1984). Dissolved organic carbon (DOC) was measured by extracting wet soil for 2 hours with ultra-pure water (1-5 ratio), on a mechanical shaker, centrifuging at 7802 g (average) (or 10 000 rpm) (model Sorvall RC-5B PLUS Centrifuge; SS-34 rotor, Kendro Scientific Ltd, St. Albans, UK) for 15 minutes, and filtering the supernatant through a glass microfibre filter (Whatman GF/F 47-mm circle, VWR International, Dorset, UK). A small aliquot (10 ml) of the extract was taken to determine the DOC

content using a Skalar SAN Analyser (model CA-14, Formacs Skalar Ltd, Wheldrake, UK). The C and N contents of the soil and the $\delta^{15}N$ values of the fertilizer were analysed using an automated continuous-flow ANCA 20/20SL system (Europa, Crewe, UK). The $\delta^{15}N$ of the applied KNO_3 fertilizer was +6.9‰ relative to N_2 in air.

4.2.4 Calculations and statistical analysis

The total emissions of N_2O, N_2 and CO_2 were estimated from the area under the curve of the recorded fluxes versus time by using the statistical package Genstat version 9 (VSN International Ltd, Hemel Hempstead, UK). The N_2 data were drift-corrected by removing the baseline of N_2 before the application of the amendment. Fluxes of N_2O and N_2 were fitted to Gaussian models, also by using Genstat (VSN International Ltd). Simple correlation analyses were used to determine whether the emissions and the N_2O/N_2 ratio were correlated with measured soil properties. Additional correlation analyses were also performed to test the relationships between the N_2O and N_2 fluxes and the isotopologues. However, the first two samplings after the application of the fertilizer were not included in the correlation analysis, as the application of the fertilizer may have displaced N_2O already accumulated in the pore space, and therefore affected the isotopic signature.

4.3 Results

4.3.1 CO_2, N_2O, N_2 fluxes and N_2O/N_2 ratios

The CO_2 emissions increased immediately after the KNO_3 and glucose amendments were applied, reaching a maximum flux of 19.0 ± 1.6 kg CO_2-C ha^{-1} day^{-1} (phase I, see Figure 4.1a above) 40 hours after the application. Thereafter, the CO_2 fluxes started to decline, but remained relatively large (> 12 kg CO_2-C ha^{-1} day^{-1}), until N_2 fluxes reached a maximum in phase II when the fluxes of both gases decreased and the CO_2 flux declined to approximately 6 kg CO_2-C ha^{-1} day^{-1}. The cumulative CO_2 emissions equalled 87.0 kg C ha^{-1}, representing 21.8% of the C applied as glucose. The CO_2 data could be best fitted to a double Gaussian model (two peaks/phased response, which explained 94.2% of the variance) (Table 4.1). In phase III, CO_2 levels decreased to a baseline value, possibly indicating the system had reached equilibrium.

The application of amendment to the incubated soils initially produced N_2O emissions for 4.5 days (mainly during phases I and II), with a maximum N_2O flux of 6.9 ± 1.8 kg N ha^{-1} day^{-1} 3 days after application (phase II) (Figure 4.1a). At the beginning of phase III, the N_2O flux decreased rapidly and was < 0.50 kg N ha^{-1} day^{-1} for the next 3 days (phase III and IV). Emissions of N_2 appeared 2 days after amendment application, with a maximum flux of 6.6 ± 3.0 kg N ha^{-1} day^{-1} on day 4, coincident with the decline in N_2O emissions (phase III). After this, a flux of N_2 > 2 kg N ha^{-1} day^{-1} was maintained for all cylinders for the remainder of the incubation (phases III-V). There was a lag between the maximum CO_2 flux and the N_2O and N_2 fluxes of 30 and 52 hours, respectively (Figure 4.1a). Immediately after the withdrawal of the O_2 supply (anaerobic period, day 7.5, phase V), both N_2O and N_2 flux increased, with a maximum on days 8 - 9 for N_2O and N_2 of 2.1 ± 0.5 and 3.3 ± 0.5 kg N ha^{-1} day^{-1}, respectively. The cumulative N_2O-N fluxes for the aerobic and anaerobic periods were 20.1 and 2.6 kg N ha^{-1}, respectively, while the cumulative N_2-N losses were 14.7 and 5.3 kg N ha^{-1} for the aerobic and anaerobic periods, respectively.

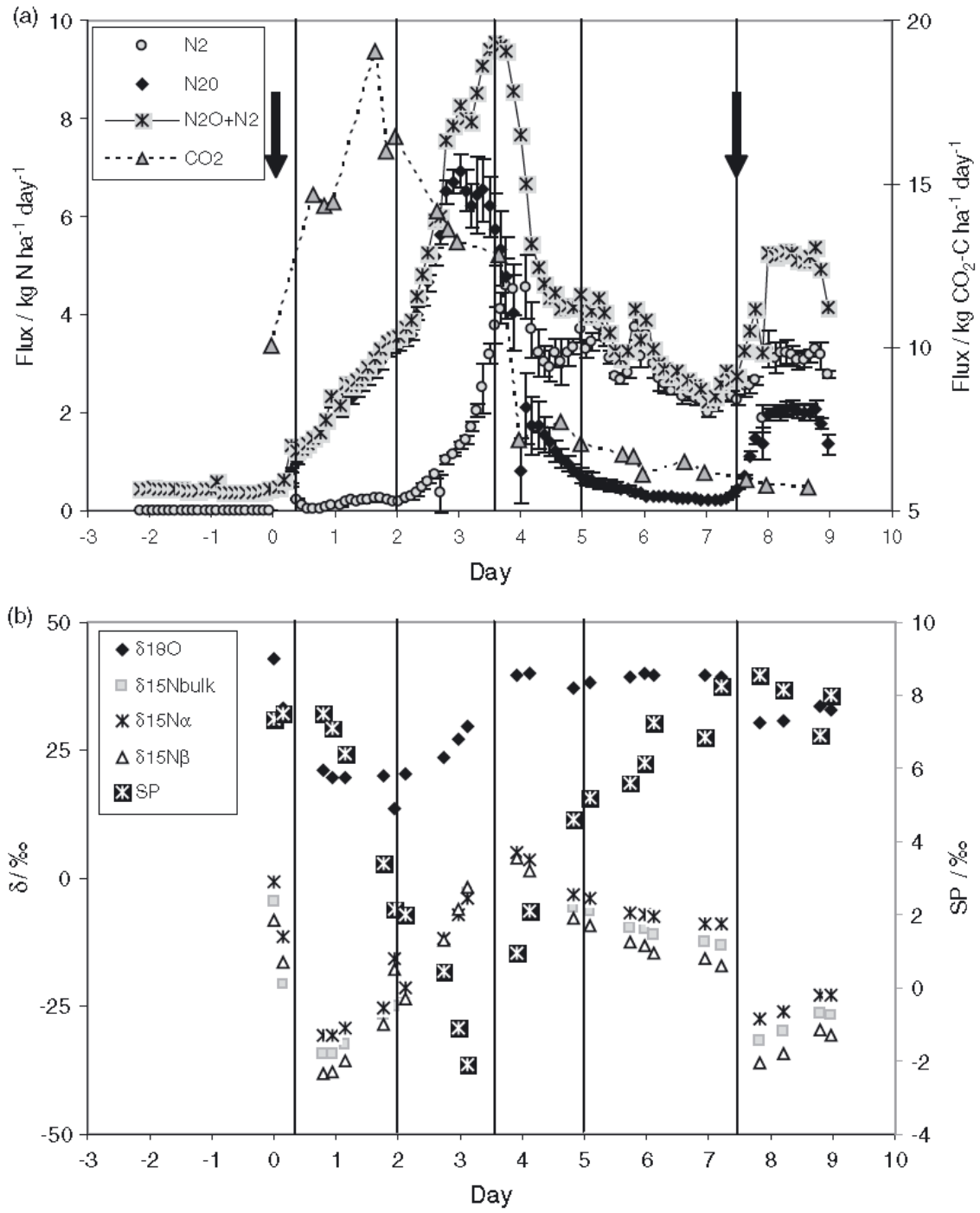

Figure 4.1: Average N_2O, N_2 and CO_2 fluxes. The vertical bars correspond to the standard errors of these averages. The left-hand side arrow corresponds to the application of the KNO_3 and glucose and the right-hand side arrow to the removal of O_2 from the system. (b) Isotopic values of the emitted N_2O, as $\delta^{18}O$, $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and SP, with vertical bars representing the six phases into which the experiment was divided, based on the isotopic signatures.

The cumulative N_2O-N and N_2-N fluxes represented 30.2 and 26.7% of the NO_3^--N applied, respectively. Clearly, a large proportion of the N applied remained in the soil. A double Gaussian model could again be fitted to N_2O data collected both before and after the removal of O_2, and to the complete set of N_2 data. The parameters for these models are shown in Table 4.1. The variance accounted for was 97.9 and 97.5%, respectively, for the N_2O and 78.7% for N_2.

The N_2O/N_2 ratio changed continuously over the incubation. During phase I the values of the ratio ranged from 1 to 75.6 and in phases III and IV it was close to zero. In phase V, the N_2 and N_2O fluxes were closer to unity and the ratio was approximately 0.5-0.7. During denitrification the mole fraction of N_2O, defined as $N_2O/(N_2O + N_2)$, values was close to 1 for phases 0 and I, ranged from 1 to 0.4 for phase II and from 0.4 to 0.01 for phase III, and was constant at 0.01 for phase IV. The removal of O_2 in phase V produced an increase in the ratio to 0.4.

Table 4.1:Values calculated for the model parameters. Fitted curve: A + B × PNORMAL (X; M; S2) + C × PNORMAL(X; M; S2). Estimates and standard errors have been included.

	N_2O							
	First peak		Second peak		N_2O		CO_2	
Parameter	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
S	12.28	0.39	7.59	0.64	1.18	0.07	1.00	0.22
M	86.79	1.54	244.53	0.67	1.48×10^5	0.07	1.48×10^5	0.23
N	129.23	0.40	260.45	0.83	1.48×10^5	0.19	1.48×10^5	0.42
B	86.43	5.13	30.52	2.95	13.31	0.93	88.90	23.00
C	295.99	7.50	32.04	2.86	9.19	0.93	40.80	16.10
A	0.45	0.05	0.15	0.09	0.02	0.13	22.60	1.48

4.3.2 Soil data

Naturally, the application of the KNO_3 and glucose amendment resulted in an increase in soil mineral N (phase 0). The increase in NO_3^--N occurred immediately after the KNO_3 application (Table 4.2), reaching a maximum of 39.7 ± 3.0 mg NO_3^--N kg dry $soil^{-1}$ in the first day (phase I). After that, NO_3^--N decreased over time. Ammonium-N increased slowly over time and reached a maximum (2.0 ± 0.6 mg NH_4^+ N kg^{-1} dry soil, Table 4.2) at the end of phase II

and at same time as N_2 emissions peaked. At the end of the incubation 13.8 ± 3.0 mg NO_3^--N kg^{-1} dry soil and 1.3 ± 0.6 mg NH_4^+-N kg^{-1} dry soil remained in the soil. Dissolved organic C increased to 300 mg C kg^{-1} dry soil after the application and then decreased to < 100 mg C kg^{-1} dry soil at the end of the incubation (Table 4.2). Soil moisture remained constant at 85% WFPS over the experimental period. No significant correlations were found between CO_2, N_2O and N_2 emissions and the mineral N concentrations in the soil.

Table 4.2: NO_3^-, NH_4^+, dissolved organic carbon (DOC) and C : N ratio in the soil at different stages of the experiment (mean ± standard error). T_0 represents the beginning of the incubation (phase 0). T_1, T_2 and T_3 represent the N_2O increase, N_2O decrease and the N_2 maximum (phases I, II and III), respectively. T_4 was the material in the soil removed at the end of the incubation (end of phase V).

	(T0 : Day 1)	(T1 : Day 1)	(T2 : Day 3)	(T3 : Day 4)	(T4 : Day 9)
mg NO_3^--N kg^{-1} dry soil	9.3±1.1	39.7±3.0	27.9±4.8	22.6±8.0	13.8±3.0
mg NH_4^+-N kg^{-1} dry soil	0.7±0.1	0.7±0.1	1.44±0.5	2.0±0.6	1.33±0.6
mg C kg^{-1} dry soil	26.3±5.9	300.9±46.2	99.3±10.6	96.6±51.8	36.8±2.1
C : N	2.6	7.4	3.4	3.9	2.4

4.3.3 Dual isotope and isotopomer ratios

Large $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values (-4.5 ± 2.4, -0.8 ± 3.1 and -8.2 ± 2.0‰, respectively) were measured 12 hours after the application of the amendment in phase 0 (Figure 4.1b). Within 1 day, early in phase I, $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values had decreased to -34.4 ± 0.01, -30.7 ± 0.3 and -38.2 ± 0.3‰, respectively (Figure 4.1b). However, on day 4 (phase III) all values had increased and $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ reached maxima (4.5 ± 1.7, 4.9 ± 1.6 and 4.0 ± 1.9‰, respectively) just after the peak in N_2 flux (day 4, phase III). Thereafter a slow decrease of the $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values was observed (phases III and IV), stabilizing at approximately -10‰ (phase IV). A further decrease in $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ was observed in phase V (-31.7 ± 2.9, -27.4 ± 2.7 and -36.0 ± 3.0‰, respectively) after the removal of the O_2 supply (days 8 - 9, end of phase IV). The $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values followed a similar trend to the $\delta^{15}N^{bulk}$ and the actual isotope values differed relatively little, with $\delta^{15}N^{\alpha}$ generally > $\delta^{15}N^{\beta}$.

The ^{15}N site preference of N_2O (SP) was 7.5 ± 0.6‰ immediately after the application of the KNO_3 and glucose amendment (phase 0), decreasing to -2.1 ± 1.4‰, which coincided with the maximum N_2O flux in phase II. An increase in SP was then observed (phase III when larger amounts of N_2 were produced), which lasted until the O_2 supply was removed in phase IV, at which point it was 8.6 ± 1.7‰. Finally, in phase V it stayed relatively constant until the end of the incubation.

The value of $\delta^{18}O$ of N_2O ($\delta^{18}O$-N_2O) decreased after the application of the KNO_3 and glucose from 43.0 ± 0.9‰ (phase 0) to 19.6 ± 0.2‰ (start of phase I), but was thereafter approximately constant (at approximately 20 - 29‰) until the beginning of N_2 formation (day 3 after application, and the middle of phase II). Subsequently, $\delta^{18}O$-N_2O values increased to a maximum of approximately 39.9 ± 0.9‰ until N_2 fluxes reached their maximum (day 4, phase III). Thereafter, $\delta^{18}O$-N_2O did not change during the incubation (phase IV) until the O_2 was removed, when it further decreased to 30‰ (phase V). Overall, the temporal trend in $\delta^{18}O$ values in phases 0, I, II and V was comparable to those of the $\delta^{15}N^{bulk}$, $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values described earlier.

When plotting $\delta^{15}N^{\alpha}$ (x) against $\delta^{15}N^{\beta}$ (y) (Figure 4.2), the data from phases I and II plotted on the 1:1 line represent a ^{15}N site preference for N_2O (SP) of 0‰. In the later phases (III-V), the values plotted under the 1:1 line but above the dotted line represent an SP of 10‰ (Figure 4.2). Plotting $\delta^{18}O$-N_2O against $\delta^{15}N^{bulk}$ of the emitted N_2O (Figure 4.3) showed that during phases I and II both isotope values increased at a ratio of approximately 1:3. In phase III we observed a decrease of approximately 20‰ in the $\delta^{15}N^{bulk}$ value, with little or no change in the $\delta^{18}O$-N_2O value. Thereafter in phases IV and V, especially when the O_2 supply was removed, both $\delta^{18}O$-N_2O and $\delta^{15}N^{bulk}$ simultaneously decreased (ratio 1:2) to values at the end of phase V that were comparable to those in the beginning of phase I. Although the $\delta^{18}O$ and $\delta^{15}N^{bulk}$ of emitted N_2O at the beginning (at the onset of denitrification) and the end (total denitrification) of the experiment were very similar, the route by which this was achieved resembled a

'hysteresis' type response, in other words the results of the forward and reverse pathways differed in their isotopic changes (Figure 4.3).

Significant correlations were found between N_2O fluxes and SP ($P < 0.01$, $r = -0.7944$), between N_2 fluxes and values of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N^{bulk}$ ($P < 0.05$) and between N_2 fluxes and $\delta^{18}O$ ($P < 0.001$, $r = -0.5031$).

4.4 Discussion

4.4.1 CO_2, N_2O, N_2 fluxes and N_2O/N_2 ratios

The large CO_2 fluxes produced immediately after application of the KNO_3 and glucose amendment (Phase 0) occurred through microbial respiration of the applied glucose. Because soil moisture conditions were established to favour denitrification, it is highly likely that denitrifiers were responsible for part of this CO_2 pulse. Furthermore, the NO_3^- added as a main substrate for denitrification, as well as glucose, should have stimulated the growth of denitrifier populations (TIEDJE ET AL., 1983). The fact that the CO_2 emissions were fitted to a double Gaussian model suggests that more than one process or source influenced CO_2 emissions.

During the first 4 days after amendment, the main product of denitrification was N_2O (phases I and II). The lag period observed between the N_2O and the N_2 peaks (19 hours) was similar to that observed by MATHIEU ET AL. (2006) of 6 - 12 hours. FIRESTONE ET AL. (1980) and DENDOOVEN & ANDERSON (1994) suggested that this lag period between the appearance of the N_2O and N_2, during the first hours of denitrification, resulted from the different lag times of the synthesis of the enzymes involved in the production and consumption of N_2O under anaerobic conditions. Another possible explanation, suggested by FIRESTONE & TIEDJE (1979), is the preferential acceptance of electrons from NO_3^- compared with N_2O. Nitrate concentrations were large during the period when N_2O was the predominant product of denitrification (phase I and II), but after the fourth day (end of phase II), N_2O emissions declined rapidly in spite of the large concentration of NO_3^- present in the bulk soil (28 mg N kg^{-1} dry soil).

This suggests that the lag was caused by enzyme synthesis, but it is also possible that local depletion of NO_3^- in denitrifying micro-sites also contributed to the decrease in N_2O emission. The continuous decline of the measured mole fraction of N_2O following the initiation of N_2 fluxes in phase I and continuing until the end of phase IV, might thus be interpreted as a result of increasing limitation of NO_3^-. The increase of the N_2O mole fraction to approximately 0.4 during phase V, when conditions were anaerobic, could result from the start of denitrification in those parts of the soil matrix that had been aerobic and thus not denitrifying during the previous time. This would be plausible because NO_3^- was probably less exhausted and denitrifying enzymes were not fully established. Isotopic evidence for the existence of several domains within the denitrifying N-pool is discussed later.

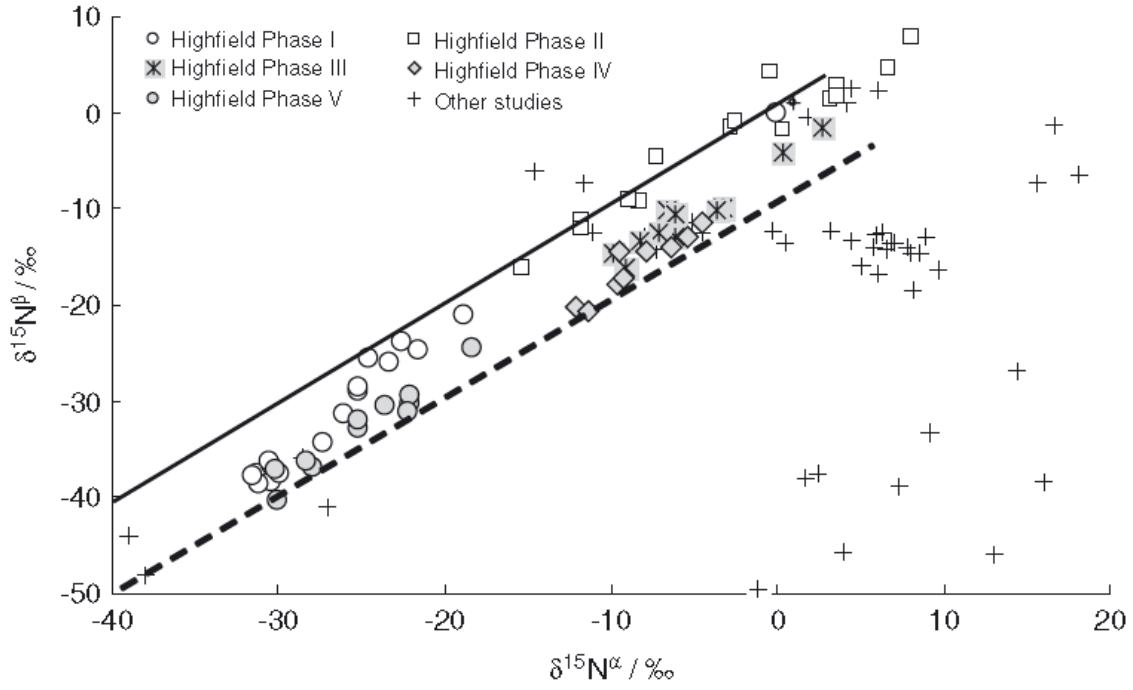

Figure 4.2: Plot of $\delta^{15}N^{\alpha}$ against $\delta^{15}N^{\beta}$ for the N_2O emitted. The phases (as defined in the text) to which the data belong are also indicated. The dashed line represents a site preference (SP) of 10‰ and the solid line the 1:1 ratio that equates to a SP of 0‰. The graph includes data from other studies from PÉREZ ET AL. (2001), BOL ET AL. (2003, 2004), WELL ET AL. (2005) and CARDENAS ET AL. (2007).

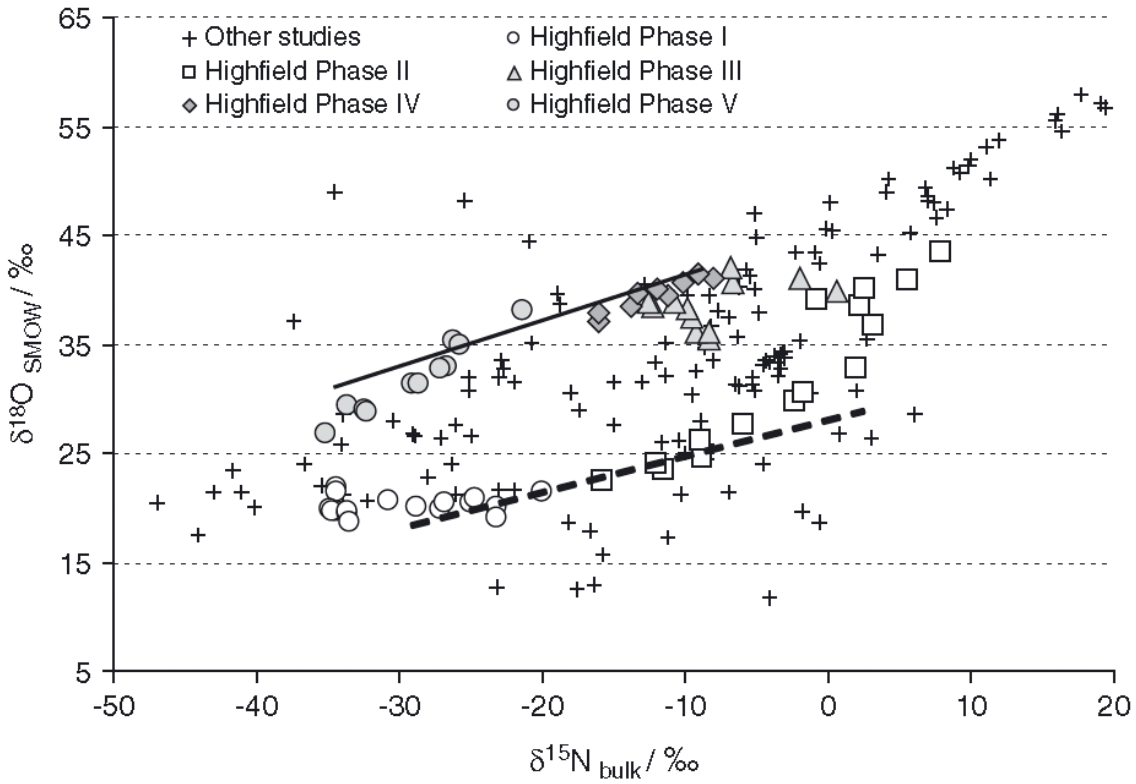

Figure 4.3: Plot of $\delta^{18}O$ against $\delta^{15}N^{bulk}$ for the N_2O emitted for the three replicates incubated until the end of the experiment. The phases (as defined in the text) to which the data belong are also indicated. The dashed line represents a 3:1 ratio and the solid line a 2:1 ratio. The graph includes data from PÉREZ ET AL. (2000, 2001), MANDERNACK ET AL. (2000), YAMULKI ET AL. (2000, 2001), BOL ET AL. (2003, 2004), TILSNER ET AL. (2003), WELL ET AL. (2005, 2006) and CARDENAS ET AL. (2007).

The N_2O peak observed in phase II (6.9 kg N ha^{-1} day^{-1}) was similar to that observed by Cardenas et al. (2003) using the same incubation technique after NO_3^- and glucose were applied, but to intact soil cores in that case. However, the present value is larger than those observed by CARDENAS ET AL. (2007) when sheep slurry was added to soil without added glucose. Although in CARDENAS ET AL. (2007) there was C supplied by the slurry, some of it would not have been available for denitrification explaining the lower fluxes (only less than 60 kg soluble C per ha were added). In the current experiment, the large denitrification rate can be explained by the addition of NO_3^- and glucose, and the optimal temperature for microbial activity. Moreover, and similarly to OSTROM ET AL. (2007), we consider that the artificial conditions imposed by homogenization of

soils, the application of large amounts of water, the activation of soil microbial communities prior to amendment application and the maintenance of anaerobic conditions in such experiments indicate that the observed rates should be considered as potential rather than actual rates. Consequently, these are likely to be in excess of what can be expected in the field. Other processes can also be responsible for emissions of N_2O and N_2. Codenitrification where one N atom from NO_2^- combines with one from a source other than NO_2^- has been reported before (TANIMOTO ET AL., 1992) but it is associated mainly with fungi (MOROZKINA & KURAKOV, 2007). We do not have evidence from our data to confirm that this was the case, but the results from the isotopomer analysis indicated (discussed later) that there may have been processes other than bacterial denitrification during the incubation experiment.

4.4.2 $\delta^{18}O$ of N_2O

During N_2O production, there are opposing inter- and intramolecular processes determining the $\delta^{18}O$-N_2O value (TOYODA ET AL., 2005;WELL ET AL., 2006). The intermolecular isotope effect resulted in depleted N_2O, because heavy NO_3^- reacts more slowly; on the other hand, the intramolecular isotope effect yields enriched N_2O, because the ^{18}O-N-bond is more stable and ^{16}O is thus removed more easily from NO_3^-. Furthermore, there is the possibility of O exchange with water during the NO_3^- to N_2O step of denitrification. CASCIOTTI ET AL. (2002) reported that incorporation of O from H_2O into N_2O during denitrification by different bacterial species may constitute up to 60 - 80% of the O in the N_2O thus produced. KOOL ET AL. (2009) proved this by using tracer studies with a large variety of soils, and a large rate of O-exchange with water was evident in most cases. Independence of $\delta^{18}O$-N_2O from $\delta^{18}O$-NO_3 during N_2O production from denitrification has been observed earlier (WELL & FLESSA, 2009a) and was attributed to O-exchange with water. Our data suggest that the combined sum of these potential and actual effects resulted in a stabilization of $\delta^{18}O$-N_2O during this first phase.

In phase II, an increase in $\delta^{18}O\text{-}N_2O$ was observed, linked to an increase of N_2 formation, which can be explained by ^{18}O accumulation in the residual N_2O during N_2O reduction. These results agree with previous observations of increasing $\delta^{18}O\text{-}N_2O$ values during N_2O reduction in laboratory studies (MENYAILO & HUNGATE, 2006B; OSTROM ET AL., 2007; VIETEN ET AL., 2007), as well as in N_2O-reducing areas in oceans (WESTLEY ET AL., 2006; YAMAGISHI ET AL., 2007). In phases III and IV, the $\delta^{18}O\text{-}N_2O$ stabilized at a large value because most of the N_2O was transformed to N_2 at more or less constant rate. Finally, in phase V the $\delta^{18}O\text{-}N_2O$ value decreased while N_2O fluxes increased much more than N_2 fluxes. This is to be expected because the increase in residual N_2O must be associated with decreasing ^{18}O enrichment.

4.4.3 $\delta^{15}N^{bulk}$ of N_2O

The ^{15}N signature can be used to discriminate between production and consumption of N_2O (BAGGS, 2008): the average ^{15}N enrichment of N_2O ($\delta^{15}N^{bulk}$) depends on the isotopic signatures of the precursor (e.g. NO_3^-) and on the balance between the production and consumption of N_2O (PÉREZ ET AL., 2006). In the absence of consumption, the total fractionation factors for the NO_3^--to-N_2O sequence of denitrification can be estimated from the difference between the $\delta^{15}N$ of the initial NO_3^- and the $\delta^{15}N^{bulk}$ (WELL & FLESSA, 2009). This value was obtained from the difference between the initial $\delta^{15}N$ of fertilizer NO_3^- (+6.9‰) and $\delta^{15}N^{bulk}$ shortly after the amendment application (phase I), giving -37‰. This is within the range of previous values determined using the same approach (WELL & FLESSA, 2009). However, $\delta^{15}N^{bulk}$ increased during phase I and reached a value of -25‰ at the end of this phase. This was probably a consequence of the increasing ^{15}N enrichment of the residual NO_3^- during on-going NO_3^- reduction. We speculate that this is a consequence of differences in the denitrification rate between microsites. In microsites with a large denitrification rate, we expect nitrate to have been rapidly depleted, resulting in larger $\delta^{15}N$ values in the residual nitrate and therefore N_2O with higher $\delta^{15}N$ being produced. When denitrifiers reduce N_2O producing N_2 (phase II), the

lighter molecules are consumed more rapidly than the heavier ones (WELL ET AL., 2006), which must further increase the difference between $\delta^{15}N^{bulk}$ and the $\delta^{15}N$ of NO_3^-. However, as we did not measure the time course of $\delta^{15}N$ of soil NO_3^-, it is not possible to differentiate between these two effects governing $\delta^{15}N^{bulk}$ during phase II.

In phase III, a decrease in $\delta^{15}N^{bulk}$ was observed. Because this is opposite to the trend observed in phase II, it is not possible to explain this by using the time course of $\delta^{15}N$ of a single N pool. In principle, N_2O could also originate from NH_4^+ via nitrification or nitrifier-denitrification, which could cause any variation in $\delta^{15}N^{bulk}$, depending on $\delta^{15}N$ of NH_4^+ and because isotope fractionation factors of nitrification are not identical to those of denitrification (PÉREZ ET AL., 2006). However, the NH_4^+ concentration in the soil was relatively small (< 2 mg N kg^{-1}) and the fraction of N_2O emitted during nitrification of NH_4^+ is typically much smaller compared with the N_2O mole fraction of denitrification (WELL ET AL., 2008B). In view of the large N_2O fluxes of > 2 kg N ha^{-1} day^{-1} it is thus not plausible that NH_4^+-derived N_2O had a significant effect on the isotopic signatures of emitted N_2O. Because N_2O originated mainly from NO_3^-, we hypothesize that the observed $\delta^{15}N^{bulk}$ pattern occurred because there were initially several NO_3^- pools, and the fraction of N_2O derived from these pools varied over time. Furthermore, nitrate diffusion between the pools was probably limited, as observed by MYROLD & TIEDJE (1985).

Pool 1 was assumed to be the NO_3^- from the KNO_3. The application of glucose with NO_3^- caused O_2 consumption, favouring the creation of more anaerobic conditions. Pool 2 could have consisted of initial soil NO_3^- that was located in sites that were not reached by the added KNO_3 and glucose, and thus initially were more aerobic with little denitrification activity. During phase III, denitrification proceeded at a slower rate. This could have resulted from the termination of denitrification in pool 1 because of NO_3^- depletion. Overall, there would have been a much slower denitrification rate in pool 2 at the sites that were not reached by the amendment. Consequently, N_2O produced during the later phase of the experiment could have come from pool 2 where the $\delta^{15}N$ of

NO_3^- and of emitted N_2O was smaller. Pool 2 would have had a strong influence on the total N flux when the first pool became depleted. This could explain the decrease in $\delta^{15}N^{bulk}$ after day 4 (phase III and beyond). The fact that the $N_2 + N_2O$ flux and $\delta^{15}N^{bulk}$ decreased in parallel supports this interpretation, because decreasing total denitrification rates can be indicative of NO_3^- depletion.

The $\delta^{15}N^{bulk}$ further decreased in phase V, after the removal of O_2. This behaviour could be explained by the assumption that pool 2 consisted of two parts: 2(i) the interior of aggregates that had been anaerobic and thus denitrifying, while O_2 was still present in the system; and 2(ii) the residual soil matrix that had been aerobic (and thus not denitrifying) until O_2 was shut off. The assumption that pool 2(ii) started to denitrify in phase V is in line with the observed increase in the N_2O mole fraction during this phase (see discussion on N_2O/N_2 ratios). This is also in agreement with an increasing N_2O flux from pool 2 (2(i) and 2(ii)) resulting from anaerobic conditions in the entire soil volume, including the previously aerobic sites. The presence of several different NO_3^- pools can also explain the double Gaussian behaviour observed in the N_2O fluxes. A study using the ^{15}N tracer technique by RUSSOW ET AL. (2009) on incubated soils provided evidence that, in the case of nitrite (NO_2^-), there were different pools and that the behaviour of the endogenous and exogenous NO_2^- was different.

Different trajectories ('hysteresis') of $\delta^{15}N^{bulk}$ and $\delta^{18}O$ during denitrification were observed: thus the ratio of the simultaneous increase (phase I and II) and decrease (phase III, IV and V) in $\delta^{15}N^{bulk}$ and $\delta^{18}O$ values differed. This could be explained by the temporal change in denitrification between the different pools. At the beginning of phase I, N_2O originated from non-fractionated NO_3^- in pool 1 while there was no reduction. In the final phase (V), the main flux might have come from pool 2(ii), which also contained non-fractionated NO_3^- initially, and thus $\delta^{15}N^{bulk}$ was similar for phases I and V. During phase V, N_2O reduction caused a positive shift in $\delta^{18}O$, which would explain the differences in $\delta^{18}O$ between phases I and V. It is also possible that a shift in the active microbial community towards species with different O-exchange with water (KOOL ET AL.,

2009) contributed to larger final $\delta^{18}O$ values. Further work is required to examine if these different 'isotope' trajectories are mediated by different microbial populations or by the existence of several N-pools.

4.4.4 ^{15}N site preference of N_2O

The ^{15}N site preference of N_2O (SP) potentially provides information about the mechanisms responsible for N_2O production in soils (TOYODA ET AL., 2005; WELL ET AL., 2006). The range in SP values in the present study is comparable to that found by BOL ET AL. (2003) under denitrifying conditions. One of the most important aspects of the current experiment was that the SP was measured during both production and partial consumption of N_2O in the same soil, and when both processes were taking place simultaneously. During phases 0 and I, N_2O consumption was absent. Thus, SP values reflected N_2O production. Initially, SP was positive with values up to 7.5‰. This is in agreement with SP values of N_2O produced in two soils with mean SPs of 3.1 and 8.9‰ (WELL & FLESSA, 2009) and thus further indicates that results from pure culture studies, reporting SPs of N_2O production by denitrifiers to be close to 0 (SUTKA ET AL., 2006), are not generally valid for soil denitrifiers. Differences in site-specific ^{15}N fractions shown by the various types of N_2O reductase among bacterial and fungal denitrifiers (SCHMIDT ET AL., 2004; SUTKA ET AL., 2008) could explain the disagreement between our data and previous pure culture studies. The decrease in SP is similar to the observations of WELL & FLESSA (2009), who found SP decreasing from 7 to 2‰ within 2 days after initiation of anaerobic conditions during a laboratory incubation of an arable soil. It was suspected that inducing denitrification by flushing with N_2 triggered a change in the community of denitrifiers and their associated enzymes.

The SP in phase I was negatively correlated with N_2O flux ($P < 0.001$, $r^2 = 0.973$). The minimum SP value was obtained when N_2O peaked. This could be because the enrichment factor (ε) of the NO_3^--to-N_2O step is known to decrease with increasing reaction rates (MARIOTTI ET AL., 1981). After N_2 production had started (phase II), the SP increased. This can be explained by N_2O reduction,

because $^{15}N^{14}N^{16}O$ is consumed with preference to $^{14}N^{15}N^{16}O$ because the ^{15}N-O bond is stronger than ^{14}N-O bond (YOSHIDA & TOYODA, 2000). This effect has also been observed by others in incubation experiments (WELL ET AL., 2005, OSTROM ET AL., 2007). At the end of the experiment the maximum SP was reached, coinciding with a relatively stable production of N_2 and with a minimum flux of N_2O.

Conversely, overall the variation of the ^{15}N site preference of N_2O (SP) was relatively small, especially when compared with N_2O fluxes from water-saturated systems (BOL ET AL., 2004; WELL ET AL., 2005). Clearly, further work needs to be performed to clarify to what extent differences in SPs between terrestrial systems are related to process conditions or to microbial community structure.

4.5 Interim conclusions

The N_2O isotopologue values reflected the temporal patterns observed in N_2O and N_2 fluxes. A concurrent increase in ^{15}N site preference and $\delta^{18}O$ of N_2O was found to be indicative of N_2O reduction to N_2. The temporal isotopic patterns of the N_2O suggested that there was a two-pool (non-homogenous) distribution of NO_3^- in the soil. Further work is needed to determine the impact of microbial communities and their enzymes on the temporal variation of isotopic signatures of emitted N_2O. This should include modelling of N_2O turnover and associated isotope fractionation in order to check potential effects of microbial dynamics and of non-homogenous distribution of N on gaseous emissions of both N_2O and N_2.

4.6 Summary of the chapter

The aim of our research was to obtain information on the isotopic fingerprint of nitrous oxide (N_2O) associated with its production and consumption during denitrification. An arable soil was preincubated at high moisture content and subsequently amended with glucose (400 kg C ha^{-1}) and KNO_3 (80 kg N ha^{-1}) and kept at 85% water-filled pore space. Twelve replicate samples of the soil were incubated for 13 days under a helium-oxygen atmosphere, simultaneously measuring gas fluxes (N_2O, N_2 and CO_2) and isotope signatures ($\delta^{18}O$-N_2O, $\delta^{15}N^{bulk}$-N_2O, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and ^{15}N site preference) of emitted N_2O. The maximum N_2O flux (6.9 ± 1.8 kg N ha^{-1} day^{-1}) occurred 3 days after amendment application, followed by the maximum N_2 flux on day 4 (6.6 ± 3.0 kg N ha^{-1} day^{-1}). The $\delta^{15}N^{bulk}$ was initially −34.4‰ and increased to +4.5‰ during the periods of maximum N_2 flux, demonstrating fractionation during N_2O reduction, and then decreased. The $\delta^{18}O$-N_2O also increased, peaking with the maximum N_2 flux and remaining stable afterwards. The site preference (SP) decreased from the initial +7.5 to −2.1‰ when the N_2O flux peaked, and then simultaneously increased with the appearance of the N_2 peak to +8.6‰ and remained stable thereafter, even when the O_2 supply was removed. We suggest that this results from a non-homogenous distribution of NO_3^- in the soil, possibly linked to the KNO_3 amendments to the soil, causing the creation of several NO_3^- pools, which affected differently the isotopic signature of N_2O and the N_2O and N_2 fluxes during the various stages of the process. The N_2O isotopologue values reflected the temporal patterns observed in N_2O and N_2 fluxes. A concurrent increase in ^{15}N site preference and $\delta^{18}O$ of N_2O was found to be indicative of N_2O reduction to N_2.

5 Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N_2O during denitrification*

* note the information in the preface and outline section (XIX)

5.1 Introduction

The greenhouse gases (GHG) methane (CH_4) and nitrous oxide (N_2O) have 298 and 25 times higher global warming potentials than that of carbon dioxide (CO_2), respectively (FORSTER ET AL., 2007). Especially N_2O is considered to be an important GHG with soils representing its major source and accounting for approximately 6% of the current global warming (IPCC, 1996) and also implicated in the depletion of stratospheric ozone (CRUTZEN, 1979). The atmospheric N_2O concentration has been increasing since the Industrial Revolution (IPCC, 2007). Agricultural soils are responsible for 10 - 12% of the anthropogenic emissions of greenhouse gases (IPCC, 2007).

Nitrous oxide can be produced by nitrification, incomplete denitrification, nitrifier denitrification and/or fungal denitrification as well as during reduction of nitrate (NO_3^-) to ammonium (NH_4^+) (YOSHIDA AND ALEXANDER, 1970; BOLLAG AND TUNG, 1972). During the bacterial process of denitrification, both production and consumption of N_2O take place simultaneously, and significant amounts of N are lost to the atmosphere as dinitrogen (N_2). As direct measurement of N_2 against a large background concentration of atmospheric N_2 is difficult, only few studies measuring both N_2O and N_2 simultaneously are found in the literature (SCHOLEFIELD ET AL., 1997a, b; BUTTERBACH-BAHL ET AL., 2002; GROFFMAN ET AL., 2006).

One way to identify the processes producing N_2O is to study the stable isotope composition of N_2O and how it changes in space and time (YOSHIDA, 1988; YOSHINARI, 1990). Because the stable isotope ratios of $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of denitrifier-derived N_2O can differ from those of nitrifier-derived N_2O (KIM AND CRAIG, 1990; WEBSTER AND HOPKINS, 1996), the isotopic composition is a tool that can be used to increase our understanding of the biological processes underlying N_2O emissions from the soil (MENYAILO AND HUNGATE, 2006). Reported values for isotopic values for ^{15}N-N_2O range from -13 to -54‰ (PÉREZ ET AL., 2006; WELL AND FLESSA, 2009a) for denitrification and up to ˉ60‰ for nitrification (YOSHIDA, 1988; PÉREZ ET AL., 2006). Denitrifying bacteria also

discriminate as a consequence of the difference in reaction rates for the isotopically light and heavy N_2O molecule, with faster rates for the light N_2O in the process of reduction to N_2, enriching the remaining N_2O in ^{15}N (BARFORD ET AL., 1999; MENYAILO AND HUNGATE, 2006; OSTROM ET AL., 2007; JINUNTUYA-NORTMAN ET AL., 2008). There are few reports on the isotopic signature in systems where both production and consumption take place simultaneously and where process rates have been measured using independent methods (WELL ET AL., 2006; CÁRDENAS ET AL., 2007).

There are several isotopologues differing in isotopic substitution of oxygen and/or the two N atoms within the N_2O molecule. Techniques to detect several isotopologues have recently been developed, allowing determination of isotopomers of N_2O, which are those differing in the peripheral (β) and central N-position (α) of the linear molecule (TOYODA AND YOSHIDA, 1999; BRENNINKMEIJER AND RÖCKMANN, 1999). The intramolecular ^{15}N site preference (SP; difference between $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$) can be used as an indicator of production processes (TOYODA ET AL., 2005). N_2O production during fungal denitrification and nitrification has been shown to produce higher SP compared to bacterial denitrification (SUTKA ET AL., 2008). In contrast to average $\delta^{15}N$ and $\delta^{18}O$ of N_2O, SP has been considered to be independent of the isotopic signature of the precursor (POPP ET AL., 2002). However, reduction during denitrification may increase the α-site enrichment in the residual N_2O, and hence also the site preference index (BOL ET AL., 2003a, OSTROM ET AL., 2007) leading to a biased evaluation of the origin of N_2O.

In agricultural systems $\delta^{15}N$ and $\delta^{18}O$ of emitted N_2O may be affected by fertilizer application as well as by endogenous sources of N present in the soil. Isotopic studies dealing with the changes in N_2O isotopologues associated with these processes used the acetylene inhibition (PÉREZ ET AL., 2006) or a ^{15}N tracer (WELL ET AL., 2006). In both cases the process rate and the associated isotope effect are determined indirectly. However, SCHOLEFIELD ET AL. (1997a, b) developed a technique that allows direct N_2 measurements and CÁRDENAS ET AL. (2003) developed an automated incubation system that allows continuous

measurements of gaseous emissions from soil cores utilizing a helium-oxygen He/O_2-atmosphere. This technique allows a direct quantification of N_2 emissions simultaneously with changes in the isotopologue composition during N_2O turnover. Despite its clear advantage, yielding more accurate results during N_2O turnover under denitrifying conditions, this method has been used only sparsely (e.g. BOL ET AL., 2003a; CÁRDENAS ET AL., 2007; MEIJIDE ET AL., 2010) for studying isotope effects during N_2O production and reduction in soils.

It is still not clear what information can be obtained from the isotopic fingerprint of soil-emitted N_2O under field conditions. WELL AND FLESSA (2009b) modelled isotope fractionation of several simultaneous processes producing or consuming N_2O using the Rayleigh equation, which was first used by MARIOTTI ET AL. (1981). It was concluded that this modelling is helpful to estimate the contribution of the various processes to the net N_2O flux from soil based on the isotopic signature of emitted N_2O.

Previous studies observed a lag period between the appearance of the N_2O and N_2 (SCHOLEFIELD ET AL., 1997a,b; CÁRDENAS ET AL., 2003; MEIJIDE ET AL., 2010). It has been suggested that this could be caused by the sequential synthesis of denitrification enzymes (FIRESTONE ET AL., 1980; DENDOOVEN AND ANDERSON, 1994). BETLACH AND TIEDJE (1981) described the accumulation of intermediates of the denitrification sequence as the result of different reaction rates rather than inhibition of a step in the reduction process. Evaluation of this effect by the use of isotopomers can be very useful. In addition, the conditions of the soil prior to the application of N and C could also produce an impact on the reaction rates/microbial activity. During a dry period, microbial biomass is destroyed. This can release C that can become available (MARIOTTI ET AL., 1981; KALBITZ ET AL., 2000) increasing reaction rates. When soils are rewetted after a dry period, a reactivation of the soil microbial communities takes place and CO_2 and N_2O pulses are produced (CABRERA, 1993; DAVIDSON ET AL., 1993). LUNDQUIST ET AL. (1999) summarized several processes which may have contributed to increased DOC and C availability after drying-rewetting: reduced microbial decomposition in dry periods, enhanced turnover of microbial biomass,

and release of available carbon by the disruption of soil aggregates. This increased C turnover is associated with an enhanced O_2 consumption which stimulates denitrification (FLESSA AND BEESE, 1995).

As a result of temporally different responses of the enzymes involved in nitrate- and N_2O reduction during denitrification the N_2O/N_2 ratio is not stable over time (FIRESTONE AND TIEDJE, 1979). Therefore, antecedent soil moisture conditions (pre-wet vs. pre-dry) could have an effect on the N_2O/N_2 ratio and thus on the isotopologue signatures of emitted N_2O.

We tested the effect of antecedent soil moisture conditions in a laboratory experiment with two different pre-treated soils under denitrifying conditions, using a He/O_2-atmosphere incubation system. One aim was to determine the impact of antecedent soil moisture on N_2 and N_2O fluxes. Moreover, we wanted to evaluate how N_2 fluxes and the N_2O/N_2 ratio are reflected by the isotopic signatures of emitted N_2O and of NO_3^- in soil and thus to test isotopologue signatures of N_2O as a tool study denitrification in soil.

5.2 Materials and methods

5.2.1 Experimental set-up

The soil was sampled from the Highfield Ley-Arable experiment (Rothamsted Research, Harpenden, UK) to a depth of 10 cm in October 2006. The soil is a well structured and free draining flinty silty clay-loam (FAO Chromic Luvisol) known locally as the Batcombe Series (GOULDING ET AL., 1993). It had been under wheat the previous season and is characterised by a pH in water of 6.3 and total C and N contents of 18.8 g C kg^{-1} and 1.9 g N kg^{-1}, respectively. The soil was air-dried (20% water-filled pore space) and plant material was removed. After the soil was sieved (< 5 mm) it was repacked to a bulk density of 1.22 g cm^{-3} into 16 cylindrical stainless steel cores (1.55 kg dry soil to each core) (143 mm diameter, 120 mm height; CÁRDENAS ET AL., 2003), were covered with a nylon mesh (1.0 mm) at the bottom to retain the soil. Two batches of 8 samples each were used. One batch was pre-treated by adding demineralised water to

achieve a moisture of 75% water-filled pore space (WFPS) being equivalent to field capacity, and the second batch was kept dry (20% WFPS). This pre-treatment lasted 4 weeks prior to the start of the incubation experiment. These batches will be referred to as "pre-wet" and "pre-dry" soils, respectively. To adjust the final moisture to 85% WFPS, the cores were placed in trays 2 days before the start of the experiment. Water was applied from the top of the core so that the soil was evenly wetted up in order to obtain homogenous soil moisture. Water leached from the bottom of the cores was kept within the tray and was added to the top of the soil column just before inserting the cores into the incubation system. After this pre-treatment 12 cores were incubated as described below, and four were kept for subsequent soil analysis.

The 12 cores were placed into a denitrification incubation system using a He/O_2-atmosphere (DENIS system) at North Wyke Research, described by CÁRDENAS ET AL. (2003). Each core was placed to an exact fit inside the incubation system, and sealed to avoid the influx of atmospheric N_2. Two spare cores of each pre-treatment were used for initial soil analysis.

Atmospheric N_2 from the headspace of the vessel and soil atmosphere was replaced with a gas mixture of He and O_2 (~10% O_2; subsequently referred to as the aerobic period) by flushing the system with a high flow rate of 30 ml min^{-1} through the soil core from the bottom (flow-through mode) in order to remove the N_2 from the soil matrix. After 36 h N_2 concentrations from the vents of the vessels were very small (99% of the N_2 was removed from the system), and the maintenance of this low N_2 concentrations indicated that N_2 had been removed from the soil matrix and that it was not diffusing from the soil. Then, the He/O_2 flux was reduced to 10 ml min^{-1} $vessel^{-1}$ and changed to a flow-over mode (i.e. He flowed across the top of each vessel). Then 50 ml of fertilizer solution containing the equivalent of 400 kg C ha^{-1} as glucose and 75 kg N ha^{-1} as KNO_3 was added to the surface of the soil via amendment vessels centrally located on the top of the incubation vessels (CÁRDENAS ET AL., 2003) leading to a final water-filled pore space of 90% in each soil core. The solution had been previously flushed with He to remove N_2 and other dissolved gases. Headspace

gases were subsequently ducted to waste or to gas chromatographs (GC) with an electron capture detector (ECD) and He ionisation detector (HID) (CÁRDENAS ET AL., 2003). The flow rate of approximately 10 ml min^{-1} $vessel^{-1}$ was regulated by the use of mass flow controllers and reduced to 5 ml min^{-1} when N_2O concentrations reached their maximum to increase sensitivity of the N_2 analysis. Detection limits at a flow rate of 10 ml min^{-1} were 2.3 for N_2O and 9.6 g N ha^{-1} d^{-1} for N_2.

After 6 days of incubation, the oxygen concentration was reduced to zero (anaerobic period) in order to achieve fully anoxic conditions. The total gas flow was kept constant and the lack of O_2 compensated with He. The samples were kept at a constant temperature of 20°C throughout the experiment via an automated temperature control unit.

Cores were removed from the system for soil analysis, one for each treatment (pre-wet and pre-dry) at 5 different stages of the experiment: directly after fertilizer application (phase 0), when N_2O fluxes increased (phase I), when N_2O fluxes were at maximum (phase II), when N_2O fluxes decreased and N_2 fluxes were at maximum (phase III), and when O_2 was shutdown (phase IV).

When a core was removed, a length of tubing was put in place to connect what was the inlet of the vessel to the outlet.

5.2.2 Gas sampling and analysis

The emissions of N_2O, CO_2 and N_2 and the isotopic signatures of emitted N_2O were measured during the incubation period. The emissions of N_2O and N_2 were measured continuously by gas chromatography and 20 ml glass vials were connected to the vents of the vessels to collect samples for CO_2 analysis. These samples were taken three times a day. CO_2 was measured with a GC equipped with flame ionization detector (FID) and a methanizer to convert CO_2 to CH_4.

For isotopic analysis of the emitted N_2O, duplicate gas samples were collected in two 115 ml septum-capped serum bottles, which were connected in line to the vent of each vessel.

Isotopologue signatures of N_2O, i.e. $\delta^{18}O$ ($\delta^{18}O$-N_2O), average $\delta^{15}N$ ($\delta^{15}N^{bulk}$-N_2O) and $\delta^{15}N$ from the central N-position ($\delta^{15}N^{\alpha}$), were analysed after cryo-focussing by isotope ratio mass spectrometry as described by WELL ET AL. (2008). ^{15}N site preference (SP) was obtained as SP = 2 * ($\delta^{15}N^{\alpha}$ - $\delta^{15}N^{bulk}$-N_2O). Isotopologue ratios of a sample (R_{sample}) were expressed as ‰ deviation from $^{15}N/^{14}N$ and $^{18}O/^{16}O$ ratios of the reference standard materials (R_{std}), atmospheric N_2 and standard mean ocean water (SMOW), respectively:

$$\delta X = (R_{sample}/R_{std} - 1) \times 1000 \qquad (1)$$

where X = $^{15}N^{bulk}$-N_2O, $^{15}N^{\alpha}$, $^{15}N^{\beta}$, or ^{18}O.

5.2.3 Soil sampling and analysis

Total C and N, $\delta^{15}N$ of total N, mineral N (NO_3^-, NH_4^+), $\delta^{15}N$ and $\delta^{18}O$ of the NO_3^-, microbial C and microbial N and the water content of the incubated soils were determined at different stages during the incubation period as described in Section 5.2.1. The removed sample was homogenised and sub samples were taken for further analysis.

Total C and N and $\delta^{15}N$ values of the soil samples were analysed using an automated continuous flow ANCA 20/20SL system (Europa, Crewe, UK). The analytical precision of the $\delta^{15}N$ measurements was < 0.2‰. Soil mineral N was analysed by automated colorimetry (SEARLE, 1984) from 2 M KCl soil extracts. The $\delta^{15}N$ and $\delta^{18}O$ of the soil NO_3^- was analysed with the method described by MCILVIN AND ALTABET (2005). Using a modified fumigation-extraction method reported in BOL ET AL. (2003b) the microbial C and N content were investigated and analysed for dissolved organic C, mineral N and microbial N (BOL ET AL., 2003B). The moisture was determined gravimetrically by drying the fresh soil overnight at 105°C.

5.2.4 Calculations and statistical analysis

The cumulative emissions of N_2O and CO_2 were estimated by calculating the area under the curve of the fluxes. The cumulative N_2 was calculated by the same method after the data were corrected by setting the N_2 zero baseline at the concentration measured before the application of the amendment. Differences between treatments were assessed by ANOVA Kruskal-Wallis tests for analysis in Statistica 8.0 (StatSoft, Inc. 2007).

5.3 Results

5.3.1 Carbon and nitrogen in the soil

Initial values for total C and total N in soil were 1.91 ± 0.07% and 0.19 ± 0.01% respectively, and did not change significantly during the experiment. $\delta^{15}N$ of total N was initially +5.7 ± 0.3‰, and also stayed stable during the incubation. The $\delta^{15}N$ of the applied KNO_3 fertilizer was ca. +6.9‰. Microbial C (C_{mic}), dissolved organic carbon (DOC) and microbial N (N_{mic}) increased after amendment and then decreased until the end of the incubation (Table 5.1). Table 5.2 shows the concentrations of ammonium and nitrate in the soil during the experiment. Ammonium concentration remained small (< 6.6 mg N kg dry soil^{-1} for pre-wet and < 7.4 mg N kg dry soil^{-1} for pre-dry) during the experimental period in both treatments while NO_3^- concentration dynamics reflected the addition of fertilizer (up to 86.7 ± 5.2 mg NO_3^--N kg dry soil^{-1} and 65.7 ± 6.9 mg NO_3^--N kg dry soil^{-1} for the pre-wet and pre-dry treatment, respectively) and the turnover during NO_3^- reduction (9.1 ± 8.1 mg NO_3^--N kg dry soil^{-1} and 18.8 ± 5.7 mg NO_3^--N kg dry soil^{-1} at the end of the incubation for the pre-wet and pre-dry treatment, respectively).

Table 5.1:Microbial C (C_{mic}), dissolved organic carbon (DOC) and microbial N (N_{mic}) [mg kg^{-1} dry soil] at different stages of the experiment. Phase 0 represents beginning of the incubation before fertilization. Phase I shows values directly after fertilization, phase II and III represent the end of different phases during the experimental time and phase IV represent the end of incubation while O_2 was shut off according to the N_2O and N_2 fluxes.

	Phase 0 day 0	Phase I day 0.9	Phase II day 2.0	Phase II/III day 3.9	Phase III day 5.9	Phase IV day 9.7
pre-wet						
C_{mic}	214.2	541.3	265.3	371.5	274.5	358.5
DOC	25.6	373.7	278.4	19.6	34.7	19.5
N_{mic}	2.1	8.9	5.0	4.2	3.1	3.4
pre-dry						
C_{mic}	259.2	497.8	432.7	297.4	297.3	242.2
DOC	46.0	235.5	182.2	65.1	37.8	51.0
N_{mic}	3.0	6.5	8.0	1.8	4.3	1.5

Table 5.2:NO_3^- and NH_4^+ contents of the soil at different stages of the experiment (Mean ± standard error). Phase 0 represents beginning of the incubation before fertilization. Phase I shows values directly after fertilization, phase II and III represent the end of different phases during the experimental time and phase IV represent the end of incubation while O_2 was shut off according to the N_2O and N_2 fluxes.

	Phase 0 day 0	Phase I day 0.9	Phase II day 2.0	Phase II/III day 3.9	Phase III day 5.9	Phase IV day 9.7
pre-wet						
mg NO_3^--N kg dry $soil^{-1}$	19.7(5.3)	86.7(5.2)	41.5(4.3)	17.8(11.1)	15.8(4.3)	9.1(8.1)
mg NH_4^+-N kg dry $soil^{-1}$	4.7(1.3)	2.3(0.9)	3.9(1.5)	3.8(2.3)	4.0(2.0)	6.6(2.6)
pre-dry						
mg NO_3^--N kg dry $soil^{-1}$	32.7(5.6)	65.7(6.9)	58.8(0.9)	14.4(3.2)	12.7(12.2)	18.8(5.7)
mg NH_4^+-N kg dry $soil^{-1}$	3.1(1.3)	1.9(1.2)	3.0(1.5)	4.6(1.8)	5.8(3.3)	7.4(3.3)

5.3.2 Gas fluxes

The emissions of N_2O, N_2, and CO_2 from the pre-wet and pre-dry treatments are shown in Figure 5.1 (day 0 concurs with fertilizer application). Due to a leakage of O_2 and N_2 into the DENIS system, data are missing between days 6 and 8. For the period of missing data, fluxes were interpolated based on the fluxes immediately before and after the leak.

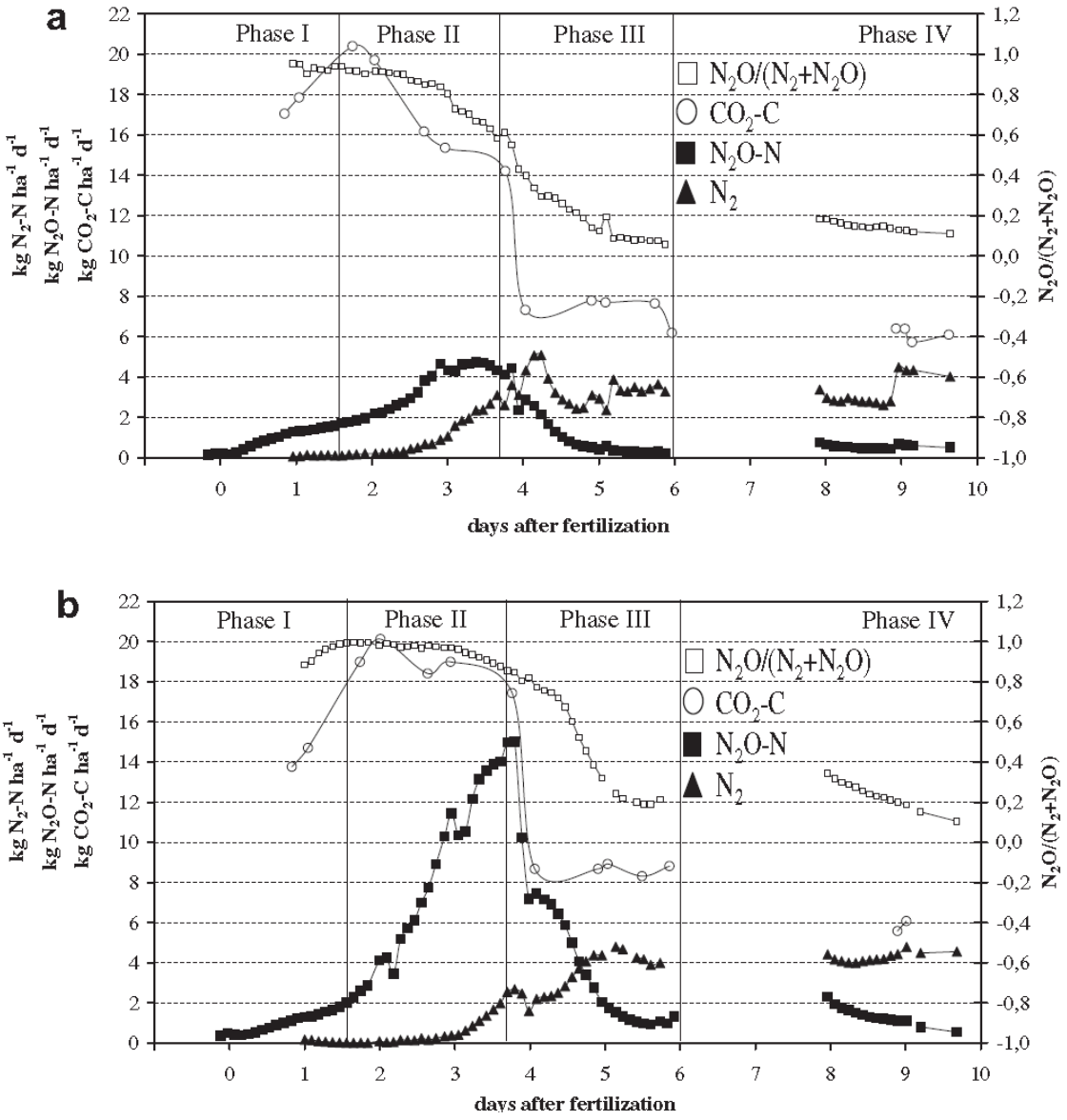

Figure 5.1: N_2O-N, CO_2-C, N_2 fluxes [kg ha^{-1} d^{-1}] and the $N_2O/(N_2 + N_2O)$ ratio in the pre-wet (a) and pre-dry (b) treatment. Data presented are the average of soil cores until they are removed for soil analysis. The vertical lines divide the different phases: increasing N_2O fluxes (phase I), maximum N_2O fluxes (phase II), decreasing N_2O fluxes and maximum N_2 fluxes (phase III) and when O_2 was shutdown (phase IV).

N_2 and N_2O fluxes exhibited characteristic but different time courses. These were used to define experimental phases 0-IV differing in flux trends and the $N_2O/(N_2 + N_2O)$ product ratios (Figure 5.1). The application of amendment immediately produced emissions of N_2O in both pre-wet and pre-dry samples

(phase I). Maximum N_2O emissions were larger for the pre-dry treatment (15.0 ± 10.6 kg N_2O-N ha^{-1} d^{-1}) compared to the pre-wet treatment (4.7 ± 1.5 kg N_2O-N ha^{-1} d^{-1}) and were obtained 3.8 and 3.4 days after the application of amendment, respectively. Emissions stayed high for about one day in the pre-wet treatment (phase II) before the emissions decreased and fluxes were smaller than 0.5 kg N ha^{-1} d^{-1} (phases III, IV). In the pre-dry treatment, N_2O emissions immediately decreased after the maximum on the fourth day reaching a rate at the end of phase III that was twice as high compared to the pre-wet treatment (about 1 kg N ha^{-1} d^{-1}).

The N_2 fluxes increased 2.5 days after amendment in the pre-wet soil and after 3 days in the pre-dry soil (phase II). Slightly higher N_2 fluxes were measured in the pre-wet treatment (5.1 ± 4.6 kg N_2-N ha^{-1} d^{-1}) compared to the pre-dry (about 4.8 ± 6.1 kg N_2-N ha^{-1} d^{-1}), whereas the N_2 maximum of the pre-wet treatment were reached 4.2 days after fertilizer application, and after 5.1 days in the pre-dry (phase III), respectively. N_2 fluxes remained at this level within the pre-dry treatment until the end of the experiment while higher fluctuations of the N_2 fluxes from the pre-wet treatment were observed (between 2.4 and 4.5 kg N_2-N ha^{-1} d^{-1}). In the pre-wet treatment, more N as N_2 (63.3%) than as N_2O (36.7%) was emitted from the soil, whereas in the pre-dry treatment the opposite was observed with less N_2 (42.4%) than N_2O (57.6%). The total gaseous N lost was about 1.6 times higher in the pre-dry treatment (59.5 kg N ha^{-1}) compared to the pre-wet (36.4 kg N ha^{-1}).

The $N_2O/(N_2 + N_2O)$ ratio continuously changed during the incubation. In phase 0 directly after fertilization, the ratio sharply increased from 0 to 1 in the pre-wet treatment within a half day whereas in the pre-dry treatment, a ratio of 1 was reached after 1.6 days. During phases I and II, N_2O was the main product but the $N_2O/(N_2 + N_2O)$ ratio decreased from 1 to 0.6 in the pre-wet treatment, and from 1 to 0.8 in the pre-dry treatment. In phase III it became even smaller with values of 0.1 in pre-wet and 0.2 in pre-dry and the N_2 flux was much larger than the N_2O flux. In phase IV the $N_2O/(N_2 + N_2O)$ ratio varied, but generally decreased, from 0.2 to 0.1 in the pre-wet and from 0.3 to 0.1 in the pre-dry. The results of

the statistical analysis showed that the areas under the curve for the averaged fluxes from each treatment were significantly different for the N_2O fluxes ($p < 0.001$) but not for the N_2 fluxes ($p > 0.1$). The total $N_2O + N_2$ flux was also significantly different for both treatments ($p < 0.001$).

CO_2 fluxes increased more or less linear upon fertilizer addition in both treatments to 20.1 ± 1.7 kg CO_2-C ha^{-1} d^{-1} in the pre-dry and to 20.4 ± 1.3 kg CO_2-C ha^{-1} d^{-1} in the pre-wet treatment within the first 2 days. When N_2O emissions of the pre-dry treatment were high (phase II), the CO_2 emissions also stayed at a higher level, with lowest fluxes of 17.3 kg CO_2-C ha^{-1} d^{-1}. The CO_2 emissions of the pre-wet samples decreased to approximately 14.2 ± 0.7 kg CO_2-C ha^{-1} d^{-1} during phase II. CO_2 fluxes of the pre-wet treatment fluctuated between 6 and 8 kg CO_2-C ha^{-1} d^{-1} for the rest of the experiment. When N_2 emissions in the pre-dry treatment were high (phase III) the CO_2 emissions were also high (between 8 and 9 kg CO_2-C ha^{-1} d^{-1}) compared to the pre-wet treatment. During the last phase IV no difference between CO_2 fluxes were observed between treatments (both were about 6.1 kg CO_2-C kg^{-1} ha^{-1}).

5.3.3 Isotopic signatures of N_2O and NO_3^-

5.3.3.1 $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3

Figure 5.2a shows the temporal pattern of the $\delta^{15}N$-NO_3 signatures. The initial mean value (pre-wet and pre-dry) before fertilization was 4.7 ± 2.2‰. After fertilization it was expected that $\delta^{15}N$-NO_3 would increase during ongoing consumption due to isotope fractionation and thus ^{15}N accumulation in the residual NO_3^-. This expectation was confirmed during phases I and II in both treatments with $\delta^{15}N$-NO_3 increasing to 19.4 ± 0.1‰ and 25.6 ± 0.5‰ in the pre-wet and pre-dry treatment, respectively. However, the further expected trend of increasing values was not observed: the $\delta^{15}N$-NO_3 signature in the pre-dry treatment remained at 25.6 ± 0.5‰ until the end of the experiment. The $\delta^{15}N$-NO_3 signatures of the pre-wet treatment decreased by 3.3‰ during phase III to 16.1 ± 0.7‰ and increased to 27.8 ± 0.4‰ in phase IV.

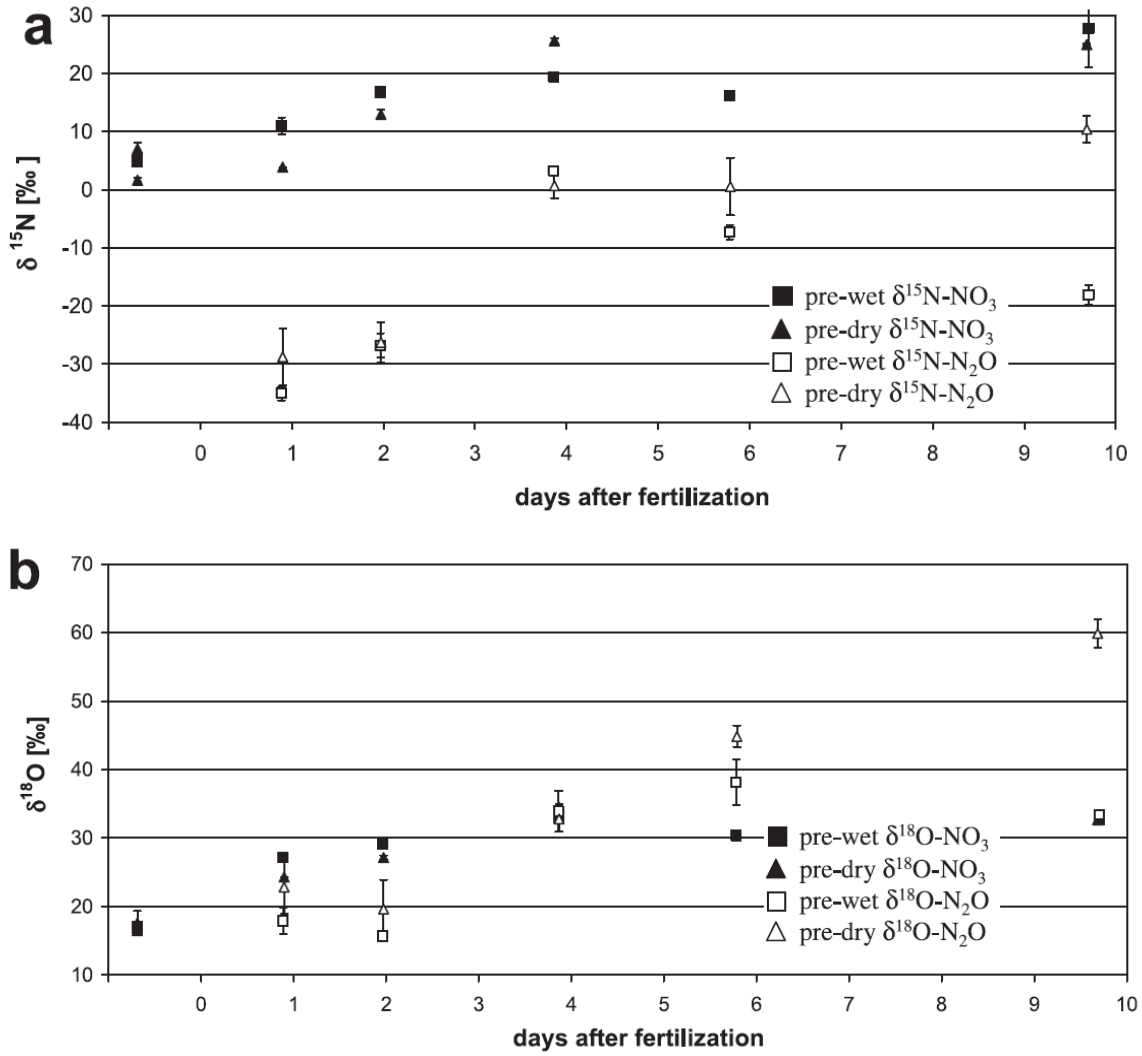

Figure 5.2: $\delta^{15}N$ (a) and $\delta^{18}O$ (b) signatures of soil NO_3^- and of N_2O which concurred with $\delta^{15}N$-NO_3 data during the incubation time. The vertical bars correspond to the standard deviation of these averages.

In Figure 5.2b the measured $\delta^{18}O$-NO_3 showed a similar pattern for both treatments. The slope of $\delta^{18}O$-NO_3 would theoretically be twice as high as $\delta^{15}N$-NO_3 if there were only intermolecular fractionation due to the fact that mass difference between heavy and light isotopes is 2 and 1, respectively. In the pre-wet treatment, the ratio between the shifts of $\delta^{18}O$-NO_3 and $\delta^{15}N$-NO_3 during phases I and II was $\Delta\delta^{15}N$-$NO_3/\Delta\delta^{18}O$-NO_3 = 0.90 (with $\Delta\delta^{15}N$-NO_3 =19.43‰ - 5.01‰ = 14.42‰; $\Delta\delta^{18}O$-NO_3 = 32.91‰ - 16.95‰ = 15.96‰). For the pre-dry

treatment, the respective ratio is 1.22 ($\Delta\delta^{15}$N-NO_3 = 25.62‰ - 4.34‰ = 21.28‰; $\Delta\delta^{18}$O-NO_3 = 34.51‰ - 17.04‰ = 17.47‰). These ratios are thus not in agreement with intermolecular fractionation during nitrate reduction as the only isotope effect. No significant differences of the δ^{18}O-NO_3 were observed during phase III and phase IV, neither in the pre-wet nor in the pre-dry treatment.

5.3.3.2 $\delta^{15}N^{bulk}$ of N_2O

Immediately after fertilization, the $\delta^{15}N^{bulk}$-N_2O of the pre-wet treatment was -35.0 ± 1.2‰ and thus 46‰ lower compared to initial δ^{15}N-NO_3, showing the expected pattern with the product N_2O being isotopically lighter than its substrate (NO_3^-) (Figure 5.2a). For the pre-dry treatment the $\delta^{15}N^{bulk}$-N_2O was -28.7 ± 4.9‰ and therefore -32‰ lower compared to initial δ^{15}N-NO_3 (Figure 5.2a).

The $\delta^{15}N^{bulk}$-N_2O was not significantly different between treatments during the first 4 days (phases I and II) but differed in phase III when N_2O reduction dominated and when O_2 was shut off (phase IV).

The $\delta^{15}N^{bulk}$-N_2O from the pre-wet treatment increased until day 4 to 8.2 ± 1.5‰ (beginning of phase III) and declined to -9.8 ± 1.2‰ until the end of phase III and to -18.1 ±1.7‰ when the O_2 supply was shut off (phase IV). In the pre-dry treatment $\delta^{15}N^{bulk}$-N_2O increased during phases I and II from -28.7 ± 4.9‰ to 0.7 ± 2.1‰ but remained constant with values around 0‰ during phase III. After the removal of O_2 the isotopic values of emitted N_2O in the pre-dry treatment increased to 10.4 ± 2.3‰. Therefore the two treatments differ in the last two stages of the experiment with about 30‰ higher $\delta^{15}N^{bulk}$-N_2O values in the pre-dry compared to the pre-wet treatment at the end.

5.3.3.3 ^{15}N site preference

The ^{15}N site preference (SP) (Figure 5.3) of both treatments showed only small differences. The SP in the pre-wet treatment decreased during phases I and II from 7.2 ± 1.0‰ to 0.8 ± 1.1‰ with a slight increase at the end of phase II to 2.1 ± 1.2‰, parallel to increasing N_2 fluxes. During phases III and IV the SP again increased continuously to 6.6 ± 0.3‰. The time course of the SP of the pre-dry treatment showed the same pattern with decreasing values from 7.8 ± 3.1‰ to -0.2 ± 2.0‰ during phases I and II. During phases III and IV the SP increased to 7.3 ± 0.7‰, similar to the pre-wet treatment.

5.3.3.4 $\delta^{18}O$ of N_2O

Figure 5.2b shows the temporal pattern of $\delta^{18}O\text{-}N_2O$ for both treatments. During phases I and II (no N_2O reduction) $\delta^{18}O\text{-}N_2O$ remained constant (about 20‰) for both treatments while the $\delta^{18}O\text{-}NO_3$ increased (see Section 5.3.3.1). When the $N_2O/(N_2 + N_2O)$ ratio is relatively constant in the pre-wet treatment (end of phase III), $\delta^{18}O\text{-}N_2O$ is also relatively constant (between 36.41 ± 3.2‰ and 38.68 ± 4.6‰) (Figure 5.1a and Figure 5.3a). In the pre-dry treatment $\delta^{18}O\text{-}N_2O$ increased (from 30.59 ± 1.0‰ to 44.84 ± 1.6‰) parallel to increasing N_2O reduction accordingly to a decreasing $N_2O/(N_2 + N_2O)$ ratio (Figure 5.2b and Figure 5.1b). This is also evident from the regression between $\delta^{18}O\text{-}N_2O$ and the $N_2O/(N_2 + N_2O)$ ratio (pre-wet: $\delta^{18}O = -25.71 \times N_2O/(N_2 + N_2O) + 43.51$, $R^2 = 0.77$; pre-dry: $\delta^{18}O = -13.65 \times N_2O/(N_2 + N_2O) + 42.61$; $R^2 = 0.95$). Also during the anaerobic phase IV similar patterns were found since $\delta^{18}O\text{-}N_2O$ slightly dropped in the pre-wet treatment (33.37 ± 0.22‰) while the $N_2O/(N_2 + N_2O)$ ratio slightly increased, and $\delta^{18}O\text{-}N_2O$ of the pre-dry treatment further increased (59.88 ± 2.02‰) while the $N_2O/(N_2 + N_2O)$ ratio further decreased compared to phase III. These observations show that there was a positive relationship between N_2O reduction and $\delta^{18}O\text{-}N_2O$ during all phases.

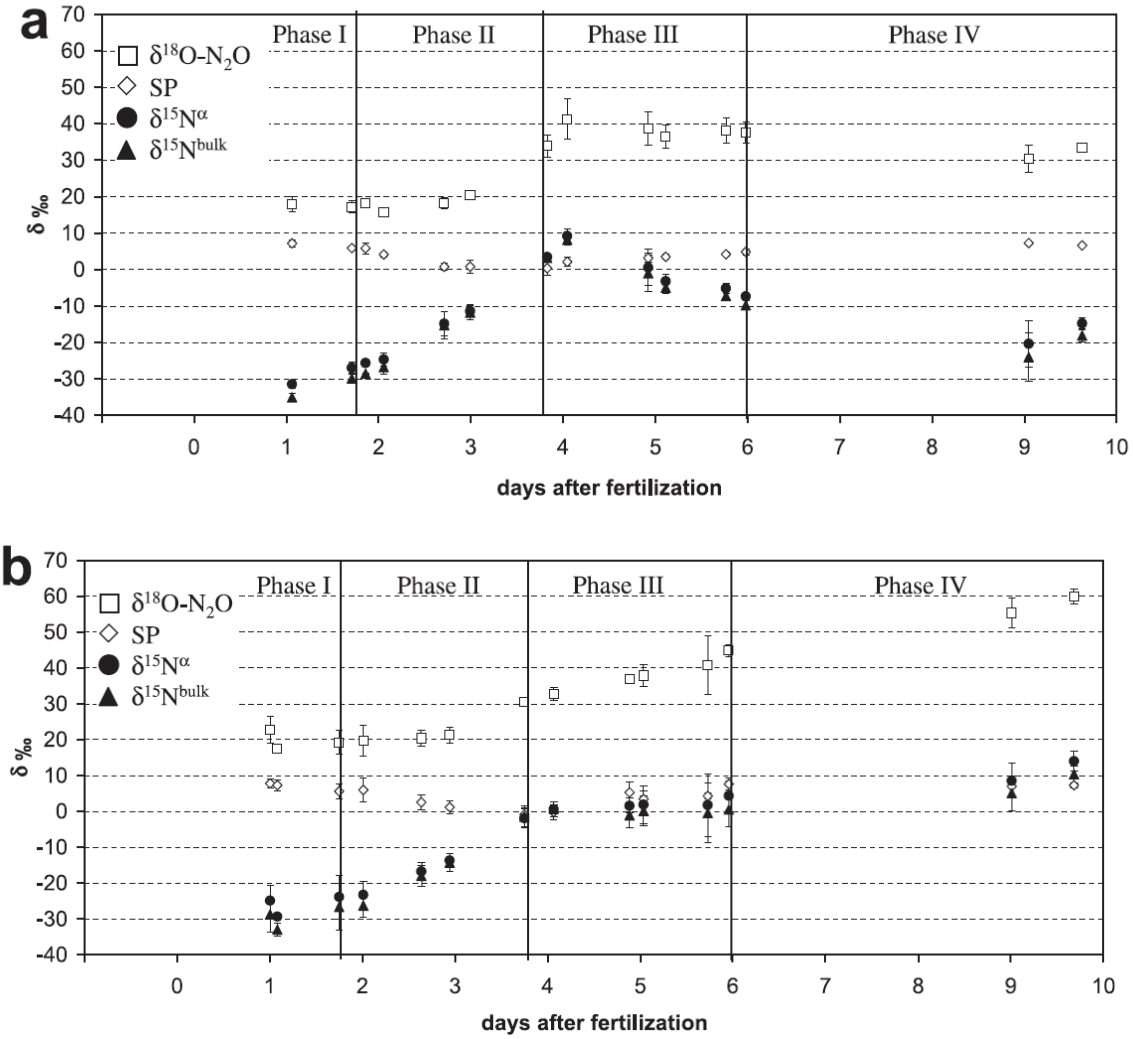

Figure 5.3: All measured isotopic values of the N_2O, as $\delta^{18}O$-N_2O, $\delta^{15}N^{bulk}$-N_2O (already presented in Figure 5.2), $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$ and SP in the pre-wet (a) and pre-dry (b) treatment. The lines correspond to the different phases: increasing N_2O fluxes (phase I), maximum N_2O fluxes (phase II), decreasing N_2O fluxes and maximum N_2 fluxes (phase III) and when O_2 was shutdown (phase IV). The vertical bars correspond to the standard deviation of these averages.

5.4 Discussion

5.4.1 N_2O and N_2 fluxes

5.4.1.1 Control of total denitrification

The soil conditions (high moisture, high NO_3^- content and addition of organic C (C_{org}) in our study were established to favour denitrification. The fast increase and high level of N_2O and N_2 fluxes in phases I to III clearly show the expected effect that nitrate and glucose stimulated the growth and activity of the denitrifier population (TIEDJE ET AL., 1983). The observed pulse in CO_2 emissions as a result of higher respiration rates after the fertilizer amendment supports this assumption. But the impact of the amendment on the time course of (N_2 + N_2O) is probably a combined effect of O_2 consumption during C_{org} respiration, high NO_3^- supply and high supply of electron donors for denitrifiers. Denitrification rates and CO_2 fluxes drastically decreased in phase III, which apparently reflects the ongoing exhaustion of glucose. N_2 and N_2O fluxes in phase IV were thus dominated by denitrification based on soil derived organic C. In the last phase of the experiment both treatments had rather similar low gaseous N production (Figure 5.1). Lack of energy was the likely reason for that because there was still nitrate for denitrification in both treatments as shown in Table 5.2. Total denitrification as given by mean (N_2 + N_2O) fluxes during the experiment was relatively high (3.67 kg N ha^{-1} d^{-1} for pre-wet and 6.27 kg N ha^{-1} d^{-1} for pre-dry).

5.4.1.2 Antecedent moisture effect on total denitrification and N_2O fluxes

Short-term N_2O pulses after rewetting dry soil have been observed in several studies (SMITH AND PARSONS, 1985; CATES AND KEENEY, 1987; RUDAZ ET AL., 1991). RUSER ET AL. (2006) also found increased C availability and associated respiratory O_2 consumption induced by soil drying and rewetting for the emissions of N_2O. Although both treatments received the same energy source and the same amount of nitrate and the soil chemical and microbial characteristics were the same, the total gaseous N loss from the pre-dry treatment was 1.6 times that from the pre-wet treatment. Also the CO_2 fluxes were almost similar

indicating similar microbial activity. We hypothesized that the pre-dry had more available C resulting from the pre-treatment. Within the soil volume reached by the amendment (pool 1), activities could have been similar in both treatments, but in the volume not reached by the amendment (pool 2), activity was higher in the pre-dry. Another possibility is that the microbial community structure could also have contributed to the pre-dry effect by a better development of biomass of facultative anaerobic (denitrifying) microbial community. Thus when the incubation began there could be differences also in the amount of denitrifiers, not only in the enzyme characteristics. It is also possible that there are different products in the two treatments that were not considered such as nitric oxide (NO) and ammonium (NH_4^+). A flush of microbial growth and net mineralization of soil N after sieved air-dried soils are rewetted have been observed on numerous occasions (BIRCH, 1960; HAYNES, 1986). Beside the increase of substrate availability (C and N mineralization) the significant larger flush of N_2O after rewetting the dry soil may be due to microbial stress (KIEFT ET AL., 1987; FIERER AND SCHIMEL, 2003), soil organic matter exposure by physical disruption of aggregates (GOEBEL ET AL., 2005), or alleviation of diffusional constraints (SCHJØNNING ET AL., 2003). The higher DOC found in the pre-dry soil at the start of the incubation in our study (phase 0, Table 5.1) supports that N_2O emissions were significantly larger than in the pre-wet soil as RUSER ET AL. (2006) suggested. This could be potentially used as a mitigation strategy for reducing N_2O emissions. We also think that the delay in the de-repression of the nitrous oxide reductase enzyme in the pre-dry treatment compared to the pre-wet could have caused the larger N_2O/N_2 ratios observed in the former treatment as SCHOLEFIELD ET AL. (1997a,b) suggested. However, at this time we don't have the data to support this.

5.4.1.3 N_2O/N_2 and $N_2O/(N_2 + N_2O)$ ratios

During the period when N_2O was the predominant product of denitrification with $N_2O/(N_2 + N_2O)$ ratios mostly > 0.6 (phases I and II), NO_3^- concentrations were highest. $N_2O/(N_2 + N_2O)$ ratios decreased during phase III when NO_3^- concentrations were lower, showing that N_2O was increasingly used as an electron acceptor, resulting in N_2 production (phase III).

The control of the $N_2O/(N_2 + N_2O)$ ratio is often explained by the balance between electron donors and electron acceptors, where a relative limitation of electron acceptors favours the reduction of N_2O. High NO_3^- concentrations have thus been shown to inhibit N_2O reductase activity (CÁRDENAS ET AL., 2003; STEIN AND YUNG, 2003; TOYODA ET AL., 2005) due to the competitive effect of NO_3^- and N_2O as electron acceptors during denitrification. The higher ratios of $N_2O/(N_2 + N_2O)$ of the pre-dry treatment can not be explained by the balance between electron donors and electron acceptors, since the ratio between available C_{org} and NO_3^- must have been larger due to higher NO_3^- consumption and more available C_{org} from the pre-dry treatment. We suspect that the higher $N_2O/(N_2 + N_2O)$ ratio is due to the higher total fluxes of the pre-dry treatment and a limitation of N_2 production by limited N_2O reductase activity. In wet soils, the N_2O reductase might have already been synthesised, and soon after the production of N_2O it could start being reduced. However, in dry soils, the N_2O reductase could have needed some time to be synthesised (FIRESTONE ET AL., 1980; DENDOOVEN AND ANDERSON, 1994), resulting in a larger $N_2O/(N_2 + N_2O)$ ratio. It has also been suggested that the different lag times of the synthesis of the enzymes involved in the denitrification of N_2O under anaerobic conditions (DENDOOVEN AND ANDERSON,1994) are responsible for the lag period between N_2O and N_2 production. Indeed, in our study we observed a delay in time for N_2 emission to appear to be around 3 days for the pre-dry compared to 2.5 days for the pre-wet treatment, and the lag between the maximum of the emissions of N_2O and N_2 was 32.4 h in the pre-dry and 20.2 h in the pre-wet treatment. The latter value is comparable to that observed for a similar pre-wetted soil in a previous study (MEIJIDE ET AL., 2010).

5.4.2 Isotopic signatures of N_2O and NO_3^- as indictors of process dynamics

To answer the question, how isotopologue signatures of observed N_2O fluxes reflect N_2 production and the N_2O/N_2 ratio, it is necessary to compare observations to the combined effect of all involved processes. In the following this question is addressed for each of the signatures, i.e. for $\delta^{15}N^{bulk}$ SP and $\delta^{18}O$.

5.4.2.1 Average $\delta^{15}N$ of N_2O and NO_3^-

During ongoing reduction of NO_3^-, $\delta^{15}N$ of residual NO_3^- must increase exponentially as a result of Rayleigh-type isotope fractionation (MARIOTTI ET AL., 1981), if the formation of new NO_3^- by nitrification is small. While N_2O reduction to N_2 is absent, $\delta^{15}N$ of emitted N_2O must be lighter compared to $\delta^{15}N$ of its NO_3^- pool. In this period (phases I and II of our study), the difference between $\delta^{15}N$ of N_2O and $\delta^{15}N$ of NO_3^- is thus equivalent to the bulk ^{15}N fractionation factor of the NO_3^--N_2O step ($\varepsilon_{1\text{-}15Nbulk}$, KENDALL, 1998). $\varepsilon_{1\text{-}15Nbulk}$ could thus be calculated for this period giving -44.8 ± 1.8‰ and -35.9 ± 4.6‰ for the pre-wet and pre-dry treatment, respectively. This is within the range of previous values determined using the same approach (WELL AND FLESSA, 2009a).

The $\delta^{15}N^{bulk}$-N_2O and $\delta^{15}N$-NO_3^- both increased during phase I which is probably a consequence of the ^{15}N enrichment in residual NO_3^- during ongoing NO_3^- reduction. However, after phase II, i.e. when N_2O fluxes decreased and N_2 fluxes increased, there was no further increase in $\delta^{15}N^{bulk}$-N_2O and $\delta^{15}N$-NO_3^-. This pattern is opposite to the expected effect of N_2O reduction, since this process must lead to a growing enrichment in $\delta^{15}N$ in the residual N_2O (MANDERNACK ET AL., 2000; WRAGE ET AL., 2005; TILSNER ET AL., 2003). It is thus not in line with a Rayleigh-type fractionation during denitrification within a single pool, i.e. a system where NO_3^- and denitrification rates are homogenously distributed. A possible explanation would be the existence of several N-pools which are different in initial N concentration and in denitrification activity, resulting in N_2O fluxes from each pool which are different in magnitude and isotopic signatures.

While the time course of isotopic signatures of the N_2O flux from each individual pool must follow the one-pool pattern described above, the combined total flux (and hence isotopic N_2O signatures) from the multiple pools together may deviate from that. Therefore, we hypothesise that, initially, there were several NO_3^- pools, and the fraction of N_2O derived from these pools varied over time. This hypothesis was tested by modelling the $\delta^{15}N^{bulk}$-N_2O from three pools.

We modelled isotopic signatures of NO_3^- and emitted N_2O for three distinct (conceptual) N-pools with differing denitrification dynamics. Pool 1 represented the top layer of the soil column (10 vol. %), where most of amendment solution (NO_3^- + glucose solution) was retained, while only a small fraction of the solution diffused into the deeper soil (pool 2). For simplicity, we assumed that pool 2 was homogenous in terms of NO_3^- and labile C, although this might have not being the case due to the possible diffusion of the amendment. Pool 2 was divided into pool 2a (45 vol. %), representing the fraction of pool 2 which was already anaerobic (and thus denitrifying) while O_2 was present in the carrier gas; and pool 2b (45 vol. %), defined as the fraction of pool 2 which was aerobic (and thus not denitrifying) while O_2 was present in the carrier gas, but which became anaerobic (and thus denitrifying) when O_2 was shut off (in phase IV). The (N_2 + N_2O) flux from pool 1, i.e. its denitrification rate was set to 50 mg N kg^{-1} d^{-1}, and 0.5 mg N kg^{-1} d^{-1} in pools 2a and 2b. Initial nitrate concentrations were 50 mg N kg^{-1} for pool 1 and 20 mg N kg^{-1} for pool 2a and 2b. A constant $N_2O/(N_2 + N_2O)$ ratio of 0.5 was assumed. In Pool 2b, denitrification started only when O_2 was shut off (phase IV). To predict the change in isotopic signatures of NO_3^- during ongoing nitrate reduction, a Rayleigh distillation equation was used: $\delta_s = \varepsilon_1 \times \ln f + \delta_{s,0}$, where δ_s and $\delta_{s,0}$ are the isotopic signatures of the residual and initial nitrate, respectively, ε_1 is the fractionation factor of the NO_3^--to-N_2O step and f is the fraction of unreacted substrate (MARIOTTI ET AL., 1981). The isotopic signature of produced N_2O from the NO_3^--to-N_2O step can be obtained by the approximation: $\delta_{denitrification,\ P} \sim \varepsilon_1 + \delta_{s,0}$, where $\delta_{denitrification,\ P}$ is the isotopic signature of produced N_2O, ε_1 is the fractionation factor of the NO_3^--to-N_2O step and $\delta_{s,0}$ is the isotopic signature of initial nitrate (MARIOTTI ET AL.,1981). The

change in the isotopic signature of N_2O during reduction to N_2 can be predicted by the Rayleigh distillation equation: $\delta_s = \varepsilon_2 \times \ln f + \delta_{s,0}$, where $\delta_{s,0}$ and δ_s are the isotopic signatures of produced N_2O and of the residual N_2O after partial reduction, respectively, ε_2 is the fractionation factor of the N_2O-to-N_2 step. For the N_2O-to-N_2 step of denitrification, f is equal to the $N_2O/(N_2 + N_2O)$ ratio of gaseous denitrification products. This assumption is only valid when mass balance is approached, i.e. after NO_3^- is exhausted and no further N_2O is produced. $\delta^{15}N$ of initial NO_3^- was assumed 0‰ in all N-pools. The fractionation factors ε_1 and ε_2 where assumed -52.4 and -10.0‰, respectively, according to literature data (OSTROM ET AL., 2007; MENYAILO AND HUNTGATE, 2006; WELL AND FLESSA, 2009a,b). With these model settings, the time courses of $\delta^{15}N\text{-}NO_3^-$ and of $\delta^{15}N^{bulk}\text{-}N_2O$ were simulated. Average $\delta^{15}N\text{-}NO_3^-$ of all N-pools and $\delta^{15}N^{bulk}$ of the total flux were obtained by a three end member mixing approach. The time courses of modelled $\delta^{15}N$ signatures are shown in Figs. 5.4 and 5.5. Each pool exhibited the Rayleigh-type behaviour, i.e. exponential increase in $\delta^{15}N\text{-}NO_3^-$ with ongoing exhaustion of the substrate (NO_3^-). For each pool the course of $\delta^{15}N^{bulk}\text{-}N_2O$ is parallel to $\delta^{15}N\text{-}NO_3^-$ where the offset results from the fractionation during NO_3^- reduction and partial N_2O reduction to N_2. In the mixed flux, initially, the $\delta^{15}N$ values of NO_3^- and N_2O are increasing in parallel since N_2O fluxes are dominated by pool 1 (simulated fluxes not shown) and the signatures behave like a one-pool system with a continuous increase. This is similar to the observed pattern during phases I and II (see Figure 5.4 and Figure 5.5). With ongoing NO_3^- exhaustion of pool 1, the contribution of the less fractionated pool 2a increases, causing decreasing $\delta^{15}N^{bulk}\text{-}N_2O$ similar to phase III of the experiment. The modelling of phase IV shows a sharp drop in $\delta^{15}N^{bulk}\text{-}N_2O$ with a subsequent increase due to beginning denitrification in pool 2b where NO_3^- is initially unfractionated, since it had been inactive until that moment. This mimics the shut off of O_2 in phase IV which initiated total anaerobiosis and thus denitrification in those parts of the soil that had been aerobic until that moment.

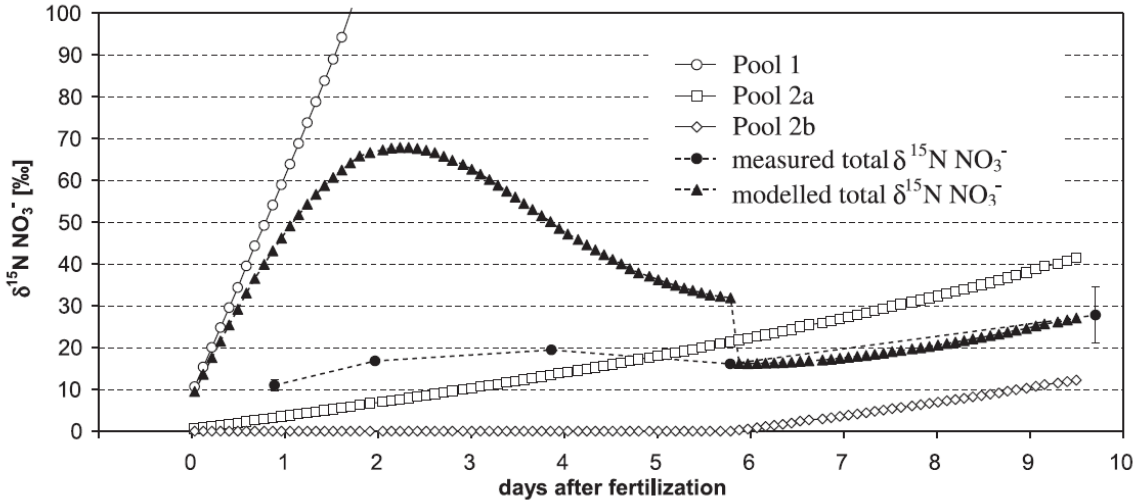

Figure 5.4: Time course of $\delta^{15}N\text{-}NO_3^-$ signatures [‰]. Modelled data of several conceptional pools (1, 2a and 2b) and of the total soil (details on the model given in the text); measured values of the total soil in the pre-wet treatment. The vertical bars correspond to the standard deviation of these averages.

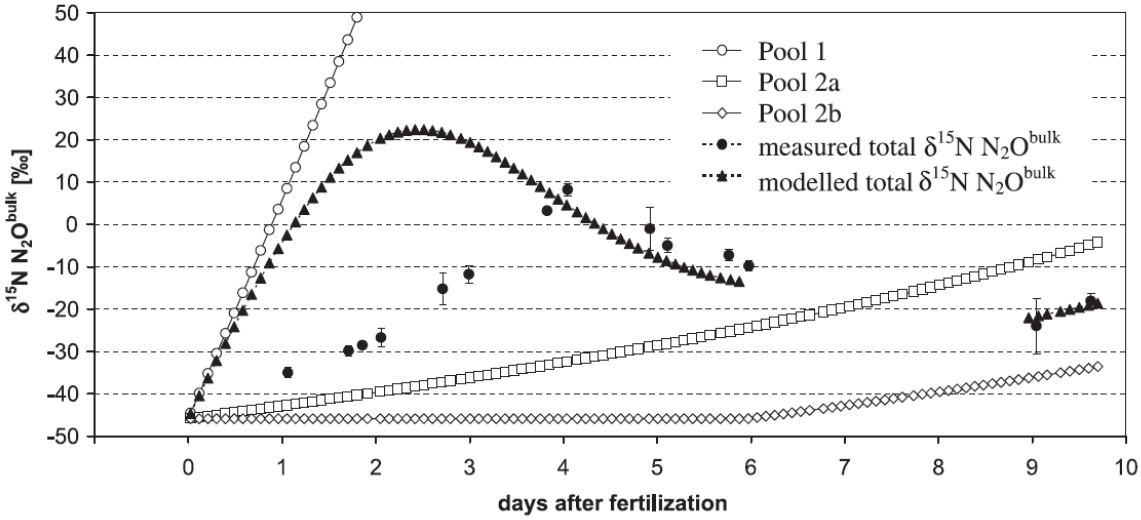

Figure 5.5: Time course of $\delta^{15}N^{bulk}\text{-}N_2O$ signatures [‰]. Modelled data of fluxes from several conceptional pools (1, 2a and 2b) and of the total soil flux (details on the model given in the text); measured values of the total flux in the pre-wet treatment. The vertical bars correspond to the standard deviation of measured averages.

But the agreement clearly shows that our hypothesis on heterogeneous distribution of substrates and denitrification activity is plausible. It shows that, while N_2O originated from several pools with different activity and NO_3^- content (day 1 - 6), the observed decrease or constancy of $\delta^{15}N^{bulk}$-N_2O over time as observed in the pre-wet and pre-dry treatments, respectively, is possible, even though $\delta^{15}N$-NO_3 increases in all denitrifying pools. The extent of this decrease or constancy depends on the size and distribution of NO_3^- and the process rates between the pools. These settings were varied to check whether the slope of $\delta^{15}N^{bulk}$-N_2O must inevitably change as a result of the multiple pool pattern described above. All tested parameter combinations (not shown) yielded a change in the slope of $\delta^{15}N^{bulk}$-N_2O, confirming the plausibility of our multi-pool hypothesis. Modelling $\delta^{15}N^{bulk}$-N_2O thus showed that the assumption of several pools with different contents of nitrate and different process rates provides an explanation of the observed time pattern of $\delta^{15}N^{bulk}$-N_2O. This suggests that isotopic signatures of N_2O reflect the heterogeneity of denitrification activity and the distribution of mineral N. Our results show that combining direct measurements of production and reduction of N_2O using the DENIS He/O_2-atmosphere system and analysing isotopic signatures of the emitted N_2O with process-based modelling is a promising tool for investigating the dynamics of N_2O in heterogeneous soils. A significant impact of substrate heterogeneity on N isotope patterns is probably not a general phenomenon but might only occur in situations where available N and C are added at the same time, e.g. by slurries from animal husbandry, biogas waste or sewage sludges.

Was the effect of antecedent moisture on denitrification reflected by $\delta^{15}N$? $\delta^{15}N^{bulk}$-N_2O differed in phase III when N_2O reduction dominated and when O_2 was shut off (phase IV) with higher values in the pre-dry treatment. This might be explained by the higher (N_2 + N_2O) fluxes during all phases which must lead to higher final enrichment in residual NO_3^-. It shows that during intense denitrification events, average $\delta^{15}N$ of NO_3^- and of emitted N_2O is an indirect indicator for the extent of NO_3^- exhaustion by denitrification.

5.4.2.2 ^{15}N site preference

The ^{15}N site preference of emitted N_2O is the result of several processes, i.e. different mechanisms of N_2O production (nitrification, bacterial and fungal denitrification) and partial N_2O reduction to N_2 (STEIN AND YUNG, 2003; SCHMIDT ET AL., 2004; OSTROM ET AL., 2007). Several studies have shown that N_2O production by denitrification, whether by NO_2^- or NO_3^- reduction, result in particularly low SP values (-5‰ TOYODA ET AL., 2005; average of 0‰, SUTKA ET AL., 2006). Because the reduction step of N_2O consists of the cleavage of NO-bonds, it is expected to cause ^{15}N accumulation on the central N-position of the residual N_2O (TOYODA ET AL., 2002; STEIN AND YUNG, 2003; SCHMIDT ET AL., 2004; OSTROM ET AL., 2007).

What information on source processes can be obtained from observed SP? During the initial phases (I and II), SP decreased from 7.2‰ to 0.8‰ and from 7.8‰ to 1.2‰ in the pre-wet and pre-dry treatments respectively, while N_2O reduction was absent, showing that the site-specific ^{15}N fractionation factor of the NO_3^--to-N_2O step was not constant. This agrees with earlier observations (WELL AND FLESSA, 2009a; MEIJIDE ET AL., 2010) that had explained the decline in SP with a decreasing contribution from fungal denitrification or by the dependence of isotope fractionation factors on N_2O production rates as shown by MARIOTTI ET AL. (1981). Theoretically, decreasing N_2O from nitrification would cause a similar trend. But it is improbable that there was initially significant N_2O from nitrification, due to enhancement of anaerobic conditions by high water content and glucose amendment (WELL ET AL., 2008). Both explanations could apply to our data. Because N_2O production drastically increased during phase II, the second explanation would be possible. Since N_2O production by some fungal denitrifiers appears to require traces of available O_2 (MOROZKINA AND KURAKOV, 2007), O_2 consumption during glucose oxidation might be an explanation for a decreasing contribution from fungal denitrification. The relatively high SP of N_2O produced by fungal denitrifiers (SUTKA ET AL., 2008) could explain why SP was initially high although there was no N_2O reduction.

To check whether N_2O reduction was reflected by SP, the phase after the beginning of the N_2 flux appearance needs to be considered. SP then increased in both treatments, which probably reflects increasing N_2O reduction to N_2. SP was thus apparently governed by two different processes successively, in the initial phase by a microbial or kinetically mediated shift and by N_2O reduction during later phases.

5.4.2.3 $\delta^{18}O$ signatures

The similar trends of $\delta^{18}O\text{-}NO_3$ and $\delta^{15}N\text{-}NO_3$ exemplify that both signatures were governed by the accumulation of heavy NO_3^- during ongoing reduction (MENYAILO AND HUNGATE, 2006; WELL AND FLESSA, 2009a). The slope of $\delta^{18}O\text{-}NO_3$ would theoretically be twice as high as $\delta^{15}N\text{-}NO_3$ if there was only intermolecular fractionation, since the mass difference between heavy and light isotopes is 2 and 1, respectively. The fact that the $\Delta\delta^{15}N/\Delta\delta^{18}O$ ratio is about 1 for both treatments can probably be explained by a combination of the opposite inter- and intramolecular isotope effects.

In phases I and II (absence of N_2O reduction), $\delta^{18}O\text{-}NO_3$ increases while $\delta^{18}O\text{-}N_2O$ is constant. This independence of the product signature ($\delta^{18}O\text{-}N_2O$) from the precursor signature ($\delta^{18}O\text{-}NO_3$) could be explained by an almost complete O-exchange with water. CASCIOTTI ET AL. (2002) reported that incorporation of O from H_2O into N_2O during denitrification by different bacterial species may constitute up to 80%. KOOL ET AL. (2009) proved this using tracer studies with a large variety of soils in which a high O-exchange with water was evident in most cases. Independence of $\delta^{18}O\text{-}N_2O$ from $\delta^{18}O\text{-}NO_3$ during N_2O production from denitrification has been observed earlier (WELL AND FLESSA, 2009a) and had been attributed to O-exchange with water.

During N_2O reduction (phase III and beyond) constant $N_2O/(N_2 + N_2O)$ ratios accompanied relatively constant $\delta^{18}O\text{-}N_2O$, while increasing $\delta^{18}O\text{-}N_2O$ showed increasing $N_2O/(N_2 + N_2O)$ ratios. This is also evident from the regressions between $\delta^{18}O\text{-}N_2O$ and the $N_2O/(N_2 + N_2O)$ ratio (see Section 5.3.3.4). This

shows that the temporal variation in $\delta^{18}O\text{-}N_2O$ was mainly governed by reduction. The close regression illustrates that $\delta^{18}O\text{-}N_2O$ might help to estimate N_2O reduction and thus N_2 fluxes.

5.5 Interim conclusions

Antecedent moisture influenced N_2O and N_2 emissions from an arable soil. Rewetting dry soil increased the emissions of N_2O but not the emissions of N_2 as compared to wetting a field moist soil. The N_2O-to-N_2 ratio was considerably lower in pre-wet soils compared to pre-dry soils. Further studies should be carried out to evaluate if wetting/irrigating of the soils before fertilizer application could be used in the field as a mitigation strategy to decrease N_2O losses.

The N_2O isotopologue values reflected the temporal pattern of observed in N_2O and N_2 fluxes. A concurrent increase in ^{15}N site preference and $\delta^{18}O\text{-}N_2O$ was found to be indicative of N_2O reduction to N_2. Modelling the isotope fractionation during production and reduction based on the measured temporal pattern of the $\delta^{15}N\text{-}N_2O$ suggested that there was a multi-pool (non-homogenous) distribution of NO_3^- in the soil. This shows that our approach of combining the measurement of N_2 and N_2O fluxes and isotopic signatures of NO_3^- and N_2O with isotope fractionation modelling gives insight into the spatial distribution of N species and microbial activity in soils. However, evaluation of isotopologues for identifying source processes was hampered by the simultaneous occurrence of several factors contributing to the time course of isotopic signatures, which could thus not be fully explained. To better estimate how N_2 fluxes and the N_2O/N_2 ratio are reflected by the isotopic signatures of emitted N_2O and of NO_3^- in soil, future studies have to include incubations with more homogenous conditions. Scaling to the field would require studies in intact cores in the lab and measurements at the plot scale.

5.6 Summary of the chapter

The present study determined the influence of initial moisture conditions on the production and consumption of nitrous oxide (N_2O) during denitrification and on the isotopic fingerprint of soil-emitted N_2O. Sieved arable soil was pre-incubated at two different moisture contents: pre-wet at 75% and pre-dry at 20% water-filled pore space. After wetting to 90% water-filled pore space the soils were amended with glucose (400 kg C ha^{-1}) and KNO_3 (80 kg N ha^{-1}) and incubated for 10 days under a He/O_2-atmosphere. Antecedent moisture conditions affected denitrification. $N_2 + N_2O$ fluxes and the N_2O-to-N_2 ratio were higher in soils which were pre-incubated under dry conditions, probably because mobilization of organic C during the pre-treatment enhanced denitrification. Gaseous N fluxes showed similar time patterns of production and reduction of N_2O in both treatments, where N_2O fluxes were initially increasing and maximised 3 - 4 days after fertilizer application, and N_2 fluxes were delayed by 1 - 2 days. Time courses of $\delta^{15}N^{bulk}$-N_2O and $\delta^{18}O$-N_2O exhibited in both treatments increasing trends until maximum N_2 fluxes occurred, reflecting isotope fractionation during intense NO_3^- reduction. Later this trend slowed down in the pre-dry treatment, while $\delta^{18}O$-N_2O was constant and $\delta^{15}N^{bulk}$-N_2O decreased in the pre-wet treatment. We explain these time patterns by non-homogenous distribution of NO_3^- and denitrification activity, resulting from application of NO_3^- and glucose to the surface of the soil. We assume that several process zones were thus created, which affected differently the isotopic signature of N_2O and the N_2O and N_2 fluxes during the different stages of the process. We modelled the $\delta^{15}N^{bulk}$-N_2O using process rates and associated fractionation factors for the pre-treated soils, which confirmed our hypothesis. The site preference (SP) initially decreased while N_2O reduction was absent, which we could not explain by the N-flux pattern. During the subsequent increase in N_2 flux, SP and $\delta^{18}O$-N_2O increased concurrently, confirming that this isotope pattern is indicative for N_2O reduction to N_2. The possible effect of the antecedent moisture conditions of the soil on N_2O emissions was shown to be important.

6 Synthesis

6.1 Synthesis and general conclusions

The world population increases rapidly and a parallel increase in N consumption to ensure the food production by fertilization is observed. Considering both, ecological and economic reasons, N loss from soils via leaching or as gaseous product (particularly N_2O) should be preferably low.

About 60% of global anthropogenic N_2O emissions (IPCC, 2007) derive from agriculture. Associated soils in turn share 15.3% of the total amount of N_2O emissions, or 41.8% of anthropic emissions (DENMAN ET AL., 2007).

Soil management practices (e.g. tillage, residues quality, N fertilization) are known to modify factors (temperature, moisture, aeration, relief position, soil characteristics, available C, C/N ratio and their interaction) which alter the net exchange of N_2O between soil and atmosphere, as well as climate does.

There are still significant questions to be addressed with respect to N transformations and losses from terrestrial ecosystems summarized by MÜLLER AND CLOUGH (2013): the interactions and impact of plants with the N cycle (KNOPS ET AL., 2002), the key processes for the N cycle, in particular denitrification (DAVIDSON AND SEITZINGER, 2006) and small-scale variability, and microsite N dynamics and interactions are unresolved to a large extent and not adequately understood.

6.1.1 Effect of long-term tillage on N_2O emissions from arable soils

6.1.1.1 Reduced tillage did not necessarily alter N_2O emissions

There are many economical and ecological reasons to adopt less intense cultivation to arable soil. Some of the economical reasons are reduced labor, saved fuel, reduced machinery wear, while ecological reasons include less erosion and thus improved water quality, improved water availability, less fossil fuel emissions and thus better air quality, improved soil structure and less risk of damage from machinery and reduced nitrogen leaching risk. Under many circumstances reduced tillage is preferable to ploughing, because it promotes soil

stability, fertility and porosity, but reductions in N_2O emissions are not necessarily concomitants of reduced tillage.

Several key factors determining the net exchange of N_2O are mentioned in literature, but key factors as well as their interactions have shown to be highly site specific in the field. Even if laboratory studies might show repeatable correlations of key factors and N_2O emissions scaling to the field is difficult.

The high complexity of the soil being a multiphase system with its mineral and organic component on the one hand and the pore system containing both soil water or soil air on the other hand, is one reason for the high uncertainties in predicting N_2O emissions from soil. Soil properties at the plot scale are highly variable in space and time and uncertainties in predicting N_2O emissions increase with increasing expansion (field, landscape, region, global).

In addition to the high variable physical, chemical and biological factors altered by climate, cropping practices and their interactions affect the microbial processes. The variety on the microbial level, including species, their quantity and their activity, contribute to differences in overall N_2O emissions. This emphasizes the need of gaining process information on the one hand and understanding the interaction of influencing variables on the other hand.

No significant effect of long-term reduced tillage at the investigated arable German soils on cumulative N_2O emissions over a period of two entire years were found (Chapter 2). The observed high spatial variability at the plot and field scale emphasizes the mentioned highly fluctuating conditions and are well documented in several studies. This variability may explain the high uncertainty in prediction of N_2O emissions from arable soils using process based models even if they are adequately calibrated (Chapter 3).

Summarizing, the effect of long-term tillage on the net exchange of N_2O was negligible and the spatial and temporal variability of flux rates were influenced by soil properties, climate and short-term management effects rather than by tillage system.

6.1.1.2 Prediction of cumulative N_2O emissions of differently tilled soils were accurate

The DNDC model was used to elucidate the role of tillage systems on crop yields and N_2O emissions for the investigated German soils (Chapter 3). Even though the site specific parameterization was successful and the prediction of annual cumulative N_2O emissions for the different tillage systems at the two studied sites were fairly accurate, the annual distributions of N_2O emissions could not be predicted.

This incident underlines the need for long-term studies to validate such models in order to improve pedotranser functions and for instance the denitrification sub-model of the DNDC. Using default values and initial data results generated by the DNDC were inappropriate for the investigated soil. Although parameters were fitted with literature data, such values (maximum grain yields, N fixation index, thermal degree days, transpiration coefficient) need to be collected emphasizing the need of field and laboratory experiments.

As a conclusion the model matched measured annual N_2O emissions and crop yields. The modeled data confirmed that differences in N_2O emissions affected by tillage system were low with one exception at one site in one year where higher emissions at the reduced tillage plots were found both, in the field and by modeling. That implies that the DNDC might be useful regarding to predict N_2O emissions from soils with differing tillage.

6.1.2 Identification of N_2O production and consumption processes during denitrification in an arable soil using stable isotope approaches

6.1.2.1 N_2O isotopologue values reflected the temporal patterns observed in N_2O and N_2 fluxes

In both laboratory experiments, the combination of flux measurements and determination of isotopologue changes during N_2O production and reduction under denitrifying conditions yielded helpful process information (chapter 4 and chapter 5). However, the surface amendment of easily degradable C and N sources has shown to hamper the evaluation of identifying source processes. The non-homogeneous distribution of C and N in the soil core probably resulted in different process zones. These pools may differ in their proportion, NO_3^- content, O_2 availability and denitrification activity. Figure 6.1 presents the assumed distribution of those different pools. The hypothesis was that the fractions of N_2O derived from those different N pools varied over time and in their isotopic signatures. A model performed with the data of the second study showed clear agreement between the modeled and measured isotopic pattern and showed that the heterogeneous distribution of substrates and denitrification activity assumed was plausible.

A concurrent increase in ^{15}N site preference (SP) and $\delta^{18}O$-N_2O was found to be indicative of N_2O reduction to N_2 within both studies.

The temporal pattern of SP might derive from two different consecutive processes: a microbial or kinetically mediated shift in the initial phase when N_2O production was the main process on the one hand and an increase in SP governed by proceeded N_2O reduction during later phases on the other hand. Concluding, further work is needed to distinguish the impact of microbial communities and their enzymes on the temporal variation of isotopic signatures of emitted N_2O.

Summarizing the results, using stable isotope approaches improved the understanding of N_2O production and consumption processes during denitrification in an arable soil.

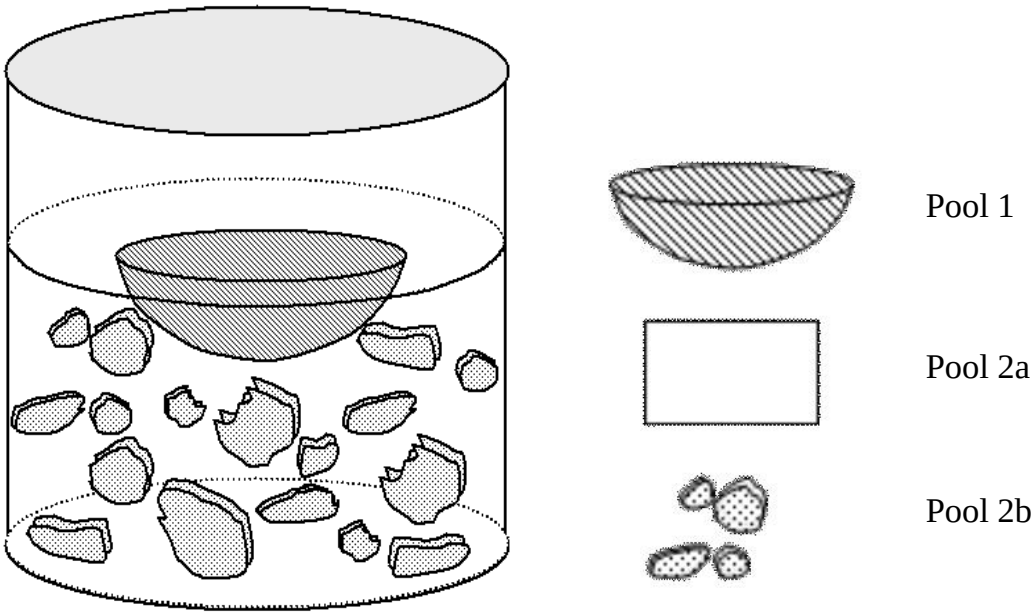

Figure 6.1: Conceptual model of different N-pools. Pool 1 is the soil volume reached by the amendment solution. Pool 2 is the volume not reached by the amendment and is subdivided into two parts. Pool 2a is the fraction of microsites which are already anaerobic, i.e. denitrifying, while O_2 is present in the carrier gas. Pool 2b is the fraction which is initially aerobic and becomes anaerobic when O_2 is shut off.

6.1.2.2 Antecedent soil moisture conditions affected emissions and isotopologue distribution of N_2O during denitrification

In general, the pre-wet treatment of the second laboratory experiment showed comparable results with respect to total denitrification (N_2 + N_2O) fluxes as well as for the temporal variation of fluxes as found in the first laboratory study. Furthermore, N_2O isotopologue values reflected the temporal patterns observed in N_2O and N_2 fluxes as monitored previously, too. As we created very similar conditions for both experiments with regard to conditions favorable for denitrification, these similar results are not surprisingly but emphasize the reproducibility and thus accuracy of results.

In accordance with the effect of rewetting soil, total denitrification and the N_2O-to-N_2 ratio have shown to be higher for the pre-dry soils, probably because mobilization of organic C during the pre-treatment enhanced denitrification.

While the temporal variability of N fluxes were similar in both treatments, the higher N_2O production found for the pre-dry soil explain the higher $\delta^{15}N^{bulk}$ values when N_2O reduction dominated. However, the question whether a shift of the microbial community structure or in the enzyme characteristic was responsible, could not be verified.

With respect to the findings of both laboratory studies we modeled the $\delta^{15}N\text{-}N_2O^{bulk}$ and $\delta^{15}N\text{-}NO_3^-$ signatures and the agreement with measured parameters showed that the heterogeneous distribution of substrates and denitrification activity assumed was plausible. Settings were varied in order to test if the different observed isotopic pattern of the pre-dry treatment could also be modeled and were thus successful. Furthermore, the higher $\delta^{15}N^{bulk}$ signatures in the pre-dry treatment may be an indirect indicator for the extent of NO_3^- exhaustion by denitrification.

The lower N_2O-to-N_2 ratio found for the pre-wet soil might be an indication of mitigation potential to decrease N_2O losses at the field scale by wetting/irrigating the soils before fertilization.

The antecedent soil moisture conditions effected emissions and isotopologue distribution of N_2O during denitrification in an arable soil. The gained process information by using stable isotope approaches may be helpful to evaluate consequences for the agricultural sector with respect to the expected climate change. For instance, warmer and longer ongoing dry periods may result in high soil-derived N_2O emissions after becoming wet.

6.2 Suggestions for future research

Influences of tillage systems might stay invisible due to opposing effects triggered by the change of various key factors. An interesting aspect for further research is for example the question if increased N_2O fluxes are due to O_2 consumption in the soil profile (conventional tillage) or due to higher N_2O production at the soil surface resulting from higher C_{org} and NO_3^- availability (reduced tillage). Moreover the high contribution of thaw induced N_2O release could be a second interesting topic for further research. Concentration profiles of N_2O in combination with flux rates and isotope measurements may fill the gap of knowledge. Using stable isotopes, the hypothesis of pronounced N_2O reduction under certain conditions may help to develop mitigation strategies to reduce N_2O emissions from arable soils.

The denitrification-decomposition (DNDC) model was used in order to better understand the effects of different long-term systems (conventional vs. reduced tillage) on crop yields and N_2O emissions. Given default values by the DNDC model were not always useful and significant changes in key soil physical and chemical parameters were partly required. The calibration of crop properties for different sites is evident as literature data only provide overviews of ranges. Gaining more data considering ongoing improvements of crop growth (better varieties, pest control, and growth promoters) may help to improve such models. Furthermore the DNDC model itself may need further enhancements (estimation of potential evapotranspiration, denitrification sub-model).

The fact that homogenized, primed and repacked soil was used and incubated at certain conditions favoring denitrification suggests that potential rather than actual flux rates were determined in the first laboratory experiment. As high amounts of water, nitrate and glucose were added and temperatures and anaerobic conditions were kept constant those fluxes were very high and are not comparable to fluxes at the field scale. Evidence was found that codenitrification

associated with fungi may have played a role next to bacterial denitrification. Further work is required to examine if the observed different 'isotope' trajectories are mediated by different microbial populations or by the existence of several N-pools. Furthermore, the determination of $\delta^{15}N$ of NO_3^- during incubation may help to differentiate between the enrichment of $\delta^{15}N$-N_2O caused by increasing $\delta^{15}N$-NO_3^- or by N_2O reduction. Clearly, further work needs to clarify to what extent differences in site preference between terrestrial systems are related to process conditions or to microbial community structure. Overall, further work should focus on the impact of microbial communities and their enzymes on the temporal variation of isotopic signatures of emitted N_2O. In order to check potential effects of microbial dynamics and of non-homogenous distribution of N on gaseous emissions of both N_2O and N_2, modeling of N_2O turnover and associated isotope fractionation should be included.

The results of the second laboratory experiment confirm the documented shift in N_2O production after rewetting soil. Further studies should be carried out to evaluate if wetting/irrigating of the soils before fertilizer application could be used in the field as a mitigation strategy to decrease N_2O losses. Furthermore, situations in which available C and N are added simultaneously (e.g. slurries from livestock farming, biogas waste and sewage sludge) should be considered. Scaling to the field would require laboratory studies with more homogeneous conditions (e.g. intact soil cores) and measurements at the field scale. Measurements at the microbial level are needed to differentiate between processes, which are accountable for the higher N_2O formation induced by rewetting. Those investigations should try to figure out if a higher microbial activity, a higher amount of denitrifiers or a shift in the microbial community or a mixture of them are mainly responsible for the higher N_2O fluxes after rewetting.

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Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbstständig und ohne unerlaubte Hilfe angefertigt habe. Ich habe keine anderen als die angegebenen Quellen verwendet und habe alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen sind, als solche kenntlich gemacht.

Die den Kapiteln 2 und 5 zugrunde liegenden Manuskripte habe ich als Erstautorin verfasst. Bei den Manuskripten der Kapitel 3 und 4 bin ich korrespondierende Autorin und maßgeblich an der Datenerhebung, Auswertung, Darstellung und Interpretation beteiligt gewesen.

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