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# Nitrous oxide emissions and mitigation strategies

# Measurements on an intensively fertilized vegetable cropped loamy soil


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Freiburg, 29. März, 2011

Helena Pfab

Ach Gott! die *Forschung* ist lang;
Und kurz ist unser Leben.
Mir wird, bei meinem kritischen Bestreben,
Doch oft um Kopf und Busen bang.
Wie schwer sind nicht die Mittel zu erwerben,
Durch die man zu den Quellen steigt!
Und eh man nur den halben Weg erreicht,
Muß wohl ein armer Teufel sterben.

O glücklich, wer noch hoffen kann,
Aus diesem Meer des Irrtums aufzutauchen!
Was man nicht weiß, das eben brauchte man,
Und was man weiß, kann man nicht brauchen.
Doch laß uns dieser Stunde schönes Gut
Durch solchen Trübsinn nicht verkümmern!
Betrachte, wie in Abendsonne-Glut
Die grünumgebnen Hütten schimmern.

Modifiziert nach Johann Wolfgang von Goethe,
Faust, 1.Teil;
kursiver Text wurde ersetzt

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# Frequently used abbreviations

| | |
|---|---|
| atom $\%^{15}N$ | $^{15}N$ abundance in atom percent |
| ASN | ammonium sulfate nitrate |
| AS | ammonium sulfate |
| $CO_2$ | carbon dioxide |
| DMPP | 3,4-dimethylepyrazole phosphate |
| DOC | dissolved organic carbon |
| ECD | electron capture detector |
| EF | emission factor |
| IPCC | Intergovernmental Panel on Climate Change |
| M | molarity |
| N | nitrogen |
| $N_2$ | dinitrogen |
| $N_2O$ | nitrous oxide |
| $NH_4$ | ammonium |
| $N_{min}$ | mineral nitrogen |
| $NO_x$ | nitrogen oxides |
| $NO_3$ | nitrate |
| $ppb_{vol}$ | parts per billion |
| WFPS | water-filled pore space |

# 1 Summary - Zusammenfassung

## 1.1 Summary:
## Nitrous oxide emissions and mitigation strategies - Measurements on an intensively fertilized vegetable cropped loamy soil

Nitrous oxide ($N_2O$) is a potent greenhouse gas which is also involved in stratospheric ozone depletion. There is consensus that a reduction in $N_2O$ emissions is ecologically worthwhile. Agricultural soils are the major source of $N_2O$ emissions in Germany. It is known that high N-fertilization stimulates $N_2O$ emissions by providing substrate for the microbial production of $N_2O$ by nitrification and denitrification in soils. However, outside the vegetation period, winter freeze/thaw events can also lead to high $N_2O$ emissions. Winter emissions constitute about 50 % of total emissions in Germany. Therefore, **annual datasets are a prerequisite for the development of $N_2O$ mitigation strategies in regions with winter frost**.

Many studies have investigated mitigation strategies for $N_2O$ emissions from agricultural soils. However, $N_2O$ release from vegetable production has seldom been studied. None of the existing **trace gas measurements on intensive vegetable production** is representative for the climatic conditions of Southern Germany. Due to the **high fertilizer N-input** (resulting in high levels of mineral N in the soil) and **N-rich residues** in late autumn, high annual $N_2O$ emissions are to be expected.

**$N_2O$ fluxes were measured** from a soil cropped with lettuce and cauliflower in Southern Germany **by means of the closed chamber method, at least weekly, for two years**. An additional study was conducted using $^{15}N$ labeled ammonium sulfate nitrate (ASN) fertilizer and exchange of labeled and unlabeled residues to obtain information about the sources (fertilizer, residues, soil internal mineralization) of $N_2O$ emissions.

Different **mitigation strategies** such as fertilizer reduction, addition of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) and banded fertilization were evaluated with respect to their reduction potential on an annual base. Fertilizer reduction is supposed to decrease the soil mineral N level, reducing the available substrate for $N_2O$ producing microorganisms. DMPP is a chemical compound which inhibits nitrification enzymatically. In banded fertilization, ammonium rich fertilizer is applied in a depot. This high concentration is also supposed to inhibit nitrification as it is toxic to microorganisms. $N_2O$ emissions should be firstly reduced directly by this inhibition of nitrification and secondly, by a lower nitrate content in soil resulting in less $N_2O$ release due to denitrification.

A high temporal variability in $N_2O$ fluxes was observed with emission peaks after N-fertilization, after the incorporation of crop residues (especially in combination with N-fertilization), after rewetting of dry soil and after thawing of frozen soil in winter.
Total **cumulative annual emissions were 8.8 and 4.7 kg $N_2O$-N $ha^{-1}$ $a^{-1}$** for the first and second experimental year in the conventionally (broadcast) fertilized treatment. This treatment was fertilized according to the German Target Value System. **$N_2O$ emission factors** were **1.6 and 0.8 %**. This is within the range of 0.3 - 3 % which is cited in the Guidelines for the Calculation of National Greenhouse Gas Inventories proposed by the Intergovernmental Panel of Climate Change (IPCC).

A **positive correlation** was found in both years between the **mean nitrate content of the top soil and the cumulative $N_2O$ emissions** of all treatments ($r^2 = 0.44$ and 0.68) as well as between the N-surpluses and the cumulative $N_2O$ emissions of the different fertilizer levels during the first year ($r^2 = 0.95$). **Fertilizer reduction** from fertilization according to good agricultural practice following the recommendations of the German Target Value System **reduced annual $N_2O$ emissions by 17 %** in the first experimental year **without yield reduction**. For the second year, the reducing effect was 10 %, but statistically not significant. Another

fertilizer reduction of a further 20 % reduced $N_2O$ emissions, but also resulted in lower lettuce yields in the first year. Therefore, an additional fertilizer reduction is not recommendable.

This work provides, **for the first time, annual datasets on** the effect of **DMPP-application** on $N_2O$ emissions. Addition of DMPP significantly **reduced annual $N_2O$ emissions by > 40 % during both years**, there was also a pronounced effect, both during the vegetation period and winter. The reason for the reducing effect in winter is not yet clear because the degradation of the active agent DMPP is temperature dependent and should take about 6 to 8 weeks under summer climatic conditions. However, we still observed significant reductions in $N_2O$ emissions in winter, about 3 months after the application. Furthermore, a reduction in $CO_2$ release was observed indicating a possible influence on heterotrophic activities or at least on their C-turnover. Due to its high $N_2O$ mitigation potential, further investigations concerning the functional and structural changes in microbial biomass after DMPP application are needed.
**Banded fertilization with ASN did not result in the expected reduction in $N_2O$ emissions** on an annual base. Even when exchanging the ASN fertilizer by nitrate-free ammonium sulfate, $N_2O$ emissions were not diminished. We assume that the high emissions were derived from the microbially intact surroundings of the depots, where nitrification was not inhibited and nitrate concentrations were probably very high, creating ideal conditions for denitrification.

**After one year, the major part of the fertilizer-$^{15}$N was found in the soil**. Only between 13 - 15 % of the fertilizer was taken up by the marketable plant parts. 1.4 % of the $^{15}$N was lost as $N_2O$-N. Total $^{15}$N recovery was 70 % after one year. The losses of non-recovered N were probably caused by nitrate leaching or as gaseous compounds such as $N_2$ or $NO_x$. Compared to cereal production systems, the N use efficiency of this vegetable production system is much lower, even with an optimized fertilization strategy.
The measurement of $^{15}$N abundances in the $N_2O$ revealed that the **most significant part of the emissions** (38 %) **was derived from the fertilizer-N** which had been taken up by cauliflower residues. $N_2O$ emissions directly derived from **lettuce and cauliflower fertilizer contributed 26 % and 20 % respectively** while $N_2O$ emissions from soil internal N pools accounted for 15 %. The contribution of lettuce residues was negligible due to their low amount of C and N.
The reason for the high importance of the cauliflower residues was ascribed to the temporarily C-limitation of the system and the provision of electron donators by organic material. Furthermore, $O_2$ is consumed during their degradation leading to the formation of anaerobic microsites when soil moisture is high. These sites offer ideal conditions for denitrification. Especially the combination of mineral N-fertilization and input of organic substance was found to increase $N_2O$ emissions. Therefore, the influence of a **de-synchronization of the incorporation of crop residues and the mineral N-fertilization** by waiting periods of up to 3 weeks was tested in an additional field trial during the cultivation of chard. The longer the waiting time between incorporation of crop residues and N-fertilizer application was, the lower were the $N_2O$ emissions. However, the effect was not statistically significant on an annual base.

In an additional microcosm incubation model study, the effect of reduced and increased input as well as of different C/N-ratios of cauliflower residues was analyzed. It was shown that due to the high nitrate level in the microcosms only the amount of residue input has an effect on the $N_2O$ emissions. The $N_2O$ emissions increased with increased amount of cauliflower residues.

Although the emission factors were within the range given by the IPCC, the **absolute annual $N_2O$ emission** was **high in intensive vegetable production** due to the high N-input. Further research is required in order to fully understand the effect of DMPP on the processes of $N_2O$ production in the field. Our study underlines the importance of avoiding N-surpluses and of strategies for residue management to reduce $N_2O$ emissions in intensive vegetable production.

## 1.2 Zusammenfassung: Lachgasemissionen und Minderungsstrategien - Messungen auf einem lehmigen Standort mit intensiver Gemüseproduktion

Lachgas ($N_2O$) ist ein klimarelevantes Spurengas, welches auch zur Ozonzerstörung in der Stratosphäre beiträgt. Es herrscht Konsens darüber, dass eine Reduktion der $N_2O$ Emissionen anzustreben ist. Hauptquelle der $N_2O$ Freisetzung in Deutschland sind landwirtschaftlich genutzte Böden. Aufgrund des hohen N-Inputs über die Düngung wird die $N_2O$-Emission stimuliert, da der Stickstoff als Substrat für die wesentlichen Prozesse der $N_2O$-Bildung in Böden wie die Nitrifikation und Denitrifikation dient. Neben den hohen $N_2O$-Emissionen während der Vegetationsperiode kann auch im Winter eine hohe $N_2O$-Freisetzung in Zusammenhang mit Frost-Tau Zyklen auftreten. Der Anteil dieser Winteremissionen an der Jahresemission beträgt in Deutschland etwa 50 %. Deshalb sind **annuelle Datensätze** eine unerlässliche **Voraussetzung für die zuverlässige Bewertung von $N_2O$-Reduktionsstrategien in Gegenden mit Winterfrost**.

Für landwirtschaftlich genutzte Böden liegt bereits eine Vielzahl an Untersuchungen zur Minderung der $N_2O$-Freisetzung vor. Jedoch wurde die $N_2O$-Freisetzung aus gemüsebaulich genutzten Böden nur selten untersucht. Keine der bisher durchgeführten **Spurengasmessungen im intensiven Gemüsebau** ist repräsentativ für die klimatischen Bedingungen Süddeutschlands. Durch den **hohen N-Düngerinput** (der zu hohen Gehalten an mineralischem Stickstoff im Boden führt) und **stickstoffreiche Ernterückstände** im Spätherbst sind hohe $N_2O$-Jahresemissionen aus diesen Flächen zu erwarten.

Im Rahmen dieser Studie wurden die **$N_2O$-Flussraten zwei Jahre lang in mindestens wöchentlicher Auflösung** auf einer Gemüsebaufläche in Süddeutschland mit der geschlossenen Kammermethode ermittelt. Während der beiden Versuchsjahre wurde jeweils ein Satz Kopfsalat und darauffolgend ein Satz Blumenkohl angebaut. Um Aufschluss über die $N_2O$-Quellen (Dünger, Ernterückstände, bodeninterne Mineralisation) zu erhalten wurde zusätzlich eine Studie mit $^{15}N$ markiertem Ammonsulfatsalpeter (ASS) und Austausch markierter und unmarkierter Erntereste durchgeführt.

Ferner wurden verschiedene **Strategien zur Reduktion der $N_2O$-Emissionen** wie Düngerreduktion, Zusatz eines Nitrifikationshemmstoffes (3,4-Dimethylpyrazolphosphat, DMPP) und eine Depotdüngung hinsichtlich ihres Potentials zur Reduktion der $N_2O$-Emissionen auf Jahresbasis getestet. Die Reduktion der $N_2O$ Emissionen sollte bei diesen Strategien wie folgt erreicht werden: Bei einer Reduktion des Dünger N-Inputs wurde eine Absenkung der Menge an mineralischem N im Boden erwartet und dadurch niedrigere Substratkonzentrationen für $N_2O$ produzierende Mikroorganismen. DMPP ist ein chemischer Hemmstoff, der die Nitrifikation auf enzymatischer Ebene inhibiert. Bei der Depotdüngung wird ammoniumreicher Dünger hoch konzentriert in Form eines Bandes im Boden abgelegt. Die hohen Ammoniumkonzentrationen sollen durch Ihre Toxizität die Nitrifikanten ebenfalls hemmen. Aufgrund der gehemmten Nitrifikation sollte einerseits die $N_2O$-Bildung während der Nitrifikation direkt vermindert und andererseits die Denitrifikation über das geringere Nitratangebot limitiert werden.

Es wurde eine sehr hohe zeitliche Variabilität der $N_2O$-Flussraten beobachtet. Ausgeprägte Emissionsmaxima traten vor allem nach N-Düngungsmaßnahmen, nach der Einarbeitung von Ernterückständen (besonders in Kombination mit der N-Düngung), nach Wiederbefeuchtung von trockenem Boden im Hochsommer sowie nach dem Auftauen von gefrorenem Boden im Winterhalbjahr auf.
Die **kumulativen Jahresemissionen** in der konventionell (breitflächig) gedüngten Variante beliefen sich im ersten und zweiten Versuchsjahr auf **8.8 und 4.7 kg $N_2O$-N $ha^{-1}$ $a^{-1}$**. Die N-Düngung erfolgte hier nach dem kulturbegleitenden $N_{min}$ Sollwertsystem. Die **$N_2O$-Emissionsfaktoren** lagen mit **1.6 % und 0.8 %** innerhalb des Unsicherheitsbereiches von 0.3 - 3 %, den der Weltklimarat (IPCC; 2006) in seinen Richtlinien zur Berechnung Nationaler Treibhausgasinventare angibt.

Es konnte ein **positiver Zusammenhang** zwischen den **mittleren Nitratgehalten des Oberbodens und den kumulativen $N_2O$-Emissionen** in den beiden Versuchsjahren ($r^2 = 0.44$ und 0.68) sowie zwischen den N-Überschüssen und den kumulativen $N_2O$ Emissionen der Düngersteigerungsreihe ($r^2 = 0.95$) im ersten Versuchsjahr nachgewiesen werden. **Eine Reduktion der N-Düngermenge** von praxisüblicher Düngung auf Düngung nach dem kulturbegleitenden $N_{min}$ Sollwertsystem führte im im ersten Versuchsjahr zu einer **Min-**

**derung der $N_2O$-Jahresemissionen um 17 %, die Gemüseerträge** wurden durch die verminderte N-Gabe **nicht beeinträchtigt**. Im zweiten Versuchsjahr wurde die mittlere $N_2O$-Emission bei reduzierter N-Gabe um 10 % gesenkt, dieser Effekt war jedoch statistisch nicht abgesichert. Eine weitere Absenkung der Düngermenge um 20 % führte zwar zu einer weiteren Minderung der $N_2O$-Emission, allerdings waren im ersten Versuchsjahr dadurch auch die Kopfsalaterträge geringer. Eine weitere Absenkung der Düngermenge ist somit nicht empfehlenswert.

Für die **DMPP**-Anwendung liegen durch diese Arbeit **erstmals Jahresdaten** zur $N_2O$-Freisetzung vor. Die Anwendung von DMPP **verringerte die $N_2O$-Emissionen in den beiden Versuchsjahren signifikant um > 40 %**. Dieser Effekt trat sowohl während der Vegetationsperiode als auch im Winter auf. Der Grund für die Emissionsminderung im Winter konnte nicht geklärt werden: Der Abbau des Wirkstoffs DMPP ist temperaturabhängig und wird unter den gegebenen Temperaturen im Sommer mit ca. 6 bis 8 Wochen veranschlagt. Die von uns beobachteten Minderungseffekte traten jedoch auch im Winter auf, also noch 3 Monate nach Applikation des Wirkstoffes. Ferner wurde eine ebenfalls verminderte $CO_2$-Freisetzung gemessen, die ein Hinweis auf einen Effekt des DMPP auf heterotrophe Mikroorganismen oder zumindest deren C-Umsatz sein könnte. Aufgrund des hohen $N_2O$-Minderungspotentials scheinen weiterführende Untersuchungen zu funktionellen und strukturellen Veränderungen der mikrobiellen Biomasse nach DMPP-Anwendung sinnvoll.

Eine **Depotdüngung mit ASS führte nicht zur erhofften Reduktion der $N_2O$ Freisetzung auf Jahresbasis**. Selbst der Ersatz von ASS durch (nitratfreies) Ammoniumsulfat führte nicht zu einer Reduktion der Emissionen. Vermutlich gehen die relativ hohen Flussraten auf die mikrobiell intakten Bereiche um die Düngerdepots zurück, in denen die Nitrifikation abläuft und in denen durch die hohen Nitratgehalte ideale Bedingungen für denitrifizierende Mikroorganismen herrschten.

**Nach einem Jahr** fand sich ein **Großteil des** mit dem Dünger ausgebrachten **$^{15}N$ im Boden** wieder. Nur 13 - 15 % wurden über die marktfähige Ware aufgenommen. 1.4 % des $^{15}N$ gingen in Form von $N_2O$-N verloren. Die Wiederfindungsrate nach einem Jahr betrug 70 %. Die Verluste an $^{15}N$ sind vermutlich auf Nitratauswaschung oder gasförmige Verluste in Form von $N_2$ oder $NO_x$ zurückzuführen. Verglichen mit dem Getreideanbau ist die N-Ausnutzung im Gemüsebau also selbst bei optimierter Düngung wesentlich niedriger. Die Messung der $^{15}N$ Häufigkeit im $N_2O$ zeigte, dass **der Hauptteil der $N_2O$-Emissionen (38 %) aus den Ernteresten des Blumenkohls stammte** (genauergesagt Dünger-N, der über die Pflanzen in die Ernteresten eingelagert wurde). **26 % und 20 %** stammten jeweils direkt **aus dem Dünger zu Kopfsalat und Blumenkohl**. Bodeninterne Quellen waren für 15 % der Gesamtemission verantwortlich, während der Beitrag der Erntereste des Kopfsalats aufgrund der geringen C- und N-Mengen vernachlässigbar gering war.

Der beträchtliche Anteil der $N_2O$-Emissionen aus den Ernteresten des Blumenkohls wurde darauf zurückgeführt, dass das System zeitweise C-limitiert war und so durch das organische Material Elektronendonatoren zur Verfügung gestellt wurden. Zudem wird beim Abbau von organischer Substanz in Böden $O_2$ verbraucht, was bei hohen Wassergehalten zur Bildung anaerober Kompartimente und so zu idealen Bedingungen für Denitrifikanten führt. Besonders der kombinierte Eintrag von organischer Substanz und mineralischem N-Dünger erhöhte die $N_2O$-Emissionen. Daher wurde in einem Zusatzversuch zu Mangold getestet, inwiefern eine **Desynchronisation der Einarbeitung von Ernteresten und der mineralischen N-Düngung** durch Wartezeiten (bis zu 3 Wochen) zu einer Emissionsminderung beiträgt. Je länger die Einarbeitung der Erntereste von der N-Düngerapplikation entfernt lag, desto geringer waren auch die $N_2O$-Emissionen, allerdings war dieser Effekt auf Jahresbasis nicht statistisch gesichert.

In einem Inkubationsversuch mit Mikrokosmen wurde der Effekt von verschiedenen C/N-Verhältnissen von Blumenkohlernteresten sowie die Einarbeitung reduzierter und erhöhter Mengen modellhaft untersucht. Es zeigte sich, dass aufgrund des generell hohen Nitratangebots in den Kosmen lediglich die verschiedenen Ernterestmengen einen Effekt auf die $N_2O$-Freisetzung zeigten. Die $N_2O$-Emission stieg mit der Menge an Ernteresten an.

Insgesamt konnte in dieser Arbeit gezeigt werden, dass **im Gemüsebau relativ hohe absolute $N_2O$-Emissionen** erwartet werden können, auch wenn der relative Anteil (Emissionsfaktoren) im Rahmen des IPCC-Unsicherheitsbereichs lag. Weitere Untersuchungen sind nötig, um die genauen Wirkungsmechanismen von DMPP auf die Bildung von $N_2O$ im Feld zu verstehen. Die vorliegende Studie belegt, dass der Vermei-

dung von N-Überschüssen und der Entwicklung von Strategien zum Ernterestmanagement im Gemüsebau große Bedeutung zur Reduktion der $N_2O$-Emissionen zukommt.

# 2 General introduction

## 2.1 State of research

Due to globalization, many problems of worldwide concern have been discussed controversially by international state conferences during the last years. One severe global challenge for the $21^{st}$ century is certainly the climate change. The Intergovernmental Panel on Climate Change (IPCC, 2007) reports that most of the observed increase in global average temperatures since the mid-$20^{th}$ century is very likely due to the observed increase in anthropogenic greenhouse gas concentrations. Global greenhouse gas emission due to human activities has grown since pre-industrial times by about 70 % between 1970 and 2004. According to the IPCC (2007), the most important anthropogenic greenhouse gases are carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulphurhexafluoride ($SF_6$). As a result of the United Nations Framework Convention on Climate Change in 1992, more than 190 countries declared their wish to stabilize global atmospheric greenhouse gas concentrations (UN, 1992). To reach this ambitious aim for nitrous oxide ($N_2O$), it is essential to quantify the strength of its sources. Nitrous oxide was discovered by Joseph Priestley in 1772 (Priestley, 1790). For the year 10000 B.C., concentrations of $N_2O$ of about 260 parts per billion by volume ($ppb_{vol}$) are reported which were measured in air entrapped in ice cores. From a preindustrial value of about 270 $ppb_{vol}$, the atmospheric concentration of nitrous oxide has been increasing to a concentration of 322 $ppb_{vol}$ in 2008 (Flückiger et al., 1999; WMO, 2009). The actual annual increase in atmospheric $N_2O$ concentration was 0.78 $ppb_{vol}$ during the last ten years (WMO, 2009) and its contribution to the total anthropogenic greenhouse gas emission is about 7.9 % (IPCC, 2007).

**Tab. 2.1:** *Sources of nitrous oxide for the 1990s (IPCC, 2007).*

| Source | $N_2O$ Tg N $yr^{-1}$ | range |
|---|---|---|
| **Anthropogenic sources** | | |
| Fossil fuel combustion & industrial processes | 0.7 | 0.2 - 1.8 |
| Agriculture | 2.8 | 1.7 - 4.8 |
| Biomass and biofuel burning | 0.7 | 0.2 - 1.0 |
| Human excreta | 0.2 | 0.1 - 0.3 |
| Rivers, estuaries, coastal zones | 1.7 | 0.5 - 2.9 |
| Atmospheric deposition | 0.6 | 0.3 - 0.9 |
| **Anthropogenic total** | **6.7** | |
| **Natural sources** | | |
| Soils under natural vegetation | 6.6 | 3.3 - 9.0 |
| Oceans | 3.8 | 1.8 - 5.8 |
| Atmospheric chemistry | 0.6 | 0.3 - 1.2 |
| **Natural total** | **11.0** | |
| **Total sources** | **17.7** | **8.5 - 27.7** |
| **Total sinks** | **12.3** | **9 - 16** |

About 11 Tg $N_2O$-N are produced yearly in soils under natural vegetation, in oceans and in the atmosphere (Tab. 2.1). Anthropogenic emission is the share of the total emission which is directly or indirectly caused by human activity. It has reached a value of 6.7 Tg $N_2O$-N per year. The annual emission of $N_2O$-N for Germany was about 38 Gg in 2008 (UBA, 2010). Besides emission from fertilizer production and biomass burning, especially agricultural soils are a major source of $N_2O$ (Moisier et al., 1998; Kroeze et al., 1999). In soils, two

microbial processes are the major sources of $N_2O$ production: nitrification and denitrification (Bremner and Blackmer, 1981). These two pathways as well as the actual understanding of other sources of $N_2O$ production of probably minor importance are shown in Figure 2.1.

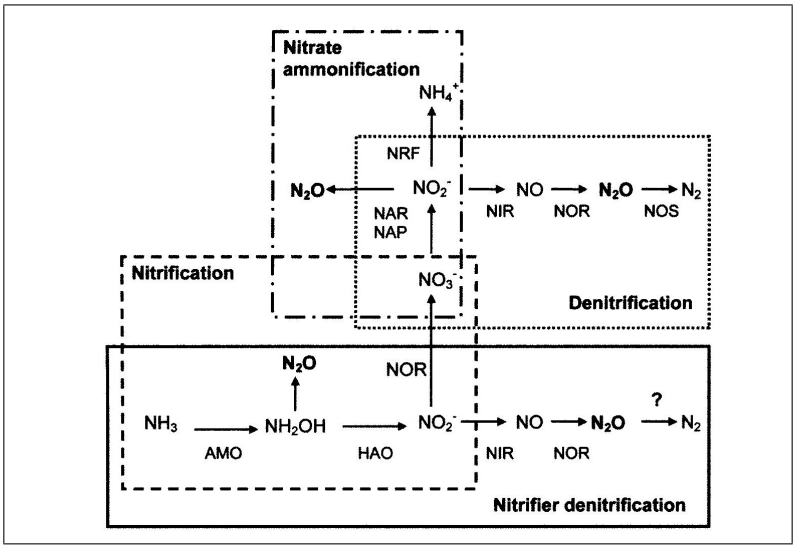


**Fig. 2.1:** *Microbial sources of $N_2O$ in soil and involved enzymes (in: Baggs and Philippot, 2010; adapted from Baggs, 2008).*

$N_2O$ is photolytically decomposed in the stratosphere. During the photolysis, NO is formed which leads to the destruction of ozone ($O_3$) molecules (Crutzen, 1981). This process is the only atmospheric sink for $N_2O$. Therefore, it has a very long atmospheric residential lifetime of 114 years (Montzka et al., 2002). Its specific global warming potential for a 100 year time horizon is about 320 as compared to the same mass of $CO_2$ (IPCC, 1994).

## Nitrification

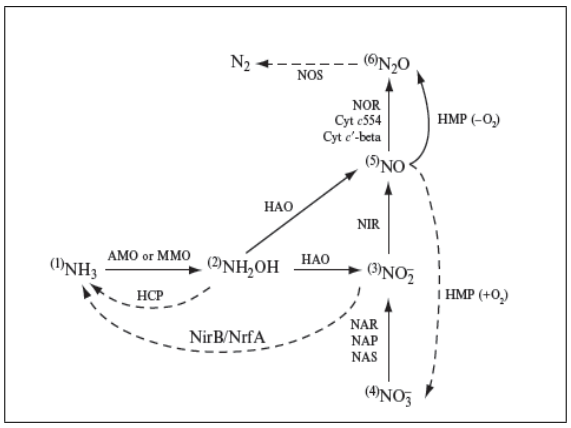


**Fig. 2.2:** *$N_2O$ production and consumption in bacteria. Solid lines: pathways that lead to $N_2O$ as an end product; dashed lines: pathways that consume $N_2O$ or remove a substrate for its production (AMO: ammonium monooxygenase, MMO methan monooxygenase, HAO hydroxylamine oxidoreductase (Stein 2011; for explanation of the other abbreviations see there)).*

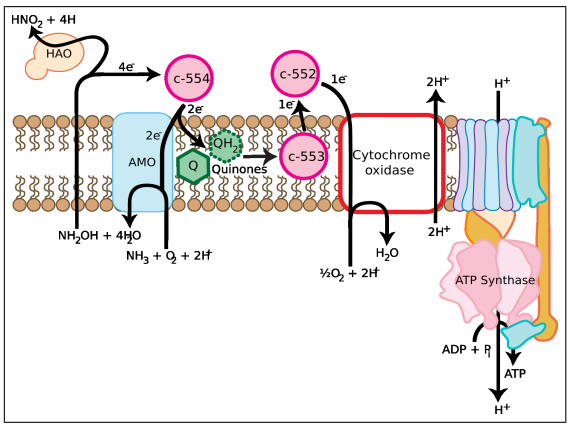


**Fig. 2.3:** *Enzymes of nitrification and energy generation in Nitrosomonas(AMO: ammonium monooxygenase, HAO hydroxylamine oxidoreductase, c: Cytochromes, Paustian, 2006).*

Nitrification was discovered and first described by the Russian microbiologist, Sergei Winogradsky at the end of the $18^{th}$ century (Winogradsky, 1892). Nitrification is the oxidation of ammonium to nitrate with nitrite as an intermediate. It is an aerobic process. Ammonium is converted via hydroxylamine to nitrite. Nitrite is then further oxidized to nitrate. $N_2O$ can be formed by two biochemical pathways (Fig. 2.2, 2.3): Firstly, as a byproduct during the ammonium oxidation, hydroxylamine is spontaneously decomposed to $N_2O$. This process is regarded as the main source of $N_2O$ from nitrification. Secondly, it can be formed by the so-called nitrifier denitrification (Arp and Stein, 2003). Here, $N_2O$ is an intermediate of the reduction of nitrite to molecular nitrogen (Wrage et al., 2004). The most intensifly studied groups of nitrifiers are the chemolitotrophic ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). The AOB are classified into three genera on the basis of their rRNA gene sequences (Head et al., 1993): *Nitrosomonas, Nitrosospina* and *Nitrosococcus*. Much less studies have been done on the classification of NOB, which are classified into four genera: *Nitrobacter, Nitrospina, Nitrococcus* and *Nitrospira* (Teske et al., 1994). Besides the chemolithotrophic bacteria, also some archaea can nitrify: expression of the ammonium monooxygenase (AMO) gene was found in a maritime Crenarchaeota (Leininger et al., 2006). However, since there are no specific inhibitors for archaea, its contribution to total nitrification rates in soils is unclear (Hayatsu et al., 2008).

### Denitrification

Denitrification is the reduction of nitrate via nitrite and $N_2O$ to $N_2$ (Fig. 2.4). It is usually an anaerobic or microaerobic, dissimilatory process coupled to electron transport phosphorylation which can be performed by numerous bacteria (for example *Paracoccus sp., Alcaligenes sp., Pseudomonas sp.*). Also several nitrifying bacteria seem to be able to denitrify (Anderson and Levine, 1986; Bremner, 1997). However, this biological process is of minor importance in cultivated soils (Bremner, 1997). Denitrification is also conducted by some funghi and archaea (Hayatsu et al., 2008), for example in woodland (Lavermann et al., 2001) and grassland (Laughlin and Stevens, 2002). Fungal denitrification was shown to be induced by significant amounts of $O_2$, but not in excess $O_2$ (Zhou et al., 2001). Another phenomenon recently discovered is "codenitrification". It indicates that a denitrifiying enzyme system uses substrates like azides and $NH_4^+$, but cannot be induced by these compounds (Shoun et al., 1992). Usually, organic carbon compounds are used as reducing agents in soils. The resulting $N_2O/N_2$-ratio depends strongly on parameters like pH, nitrate, $O_2$ availability et cetera. The contribution of codenitrification to $N_2O$ emissions from soils is unknown (Baggs and Philippot, 2010).

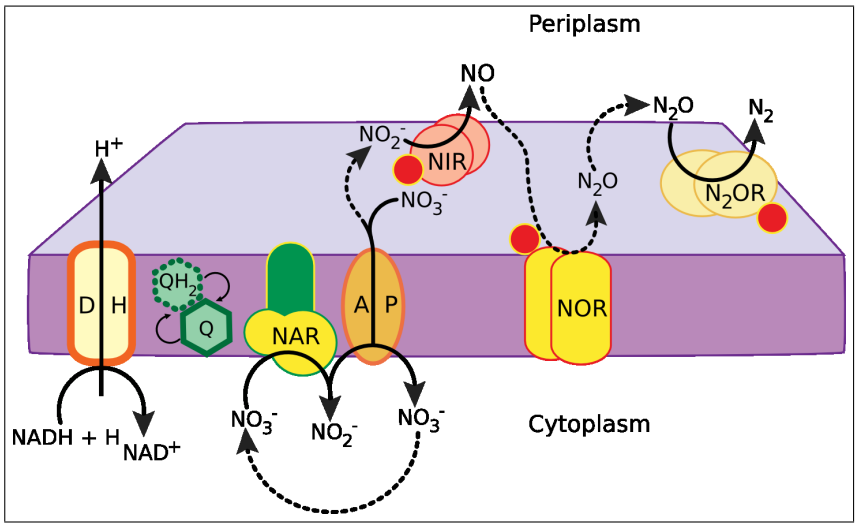


**Fig. 2.4:** *Formation of $N_2O$ during denitrification and involved enzymes (Paustian, 2006).*

It has further been shown that during assimilatory nitrate reduction, small amounts of $N_2O$ can be formed (Bleakley and Tiedje, 1982). Also nonbiological reactions (chemodenitrification) can contribute to $N_2O$ production in soils. It is most significant when $NO_2$ accumulates and reacts with organic compounds to produce NO and $N_2O$. Little is known about its contribution to total $N_2O$ production in soils, but it is probably most significant in acidic soils (Baggs and Philippot, 2010). Of all described reactions, aerobic nitrification and denitrification are considered to be the major processes producing $N_2O$ in soils (Bouwman et al., 2010). In the following, the study will therefore focus on these two processes.

In the field usually only net $N_2O$-production rates are measured. These are a result of all $N_2O$ producing and consuming processes. A scheme of these processes was published by Davidson (1991) and is known as "hole-in-the-pipe"-model (Fig. 2.5): both nitrification and denitrification are visualized as pipes with holes. The rate of net $N_2O$ production depends on the process rates (flow through the pipes), the sizes of the leaks (holes

in the pipes) and the diffusion and consumption of $N_2O$ before its transition from the soil to the atmosphere.

Because many enzymatic reactions are responsible for the rate of production of $N_2O$ in soils, there are many parameters which influence the net $N_2O$ emission rates in soil. Some of the most important are briefly mentioned in the following. Remember that the net $N_2O$ rate is always a result of the interplay between many influencing parameters.

### Soil nitrate and ammonium content

The relationship between N-input and $N_2O$ emissions is discussed in detail in the following chapters. The level of mineral nitrogen in soil influences the rate of $N_2O$ emissions. Ammonium and nitrate are substrates for nitrification and denitrification. They mainly stem from mineralization (e.g. from soil, N-rich residues), from atmospheric deposition and from fertilizer input. While ammonium is usually bound to clays and humus through ion exchange, nitrate is highly mobile. Usually, ammonium stimulates nitrification and nitrate increases both denitrification rate and $N_2O/N_2$ ratio. Of course this observation is only pronounced as long as the substrates are the only process limiting parameters. Application of inorganic N-fertilizer was shown to cause short-term peaks in $N_2O$ emissions in many studies (e.g. Moisier et al., 1983; Ryden 1983; Flessa et al., 1998; Samson et al., 1990; Ruser et al., 2001; Velthoff et al., 2002). Overall, a linear increase of $N_2O$ emissions with increasing N-input has been observed (Eichner 1990; Bouwman, 1996; Stehfest and Bouwman, 2006). Generally, emissions from mineral fertilizer seems similar up to a certain degree independent from the type of fertilizer. From these observations, the IPCC emission factor was derived (see Chapter 6).

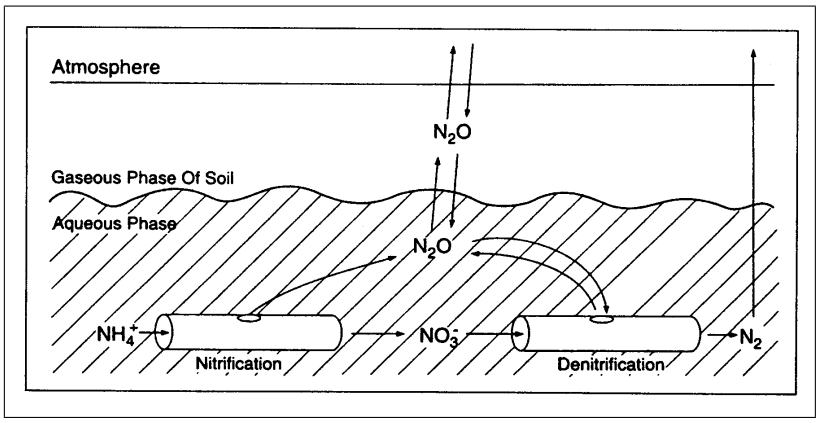


**Fig. 2.5:** *Production and release of $N_2O$ according to the hole-in-the-pipe-model (Davidson, 1991; modified).*

### WFPS and oxygen availibility

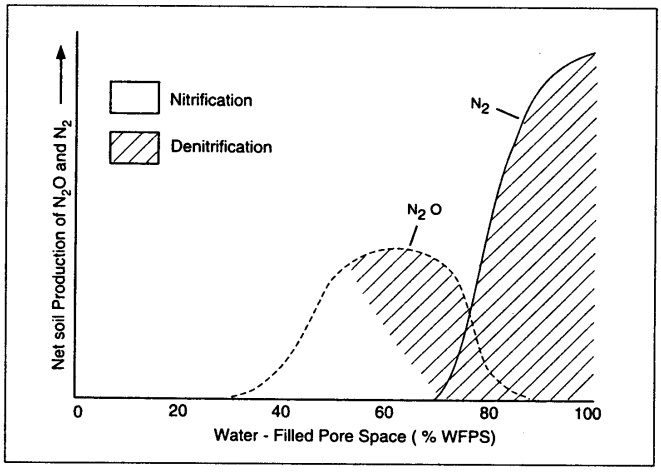


**Fig. 2.6:** *Relationship between water-filled pore space and net production of nitric oxide (NO), nitrous oxide ($N_2O$) and dinitrogen ($N_2$, Davidson, 1992).*

Soil moisture is a very important parameter influencing $N_2O$ emissions. An even closer relationship was found between water-filled pore space (WFPS) and $N_2O$ emissions since this value also takes total pore space into account. Water is essential for microbial life and enzymatic reactions, but the main reason for the influence of water on $N_2O$ production is that it constraints the diffusion of oxygen: in water, it diffuses by a factor four slower than in air (Heincke and Kaupenjohann, 1999). Both nitrification and denitrification rates increase up to a certain WFPS and then decrease, but this optimum WFPS is different for both processes. Since nitrification is an aerobic process, its optimum WFPS is at about 60 % (strongly depending on the soil type).

For higher values, oxygen gets more and more limited for microorganisms. For denitrification as a micro- or anaerobic process, optimum WFPS is much higher, values of about 70 % were reported (Ruser et al., 2006).

For higher WFPS, reduction potential further increases and reduction of $N_2O$ proceeds to $N_2$. For example in waterlogged soils, nitrate can almost completely be reduced to $N_2$ (Granli and Bøckmann, 1994). Davidson (1991) first derived a curve for the dependence of net $N_2O$ production on WFPS which peaked at about 60 % (Fig. 2.6). However, it has been shown that in arable systems, this threshold value can also be higher (Ruser et al., 2006). It is important to keep in mind that both nitrification and denitrification proceed simultaneously in soils, while denitrifiers use the product of nitrification as their substrate. This led to the hypothesis that conditions are ideal for $N_2O$ production if both aerobic and anaerobic microsites on a small scale allow for the coexistence of both groups in the soil.

A particular case are dry-wet cycles, which have been shown to cause peaks in $N_2O$ emissions (Davidson, 1992; Hütsch et al., 1999; Ruser et al., 2006). However, since vegetable systems are usually irrigated, it is not very probable that dry-wet peaks will be of major importance.

#### Input of organic material

It has been illustrated that the input of organic material to soils, e.g. as residues or other organic fertilizer, has stimulated $N_2O$ emissions. This is mainly the case if denitrification is the main process of $N_2O$ formation. There are several reasons for this: First, arable soils in Germany usually show rather low contents of organic carbon. Therefore, an input of easily mineralizable carbon can provide reducing agents (electron donators) for denitrification (deCatanzaro and Beauchamp, 1985; Granli and Bøckmann, 1994). Second, organic material is substrate for respiration and may induce $O_2$ limitation. Especially in close proximity to the organic substances, ideal conditions for denitrifiers can arise. Third, organic carbon increases the water-holding capacity of a soil resulting in a high WFPS and the stimulation of $N_2O$ emission from denitrification. The influence of the C/N ratio of the organic material and simultaneous input of N-fertilizer on $N_2O$ emissions are discussed in detail in Chapter 11.

#### Temperature

It has been seen that many enzymes of nitrification and denitrification are temperature dependant. However, a clear temperature dependence of $N_2O$ production such as can be found for $CO_2$ production was rarely encountered. If so, it is most probable for natural ecosystems which are dominated by nitrification (e.g. Dong et al., 2003). Denitrification is performed by numerous different microorganisms with different temperature optima. This might be a reason why a liner correlation between temperature and $N_2O$ emission was seldom found. Flessa et al. (2002) cite the diurnality of soil respiration and following creation of anaerobic conditions as a reason for diurnal cycles of $N_2O$ emissions.

Very low temperatures in winter usually decrease $N_2O$ emissions. When temperatures slowly rise, the low temperatures may suppress the synthesis of $N_2O$ reductase at a time where $N_2O$ is still being produced or production has started again (Vinther 1990). This was seen as one major reason for high emission after freeze/thaw cycles (Dörsch et al, 1993; Teepe et al., 2001, Mørkved et al., 2006). In these studies, high peaks were also measured after freezing and thawing of soil.

#### Further parameters

Several other factors influence $N_2O$ emissions as well. For example pH influences both nitrification and denitrification, but only one site has been included in this study and therefore changes in pH should be of minor importance. The same applies to soil type and structure.

In this study, emphasis was on direct $N_2O$ emissions from soil, this means the release of $N_2O$ from soil into the atmosphere directly at the site of its production. Further investigations on indirect emissions were done on the same site but are outside the focus of this work.

#### Nitrous oxide and agriculture

Agriculture is known to be the major anthropogenic source of $N_2O$. Isermann (1994) assumes that more than 80 % of total anthropogenic $N_2O$ emissions are derived from agriculture, of which more than 75 % would be derived from cultivated soils. Duxbury et al. (1993) even estimated that more than 92 % of the total anthropogenic $N_2O$ emissions are released from agriculture. New modeling approaches by Bouwman et al. (2010) estimate a release of about 4 Tg $N_2O$-N. However, they do not give a value for total anthropogenic $N_2O$ emissions. One

main reason for the high $N_2O$ emissions from agricultural soils is the high N-fertilizer input, often in combination with carbon-rich residues. For the estimation of direct emissions from managed soils, the IPCC has published a default value which is called emission factor 1 (EF1). It assumes that 1.0 % (range 0.3 - 3 %) of the total N additions from mineral fertilizers, organic amendments and crop residues as well as N mineralized from mineral soil as a result of loss of soil carbon are lost as direct $N_2O$ emissions (IPCC, 2006).

#### Vegetable production

Worldwide, an estimate of $52 \cdot 10^6$ ha of agricultural soil are covered by vegetable cropped soils (Rabobank, 2006). More than $1.5 \cdot 10^6$ ha are located in Europe (Eurostat Online Database, 2007) and about $10^5$ ha in Germany (LEL, 2010). Information on $N_2O$ emission from these areas is therefore of interest for greenhouse gas inventories.

Vegetable cropped soils are especially associated with high N-fertilizer inputs. At the time of harvest the plants are often in the vegetative growth phase. This often also results in great N-surpluses and high $N_{min}$ contents after harvest. Measurements indicated amounts of up to 164 kg N $ha^{-1}$ on harvesting cauliflower in 0 - 90 cm depth (Rahn et al., 1992). Additionally, considerable amounts of N can be mineralized from plant residues which are left on the vegetable fields (De Neve et al., 1996). The resulting high $N_{min}$ contents lead to the assumption of relatively high $N_2O$ emissions from vegetable fields. Despite this, hardly any studies have measured $N_2O$ emission from vegetable fields. Some measurements have been carried out in China (Xiong et al., 2006; Pang et al., 2009; Li and Wang, 2007), but mainly focused on glasshouses or only covered part of the year. Two studies have been conducted on onion fields (Van der Weerden et al., 2000; Duxbury et al., 1982). Dobbie et al. (1999) measured $N_2O$ emission in Scotland from a soil cropped with broccoli. The emissions determined by Dobbie et al. during the vegetation period of Brassicas in Scotland were between 9 and 12 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$. Van der Weerden et al. measured a release of up to 8 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ during 8.5 months of onion cultivation in New Zealand. The measurements of Duxbury et al. (1982) were carried out on drained peatlands. However, these studies are not representative for our study region and its climatic conditions with winter frost.

#### Annual datasets

Annual datasets are a prerequisite for the comparison of greenhouse gas emissions from different regions (Bouwman, 1996). Despite this, measurements are very often only conducted for several weeks or months. Winter emissions can contribute up to 76 % to total $N_2O$ emissions (Sylväsalo, 2004; Flessa et al., 1995, 1998; Röver et al., 1998; Kaiser et al., 1998). Many authors reported constantly high emissions even when temperatures were very low or even when soil was frozen (Sommerfeld et al., 1993; Kammann et al., 1998; Teepe et al., 2002; Regina et al., 2004). Especially in regions with strong winter frost, freeze-thaw cycles can cause high emission peaks outside the cropping season (Flessa et al., 1995; Wagner-Riddle and Thurtell, 1998; Teepe et al., 2002; Dörsch et al., 2004; Singurindy et al., 2009). These emissions might be due to the release of substrates for denitrification by mechanical breakdown of aggregates by freezing (Christensen and Christensen, 1991). Further substrate can be provided by microorganisms which are killed during soil freezing (Skøgland et al., 1988, Herrmann and Witter, 2002; Müller et al., 2002): This organic material enhances the activity of the surviving microorganisms (Christensen and Christensen, 1991). Another explanation for high $N_2O$ emissions after freeze-thaw cycles is that during freezing, the ice layer serves as diffusion barrier. However, beneath this ice layer, $N_2O$ can be produced by denitrifiers in unfrozen soil water (Teepe et al., 2001). $N_2O$ production during freezing of soil can also take place in deeper soil layers while a continuous ice layer in the upper soil inhibits its release (Burton and Beauchamp, 1994). The freezing of soil water also increases the concentration of substrates in the remaining liquid. When temperature rises, the produced $N_2O$ is released at once, causing the observed emission peaks.
Of the few studies dealing with $N_2O$ emissions from vegetable fields mentioned above, none was conducted in a region with freeze-thaw cycles in winter. This study provides, for the first time, a complete two-year dataset for vegetable cropped soil in a region with strong winter frost (Southern Germany).

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# 3 Overview and Hypotheses

In the following, the hypotheses of the study will be described briefly to give an overview of the different Chapters:

## 3.1 Metholodogy: Measurements with high spatial and temporal resolution

**Hypothesis: A weekly sampling strategy with event-related extra samplings using circular chambers provides results of acceptable accuracy that is to say no significant difference to measurements with higher spatial and temporal resolution** (Chapter 5)

Since the parameters regulating $N_2O$ fluxes vary on a small scale in the field and are also variable in time, high variability in $N_2O$ fluxes has often been observed for $N_2O$ measurements. In the study described in Chapters 6 to 10, a strategy with weekly measurement was chosen which was supplemented by event-related measurements according to Flessa et al. (2002) e.g. after rewetting of dry soil or freeze-thaw cycles. For the measurement of plots with broadcast and depot fertilization, circular PVC chambers were used. In the plots with depot fertilization, the rings partly covered the fertilizer depot according to the portion of area above the depot band to the total plot area. To test if this strategy was adequate, additional high resolution measurements were carried out. Furthermore, we wanted to find out if spatial heterogeneity concerning for example soil mineral N, WFPS and soil compaction had an influence on $N_2O$ fluxes, i.e. if differences between various areas of the field (areas next to plants, tractor compacted interrows) could be detected.

The following additional measurements were conducted to test our methodology:

(i) High temporal resolution measurements from plots with broadcast fertilization;
(ii) High spatial resolution measurements from plots with broadcast fertilization (influence of plants and soil compaction);
(iii) High spatial resolution measurements with tripartite chambers as compared to conventional circular chambers.

It was assumed that our sampling strategy would be adequate and not result in significant differences in cumulative $N_2O$ emissions as compared to the measurements with higher resolution. We further expected lower $N_2O$ emissions from chambers where plants were included (due to lower soil moisture and soil mineral N owed to plant uptake) and higher $N_2O$ emissions from tractor compacted interrows due to higher soil moisture.

## 3.2 Field study: $N_2O$ emissions and field management

**Hypothesis: N-Fertilizer reduction decreases $N_2O$ emissions but is limited by yield depression** (Chapter 6)

It is known that the input of N-fertilizer to soils can increase $N_2O$ emissions (Eichner, 1990; Bouwman, 1996; Ruser et al., 1998). Higher N-fertilizer input usually leads to higher levels of soil mineral N (Ruser et al., 2001). Both ammonium and nitrate can serve as substrate for nitrifiying and/or denitrifying microorganisms and thus increase $N_2O$ emission. Furthermore, the $N_2O/N_2$ ratio during denitrification can be increased by high nitrate levels. This incomplete reduction emerges especially in combination with good $O_2$ availability in times of low soil moisture (Granli and Bøckmann, 1994).

Although high $N_2O$ emissions should be avoided, a strong fertilizer reduction beneath the plant demand can also lead to a decrease in marketable yield. For farmers, it is very important to ensure that beneficial environmental effects by fertilizer reduction do not cause financial losses for them.

In this study, $N_2O$ emissions and yields were determined for three fertilizer levels for a lettuce-cauliflower rotation (for details see Material and Methods)

(i) the highest N-fertilizer level corresponding to good agricultural practice;
(ii) an intermediate fertilizer level according to the value recommended by a target value system which was developed for agricultural consultants in vegetable production;
(iii) the lowest fertilizer level with a further reduction of 20 % compared to (ii) to take the soil internal mineralization into account.

It was hypothesized that each reduction in the amount of N-fertilizer would lead to a decrease in total annual $N_2O$ emission, as this linear relationship has been seen in numerous studies. Because of the high mineralization potential of the soil and the high input of mineral N-fertilizer and organic material by crop residues, it was assumed that only at the lowest N-fertilization level yields could be affected by a reduction in N-fertilization.

## 3.3 Catch crops and waiting times

**Hypothesis: Field management like the use of catch crops as well as waiting time between plowing and following N-fertilization can decrease $N_2O$ emission** (Chapter 7)

In many studies, the importance of soil mineral N levels for $N_2O$ emissions has been described (Ryden, 1983; Eichner, 1990; Ruser et al., 1998). Especially in vegetable production systems with their high mineral and organic N-input and harvest in the vegetative growth state, there is a risk of high residual N in autumn and winter. This high N level is assumed to cause high $N_2O$ emission, providing substrate for nitrification and denitrification. Cover crops can absorb substantial amounts of N (Isse et al., 1999; Collins et al., 2007) and thereby minimize N losses by leaching and $N_2O$ emission. However, as soon as the plants are incorporated, they are exposed to mineralization and release organic C and N (De Neve et al., 1996). Therefore it is probable that the type of cover crop is of importance to have beneficial effects of $N_2O$ emission. Off-freezing catch crops might not succeed in a reduction of $N_2O$ emissions because nitrogen is released in autumn and can then increase soil mineral N levels. It is known, that denitrification can take place at low temperatures around 0°C (Röver et al., 1998) and lead to $N_2O$ emission in winter. Winter-hard cover crops seem to be more appropriate to reduce soil mineral nitrogen for a longer time and effectively decrease cumulative $N_2O$ emissions on an annual base.

Besides the beneficial effects that catch crops can have on $N_2O$ emissions in winter, also the beginning of the next vegetation period has to be investigated: while nitrogen and carbon are fixed in the cover crops outside the cropping season, the organic material can be decomposed quickly after its incorporation. If nitrogen is released from the incorporated cover crops before there is a substantial demand of the following crops, it might further increase soil mineral N level and increase $N_2O$ emission. Therefore, it could be appropriate to avoid extended waiting times between the incorporation of cover crops, but to incorporate them late so that the released nitrogen can be taken up by the following crop. Aim of this study was

(i) to evaluate the effect of black-fallow, an off-freezing and a winter-hard catch crop on $N_2O$ emissions;
(ii) to compare the effect of different waiting periods (7 - 35 days) between incorporation of cover crop residues and the following N-fertilization on $N_2O$ emissions from the consecutive crop.

It is assumed that only a winter-hard catch crop has the potential to mitigate $N_2O$ emissions and that the highest cumulative emissions are released from the black fallowed soil. It is further assumed that a longer waiting time between the incorporation of the catch crops and planting and of the next crop will increase $N_2O$ emissions due to increased mineral N levels in the soil which are not reduced by immediate plant uptake.

## 3.4 Field study: $N_2O$ emissions and nitrification inhibitory effects

**Hypothesis: Addition of a nitrification inhibitor and placed N-fertilization decrease $N_2O$-emissions during the vegetation period, but not on an annual base** (Chapter 8)

To reduce $N_2O$ emissions from arable land, the inhibition of nitrification is a potent measure (Akiyama et al., 2010). Ammonium is stabilized by the retardation of its oxidation to $NO_2$. Hence $N_2O$ emissions from nitrification are reduced on the one hand and on the other hand, while nitrification is inhibited, less nitrate is produced. Nitrate is not only substrate for denitrification but also more susceptible to be lost by leaching. Many studies have measured decreased $N_2O$ emissions after addition of nitrification inhibitors from grassland (Merino et al., 2005) and arable soil (Weiske et al., 2001).

3,4-dimethylpyrazole phosphate (DMPP) is a relatively new nitrification inhibitor. In 2001, Zerulla et al. (2001) described its advantageous characteristics such as high effectiveness and low risk of translocation in soil. DMPP specifically inhibits the enzyme ammoniummonooxygenase (Weiske et al., 2001), which catalyzes the first step of nitrification: the oxidation of ammonium to hydroxylamine. Addition of DMPP to N-fertilizer and slurry has been shown to reduce $N_2O$ emissions during the cropping period (Weiske et al., 2001; Merino et al., 2005). For the winter season, no data on $N_2O$ emissions is available up to now.

Controlled Uptake Longterm Ammonium Nutrition (CULTAN) is an N-placement fertilization strategy with nitrification inhibiting effect. Fertilizers with high ammonium contents are placed into the soil as a depot (Sommer, 2005). The resulting high concentrations of ammonium (osmotic values > 3000 ppm) unfavorable for microorganisms and therefore inhibit nitrification (Wetselaar et al. 1972) inside the depots.

The inhibition of nitrification and stabilization of ammonium brings about a lower risk of nitrate leaching. This allows the application of simplified fertilization strategies with less fertilizer applications (Fettweis et al. 2001; Serna et al., 2000). It is still possible that the stabilization of ammonium might also lead to a higher N uptake of the crop due to lower N-losses by denitrification and leaching. Consequently, the C/N ratio of the crop residues could be lower. In vegetable production, plant residues are often left on the field and incorporated into the soil. During the winter season, mineralization might then set the nitrogen free and enhance $N_2O$ emissions from denitrification.

Up to now, the influence of DMPP and N-depot fertilization on $N_2O$ emissions has never been tested on a vegetable cropped soil in an annual study. Aim of this study was thus to compare $N_2O$ emissions on an annual base from

(i) conventional N-fertilization split in two doses with ammonium sulphate nitrate (ASN) with
(ii) the same amount of ASN in one dose with addition of nitrification inhibitor DMPP and
(iii) the same amount of ASN in one dose in placed N-fertilization.

We hypothesize that the addition of a nitrification inhibitor as well as placed N-fertilization decrease $N_2O$ emissions during the vegetation period, even in a fertilization strategy with less applications. However, on an annual basis, $N_2O$ emissions will not be decreased.

## 3.5 $N_2O$ emissions as influenced by placement and fertilizer nitrate-N

**Hypothesis: The use of fertilizer without nitrate for depot fertilization decreases $N_2O$ emission, but also vegetable yield** (Chapter 9)

For the effectiveness of placed fertilization, a high ammonium content of the fertilizer is essential to ensure the inhibition of microorganisms. Stark et al. (1996) reported a reduction in oxidation activity of nitrifying microorganisms at concentrations of 1.6 mM $NH_4$-N in the soil solution. However, vegetable plants depend on sufficient nitrate for their growth, especially at an early stage (Haynes and Goh, 1978) and if their vegetation period is short. For this reason fertilizers like ammonium sulphate nitrate with a content of nitrate-N of 7.5 % are used to assure the desired vegetable yield (Sommer, 2005). For vegetable production, $N_2O$ emissions and vegetable yield were compared between

(i) placed N-fertilization without nitrate (ammonium sulfate) and
(ii) placed N-fertilization with ammonium sulfate nitrate containing a considerable nitrate content (7.5 % nitrate-N).

It is assumed that application of an N-fertilizer without nitrate like ammonium sulphate will decrease $N_2O$ emission (due to substrate inhibition for the nitrifiers and less nitrate as substrate for denitrifiers), but at the same time will probably decrease vegetable yield.

## 3.6 $^{15}N$ field study: sources of $N_2O$ emissions

**Hypothesis: Fertilizer-N recovered by plant residues contributes substantially to total $N_2O$ emission; the emission factor for N from residues in vegetable production systems is higher than the emission factor for mineral N input** (Chapter 10)

For the development of optimized N-fertilization strategies, the reduction of $N_2O$ emissions and an increase in N use efficiency, it is essential to know about the fate of N in soils. Addition of $^{15}$N-labelled fertilizer allows to trace the fertilizer-N in soil, $N_2O$ and plants. By the exchange of labeled and unlabelled residues and the measurement of $^{15}N$-$N_2O$, the share of $N_2O$ from fertilizer and residues to direct emissions can be calculated (for details see Material and Methods, Chapter 10).

Residues from vegetable production are known to contain substantial amounts of nitrogen of up to 140 kg N $ha^{-1}$ (Everaarts, 2000; Akkal-Corfini et al., 2010) and are usually left on the field after harvest. For example, Porter et al. (1996) found that 26 % of the fertilizer was taken up by plant residues. It has further been seen that mineralization of vegetable residues can be very fast (De Neve, 1996), providing organic nitrogen and especially carbon to $N_2O$ producing microorganisms. The degradation of organic material can stimulate respiration which causes the formation of anaerobic microsites. These microsites offer ideal conditions for denitrification (deCatanzaro and Beauchamp, 1985).
Direct fertilizer N input is indeed also easily available to microorganisms, but a lack in carbon might often limit denitrification due to lack of electron donators. In fact, many studies found increased $N_2O$ emissions from combined addition of mineral and organic N as compared to the single applications (Aulakh et al., 1984; Sarkodie-Addo et al., 2003). Overall, it is probable that total $N_2O$-N loss derived from fertilizer N which has been recovered by residues is higher than the loss from mineral N input, especially in a C-limited system.

The aims of this simultaneously conducted $^{15}$N study were

(i) to trace the fate of fertilizer $^{15}$N applied to soil in the vegetable production system by measuring the recovery in soil, marketable yield and plant residues;
(ii) to quantify the contribution of fertilizer and residue derived N to total $N_2O$ emissions and
(iii) to calculate emission factors for the residue derived emissions and compare them with the IPCC default value of 1 %.

It is assumed that substantial amounts of N are taken up by residues and contribute to $N_2O$ emission after their incorporation. It is possible that $N_2O$ emissions from residue derived fertilizer nitrogen are even higher than direct fertilizer derived emissions due to the simultaneous carbon input. It is further hypothesized that residue derived emission factors are as high or even higher than $N_2O$ emissions directly derived from mineral N.

## 3.7 Laboratory incubation study: $N_2O$ emissions as influenced by C/N ratio and amount of residue addition

**Hypothesis: $N_2O$ emissions are positively correlated with the mass and negatively correlated with the C/N ratio of residues added to soil. For a correct experimental setup, all residues must be taken from the same plant species** (Chapter 11)

When plant residues are added to soil, a negative correlation of the C/N ratio of residues added to soil and resulting $N_2O$ emission was usually reported: Several authors reported same or even decreased $N_2O$ emissions due to immobilization after the addition of residues with high C/N ratios like straw (e.g. Chaves et al., 2005; Toma and Hatano, 2007) because of immobilization of mineral N. Vigil and Kissel (1991) reported a critical C/N ratio of organic material of 40 as a breaking point between immobilization and mineralization. The incorporation of residues with low C/N ratios usually caused an increase in $N_2O$ emissions (Aulakh et al., 2001; Baggs et al., 2000; Velthoff et al., 2002).

A drawback of all studies dealing with residues with different C/N ratios is a methodical problem: for investigations on the influence of C/N ratios on $N_2O$ emission, to the author's knowledge there are only studies using material of different plant species exemplary for different C/N ratios like e.g. different cereals for high or brassicaceae for low C/N ratios and compared the resulting $N_2O$ emissions. It is thus well-known that residues of different plants can contain various chemical compounds with different properties which can influence soil microorganisms. Even if C and N contents are similar, it can be bound in chemically different substances. For example, differences in lignin, polycellulose content and protein binding capacity can influence $N_2O$ emissions from residues (Millar and Baggs, 2004; Garcia-Ruiz and Baggs, 2007). To find out only about the influence of the C/N ratio and to eliminate biases due to different secondary plant compounds, it is thus essential to use residues from the same species with different C/N ratios. Therefore in this study only cauliflower residues from the same field, but with different fertilization levels and thus C/N ratios were used. It is still probable that the

hypothesis of a negative correlation between $N_2O$ emissions and C/N ratio of the residues could hold true due to easier mineralization of residues with low C/N ratio.

To reduce $N_2O$ emissions from residues in the field, but also to use their unexploited energy potential, one possible strategy is to remove part of the residual biomass from the field during or after harvest and to use them for anaerobic digestion in biogas facilities (Stinner et al., 2008; Möller et al., 2009). This decreases the total residue input in autumn. Contrary, it is also interesting to know about increased residue input and the effect of more vegetable biomass due to excessive N-fertilization. Therefore it is important to find out about the correlation of $N_2O$ emission and biomass amount. Velthoff et al. (2002) conducted a study where sugar beet residues were added to soil. They included a treatment with fourfold increased residue amounts to simulate uneven distribution in the field. This fourfold increase in amount led to a disproportionately high increase in $N_2O$ emissions (more than fourfold) in the clay soil. Aim of our study was to use 3 mass levels of cauliflower residues, corresponding to reduced and increased input of residual N in the field.

Aim of this study was to verify the influence on $N_2O$ emissions of

(i) residues with varying C/N ratios of the same plants (cauliflower) to exclude methodical biases due to the influence of secondary plant metabolites;
(ii) increased and reduced input of residues to simulate different management practices.

It is assumed that lower C/N ratios as well as higher amounts of cauliflower residue input increase $N_2O$ emissions due to easier decomposition and increased substrate availability.

## 3.8 References


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# 4 Material and Methods

## 4.1 Study site and experimental set-up

In this Chapter, a general overview on Material and Methods will be given. For detailed information like soil characteristics, climatic conditions et cetera be referred to Material and Methods, Chapter 6, 8 and 10. The experimental site was located on Universität Hohenheim's "Heidfeldhof", 13 km south of Stuttgart, Germany (48° 43' 00" N; 9° 11' 40" E). Figure 4.1 shows the position of Hohenheim on a national map. Figure 2.1 shows the fields belonging to the experimental farm. Our study site was located in the East of the farm (indicated by an arrow, "Schlag 25"). All studies except the laboratory incubation study (Chapter 11) were carried out at this location. Furthermore, Table 4.1 gives an overview of the 10 treatments which are described in detail in Chapters 5 to 10. All treatments were arranged in a complete randomized block design in 4 replications as shown in Figure 4.2. Fertilization was carried out according to the German Target Value Systems as proposed by Feller et al. (2001). The target values for the vegetables which were planted are shown in Table 4.2.

**Tab. 4.1:** *Overview of the treatments of this study: name, N-fertilization, level of N-fertilization, use of nitrification inhibitory strategies and isotopic labeling, catch crop type and chapter reference in this work; abbreviations: ASN = ammonium sulfate nitrate; DMPP = 3,4-dimethylpyrazole phosphate; WW = winter wheat; GR = green rye).*

| No | Name | N-fertilization | Amount of N-fertilizer [% of Target Value] | Inhibition of nitrification | Isotopic labelling | Catch crop | For further information see Chapter…. |
|---|---|---|---|---|---|---|---|
| 1 | control | - | 0 | - | - | WW/GR | 5 – 10 |
| 2 | depot | ASN | 100 | depot | | WW/GR | 8, 9 |
| 3 | L+/C+ | ASN | 100 | no | $^{15}$N-labelling | WW/GR | 10 |
| 4 | LOW | ASN | 80 | no | | WW/GR | 6 |
| 5 | L-/C- | ASN | 100 | no | transfer of $^{15}$N - labeled residues | WW/GR | 10 |
| 6 | OPT = CONV | ASN | 100 | no | | black fallow | 6, 7, 8 |
| 7 | OPT = CONV | ASN | 100 | no | | Phacelia | 6, 7, 8 |
| 8 | - | ASN + DMPP | 80 | DMPP | | WW/GR | unpublished |
| 9 | NI | ASN + DMPP | 100 | DMPP | | WW/GR | 8 |
| 10 | GP | ASN | 130 | no | | WW/GR | 6 |

**Tab. 4.2:** *Target Values for N-fertilization for lettuce, cauliflower and chard according to the German Target Value System (Feller et al., 2001)*

| vegetable | target value [kg N ha$^{-1}$] |
|---|---|
| lettuce | 150 |
| cauliflower | 286 |
| chard | 150 |


**Fig. 4.1:** *Position of the study site (red arrow)*

| | | | | | |
|---|---|---|---|---|---|
| 3 | 8 | 2 | 9 | 5 | 7 |
| 4 | 5 | 7 | 1 | 10 | 6 |
| 1 | 10 | 6 | 4 | 2 | 3 |
| 6 | | 9 | 8 | | 4 |
| 2 | 9 | 1 | 5 | 8 | 3 |
| 8 | 4 | 10 | 7 | 9 | 2 |
| 7 | 3 | 5 | 10 | 6 | 1 |

**Fig. 4.2:** *The fully randomized block design*

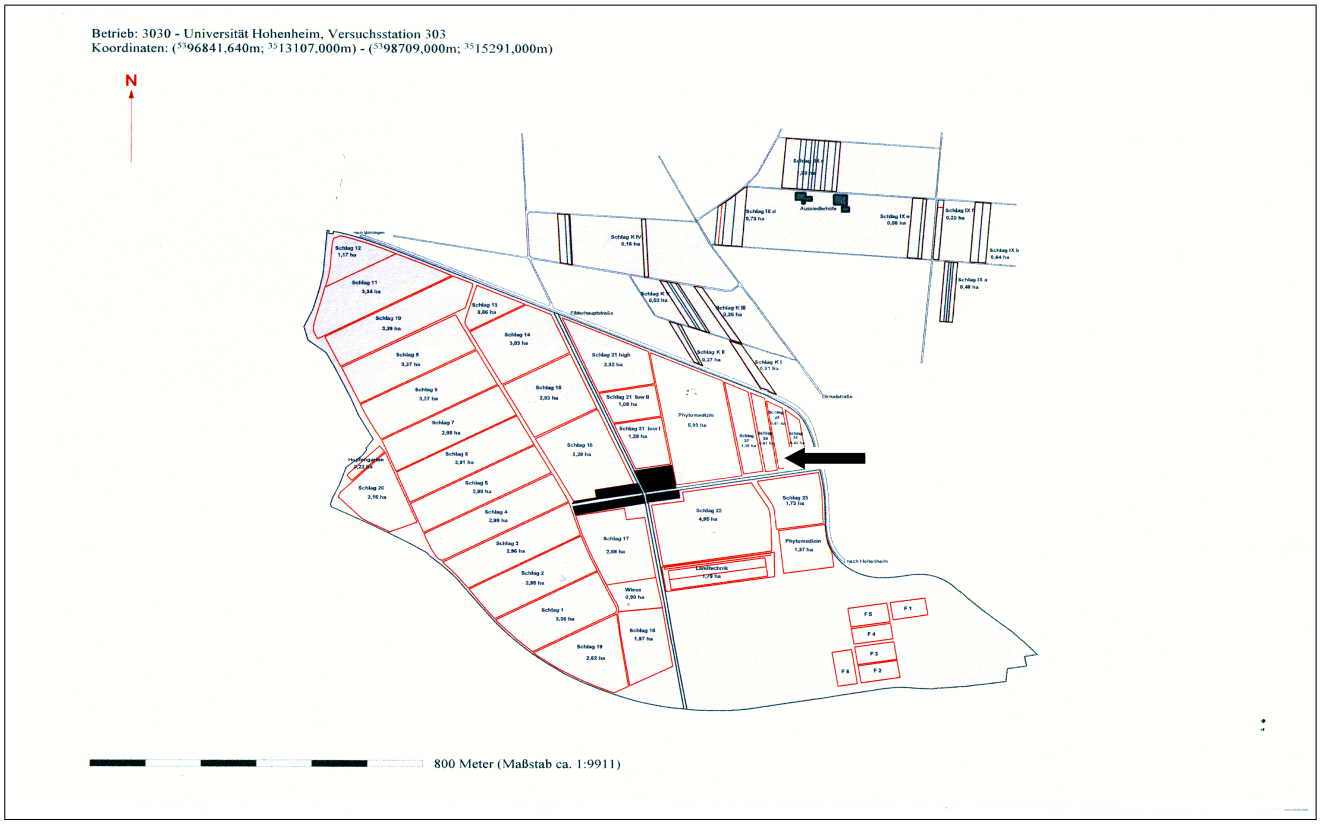


**Fig. 4.3:** *Map of the experimental farm belonging to Universität Hohenheim and compartition in fields (study site indicated by an arrow)*

## 4.2 References

Feller, C., Fink, M., Laber, H., Maync, A., Paschold, P.-J., Scharpf, H.-C., Schlaghecken, J., Strohmeyer, K., Weier, U., Ziegler, J., 2001. Düngung im Freilandgemüsebau. Gartenbauliche Berichte. Institut für Gemüse- und Zierpflanzenbau Grossbeeren, Erfurt, *in German*.

# 5 Methodology: Measurements with high spatial and temporal resolution

Results of the following Bachelor Theses are included in this chapter:
"Lachgasemissionen auf einem Gemüsefeld mit platzierter N-Düngung (CULTAN)"; Spengler, J., 2009
"Räumliche und zeitliche Variabilität der $N_2O$-Emissionen im Feldgemüsebau"; Winkler, B., 2009
"Einfluss der Düngerplatzierung und Düngerform auf die $N_2O$-Emissionen im Feldgemüsebau"; Kesenheimer, K., 2010

## 5.1 Introduction

A high variability of the measurement data is a challenge that has to be dealt with in most field studies. For nitrous oxide ($N_2O$), it is known that fluxes can be highly variable: in many studies, coefficients of variation > 100 % have been reported (Ambus and Christensen, 1994; Yates et al, 2006). The high temporal and spatial variability of $N_2O$ emissions hinders efforts to develop valuable estimates of $N_2O$ emissions and therefore it is important to choose an adequate methodological approach for measurements.

$N_2O$ emissions are mainly derived from two microbial processes in soils: nitrification and denitrification. The microorganisms producing $N_2O$ are influenced by a complex interaction of many environmental parameters such as temperature, moisture regime and concentrations of mineral and organic substrates (see Chapter 2).

Because these conditions may change within relatively short time frames, $N_2O$ production rates are also highly variable in time, leading to high **temporal variability**. Very frequent measurements are therefore desirable. On the one hand, each data collection is cost and (especially if carried out manually) labor intensive. On the other hand it must be ensured that enough data is collected to obtain reliable estimates for the calculation of representative cumulative $N_2O$ emissions. In some studies, sampling strategies with high and lower temporal resolution were compared to find out about the required sampling frequency to obtain estimates within a certain precision level (Loftfield et al., 1992; Smith and Dobbie, 2001; Parkin, 2008; Laville et al., 2010). Scott et al. (1999) measured $N_2O$ fluxes manually twice a week and additionally took six samples per day with an automated system for 36 days after incorporation of sewage slurry. Cumulative $N_2O$ emissions were underestimated by about 40 % by the manual sampling schedule as compared to the near-continuous, because peaks e.g. after strong precipitation were not detected. To reduce errors by weekly sampling in times of high flux rates, Flessa et al. (2002) proposed an extended sampling schedule with additional measurements after heavy precipitation and after strongly increased emissions. Thereby they could reduce the error of cumulative flux rates by temporal variability to less than 10 % in their study.

Besides the temporal, the high **spatial variability** of $N_2O$ emissions has to be taken into account for the choice of an adapted sampling strategy as well. Differences in parameters like bulk density, moisture regime, supply with mineral and organic substrates etc. might occur on a small spatial scale and influence substrate and oxygen supply and thereby activity of microorganisms. Parkin (1987) reported that denitrification exhibited a highly spatial discontinuity with "hot-spots" of increased activity randomly distributed over the field. 25 - 85 % of the denitrifying activity of soil columns could be ascribed to pieces of organic material which were less than 1 % of total column weight.

**Plants** growing on arable land influence the physical, chemical and biological properties of soils in many ways. Thereby they might influence the $N_2O$ producing microorganisms and consequently $N_2O$ emission. Plants can enhance denitrification activity by rhizodeposition, the release of organic compounds which can be used as electron donators (Miller et al., 2008). Substantial rhizodeposition of up to 200 to 500 kg C $ha^{-1}$ was reported for wheat and barley (Van Noordwijk et al, 1994; Swinnen et al., 1995). Opposite effects could be expected by the

uptake of mineral nitrogen by plants because of the positive relationship between $N_{min}$ levels and $N_2O$ emissions.

In irrigated vegetable fields, WFPS is often above the threshold value for increased denitrification which was shown to be 60 % (Davidson, 1991) or even higher for grassland or arable land (De Klein and Van Logestijn, 1996; Ruser et al., 2006). If water is taken up by plants, WFPS might temporarily fall below the critical value and plants would thereby decrease the cumulative $N_2O$ emissions. However, vegetable leaves can protect the soil from excessive evaporation. This may keep WFPS above the threshold value and increase $N_2O$ emissions or even decrease $N_2O$ emissions following rewetting after irrigation as reported in several studies (Davidson, 1992; Hütsch et al., 1999; Ruser et al., 2006). Finally, several plants are known to transport soil $N_2O$ to the atmosphere via transpiration (Chang et al., 1998) or release small amounts of plant produced $N_2O$ (Chen et al., 2002). Another factor which might lead to a high spatial variability on arable land is **soil compaction** by tractor passages. It is known that an increase in bulk density leads to higher WFPS values and might therefore locally increase $N_2O$ emission (Bakken et al., 1987; Hansen et al., 1993). Ruser et al. (1998) investigated the influence of soil compaction on potato fields and reported extremely increased fluxes from the tractor lines. It is probable that in vegetable fields, the soil compaction in the interrows influences also $N_2O$ emissions.

A close relationship between soil mineral N contents and $N_2O$ emissions has often been found in studies from various sites (Eichner et al., 1990; Bouwman et al., 2002; Stehfest and Bouwman, 2006). In fields with placed fertilization, the spatial variability of mineral N concentrations in the soil is very high. Depots are characterized by extreme concentrations of mineral N. These fertilizer depots are supposed to inhibit nitrification (for more details, refer to Chapter 8 and 9). Nevertheless, Parkin (2008) observed a higher $N_2O$ emissions from fertilizer bands of anhydrous ammonia than from more distant from of the fertilizer band. But the chambers that were used in this study were almost as broad as the distance between two fertilizer bands. In order to measure $N_2O$ fluxes from fields with **depot fertilization**, measurements with high spatial resolution are essential. Extrapolations can only be calculated correctly, if the fluxes from bands and outside bands are determined precisely. Therefore, smaller chambers and bases covering specifically the areas of interest are needed. Larger conventional chambers might provide the same results, but first it must be verified that this sampling strategy is appropriate. For a comparison of data from measurement with larger conventional chambers, it is useful to measure fluxes from three areas with smaller chambers: from the soil directly over the fertilizer depots, from the area around the fertilizer bands which might be influenced by the depot and from the areas which are far enough from the fertilizer depot that they are probably not influenced by the band.
To ensure that temporal and spatial resolution of measurements were adequate in this study (Chapter 6 to 10), additional measurements were conducted simultaneously to find out about an appropriate sampling protocol.

## 5.2 Material and Methods

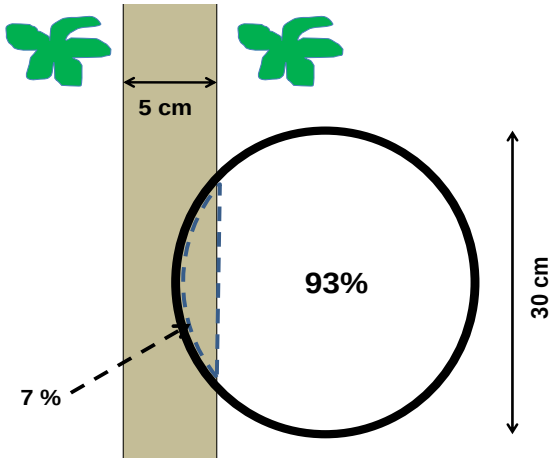


**Fig. 5.1:** *Size of the circular chamber and position on the fertilizer band (grey area); numbers indicate the area above and outside the fertilizer band.*

All gas measurements were carried out as described in Chapter 6 and 8 with the closed chamber method. A weekly sampling protocol was chosen. As recommended by Flessa et al. (2002), it was supplemented by additional measurements after heavy precipitation (> 10 mm) and after freeze-thaw events. For the measurements of $N_2O$ fluxes of plots with broadcast fertilization, circular PVC bases with an inner diameter of 30 cm and a height of 10.5 cm were inserted in the middle of each plot into the uncompacted soil between the vegetable plants. For the measurements of $N_2O$ fluxes of plots with placed fertilization, the same circular base frames were used. The base frames partly covered the fertilizer depot according to the portion of area above the depot band to the total plot area (7 % of the chamber area directly covered the depot, see Figure 5.2). For further details see Chapter 6, 6.3 and Chapter 8. To test if the flux rates measured with this temporal and spatial resolution are reliable, additional manual measurements with higher temporal and spatial resolution were carried out. The study site is described in Chapter 4 and 6.

### 5.2.1 High temporal resolution measurements from plots with broadcast fertilization

For high temporal resolution measurements from plots with broadcast fertilization, additional manual measurements with higher temporal resolution were carried out on plots with broadcast fertilization (treatments "CONV" in Table 4.1) during the vegetation period of lettuce 2009. For 10 days, samples for the measurement of $N_2O$ flux rates were taken daily additionally to the weekly sampling procedure (Winkler, 2009). For another two weeks, samples were taken twice a week and then another two samplings were conducted at intervals of one week. Samples were taken at the same time of day and in the exact same manner as weekly sampling to ensure the comparability of data.

### 5.2.2 High spatial resolution measurements from plots with broadcast fertilization: influence of plants and soil compaction

For the high spatial resolution measurements from plots with broadcast fertilization, additional measurements were carried out following the same protocol, but at additional regions of the field during the vegetation period of lettuce 2009. The high temporal resolution (daily, then twice a week, then weekly) was chosen. To test if the $N_2O$ emission from soil influenced by plants would differ from unaffected regions, additional circular chamber bases were inserted in four replicates which included one lettuce seedling in the middle of each chamber base. Another set of circular chamber bases was placed in four replicates onto the tractor compacted interrow (Winkler, 2009).

### 5.2.3 High spatial resolution measurements with tripartite chambers as compared to conventional circular chambers

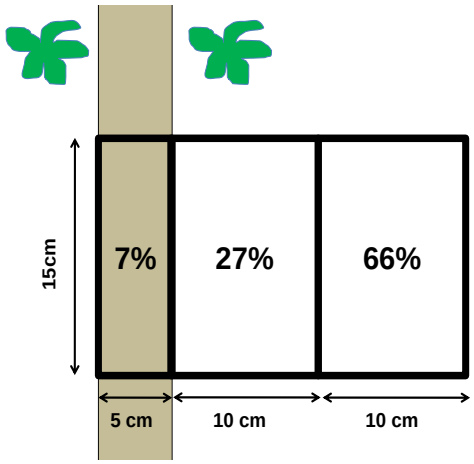


**Fig. 5.2:** *Size and partitioning of the tripartite chamber and position on the fertilizer band (grey area).*

For high spatial resolution measurements from plots with placed fertilization, rectangular tripartite chambers ("depot-chambers") were constructed in order to measure $N_2O$ emissions (see Fig. 5.2). One compartment with a width of 5 cm was placed directly onto the fertilizer band, the second compartment with a width of 10 cm was located next to the fertilizer band and the third with a width of 10 cm as well had the maximum distance from the fertilizer band. The volume of the smaller compartment was 1.8 liter and 3.6 liter for the two bigger compartments. A trench on top of the bases was filled with water to allow the airtight attachment of the compatible chambers during the measurement period. Like the circular chambers, a vent was inserted to allow pressure equalization. The "Depot"-chambers were used in plots with placed fertilization in eight replicates (additionally to the four replicates of circular bases) and in plots with broadcast fertilization in four replicates during the vegetation period of cauliflower in 2009 (Spengler, 2009) and during the vegetation period of chard in 2010 (Kesenheimer, 2010).

### 5.2.4 Calculations

All calculation and statistics were carried out as presented in 6,6.3.4 to ensure comparability of all results. It was determined that the depot area on the fertilizer band covered 7 % (6 fertilizer bands in each plot, 5 cm width each band), the area next to the depot 27 % and the area at a greater distance from the depot the remaining 66 % of the total plot area (4.5 m · 6 m). Note that in Figures 5.5, 5.6 and 5.7, the compartments are named according to these contributions to the total plot size. Total cumulative flux rates of the "Depot"-chambers pro rata were calculated by multiplication of the single flux rates of the three compartments with their area A/100 and addition of these three values.

## 5.3 Results and Discussion

### 5.3.1 High temporal resolution measurements from plots with broadcast fertilization

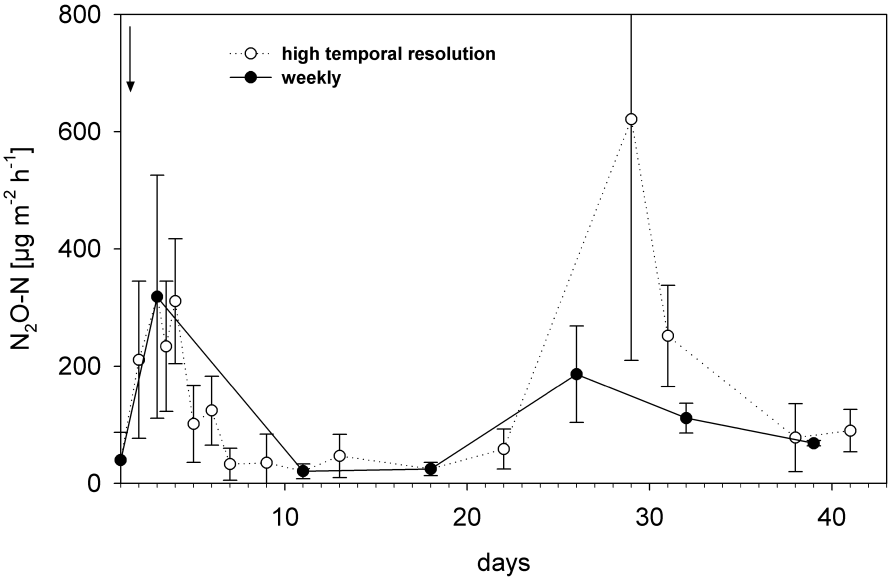


**Fig. 5.3:** *Mean $N_2O$ flux rates and standard deviations ($n = 4$) from measurements with conventional temporal resolution according to the extended sampling protocol described by Flessa et al. (2002) and from measurement with high temporal resolution after fertilizer application to lettuce (arrow); (Winkler, 2009)*

The temporal pattern of the $N_2O$ flux rates during the measurement is shown in Figure 5.3. The initial peak after fertilization on day one was well captured by the conventional method due to an additional measurement after fertilization. In fact, this led to a slight overestimation by the assumption of constant flux rates until the next samplings. However, this overestimation was counterbalanced because the peak on day 29 was not captured by the conventional method. Overall, both measurement protocols led to similar results: Cumulative $N_2O$ emission during the vegetation period of lettuce from measurements with high temporal resolution was only 130 g $N_2O$-N higher as compared to measurement according to the conventional method (Tab. 5.1). This corresponded to a difference of 8.9 % and was statistically not significant.

**Tab. 5.1:** *Mean cumulative $N_2O$ emission with conventional temporal resolution (standard deviation, $n = 4$) from measurement with conventional temporal resolution according to the extended sampling protocol described by Flessa et al. (2002) and from measurement with high temporal resolution as well as difference between the two methods in gram and in percent. Statistically significant groups are indicated by different superscript letters (Student-Newman-Keuls Test; $p < 0.05$); (Winkler, 2009).*

| | cumulative emissions ± SD | difference | |
|---|---|---|---|
| | g $N_2O$-N (vegetation period)$^{-1}$ | g | % |
| conventional method | 1329 ± 461[a] | 130 | 8.9 |
| high temporal resolution | 1459 ± 214[a] | | |

### 5.3.2 High spatial resolution measurements from plots with broadcast fertilization: influence of plants and soil compaction

The temporal pattern of the $N_2O$ flux rates during the measurement is shown in Figure 2.4. The inclusion of a plant into the closed chambers did not have striking effects on the general course of the $N_2O$ flux rates (Fig. 5.4). The flux rates were slightly lower for most of the samplings, but this difference was statistically not significant. No flux rates are shown for $CO_2$ for the treatment "with plant". During the sampling, the plants are covered by the dark chambers which leads to unnatural conditions. While photosynthesis is inhibited, mitochondrial respiration can still progress producing significant amounts of $CO_2$ release. This leads to the artefact of an

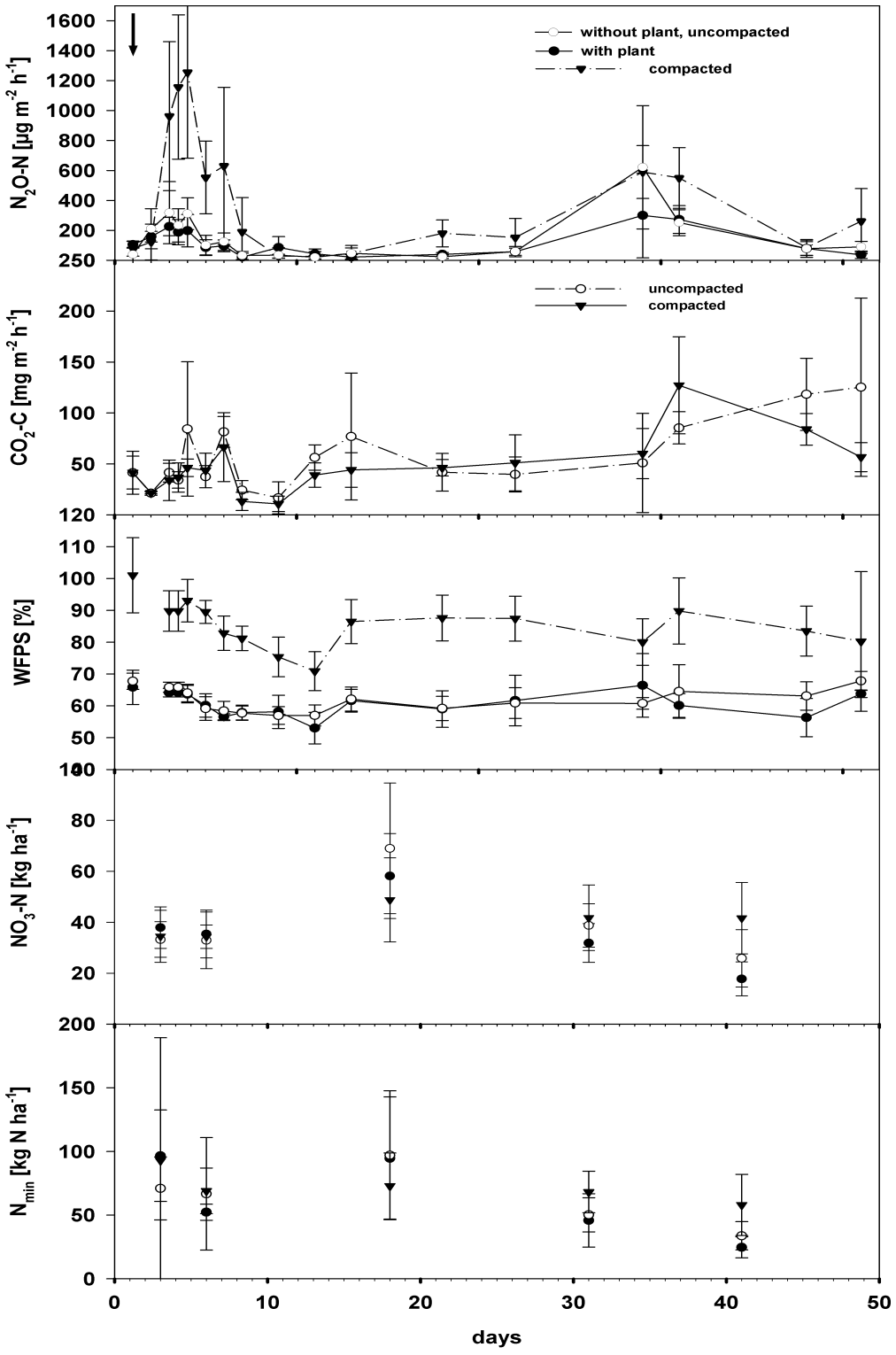


**Fig. 5.4:** *Mean $N_2O$ and $CO_2$ flux rates, WFPS, soil mineral nitrate (0 - 25 cm) and total soil mineral N (0 - 25 cm) and standard deviations (n = 4) from uncompacted soil without plant, from uncompacted soil with plant and from tractor-compacted soil after fertilizer application to lettuce (arrow). $CO_2$ fluxes are only shown for the chamber measurements without plants since we used dark chambers (see Chapter 6); (Winkler, 2009).*

overestimation of $CO_2$ flux rates. No significant difference was found between the $N_{min}$ and nitrate level of soil with and without plants neither for WFPS (Fig. 5.4). Only during the last 10 days of measurement when lettuce plants were already fully developed, lower values for soil moisture and mineral N were measured in the treatment with plant. The reason for the analogousness of WFPS values of the soil with and without plants was probably that the increased water uptake by plants was counterbalanced by a reduced evaporation from soil due to the shade of the lettuce leaves. The similar soil mineral N contents could be explained in the same way: the plants might have taken up more nitrogen from the soil next to their roots and decreased the $N_{min}$ concentration, but at the same time rhizodeposition from the roots stimulated the microorganisms leading to additional nitrogen supply from increased mineralization. Kuzyakov et al. (2002) reported that about 120 - 160 kg C $ha^{-1}$ were translocated from the roots by rhizodeposition from a lettuce crop which was fertilized with 160 kg N $ha^{-1}$. During the whole vegetation period, cumulative $N_2O$ emission was not different between soil with and without plants. As in this study, Ernfors et al. (2010) observed that exclusion of roots had also no effect on $N_2O$ emissions on drained organic forest soil. Soil compaction by tractor-traffic caused a high peak especially during the week after fertilizer application. This is also reflected after cumulation of the $N_2O$ emissions (Tab. 5.2): emission from the compacted areas was significantly higher than from the uncompacted ones. No difference was found for the cumulative $CO_2$ emissions (data not shown).

The emission factor of the uncompacted area was 1.0 %, which is exactly the IPCC default value, while the emission factor for the compacted area was more than double (2.2 %, Tab. 5.2). The results can be used to extrapolate these higher emission factors on the whole field basis. If two wheel compacted areas with a width of 0.15 m (half breadth of a wheel) each are assumed from each tractor passage, 20 % of the field is compacted. This leads to an underestimation of the total field $N_2O$ emissions of 24 %. It is important that our data is comparable with other studies which neglected the effect of tractor-compaction. Therefore for the calculation of IPCC emission factors, this information was not used in the following chapters. But it must still be kept in mind that emission factors are even higher when taking soil compaction into account.

**Tab. 5.2:** *Mean cumulative $N_2O$ emission (standard deviation) and emission factor (percental $N_2O$-N loss of fertilizer N input) from uncompacted soil without plant, from uncompacted soils with plant and from tractor-compacted soil (n = 4); mean emission factor (EF, % $N_2O$-N-loss of fertilizer N input) from all measurements from uncompacted soil ( = with and without plant) and for the whole plot ("total"). Statistically significant groups are indicated by different superscript letters (Student-Newman-Keuls Test; $p < 0.05$); (Winkler, 2009).*

| compacted | plant | $N_2O$-N kg $N_2O$-N $ha^{-1}$ vegetation period-1 | emission factor (EF) % of Input | mean EF uncompacted |
|---|---|---|---|---|
| - | - | 1.46 (0.21)[a] | 1.08[a] | 0.98[a] |
| - | + | 1.19 (0.37)[a] | 0.88[a] | |
| + | - | 2.93 (0.66)[b] | 2.17[b] | |
| **total** | | | 1.22 | |

### 5.3.3 High spatial resolution measurements with tripartite chambers compared to conventional circular chambers

For placed fertilization, emissions were significantly higher from the areas directly over the fertilizer band and adjacent to the fertilizer band than from at a greater distance from the fertilizer band which points to a high spatial variability due to different soil mineral N concentrations (Fig. 5.6 and 5.7). For example, 68 % of the total $N_2O$ release was derived from 34 % of the area (over and next to the fertilizer band) for the placed fertilization to cauliflower in 2009.

That the use of the tripartite chamber on the plots with broadcast fertilization shows the same results as the circular chamber indicates that the design of the tripartite chambers (concerning area/height et cetera) is adequate (Fig. 5.5).

For all measurements, no significant differences in emissions (Fig. 5.5, 5.6, 5.7) or emission factors (Tab. 5.3) were observed between measurement with high spatial resolution (tripartite chamber) and conventional method

(circular chamber). This indicates that measurement with circular chambers is adapted for the determination of flux rates also on plots with banded fertilization and due to the lower workload even more recommendable.

**Tab. 5.3:** *$N_2O$ emission factors for measurement with tripartite and circular chambers for broadcast and placed fertilization to cauliflower and for placed fertilization to chard. Statistical differences between tripartite and circular chambers are indicated by different superscript letters (Student-Newman-Keuls Test; $p < 0.05$); (Spengler, 2009 and Kesenheimer, 2010).*

| crop | fertilization | chamber type | loss<br>% fertilizer-N input |
|---|---|---|---|
| cauliflower 2009 | broadcast | tripartite | 0.4[a] |
| | | circular | 0.5[a] |
| | placed | tripartite | 0.9[a] |
| | | circular | 0.8[a] |
| chard 2010 | placed | tripartite | 6.0[a] |
| | | circular | 4.6[a] |

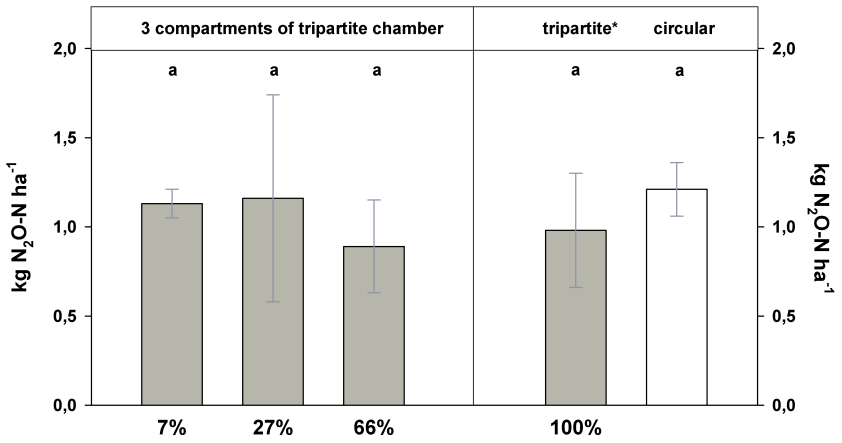


**Fig. 5.5:** *left: Total cumulative $N_2O$ emission during the vegetation period of cauliflower with **broadcast** fertilization for measurement with a tripartite chamber from the compartment directly above the fertilizer band which covers 7 % of the total plot area ("7 %"), from the compartment next to the fertilizer band ("27 %") and from the compartment at a greater distance from the fertilizer band ("66 %");*
*right: Total cumulative pro rata emission ("100 %") from measurement with tripartite and circular chambers ($n = 4$ for each; *for calculation see Material and Methods). Statistically significant groups are indicated by different superscript letters (Student-Newman-Keuls Test; $p < 0.05$); (Spengler, 2009).*

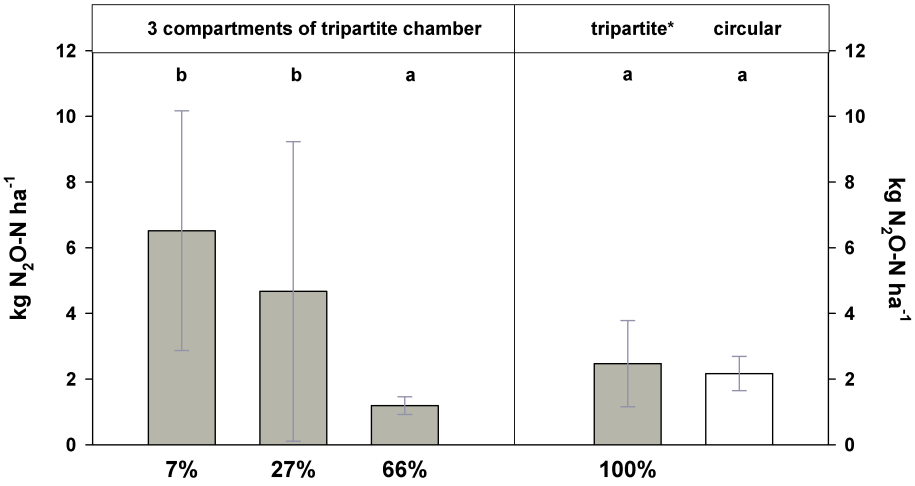


**Fig. 5.6:** *left: Total cumulative $N_2O$ emission during the vegetation period of* ***cauliflower with placed*** *fertilization for measurement with a tripartite chamber from the compartment directly above the fertilizer band which covers 7 % of the total plot area ("7 %"), from the compartment next to the fertilizer band ("27 %") and from the compartment at a greater distance from the fertilizer band ("66 %");*
*right: Total cumulative pro rata emission ("100 %") from measurement with tripartite and circular chambers (n = 4 for each; *for calculation see Material and Methods). Statistically significant groups are indicated by different superscript letters (Student-Newman-Keuls Test; p < 0.05); (Spengler, 2009).*

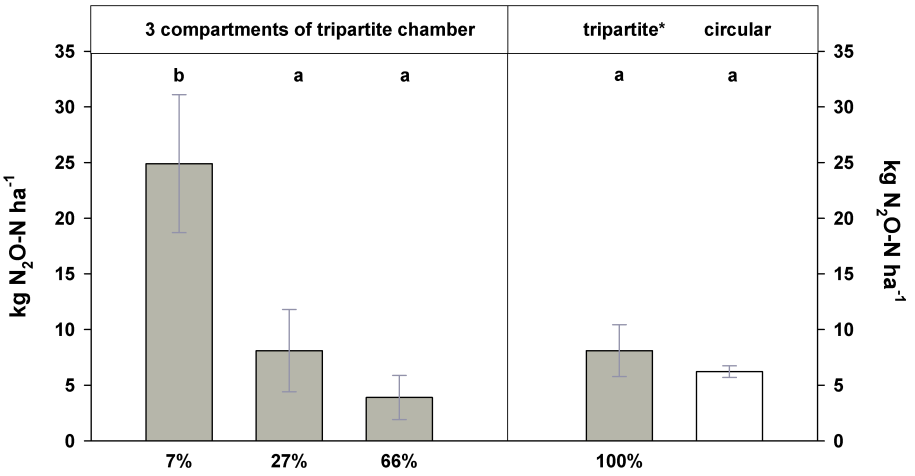


**Fig. 5.7:** *left: Total cumulative $N_2O$ emission during the vegetation period of* ***chard with placed fertilization*** *for measurement with a tripartite chamber from the compartment directly above the fertilizer band which covers 7 % of the total plot area ("7 %"), from the compartment next to the fertilizer band ("27 %") and from the compartment at a greater distance from the fertilizer band ("66 %");*
*right: Total cumulative pro rata emission ("100 %") from measurement with tripartite and circular chambers (n = 4 for each; *for calculation see Material and Methods). Statistically significant groups are indicated by different superscript letters (Student-Newman-Keuls Test; p < 0.05); (Kesenheimer, 2010).*

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# 6 $N_2O$ fluxes from a Haplic Luvisol under intensive production of lettuce and cauliflower as affected by different N-fertilization strategies

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## 6.1 Abstract

Vegetable production systems often show high soil mineral nitrogen contents and thus are potential sources for the release of the climate relevant trace gas $N_2O$ from soils. Despite numerous investigations on $N_2O$ fluxes, information on the impact of vegetable production systems on $N_2O$ emissions in regions with winter frost is still rare. This present study aimed to measure the annual $N_2O$ emissions and the total yield of a lettuce-cauliflower rotation at different fertilization rates on a Haplic Luvisol in a region exposed to winter frost (Southern Germany). We measured $N_2O$ emissions from plots fertilized with 0, 319, 401 and 528 kg N $ha^{-1}$ (where the latter three amounts represented a strongly reduced N-fertilization strategy, a German target value system, and the N-amount fertilized under good agricultural practices). The $N_2O$ release from the treatments was 2.3, 5.7, 8.8 and 10.6 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ respectively. The corresponding emission factors calculated on the basis of the total N-input ranged between 1.3 and 1.6 %. Winter emission accounted for 45 % of the annual emissions, and a major part occurred after the incorporation of cauliflower residues. The annual $N_2O$ emission was positively correlated with the nitrate content of the top soil (0 - 25 cm) and with the N-surpluses of the N-balance. Reducing the amount of N-fertilizer applied significantly reduced $N_2O$ fluxes. Since there was no significant effect on yields if fertilization was reduced from 528 kg N $ha^{-1}$ according to "good agricultural practice" to 401 kg N $ha^{-1}$ determined by the German Target Value System, we recommend this optimized fertilization strategy.

## 6.2 Introduction

Nitrous oxide ($N_2O$) is a very potent greenhouse gas, which contributes to radiative forcing and is involved in stratospheric ozone depletion (Crutzen, 1981; IPCC, 2007). More than 70 % of the total anthropogenic $N_2O$ emission is related to agricultural practices (Cole et al., 1997), more than half of the anthropogenic $N_2O$ emissions result from soil emissions (IPCC, 2001). In soils, $N_2O$ is mainly formed during nitrification or denitrification, both of which are stimulated by the input of N-fertilizers. Several investigations indicated increasing $N_2O$ fluxes from arable and grassland soils with increasing amounts of N-fertilization (Eichner, 1990; Stehfest and Bouwman, 2006). If fertilizer doses are reduced below the optimum range for plant growth in order to reduce $N_2O$ fluxes, plant yield will also decrease as a result of the insufficient N supply. Despite numerous investigations on the effect of varied N rates on $N_2O$ fluxes, almost no information concerning plant yield has been provided. To be accepted by the farmers, the development of $N_2O$ mitigation also requires information on yield stability. This can be ensured with the help of an $N_{min}$ Target Value System ($N_{min}$-Sollwertsystem) as proposed by Feller et al. (2001). This system was developed for fertilizer consultants in vegetable production. It recommends target values for available mineral N during the cropping season of different vegetable crops. A more detailed description was given by Lorenz et al. (1989).

Vegetable production covers more than $1.5 \cdot 10^6$ ha of arable land in Europe (Eurostat Database, 2007). This land use is partly associated with a high level of N-fertilization and results in high N surpluses since vegetables such as cauliflower and broccoli are harvested in the vegetative growth stage (Krug et al., 2002). For these vegetables, high amounts of mineral N remain in the soil after harvest, measurements indicated amounts of up to 164 kg N $ha^{-1}$ (Rahn et al., 1992). Between 94 and 140 kg N $ha^{-1}$ are additionally provided by the decomposition of plant residues (Everaarts, 2000; Akkal-Corfini et al., 2010). Especially brassica residues have a narrow C/N-ratio (e.g. 13.7 for broccoli shoots, Velthof et al., 2002) thus favoring the decomposition and the release of mineral N and easily available C. The microbial respiration of C leads to an increased oxygen consumption and may thus enhance denitrification (Flessa and Beese, 1995; Ambus, 1996). Baggs et al. (2000) found increasing $N_2O$ emissions with decreasing C/N-ratio of plant residues incorporated into different soils. A similar relationship between the $N_2O$ emissions during the winter season and the ratio of dry matter to N of crop residues has been demonstrated in a field study by Kaiser et al. (1998). In the winter season, freeze-thaw cycles are known to cause high $N_2O$ fluxes, especially during the thawing of frozen soil (Dörsch et al., 2004; Singurindy, 2009). These high emissions can contribute up to 76 % of the total annual $N_2O$ loss (Flessa et al., 1995), the mean contribution for six German arable fields was 50 % (Kaiser and Ruser, 2000).

The high contribution of $N_2O$ emissions during winter clearly indicates that annual data sets are needed for reliable estimations of $N_2O$ inventories. Bouwman (1996) stressed that annual data sets were a prerequisite for the comparison of flux data from different regions. Despite numerous field experiments on $N_2O$ fluxes from agricultural soils, information on $N_2O$ dynamics in vegetable cropped soils is still rare. Some $N_2O$ measurements from vegetable cropland have been carried out in China (Pang et al., 2009), but they either covered only part of the year or focused on emissions in greenhouses.

Dobbie et al. (1999) detected high $N_2O$ emissions from a field cultivated with broccoli in Scotland. Among the huge number of data sets summarized and provided online by Stehfest and Bouwman (2006), only two studies investigated the $N_2O$ fluxes from vegetable fields. Van der Weerden et al. (2000) measured $N_2O$ emissions from onion production in an organic farming system in New Zealand and Duxbury et al. (1982) reported flux measurements from an onion field in Florida. All investigations were carried out in regions with no freeze-thaw events during winter. Therefore, the data sets do not represent the climatic conditions in Southern Germany which are characterized by temperate climate with frost periods during winter and mineral soils developed from loess sediments.

For the calculation of national greenhouse gas inventories, the IPCC (2006) recommends a default emission factor of 1 of the total annual N input for direct $N_2O$ emissions (EF 1). Currently, it has not been proven that the application of this emission factor is valid for soils in Southern Germany under vegetable production.

The first objective of our study was thus to provide annual field data on $N_2O$ emissions for a vegetable field in Southern Germany, i.e. in the temperate zone with winter freeze-thaw cycles. We hypothesize that the corresponding emission factors would be higher than the default emission factor of 1 % due to the comparatively

high N-inputs, the harvest in the vegetative growth state and the expected high emissions during winter due to freeze-thaw cycles. Secondly, we postulate a mitigation potential for $N_2O$ emissions of two reduced fertilization strategies as compared to N-fertilization measures under good agricultural practices. For the first reduced fertilization strategy, N-fertilization was adapted to plant demand according to a target value system to avoid N-surpluses. For the second strategy, a further reduction was carried out assuming that this would not lead to any reduction in yields due to soil internal N mineralization.

## 6.3 Material and Methods

### 6.3.1 Experimental site and crop management

The experimental site was located on Universität Hohenheims' "Heidfeldhof", 13 km south of Stuttgart, Germany (48° 43' 00" N; 9° 11' 40" E). The research farm is located 410 m above sea level. The mean annual precipitation is 686 mm with a mean annual temperature of 8.8°C. The soil type was a Haplic Luvisol derived from periglacial loess. The top soil (0 - 25 cm) had a pH of 5.5 ($CaCl_2$) and an organic C content of 1.8 %. The soil texture of the stone free top soil consisted of 2 % sand, 68 % silt and 30 % clay.

The experimental design was a fully randomized block design with four replicates. The plot size was 6 m · 4.5 m (corresponding to the breadth of three vegetable patches, which were each 1.5 m wide). The experiment was established in 2007 with lettuce (*Lactuca sativa* var. *capitata* L., variety "Gisela") planted mid May and harvested at the end of June, followed by green rye (*Secale cereale* L.) as a cover crop sown in late autumn. Due to unfavorable weather conditions in April 2008, the green rye was incorporated using rotary tillage only three days before planting the succeeding lettuce on May 2 (variety "Gisela", 110,000 plants $ha^{-1}$) and the start of the gas flux measurements. The lettuce was harvested on June 25. For the determination of the yield, 25 representative plants were cut out of the inner vegetable patch, cleaned and weighed. As it is common practice in market gardening to leave residues on the field, all organic material besides the marketable heads was put back onto the field immediately after weighing. Cauliflower (*Brassica oleracea* var. *botrytis L.*, variety "Dexter", 20,000 plants $ha^{-1}$) was planted on July 15 and harvested in three campaigns on October 17, 24, and 30 because not all heads were mature on the first date. At each sampling, all heads of cauliflower with a diameter larger than 11 cm were cut, weighed, cleaned by removing all leaves beside the innermost and weighed again. All residues were returned to the field after determining total weights. After November 11, the field lay fallow as the temperature was too low for the cultivation of a catch crop. Irrigation was carried out according to the irrigation tool "agrowetter" provided by the German meteorological service (Deutscher Wetterdienst, Offenbach, Germany).

Nitrogen was broadcast as ammonium sulfate nitrate ($2NH_4NO_3 \cdot (NH_4)_2SO_4$), at rates shown in Table 6.1. N-fertilization of cauliflower was split into two doses. Immediately before planting, the mineral N content of the upper horizon (0 - 25 cm) was determined and subtracted from the target value of the first N-application for each crop. In contrast, the mineral N in the soil was not considered for the second N-application to cauliflower to ensure comparability with other treatments (not further specified here) where the application of N was not split.

We investigated the following fertilization treatments:
(i) in total 528 kg N $ha^{-1}$ $yr^{-1}$, which corresponded to the amounts commonly used by farmers following good agricultural practice (GP);
(ii) an optimized fertilization strategy (OPT) adopted to the plant demand using a target value of 401 kg N $ha^{-1}$ $yr^{-1}$ as recommended by Feller et al. (2001);
(iii) a reduced fertilization strategy (LOW) with a further reduction by 20 % to 319 kg N $ha^{-1}$ $yr^{-1}$, assuming that due to soil internal N mineralization this would not have any negative effect on yields; and
(iv) an unfertilized control treatment (control).

**Tab. 6.1:** *N-fertilization (kg N $ha^{-1}$) to lettuce and cauliflower, soil mineral N (0 - 25 cm, in brackets), total fertilization and total plant available N in the control treatment, in the treatment with low (LOW) and optimized (OPT) fertilization and in the treatment with fertilization according to good agricultural practice (GP).*

| | date | control | LOW | OPT | GP |
|---|---|---|---|---|---|
| lettuce | 02.05.08 | (15) | 105 + (15) | 135 + (15) | 180 + (15) |
| cauliflower | 15.07.08 | (15) | 100 + (14) | 123 + (20) | 162 + (24) |
| | 09.09.08 | | 114 | 143 | 186 |
| **total fertilization** [kg $ha^{-1}$] | | | **319** | **401** | **528** |
| **total available N** [kg $ha^{-1}$] | | **30** | **348** | **436** | **567** |

### 6.3.2 Gas flux measurements and soil sampling

Trace gas fluxes were measured at least once a week in the morning using the closed chamber method (Hutchinson and Livingston, 1993). Periodic sampling with a weekly sampling design provides emission data within about ± 20 % of the actual emission (Parkin, 2008). We supplemented this design by event-oriented measurements (e.g. after rewetting of dry soil or after thawing of frozen soil) to improve the reliability of our measurements. As shown by Flessa et al. (2002), using this extended gas sampling schedule decreases the error of cumulative flux estimations below 10 %.

The circular PVC-chambers had an inner diameter of 30 cm and consisted of a base frame with a height of 15 cm and a chamber with a height of 10.5 cm (Flessa et al., 1995). The base frames were inserted in the middle of each plot at a depth of 10 cm. During the vegetable growth, the chambers were placed between plants. The base frames were only removed prior to and re-installed immediately after soil management practices.
Evacuated glass vials (V = 22.4 ml) and a double-sided cannula were used to take gas samples from the chamber through a rubber septum. At each sampling date, four gas samples were taken from each chamber at 10 - 20 min. intervals depending on the expected trace gas flux rate with the first sample being taken immediately after closing the chamber. We evaluated our sampling system according to the quality protocol published by Rochette and Eriksen-Hamel (2008). Our methodology was rated as "very good" in the categories chamber design, seal on soil surface and air sample handling and storage.

Soil sampling (0 - 25 cm) and the determination of soil temperature were carried out simultaneously to each flux measurement. For each of the replicates, six soil samples were taken randomly ensuring a safety distance of 0.5 m from the base frames. In contrast we tested a modified strategy for soil sampling with one pooled sample from all four replicates between July 22 and August 8. Consequently, no standard deviations can be calculated for this period. Soil temperature was measured weekly with a portable thermometer that was inserted into the soil (7 cm depth). Bulk density ($A_p$ horizon, 0 - 15 cm) was determined once during the lettuce and cauliflower vegetation period and during the winter season using stainless steel cylinders (V = 100 $cm^3$). Precipitation and air temperature data were provided by the university's meteorological station, which is located approximately 500 m from the experimental site.

### 6.3.3 Laboratory analysis

$N_2O$ in the gas samples was analyzed using a gas chromatograph equipped with a $^{63}Ni$ electron capture detector (ECD) (5890 series II, Hewlett Packard) and an autosampler (HS 40, Perkin Elmer). $N_2$ was used as carrier gas. Three standard gases with concentrations of 405 ppb, 1500 ppb and 3000 ppb $N_2O$ were used for calibration.
Soil moisture was analyzed gravimetrically after drying the soil at 105°C for 24 h. The water-filled pore space (WFPS) was calculated as described by Ruser et al. (1998).
For the quantification of mineral N contents, 20 g of fresh soil were extracted with 40 ml of a 0.5 M $K_2SO_4$

solution. Concentrations of nitrate ($NO_3^-$) and ammonium ($NH_4^+$) in the extracts were determined using a flow injection analyzer (3 QuAAtro, SEAL Analytical, UK).
Plant samples of lettuce and cauliflower for each of the four replicate plots were dried at 60°C and ground. Heads and residues were prepared and measured separately. C- and N-analyses were conducted (two pseudoreplicates) using an elemental analyzer (vario MAX CN, Elementar Analysensysteme, Hanau).

### 6.3.4 Calculations and statistical analyses

$N_2O$ flux rates were calculated using the slope of the temporal change in concentration within the closed chamber (Flessa et al., 1995). As a criterion for the linearity of the trace gas enrichment in the chamber's atmosphere, we used the coefficient of determination ($r^2$). If the value was below the threshold of 0.8, the $N_2O$ flux was set to zero. Low values for $r^2$ were only found in case of small $N_2O$ concentration differences among the samples from one chamber that were within the range of the accuracy of our gas chromatograph (±10 ppb).

Cumulative $N_2O$ emissions were calculated assuming constant flux rates between two sampling dates. For all calculations and analyses, the period from May 2 until July 14 is referred to as the vegetation period of lettuce ("lettuce"), the period from July 15 until November 3 as vegetation period of cauliflower ("cauliflower") and the period from November 4 (2008) until April 30 (2009) as the period outside the cropping season ("winter").
For the C- and N-ratios, means of the two pseudoreplicate samples were used for statistical analyses. The N-balance was calculated as N-fertilizer input minus the N-removal from the field for the two crops. $N_2O$ emission factors (EF1) were calculated according to the IPCC methodology for direct emissions (IPCC, 2006).

Statistical analyses were carried out using the Statistical Software package SigmaStat 3.5. Depending on the distribution of the data, a One Way Anova or a Kruskal-Wallis One Way Anova on Ranks was performed to detect differences between the treatments concerning yield, N-contents and cumulative emissions. Significant differences were determined using a pairwise multiple comparison procedure (Student-Newman-Keuls Test, $p < 0.05$). The data is presented as arithmetic means with standard deviation.

## 6.4 Results and Discussion

### 6.4.1 Lettuce and cauliflower yield

Lettuce yields (Tab. 6.2) ranged from 5 Mg ha$^{-1}$ in the control treatment to 40 Mg ha$^{-1}$ marketable yield in the treatment following good agricultural practice (GP). The percentage of marketable goods was almost 100 % of the total yield for all fertilized treatments. The reduction of the amount of N-fertilizer from the GP treatment to the optimized level (OPT) did not lead to a significant yield reduction (40 Mg ha$^{-1}$ GP and 39 Mg ha$^{-1}$ OPT). In contrast, the further reduction of the N amount by 20 % (OPT versus LOW) significantly reduced the lettuce yield.

The mean N-uptake of lettuce heads was 9 kg N ha$^{-1}$ in the unfertilized control treatment. For the marketable yield in the fertilized treatments, it varied between 43 and 62 kg N ha$^{-1}$. The N content of the lettuce residues varied between 9 and 25 kg N ha$^{-1}$ corresponding to between 26 and 50 % of the plants' total N-uptake, respectively. The median of the C/N-ratio of the residues was 17.

Marketable yield of cauliflower in the fertilized treatments ranged between 30 Mg ha$^{-1}$ in the OPT treatment and 34 Mg ha$^{-1}$ in the GP treatment (Tab. 6.2). No marketable cauliflower heads were produced in the unfertilized control treatment. The mean percentage of marketable heads in the fertilized treatments was 88 % (data not shown). The reason for this low portion of marketable yield of cauliflower was the occurrence of precocious flower buds; the high air temperature after planting followed by a cold period with temperatures below 0°C (Fig. 6.1) in September induced vernalisation and the resulting flowering.

**Tab. 6.2:** *Mean marketable yield and mean N-content of the heads and the residues of lettuce and cauliflower in the control treatment without N-fertilization (control), in the treatment with low (LOW) and optimized (OPT) fertilization and in the treatment with fertilization according to good agricultural practice (GP)± SD, n = 4). Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, p < 0.05).*

| | | control | LOW | OPT | GP |
|---|---|---|---|---|---|
| **marketable yield** | lettuce | **5** ± 3$^{a}$ | **26** ± 7$^{b}$ | **39** ± 8$^{c}$ | **40** ± 5$^{c}$ |
| [Mg ha$^{-1}$] | cauliflower | **0** $^{a}$ | **31** ± 1$^{b}$ | **30** ± 4$^{b}$ | **34** ± 1$^{b}$ |
| **N-content of heads** | lettuce | **9** ± 7$^{a}$ | **43** ± 9$^{b}$ | **61** ± 15$^{b}$ | **62** ± 11$^{b}$ |
| [kg N ha$^{-1}$] | cauliflower | **0** $^{a}$ | **75** ± 3$^{b}$ | **78** ± 7$^{b}$ | **87** ± 7$^{b}$ |
| **N-content of residues** | lettuce | **9** ± 4$^{a}$ | **22** ± 3$^{ab}$ | **22** ± 4$^{ab}$ | **25** ± 2$^{b}$ |
| [kg N ha$^{-1}$] | cauliflower | **18**$^{x}$ ± 3$^{a}$ | **100** ± 11$^{b}$ | **116** ± 35$^{b}$ | **136** ± 24$^{b}$ |

$^{x}$ residues and non-marketable cauliflower heads

We found no statistical differences in cauliflower yield between the fertilized treatments, indicating that a reduction in the amount of N-fertilizer has no negative effect on yield. The mean N-uptake of marketable cauliflower heads varied between 75 and 87 kg N ha$^{-1}$ in the fertilized treatments (Tab. 6.2). The N-content of the residues was 100 kg N ha$^{-1}$ for the LOW, 116 kg N ha$^{-1}$ for the OPT and 136 kg N ha$^{-1}$ for the GP treatment (statistically not significant). The total plant N uptake of the fertilized treatments therefore ranged between 175 and 223 kg N ha$^{-1}$. The median C/N-ratio of the plant residues was 10. The N content of the residues of the fertilized plots varied between 57 and 61 % of the total N-uptake. This is in accordance with the results published by Akkal-Corfini et al. (2009) who reported that 51 - 59 % of the total plant N was found in the residues of an unfertilized cauliflower planted after intensively fertilized potatoes. Likewise, the N uptake of the cauliflower residues produced in our study is similar to the 170 - 300 kg N ha$^{-1}$ found by Akkal-Corfini et al. (2009). Kage et al. (2003) reported an N uptake of about 230 kg N ha$^{-1}$ for cauliflower fertilized following the German Target Value System on a loess soil near Hannover, Germany.

### 6.4.2 Temporal pattern of the $N_2O$ flux rate

The $N_2O$ flux rates during the vegetation period showed a highly variable temporal distribution with mainly low fluxes and few high flux rates after N-fertilization measures with simultaneous high soil moisture conditions and during cauliflower harvest (Fig. 6.1 and 6.2). The highest flux rate with 1705 µg $N_2O$-N m$^{-2}$ h$^{-1}$ was measured during lettuce cultivation in the treatment GP (Fig. 6.2d). It coincided with a period of high ammonium and nitrate concentrations (Fig. 6.2e,f) providing substrate for $N_2O$ producing microorganisms in soils. Increased $N_2O$ flux rates after N-applications in combination with high soil water contents have often been reported from arable soils as well as from grassland soils (Rochette et al., 2008; Hernandez-Ramirez et al., 2009).

Although cauliflower and lettuce received similar single N-doses, no strong response to the N-application was observed for cauliflower. Maximum flux rates after N-fertilization to cauliflower were by a factor 16 to 68 lower than the maximum rate during the cultivation of lettuce. We assume that the late incorporation of green rye three days before seed bed preparation for lettuce induced $O_2$ depletion in the top soil due to the mineralization of easily available C from the rye residues. The consumption of $O_2$ can enhance the formation of anaerobic microsites in the soil and thereby increase denitrification rates (e.g. Flessa and Beese, 1995; Miller et al., 2008). In periods with high amounts of easily available C as provided by the incorporated green rye, the addition of mineral N increases $N_2O$ emissions and should therefore be avoided (Clayton et al., 1997; Dittert et al., 2005). Several studies found an increase in $N_2O$ flux rates after the addition of crop residues, especially for residues with low C/N-ratio (Kaiser et al., 1998; Velthof et al., 2002). The crop residues from cauliflower had a narrow C/N-ratio, thus favoring a fast turnover of the organic. This might have led to a strong $O_2$ depletion and resulted in high, long-lasting $N_2O$ fluxes after harvest and incorporation of the residues.

The mean $N_2O$ flux rates from the fertilized treatments exceeded 100 µg $N_2O$-N m$^{-2}$ h$^{-1}$ for more than seven weeks prior to the beginning of soil frost at the end of December (Fig. 6.2a,d and 6.1b) with exception

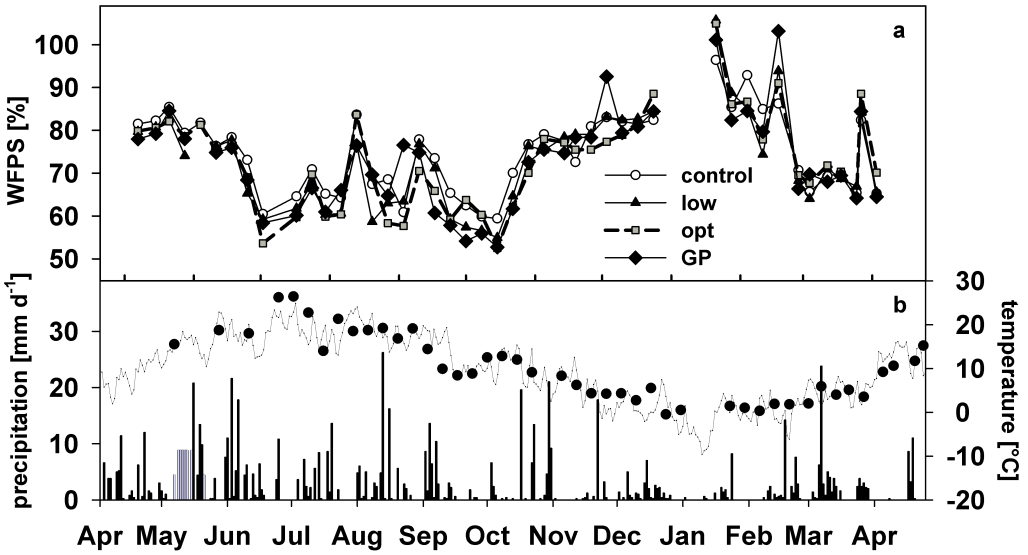


**Fig. 6.1:** *(a) Mean water-filled pore space (n = 4) in the control treatment, in the treatments with low fertilization (LOW), optimized fertilization (OPT) and in the treatment with fertilization according to good agricultural practice (GP). (b) Mean air (solid line) and soil temperature in 7 cm depth (circles), daily precipitation (black bars) and irrigation (dotted grey bars).*

of only four samplings from the treatment with reduced fertilization. Soil freezing slightly reduced $N_2O$ fluxes. However, the fluxes were still higher than the $N_2O$ baseline between July and October. Elevated $N_2O$ fluxes during frost periods have been reported elsewhere (Kammann et al., 1998; Regina et al., 2004) and explained by the production of $N_2O$ during microbial denitrification in a thin water film on soil aggregates with high concentrations of easily available N and C compounds (Teepe et al., 2001). The increased $N_2O$ fluxes at the end of January (up to 394 µg $N_2O$-N $m^{-2}$ $h^{-1}$ in the GP treatment) coincided with thawing of the frozen soil, but only down to a depth of about 5 cm. Due to melting of the snow, water-logging occurred because the ice layer hindered water infiltration. The calculated water-filled pore space during this period reached nearly 120 % (Fig. 6.1a). This theoretical supersaturation is due to our assumption of a constant soil bulk density of 1.40 Mg $m^{-3}$ outside the cropping period. This density was used to calculate the pore space. It was measured in autumn and did not allow for the transient increase in soil porosity during frost periods (Kay et al., 1985). High fluxes during soil thawing have frequently been reported from regions with pronounced freeze-thaw cycles (Ruser et al., 2001; Jungkunst et al., 2006). The reasons for these high flux rates are still speculative. Burton and Beauchamp (1994) assumed an accumulation of $N_2O$ from denitrification beneath the frozen layer in soils. In contrast, Christensen and Tiedje (1990) as well as Christensen and Christensen (1991) showed increased denitrification in the upper thawed centimeters of the soil as a result of the supply of nutrients by the lysis of soil microbes and aggregate disruption. It seems most plausible that also in our study, the emitted $N_2O$ was produced in the upper unfrozen centimeters of the top soil.

## 6.4.3 Cumulative $N_2O$ emission

### $N_2O$ emission during the cropping season

Cumulative emissions during the cropping period showed a positive correlation with the amount of fertilizer input ($r^2 = 0.95$, data not shown); all differences, except between OPT and GP, were significant. The emission from the soil in the unfertilized control treatment during this period was 1.1 kg $N_2O$-N $ha^{-1}$. For the soil in the LOW, OPT and GP fertilizer treatments, emissions were 3.2, 4.7 and 5.8 kg $N_2O$-N $ha^{-1}$, respectively (Tab. 6.3). For the cropping season, we found a positive correlation between the mean soil nitrate contents (0 - 25 cm depth) and the cumulative $N_2O$ emissions ($r^2 = 0.98$). Furthermore, the mean soil moisture for all investigated treatments during the cropping season ranged between 68 and 72 % WFPS. These values are well

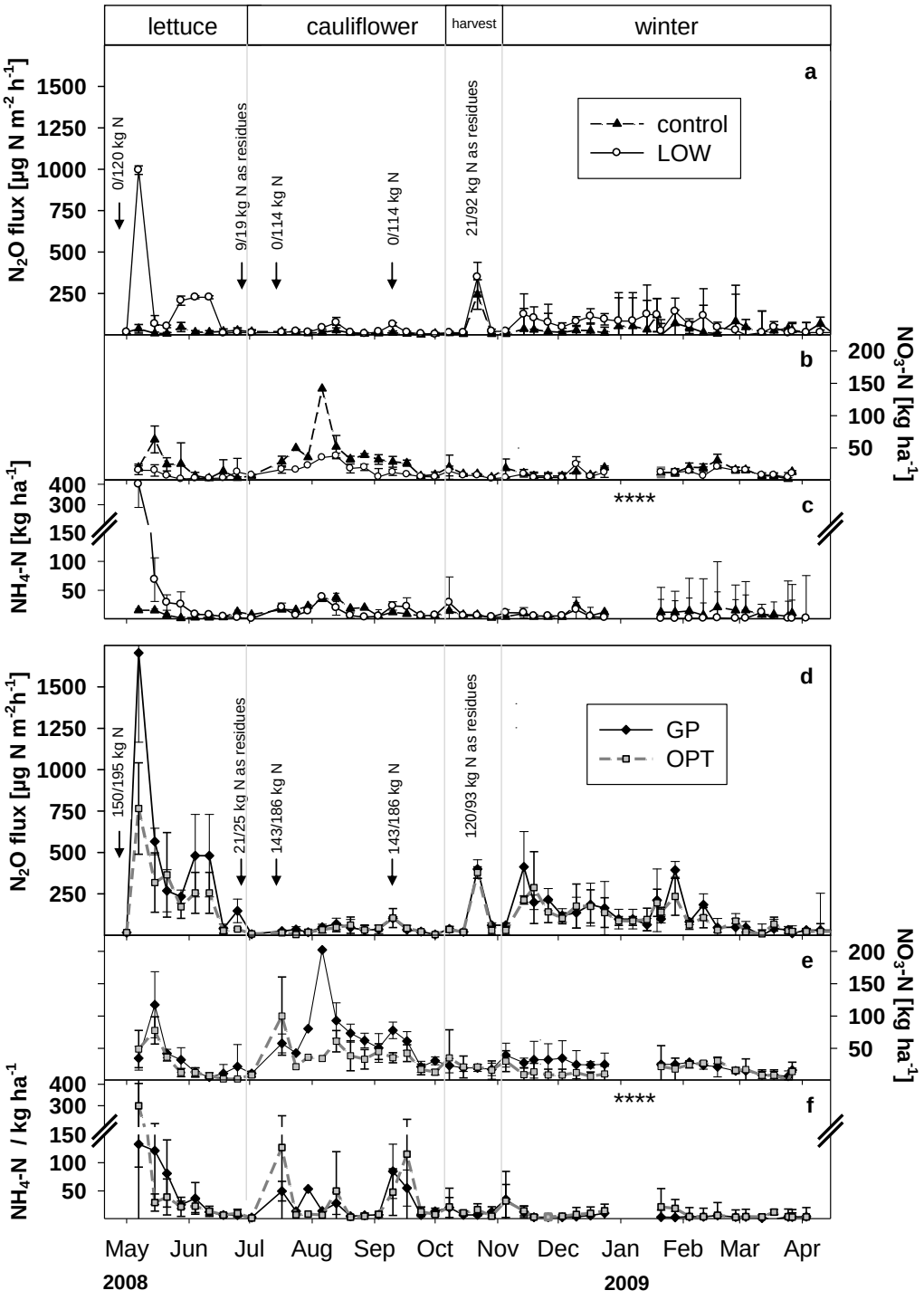


**Fig. 6.2:** *Mean $N_2O$ flux rates and mean ammonium-N and nitrate-N (0 - 25 cm depth, n = 4) in the control treatment, in the treatments with low fertilization (LOW) (a - c), optimized fertilization (OPT) and in the treatment with fertilization according to good agricultural practice (GP) (d - f). Arrows indicate N-fertilization measures, the frost period is specified by the bar on top. Error bars indicate standard deviations.*

above the threshold value of approximately 65 % for an increased production of $N_2O$ from denitrification in soils (Ruser et al., 2006; Well et al., 2006). The positive correlation of soil nitrate content and high soil moisture suggests that denitrification was the major source for the $N_2O$ fluxes from our experimental plots.

#### $N_2O$ emission outside the cropping season

Between 54 % (control) and 44 % (LOW) of the $N_2O$ emission occurred outside the cropping season (winter in Tab. 6.3). For other study sites in Germany, Kaiser and Ruser (2000) and Jungkunst et al. (2006) also reported a similar contribution of winter emissions to annual emissions. They explained the high winter emissions with the occurrence of freeze-thaw cycles, whereas in our study between 41 and 70 % of the winter emission occurred in the time span between harvest and the first frost event.

#### Annual $N_2O$ emission, emission factors and yield related $N_2O$ emission

The annual $N_2O$ release from the unfertilized control plots was 2.4 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ (Tab. 6.3). This background emission is in the upper range as compared to the $N_2O$ emissions from unfertilized arable soils in Germany (0.04 and 2.8 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$; Jungkunst et al., 2006). One reason for the high background emission might be the input of crop residues. Compared to other crops such as cereals, residues in the unfertilized control provided relatively high amounts of organic C and N (328 kg C $ha^{-1}$ and 19 kg N $ha^{-1}$ in 2008, data not shown). A further reason might be the elevated soil moisture content due to irrigation, as suggested by Mosier and Hutchinson (1981).
The annual emissions in the fertilized treatments ranged from 5.7 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ in the LOW treatment to 10.6 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ in the GP treatment, whereas the annual emission from the OPT treatment was on an intermediate level of 8.8 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$. All differences were statistically significant (Tab. 6.3). These emissions are in the upper range of the values specified by Jungkunst et al. (2006). As for the emissions during the cropping season, we found a close relationship between the annual $N_2O$ emission and the mean soil nitrate content in the top soil ($r^2 = 0.83$, data not shown).

**Tab. 6.3:** *Mean annual cumulative $N_2O$ emissions during the vegetation periods of lettuce and cauliflower, during winter and annual emissions (± SD, n = 4), N-Input from fertilizer and residues, the sum of both (total) and the $N_2O$ emission factor in % of the input. Data are given for the control treatment, in the treatments with low (LOW) and optimized (OPT) fertilization and for the treatment with fertilization according to good agricultural practice (GP). Superscript letters indicate statistical groups (Student-Newman-Keuls Test, p < 0.05).*

| | | control | LOW | OPT | GP |
|---|---|---|---|---|---|
| **$N_2O$ emission** [kg $N_2O$-N $ha^{-1}$ $yr^{-1}$] | **lettuce** | 0.4 ± 0.1$^{a}$ | 2.0 ± 0.7$^{a}$ | 3.2 ± 0.7$^{ab}$ | 4.1 ± 1.2$^{b}$ |
| | **cauliflower** | 0.7 ± 0.1$^{a}$ | 1.2 ± 0.1$^{b}$ | 1.5 ± 0.2$^{c}$ | 1.7 ± 0.3$^{c}$ |
| | **winter** | 1.3 ± 0.1$^{a}$ | 2.5 ± 0.6$^{a}$ | 4.1 ± 1.1$^{b}$ | 4.8 ± 1.0$^{b}$ |
| | **annual** | **2.4 ± 0.2$^{a}$** | **5.7 ± 1.2$^{b}$** | **8.8 ± 0.9$^{c}$** | **10.6 ± 0.7$^{d}$** |
| **N-Input** [kg N $ha^{-1}$] | **fertilizer** | 0 | 319 | 401 | 528 |
| | **residues** | 27 | 122 | 138 | 161 |
| | **total** | 27 | 441 | 539 | 689 |
| **$N_2O$ Emission factor*** [% N-Input] | | | 1.3 | 1.6 | 1.5 |

* $N_2O$ Emission factor = ($N_2O$-N emission/ total N-Input ) · 100

The reduction of fertilizer from the GP to the OPT level led to a reduction in annual $N_2O$ emissions by 1.8 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$. We could not determine significant effects on the yields of lettuce or cauliflower by this reduction. This generally confirms our hypothesis of the $N_2O$ mitigation potential by a reduced N-input although a too strong reduction like from the OPT to the LOW N-level led to lower crop yield.
Based on the N-input through fertilizer and crop residues, we calculated the emission factor EF1 for all fertilized treatments in accordance to the guidelines of the IPCC (2006). The emission factors ranged between 1.3 and

1.6 % (Tab. 6.3). They were higher than the default value of 1.0 % recommended by the IPCC (2006), but in contrast to our hypothesis of an increased emission factor, it lay within the proposed range (0.3 - 3 %). These results are in agreement with emission factors calculated by Dobbie et al. (1999) who related the annual $N_2O$ emission to the amount of N-fertilizer applied to potatoes and broccoli.

As shown in Figure 6.3, the annual $N_2O$ emission increased with increasing N-surpluses on the field level. Consequently, the reduction of N-surpluses by minimizing the amounts of applied fertilizer-N plays a key role in $N_2O$ mitigation strategies in vegetable production systems. Similar relationships between N-surpluses or the N use efficiencies and the $N_2O$ emissions have been reported by Kaiser and Ruser (2000) and by van Groenigen et al. (2004) for arable farming.
A reduction in N-fertilization is also highly desirable for farmers and helps them to maximize their profits if it does not impede the yields.

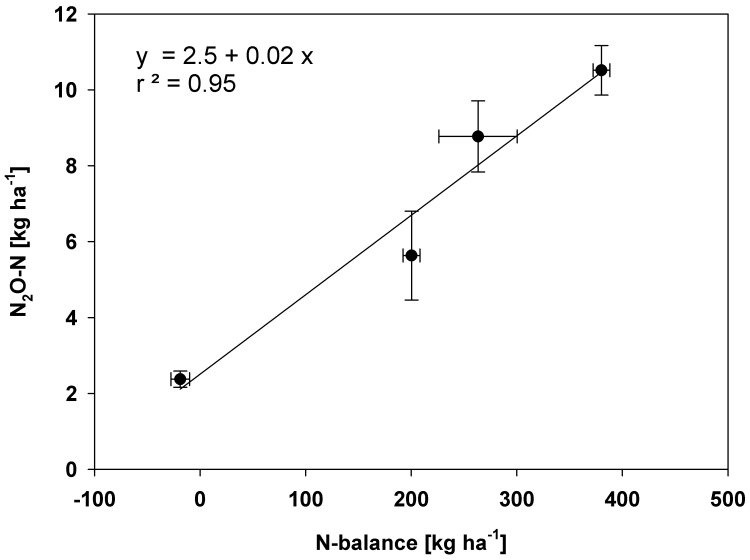


**Fig. 6.3:** *N-balance and cumulative annual $N_2O$ emissions in the control treatment, in the treatments with low fertilization, optimized fertilization and in the treatment with fertilization according to good agricultural practice (in this order, $n = 4$), error bars indicate standard deviations.*

## 6.5 Conclusions

In addition to the events that have frequently been shown to stimulate $N_2O$ emissions from soil like N-fertilization and rainfall, high fluxes were attributed to an increased carbon availability. Emission peaks occurred after the incorporation of the catch crop in spring and during the mineralization of the cauliflower residues.

Our data clearly demonstrate the huge reduction potential for $N_2O$ emissions from vegetable fields by optimizing the N-fertilizer amount as proposed by the German Target Value System (Feller et al., 2001). Reducing the N-addition by approximately 24 % (corresponding to 127 kg N $ha^{-1}$ $yr^{-1}$) from the amount used following good agricultural practice led to a saving of 1.8 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ without any negative effect on the vegetable yield. To minimize $N_2O$ emissions from vegetable production, an ecologically sound management strategy must include the avoidance of high N-surpluses.

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# 7 Influence of catch crops as well as of a waiting period between catch crop incorporation and following N-fertilization on $N_2O$ emissions

Results of the following Bachelor Thesis are included in this chapter:
"Einfluss von Zwischenfrucht und Umbruchtermin auf die $N_2O$-Emissionen der Folgefrucht"; Ebinger, K. 2010

## 7.1 Introduction

One field management measure which has been proposed to reduce N loss from arable land is the growing of cover crops. This might also be beneficial in vegetable production systems, where high nitrate levels of up to 50 kg N $ha^{-1}$ were measured (Chapter 6). This $NO_3$-N is on the one hand susceptible to leaching by means of gravitational water (Martinez and Guiraud, 1990; Isse et al., 1999) and on the other hand it can increase the N loss via denitrification during winter. Up to 4.8 kg N $ha^{-1}$ were lost as $N_2O$ during the winter season (Chapter 6) from the lettuce-cauliflower rotation of this study.

Furthermore, catch crops have beneficial effects, for example the improvement of soil structure (Roberson et al., 1991; Sainju et al., 2005), weed suppression (Campiglia et al., 2009), and reduction of soil compaction (Chen and Weil, 2010). More beneficial effects of the implementation of cover crops such as e.g. increase in soil organic matter and reduction of soil erosion are described in more detail in Sarrantonio and Gallandt (2003). By means of water uptake and evapotranspiration, the growing of catch crops in winter reduces the amount of leachate and nutrient losses by percolation. Decreases in WFPS can in turn decrease $N_2O$ emissions.

The German Ordinance on Fertilization ("Düngeverordnung") approves these positive effects in vegetable production systems: if cover crops are grown, an additional 40 kg N $ha^{-1}$ may be left in the field after harvest as "inevitable N surplus" (BGBl., 2006) for a certain group of vegetable species ("Gemüsekategorie III"). This group includes several Brassica species such as broccoli, cauliflower and savoy cabbage as well as sweet corn, green squash and runner beans. However, it is not further specified what type of catch crops should be chosen. Catch crops like rape, mustard, *Phacelia* and also cereals have a high potential to conserve nutrients. However, it is obvious that off-freezing catch crops such as *Phacelia* immobilize nitrogen for only a limited period of time. Considerable amounts of nitrogen can be released after freezing if the weather becomes mild and humid (Schmaler et al., 1992; Berger et al., 1993). In this case, decomposition and denitrification as well as leaching can cause considerable N-losses during winter. Another advantage of winter-hard catch crops such as green rye is that they can take up much higher amounts of nitrogen than herbs like *Phacelia* due to their quick growth, high cold persistence and a fibrous deep root system (Sarrantonio and Gallandt, 2003), especially also in spring when again precipitation and higher temperatures increase the risk of N losses.

Besides the risk of losses in winter before incorporation, the period after incorporation of the catch crops in the next vegetation period is also of interest. It is assumed that N from plants is released slowly and can be taken up by the growing plants (Isse et al., 1999) so that most of the nitrogen is recovered. Therefore, it was claimed to incorporate the catch crops as close as possible before planting the next crop. Despite this, high losses of $N_2O$ (> 4 kg $N_2O$-N during 4 weeks) were observed after the incorporation of green rye in this study (Chapter 6) with a period of only 4 days between tilling of the chopped green rye and the planting and initial N-fertilization of the main crop. In the second experimental year however, no catch crop was implemented and no such high emissions were observed. The decomposition of residues after their incorporation can release considerable amounts of nitrogen and carbon (Collins et al., 1990; Entry et al., 1996). If the next crop is not planted immediately, an increase in soil mineral N can be assumed for residues with low C/N ratio. Green

rye is usually incorporated in spring when its C/N ratio is still relatively low. Lenzi et al. (2009) for example reported a C/N ratio of 24 for green rye before its incorporation. Therefore it is possible that an increase in mineral soil N might lead to increased $N_2O$ emissions: the mineral N level is elevated by the mineralization of the catch crops. Furthermore, mineralization can cause $O_2$ depletion and therefore stimulate $N_2O$ production by denitrification. After N-fertilization nitrogen surpluses can arise because the N-uptake of the subsequent crop is low immediately after planting. Taken together, the waiting time can therefore increase cumulative $N_2O$ emission.
For this reason it is important to investigate the influence of the waiting time between incorporation of the cover crop and N-fertilization of the succeeding crop.

To evaluate the effects of catch crops systematically, we wanted to

(i) test the influence of different catch crops (winter-hard, off-freezing) on $N_2O$ emissions as compared to a bare fallow and
(ii) test weather waiting periods between the incorporation of the catch crops and the N-fertilization of the next crop influence the $N_2O$ emissions from the following crop.

It is assumed that due to the reduction of soil $N_{min}$, $N_2O$ emissions is decreased in winter when a winter-hard catch crop is grown as compared to a bare fallow. The implementation of an off-freezing catch crop probably leads to intermediate $N_2O$ emissions because soil $N_{min}$ is immobilized at least temporarily. It is further assumed that a short waiting time between the incorporation of the catch crops and planting and of the next crop will decrease $N_2O$ emissions due to a better synchronization with plant uptake.

## 7.2 Material and Methods

### 7.2.1 Study site and field management

**Tab. 7.1:** *Overview of the treatments showing type of catch crop implementation, date of catch crop incorporation and waiting time between catch crop incorporation and fertilization (d = days)*

| treatment | catch crop | date of catch crop incorporation | waiting time between incorporation and fertilization |
|---|---|---|---|
| control (unfertilized) | green rye | 29 Apr | 7 d |
| bare fallow | - | 29 Apr | 7 d |
| *Phacelia* | *Phacelia tanacetifolia* | 29 Apr | 7 d |
| early | green rye | 25 Mar | 35 d |
| medium | green rye | 15 Apr | 21 d |
| late | green rye | 29 Apr | 7 d |

The study was conducted at the site described in Chapter 4 and 6. On September 23 in the second year of measurement, the following three treatments with different catch crops were established (4 replicates) on plots which had been fertilized according to the German target value system for the precedent two years:

(i) *Phacelia tanacetifolia* was sown at 12 kg seed $ha^{-1}$ ("Phacelia");
(ii) green rye was sown at 160 kg seed $ha^{-1}$ ("green rye") and
(iii) no catch crop ("bare fallow") was sown.

*Phacelia tanacetifolia* reached a height of about 0.25 m on December 9 and then froze due to a decrease in temperature. The green rye plants developed well and did not freeze off during winter. To compare the effect of different waiting times between catch crop incorporation and N-fertilization, two more treatments with green rye (4 replicates each) were installed:

(iv) one treatment which was tilled 14 days before the last tilling and
(v) one treatment which was tilled 27 days the last tilling (Tab. 7.1).

One day before each management measure, one square meter of cover crop plants was cut, plant material was weighed and packed for dry matter determination and C/N analyses. Chard (*Beta vulgaris* sub. *vulgaris*) was planted on 17 May with 25 · 30 cm in 6 rows per plot (4.5 m broad), resulting in a density of 80,000 plant $ha^{-1}$. N-fertilization was carried out broadcast on 5 May with ammonium sulfate nitrate: An $N_{min}$ analysis showed that 15 kg N $ha^{-1}$ were still present in the soil. This value was subtracted from the target value of 120 kg N $ha^{-1}$ for the first N-fertilization. The second fertilization (30 kg N $ha^{-1}$) was carried out on 20 June. Chard was harvested on 8 July. 20 representative plants were taken from each plot and fresh weights were determined. 300 g of fresh leaves were packed for dry matter determination and C/N analyses.

### 7.2.2 Gas measurement

$N_2O$ was measured as described in Chapter 6 from the first incorporation of green rye on 25 March until 21 June (This work was done in line with a bachelor thesis (Ebinger, 2010) which did not allow for a measuring period covering the whole chard season).

### 7.2.3 Soil sampling and laboratory analyses

Soil sampling, manual temperature measurement, measurement of nitrate and ammonium concentrations, determination of water-filled pore space and C and N analyses of the chard leaves were carried out as described in Chapter 6. Bulk density was determined once during the vegetation period.

## 7.3 Results and Discussion

### 7.3.1 Influence of cover crops on $N_2O$ emissions

*$N_2O$ emissions and controlling parameters during the winter season*

As it is typical for vegetable cropping systems, $N_{min}$ levels were relatively high after harvest. They were above 50 kg N $ha^{-1}$ (0 - 25 cm) and even increased within the next 2 months to values > 100 kg $N_{min}$ $ha^{-1}$ for all treatments except the unfertilized control. This might be due to the mineralization of cauliflower residues. De Neve et al. (1996) studied N-mineralization from cauliflower residues. Depending on the temperature, mineralization took between 5 weeks and up to more than 3 months. During this time, a total of 67 and 82 % of total stem and leaf-blade nitrogen was mineralized, respectively. Considerable amounts of up to 8 kg N $ha^{-1}$ per week were released.

The highest $N_{min}$ values were measured in the bare fallow treatment and the lowest in the green rye treatment for most samplings during November and December. This is not surprising because rye has a high potential for N uptake (Sarrantonio and Gallandt, 2003), whereas with the bare fallow, all N from mineralization remained in the soil. In the bare fallow treatment, the highest $N_{min}$ levels of > 150 kg N $ha^{-1}$ (± 17) were measured in December. However, at the next sampling one week later, all three treatments showed a steep decrease in $N_{min}$ level (Fig. 7.1). $N_{min}$ levels declined to a background level of < 40 kg N $ha^{-1}$. In the week between the samplings, temperature had dropped to < 0°C, but no precipitation was measured at the climate station. Yet is possible that the rainwater collector device or the datalogger did not work well in that week. Especially the rainwater collectors used at the metereological station which measure the water that is passing through are sometimes blocked e.g. by leaves which fall in from the top.
However at the next sampling in 50 - 75 cm, the $N_{min}$ level had risen by about 40 kg N $ha^{-1}$ in that depth indicating considerable N leaching (data not shown). It is also possible that due to spatial variability on a small scale, it might have rained more on the study site than at the meteorological station. This is further supported by data from another meteorological station (meterological station belonging to the Institute of Product Quality of Speciality Crops, Universität Hohenheim) which reported 22 mm of precipitation in that week. The data illustrates the risk of N-leaching during periods of high precipitation in especially in fallow plots.

*Phacelia* plants grew quickly to a height of about 0.25 m until the beginning of December, while the green rye developed slowlier. *Phacelia* plants significantly reduced the soil mineral N level as compared to the bare fallow from the middle of October (when they had developed) until they froze off in the second week of December (Student-Newman-Keuls Test, $p < 0.05$). At this time, the C/N ratio of the *Phacelia* residues was 6.2. No obvious increase in soil $N_{min}$ was observed by its mineralization directly after the off-freezing of *Phacelia*. Maybe

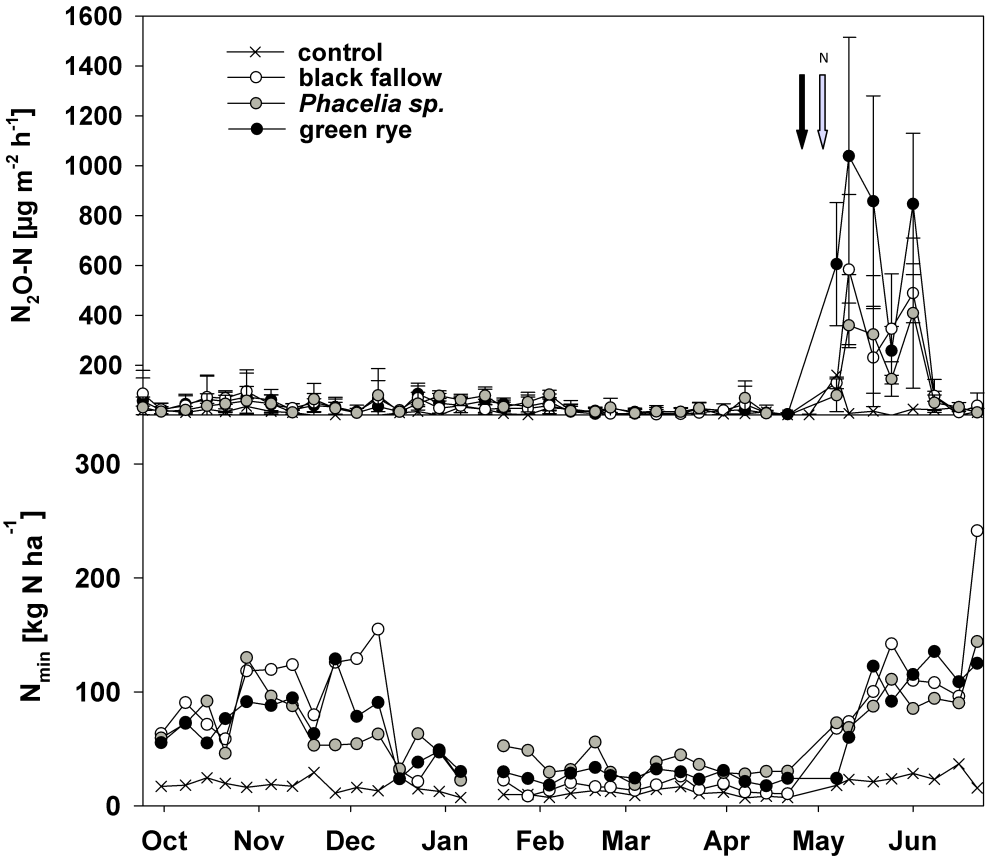


**Fig. 7.1:** *Mean $N_2O$ flux rates and standard deviations (a) and mean soil mineral N contents (b) from the unfertilized control treatment, from the bare fallow treatment, from the treatment with Phacelia as a catch crop and from the treatment with green rye as a catch crop. The black arrow indicates ploughing of the soil and incorporation of the catch crops if available, the grey arrow indicates N-fertilization (not for the control). Standard deviations for soil mineral N contents are omitted due to clarity. Some values are missing due to soil frost; (Ebinger, 2010).*

mineralization was delayed due to the low temperatures and soil freezing. In January and February however, the highest $N_{min}$ levels were measured in this treatment, although the differences were not significant.

The differences in $N_{min}$ level caused by the differences in plant cover were not reflected in the $N_2O$ flux rates in winter: During the whole winter season, $N_2O$ emissions were similarly low from all treatments, even at the time of very high $N_{min}$ levels > 100 kg N $ha^{-1}$. No correlation between soil mineral N or nitrate-N and $N_2O$ flux rates was found. One explanation for the lack of response to high soil mineral N levels might be a relatively dry period of two weeks with WFPS values < 60 % in the middle of October (Chapter 8; Fig. 8.2).
The explanation that nitrogen was not limiting for the production of $N_2O$ is more convincing since the soil was temporarily C-limited. This had already been proven during the vegetation period of cauliflower: additional chamber bases had been installed and were irrigated either with 4 mm glucose solution (0.14 M) or distilled water. The addition of glucose resulted in $N_2O$ emissions which were 32-times higher than from plots which received only water (Fig. 7.2)

This points to denitrification as a major source of $N_2O$. It agrees further with the statistically significant positive correlation between $N_2O$ and $CO_2$ flux rates ($r^2 = 0.29$) which has been found for the period from March 25 (first incorporation) until the end of the study. $CO_2$, WFPS and temperature explained a 48 % of the variation in $N_2O$ flux rates. Assuming that carbon was likewise limited in all plots, it is therefore not surprising that the cumulative $N_2O$ emission before incorporation of the catch crops was low (see Fig. 7.3) and not significantly different regardless of weather the soil was bare or directly after the off-freezing of *Phacelia* or green rye were grown.

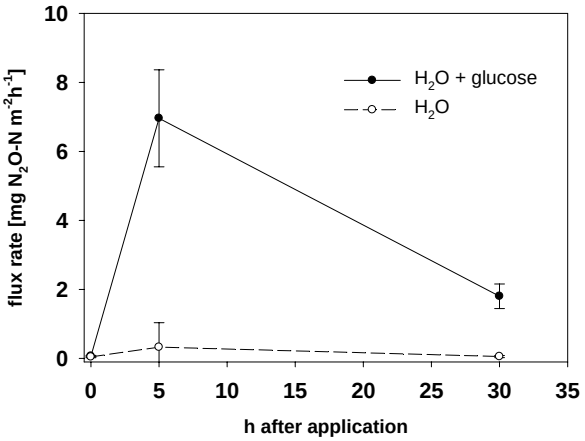


**Fig. 7.2:** *Mean $N_2O$ flux rates and standard deviations (n = 4) after application of 4 mm of 0.14 M glucose solution or water.*

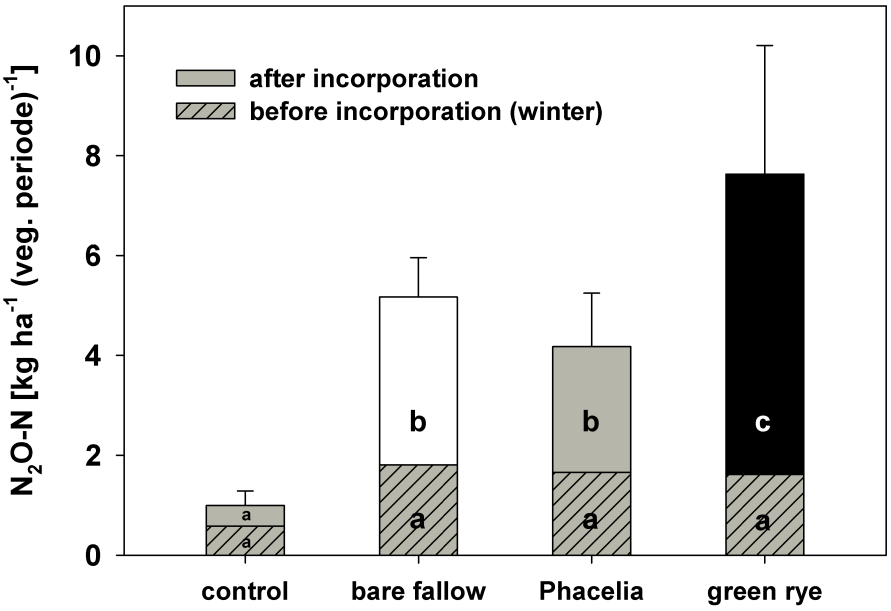


**Fig. 7.3:** *Cumulative $N_2O$ emission (n = 4) from the unfertilized control treatment, from the bare fallow treatment, from the treatment with Phacelia as a catch crop and from the treatment with green rye as a catch crop before catch crop incorporation during the winter season after the harvest of cauliflower (shaded) and after incorporation during the vegetation period of chard. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, p < 0.05); (Ebinger, 2010).*

### 7.3.2 $N_2O$ emissions and controlling parameters during the subsequent crop

After the incorporation of the catch crops and N-fertilization 7 days later, a steep increase in soil $N_{min}$ as well as $N_2O$ emissions was measured. Flux rates were greatest from the treatment with green rye and reached maximum values of 1038 µg $N_2O$-N $m^{-2}$ $h^{-1}$. This concurred with the results obtained in the first experimental year when green rye had been incorporated few days before the N-fertilization to lettuce (Chapter 6, Fig. 6.2). Cumulative $N_2O$ emission from the incorporation of the catch crops on 29 April until the end of the experiment was significantly higher from the green rye treatments (6.0 kg $N_2O$-N) than from the *Phacelia* (2.5 kg $N_2O$-N) and bare fallow treatment (3.4 kg $N_2O$-N). However, no difference was found in soil mineral N values.

As during the winter season, carbon was more important and the decisive factor for the total $N_2O$ emission: the green rye provided 3218 kg C and 146 kg N $ha^{-1}$ (Fig. 7.6). In combination with the high mineral N input from fertilization, this increased $N_2O$ emission. Similar results have been reported in several studies, where organic material stimulated $N_2O$ emissions. In combination with N-fertilization, the input of organic carbon has also been seen to increase $N_2O$ emissions (Aulakh et al., 1984; Sarkodie-Addo et al., 2003; Garcia-Ruiz and Baggs, 2007). Two main reasons can be specified: Firstly, carbon can serve as an electron donator for

denitrification (Weier et al., 1993). Secondly, decomposition of the residues can increase $O_2$ consumption and thus create more anaerobic microsites which are ideal for denitrification (Tiedje et al., 1984; Thomson et al., 1997). Net immobilization could not be observed due to the low C/N ratio of the green rye manure and the N-fertilization. Enough nitrogen was provided to meet the demand of the microorganisms.

The *Phacelia* residues only contained 29 (± 4) kg N $ha^{-1}$ und 178 (± 13) kg C $ha^{-1}$ before they froze off. The carbon and nitrogen might have already been partly mineralized during the winter season. On the bare fallow plots, only some weeds might have provided low amounts of carbon (not quantified). Therefore, cumulative emission from both of these treatments was lower than from the green rye treatment. This means that adherence of the German ordinance on fertilization concerning the growing of winter cover crops does not necessarily decrease cumulative $N_2O$ emission in our Haplic Luvisol if the time of N-fertilization is too close to the input of the organic material. Nitrate leaching was determined in this study by a second project dealing with the quantification of nitrate leaching and indirect $N_2O$ emissions. First estimations with the model WINEPIC show that nitrate leaching of about 20 kg N $ha^{-1}$ occurred in the treatment with conventional fertilization and green rye (Palmer 2011, personal communication). This is less than the values observed on sandy soils, but still considerable for this ecosystem. Therefore, attention should be paid to the choice of the catch crop to minimize these losses as far as possible.

### 7.3.3 Influence of a waiting period on $N_2O$ emissions

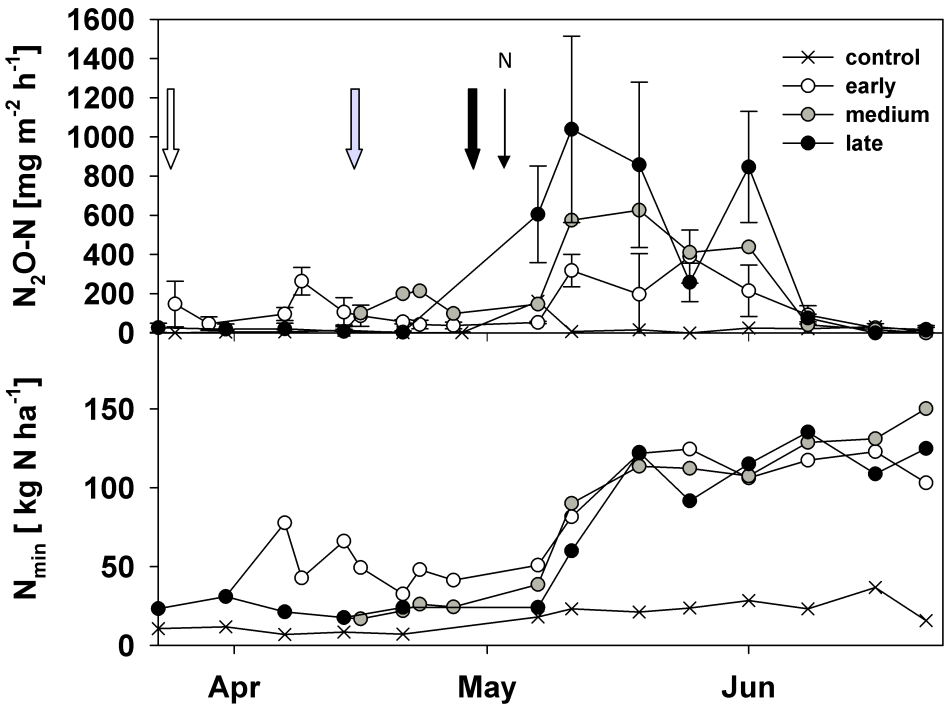


**Fig. 7.4:** *Mean $N_2O$ flux rates and standard deviations (n = 4) and mean soil mineral N contents (n = 4) from the unfertilized control treatment (late catch crop incorporation) and from the treatment with early (white arrow) ploughing, medium (grey arrow) catch crop incorporation and late (black arrow) catch crop incorporation of plots sown with green rye as catch crop. "N" indicates the time of N-fertilization. Standard deviations are omitted for soil mineral N due to clarity; (Ebinger, 2010).*

The incorporation of organic material with a high C/N ratio like catch crops can immobilize nitrogen and lead to lower plant nitrogen availability (Chaves et al., 2007) as well as to reduced $N_2O$ emissions (Kaewpradit et al., 2008). However, in our study the C/N ratio of the incorporated green rye was 10, 13 and 23 for the early, medium and late incorporation. This is a rather low value which is not supposed to cause immobilization of nitrogen (Vigil and Kissel, 1991). For the same reasons as described above, $N_2O$ loss by denitrification was stimulated by the input of green rye.

In our study, a waiting period between incorporation of the catch crops and N-fertilization obviously prevented the synchronic availability of carbon and nitrogen and reduced cumulative $N_2O$ emission as compared to the shorter waiting times (Fig. 7.4). However, this finding is in contrast to many recommendations. In England,

the ministry of Agriculture, Fisheries and Food even recommends that incorporation of green manure should be delayed until just before the next crop is sown (MAFF, 1991). This probably focuses mainly on the avoidance of N loss by leaching. Of course, the importance of N leaching depends on precipitation during the waiting period and could be more pronounced in other years and on lighter soils with higher sand content.

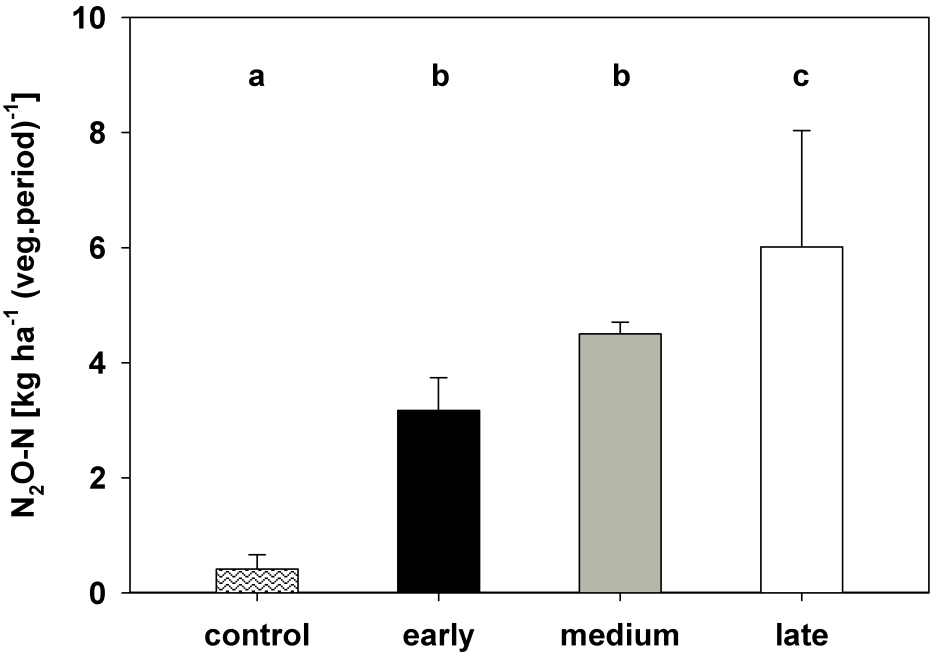


**Fig. 7.5:** *Cumulative $N_2O$ emission and standard deviations (n = 4) for the period between the first catch crop incorporation until the end of the measurement during the vegetation period of chard from the unfertilized control treatment and from the treatments with early, medium and late incorporation of green rye cover crops. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, p < 0.05); (Ebinger, 2010).*

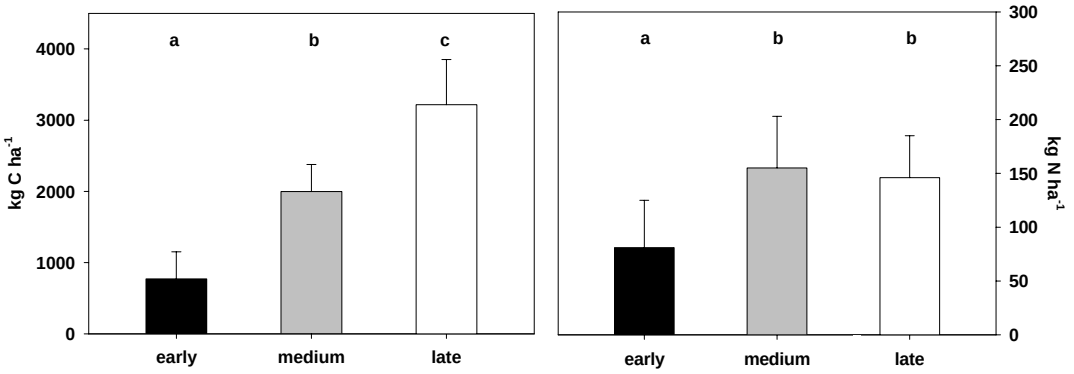


**Fig. 7.6:** *Mean carbon and nitrogen input and standard deviations (n = 4) of green rye at the time of incorporation. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, p < 0.05); (Ebinger, 2010).*

The early date and a 35-day waiting period until the N-fertilization resulted in an increase in $N_{min}$ and $N_2O$ flux rates (up to 263 µg $N_2O$-N $m^{-2}$ $h^{-1}$). The medium date showed less effect both on $N_{min}$ as well as on $N_2O$ flux rates (maximum 213 µg $N_2O$-N $m^{-2}$ $h^{-1}$). Only after the third date in combination with the N-fertilization, major effects were measured: soil $N_{min}$ levels increased equally for all 3 treatments. $N_2O$ flux rates also peaked, with distinctive peak-height depending on waiting time: maximum fluxes of 1038, 583 and 360 µg $N_2O$-N $m^{-2}$ $h^{-1}$ were measured from the treatments with late, medium and early incorporation of catch crops. High peaks after incorporation of residues have been reported elsewhere (Ruser et al., 1998; Toma and Hatano, 2007). However, our results further indicate that a waiting period of more than three weeks can significantly reduce cumulative $N_2O$ emission during the following crop (Fig. 7.5). Two explanations can be cited: firstly, green rye provided much more organic carbon when it could keep on growing for another 35 days

as compared to the early plowing (Fig. 7.6), resulting in ideal conditions for denitrifiers as explained before. Another reason is that for the two earlier dates, the organic compounds were not released simultaneously with the input of mineral N-fertilizer. At the time when the mineral nitrogen was added, the major part of the carbon was probably already degraded. The creation of anaerobic conditions by the organic material probably did not coincide with the mineral N-input and therefore there was less stimulation of denitrification. Similar results for higher $N_2O$ emissions when combining organic and mineral inputs were found e.g. by Aulakh et al. (1984), Sarkodie-Addo et al. (2003) and Garcia-Ruiz and Baggs (2007). However, a recommendation for a 3-week waiting period is only reasonable if the risk of leakage caused by precipitation is low. For example on sandy soil in regions with a high risk of precipitation in spring it is questionable if waiting periods should be so prolonged. Further research on the length of waiting periods on sandy soils would be needed.

## 7.4 Conclusions

An increased risk for N leaching was detected in winter and was most pronounced in the bare fallow treatment. The low $N_2O$ emissions during the winter period independent of the catch crop management as well as the extraordinarily high $N_2O$ emission after the incorporation of green rye imply that growing a cover crop can even increase $N_2O$ emissions from this C-limited vegetable production system due to high emissions in spring. Since organic C-input in combination with mineral N-fertilization stimulates $N_2O$ release, a 3-week waiting period can significantly reduce $N_2O$ emissions. Adherence to the German regulation which prescribes the growing of a catch crop for certain vegetables (but no waiting periods) can therefore even increase cumulative $N_2O$ emission, if C-rich cover crops are incorporated shortly before the next N-fertilization.

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# 8 Influence of a nitrification inhibitor and placed N-fertilization on $N_2O$ fluxes from a vegetable cropped loamy soil

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Authors: Pfab, H., Palmer, I., Buegger, F., Fiedler, S., Müller, T. and Ruser, R.

## 8.1 Abstract

Arable soils are a major source of the climate relevant trace gas nitrous oxide ($N_2O$). Although $N_2O$ emissions from soils increase with the amount of N-fertilizer, there is still a lack of data for intensively fertilized systems, such as vegetable production. We investigated the effect of a nitrification inhibitor and placed N-fertilization on $N_2O$ fluxes and yields as compared to conventional broadcast fertilization from a lettuce-cauliflower rotation over two years of measurement. Except for a lower cauliflower yield in the second experimental year with placed fertilization, no differences in yields between the fertilized treatments were observed. Annual cumulative $N_2O$ emissions of the conventionally fertilized treatment (ammonium sulfate nitrate), were 8.8 and 4.7 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$, indicating a high inter-annual variability.

The addition of the nitrification inhibitor 3,4-dimethylepyrazole phosphate (DMPP) significantly reduced $N_2O$ emissions during the cropping season and also during the winter period, resulting in an annual reduction of 45 and 40 % as compared to the conventionally fertilized treatment. Generally, the reason for the lower $N_2O$ release from the DMPP treatment remained unclear since we did not find any significant differences in the mineral N pool in periods with distinctive inhibition. For the post-harvest period in the first experimental year, we found lower soil respiration rates covering a time frame of several months which started about six weeks after fertilizer application.

In contrast to the treatment with nitrification inhibitor, the placed fertilization as an N-depot did not help to reduce annual $N_2O$ emissions, although the ratio of ammonium-N to nitrate-N in the first weeks after N-application implied a nitrification inhibitory effect in the fertilizer depot. We assume that, even though ammonium concentrations in the depots were high, toxicity was not sufficient for a complete inhibition of the microbial population in the surrounding of the depots, resulting in considerable $N_2O$ production from this area. The emission factors calculated for the fertilized treatments ranged between 0.5 and 1.6 % and were thus within the range proposed by the guidelines of the IPCC (2006).

However, the absolute $N_2O$ emissions from intensively fertilized vegetable fields are high. For effective, but environmentally sound vegetable production, a deeper understanding of both nitrification inhibitory strategies is necessary.

## 8.2 Introduction

The concentration of the greenhouse gas nitrous oxide ($N_2O$) has been continuously increasing over the last decades (Prather et al., 1995; IPCC, 2007). In soils, $N_2O$ is produced to a major part by the two microbial processes; nitrification and denitrification (Bremner and Blackmer, 1981; Davidson, 1991). Intensive N-fertilization fuels these processes as it provides substrate for nitrifying and denitrifying microorganisms.

Nitrous oxide is produced as a by-product during nitrification and as an intermediate during denitrification. Nitrification is the microbial oxidation of ammonium into nitrite which is further oxidized to nitrate (Granli and Bøckman, 1994). The provided nitrate serves as substrate for denitrification. N-fertilization with ammonium rich fertilizers and the inhibition of nitrification is proposed as a potent measure to reduce $N_2O$ losses from arable as well as from grassland soils (Bremner and Blackmer, 1978; Moisier, 1996; DeKlein et al. 1996, Akiyama, 2010). 3,4-dimethylpyrazole phosphate (DMPP) is a relatively new nitrification inhibitor (NI) which has undergone standard toxicologic and ecotoxicologic tests (Roll, 1999; Andreae, 1999). Zerulla et al. (2001) described its advantageous properties such as high efficiency (0.5 - 1.5 kg DMPP $ha^{-1}$) as compared to other inhibitors like dicyandiamide (DCD) and its low risk for translocation. The addition of the nitrification inhibitor DMPP is expected to reduce $N_2O$ emissions during the vegetation period through the inhibition of the first reaction of nitrification. This is achieved by the inhibition of the enzyme ammonia monooxygenase (Vannelli and Hooper, 1992) which catalyzes the conversion of ammonium to hydroxylamine. The inhibition causes a stabilization of ammonium and a strong delay in the production of nitrate which was often shown to reduce leaching (Chaves et al., 2006; Diez et al., 2010). The stabilization of ammonium ($NH_4^+$) by NI with lower risk of N-leaching allows for simplified fertilization strategies with less fertilizer applications (Serna et al., 2000; Fettweis et al., 2001).

Several studies have been published on the reductive effect of DMPP on $N_2O$ emissions: Weiske et al. (2001a) measured $N_2O$ fluxes from arable soil for three years during the cropping season and found decreases in the cumulative $N_2O$ emissions of up to 53 % for this period. A decrease of $N_2O$ emissions for DMPP was also reported for winter wheat by Linzmeier et al. (2001). They used fertilizer with DMPP in simplified fertilizer strategies (fewer applications) and compared it to conventional fertilization in two following years during experimental periods covering the cropping season. For the four weeks after fertilizer application, fertilizer-derived losses were reduced by about 50 % in the first year of their measurements. Menendez et al. (2006) found a reduction in $N_2O$ emissions from grassland when adding DMPP to slurry, but not when adding DMPP to ammonium sulphate nitrate (ASN). In contrast, Belastegui Macadam et al. (2003) found a reductive effect of DMPP for both slurry and mineral fertilizer.

None of the published studies covered a continuous measuring period of a whole year, although the importance of annual datasets for the measurement of $N_2O$ emissions is known and a contribution of up to 89 % of winter emissions to total annual emissions has been reported for study sites with winter frost in Germany (Flessa et al., 1995; Kaiser and Ruser, 2000). Therefore, measures which aim to reduce the impact of agricultural activities on the earth's climate need to be verified on an annual base, at least in regions with distinctive freezing/thawing cycles during the winter season. In terms of nitrification inhibitors it could be assumed that lower nitrate leaching losses would result in a higher fertilizer use efficiency which might decrease the C/N-ratio of plant residues thus stimulating their mineralization in fall. Kaiser et al. (1998) and Baggs et al. (2000) found increasing $N_2O$ emissions during the winter period with decreasing C-to-N-ratio of crop residues. In addition, Ruser et al. (2001) found a strong correlation between the nitrate contents of the top soil and the $N_2O$ emissions during the winter season. Up to now, no study has measured the effect of DMPP on $N_2O$ emissions during a whole year including the winter season. Therefore it is still unclear whether the reduction of $N_2O$ emissions by the use of a nitrification inhibitor is also valid on an annual base.

Aside from the addition of synthetic NIs, nitrification can also be inhibited by the creation of unfavorable conditions for nitrifying organisms. Controlled Uptake Longterm Ammonium Nutrition (CULTAN) is an N placement fertilization strategy where N-fertilizer with a high ammonium portion is placed in the soil as a depot (Sommer, 2005). In the N-depots, ammonium concentrations are extremely high. It is known that these high osmotic values (e.g. ammonium-N concentrations in the soil solution > 3000 ppm) can completely inhibit nitrification (Wetselaar et al. 1972). This leads to a stabilization of ammonium similar to the use of NI. To ensure an easily available N supply for vegetable crops with a short growing period, the CULTAN-method can be modified with additional nitrate which should cover this early N demand. These so-called pseudo-CULTAN

strategies use fertilizer depots with up to 30 % of the total N as nitrate (Sommer, 2005).

Vegetable production is associated with high N surpluses due to the harvest during the vegetative growth phase (Krug et al., 2002). The resulting high mineral N contents of the soil in combination with high soil moisture due to irrigation during dry summer conditions offer ideal conditions for denitrifying organisms. Therefore, comparably high flux rates can be expected for vegetable fields. Furthermore, only few datasets for vegetable production have been provided so far (Dobbie et al., 1999; Van der Weerden et al., 2000). None of these investigations were carried out in a region with freeze-thaw cycles, which are known to contribute a major part of the total annual emissions (Flessa et al., 1995, Ruser et al., 2001). For these reasons, we chose a lettuce-cauliflower rotation in a temperate region with strong winter frost (Southwest Germany) for our investigations.

Several studies have reported increasing yield or yield quality both for the use of NI (Pasda et al., 2001) and for N-depot fertilization (Sommer, 2005) compared to broadcast fertilizer application. We hypothesize that the nitrification inhibiting effect (either with an inhibiting compound or with the CULTAN-technique) would reduce $N_2O$ emissions during the vegetation period without any negative effects on yield. An increase in emissions may be expected for the winter season. Therefore, on an annual basis, no significant difference in $N_2O$ emissions from conventionally fertilized soils can be expected.

The aims of our study were:

(i) to quantify the impact on $N_2O$ emissions by the addition of a nitrification inhibiting compound (DMPP) as compared to conventional N-fertilization;
(ii) to quantify the effect of N-fertilization according to the CULTAN-technique on $N_2O$ emissions as compared to conventional N-fertilization and
(iii) to calculate emission factors for direct emissions (EF1) for all treatments according to the IPCC (IPCC, 2006).

## 8.3 Material and Methods

### 8.3.1 Study site

The field trial was established on the experimental farm "Heidfeldhof", which belongs to the Universität Hohenheim. It is located 13 km south of Stuttgart, Germany (48° 43' 00" N; 9° 11' 40" E) in an absolute altitude of 410 m. The mean annual precipitation is 686 mm, the average air temperature 8.8°C. The soil type is a Haplic Luvisol derived from periglacial loess. $C_{org}$ and $N_t$ content of the top soil was 1.8 % and 0.16 %. Texture consisted of 2 % sand, 68 % silt and 30 % clay, the initial soil pH was 5.5 and the gravel content was < 1 %.

### 8.3.2 Field management and experimental set-up

**Tab. 8.1:** *Management of the vegetable field for both experimental years and periods for the calculation of the cumulative $N_2O$ emissions; *periods for the calculation of cumulative $N_2O$ emission slightly modified in contrast to Pfab et al., 2011; ** $2^{nd}$ fertilization only for the treatment with broadcast fertilizer application.*

| | planting/sowing | 1st N-fertilization (2nd N-fertilization)* | harvest | period for the calculation of cumulative $N_2O$ emissions |
|---|---|---|---|---|
| **lettuce** | 02.05.08<br>15.04.09 | 02.05.08<br>15.04.09 | 25.06.- 26.06.08.<br>29.05.09 | 02.05.-26.06.08<br>17.04.-09.06.09 |
| **cauliflower** | 05.07.08<br>10.06.09 | 15.07.08 (09.09.08)<br>10.06.09 (20.07.09) | 17.10. - 28.10.08<br>21.08.- 26.08.09 | 27.06.-30.10.08<br>10.06.-10.09.09 |
| **"winter"** | | | | |
| *2008* bare fallow | 11.11.08 | - | - | 31.10.-16.04.09 |
| *2009* green rye | 23.09.09 | - | - | 11.09.-04.05.10 |

For the investigation, a lettuce-cauliflower rotation was established in both experimental years. In the first year of measurement, the field lay fallow in winter, while in the second year of measurement green rye was sown as a catch crop. The field management was conducted according to Table 8.1 and is described in detail in Pfab et al. (2011). For irrigation practices, we followed the advices of the irrigation tool "agrowetter" provided by the German meteorological service (Deutscher Wetterdienst, 2008, Onlineservice, Offenbach, Germany).

All treatments were arranged in a fully randomized block design with four replicates. Plot size was 6 · 4.5 m. Table 8.2 shows the rates of nitrogen supplied as ammonium sulfate nitrate (ASN). Recently, we reported results from $N_2O$ flux measurements from experimental plots which received different amounts of ASN (Pfab et al., 2011). These treatments were integrated in the experimental setup. Here we use the so-called optimum treatment (ASN, conventional broadcast application, "CONV") for comparison and the unfertilized treatment as controls for our investigations on nitrification inhibition. Two treatments were tested:

(i) a treatment with addition of the nitrification inhibitor DMPP (trade name "Entec 26®"; "NI") and
(ii) an N-depot treatment ("Depot"). In the N-depot treatment, a side dressing at about 5 cm from the seedlings was conducted for lettuce in 2008.

For all other fertilizations, a furrow was drawn at a depth of 10 cm and fertilizer granules were spread evenly within one day before planting. This band had a breadth of 5 cm and was re-covered with soil afterwards. It was located directly underneath the plants for cauliflower, for lettuce the band was placed between the plant rows.

The mineral N content of the top soil (0 - 25 cm) was determined before fertilization and subtracted from the target value. Following good agricultural practice, the fertilizer application was split in two doses for the treatment "CONV". In the two treatments with nitrification inhibiting effect (NI, Depot) only a single fertilization was carried out. To ensure comparability of all treatments in terms of the amount of applied N-fertilizer, the mineral N content of the soil was not taken into account for the second fertilization measure of treatment "CONV".

**Tab. 8.2:** *N-fertilization and soil mineral N (0 - 25 cm, in brackets) before lettuce and cauliflower planting, total N-fertilization and total plant available N ("total available N") in the control ("cont"), in the treatment with broadcast fertilizer application ("CONV"), in the treatment with addition of a nitrification inhibitor ("NI") and in the treatment with placed fertilization ("Depot"); fertilization was only split for the treatment "CONV". The values were same for both experimental years.*

| treatment | lettuce [kg N ha$^{-1}$] | cauliflower [kg N ha$^{-1}$] | | total fertilization [kg N ha$^{-1}$] | total available N [kg N ha$^{-1}$] |
|---|---|---|---|---|---|
| | | 1$^{st}$ fert. | 2$^{nd}$ fert. | | |
| cont | 0 + (15) | 0 + (15) | 0 | 0 | 30 |
| CONV | 135 + (15) | 123 + (20) | 143 | 401 | 436 |
| NI | 135 + (15) | 266 + (20) | 0 | 401 | 436 |
| Depot | 135 + (15) | 266 + (20) | 0 | 401 | 436 |

### 8.3.3 Gas flux measurement and soil sampling

Fluxes of $N_2O$ were measured at least weekly in the morning using the closed chamber method. We used round PVC-chambers with an inner diameter of 30 cm and a height of 10.5 cm (Flessa et al., 1995). According to Rochette and Eriksen-Hamel (2008) the criteria for assessing the quality of $N_2O$ flux measurements can be evaluated with the help of four groups of characteristics. For this purpose, grades between "very poor" and "very good" can be reached. Our methodology "very good" in the categories "chamber design", "seal on the soil surface" and "air sample handling and storage" and "good" in the category "determination of dC/dt".

The base frames were inserted between the plants in the middle of each plot. In the depot plots, the rings partly covered the fertilizer depot according to the portion of area above the depot band to the total plot area

(7 % of the chamber area directly covered the depot).

For soil management practices, the basements were removed and reinstalled at the same location immediately afterwards. For gas sampling, glass vials (22.4 ml) were evacuated and four gas samples at intervals of 10 to 20 minutes were taken from each chamber with a cannula. Measurement intervals were varied depending on the expected $N_2O$ flux rate.
Gas samples were measured within one week using a gas chromatograph equipped with a $^{63}$Ni electron capture detector (ECD) (5890 series II, Hewlett Packard) and autosampler (HS 40, Perkin Elmer).
$N_2O$ fluxes were calculated from the slope of the linear temporal change in the concentrations of the chambers' atmosphere. Simultaneously with the determination of the trace gas fluxes, soil sampling of the plough layer (0 - 25 cm) was conducted for the determination of the mineral N content ($N_{min}$). On the plots with N-depot fertilization, samples were taken separately from the fertilizer bands and from the area outside the fertilizer bands during the cultivation period.
The bulk density was determined using stainless steel cylinders of 100 $cm^3$ in ten replicates for each crop. Climate data were by courtesy of the university's meteorological station which is located only 500 m from the study site.

### 8.3.4 Laboratory analyses

Soil moisture was analyzed gravimetrically after drying the soil at 105°C for 24 h, for the calculation of the water-filled pore space (WFPS) see Ruser et al. (1998).
Extraction for $N_{min}$ analysis was carried out using 0.5 M $K_2SO_4$ solution. Concentrations of nitrate ($NO_3^-$) and ammonium ($NH_4^+$) in the extracts were determined using a flow injection analyzer (3 QuAAtro, SEAL Analytical, UK).
For the analysis of the plants, samples were dried at 60°C, ground and analyzed for C and N (two replicates) using an elementar analyzer (vario MAX CN, Elementar Analysensysteme, Hanau, Germany).

### 8.3.5 Statistics and Calculations

$N_2O$ flux rates were calculated using the slope of the temporal change in concentration within the closed chamber (Flessa et al., 1995). Cumulative $N_2O$ emissions were calculated assuming constant flux rates between two sampling dates. For the cumulation of the different vegetation periods and the time outside the cropping season ("lettuce", "cauliflower" and "winter") the periods given in Table 8.1 were used.

For the comparison of the cumulative emissions of a crop in different years it is ideal if the cumulated periods are of similar length. For lettuce, it is possible to adapt the length to 52 days for both years. The cumulation is then carried out for the first year from planting until the last harvest and for the second year from the first sampling after planting of lettuce until the day before planting the cauliflower. For cauliflower, the cropping periods were very different in length because for the second measuring year another cauliflower variety had been chosen (to prevent delays in flowering like they were seen in the first experimental year). It is thus not possible to cumulate over equal periods: in the first year, the cumulation was done over 125 days from the end of the lettuce season until the end of the cauliflower harvest, in the second year over 93 days from planting until the end of the cauliflower harvest. It must further be noted that the first experimental year (period from the first to the second planting of lettuce) was 31 days shorter than the second experimental year (second planting of lettuce until end of measurements).
The N-balance was calculated as N-fertilizer input minus the N-removal from the field for the two crops. $N_2O$ emission factors were calculated according to the IPCC methodology for direct emissions, EF1 (IPCC, 2006).

Statistical analyses were carried out using the Statistical Software packet SigmaStat 3.5. Before calculation, all data was checked for normality. Depending on the distribution of the data, a One Way Anova or a Kruskal-Wallis One Way Anova on Ranks was performed to detect differences between the treatments concerning marketable yield (for lettuce 2008 log transformed data), C/N-ratios, N-uptake, ammonium content of the soil and cumulative emissions. For the determination of significant differences, we used a pairwise multiple comparison procedure (Student-Newman-Keuls Test, $p < 0.05$). For all normally distributed data, arithmetic means with standard deviations are shown. The C/N-ratios were not normally distributed and therefore the medians are presented.

## 8.4 Results and Discussion

**Tab. 8.3:** *Mean marketable yield (n = 4) of lettuce and cauliflower in the two experimental years ± standard deviation in the control treatment, in the treatment with conventional broadcast fertilization ("CONV"), in the treatment with addition of nitrification inhibitor ("NI") and in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot"). *Data 2008 from Pfab et al., 2011. Statistically different groups are indicated by different letters (Student-Newman-Keuls Test, p < 0.05).*

| | | control* | CONV* | NI | Depot |
|---|---|---|---|---|---|
| | | | Mg ha$^{-1}$ | | |
| lettuce | **2008** | **5** ± 3$^{a}$ | **39** ± 8$^{bc}$ | **42** ± 2$^{c}$ | **32** ± 2$^{b}$ |
| | **2009** | **0**$^{a}$ | **37** ± 4$^{b}$ | **38** ± 3$^{b}$ | **38** ± 8$^{b}$ |
| cauliflower | **2008** | **0**$^{a}$ | **30** ± 4$^{b}$ | **30** ± 3$^{b}$ | **32** ± 1$^{b}$ |
| | **2009** | **0**$^{a}$ | **27** ± 3$^{c}$ | **26** ± 3$^{c}$ | **22** ± 1$^{b}$ |

**Tab. 8.4:** *Median C/N-ratios (n = 4) of the lettuce and cauliflower residues in the two experimental years in the control treatment ("control"), in the treatment with conventional broadcast fertilization ("CONV"), in the treatment with addition of nitrification inhibitor ("NI") and in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot"). Statistically different groups are indicated by different letters (Student-Newman-Keuls Test, p < 0.05).*

| | | control | CONV | NI | Depot |
|---|---|---|---|---|---|
| lettuce | **2008** | 18.7$^{a}$ | 15.4$^{a}$ | 15.3$^{a}$ | 16.2$^{a}$ |
| | **2009** | 15.8$^{b}$ | 11.2$^{a}$ | 10.8$^{a}$ | 10.3$^{a}$ |
| cauliflower | **2008** | 18.9$^{c}$ | 10.4$^{a}$ | 11.6$^{b}$ | 10.0$^{a}$ |
| | **2009** | 16.3$^{b}$ | 8.7$^{a}$ | 9.1$^{ab}$ | 9.3$^{ab}$ |

For both years, the marketable yield ranged between 0 and 42 Mg fresh matter ha$^{-1}$ for lettuce and between 0 and 38 Mg fresh matter ha$^{-1}$ for cauliflower (Tab. 8.3). We did not find any significant difference between the fertilized treatments for cauliflower in 2008 and for lettuce in 2009, neither for total biomass (data not shown) nor for total marketable yield. As compared to the broadcast N-fertilization treatment without nitrification inhibitor, the addition of DMPP increased the yield by 3 Mg ha$^{-1}$ for lettuce in 2008, this increase was not statistically significant. Similarly, Pasda et al. (2001) reported no significant increase in lettuce yield when using ASN with DMPP. Contrary to our results, cauliflower yields were significantly increased in their study. Hähndel and Zerulla (1999) investigated the effect of DMPP on vegetable yield in several field experiments. They found at least comparable or higher yields for lettuce and cauliflower when adding DMPP to the fertilizer. Independent of the plant yields the reduction in the number of fertilizer doses decreases workload for farmers (Ebertseder and Kurpjuweit, 1999). A reduction in fertilizer applications also leads to less tractor passages and associated $CO_2$ emissions from diesel consumption and it further decreases the risk of soil compaction (Hansen et al., 1993). As shown by Ruser et al. (1998), soil compaction might strongly increase $N_2O$ fluxes from arable fields.

The N-depot fertilization caused lower yields for lettuce in 2008 (Depot: 32 Mg ha$^{-1}$ and CONV: 39 Mg ha$^{-1}$) and for cauliflower in 2009 (Depot: 22 Mg ha$^{-1}$ and CONV: 27 Mg ha$^{-1}$), this decrease was statistically significant for the latter. In contrast, Vorsatz (2000) compared vegetables fertilized with urea-ammonium-sulfate

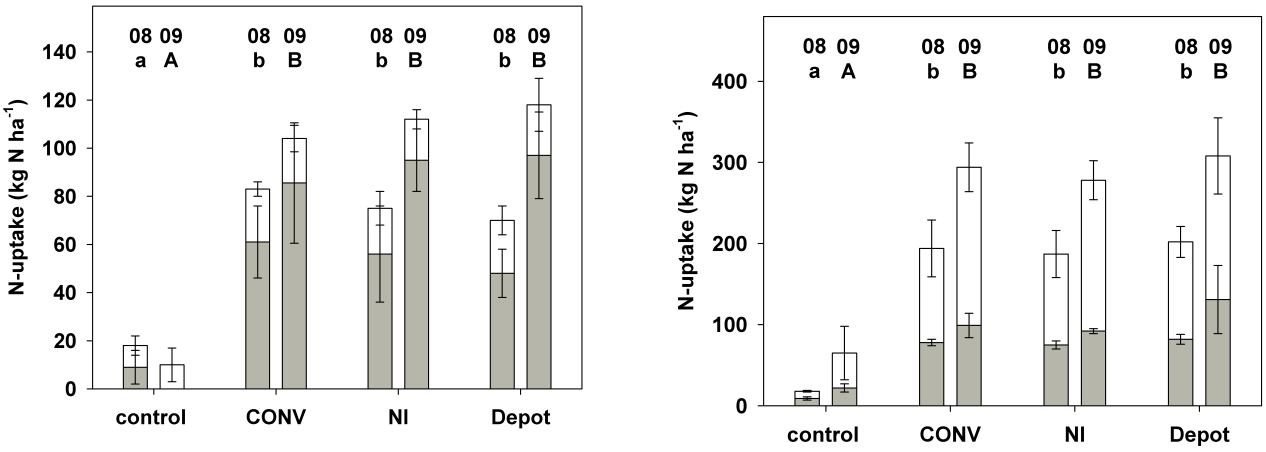


**Fig. 8.1:** *left: Mean N-content of marketable yield (grey) and residues (white) of* ***lettuce*** *in the control treatment ("control"), in the treatment with conventional broadcast fertilization ("CONV"), in the treatment with addition of nitrification inhibitor ("NI") and in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot") in 2008 and 2009 (n = 4); not marketable plants were treated as residues;*
*right: Mean N-content of marketable yield (grey) and residues (white) of* ***cauliflower*** *in the control treatment, in the treatment with conventional broadcast fertilization ("CONV"), in the treatment with addition of nitrification inhibitor ("NI") and in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot") in 2008 and 2009 (n = 4); plants of the control were not marketable. Letters indicate statistical groups of the total plant N-uptake for 2008 (minuscule) and 2009 (capital letter) (Student-Newman-Keuls Test, $p < 0.05$). Error bars indicate standard deviations. The data from the CONV treatment 2008 was taken from Pfab et al., 2011.*

solution as a depot with conventional calcium ammonium nitrate fertilization and found no differences in yields for lettuce and cauliflower.

Table 8.4 shows the median C/N-ratio of the crop residues of lettuce and cauliflower for both years. The median C/N-ratios were calculated because the data were not normal distributed. For the fertilized lettuce treatments they varied between 15.4 and 16.2 in 2008 and between 10.3 and 11.2 in 2009. The lower C/N-ratios in 2009 were the result of the high N-uptake in the second experimental year. For cauliflower, the C/N-ratios varied between 8.7 and 11.6 during the whole experimental period. They thus were in the same order of magnitude than values given by other authors (Baggs et al., 2000; Velthoff et al. 2002; Chaves et al., 2007). Apart from cauliflower in 2008, no statistically significant difference in the C/N-ratio of the residues was found between the differently fertilized treatments. The hypothesis of a higher N-uptake from treatment NI and therefore lower C/N-ratios from plant residues thus could not be proven.

The mean N-uptake of the plants is shown in Figure 8.1. In 2009, the mean total N-uptake in the fertilized treatments (lettuce: 109 kg N $ha^{-1}$; cauliflower: 292 kg N $ha^{-1}$) was higher than in 2008 (lettuce: 75 kg N $ha^{-1}$; cauliflower: 194 kg N $ha^{-1}$). We assume that the reason for the higher N-uptake in 2009 was a more N-efficient cauliflower variety and, in the case of lettuce, that both mean temperature and total precipitation were higher in 2009 (by 0.4°C and about 50 mm respectively, see also Figure 3), creating better conditions for growth and N-uptake. No statistically significant difference was found between the fertilized treatments. Similar N-uptake has been reported for lettuce (Jackson et al., 1994; Feller et al., 2001) and cauliflower (Kage et al., 2003; Akkal-Corfini et al., 2010).

### 8.4.1 Temporal $N_2O$ flux dynamics and environmental conditions

The $N_2O$ flux rates (Fig. 8.4 and 8.5) showed the typical high temporal variability as often reported for study sites in Southern Germany (Flessa et al., 1995; Ruser et al., 2001). Remarkably high flux rates of up to 812 µg $N_2O$-N $m^{-2}$ $h^{-1}$ were measured in 2008 and up to 355 µg $N_2O$-N $m^{-2}$ $h^{-1}$ in 2009. As in many other studies,

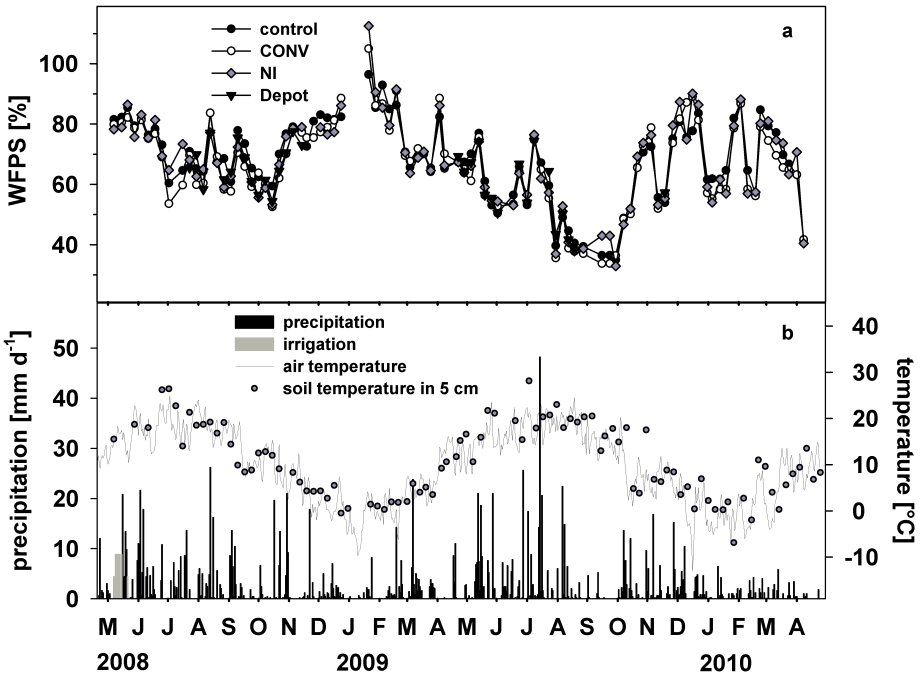


**Fig. 8.2:** *(a) Mean water-filled pore space (n = 4) in the control treatment, in the treatment with conventional broadcast fertilization ("CONV"), in the treatment with addition of nitrification inhibitor ("NI") and in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot").*
*(b) Mean air and soil temperature in 5 cm depth, daily precipitation and daily irrigation.*

these high flux rates occurred especially after N-fertilization measures (Eichner, 1990; Stehfest and Bouwman, 2006), after the re-wetting of dry soil (Davidson, 1992; Hütsch et al., 1999; Ruser et al., 2006) and after the incorporation of residues (Kaiser et al., 1998; Velthoff et al., 2002). Especially high $N_2O$ fluxes were observed after the fertilization of lettuce only few days after the incorporation of cauliflower residues in 2008. Despite high nitrate concentrations as a result of the mineralization of cauliflower residues at the beginning of the winter season in 2009, the $N_2O$ fluxes remained on a low level. As frequently reported in studies in Germany (Flessa et al., 1995; Kaiser and Ruser, 2000; Ruser et al., 2001; Sehy et al., 2003; Jungkunst et al., 2006), high flux rates occurred during freeze-thaw cycles in December and January in the first year whereas the fluxes in the second winter remained low. Generally, the flux rates were lower in 2009 than in 2008.

### Addition of nitrification inhibitor DMPP

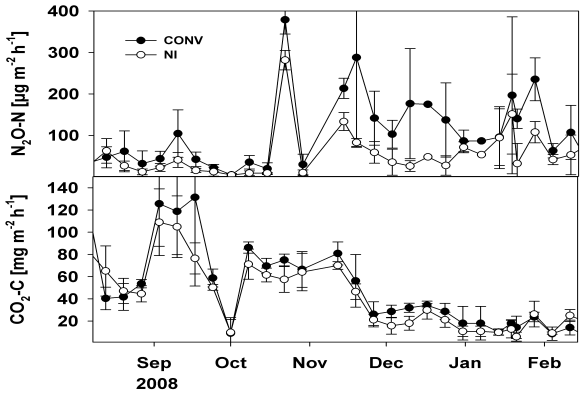


**Fig. 8.3:** *Mean $N_2O$-N and $CO_2$-C fluxes (n = 4) with standard deviations during several months when differences for $N_2O$ fluxes were detected between the treatment with conventional broadcast fertilization ("CONV") and the treatment with addition of nitrification inhibitor ("NI").*

The addition of the nitrification inhibitor DMPP reduced $N_2O$ emissions for both vegetation periods of lettuce (Fig. 8.4). In 2008, the expected effect on soil ammonium and nitrate levels could not be seen clearly. In 2009 however, the treatment NI showed higher concentrations of soil ammonium compared to the treatment with conventional fertilization. In contrast to the split N fertilizer application in the conventional treatment, the N fertilizer in the treatment with DMPP was applied in only one pass (Tab. 8.2). In the NI treatment this led to nitrate levels up to twice as high as compared to the treatment with conventional fertilization in the weeks after fertilization in both years.

As compared to the fluxes from the CONV treatment, the fluxes from the NI treatment were distinc-

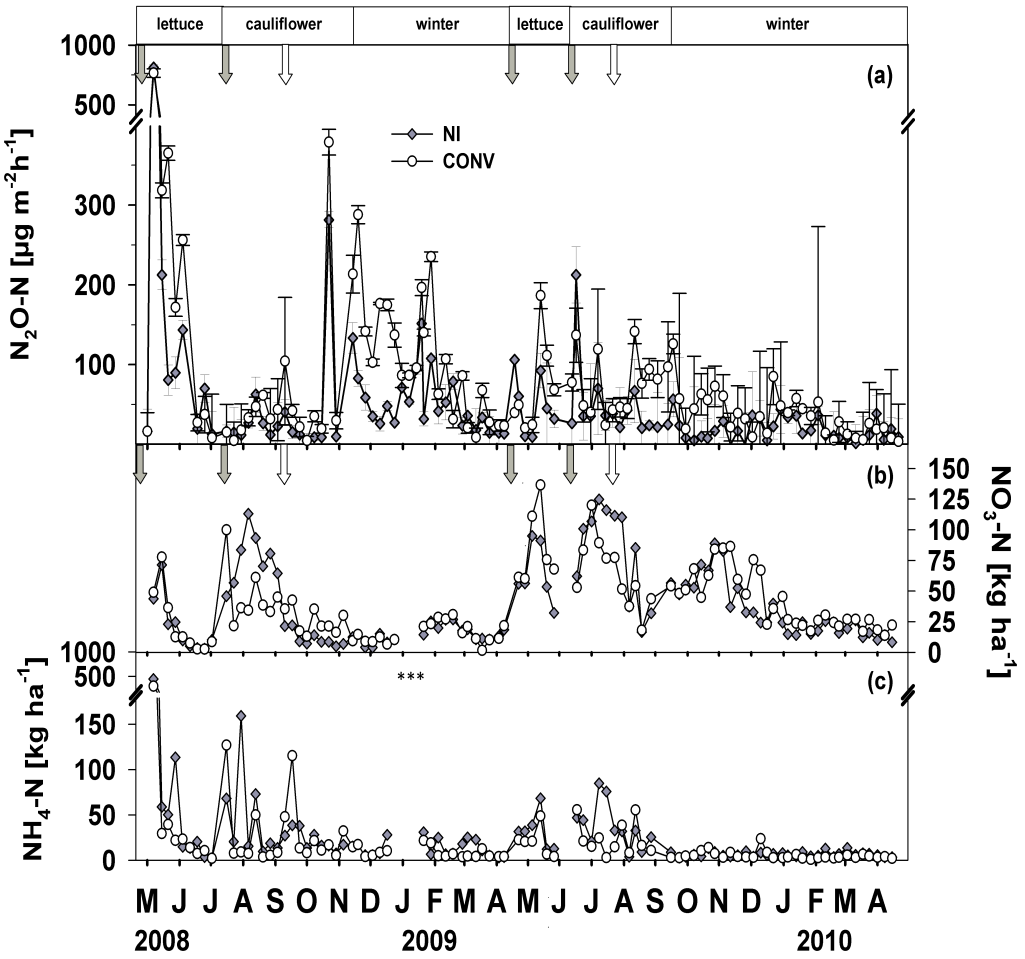


**Fig. 8.4:** *Mean $N_2O$ fluxes in the treatment with conventional broadcast fertilization ("CONV") and in the treatment with addition of nitrification inhibitor ("NI") (a, n = 4). Mean ammonium-N (b) and nitrate-N (c, each 0 - 25 cm depth) in the treatments "CONV" and "NI". Arrows show fertilization measures to all treatments (grey) and to the treatment "CONV" only (white). Stars (*) indicate that soil was frozen (no soil sampling).*

tively lower during the growing period of lettuce in both years (Mann-Whitney-U-Test; $p < 0.05$; first sampling after fertilization excluded) and during the vegetation period of cauliflower during the second year (Mann-Whitney-U-Test; $p < 0.05$). In contrast to several investigations on the effect of NIs (Linzmeier et al., 2001; Weiske et al., 2001a) we could not generally relate the lower $N_2O$ emissions which resulted from the use of nitrification inhibitor to the concentrations of ammonium and nitrate in soil. Further investigations have shown that the carbon availability is of major importance for the $N_2O$ fluxes from our site indicating a temporary carbon limitation for heterotrophic microbial activity (data not shown). This was also confirmed by a positive correlation between the $CO_2$ and $N_2O$ fluxes during periods with differences in flux rates between the treatments CONV and NI. Using a stepwise multiple linear regression, the $CO_2$ flux rates were the best parameter to explain the variability of $N_2O$ fluxes during the vegetation period of cauliflower in the two experimental years (19 and 26 % of the variability of $N_2O$ flux rates could be explained, respectively). Obviously, the NI reduced carbon mineralization which was indicated by the lower $CO_2$ fluxes in this treatment, even though this effect was only pronounced during part of the cauliflower cropping and winter season 2008/2009 (Fig. 8.3). Weiske et al. (2001b) reported decreased $CO_2$ release following the application of DMPP during the vegetation period. Although the reason for this phenomenon still remains unclear, the effect was also confirmed by a separate lab experiment (data not shown). Surprisingly, the $CO_2$ flux rates from the NI treatment were still reduced at the time when the NI should already have been degraded. As reported by Zerulla et al. (1999), the degradation of DMPP in soils is temperature dependent. Generally at 20°C it lasts around six weeks, but under cool conditions

(5°C), the inhibition has been reported to last up to 20 weeks. Nevertheless, in our study the NI application was carried out on 15 July in the first and on 12 June in the second experimental year. Mean monthly air temperatures for July and August varied between 18 and 20°C in the two investigated years and were thus favoring the degradation rate of DMPP. Therefore an inhibitory effect of DMPP after the harvest of cauliflower, 10 to 15 weeks after fertilization, cannot be expected under our climatic conditions. Surprisingly, $N_2O$ fluxes from the NI treatment at the beginning of both winter seasons were lower than in the CONV treatment (T-test, $p < 0.001$).

### Sidedressing and placed fertilization

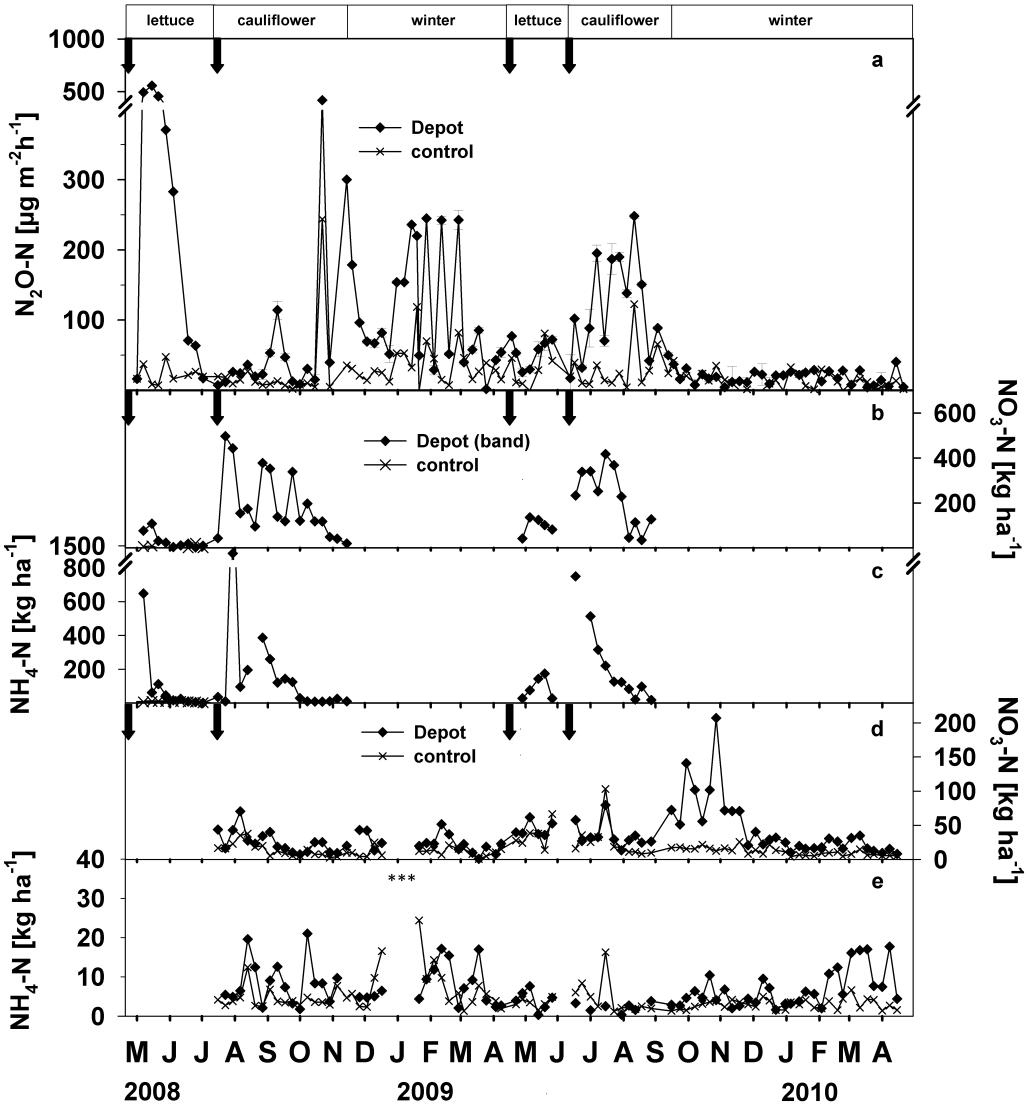


**Fig. 8.5:** *Mean $N_2O$ fluxes ($n = 4$) in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot", 4a). Mean ammonium-N (4b) and nitrate-N (4c, each 0 - 25 cm depth) in the treatment "Depot" from the area of the fertilizer band. Mean ammonium-N (4d) and nitrate-N (4e, each 0 - 25 cm depth) from the area outside the fertilizer band and outside the cropping season for the whole field ("Depot") and in the unfertilized control treatment ("control"). Arrows show fertilization measures to the treatment "Depot". Stars (*) indicate that soil was frozen (no soil sampling).*

For the depot-treatment, soil ammonium and nitrate levels were determined separately for the fertilizer bands and for the area outside the bands (Fig. 8.5). Due to the extremely high fertilizer concentrations in the depots,

we measured extraordinary high mineral N concentrations of up to 1332 kg $NH_4$-N $ha^{-1}$ and 498 kg $NO_3$-N $ha^{-1}$ in the bands. For the area between the fertilizer depots, the corresponding values were 21 kg $NH_4$-N $ha^{-1}$ and 79 kg $NO_3$-N $ha^{-1}$ during the vegetation period. The reported ammonium contents represent an integrative value over the sampled 0 - 25 cm of the top soil. Therefore it was not possible to calculate the ammonium concentration of the soil solution in the depot. However, these values of up to 1500 mg $NH_4$-N (l soil solution)$^{-1}$ were in the same order of magnitude or even higher than the threshold values for the inhibition of nitrification in soils (Wetselaar et al., 1972) or in aqueous systems (Arp and Stein, 2003; Sanchez et al., 2005; Rozich and Castens, 1986), which clearly indicates that inhibition of nitrification must have occurred after the establishment of the depots.

The ratio of ammonium-N to nitrate-N in the depot treatment as compared to the broadcast treatment gives information on the inhibition of nitrification through placed fertilization. During the six weeks after fertilization of cauliflower, for example, $NH_4$-N/$NO_3$-N ratios were 2 and 4fold higher for the depot treatment than for the broadcast treatment in 2008 and 2009, respectively. The effect was statistically significant for 2009 (T-test, $p < 0.05$). It indicated that, at least during the weeks after fertilization, ammonium was stabilized in the depot treatments due to the placed application.

The $N_2O$ fluxes from the depot-treatment showed the same temporal dynamics as the other fertilized treatments. High emissions occurred after N-fertilization, after harvest and during freeze-thaw cycles in winter (Fig. 8.5). $N_2O$ emissions were higher during the cauliflower season in the second experimental year as compared to the first year. High daily precipitation and high amounts of nitrate in the depots resulted in increased $N_2O$ emissions of up to 248 µg $N_2O$-N $m^{-2}$ $h^{-1}$ in the second experimental year during July.

Although only 14 % of the variability of the $N_2O$ fluxes could be explained by soil respiration during the vegetation periods of both experimental years, the $CO_2$ release was again the best predictor among the tested variables (Stepwise Forward Regression).

### 8.4.2 Cumulative $N_2O$ emission

The cumulative emissions for the first year of measurement were 2.3 kg (first year) and 1.8 kg (second year) $N_2O$-N $ha^{-1}yr^{-1}$ for the control and 8.8 kg (first year) and 4.7 kg (second year) $N_2O$-N $ha^{-1}$ $yr^{-1}$ for the conventionally fertilized CONV treatment (Tab. 8.5).

The high $N_2O$ fluxes during the cultivation of lettuce and after the incorporation of cauliflower residues during the first experimental year resulted in very high annual $N_2O$ emissions in the first year. They were nearly twice as high as the emissions during the second experimental year. A high inter-annual variability in $N_2O$ of the same range as in our study was often observed and mainly explained by differences in weather conditions between the years (Kaiser and Ruser, 2000; Dobbie et al., 2003; Wagner-Riddle et al., 2007). As often reported, we also observed a high proportion of winter emissions to the total annual emissions of up to 47 % for the fertilized treatments during both experimental years.

The addition of DMPP led to lower cumulative $N_2O$ emissions compared with the conventionally fertilized treatment during the season of lettuce and cauliflower; this reduction in $N_2O$ emissions was statistically significant for cauliflower in 2008 (Tab. 8.5). This decrease in $N_2O$ emisssions occurred even though the fertilizer application had not been split into two doses as for broadcast fertilization. These results are in agreement with the reductive effect of DMPP reported by Linzmeier et al. (2001). Weiske et al. (2001a) measured trace gas fluxes in three consecutive vegetation periods of summer barley, maize and winter wheat and found reductions in $N_2O$ emissions similar to our results, ranging between 41 and 53 %. These data are also in very good agreement with the report from a meta-analysis calculated by Akiyama et al. (2010).

Among 85 field studies on the effect of nitrification inhibitors on the $N_2O$ fluxes, Akiyama et al. (2010) cited 12 studies for DMPP as nitrification inhibitor. As compared to the conventional fertilization treatment, cumulative $N_2O$ emissions were reduced by a mean of 50 %. However, none of these 12 datasets covered a whole experimental year including intensive freeze-thaw cycling during the winter season. Contrary to our expectations, we also observed a reducing effect on $N_2O$ emissions during the winter season. The application of DMPP reduced winter emissions by 51 and 50 %, this reduction was statistically significant for the first experimental year.

Cumulative $N_2O$ emissions from the depot-treatment were generally higher than from the CONV treatment with broadcast fertilization. This increase in $N_2O$ emissions was significant for cauliflower in 2009 (Tab. 8.5). On an annual base, depot fertilization surprisingly increased $N_2O$ emissions by 19 % during the first experimental year. For the second year, total annual cumulative emissions did not differ significantly.

Our results are in good agreement with the investigations by Cheng et al. (2002) who did not find any effect of sidedressing on the $N_2O$ emissions during the vegetation period of Chinese cabbage as compared to the broadcast N-application. Parkin (2008) compared the emissions from soil directly above and from soil between anhydrous ammonia bands injected in 20 cm depth. He observed higher emissions above the bands. However, Engel et al. (2010) did not find any difference in the $N_2O$ emissions from a broadcast treatment and an urea injection treatment. Additionally, the depth of the fertilizer application might influence the $N_2O$ emissions. Better aeration for depots in two centimeter depths was mentioned as the reason for the lower $N_2O$ emissions as compared to N-injection in 10 cm depth (Drury et al., 2006). Liu et al. (2006) also found lower emissions from surface placement of liquid urea-ammonium nitrate than from placement in 5 cm depth. However, emissions were lower for depots in 10 cm depth as compared to 5 cm. In contrast, Khalil et al. (2009) did not observe any difference between the emissions from urea granules placed in 5 or 7.5 cm depth.

Although we observed a delay in nitrification after N-fertilization to cauliflower as indicated by the higher $NH_4$-N/$NO_3$-N ratios, we did not find a reduction in cumulative $N_2O$ release. We assume that $N_2O$ was produced in the microbially intact surrounding of the depots. High nitrate concentrations and/or elevated ammonium concentrations below the toxic level established ideal conditions for $N_2O$ production in these hot spots.

**Tab. 8.5:** *Mean cumulative $N_2O$ emissions ± standard deviation ($n = 4$) for the vegetation period of lettuce and cauliflower, during winter and annual emissions for the first and second experimental year, $N_2O$ emission factors in the control treatment ("control"), in the treatment with broadcast fertilization ("CONV"), in the treatment with addition of nitrification inhibitor ("NI") and in the treatment with sidedressing (lettuce first year) and depot fertilization (lettuce second year and cauliflower both years) ("Depot"). Statistically different groups are indicated by different letters (Student-Newman-Keuls Test, $p < 0.05$); *Calculation according to IPCC, 2006.*

| | | control | CONV | NI | Depot |
|---|---|---|---|---|---|
| **1st year of measurement** | | | | | |
| **$N_2O$ emission [kg $N_2O$-N $ha^{-1}$ $year^{-1}$]** | **lettuce** | $0.4 \pm 0.1^{a}$ | $3.2 \pm 0.7^{bc}$ | $1.7 \pm 0.6^{ab}$ | $4.4 \pm 2.4^{c}$ |
| | **cauliflower** | $0.7 \pm 0.1^{a}$ | $1.5 \pm 0.2^{b}$ | $1.0 \pm 0.1^{a}$ | $1.5 \pm 0.2^{b}$ |
| | **winter** | $1.3 \pm 0.1^{a}$ | $4.1 \pm 1.1^{b}$ | $2.0 \pm 0.2^{a}$ | $4.5 \pm 1.3^{b}$ |
| | **annual** | **$2.4 \pm 0.2^{a}$** | **$8.8 \pm 0.9^{c}$** | **$4.8 \pm 0.7^{b}$** | **$10.4 \pm 1.5^{d}$** |
| **$N_2O$ Emission factor* [% N-Input]** | | **$14 \pm 3.9^{c}$** | **$1.6 \pm 0.2^{b}$** | **$0.9 \pm 0.3^{a}$** | **$2.0 \pm 0.2^{b}$** |
| **2nd year of measurement** | | | | | |
| **$N_2O$ emission [kg $N_2O$-N $ha^{-1}$ $year^{-1}$]** | **lettuce** | $0.4 \pm 0.1^{a}$ | $0.9 \pm 0.1^{b}$ | $0.6 \pm 0.2^{ab}$ | $0.7 \pm 0.2^{ab}$ |
| | **cauliflower** | $0.6 \pm 0.3^{a}$ | $1.6 \pm 0.2^{b}$ | $1.1 \pm 0.4^{ab}$ | $2.5 \pm 0.6^{c}$ |
| | **winter** | $0.9 \pm 0.5^{a}$ | $2.2 \pm 1.7^{a}$ | $1.1 \pm 0.3^{a}$ | $1.3 \pm 0.4^{a}$ |
| | **annual** | **$1.9 \pm 0.5^{a}$** | **$4.7 \pm 1.6^{b}$** | **$2.8 \pm 0.5^{a}$** | **$4.5 \pm 0.5^{b}$** |
| **$N_2O$ Emission factor* [% N-Input]** | | **$3.1 \pm 1.8^{b}$** | **$0.8 \pm 0.2^{ab}$** | **$0.5 \pm 0.2^{a}$** | **$0.8 \pm 0.1^{ab}$** |

## 8.4.3 $N_2O$ emission factors

The emission factors calculated in accordance with the IPCC guidelines (IPCC, 2006) ranged between 0.5 and 1.9 % (Tab. 8.5). Due to the high inter-annual variability, the factors for the first experimental year were approximately twice as high as for the second year. All factors from fertilized treatments were within the range of uncertainty (0.3 - 3 %) published by the IPCC. As for the cumulative emissions, DMPP reduced the emission

factor significantly as compared to the treatment without nitrification inhibitor. To our best knowledge, our measurements represent the first annual dataset on the effect of DMPP on $N_2O$ fluxes from a fertilized arable soil in a region with intensive freeze-thaw cycles. However, the impact of a long-term use of a nitrification inhibitor on the agroecosystem still remains unclear.

## 8.5 Conclusion

Especially in vegetable production, high N-fertilizer input, high amounts of residue bound N and its mineralization result in high soil mineral N contents which can serve as a source for $N_2O$ and nitrate losses. Minimization of these losses is a prerequisite for an environmentally sound agricultural land use. For this purpose, two strategies with nitrification inhibitory effect were tested.

For the first time, the effect of DMPP on $N_2O$ emissions was tested on an annual basis; using DMPP as nitrification inhibitor resulted in a high reduction potential of 45 and 40 % for the two experimental years. A significant reduction was also observed in the winter season; the reason for that phenomenon is still unclear. The contradiction between the very high reduction potential and the lack of knowledge on the underlying processes clearly points out the demand for further research prior to large-scale application.

Placed N-fertilization with ammonium-rich N-fertilizers is also supposed to inhibit nitrification in soils. Currently, in Germany the so-called CULTAN technique is in the focus of interest, mainly to reduce nitrate losses via the leaching path. Though in our study placed fertilization did not succeed in $N_2O$ mitigation, presumably due to favorable conditions for $N_2O$ producing microorganisms in the microbial intact surrounding of the fertilizer depot.

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# 9 $N_2O$ emissions as influenced by placement and fertilizer nitrate-N

Results of the following Bachelor Theses are included in this chapter:
"Einfluss der Düngerplatzierung und Düngerform auf die $N_2O$-Emissionen im Feldgemüsebau"; Kesenheimer, K., 2010
"Einfluss von Zwischenfrucht und Umbruchtermin auf die $N_2O$-Emissionen der Folgefrucht"; Ebinger, K. 2010

## 9.1 Introduction

Although placed N-fertilization is said to reduce N losses in vegetable production systems according to Sommer (2005), no reduction in $N_2O$ emissions could be proven when using ammonium nitrate sulfate (ASN) fertilizer (Chapter 8). This fertilizer contains 7 weight percent of nitrate. Nitrate is more mobile in soils than ammonium. From the results in Chapter 8, it was concluded that no reduction in $N_2O$ emissions was observed because nitrate diffused from the N-depot into the microbially intact soil close to the fertilizer band and promoted ideal conditions for increased denitrification from this area ("hot spots"). The reason for the choice of a fertilizer with a certain nitrate content was olericultural. Ammonium in placed fertilization is supposed to be relatively immobile. If the fertilizer does not contain the more mobile nitrate and nitrification is inhibited there might not be enough plant available nitrogen. This is especially a problem at the beginning of the growth phase and has mostly importance for crops with a short vegetation period. Generally some vegetables seem to prefer certain ions to others: Santamaria et al. (1999) compared chard grown hydroponically with different $NH_4/NO_3$ ratios of the solution. Growth was inhibited by $NH_4$ nutrition and reached the highest values with the $NH_4/NO_3$ ratio 0:100. It is also well-known that, in particular, vegetables need nitrate for growth and for a healthy appearance, especially in the initial phase after planting (Haynes, 1987; Marschner, 1987). For that reason, it seemed probable that a pure ammonium fertilization could not be adapted for the cultivation of chard. It was still one of our aims to find out if the fertilizer's nitrate content was the reason for the high $N_2O$ emissions from placed fertilization.

A comparison of $N_2O$ emissions from depot fertilization using fertilizer with and without nitrate was therefore conducted. We compared the $N_2O$ fluxes and yields after the application of ammonium sulfate (AS) or of ammonium sulfate nitrate (ASN). In contrast to the nitrate-free AS, ASN contains approximately one third of the total fertilizer-N as $NO_3$-N. In order to determine the effect of placed fertilization, treatments with broadcast fertilizer application were included. It was hypothesized that placed fertilization with AS would be able to decrease $N_2O$ emissions as compared to broadcast fertilization due to an nitrification inhibitory effect. In contrast, depot fertilization with ASN was expected not to show a decrease in $N_2O$ emissions due to its initial nitrate content. Vegetable yields were assumed to be reduced for both ammonium sulfate treatments as compared to the corresponding N-fertilization treatment when using ammonium sulfate nitrate.

## 9.2 Material and Methods

The study was conducted in the chard field described in Chapter 4. The site as well as field management are described in Chapter 6 and 8. The target value for fertilization was calculated according to the German Target Value System. An $N_{min}$ analysis showed that 15 kg N $ha^{-1}$ were present in the soil in the week before fertilization. This value was subtracted from the target value for the first N-fertilization. To ensure comparability of treatments with one and two fertilizer applications, the soil $N_{min}$ was not considered for the second fertilization. The following treatments were established (Tab. 9.1):

(i) an unfertilized control

(ii) two treatments with broadcast fertilization (nitrate-containing = ASN and nitrate-free = AS) with fertilization split in two applications and
(iii) two treatments receiving the same amount of fertilizer (ammonium sulfate nitrate and ammonium sulfate as well) in one dose as a depot fertilization. For these depots, furrows were drawn at a depth of 10 cm and fertilizer granules were spread evenly within. The band had a breadth of 5 cm and was covered again with soil after the granule application. Following good agricultural practice, the bands were located betweenthe chard plant rows to avoid damage of the vegetable roots due to high fertilizer concentrations in the soil.

20 representative plants were taken from each plot at harvest and fresh weights were determined. 300 g of fresh leaves were packed into bags for C/N analyses.

**Tab. 9.1:** *Characteristics of the established treatments regarding fertilizer type, nitrate content of the fertilizer, mode of fertilizer application, amount of fertilizer-N applied by the first and second application (and residual soil $N_{min}$) as well as date of fertilization measures and total available N in the treatments; (Kesenheimer, 2010). ASN = ammonium sulfate nitrate; AS = ammonium sulfate; $N_{min}$ = soil mineral N; *$N_{min}$ not considered.*

| treatment | fertilizer | $NO_3$-content of fertilizer | fertilizer application | 1st fertilization ($N_{min}$) | 2nd fertilization* | Total available N |
|---|---|---|---|---|---|---|
| | | | | kg N $ha^{-1}$ 5 May | kg N $ha^{-1}$ 20 June | kg N $ha^{-1}$ |
| +$NO_3$ broadcast | ASN | 7% | broadcast | 105 (+15) | 30 | 150 |
| +$NO_3$ depot | ASN | 7% | depot | 135 (+15) | 0 | 150 |
| - $NO_3$ broadcast | SSA | - | broadcast | 105 (+15) | 30 | 150 |
| - $NO_3$ depot | SSA | - | depot | 135 (+15) | 0 | 150 |

Gas measurements were carried out with circular chambers as described in Chapter 6. The validity of this method was proven as described in Chapter 5, as well as the soil sampling and the laboratory analyses.

## 9.3 Results and Discussion

The use of AS did not decrease cumulative $N_2O$ emission as compared to ASN (Tab. 9.2). Cumulative $N_2O$ emissions from all fertilized treatments were relatively high ranging between 4.6 and 9.3 kg $N_2O$-N $ha^{-1}$ during the 45 days of measurement. Figure 9.1 illustrates the temporal pattern of the $N_2O$ flux rates. The two broadcast as well as the two depot treatments show a similar temporal pattern and height of the flux rates, no matter which fertilizer was used (with or without nitrate). Mean maximum emission was even higher from -$NO_3$ depots than from +$NO_3$ depots. Both depot fertilization treatments showed maximum $N_2O$ flux rates in the 5 weeks after fertilization with 1053 ± 974 and 1501 ± 795 µg $N_2O$-N $m^{-2}$ $h^{-1}$ for +$NO_3$ and -$NO_3$ respectively. Similarly, in the study described in Chapter 8, maximum flux rates had also been measured 5 weeks after fertilization of cauliflower in the treatment +$NO_3$ from the area over the bands (flux rates not shown).

As shown in Figure 5.7 (Chapter 5), the emissions quantified with our circular chamber did not statistically differ from the ones measured with the segmented chambers during that period. No differences were found between the cumulative $N_2O$ emissions of all fertilized treatments (Tab. 9.2). Contrary to our hypothesis, cumulative $N_2O$ emission from the depot treatments even tended to be higher than from the broadcast treatments.

While broadcast fertilization led to increased emission directly after fertilizer application, this peak occurred with a retard of approximately 5 weeks for depot fertilization. Table 9.2 shows the fraction of cumulative $N_2O$ emission which had been released in the first half of the study (21 days) as compared to the second half (24 days): The portion of the total $N_2O$ emission released during the second half of the measurement ranged between 71 and 83 % for the depot fertilization and between 24 and 34 % for the broadcast fertilization, indicating that the depot fertilization delays the release of $N_2O$.

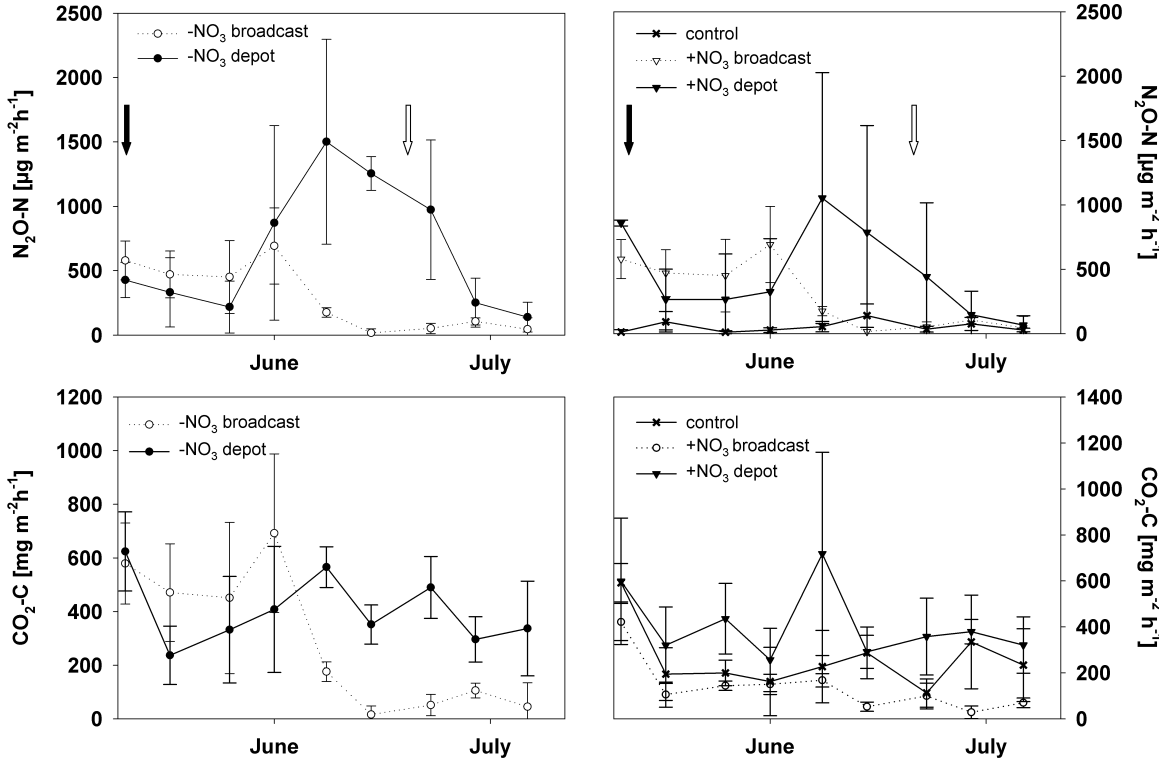


**Fig. 9.1:** *Mean $N_2O$-N and $CO_2$-C fluxes and standard deviations (n = 4) in the treatments with depot and broadcast fertilization with nitrate-free fertilizer (left, "-$NO_3$") and in the unfertilized control treatment as well as in the treatments with depot and broadcast fertilization with nitrate-containing fertilizer (right, "+$NO_3$"); (Kesenheimer, 2010).*

This is in accordance with the temporal pattern of the soil mineral N: the initially high ammonium concentrations decreased, while the nitrate level increased in all fertilized treatments. Maximum nitrate levels were reached much later for the depot fertilization treatments and obviously nitrification was inhibited during the first weeks in the depot treatments. After 3 and 5 weeks respectively, a steep increase in soil nitrate was measured in the bands, indicating that the inhibitionary effect was diminishing. It is assumed that beginning from the border areas, nitrate was produced from the ammonium in the AS treatments. It then diffused into surrounding soil areas, dissolving the depot and creating "hot spots" with ideal conditions for denitrifiers which then produced $N_2O$. This effect on $N_2O$ emissions is comparable for both fertilizers when regarding the whole vegetation period, indicating that it does not matter if the nitrate is initially provided by the ammonium fertilizer or produced by nitrification. It seems that the total nitrogen input is the decisive parameter for the cumulative $N_2O$ emission for the period covered by our measurements.

As expected, ammonium concentration in the broadcast fertilization treatments was lower than in the depot treatments. For the first sampling on May 11 (week after first fertilization) however, one would have expected a higher mineral N level in the broadcast treatment since 135 kg N $ha^{-1}$ were applied. The low value might underestimate the actual soil ammonium content due to the sampling strategy: the fertilizer was applied as granules which might not yet have been completely dissolved. If samples were taken without including the granules, an underestimation of total soil N in the first week is probable.

A stepwise multiple regression was calculated for a parameterization of the $N_2O$ flux rates including soil ammonium and nitrate, temperature, WFPS and $CO_2$ as independent variables. Only the $CO_2$ flux rates were included in the model and explained 19 % of the variability in $N_2O$ fluxes. This is a further indication that heterotrophic microorganisms i.e. denitrifiers were mainly responsible for the production of $N_2O$. A temporarily C-limitation of the system could be a further explanation why the depot fertilization (which only causes differences in soil nitrogen level) did not have the expected effect on $N_2O$ emissions.

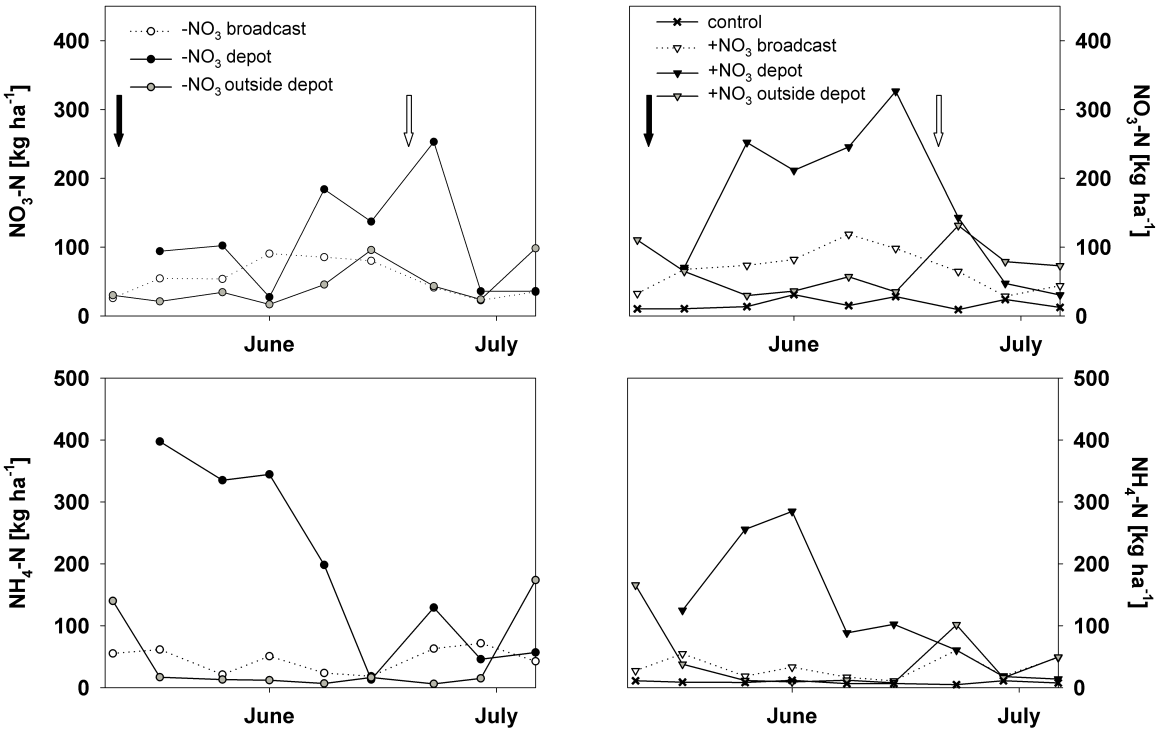


**Fig. 9.2:** *Mean soil nitrate-N and ammonium-N in the treatments with depot and broadcast fertilization with ammonium sulfate (left, "-$NO_3$") and in the unfertilized control treatment as well as in the treatments with depot and broadcast fertilization with ammonium nitrate sulfate fertilizer (right, "+$NO_3$"). Standard deviations are omitted due to clarity. Arrows indicate N-application in the broadcast treatment; (Kesenheimer, 2010).*

For an evaluation of depot fertilization, a balance must also be drawn for the vegetable yield: The portion of marketable vegetable was similar from all fertilized treatments (approximately 95 %), while no marketable yield was produced in the unfertilized control treatment. Also for the N-uptake, no difference was found between the treatments with a mean of 138 kg N $ha^{-1}$. Surprisingly, the application of AS tended to permit higher yields than ASN, although this difference was statistically not significant (Fig. 9.3). It is obvious that the initial mineral soil level of 15 kg N $ha^{-1}$, the mineralization potential of the soil and the nitrogen uptake from the depot were sufficient to meet the nitrogen demand of the chard plants.

**Tab. 9.2:** *Mean total cumulative $N_2O$-N loss ± standard deviation (11.05. - 06.07.) in the depot treatments after fertilization with ammonium nitrate sulfate ("+$NO_3$") or with ammonium sulfate ("-$NO_3$"), in the treatments with broadcast fertilization and in the unfertilized control ("control"); percentage contribution of the first (11.05. - 01.06.) and second (02.06. - 06.07.) half of the experiment of total $N_2O$-loss and total $N_2O$-loss in % of the applied fertilizer; (Kesenheimer, 2010).*

| treatment | depot | | broadcast | | control |
|---|---|---|---|---|---|
| | ANS | AS | ANS | AS | |
| **$N_2O$-N loss** (kg $N_2O$-N $ha^{-1}$ veg.period$^{-1}$) | $6.22^{b}$ ± 0.52 | $9.32^{b}$ ± 0.85 | $6.21^{b}$ ± 1.05 | $4.63^{b}$ ± 0.58 | $0.8^{a}$ ± 0.09 |
| 11.05. – 01.06. | 29% | 17% | 76% | 66% | 27% |
| 02.06. – 06.07. | 71% | 83% | 24% | 34% | 73% |
| **$N_2O$-N loss** (%- applied N-fertilizer) | 4.6 | 6.9 | 4.6 | 3.4 | - |

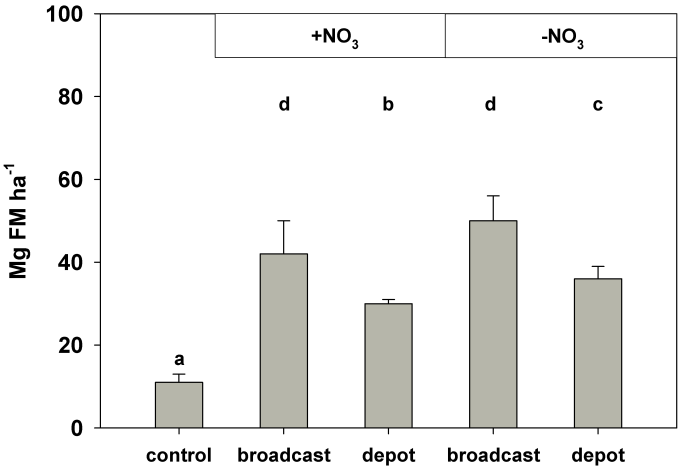


**Fig. 9.3:** *Mean marketable chard yield and standard deviation (n = 4) in the unfertilized control ("control"), in the treatments with broadcast and depot fertilization with ammonium nitrate sulfate ("+$NO_3$") and in the treatments with broadcast and depot fertilization with ammonium sulfate ("-$NO_3$"). Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, $p < 0.05$); FM = fresh matter; (Kesenheimer, 2010).*

Mean total plant yields were thus well in the expected range (Wonneberger and Keller, 2004). The influence of depot fertilization on total fresh yield was unexpected, because it decreased yields significantly for both AS and ASN. This contrasts the results reported by Vorsatz (2000) and Sommer (2005). The highest fresh matter yield (50 $Mg^{-1}$) was measured from broadcast fertilization without nitrate. However, that depot fertilization had a negative effect on plant yields is contradictory to the yield data of lettuce and cauliflower during the two preceding experimental years. No effects had been found as compared to broadcast fertilization for these vegetables. The reason for the different results could not be clarified.

## 9.4 Conclusion

Since no difference was found in total cumulative $N_2O$ emission between all fertilized treatments, depot fertilization is obviously not an adapted mitigation strategy for $N_2O$ emissions in vegetable production. In addition, when using fertilizers without nitrate, depot fertilization is not effective in the inhibition of nitrification during the whole vegetation period under field conditions. However, it inhibits nitrification and might therefore have other positive environmental effects such as decreased nitrate leaching and less tractor passages. Depot fertilization even decreased chard yields and therefore cannot be recommended for vegetable production under these climatic and soil conditions. The total nitrogen input seems decisive for $N_2O$ production during the investigated period independent of nitrate content and placement of the fertilizer.

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# 10 Assessing the contribution of fertilizer and crop residues to $N_2O$ emissions from a vegetable production system

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Authors: Pfab, H., Palmer, I., Buegger, F., Fiedler, S., Müller, T. and Ruser, R.

## 10.1 Abstract

In order to investigate $N_2O$ emissions from vegetable production, we conducted a $^{15}N$ experiment on loamy soil. The fertilizer for lettuce and cauliflower was labeled with $^{15}N$ separately, and the labeled and unlabeled crop residues were exchanged before the following crop. The $N_2O$ fluxes and the $^{15}N$ abundances in the samples were monitored over two years. The addition of $^{15}N$ labeled fertilizer revealed that $>50\,\%$ of the applied mineral N-fertilizer was found in the soil after one year, while only 13 - 15 % of the applied fertilizer N to lettuce and cauliflower were recovered in the marketable yield. This partitioning is different from recoveries from grain production with a higher portion of N in the plants and the result of a lower N use efficiency in vegetable production. Comparison of apparent recovery and $^{15}N$ recovery fraction in total plant biomass revealed that considerable amounts of nitrogen were derived from mineralization. Cumulative annual $N_2O$ emissions were 6.4 and 4.2 kg $N_2O$-N $ha^{-1}\,yr^{-1}$ for the two experimental years. Especially organic N-input by cauliflower residues was of high importance, contributing 38 % to total $N_2O$ emission. Lettuce and cauliflower fertilizer were responsible for 26 % and 20 % of the total $N_2O$ emissions. For the reduction of $N_2O$ emission, especially the management of residues rich in organic C and N is essential.

## 10.2 Introduction

Nitrous oxide ($N_2O$) is a trace gas which contributes to the greenhouse effect (IPCC, 2007) and is involved in stratospheric ozone depletion (Crutzen, 1981). More than 70 % of the anthropogenic $N_2O$ emissions are derived from agriculture (Cole et al., 1997). The Intergovernmental Panel of Climate Change (IPCC) estimates that 1 % of the N-input is lost as direct $N_2O$ emissions. Since the results on the effect of organic material on $N_2O$ emissions are inconsistent, no differentiation is made between N-input from synthetic N-fertilizers, organic N applied as fertilizer or organic N in crop residues (IPCC, 2006). This has been criticized by authors, claiming crop specific emission factors (Delgado et al., 2010). But still data on the share of residues to $N_2O$ emissions is rare. For the development of mitigation strategies for the reduction of $N_2O$ emissions from agricultural soil, a better understanding of N-cycling and the mechanisms of their production is essential. This objective can be approached by studies allowing to trace N in soil and gaseous losses.

In contrast to cereal production systems, N-cycling studies for vegetable production are scarce. Vegetable production systems cover more than $1.5 \cdot 10^6$ ha of arable land in Europe (Eurostat Database, 2007) and are associated with high mineral N levels at the time of harvest. Additionally, high amounts of up to 140 kg N $ha^{-1}$ are taken up by plant parts which are left on the field as residues (Rahn et al., 1992; Everaarts, 2000; Akkal-Corfini et al., 2010). These residues have much lower C/N-ratios as compared to straw and are often incorporated into the soil. Still it is not clear, to which extent they contribute to total $N_2O$ emissions from vegetable systems. Emissions from these residues might significantly exceed the default emission factor of 1 % which is currently used for the estimation of $N_2O$ emissions.

Several laboratory studies investigated the effect of crop residues or other organic materials on $N_2O$ emissions from agricultural soils (Aulakh et al., 1984; Flessa and Beese, 1995; Miller et al., 2008). Emission factors of up to 14 % have been found for broccoli residues in a sandy soil (Velthoff et al., 2002). A drawback of all laboratory studies is thus that it is not sure whether the results are valuable for the conditions for $N_2O$ production in the field. Furthermore, these studies usually do not include plants and cover only short-term periods.

However, similar results have been reported in several field studies. Kaiser et al.(1998) showed that $N_2O$ emissions during the winter season increased with decreasing C/N-ratio of the residues. Vinther et al. (2004) measured $N_2O$ fluxes on a Typic Haplodult after the incorporation of crop residues with C/N-ratios between 30 and 43. The resulting emission factors, calculated for a time span of 140 days varied between 1.5 and 14 %. Toma and Hatano (2007) reported lower emission factors $< 1$ % for residue derived $N_2O$ emissions from a Gray Lowland soil in Japan, but the measurement period covered only two months.
Usually incorporation of plant material with low C/N-ratio enhances $N_2O$ emissions (Flessa and Beese, 1995; Hood et al., 2000, Ruser et al., 2001; Flessa et al., 2002). For example, Baggs et al. (2000) measured $N_2O$ emission for 79 days after incorporation of lettuce residues and reported that 65 % of total emissions were released during the 14 days after incorporation. The increase in $N_2O$ production was probably due to the supply of easily available organic C (Toma and Hatano, 2007) which is used as substrates or as electron donator by heterotrophic microorganisms. Furthermore, the enhanced microbial activity during mineralization of the organic input leads to an increase in $O_2$ consumption and the creation of anaerobic microsites which are ideal for denitrifiers (Miller et al., 2008).

Unfortunately, in most field studies no controls without addition of residues were included because the influence of residues was usually not the main subject of the studies. Moreover, the share of $N_2O$ emissions derived from mineral N and organic N from residues could often not be quantified separately.

Especially the simultaneous addition of mineral N and fresh organic matter which provides considerable amounts of C is known to stimulate disproportionate high $N_2O$ emissions (Sarkodie-Addo et al., 2003; Garcia-Ruiz and Baggs, 2007). This amplification was also found for green rye in vegetable production (Pfab et al., 2011). High emission peaks were found after the incorporation of cauliflower residues and green rye manure. The increased emission after the incorporation of cauliflower residues surprisingly covered the major part of the winter season. It is generally interesting to find out if the released $N_2O$ was derived from the fertilizer, the residues or the soil pool.

For a better understanding of the fate of N in soil and for the quantification of residue derived emission,

the use of $^{15}N$ is a potent tool. Dourado-Neto et al. (2010) measured multiseason recoveries from mineral and organic N in nine tropical countries. Delgado (2004) and Collins (2007) exchanged labeled and unlabeled residues in the field and quantified the contribution of crop residues to the N uptake of the succeeding potato crops. These studies were conducted on small grain-potato and mustard-potato rotations. Many other authors used $^{15}N$ labeled fertilizer in field trials to quantify recovery of residue N (e.g. Harris et al., 1994; Akkal-Corfini et al., 2009). But $N_2O$ emissions were not quantified in these studies and mostly residues with high C/N-ratios were used so that it is not sure if the results can be transferred to vegetable production systems. To the author's best knowledge, no annual $^{15}N$ field study with measurement of fertilizer and residue derived $N_2O$ emissions has been reported from vegetable cropped soil so far. For the comparison of $N_2O$ emissions from different regions and crop rotations, it is essential to use annual datasets. Up to 89 % of total $N_2O$ emissions from agricultural soils were shown to be produced in winter (Flessa et al., 1995; Wagner-Riddle et al., 1998; Kaiser et al., 1998; Kaiser and Ruser, 2000). Especially freeze-thaw cycles lead to emission peaks in winter which contribute considerably to total $N_2O$ emissions. For these peaks, several reasons have been proposed: freezing can destroy soil aggregates, releasing considerable amounts of organic substrates for microorganisms (Christensen and Christensen, 1991). Furthermore, microorganisms killed by soil frost are a source of substrate for the remaining population (Skøgland et al., 1988, Herrmann and Witter, 2002, Müller et al., 2002). Finally the ice layer can also serve as a diffusion barrier. When the ice melts, $N_2O$ produced beneath in thin waterfilms (Teepe, 2001) or in deeper soil layers (Burton and Beauchamp, 1994) would then be released.

We recently published the results of $N_2O$ flux measurements on a loamy soil in Southern Germany (Pfab et al., 2011). The annual emissions from the vegetable field of two consecutive years were 8.8 and 4.7 kg $N_2O$-N with contributions of about 45 % of winter emissions. High and long-lasting peaks were measured after the incorporation of cauliflower residues. The aims of this simultaneously on the same study site conducted $^{15}N$ study were

(i) to trace the fate of fertilizer $^{15}N$ applied to soil in the vegetable production system by measuring the recovery in marketable yield, residues, $N_2O$ and soil;
(ii) to quantify the contribution of fertilizer and residue derived N to total $N_2O$ emissions and
(iii) to calculate emissions factors for the residue derived emissions and compare them with the IPCC default value of 1 %.

We assume that fertilizer N recovered by plant residues contributes substantially to total $N_2O$ emissions. The emission factor for N from residues in vegetable production systems might even be higher than the emission factor for mineral N input due to the oxygen depletion during mineralization of the organic material.

## 10.3 Material and Methods

**Tab. 10.1:** *Management of the vegetable field for both experimental years and periods for the calculation of the cumulative $N_2O$ emissions.*

| | planting/sowing | 1st N-fertilization (2nd) | harvest |
|---|---|---|---|
| **lettuce** | 02.05.08<br>15.04.09 | 02.05.08<br>15.04.09 | 25.06.-26.06.08.<br>29.05.09 |
| **cauliflower** | 05.07.08<br>10.06.09 | 15.07.08 (09.09.08)<br>10.06.09 (20.07.09) | 17.10. - 28.10.08<br>21.08.- 26.08.09 |
| **"winter"** | | | |
| *2008 Black fallow* | 11.11.08 | - | - |
| *2009 Secale cereale* | 23.09.09 | - | - |

The field study was established in May 2007 on Universität Hohenheim's "Heidfeldhof", which is located 13 km south of Stuttgart, Germany (48° 43' 00" N; 9° 11' 40" E). It is 410 m above sea level. The mean annual precipitation is 686 mm and the mean annual temperature 8.8°C. The soil in the experiment is a Haplic Luvisol

derived from periglacial loess, $C_{org}$ and $N_t$ content of the top soil (0 - 25 cm) was 1.8 % and 0.16 %. Texture consisted of 2 % sand, 68 % silt and 30 % clay, the initial soil pH was 5.5 and the gravel content was < 1 %.

The four replicates of each treatment were arranged in a fully randomized block design. The plot size was 6 x 4.5 m, consisting of three patches which had a broad of 1.5 m. Only the middle patch was used for the $^{15}N$ study and divided into 2 sub-treatments (Fig. 10.3). In one subplot lettuce was fertilized with conventional ammonium sulfate nitrate (ASN) granules ($2NH_4NO_3 \cdot (NH_4)_2SO_4$). In the other subplot, granulated ASN with 20 atm % $^{15}N$ in both N pools (ammonium and nitrate) was applied. These granules were compacted by the BASF company (Germany). To avoid any lateral carryover, panel sheets made of stainless steel were inserted into the soil laterally around all subplots up to a depth of 1 m. Another treatment with two sub-treatments was established accordingly for cauliflower in autumn. Detailed information on the field mangement is given in Table 10.1. In the year prior to our measurements, cultivation of lettuce was already practiced, followed by green rye (*Secale cereale* L.) sown in autumn. We started the planting and our measurement immediately after the incorporation of the green rye in 2008. Lettuce (*Lactuca sativa* var. *capitata* L., variety "Gisela") was planted with 50 heads per subplot and cauliflower (*Brassica oleracea* variety *botrytis* L., variety "Dexter") with 10 heads per subplot. In the second year of measurement, another variety of cauliflower ("Fremont") was chosen. Irrigation was carried out according to the irrigation tool "agrowetter" provided by the German meteorological service (Deutscher Wetterdienst, 2008, Onlineservice, Offenbach, Germany).

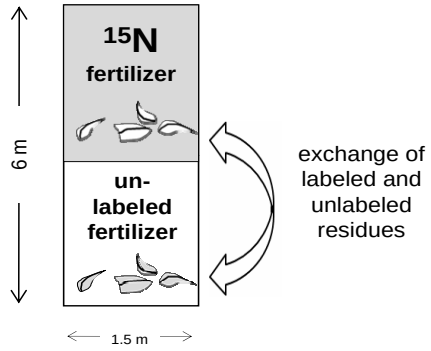


**Fig. 10.1:** *Plot design and $^{15}N$ labeling of lettuce: $^{15}N$ fertilizer (20 atm. %) was applied to one subplot (grey background). Another unlabeled subplot (white background) was used to exchange the labeled with unlabeled residues. Accordingly, subplots were established for cauliflower. All subplots were embedded in larger plots with 4.5 · 6 m.*

At each harvest, marketable heads and residues were weighted. While the marketable goods were removed, the residues were brought back onto the subplots in the first year: The labeled residues were dried, ground and were distributed on the unlabeled subplots homogeneously. Accordingly the unlabeled residues were put back onto the labeled plots. The incorporation was done by a manual rotary tiller, starting with the unlabeled plots to avoid carryover of $^{15}N$. Between the incorporation of residues from different subplots, the machine was systematically cleaned.

### 10.3.1 Fertilization

**Tab. 10.2:** *Names of the subplots and labeling of the applied fertilizer of lettuce and cauliflower and the residues of lettuce and cauliflower after their exchange (n = 4).*

| subplots | labeling of N-fertilizer | | residues after exchange | |
|---|---|---|---|---|
| | lettuce | cauliflower | lettuce | cauliflower |
| L+ | +$^{15}N$ | unlabeled | unlabeled | unlabeled |
| L- | unlabeled | unlabeled | $^{15}N$ | unlabeled |
| C+ | unlabeled | +$^{15}N$ | unlabeled | unlabeled |
| C- | unlabeled | unlabeled | unlabeled | $^{15}N$ |

All of the subplots received the same amounts of granulated ASN as broadcast fertilization. Default values were calculated with the $N_{min}$ Target Value System as proposed by Feller et al. (2001). This system recommends default values for available mineral N (see Lorenz et al., 1989). Soil mineral N was analyzed one week before fertilization and the resulting values were subtracted from the default value. For lettuce, 15 kg mineral N $ha^{-1}$

were found in the soil (0 - 25 cm) before fertilization. N-fertilization to lettuce was 135 kg N $ha^{-1}$ for all plots. For cauliflower, soil mineral N content was 20 kg N $ha^{-1}$ before fertilization and the target value was 286 kg N $ha^{-1}$; N-fertilization was split into two doses. Total fertilization was hence 401 kg N $ha^{-1}$ and total available N (fertilizer-N + soil mineral N) 436 kg N $ha^{-1}$.
Of the 16 subplots, four received $^{15}N$ enriched ASN to lettuce (L+) and another four to cauliflower (C+). For these eight subplots, a mirror set of subplots was established (L- and C-) which was managed identically but fertilized with unlabeled fertilizer. After harvest, the residues of the labeled and unlabeled plots were exchanged (Tab. 10.2, Fig. 10.3).

### 10.3.2 Gas and soil sampling

Trace gas fluxes were measured at least weekly with the closed chamber method (Hutchinson and Livingston, 1993). This sampling design was supplemented by extra measurements e.g. after rewetting of dried soil in summer or during freeze-thaw cycles in winter. We used dark circular PVC-chambers as described by Flessa et al. (1995).
Gas samples were taken periodically out of the chamber's atmosphere with evacuated glass vials with the help of a double-sided cannula through a septum. For the measurement of the $N_2O$ concentration, four gas samples were taken, one of them immediately after closure of the chamber and the others at intervals of 10 minutes. Additional samples (volume 100 ml) were taken out of the chamber immediately after closing and after the last gas sampling to determine the $^{15}N$ abundance of the $N_2O$.
The quality of our sampling procedure was evaluated according to the protocol published by Rochette and Eriksen-Hamel (2008). For the categories "chamber design", "seal on soil surface" and "air sample handling and storage", our methodology was rated as "very good" and for the category "determination of dc/dt" as "good".

$N_2O$ flux rates have been published from other plots with the same fertilization on this site in Pfab et al. (2011). A linear regression between the data from this study and the treatments of Pfab et al. (2011) had an $r^2$ of 0.74 and a slope of 0.9 which proves that the fluxes were very similar and that the use of the stainless steel boundaries had no influence on the $N_2O$ fluxes (data not shown).
Simultaneously to the gas sampling, we took soil samples (0 - 25 cm) separately from each of the four replicate plots (six randomly distributed samples). Between sampling of different treatments, all tools were cleaned thoroughly with HCl (5 %) and water. Attention was payed during the whole experiment to avoid carryover of soil between the differently labeled treatments. In order to exclude $^{15}N$ translocation over the sampling holes in deeper soil layers, the holes were filled with quartz sand after sampling.
Bulk density ($A_p$ horizon, 0 - 15 cm) was determined once during each lettuce and cauliflower vegetation period and once during each winter season using stainless steel cylinders ($V = 100\,cm^3$).

### 10.3.3 Laboratory analysis

$N_2O$ concentrations were measured with a gas chromatograph as described in Pfab et al. (2011). $^{15}N$ abundances in $N_2O$ were measured with an Isotope Ratio Mass Spectrometer (IRMS) delta plus (Finnigan MAT, Bremen, Germany) coupled with a fully automated PreCon-Interface for preparing the $N_2O$ from the air sample (Brand, 1995). The minimal detection limit for the gas chromatograph was determined by 20 repeated measurements of a calibration gas with an ambient concentration. The mean standard deviation of these series was 20 ppb and taken as the minimal detectable concentration difference. This results in an estimate of a minimal detectable flux rate of 27 µg $N_2O$-N $m^{-2}$ $h^{-1}$.
To determine the soil moisture content, samples were dried at 105°C for 24 h and analyzed gravimetrically. For the calculation of the water-filled pore space see Ruser et al. (1998).
For the soil extraction, 80 ml $K_2SO_4$ were shaken with 40 g of fresh soil for one hour and filtered. The concentrations of nitrate ($NO_3^-$) and ammonium ($NH_4^+$) in the solutions were measured using a flow injection analyzer (3 QuAAtro, SEAL Analytical, UK). The C and N content of the top soil was determined using an elemental analyzer (vario MAX CN, Elementar Analysensysteme, Hanau). For $^{15}N$ analysis of the plants, lettuce and cauliflower samples of each of the four treatments were dried at 60°C and grinded. Delta $^{15}N$ values were measured with a CN-elemental analyzer (EuroVector, HEKAtech, Wegberg, Germany) coupled with an IRMS-mass spectrometer (Delta plus Advantage, Thermo Finnigan, Bremen, Germany).

### 10.3.4 Calculations

The $^{15}N_2O$-content of the emission is a mixture from the $N_2O$ produced from the unlabeled soil and from the labeled fertilizer or residues. The share of the different sources (lettuce and cauliflower fertilizer/residues) to the total $N_2O$ emission can be calculated taking the atm % $^{15}N$ abundances of the different sources into account: The $^{15}N$ content of the fertilizer to lettuce as well as to cauliflower was 20 atm % $^{15}N$ and $^{15}N$ content of the residues was 10.3 atm % $^{15}N$ for the lettuce residues and 9.8 atm % $^{15}N$ for the cauliflower residues. For the unlabeled soil, a natural $^{15}N$ abundance of 0.368 was assumed.
The sampled $N_2O$ is a mixture of $N_2O$ from the air, not labeled $N_2O$ released from the soil pool and labeled $N_2O$ from the fertilizer or residue pool. In each of these pools for itself the masses 44, 45 and 46 are randomly distributed, but not for the mixture. Because of that, measured $d45N_2O$ values lead to calculation of wrong ratios of labeled and non labeled $N_2O$. Therefore the expected $d45N_2O$ values were calculated for different mixtures of labeled and non labeled $N_2O$. With these "wrong" values and the values assuming random distribution, a correlation can be established for a known enrichment of the fertilizer or the residue pool, respectively.
The share y of the different sources (lettuce and cauliflower fertilizer/residues) to the total $N_2O$ emission for the whole year and all other time periods was calculated by multiplication of y with the total $N_2O$ flux rate of the sampling and cumulation of the results. This cumulation was done assuming constant flux rates and y-values between two samplings.

For the analysis of cumulative $N_2O$ emission data, it is advantageous to compare periods of similar length. Therefore, the vegetation period of lettuce was defined from planting until the last harvest in the first year of measurement, whereas in the second year of measurement the days until the planting of the cauliflower were included. For cauliflower, the length of the vegetation periods in the first and second year of measurement differed very much due to the weather conditions. Consequently, the vegetation period of cauliflower used for cumulation was 32 days shorter in the second year.

The quantity of $^{15}N$ in the soil top (0 - 25 cm) was calculated by multiplication of the atm % $^{15}N$ excess in the soil and the total N content. The total N content for our soil was 6062 kg N $ha^{-1}$ for the plough layer (0 - 25 cm), 2878 kg N $ha^{-1}$ in 25 - 50 cm and 2480 kg N $ha^{-1}$ in 50 - 75 cm. The quantity of $^{15}N$ in the plant samples was calculated by multiplication of the atm % $^{15}N$ excess in the samples with the total dry matter weight of the marketable plants.

The percent recovery of labeled N was calculated by dividing the sum of additional $^{15}N$ in soil, gas and plant samples by the amount of $^{15}N$ applied in fertilizer.

## 10.4 Results and Discussion

### 10.4.1 $^{15}N$ and nitrogen uptake of lettuce and cauliflower

**Tab. 10.3:** *Mean N-uptake ( ± standard deviation) and total biomass for lettuce and cauliflower in all subplots. Values calculated from 16 replicates, except for lettuce in 2008 (four replicates only).*

| | | N-uptake marketable yield ± SD | N in residues ± SD | total biomass ± SD |
|---|---|---|---|---|
| | | kg N $ha^{-1}$ | | Mg $ha^{-1}$ |
| 2008 | lettuce | 47 ± 12 | 20 ± 5 | 40.2 ± 3.5 |
| | cauliflower | 77 ± 19 | 116 ± 28 | 60.8 ± 10.0 |
| 2009 | lettuce | 91 ± 20 | 14 ± 6 | 35.0 ± 4.0 |
| | cauliflower | 102 ± 47 | 235 ± 65 | 54.2 ± 6.5 |

Total biomass and total N-uptake of lettuce and cauliflower are shown in Table 10.3. Probably due to different varieties of cauliflower and different weather conditions in the two experimental years, N-uptake was higher in the second year than in the first. Labeled residues of lettuce (from subplot L+) which were used for exchange with unlabeled residues (from subplot L-) contained 20 kg N with 10.3 atm % $^{15}$N (± 1.5 atm %). Labeled cauliflower residues (from subplots C+) contained 101 kg N with 9.8 atm % $^{15}$N (± 2.7 atm %). The $^{15}$N label of the residues indicated that about half of the nitrogen that they took up was derived from the N-fertilizer which had 20 atm % $^{15}$N excess.

### 10.4.2 Temporal pattern of the $N_2O$ fluxes

The $N_2O$ flux rates showed high temporal variability in both experimental years (Fig. 10.2a). High flux rates were found after N-fertilization, especially in combination with high soil moisture values and with input of organic C (Fig. 10.2 a,c,d). For example this was the case in the first year of measurement when the N-fertilization was carried out only three days after the incorporation of green rye. In the second year of measurement, no such strong peak was observed for lettuce because no catch crop was grown. Although there was no such high peak during lettuce cultivation, emissions were generally higher during the vegetation period of cauliflower during the second year. In both winters, elevated $N_2O$ flux rates were found for several weeks after the incorporation of the cauliflower residues. A possible explanation for this emission pattern is that the system was temporarily C-limited (Pfab et al., 2011). $N_2O$ producing heterotrophic microorganisms use C-rich material as electron donators. Also the $O_2$ depletion caused by the turnover of the organic matter might have led to the formation of more anaerobic microsites and thus favored the $N_2O$ production (de Catanzaro and Beauchamp, 1985).

### 10.4.3 Contribution of fertilizer and residues to $N_2O$ emissions

Figure 10.2b shows the contribution of the fertilizer and residue derived N to the total $N_2O$ fluxes. After the fertilization of lettuce in the first year of measurement, between 22 and 96 % of the total $N_2O$ fluxes were derived from the fertilizer N. Although this was quite a high share, it also indicates that between 4 and 78 % of the $N_2O$-N were provided by soil internal sources. This is in agreement with Linzmeier et al. (2001) who determined that the portion of $N_2O$ emission derived from soil-N was between 40 and 60 % during the cultivation of winter wheat.

After the incorporation of the lettuce residues and the first N-fertilization to cauliflower on 15 July, the absolute $N_2O$ fluxes stayed rather low. The major source of the $N_2O$ fluxes shifted to the cauliflower fertilizer with a contribution of up to 56 %, while the lettuce fertilizer and residues contributed only up to 10 and 6 %, respectively. This minor importance of the lettuce residues for the total emissions might be due to the low absolute N-input of the residues and a mean C/N-ratio of 17, which is significantly higher than the value of 10 which we found for the cauliflower residues. As shown by several authors, the $N_2O$ emission increases with decreasing C/N-ratio (Kaiser et al., 1998; Baggs et al., 2000; Velthoff et al., 2002; Millar and Baggs, 2005; Toma and Hatano, 2007). After a decline in the share of cauliflower fertilizer to the $N_2O$ fluxes, the second N-application again induced an increase in the share of the cauliflower fertilizer to 56 %. On 7 November, cauliflower residues were incorporated into the field. During the next four month, they were responsible for an average of 55 % of the total $N_2O$ fluxes.

The mean annual cumulative $N_2O$ emission from all subplots was 6.4 ± 2.1 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ in the first year of measurement. No statistically significant differences were found between the four treatments. On that annual base, soil accounted for 15 % of the total $N_2O$ emissions in the first year of measurement. Because of the high share of emissions derived from cauliflower residues in winter and the relatively high fluxes during this time ($<$ 100 µg $N_2O$-N $m^{-2}$ $h^{-1}$ for more than seven weeks), they contributed 38 % to total $N_2O$ emission and were thus the most important source. The lettuce fertilizer was responsible for 26 % of the total $N_2O$ emission, followed by 20 % from cauliflower fertilizer and 1 % derived from the lettuce residues. These results indicate that especially N in cauliflower residues (which was provided by the fertilizer, of course) has a high potential for elevated $N_2O$ emission. The source strength of residues is due to the combination of input of organic C and N. Especially the simultaneous input of N and C can cause increased $N_2O$ emission (Aulakh et al., 1984; Sarkodie-Addo et al., 2003; Garcia-Ruiz and Baggs, 2007). Therefore, not only the total amount of N input, but also the type (mineral N, organically bound N) is of importance. Especially in a temporarily C-limited system, the contribution to $N_2O$ emissions of N bound in residues with low C/N- ratio is obviously higher than from mineral N.

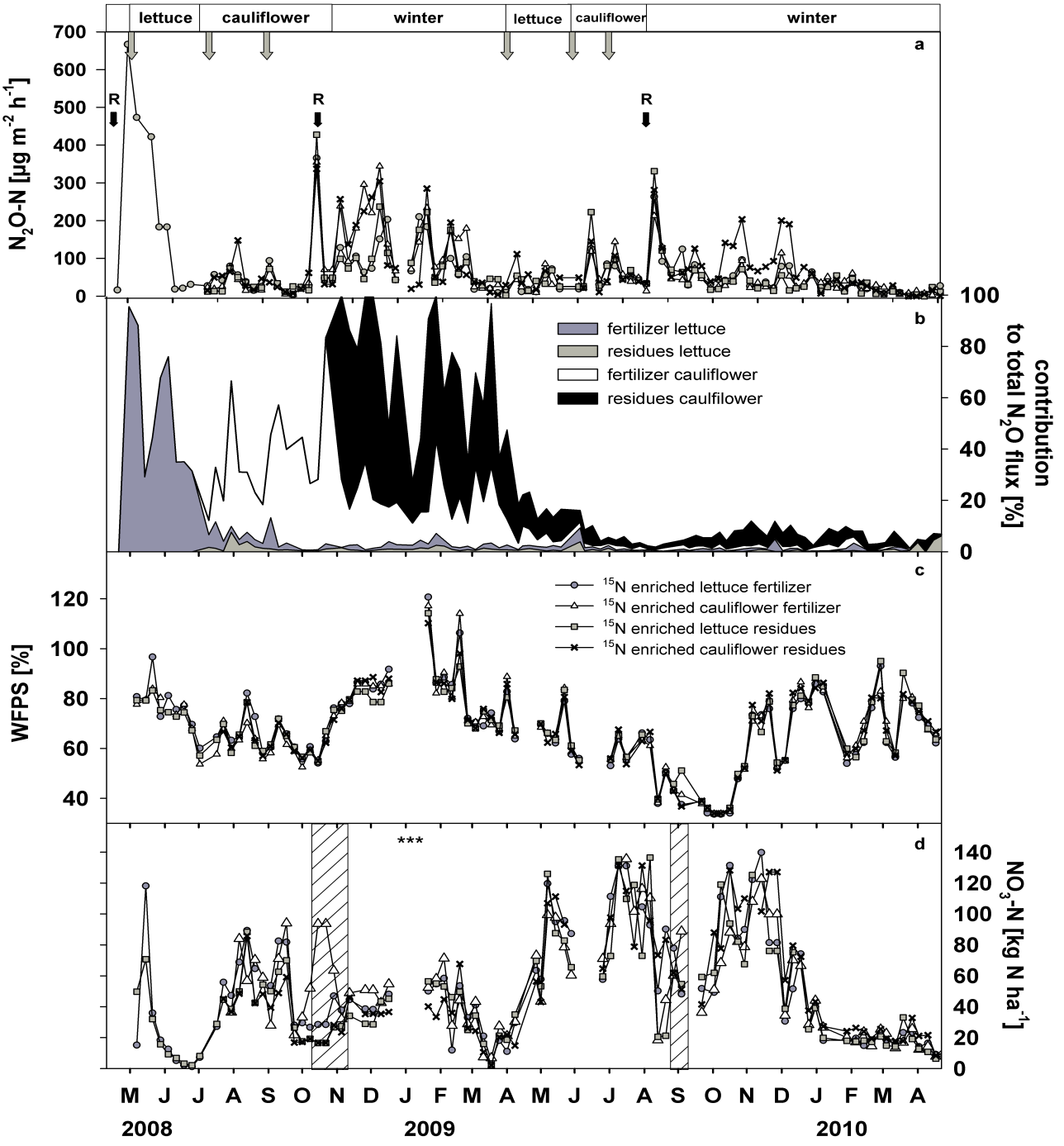


**Fig. 10.2:** *(a) Mean $N_2O$-fluxes and standard deviations ($n = 4$) for the two years of measurement for the treatments with $^{15}N$ enriched lettuce and cauliflower fertilizer and with $^{15}N$ enriched lettuce and cauliflower residues.*
*(b) Percental contribution of lettuce and cauliflower fertilizer and of lettuce and cauliflower residues to the total $N_2O$ fluxes ($n = 4$).*
*(c) Mean WFPS ($n = 4$) for the treatments with $^{15}N$ enriched lettuce and cauliflower fertilizer and with $^{15}N$ enriched lettuce and cauliflower residues.*
*(d) Mean soil nitrate-N ($n = 4$) for the treatments with $^{15}N$ enriched lettuce and cauliflower fertilizer and with $^{15}N$ enriched lettuce and cauliflower residues; shaded frames indicate the harvest periods of cauliflower.*

In the second year of measurement, a total of 4.2 ± 0.8 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ was released from all subplots. Again there was no significant difference between the treatments. The lower total $N_2O$ emission as compared to the first experimental year was assigned to different weather conditions (Chapter 8, Fig. 8.2). In 2009, precipitation was about 50 mm and mean temperature about 0.4°C higher. During the second year of measurement, 4.7 % of the first year's fertilizer N-input was released as $N_2O$-N. A total loss of 0.1 % of the N-fertilizer input of the previous year was observed during the second year.

### 10.4.4 $N_2O$ emission factors

The total N-input was 532 ± 28 and 649 ± 70 kg N $ha^{-1}$ $yr^{-1}$ for the first and second year of measurement. Related to this input, we calculated emission factors of 1.2 and 0.7 % respectively. In the Guidelines for the calculation of National Greenhouse Gas Inventories the IPCC proposes that 1 % of the total N-input is lost as direct $N_2O$ emissions from soil (emission factor EF1, IPCC, 2006). The values calculated for our study are well within the range of 0.3 to 3 % suggested by the IPCC.

Due to the $^{15}N$ labeling we were able to calculate separate emission factors for the residues, which were 0.4 % for lettuce and 2.7 % for cauliflower. So both emission factors were within the range of the IPCC. However, the 8 times higher emission factor for cauliflower clearly illustrates that the $N_2O$ emissions in the field might vary depending on the specific crop. These differences might mostly be a result of the differences in the C/N-ratios of the two crops.

### 10.4.5 Total $^{15}N$-recovery

The total recovery rates after one year (Tab. 10.4 and 10.5) were 79 % and 65 % for the fertilizer N applied to lettuce and cauliflower. In our study, the N losses were probably caused by nitrate leaching or as gaseous compounds such as $N_2$ or $NO_x$ or to a negligible part by the weekly soil removal for laboratory analysis. These recoveries are well in the range of recoveries of other field investigations (Jensen et al., 1997; Garza et al., 2009; Zhang et al., 2010) ranging between 60 and 85 %. In some studies, even higher recoveries were reported; Fredrickson et al. (1982) fertilized spring wheat with $^{15}N$ labeled ammonium sulfate. He reported recovery rates for plant and soil of even > 100 %. However, this study was conducted in a region with less precipitation. Our main pathways for N-loss (leaching and denitrification) would also have been of minor importance under drier conditions. Relatively high nitrogen losses as $N_2$ can be expected from denitrification in our irrigated soil. The $N_2/N_2O$ ratio of denitrification can vary widely depending e.g. on the aeration of the soil. $N_2$ can even be the only product of denitrification at very high soil water contents (Granli and Bøckman, 1994). Liu et al. (2007) measured that in an incubation study with different tillage simulations, almost as much nitrogen was lost as $N_2$ than as $N_2O$ at WFPS values of 60 and 75 %. Teira-Esmatges et al. (1998) reported even up to 22 times higher losses as $N_2$ than as $N_2O$ from three irrigated arable soils. Therefore, the recovery rates found in our study are not surprising.

### 10.4.6 $^{15}N$-recovery in soil

The major part of the fertilizer-N input was found in the soil (0 - 75 cm) after one year (Tab. 10.4). Several $^{15}N$ studies on cereal and corn fields found much lower values of N recovery in soil, ranging roughly between 20 and 40 % (Harris et al., 1994; Seo et al., 2006; Delgado et al., 2009). The main reason for the lower recovery in the soil as compared to our study might be the higher N-efficiency of the cereals and corn systems, resulting in a higher portion of the N-fertilizer in the plants. Contrary to cereals, vegetables are harvested at a time where they are still in the vegetative growth phase (Krug et al., 2002).

It is known that especially in vegetable production, high levels of mineral N remain in the soil after harvest (Rahn et al., 1992). Furthermore, considerable amounts of N are returned as crop residues. Between 94 and 140 kg N-uptake $ha^{-1}$ were reported for cauliflower residues (Everaarts, 2000; Akkal-Corfini et al., 2009) and up to 150 kg N $ha^{-1}$ for broccoli residues (Everaarts and Willigen, 1999). In our study, about 50 % of the N bound in crop residues was derived from the N-fertilizer. After the mineralization of this organic material, approximately 60 % of the N in the residues is released as mineral N (De Neve al., 1996). This N is assigned to the soil pool

after one year, explaining the high portion of soil N (Tab. 10.4). The $^{15}$N recovery in the soil strongly decreased between the first and the second year (Tab. 10.5). We assume that these losses were due to nitrate leaching.

### 10.4.7 N in plants

However, it is known that results from laboratory studies are not always reproducible under field conditions (Kampichler et al., 2001). It was further shown that in laboratory experiments N-mineralization can be strongly stimulated by the soil disturbance (Raison et al., 1987). The generally higher temperature and differences in nutrient contents and availability in pot experiments might also have caused differences in N-uptake.
Only a very small share of total fertilizer N, 13 and 15 % were found in the marketable parts of the cauliflower and lettuce, respectively (Tab. 10.4). This low portion is in contradiction to much higher values of between 40 and 80 % found for broccoli and lettuce in pot experiments by Holness et al. (2008). At least for lettuce, this is surprisingly high, and also for broccoli more similarity of the result would have been expected.

### 10.4.8 N use efficiency

Increasing nitrogen use efficiency (NUE) is an important objective in vegetable production. An apparent recovery fraction is usually calculated by the so-called difference method from the N-uptake by fertilized and unfertilized crops: ($N_{sample}$-$N_{control}$)/N fertilizer input · 100. With the help of $^{15}$N data, a $^{15}$N recovery fraction can also be calculated from isotope-ratio analysis and N uptake by fertilized crops (Harmsen, 2003). These two methods do not necessarily lead to the same result. Jokela et al. (1997) reported a factor 2 to 10 between the results from the difference method and the $^{15}$N data. However in their study, mineral N in the soil was adequate so that N-fertilization hardly increased yield. Consequently, the difference method resulted in very low recoveries. But still $^{15}$N fertilizer was the preferred N-source so that the recovery calculated by the $^{15}$N method was much higher.

In our study, the same tendency was observed, but less pronounced and probably for other reasons. For example for cauliflower in 2009, the apparent recovery fraction of fertilizer is 59 %, which corresponded to 34 kg $^{15}$N. The $^{15}$N recovery fraction actually measured was only 16 kg. As cited by Harmsen (2003) an increase in soil internal mineralization could release additional $^{14}$N from the soil pool and immobilization-mineralization could also provide additional $^{14}$N from soil organic N. We assume that in our soil especially the increase in N-mineralization is responsible for the lower $^{15}$N recovery fraction in plant biomass. However, this subject cannot be clarified with the help of the $^{15}$N method. Especially in tropical soils, differences were also ascribed to elevated immobilization-mineralization (MacKown and Sutton, 1997).

**Tab. 10.4:** *Recovery of $^{15}N$ from labeled fertilizer to lettuce and cauliflower in yield, $N_2O$ and soil (in kg $^{15}N\ ha^{-1}$) in the first experimental year in plots with $^{15}N$ labeled fertilizer or $^{15}N$ labeled residues; recovery of fertilizer applied to lettuce and cauliflower as well as total fertilizer recovery (in %) after one year (n = 4 for all values).*

| | unit | $^{15}$N in fertilizer | marketable yield lettuce | marketable yield cauliflower | $N_2O$-N | soil after 1 year (0-75 cm) | total |
|---|---|---|---|---|---|---|---|
| plots with $^{15}$N labeled fertilizer to lettuce | kg $^{15}$N ha$^{-1}$ | 27 | 4.0 ± 0.4 | 0.5 ± 0.1 | 0.4 ± 0.1 | 15 ± 4.1 | |
| plots with $^{15}$N labeled lettuce residues | kg $^{15}$N ha$^{-1}$ | | | 0.1 ± 0.02 | 0.01 ± 0.1 | 2.5 ± 0.5 | |
| **recovery lettuce fertilizer (%)** | **%** | **≙ 100** | **15 ± 1.5** | **1.7 ± 0.5** | **1.4 ± 0.5** | **61 ± 16** | **79 ± 17** |
| plots with $^{15}$N labeled fertilizer to cauliflower | kg $^{15}$N ha$^{-1}$ | 53 | | 7.0 ± 2.4 | 0.3 ± 0.1 | 18 ± 3.9 | |
| plots with $^{15}$N labeled cauliflower residues | kg $^{15}$N ha$^{-1}$ | | | | 0.5 ± 0.1 | 8.2 ± 1.9 | |
| **recovery cauliflower fertilizer (%)** | **%** | **≙ 100** | | **15 ± 2.6** | **1.4 ± 0.4** | **50 ± 8.4** | **66** ± 10 |
| **total recovery (%)** | **%** | | **5.1 ± 0.6** | **11 ± 2.1** | **1.4** ± 0.4 | **53 ± 3.9** | **70** ± 2.6 |

**Tab. 10.5:** *Recovery of $^{15}N$ from labeled fertilizer to lettuce and cauliflower in yield, $N_2O$ (in kg $^{15}N$ $ha^{-1}$) in the second experimental year in plots with $^{15}N$ labeled fertilizer or $^{15}N$ labeled residues; recovery of fertilizer applied to lettuce and cauliflower (in %, n = 4 for all values).*

| | | marketable yield | | $N_2O$-N |
|---|---|---|---|---|
| | unit | lettuce | cauliflower | |
| plots with $^{15}N$ labeled fertilizer to lettuce | kg $^{15}N$ $ha^{-1}$ | 0.2 ± 0.1 | 0.1 ± 0.04 | 0.01 ± 0.01 |
| plots with $^{15}N$ labeled lettuce residues | kg $^{15}N$ $ha^{-1}$ | 0.05 ± 0.0 | 0.06 ± 0.04 | 0.01 ± 0.01 |
| **recovery lettuce fertilizer (%)** | **%** | **1.0 ± 0.4** | **0.6 ± 0.2** | **0.02 ± 0.01** |
| plots with $^{15}N$ labeled fertilizer to cauliflower | kg $^{15}N$ $ha^{-1}$ | 0.4 ± 0.1 | 0.5 ± 0.2 | 0.01 ± 0.01 |
| plots with $^{15}N$ labeled cauliflower residues | kg $^{15}N$ $ha^{-1}$ | 0.6 ± 0.2 | 0.3 ± 0.06 | 0.02 ± 0.01 |
| **recovery cauliflower fertilizer (%)** | **%** | **2.0 ± 0.4** | **1.5 ± 0.4** | **0.06 ± 0.02** |
| **total recovery (%)** | **%** | **1.8 ± 0.5** | **1.4 ± 0.4** | **0.06 ± 0.1** |

## 10.5 Conclusion

The nitrogen use efficiency (NUE) was very low in the investigated vegetable production system. High levels of mineral N were found in the soil due to the high N-fertilizer input and the mineralization of N-rich residues. Although there is relatively low vertical water transport in the loamy soil, the high portion of N remaining in the soil is susceptible to leaching and gaseous N-losses.

The high relevance of cauliflower residues for the $N_2O$ emissions underlines the need for mitigation strategies such as the optimization of the catch crop management, the use of crop residues with low C/N-ratio for biogas digestion or the immobilization of N surpluses of organic material with a wide C/N-ratio (for example cereal straw).

Our measurements suggest that also during the second year there are still losses from the fertilizer N-input of the preceding year. Although clearly lower than during the first year, we also showed fertilizer induced losses during the second year, accounting for about 0.1 % of the fertilizer input.

## 10.6 Acknowledgement

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# 11 Laboratory incubation study: $N_2O$ emissions as influenced by C/N ratio and amount of residue addition

## 11.1 Introduction

As a means of reducing the emission of the trace gas nitrous oxide ($N_2O$), agricultural field management strategies are a potent and promising tool, since > 70 % of total $N_2O$ emission is derived from agriculture (Cole et al., 1997). In particular, crop residue management might play a key role in the release of $N_2O$ from soils (Chapter 7). In vegetable production systems, high amounts of residues with nitrogen contents of up to 140 kg N $ha^{-1}$ are often left on the field after harvest and are incorporated into the soil afterwards (Everaarts, 2000; Akkal-Corfini et al., 2010). High emission factors of up to 14 % (Vinther et al., 2004) have been reported for low-input crop rotations with residue incorporation of cereal straw and other green manures. In the production system described in Chapter 6, cauliflower residues contributed significantly to total $N_2O$ emission: It has been shown that up to 38 % of total annual $N_2O$ emission was derived from cauliflower residue nitrogen in the lettuce-cauliflower rotation. For the development of mitigation strategies, it is indispensable to know about the properties of the residues which favor these high emissions.
One possible mitigation strategy is the removal of crop residues during harvest instead of their incorporation into the soil. It was for example proposed to harvest and digest residues in a biogas fermenter (Möller and Stinner, 2009). Since this is very labor intensive, it must be assured that a removal of crop residues is effective in reducing $N_2O$ emissions in vegetable cropping systems at all. Möller and Stinner (2009) found a reduction of 38 % for $N_2O$ emissions in trial series including several crops like cereals and legumes, but no vegetables were included.

Incubation experiments have also tested the influence of different amounts of residue addition on $N_2O$ emissions. For example, Velthof et al. (2002) investigated one treatment with residue input according to agricultural practice and one treatment with fourfold input to simulate heterogeneous distribution of residues in the field. In their study on a sandy and a clay soil, emissions were over-proportionally higher for the high input treatment. To the best of the author's knowledge, no study, which also includes a treatment with reduced residue input, has yet investigated the effect of different amounts of cauliflower residues on $N_2O$ emissions. Especially for vegetable systems, it is important to obtain more information about possible strategies to reduce the high emissions from residues. For the development of effective reduction strategies, it is essential to know about the relationship between total residue input and $N_2O$ emissions.

A lot of attention has been paid to the C/N ratio of residues and on its effect on $N_2O$ emissions. It seems that the influence of residues on $N_2O$ emissions depends on their nitrogen content, their exposition to mineralization as well as on the nutrient status and mineralization potential of the soil (Vigil and Kissel, 1991; Kaiser et al., 1998; Velthoff et al., 2002).

It is known that after incorporation of residues with high C/N ratios (corresponding to high C contents) immobilization can have no effect or can even reduce cumulative $N_2O$ emission due to the reduction in soil mineral N levels (Vigil and Kissel, 1991; Chaves et al., 2005, 2007). However, in combination with application of mineral N fertilizer, increases in $N_2O$ emissions were seen which even exceeded the emissions of single applications of mineral or organic fertilizer (Aulakh et al., 1984; Sarkodie-Addo et al., 2003; Garcia-Ruiz and Baggs, 2007). Vigil and Kissel (1991) reported a critical value of 40 as breakpoint between immobilization and mineralization for their soil. For the input of residues with low C/N ratios, numerous authors found a negative correlation with $N_2O$ emissions. This was ascribed to the faster mineralization of the residues with lower C/N ratio. In these studies, usually residues from different plant species and from different plant families were used as representatives for different C/N ratios as they are easily available (Kaiser et al., 1998; Baggs et al., 2000;

Millar and Baggs, 2005; Toma and Hatano, 2007; Velthoff et al., 2002).

Although it is convincing that there is probably a relationship between C/N ratio and cumulative $N_2O$ emission after residue input, a different chemical composition of residues could also influence the $N_2O$ emissions. It is well understood that many secondary plant metabolites slow down degradation or inhibit microorganisms involved in the mineralization process (Perumal Samy and Gopalakrishnakone, 2008). For example, juice of *Brassica oleracea* sp. has been found to have antimicrobial effects which are ascribed to its glucosinolate-derived isothiocyanates (Brandi et al., 2006). Lignin and polyphenol contents of plant residues are also known to play a role in mineralization kinetics of residues and $N_2O$ emissions. Lignin can be degraded to polyphenols (Haynes, 1986). Polyphenolic compounds are in turn able to bind to various forms of N depending on their protein binding capacity. The resulting complexes are then less available to the attack by microbial enzymes (Palm and Sanchez, 1991). The subsequentially lower levels of mineral N during residue mineralization might then reduce $N_2O$ emission during residue degradation. A negative relationship between $N_2O$ fluxes and polyphenol content, protein binding capacity as well as (lignin+polyphenol/N)-ratio has been reported by Millar and Baggs (2004). Because of this importance of the chemical composition of the residues, it is thus essential to pay attention to a correct experimental setup and to use residues from the same plant with different C/N ratios to verify the information on their influence on $N_2O$ emissions, even if they are not as easily available as residues from different species.

In vegetable cropped soils, high N-fertilizer input and removal of organic biomass as well as few input of organic fertilizer can cause massive C-limitation for the denitrifiying microorganisms in the soil. This was also demonstrated for the soil of our study (Chapter 7, Fig. 7.2). The C content of plant residues is usually relatively constant independently of their C/N ratio (Vigil and Kissel, 1991). In systems with high soil nitrate contents, nitrogen is not supposed to be limited for microorganisms. It is probable that a treatment with residues with lower C/N ratio will not show an increase in $N_2O$ emissions because only changes in $N_{min}$ level, but not in the limiting available C content of the soil can be expected. An increase in the total input of residues with low C/N ratio like cauliflower residues will thus probably enhance emissions (if $N_{min}$ concentrations are high enough to prevent immobilization) because more of the limiting carbon is provided for soil microorganisms. Aims of this study were to

(i) test the influence of reduced ($\frac{1}{3}$) and increased (3-fold) input of cauliflower residues on $N_2O$ emissions and
(ii) to verify the reported negative relationship between C/N ratio and $N_2O$ emissions.

It was assumed that both an increase in total residue input by factor 3 as well as a decrease in C/N ratio would increase $N_2O$ emission. Due to the C-limitation of the system it is probable that a threefold total residue amount will result in a much stronger effect than incorporation of residues with lower C/N ratio.

## 11.2 Material and Methods

### 11.2.1 Experimental setup

**Tab. 11.1:** *Names and characteristics of the established treatments regarding nitrogen (N) and carbon (C) content of the residues, C/N ratio of the residues and application rate per microcosm.*

| CN of applied residues | application rate residues | name | N % | C % | C/N | application rate g (microcosm)$^{-1}$ |
|---|---|---|---|---|---|---|
| control | | control | - | | | 0 |
| low | | low CN | 4.9 | 39.8 | 8.2 | 3.6 |
| medium | low | low mass | 3.0 | 39.7 | 13.2 | 1.2 |
| medium | medium | medium mass & CN | 3.0 | 39.7 | 13.2 | 3.6 |
| medium | high | high mass | 3.0 | 39.7 | 13.2 | 10.7 |
| high | | high CN | 2.2 | 38.0 | 17.1 | 3.6 |

$N_2O$ and $CO_2$ samples were taken from soil columns over a period of 24 days. The soil columns were part of a microcosm system which allowed continuous flushing of the system with constant gas flow rates. Temperature was kept at a steady 20°C with the help of a climate control unit. Because it is known that both rewetting or dried soil as well as aggregate disruption (which could have happened during sieving) can lead to short time $N_2O$ peaks (Letey et al., 1980; Gregorich et al., 1989; Ruser et al., 2006; Bergstermann et al., 2011), a preconditioning phase preceded the measurement.
After 3 weeks, constant flux rates were measured and the following treatments were realized (see Tab. 11.1):

(i) a control treatment without addition of plant residues;
(ii) three treatments with input of ground cauliflower residues with low, medium and high C/N ratios and
(iii) three treatments with low, medium and high addition of residue input with medium C/N ratio.

The treatment with the medium addition of residues was identical with the medium C/N ratio treatment, resulting in 6 different treatments. 4 replicates were prepared for each of the treatments.

Soil was taken from the upper horizon (0 - 25 cm) of the field described in Chapter 4 - 10, air dried and sieved through a 5 mm sieve. Plexiglas cylinders (height 30 cm, inner diameter 14.4 cm) were packed up to a height of 25 cm with soil compacted to a bulk density of 1.3 g $cm^{-2}$ (corresponding to 5 kg of dry soil for each column). The microcosms were sealed airtight by a removable top lid and a bottom lid with a Cellulose Acetat membrane filter (pore size 0.2 µm, Whatman, UK). An opening with an airtight screw-on cap was placed at the top of each cylinder to permit irrigation. The microcosm system was continuously flushed with atmospheric air. Several samples of ambient air were taken from the system inlet and from the inlet of each treatment during each measurement. The concentrations of these samples were considered for the calculation of flux rates. For each treatment, the gas flux could be separately regulated with a needle valve to keep the gas concentration above the soil column in an optimal range concerning the GC sensitivity. Exhaust air left the system through an outflow tube connected to a wash bottle which avoided entrance of ambient air into the system from this side. At each sampling, flow rates were measured at the exhaust using a high precision digital flow meter (Thermo Scientific, Langenselbold, Germany).

For the gas sampling, 22.4 ml vials with crimp-cap septa (Häberle, Lonsee-Ettlenschieß, Germany) were connected to the exhaust air of the microcosm system and to the washing bottles by means of tubes, needle valves and capillary tubes. This enabled the flushing of vials with exhaust air. $N_2O$ and $CO_2$ in the gas samples was then analyzed using a gas chromatograph equipped with a $^{63}Ni$ electron capture detector (ECD) (5890 series II, Hewlett Packard) and autosampler (HS 40, Perkin Elmer).

Fresh cauliflower leaves were dried, ground and C/N ratios were determined using an elemental analyzer (vario MAX CN, Elementar Analysensysteme, Hanau). The amount "medium input" corresponds to a fresh weight of 6.67 Mg fresh weight (75 kg N $ha^{-1}$ and 800 kg C $ha^{-1}$ dry matter) which was calculated to be the mean input of cauliflower on the vegetable field from which the soil was taken. The mean C/N ratio of the cauliflower residues used for the study was 13.2. Due to different fertilization levels in this field study, cauliflower residues with lower (8.2) and higher (17.1) C/N ratios were also available. Amounts, C/N ratios and abbreviations of the treatments are shown in Table 11.1. Residues were carefully mixed into the upper 5 cm of the soil columns. The upper layers of the control soil columns were treated in the same manner but without addition of residues.

Water-filled pore space (WFPS) was adapted to a value of 75 % with $10^{-2}$ M $CaCl_2$ solution at the beginning of the study and kept at this level by periodically weighing and readjusting the WFPS value gravimetrically. Therefore, the lid was opened and a precise irrigation was carried out with the help of a jet-nozzle which fitted into the opening at the top of the cylinders. After 14 days, WFPS was increased to 85 % and after another 2 days to 90 %. The study was completed after a total of 24 days. Soil moisture was analyzed gravimetrically after drying the soil at 105°C for 24 h. Samples from all microcosms were taken at the beginning, when increasing the WFPS and at the end of the study. The water-filled pore space (WFPS) was calculated as described by Ruser et al. (1998).

For the quantification of mineral N contents, 20 g of fresh soil were extracted with 40 ml of a 0.5 M $K_2SO_4$ solution. At the beginning of the study, only concentrations of soil nitrate were measured using a quick-check (RQ easy Nitrate test, Merck, Darmstadt). At the end of the study, the microcosms were opened and samples were taken of the upper layer (0 - 5 ) and the lower layer (5 - 25 ). Concentrations of nitrate ($NO_3^-$)

and ammonium ($NH_4^+$) in the extracts were determined using a flow injection analyzer (3 QuAAtro, SEAL Analytical, UK). Furthermore, concentrations of total dissolved organic carbon (DOC) were measured according to method introduced by Ruser et al. (2008) with a fully automated "multi N/C 2100S" analyzer (Analytic Jena, Jena, Germany).

### 11.2.2 Calculation and statistics

Due to technical problems, 3 of the 24 microcosms could not be sealed perfectly and showed only ambient gas concentrations during the whole experiment (one from treatment "low CN" and two from treatment "medium mass and CN"). They were therefore excluded for the calculation of the mean gas fluxes. Since the soil nitrate contents should not be influenced by the air tightness (WFPS was periodically adjusted), they were included for these calculations.

$N_2O$-N and $CO_2$-C fluxes per microcosm were calculated using gas flow rates, gas concentrations of the samples and gas concentrations of the blank samples from the atmospheric air using the following formular:

$$N_2O \text{ flux rate } (N_2O\text{-N } m^{-2}h^{-1}) = \frac{((c_s - c_c) \cdot k_f \cdot \frac{273.15}{T} \cdot k_A)}{22.4} \quad (11.1)$$

$c_s$: concentration in the sample (ppb)
$c_c$: concentration in the blank sample from atmospheric air (ppb)
$k_f$: air flow in the microcosm system in l $h^{-1}$
T: temperature in microcosm system
$k_A$: 61.4 (factor for extrapolation to 1 $m^{-2}$ )

Cumulative $N_2O$ emission was calculated assuming constant flux rates between two sampling dates. Statistical analyses were carried out using the Statistical Software packet SigmaStat 3.5. Depending on the distribution of the data, means (gas flux rates, nitrate concentrations, total C and N concentrations, C/N ratios) or medians (cumulative gas emission) are presented. Differences between the treatments were detected using a One Way Anova or a Kruskal-Wallis One Way Anova on Ranks. Significant differences were determined using a pairwise multiple comparison procedure.

## 11.3 Results and Discussion

### 11.3.1 Temporal pattern of $N_2O$ flux rates

$N_2O$ flux rates generally increased markedly after the addition of the crop residues except for the unamended control (Fig. 11.1). When comparing the flux rates of the control with the flux rates from the treatments with residue input, this difference was statistically significant (T-test, $p < 0.05$). The effect of increased $N_2O$ emissions after the addition of crop residues has often been observed (Flessa and Beese, 1995; Millar and Baggs, 2004). Both the substrate input serving as electron donator and the creation of anaerobic microsites during the decomposition of the organic material improve the conditions for denitrification (Beauchamps et al., 1989; Miller et al., 2008).

Both the initial input of crop residues as well as the later increases in WFPS led to clear peaks in $N_2O$ flux in treatments with residue addition. The increases in flux rates due to an increase in WFPS is in accordance with several studies where the highest flux rates from microcosms occurred in the treatments with the highest soil moisture content (Dobbie and Smith, 2001; Ruser et al., 2006; Well et al., 2006).

The high flux rates at the end of the incubation were probably due to a period of very high temperatures outside the chamber which could not be compensated for by the climate control unit and resulted in a temperature of about 24°C. Since many processes in soil are temperature dependent, an increase in microbial activity and mineralization might have provided additional substrate for the microorganisms.

#### Amount of residue addition

Flux rates after addition of different amounts of residue input varied between 0 and 9683 µg $N_2O$-N $m^{-2}$ $h^{-1}$ (the latter measured in the treatment with highest amount of residue addition). The highest peaks were seen at

the beginning of the experiment, followed by another two peaks after each increase in WFPS. When comparing all single measured flux rates, reduction of residue input to a third of the medium input led to significantly lower fluxes. Addition of the threefold amount of residues resulted in immense initial peaks, but they declined rapidly and fluxes decreased under the level of the medium treatment after two days.

#### C/N ratio of added residues

Fluxes from the treatments with addition of residues with different C/N ratio were also hugely variable and ranged between 0 and 8687 µg $N_2O$-N $m^{-2}$ $h^{-1}$ (the latter measured in the treatment with addition of residues with low C/N ratio). The temporal pattern of $N_2O$ flux was similar to as described above with the maximum $N_2O$ peak at the beginning of the measurements and two more peaks after each increase of the WFPS. At the beginning, fluxes were most elevated from the treatment with addition of residues with lowest C/N ratio. When comparing all measured flux rates of all four treatments, the high C/N ratio of the residues led to a significant decrease in $N_2O$ flux as compared to the other two treatments with residues with low and medium C/N ratio.

### 11.3.2 Temporal pattern of $CO_2$ flux rates

Also for $CO_2$, high temporal variation with CVs of up to 395 % was found (Fig. 11.1). Flux rates ranged from 2 to 1472 mg $CO_2$-C $m^{-2}$ $h^{-1}$ for the control and from 0 to 5474 mg $CO_2$-C $m^{-2}$ $h^{-1}$ for the treatments with

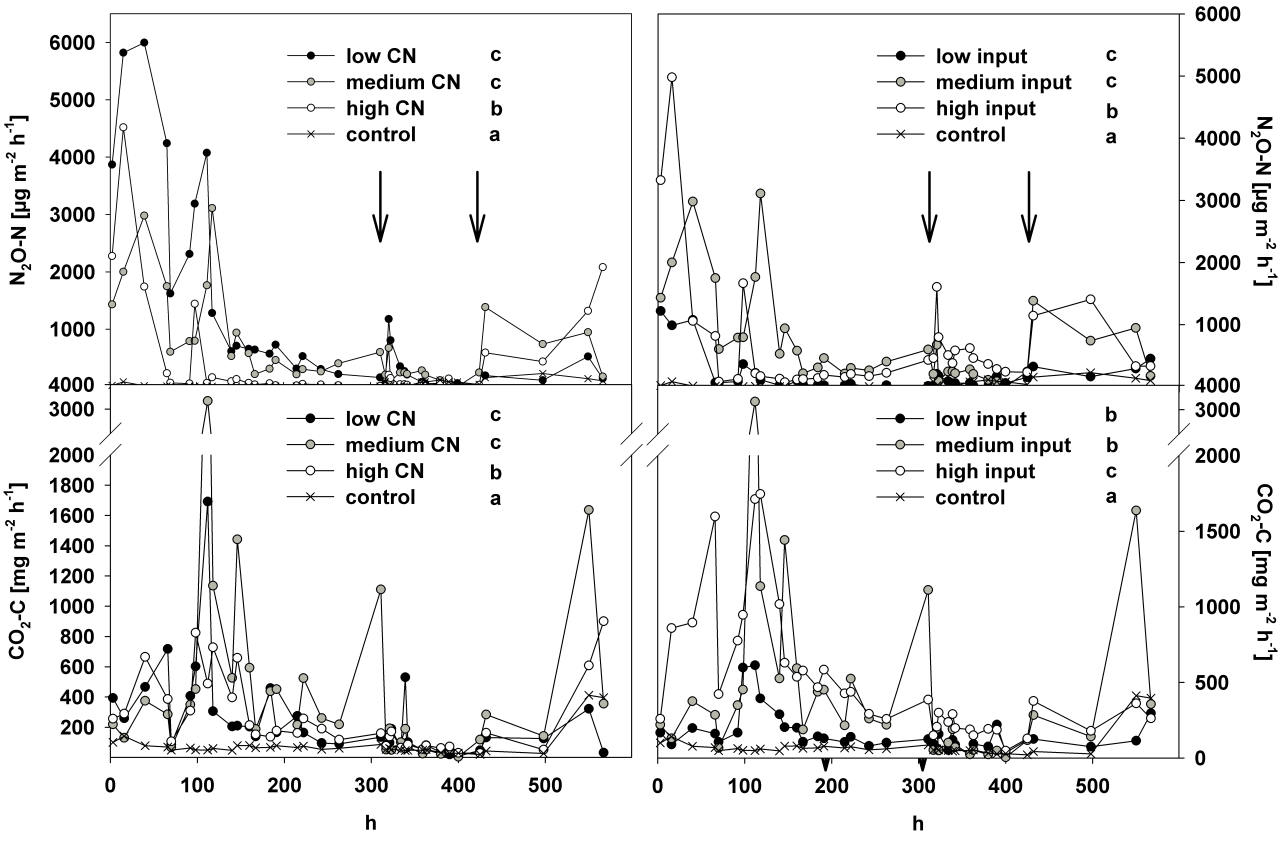


**Fig. 11.1:** *Mean $N_2O$-N and $CO_2$-C fluxes (n = 4) measured in a microcosm system during the 24 days after addition of cauliflower residues in the control treatment (shown twice), in the treatments with low, medium and high C/N ratio of residues and in the treatments with low, medium and high input mass of residues. The medium treatment is shown in both contexts. Different superscript letters indicate statistically significant differences between the flux rates of the treatments (Student-Newman-Keuls Test, p < 0.05). Standard deviations are omitted due to clarity. Arrows indicate increases in WFPS.*

residue addition. The addition of residues increased $CO_2$ fluxes significantly as compared to the control when all single measured flux rates are taken into account (Mann-Whitney-U Test, $p < 0.05$). The flux rates were quite high, but still of about the same order of magnitude as flux rates reported by other authors. Vinther et al. (2004) observed flux rates in the field from bare soil between about 200 and almost 900 µg $m^{-2}$ $h^{-1}$ from crop rotations with winter wheat or pea/barley and C-input of about 2000 kg C $ha^{-1}$. Toma and Hatano (2007) incorporated residues with C/N ratios between 12 and 110 and measured $CO_2$ flux rates of up to about 500 mg C $m^{-2}$ $h^{-1}$ with similar C-application rates as in our study.

The temporal pattern of the $CO_2$ flux rates was different from the $N_2O$ flux rates: after the addition of the residues the highest peak in $CO_2$ flux did not start immediately, but after a delay of about 100 hours. $CO_2$ is not only produced during denitrification, but is an end product of various dissimilatory processes and generally an indicator of high microbial activity. One explanation for the lag phase for increased $CO_2$ fluxes is that mineralization of the organic material and the following increased respiration activity needs a certain time to establish (e.g. for multiplication of microorganisms and synthesis of their enzymes). Statistical comparison of all flux rates resulted in very similar results than for $N_2O$.

### 11.3.3 Soil nitrate

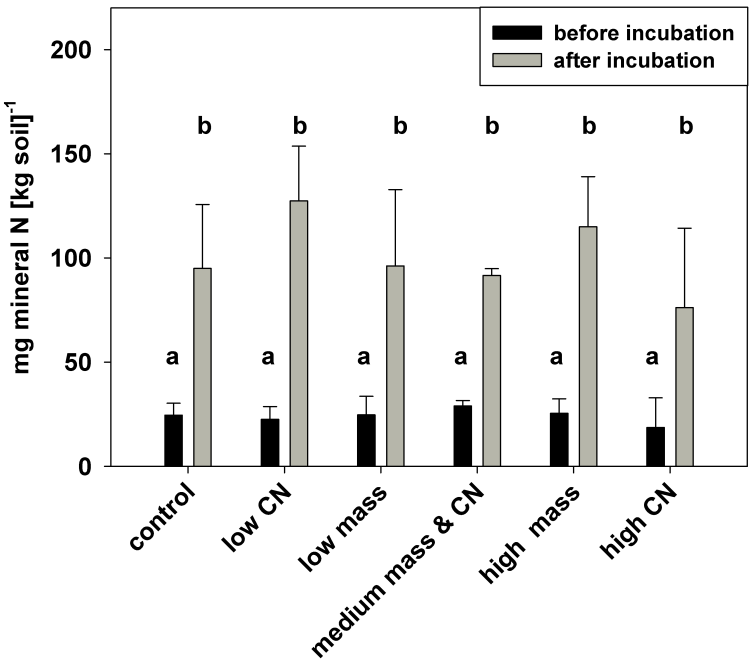


**Fig. 11.2:** *Mean $N_{min}$-content ($n = 4$) before and after the incubation in the different treatments (explanation of the names see Table 11.1). Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, $p < 0.05$).*

No differences in nitrate concentrations were measured between all treatments before start of the incubation, with mean nitrate concentrations of 24 ± 8 mg $NO_3$-N ($kg^{-1}$ soil). Ammonium analysis was not carried out before the incubation, but analysis of mineral N during the field study allowed an estimation of approx. 0.5 mg $NH_4$-N ($kg^{-1}$ soil). These values are similar to the values of mineral N reported in other incubation studies (e.g. Aulakh et al., 2001). The mineral N content of the soil columns after the incubation (Fig. 11.2) was significantly higher than before the incubation. With a mean of 103 ± 31 mg N (kg $^{-1}$ soil) it was higher than the values reported for example by Aulakh (2001) and Sarkodie-Addo (2003). One reason for this relatively high content which was also found in the control treatment was that we used a static approach without percolation and that nitrogen from mineralization accumulated in the columns as nitrate. Flessa and Beese (1995) reported concentrations of up to 25 mg $NO_3$-N (l leachate)$^{-1}$, showing that a considerable amount of nitrate can leave the system by this path if the experimental setup allows for leaching. It would have been probably better to install a possibility for percolation to eliminate the danger of masking by nitrate accumulation the effect of the residue input. Still the mineral N values measured in our study were lower than the up to 200 mg N (kg $^{-1}$

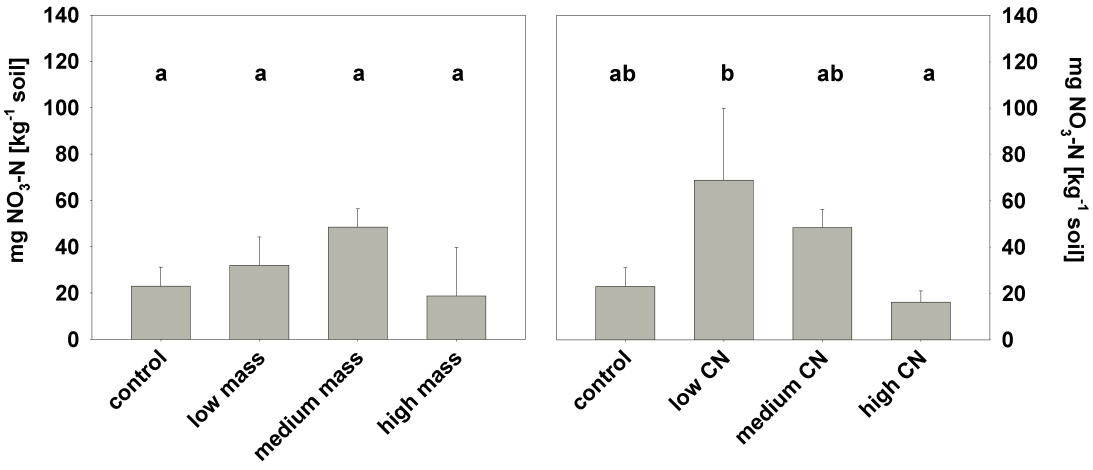


**Fig. 11.3:** *left: Mean nitrate-N content per kg soil (n = 4) in the control and in the treatment with low, medium and high* ***amount of residue input****. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, p < 0.05).*
*right: Mean nitrate-N content per kg soil (n = 4) in the control and in the treatment with low, medium and high* ***C/N ratio of applied residues****. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, p < 0.05).*

soil) reported by Velthoff et al. (2002).

It has been shown that for the emission of $N_2O$, the upper centimeters of a soil can be the major source (data not shown). Significant differences after the incubation were found for the nitrate-N in the upper soil layer (0 - 5 cm). A higher soil nitrate level was measured for the treatment with residue input with low C/N ratio as compared to residues with high C/N ratio (Fig. 11.3). Similar results have been reported in other studies. For example, Garcia-Ruiz and Baggs (2007) found a correlation between net mineralization and total N content of the residues (which increases with decreasing C/N ratio). That no significant change in cumulative $N_2O$ emission was observed due to the C/N ratios indicates that the soil nitrate content was obviously not a limiting factor for the $N_2O$ production. No significant difference in soil nitrate was found for the different amounts of residue input (Fig. 11.3).

### 11.3.4 Dissolved organic carbon

Dissolved organic carbon concentrations were not different neither between the treatments nor between the upper and lower layer after the incubation. Mean concentrations of DOC were 64 ± 15 µg C g soil$^{-1}$ for 0 - 5 cm and 62 ± 15 µg C g soil$^{-1}$ for 5 - 25 cm. The mean total C content (0 - 25 cm) of all treatments with residue input corresponded to 410 kg C ha$^{-1}$. No analysis had been done before the incubation. It is probable that there was no difference in C-contents before incubation between upper and lower layer (since the sieved soil was mixed well), but still an assumption. No tendency to vary, because of the specific amounts of residue input, was obvious after the incubation.

### 11.3.5 Cumulative $N_2O$ emission

For the evaluation of the effect of residue mass and C/N ratio on $N_2O$ emissions, the cumulative $N_2O$ emission is more relevant than the comparison of the single flux rates. The median cumulative $N_2O$ emission (data was not normally distributed) is shown in Figure 11.4. For both $N_2O$ and $CO_2$ the control showed a tendency towards lower emissions as compared to the treatment with residue input, which was significant for some of the treatments.

#### Amount of residue input

Reduction of the residue input by $\frac{2}{3}$ resulted in a significant reduction in cumulative $N_2O$ emissions. Since no difference was found concerning the nitrate level, the availability of N can be excluded as a reason. But probably the lower C-input in the treatment with less residue input was the reason for the observed differences. This is in good accordance with the results described in Chapter 6 and Chapter 7, where a temporal C-limitation of the soil was reported. The observation of reduced $N_2O$ emissions for the treatment with reduced residue input shows that the removal of residues after vegetable harvest could be an interesting mitigation option.

#### C/N ratio of the residues

No correlation between the C/N ratio of the residues and the cumulative $N_2O$ emission was found. Maybe one reason for that is also the comparable C-input of the residues which stimulated activity of soil microorganisms comparably and independently of the N content of the input. For the $N_2O$ emissions, only a tendency to lower emissions from plots with higher C/N ratio was observed, however this was statistically not significant. Besides many studies which found a significant difference using plant material from different plant species, there is one study in literature which compared emissions after the incorporation of three agroforestry residues with high and low (water soluble) C/N ratio respectively (Millar and Baggs, 2005). Although differences in chemical composition can be excluded, they found higher $N_2O$ emissions from residues with lower C/N ratio, but only for the 8 days after incorporation. Contrary, no differences were found for the 24 days of this study. Obviously the faster decomposition of residues with low C/N ratio and the resulting higher N availability (Vigil and Kissel, 1991; Eiland et al., 2001) increases $N_2O$ emissions if nitrogen is limiting for microorganisms. In our lab experiment, C is considered as the limiting factor (Chapter 7), while nitrate was abundant in the soil (Fig. 11.3). Therefore $N_2O$ emissions were not influenced by the higher C/N ratio of the residues which is equivalent to a higher N content (since the C content of the treatments was about the same, Tab. 11.1, Vigil and Kissel, 1991). Nitrate levels were extremely high in the microcosms due to high soil internal mineralization. No nitrogen was removed by percolate water, this may have ruled out any effect of C/N ratio on $N_2O$ emissions that has been reported from microcosms from other soils (Velthoff et al., 2002; Toma and Hatano, 2007). But since only 2 data-points were available for medium C/N ratio and the study was carried out in the laboratory, a verification of the result with more replicates and under field conditions is necessary.

### 11.3.6 Cumulative $CO_2$-C emission

In the control treatment without input of residue, 40 g $CO_2$-C $m^{-2}$ were released during the incubation. The absolute cumulative $CO_2$-C rates from the treatments with residue input ranged between 66 and 401 g $CO_2$-C $m^{-2}$ during the 24 days of the incubation with a mean of 160 g $CO_2$-C $m^{-2}$. This is in accordance with measurements on the field from which the soil was taken, where up to 2500 kg C $ha^{-1}$ were released during a year with increases in $CO_2$ release e.g. after the incorporation of green rye. However, the temperature dependence of $CO_2$ as well as the $CO_2$ release from dark respiration (for example from the catch crop green rye) during the measurement with the dark closed chambers also can cause substantial differences to the measurement in the laboratory. An increase in $CO_2$ fluxes is sometimes also observed due to physical disturbance as it is the case for tillage, but also for soil sieving. Drying and wetting of the soil seems to be a major cause for this effect (Denef et al., 2001). In our study however, this effect was excluded: a waiting time until the fluxes reached background level ensured that the peak after rewetting did not falsify the results. The cumulative $CO_2$-C emission from the control treatment was relatively low as compared to the other treatments, indicating that the soil sieving did not cause exorbitant $CO_2$ emissions. The absolute cumulative $CO_2$-C rates are somewhat higher, but still in the range of the values reported by other studies. For example, Flessa and Beese (1995) measured more than 120 g $CO_2$-C $m^{-2}$ during an experiment of 45 days after incorporation of sugarbeet leaves with an input rate of 3245 kg C $ha^{-1}$. Millar and Baggs (2004) reported flux rates between 25 and 48 g $CO_2$-C $m^{-2}$ during 29 days after incorporation of agroforestry residues. Also Millar et al. (2008) gave comparable numbers of cumulative $CO_2$-C emission. They were expressed in mg C per kg soil and ranged between 61 and 307 after addition of up to 500 mg glucose-C and up to 50 mg nitrate-N per kg soil (corresponding about to our low input treatment). Our mean C-loss expressed in this unit was 543 mg C per kg soil and is thus of the same order of magnitude.

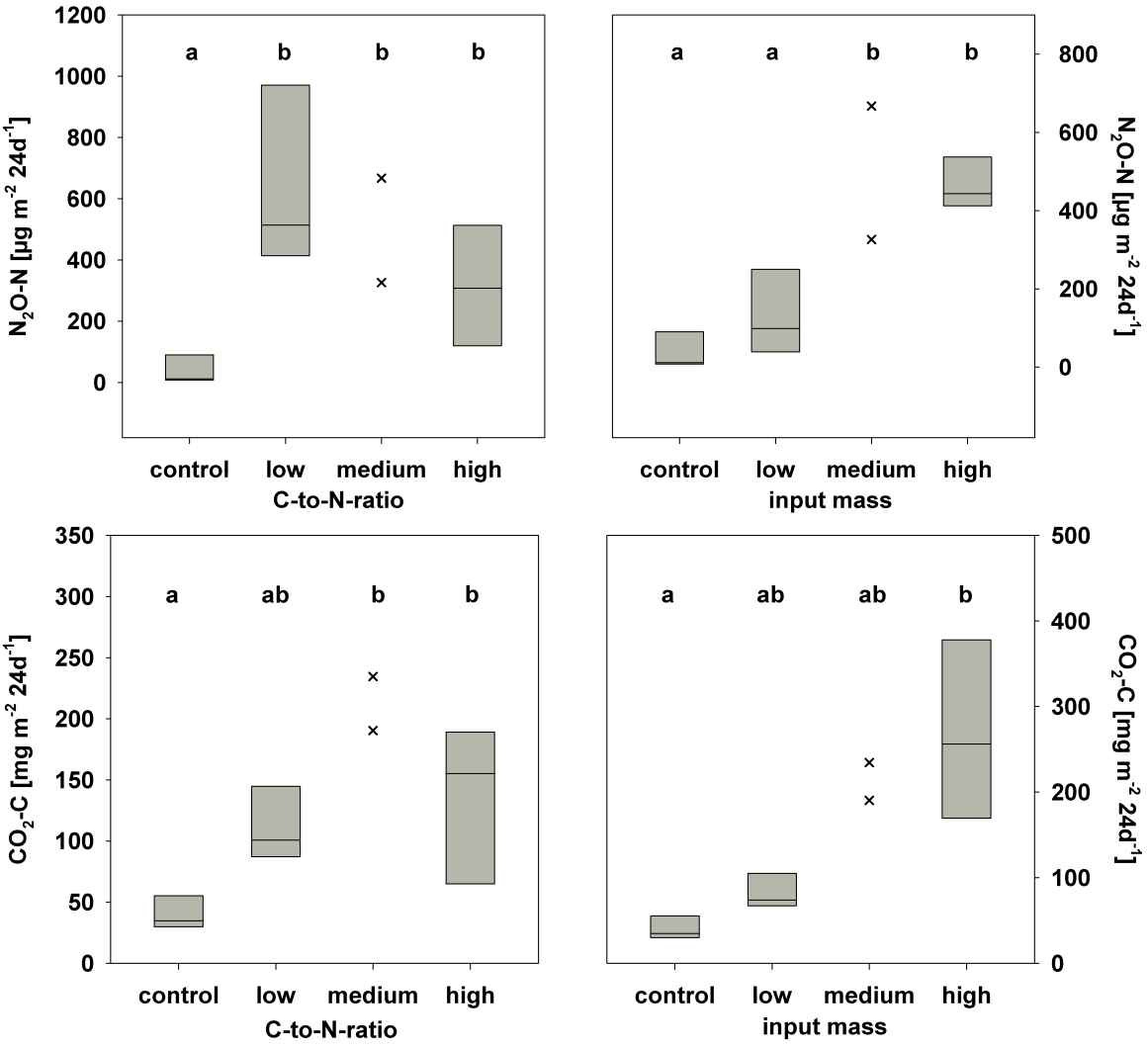


**Fig. 11.4:** *Median cumulative $N_2O$-N and $CO_2$-C emissions (n = 4) measured in a microcosm system during the 24 days after addition of cauliflower residues in the control treatment (shown twice), in the treatments with low, medium and high C/N ratio of residues and in the treatments with low, medium and high input mass of residues. The medium treatment is shown in both contexts and only displayed as crosses due to missing data. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, $p < 0.05$).*

### Amount of residue input

Input of lower amounts of residues decreased cumulative $N_2O$ as well as $CO_2$ emission. This was only significant for $N_2O$ where a reduction in residue input decreased cumulative emission as compared to the treatment with medium input (Fig. 11.4). The soil mineral N contents of the treatments with different amounts of residue input were similar at the end of the incubation and can not be responsible for the increase in $N_2O$ production. Input of residues is known to increase the microbial $O_2$ consumption, so that the creation of anaerobic microsites conducive for denitrification was probably extended (Beauchamp et al., 1989; Miller et al., 2008). In these hot spots, disproportionately high amounts of $N_2O$ can be released. The tendency to higher cumulative $CO_2$ losses points to a high heterotrophic activity as explanation, but the differences for $CO_2$ were not significant.

Another explanation for the importance of residue amount is the C-limitation of the system: if nitrogen is abundant, a higher carbon supply with a higher input of residue amount provides more electron donators

which are necessary for denitrification. This agrees with not finding any difference for varying C/N ratios. The residues with different C/N ratio only showed negligible differences in total C contents (Tab. 11.1). The removal of $\frac{2}{3}$ of the residues significantly decreases $N_2O$ emissions: Cumulative emission of about 3.7 kg $N_2O$-N $ha^{-1}$ could be saved. This management strategy has been proposed by several authors (Clemens et al., 2006; Möller and Stinner, 2009) and can therefore be an interesting mitigation option. However its effectiveness depends on the emissions during the further treatment of the removed residues and on the emissions during contingent reallocation of the effluents on the field.

#### C/N ratio of residue input

As already mentioned, no difference in cumulative $CO_2$ emissions was found for the different C/N ratios of the residues. The residues all supplied equal amounts of C. Assuming that the system was C-limited and that mostly the amount of C-input ruled the total $N_2O$ emission explains why no difference was observed for the C/N ratios.

### 11.3.7 Percentage loss of residue input

#### Percentage loss of C-Input from cauliflower residues

A mean corresponding to about 1700 kg C $ha^{-1}$ was lost during the incubation period as $CO_2$-C, while the C-input corresponded to 800 kg C $ha^{-1}$ for the mean input treatment. Therefore, when calculating the percentage loss of C provided by the residue input (Tab. 11.2), some of the treatments show values > 100 %. If the background emission from the control is taken into account, the values are reduced to between 80 and 197 %. This is relatively high as compared to the relative values reported in other studies. Toma and Hatano (2007) reported values of up to 45 % loss of the C-input from onion leaves. Flessa and Beese (1995) reported 30 % for sugarbeet leaves. De Neve et al. (1996) investigated C- and N-mineralization from cauliflower residues and found that only 55 % of total C-input was released within 3 weeks. Also over a longer period, not all of the carbon, but only 60 % were susceptible to degradation. Both of these observations indicate that part of the $CO_2$ was derived from carbon stocks from the soil pool. This has been reported in other studies after the input of organic material (Bol et al., 2003). In our study, a strong priming effect has been found for N (Chapter 10). However, the cumulative $CO_2$ release is unexpectedly high and cannot be explained easily.
Guenet (2010) reported that priming effects were following a saturating function of fresh organic matter inputs and decreasing with increasing fresh matter input. However, in our study also no clear trend was found to support this observation and no significant differences were found for the emission factors between the different treatments due to high standard deviations.

**Tab. 11.2:** *Mean percentage loss of N-input as $N_2O$ and of C-input as $CO_2$ of all treatments. Different superscript letters indicate statistically significant differences between groups (Student-Newman-Keuls Test, $p < 0.05$). *($CO_2$-$C_{treatment}$-$CO_2$-$C_{control}$)/(C-Input from residues)*

| CN of applied residues | application rate residues | name | EF($N_2O$) (% loss of N-input) | EF ($CO_2$)* (% loss of C-input) |
|---|---|---|---|---|
| control | | control | - | |
| low | | low CN | 6.0[a] | 81[a] |
| medium | low | low mass | 6.0[a] | 144[a] |
| medium | medium | medium mass & CN | 6.0[a] | 197[a] |
| medium | high | high mass | 2.4[a] | 87[a] |
| high | | high CN | 6.5[a] | 155[a] |

#### Percentage loss of N-Input from cauliflower residues

All emission factors ( % $N_2O$-N loss from residue-N input) besides one were at least 6 % when calculating for the 24 days of the incubation (Tab. 11.2). The results provide an indication that cauliflower residues can substantially increase the emission factors of vegetable cropping systems. These values are much higher than

data reported by Velthoff et al. (2002) who found only emission factors < 1 % for several residues with comparable C/N ratio including broccoli and Brussels sprouts in an incubation study from a clay soil. For a sandy soil however, they even reported higher values of up to 14 %. Also Toma and Hatano (2007) reported much lower emission factors for onion leaves and soybean residues (C/N ratio 12 and 15) from a Gray lowland soil in a field study. Our investigation proved that the management of cauliflower residues is obviously of immense importance for the development of potent mitigation strategies for vegetable production systems.

## 11.4 Conclusion

High emission factors of up to 6.5 % for $N_2O$ were found after the input of cauliflower residues in the laboratory incubation study over a period of 24 days. It indicates the importance of residue management in vegetable production systems. A reduction in residue input by $\frac{2}{3}$ led to a significant decrease in cumulative $N_2O$ emissions, probably due to the C-limitation of the system. Due to high nitrate accumulation after the incubation following the residue input, neither a difference in nitrate content nor a significant effect on the cumulative $N_2O$ emission was found due to the C/N ratio of the residues. For a generalization of this result, a repetition of the study with increased number of replicates and verification under field conditions would be desirable.

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# 12 General Discussion

## 12.1 Parameters controlling $N_2O$ emissions

**Tab. 12.1:** *Results from a stepwise multiple forward regression with the dataset of the **first** experimental year (n = 2208).*

| parameter to enter | $r^2$ | p |
|---|---|---|
| $CO_2$ | 0.07 | < 0.001 |
| WFPS | 0.13 | < 0.001 |
| precipitation | 0.14 | < 0.001 |
| soil temperature | 0.15 | < 0.001 |
| ammonium-N | 0.16 | < 0.001 |

**Tab. 12.2:** *Results from a stepwise multiple forward regression with the dataset of the **second** experimental year (n = 2808).*

| parameter to enter | $r^2$ | p |
|---|---|---|
| nitrate-N | 0.10 | < 0.001 |
| soil temperature | 0.14 | < 0.001 |
| $CO_2$ | 0.15 | < 0.001 |

**Tab. 12.3:** *Results from a stepwise multiple forward regression with the dataset of the **first and second** experimental year (n = 5016).*

| parameter to enter | $r^2$ | p |
|---|---|---|
| soil temperature | 0.028 | < 0.001 |
| WFPS | 0.044 | < 0.001 |
| nitrate-N | 0.054 | < 0.001 |
| $CO_2$ | 0.059 | < 0.001 |
| ammonium-N | 0.063 | < 0.001 |
| precipitation | 0.065 | <0.013 |

Since Chapters 5 to 10 only deal with parts of the dataset, it is also interesting to analyse the whole dataset in regard to the parameters influencing $N_2O$ fluxes (Tab. 12.1 and 12.2). Due to the high variability of the dataset, only a relatively small portion of the variability of $N_2O$ fluxes of up to 6.5 % could be explained with this approach (Tab. 12.3). All coefficients were positive. Altogether, especially the positive correlation with the variables "nitrate-N" and "$CO_2$" included in the model point to denitrification as major source of $N_2O$ emissions. However, a correlation with the ammonium-N also underlines the importance of nitrification. For an additional assessment, it is possible to have a further look at the data using mean values.

### 12.1.1 Nitrate

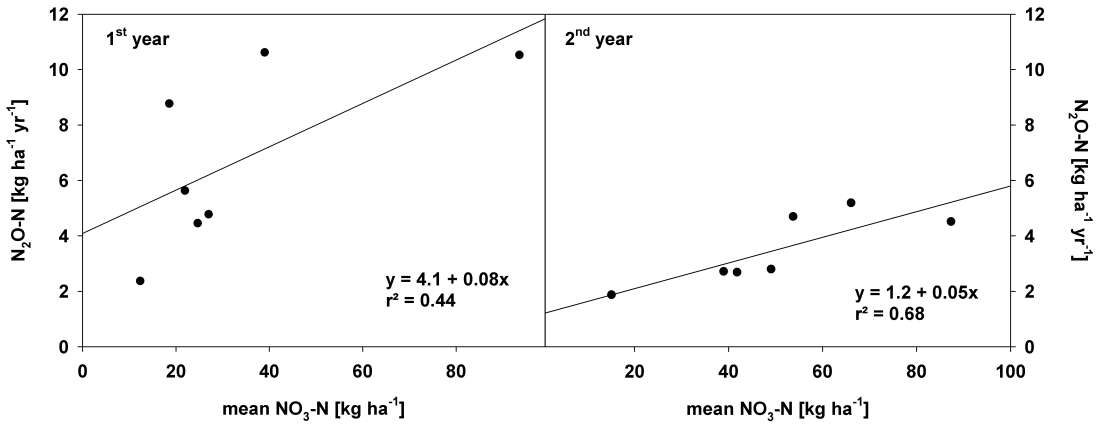


**Fig. 12.1:** *Mean cumulative $N_2O$-N emission and mean $NO_3$-N content of the top soil (0 - 25 cm) in the first and second experimental year (all treatments included)*

The mean values of nitrate-N can be separately correlated with the cumulative $N_2O$ emission. Since the nitrate level was higher in the second year than in the first, it is not reasonable to calculate mean values over both years. If the two years are treated separately, relative good positive correlations ($r^2 = 0.44$ and 0.68) for the first and second experimental year are found, respectively (Fig. 12.1.1). This observation points again to denitrification as the major source of $N_2O$ emissions and has already been found in numerous studies (e.g. Ruser et al., 2001; Sehy et al., 2003).

### 12.1.2 WFPS

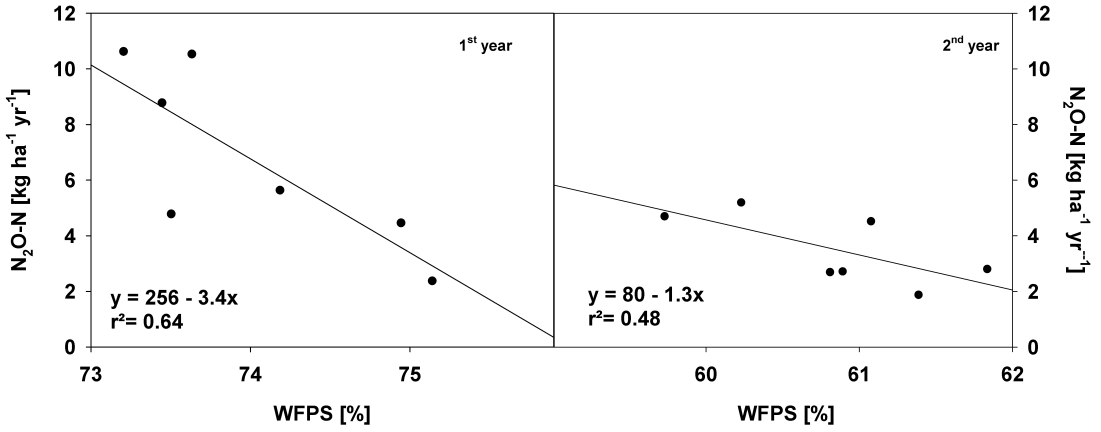


**Fig. 12.2:** *Mean cumulative $N_2O$-N emission and mean water-filled pore space (0 - 25 cm) in the first and second experimental year.*

The mean WFPS values for both the single experimental years as well as for the whole study show a good negative relationship with the cumulative $N_2O$ emission ($r^2 = 0.64$ for the first year; $r^2 = 0.48$ for the second year; $r^2 = 0.71$ for both years, Fig. 12.1.2). Usually positive correlations are reported for non-irrigated systems, but in irrigated systems like vegetable production the WFPS is usually higher than the threshold value for strongly increased $N_2O$ production. Further increases in WFPS are known to decrease the $N_2O/N_2$ ratio. The reason is an elevated reduction potential in the soil due to decreased oxygen levels because water serves as a diffusion barrier for oxygen.

### 12.1.3 Carbon availibility

A temporary C-limitation of $N_2O$ production is probably less common than N-limitation, but has been seen in other ecosystems like temperate forest soils (Perez, 2010), humid tropical forest soils (Garcia-Montiel et al., 2003) and compacted grassland (Abbasi and Adams, 1999). There are many observations in our study which point to the major influence of carbon as a parameter controlling $N_2O$ emissions. For example the correlation of $N_2O$ and $CO_2$, the increased emission after incorporation of carbon rich substrate and the 32fold increase in $N_2O$ flux rates after the addition of glucose (Chapter 7). One possibility cited in literature to find out more about carbon availability is the measurement of dissolved organic carbon (DOC). DOC is defined as the organic carbon fraction in solution that passes through a 0.45 µm filter. DOC is the most mobile and active cycling organic carbon fraction as compared to the fraction immobilized in organic compounds (Bolan et al., 2011). In our study, DOC was analyzed for the first experimental year. However, due to practical constrictions, soil was frozen before extraction. That was probably the reason why the data was not very plausible. Furthermore, the total amount of DOC does not necessarily reflect the in situ availability of DOC in the soil (Sehy et al., 2004). For example, DOC in macropores might not be used for denitrification because of the good aeration status (Zsolnay, 2003). But even though DOC was not a good predictor for $N_2O$ fluxes, many other indications show that carbon availability is of very high importance in vegetable production (see also Chapter 7).

## 12.2 $N_2O$ mitigation strategies

In Chapter 6 it has been shown that even though IPCC emission factors for $N_2O$ emissions from vegetable fields are within the range proposed by the IPCC (0.3 - 3 %); absolute $N_2O$ emission can be > 10 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$. If the higher emissions from the tractor compacted areas are taken into account, these values would even increase to > 12.5 kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ (see Chapter 5). The main reasons for these high emissions include the high fertilizer N input as well as the high amounts of organic nitrogen and carbon which are provided by plant residues and usually incorporated into the field after harvest.

**Tab. 12.4:** *Different strategies for the mitigation of $N_2O$ emissions from intensive vegetable production: percentage reduction of annual $N_2O$ emissions in % and kg $CO_2$-equ as well as converted to km of a passenger car (102 g $CO_2$-consumption (100 km)$^{-1}$) for the first and second years of measurement. An asterix (*) indicates a significant reduction in $N_2O$ emissions by a certain mitigation strategy in our study.*

| Strategy | % | | kg $CO_2$-equ $ha^{-1}$ | | km | |
|---|---|---|---|---|---|---|
| | 1st year | 2nd year | 1st year | 2nd year | 1st year | 2nd year |
| Fertilizer reduction | 17 * | 10 n.s. | 901 | 241 | 8829 | n.s. |
| Addition of DMPP | 45 * | 40 * | 1946 | 926 | 19077 | 9074 |
| Desynchronization of C- and N-input | | 29 n.s. | | 1385 | | n.s. |
| Replacing green rye by Phacelia | | 56 * | | 2634 | | 25824 |
| Replacing green rye by bare fallow | | 44 * | | 2064 | | 20239 |

Several strategies have been tested in regard to their efficiency in reducing the elevated $N_2O$ emissions from intensive vegetable production and are summarized in Table 12.4. For the desynchronization of C- and N-input, the cumulation for a whole year is not described in Chapter 7, but was achieved by combining data of the chard period and the preceding measurements on the same site, starting from the planting of cauliflower in 2009. These reduction potentials are shown expressed in percentage and were then converted into $CO_2$ equivalents (equ), using the conversion factor of 296 which is the Global Warming Potential of $N_2O$ on a 100 year time frame (IPCC, 2001). To make these numbers more vivid, the kg $CO_2$-equ $ha^{-1}$ were also converted to kilometers which can be driven by car with this amount. Therefore, a relatively economical car was chosen with a consumption of 102 g $CO_2$ (100 km)$^{-1}$. It must be kept in mind that these numbers refer to only one ha and that about $10^6$ ha of Germany are covered with intensive vegetable production (Statistisches Bundesamt, 2010).

### 12.2.1 Fertilizer reduction

In Chapter 6 it has been shown that fertilizer reduction is a very effective tool in the reduction of total $N_2O$ emission from vegetable cropped sites. This strategy functions, as it has been described in many studies before,

via the concentrations of mineral N in the soil. When only comparing the three fertilization levels and the control as described in Chapter 6, a strong correlation was found between the mean nitrate content of the top soil (0 - 25 cm) and the cumulative $N_2O$ emission on an annual basis in the first and second experimental years ($r^2 = 0.98$ and 0.89 respectively).

However, in vegetable cropping systems a relatively high N-fertilizer input, as well as a rather high soil mineral N level, is necessary for effective production. A fertilizer reduction by 20 % beneath the level prescribed by the German Target Value System led to lower lettuce yields in 2008. N-fertilizer reduction is therefore relatively restricted by plant demand. In the second year, no yield reduction was observed for further fertilizer reduction. For farmers however, the monetary aspect is of most importance. Since yield declines were observed for one out of two years, it should not be recommended to farmers as a mitigation option. For our data it can be concluded that farmers can be recommended to apply N-fertilizer according to the German Target Value System. After all, we measured a reduction potential of up to 17 %, but also no reduction in yields for the second year of measurement.

### 12.2.2 Addition of DMPP

Addition of DMPP has been discussed as a mitigation option for $N_2O$ emissions in detail in Chapter 8. With a reduction potential of 45 and 40 % it is even more effective than fertilizer reduction and no decrease in total yields was observed. However, it is questionable whether a large-scale application of an additional chemical compound is recommendable from an ecological point of view. DMPP has been tested in several model trials (Andreae, 1999; Roll, 1999). Roll (1999) reported that no indication was found for example for acut oral or subcutaneous toxicity in rats and no skin irritations in rabbits. However, chromosome aberrations were found in vitro in mammal cells. No further attention was given to this aspect however, since all *in vivo* tests were negative. He summarized that no toxic effects could be expected from DMPP. Andreae (1999) summarized that also no ecotoxicology was detected, for example no toxicity was found in fish, daphnia or earthworms. However, they could not prove biological degradation of DMPP in aqueous systems, but argued that DMPP had been seen to be degraded in soil and would not infiltrate into deeper soil layers. All in all, DMPP was judged as "not harmful to the environment" according to the European criteria EEC 93/21 (Andreae, 1999).
However, in both studies no long-term experiments were included and the use of model organisms does not exclude that further adverse effects could arise for other organisms. Up to the present, empiric studies have shown that obviously the ammonium monooxygenase is inversely inhibited by DMPP. Yet the mechanism is not fully understood. At least no inhibition of the structurally similar methanmonooxygenase has been found which might have negated the lower $N_2O$ emissions by lower $CH_4$ oxidation (Weiske et al., 2001). Of course, it is very difficult to study the effects of inhibition of nitrification on the complete ecosystem. Many correlations exist between the organisms of an environment and in such complex systems it is almost impossible to evaluate the effects on the complete system.

### 12.2.3 Desynchronization of C-and N-input

#### Waiting period

In Chapter 7, the effect of an optimization of N-fertilizer timing relative to catch crop incorporation was evaluated by investigating different waiting periods between incorporation and N-fertilization. Although a significant effect was seen if only the time from the catch crop incorporation until the harvest of chard are taken into account, this effect was not significant if annual datasets were compared (beginning from the cauliflower harvest in 2009). It has often been emphasized that for the evaluation of a strategy for the mitigation of $N_2O$, whole annual datasets are essential (Flessa et al., 1995; Bouwman et al., 1996). However, if these strategies are for example combined with further strategies or a waiting time would have been realized for all of the crops, the combined effects could get significant.

#### Replacing green rye by Phacelia or bare fallow

The effects observed for different waiting times between the incorporation of green rye and N-fertilization are very strong, due to the temporary C-limitation of the soil. Relatively high and significant reductions of up to 56 % in annual $N_2O$ emissions have been found if green rye was replaced by *Phacelia* or bare fallow. This is due to the lower carbon input before N-fertilization in spring. However, also the risk of N-losses by leaching should

not be disregarded. Green rye was still most effective in the uptake of nitrogen. Even in our loamy soil, it can be reckoned, from the mineral N in autumn (Chapter 7, Fig. 7.1), that leaching losses occurred and would be stronger still in a sandy soil. Also in spring there is a substantial risk of leaching when precipitation occurs. Since the rainfall cannot be predicted for a period of as long as the waiting time, it is therefore questionable if waiting times of 3 weeks are really a good recommendation. However, on our soil and under the climatic conditions of the experimental years 2008 to 2010, the desynchronization of C- and N-input was the most effective strategy for $N_2O$ reduction (Tab. 12.4).

## 12.3 Relevance of the $N_2O$ reduction strategies on a national base

The area cropped with cauliflower was 4491 ha and 2259 ha for lettuce in 2010 in Germany. The total area used for intensive vegetable production is about $10^6$ ha in Germany (Statistisches Bundesamt, 2011). It is difficult to calculate the $N_2O$ mitigation potential of the strategies for all vegetable cropped soils in Germany because there might be differences depending on the plant species and on the soil types. For a rough extrapolation, it is assumed here that the potential for the reduction of $N_2O$ emissions is similar for a third of the vegetable cropped soils. When using the data from the first experimental year, this reduction corresponds to 96 Mg $N_2O$ for the fertilizer reduction strategy and to 207 Mg $N_2O$-N for the use of DMPP. The Umweltbundesamt (2010) quotes that in 2007 a total of $96 \cdot 10^3$ Mg $N_2O$-N was released from agriculture. Fertilizer reduction and use of DMPP would therefore decrease these $N_2O$ emissions by only 0.1 and 0.2 % respectively However, the assumption that the reduction is possible on one third of the vegetable is more or less accidental and should be verified with the help of information on soil use, soil type and climate.

This sounds rather disillusioning. But it is much more ostensive if the reduction potential **per hectare** is expressed as kilometers driven by a car. Between roughly 9000 and 26000 km can be driven with an economical car for the mitigation potential of the different strategies per hectare. This is a result which can help to make the results interesting for a broader audience and also to be more motivating for farmers. The reduction potential by fertilizer reduction per hectar also corresponds to about half of the annual $CO_2$ release by consumption of electricity in a two-person-household in Germany (which is 2215 kg $CO_2$, BDEW, 2010).

Generally, it is not new that there is no simple cure-all for environmental protection and that it needs many motivated and conscious participants and also many different strategies for long-term improvements.

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# 13 Curriculum Vitae

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10/2003–07/2006 Diploma Thesis at the Albert-Ludwigs-Universität Freiburg; Title: Diasporenbank und Epiphytenvegetation in Wäldern unterschiedlichen historischen Alters

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10/2010 Poster: Beitrag von N-Düngung und Ernterückständen zu den $N_2O$ Emissionen einer Gemüsebaufläche; KTBL-Tagung (Bad Staffelstein)

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06/2009 Presentation: $N_2O$ emission from a high N-input system as influenced by fertilizer amount and type; Nitrogen Workshop (Turin)

04/2009 Poster: Nitrogen loss from high N-input vegetable fields - a) direct emissions: European Geoscience Conference (EGU) (Vienna)

## Supervision of Bachelor Theses

| | |
|---|---|
| 09/2009 | *Bastian Winkler*: Räumliche und zeitliche Variabilität der $N_2O$-Emissionen im Feldgemüsebau |
| 09/2009 | *Julia Spengler*: Lachgasemissionen auf einem Gemüsefeld mit platzierter N-Düngung (CULTAN) |
| 08/2010 | *Kim Ebinger*: Einfluss von Zwischenfrucht und Umbruchtermin auf die $N_2O$-Emissionen der Folgefrucht |
| 09/2010 | *Katharina Kesenheimer*: Einfluss der Düngerplatzierung und Düngerform auf die $N_2O$-Emissionen im Feldgemüsebau |

## Language skills

| | |
|---|---|
| Languages | English and French (written and oral: very good);<br>Italian (written and oral: good);<br>advanced Latin proficiency exam |

## Travel experience

| | |
|---|---|
| 07/2010–10/2010 | Backpacking in India |
| 01/2001–09/2001 | Language school, Perugia (Italy)<br>WWOOF: Willing work on Organic Farms (Italy) |
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## Hobbies

| | |
|---|---|
| Sports | Hiking on viae ferratae; Mountainbiking |
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Hohenheim, March 29, 2011

# 14 Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on ammonia-oxidizing bacteria and archaea in rhizosphere and bulk soil

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Authors: Kleineidam, K., Košmrlj, K., Kublik, S., Palmer, I., Pfab, H., Ruser, R. Fiedler, S., Schloter, M.
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## 14.1 Abstract

In agricultural plant production nitrification inhibitors like 3,4-dimethylpyrazole phosphate (DMPP) are used to retard the microbial nitrification process of fertilized ammonium to enhance the nitrogen supply for cultivated crops and to reduce nitrogen losses from the production system. Besides the well-known ammonia-oxidizing bacteria (AOB) it is known for a few years that also ammonia-oxidizing archaea (AOA) are able to perform the first step in nitrification, hence being also a target for a nitrification inhibitor. However, so far no information are available concerning the effectiveness of DMPP and its extent towards AOB and AOA, neither in bulk soil nor in the root-rhizosphere complex. We investigated in a field experiment performed according to agricultural practice the effect of DMPP on the abundance of AOB and AOA two, four and eight weeks after fertilization. We observed impaired abundances of AOB but not of AOA in both soil compartments that were still visible eight weeks after application, possibly indicating a reduced effectiveness of the nitrification inhibitor in our study.

## 14.2 Introduction

Nitrification inhibitors (NI) are chemical compounds which are able to delay the stepwise microbial oxidation of ammonium via nitrite to nitrate in soil. For example, dicyandiamide (DCD) or nitrapyrin retard ammonium oxidation for several weeks and consequently reduce the risk for contamination of ground- and drinking water by nitrate leaching and for large emissions of nitrous oxide ($N_2O$), a greenhouse gas contributing to climate warming and ozone destruction (Amberger, 1986; Slangen and Kerkhoff, 1984). NI are frequently used in agricultural and horticultural practice in regions notably with sandy soils to increase the fertilizer use efficiency of the plants and to facilitate the timing of fertilizer application as well as the application frequencies.

In recent years the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) has come to the market. DMPP is used in combination with mineral ammonium nitrate fertilizers in application rates less than one tenth compared to DCD and able to delay nitrification over a period of four to ten weeks depending on the climatic conditions and site characteristics (Barth et al., 2001; Barth et al., 2008; Chen et al., 2010; Pasda et al., 2001; Zerulla et al., 2001). Moreover, DMPP may increase crop yield and/or improve yield quality (Pasda et al., 2001), reduce nitrate concentration in soil and leachate (Chen et al., 2010; Li et al., 2008), and decrease the release of $N_2O$ from soil (Weiske et al., 2001). DMPP is thought to only block the first step in nitrification,

the oxidation of ammonia to hydroxylamine, while the second step, the oxidation of toxic nitrite, remains unaffected (Li et al., 2008; Weiske et al., 2001). Since ammonia oxidation is considered to be the rate-determining step in nitrification, it is regarded as "pinhole" of this transformation process. Only a few selected groups of microorganisms are able to perform this oxidation despite the huge phylogenetical and physiological diversity of microbes.

Traditionally, beta- and gamma-proteobacteria have been considered as exclusive contributors to ammonia oxidation (Bock and Wagner, 2006), however recently, also ammonia oxidizers belonging to the domain of archaea were identified in terrestrial and marine ecosystems (Hallam et al., 2006; Könneke et al., 2005; Schleper et al., 2005; Treusch et al., 2005). Quantification of the *amoA* gene that encodes a subunit of the key enzyme ammonia monooxygenase showed that in different soils archaea (ammonia-oxidizing archaea, AOA) were conspicuously more abundant than bacteria (ammonia-oxidizing bacteria, AOB) (Adair and Schwartz, 2008; Boyle-Yarwood et al., 2008; Chen et al., 2008; He et al., 2007; Leininger et al., 2006). Although transcriptional activity of archaea was demonstrated in situ (Leininger et al., 2006; Treusch et al., 2005), their share of ammonia oxidation in soil is still unclear. Recent studies with different agricultural soils revealed both, indications for archaea being major contributors (Offre et al., 2009; Tourna et al., 2008) and hints that bacteria were functionally more important in ammonia oxidation (Di et al., 2009; Jia and Conrad, 2009). As AOB and AOA might presumably exhibit different cellular biochemistry, they might also be differently susceptible to inhibitory compounds. However, no information is available concerning the effectiveness of DMPP in relation to the two different groups of soil ammonia oxidizers. Therefore, we investigated in an established field experiment the impact of DMPP on the target organisms AOB and AOA in bulk soil and the root-rhizosphere complex (RRC) of cauliflower plants, respectively, two, four and eight weeks after its application. The *amoA* gene, coding for the enzyme ammonia monooxygenase, served as indicator for the abundance of AOB and AOA and was quantified by real-time PCR based on DNA extracts obtained from the different soil compartments. The achieved data were compared with ammonium and nitrate concentrations in soil.

## 14.3 Material and Methods

### 14.3.1 Study site

The field experiment was conducted at the Heidfeldhof, the research farm belonging to the University of Hohenheim, Germany (48°43'5.00" N, 9°11'40" E, 410 m above sea level). The soil is classified as Haplic Luvisol consisting of a loamy silt texture (silt, clay, sand = 73, 25, 2 %, respectively) with a stone content of < 1 %, 1.8 % organic carbon and a pH value ($CaCl_2$) of 5.5 in the topsoil (0 - 25 cm). The mean annual temperature accounts for 8.8°C, annual precipitation amounts to 686 mm (University of Hohenheim, Department of Physics and Meteorology, 2009). Data concerning the sum of weekly precipitation and mean weekly air temperature during the investigation period as well as air and soil temperature in 2 and 5 cm soil depth at the sampling days, respectively, are shown in Fig.14.1.

### 14.3.2 Experimental set up

The experiment was carried out using an established field trial with a crop rotation of rye (*Secale cereale*) as catch crop, lettuce (*Lactuca sativa* var. *capitata* L., variety 'Gisela'), cauliflower (*Brassica oleracea* var. *botrytis* L., variety "Dexter") and bare fallow. At the beginning of the experiment in the mid of July 2008 cauliflower was planted (20,000 plants $ha^{-1}$) as crop of investigation. Grubbing, harrowing and plant bed preparation were performed one week before. Insecticide applications were conducted 3 and 7 weeks after planting using "Karate®" (75 ml $ha^{-1}$) and "Steward®" (85 g $ha^{-1}$), respectively. Mechanical hoeing was carried out after 4 weeks. Two fertilizer treatments were comparatively investigated: ENTEC®, a granulated ammonium sulphate nitrate (ASN) fertilizer being formulated with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) and a common treatment of pure ASN (18.5 % $NH_4^+$-N, 7.5 % $NO_3^-$-N, 13 % S). In both treatments 266 kg N $ha^{-1}$ were supplied in broadcast application (resulting in 1.94 kg DMPP $ha^{-1}$ and 0.86 kg P $ha^{-1}$ in the ENTEC treatment, respectively). While, according to agricultural practice, the amount of ASN was split in an application of 50 % immediately after planting and a second application nearly 8 weeks later, ENTEC was given in one application directly after planting. Per fertilizer treatment four independent replicate plots were prepared in a randomized block design that were sampled just before and 2, 4, and 8 weeks after planting and

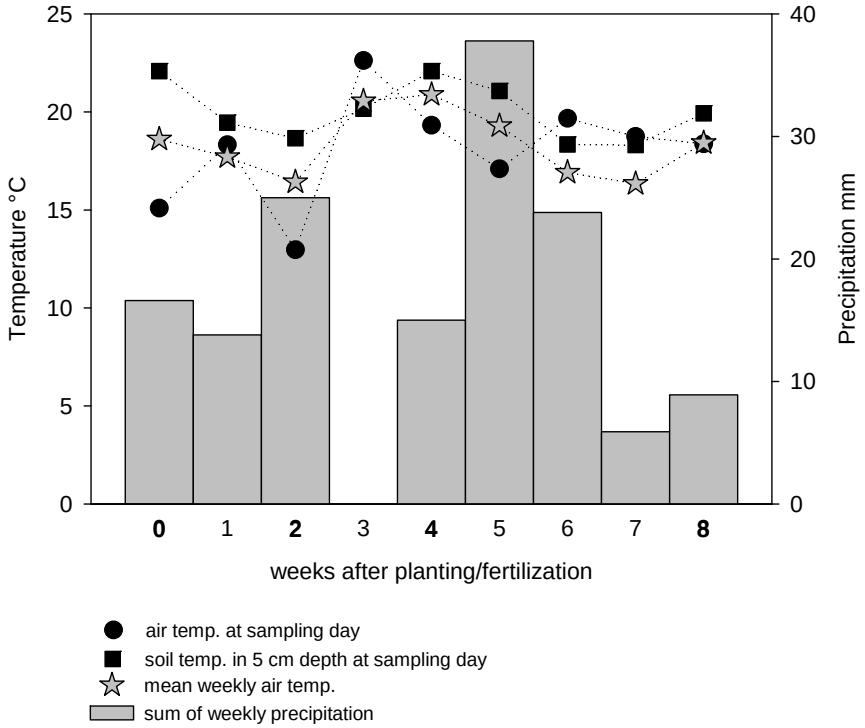


**Fig. 14.1:** *Sum of weekly precipitation, mean weekly air temperature, as well as air temperature and soil temperature in 5 cm soil depth at the sampling days, respectively, during the investigation period; bold numbers indicate sampling time points for quantification of AOB and AOA.*

fertilization, as the nitrification-inhibiting effect of DMPP was assumed to last for 4 to 6 weeks.

### 14.3.3 Ammonium and nitrate measurements in bulk soil

Per replicate plot 6 bulk soil samples were taken from 0 - 25 cm soil depth with a Pürckhauer boring rod (3.5 cm diameter), well homogenized and divided into two sub-samples. The part for DNA extraction was immediately frozen in liquid nitrogen and stored at -80°C, the part for mineral nitrogen determination was stored at -20°C. Samples of the root-rhizosphere complex (RRC) of cauliflower plants (roots with adhering soil) were taken from 5 plants per replicate plot and time point and treated separately. After vigorous shaking the RRC was also immediately frozen in liquid nitrogen and stored at -80°C.

### 14.3.4 Real-time PCR assays

DNA was extracted from 0.4 g bulk soil and root-rhizosphere complex, respectively, using the FastDNA® Spit Kit for Soil (MP Biomedicals, France) and the Precellys®24 lyser/homogeniser (Bertin Technologies, France) in accordance to the manufacturer's instruction. Quality and quantity of the extracted DNA were checked with a spectrophotometer (Nanodrop, PeqLab, Germany). Afterwards, extracts were stored at -20°C until use.

Abundance of *amoA* genes of ammonia-oxidizing bacteria (AOB) and archaea (AOA) in bulk soil and the root-rhizosphere complex was assessed by quantitative real-time PCR using SybrGreen as fluorescence dye. Real-time PCR was carried out on a 7300 Real-Time PCR System in 96 well plates (both Applied Biosystems, Germany). The reaction volumes of 25 µl consisted of 12.5 µl Power SybrGreen Master Mix (Applied Biosystems, Germany), 0.5 µl of each primer (10 pmol $\mu l^{-1}$, Thermo Fisher Scientific, Germany), 0.5 µl bovine serum albumin (3 %, Sigma, Germany), 0.625 µl dimethyl sulfoxide (Sigma, Germany), 2 µl template and 8.375 µl DEPC-treated

water. For targeting AOB primers *amoA*-1F and *amoA*-2R (Rotthauwe et al., 1997) were used, whereas for AOA primers amo19F (Leininger et al., 2006) and CrenamoA16r48x (Schauss et al., 2009) were applied. Serial dilutions of cloned *amoA* gene fragments derived from *Nitrosomonas* sp. and fosmid clone 54d9 (Treusch et al., 2005) ranging from 101 to 105 gene copies $\mu l^{-1}$ were used as standards. Samples beyond the standard curve were not considered. The detection limit was assigned to 68 and 46 gene copies $\mu l^{-1}$ for AOB and AOA, respectively. All samples, standards and non-template controls were analyzed in triplicates. To avoid inhibition of the PCR reactions dilution series of the samples were tested in advance resulting in optimal dilutions of 1:50 for bulk soil and 1:100 for rhizosphere extracts.
Each PCR run started with a hot start at 95°C for 10 min and continued with 40 cycles of 95°C for 45 s, 58°C/55°C (AOB/AOA) for 45 s and 72°C for 45 s. To confirm the specificity of the amplicons after each PCR run a melting curve and a 2 % agarose gel were conducted. Amplification efficiencies were calculated with Eff = [10(1/-slope) - 1] and resulted in values of 88.0 - 90.9 for *amoA* AOB and 84.4 - 86.9 for *amoA* AOA.

### 14.3.5 Statistics

Significant effects of DMPP compared to the pure ASN treatment on the abundance of AOB and AOA *amoA* genes were checked by t-test in bulk soil and root-rhizosphere complex at a given time point, respectively ($p < 0.05$).

## 14.4 Results

### 14.4.1 Soil ammonium and nitrate concentrations

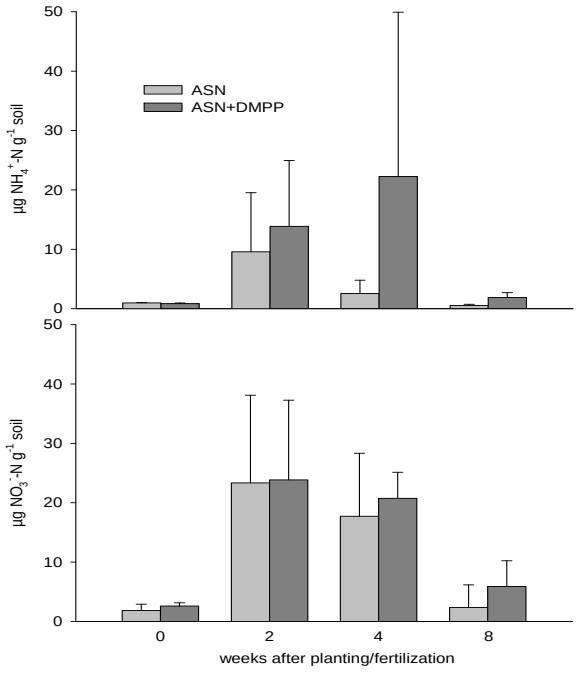


**Fig. 14.2:** *Ammonium and nitrate concentrations in 0 - 25 cm bulk soil in the two fertilizer treatments pure ASN (split application) and ASN+DMPP before (week 0) and 2, 4, and 8 weeks after planting/fertilization of cauliflower; error bars indicate standard deviation of mean (n = 4).*

Two and four weeks after application increased soil ammonium (from 1 to 10 and 22 µg N $g^{-1}$ soil, respectively) and nitrate (from 2 to 23 g N $g^{-1}$ soil) values were determined in both fertilizer treatments (Fig. 14.2). After eight weeks the mineral nitrogen concentrations had declined to pre-fertilization levels, only a slightly higher nitrate content was observed in the DMPP treatment. At the different sampling time points no significant differences between the two treatments were determined although, according to agricultural practice, the ASN application was split whereas ASN+DMPP was given in one application at the beginning of the experiment.

### 14.4.2 Abundance of AOB *amoA* and AOA *amoA* genes

In the bulk soil quantification of *amoA* AOB genes by real-time PCR revealed increasing copy numbers in both treatments in the range of $4.2 \cdot 10^5$ to $3.6 \cdot 10^6$ $g^{-1}$ soil during the sampling period (Fig. 14.3a). At every sampling date lower gene copy numbers were observed in the DMPP compared to the pure ASN treatment, being significant 8 weeks after application. The abundance of AOA *amoA* genes also increased in both treatments exhibiting copy numbers between $8.8 \cdot 10^6$ and $3.0 \cdot 10^8$ $g^{-1}$ soil, however, no trend of reduced or elevated gene copies due to the nitrification inhibitor was visible (Fig. 14.3a).

Thus, in bulk soil higher AOA than AOB *amoA* copy numbers were found in both treatments, but with wider AOA:AOB ratios in the DMPP-treated samples. In the root-rhizosphere complex (RRC) of cauliflower

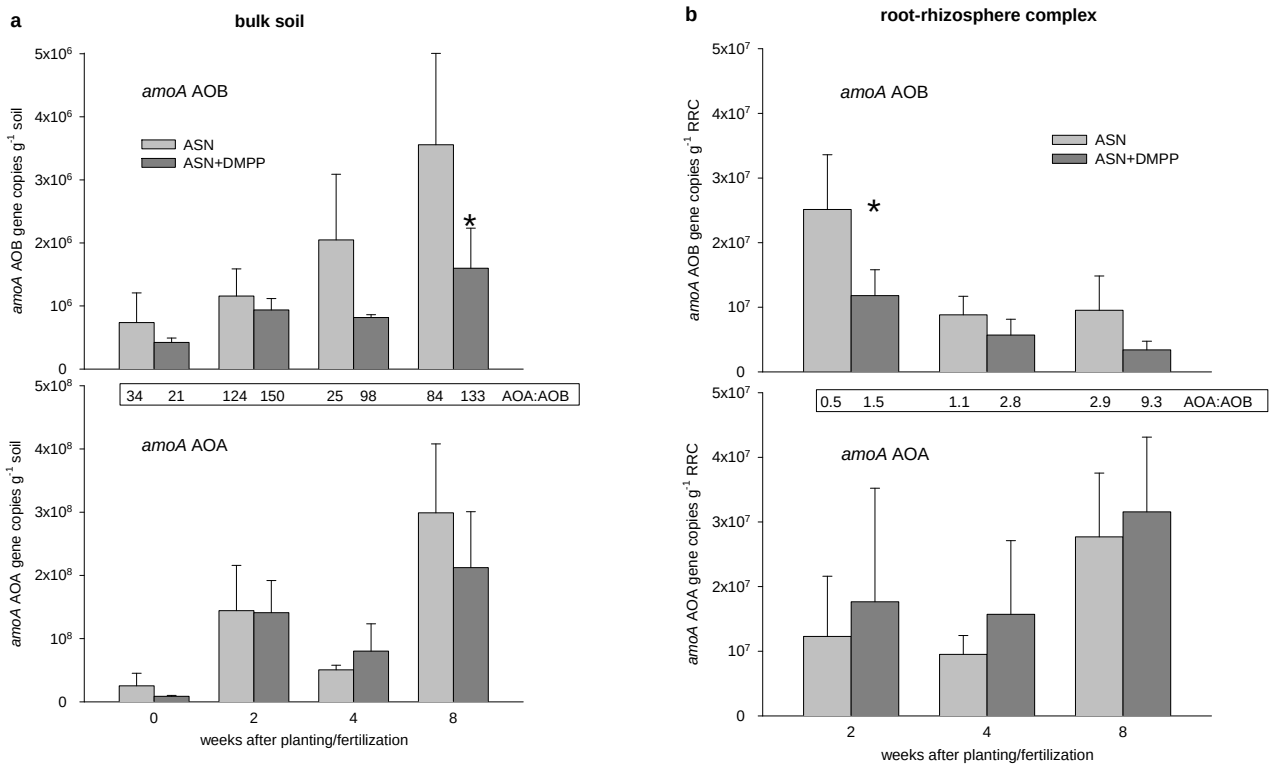


**Fig. 14.3:** *Abundances of amoA genes of ammonia-oxidizing bacteria (AOB) and archaea (AOA) before (week 0) and 2, 4, and 8 weeks after planting of cauliflower in 0 - 25 cm bulk soil (3a) and the root-rhizosphere complex (RRC, 3b); error bars indicate standard deviation of mean (n = 4); stars indicate significant difference between the two fertilizer treatments ASN and ASN+DMPP at a given time point ($p < 0.05$); ratios of AOA:AOB within a fertilizer treatment at a given time point are shown in boxes.*

decreasing numbers of AOB *amoA* genes were measured in both treatments which ranged from $2.5 \cdot 10^7$ to $3.4 \cdot 10^6$ $g^{-1}$ soil (Fig. 14.3b). As observed for AOB in bulk soil, lower copy numbers were found in the DMPP treatment in comparison to the pure ASN treatment being significant 2 weeks after application. In contrast to AOB, increasing copy numbers of AOA *amoA* were shown in the RRC accounting for $9.5 \cdot 10^6$ to $3.2 \cdot 10^7$ $g^{-1}$ soil (Fig. 14.3b). Interestingly, at all sampling dates a trend of increased gene copies in the DMPP treatment compared to ASN was detected. As in bulk soil, more AOA than AOB *amoA* copies were observed in the RRC, but in clearly narrower ratios that increased over time notably in the DMPP-treated soil. Comparing the AOB *amoA* gene abundance pattern between the two soil compartments, higher copy numbers were assessed in the RRC than in bulk soil in both treatments, but with decreasing tendency during the sampling period. In contrast, more AOA *amoA* genes were determined in the bulk soil compared to the RRC independent of the treatment.

## 14.5 Discussion

As fertilization in our field experiment was performed according to agricultural practice, the pure ASN fertilization was split in two applications, whereas ASN+DMPP was given in only one application. Consequently, the amount of mineral nitrogen applied after planting in the DMPP treatment was twice as much as in the common split ASN treatment. However, the different fertilizing strategies were not reflected in significant differences between the treatments, even not for ammonium although most of the nitrogen introduced through ASN reached the soil in form of $NH_4^+$-N which should have been prevented from oxidation by the NI. This could indicate a reduced effectiveness of DMPP in our experiment, which might be explained by the high share of the presumably less sensitive AOA and their contribution to ammonia oxidation in this arable soil (see below). However, also the lower mobility of ammonium compared to nitrate in the soil in combination with the sampling

approach by coring (relatively small diameter) might have contributed to the results (Azam et al., 2001).

While no pattern of increased/decreased soil mineral nitrogen concentrations could be determined between the two fertilizer treatments, the molecular analyses clearly indicated that DMPP impaired AOB in both soil compartments, whereas AOA were not affected or even increased in population size over time. Statistically significant effects were only confirmed at two time points for AOB, which is however frequently found in field studies due to a higher variability between the replicates under environmental conditions compared to laboratory experiments with, e.g., homogenized, sieved soil, identical water content and identical climatic conditions.

Our observations are in accordance with results of DCD revealing in urine-fertilized pasture soils an inhibiting effect of the NI on AOB *amoA* gene copy numbers, whereas AOA remained unaffected (Di et al., 2010; O'Callaghan et al., 2010). Comparatively findings related to the NI acetylene were reported by Jia and Conrad (2009). Offre and colleagues (2009) postulated that active ammonia oxidation was mostly due to archaea in acetylene-treated arable soils. In our study the even slightly increased AOA *amoA* gene copies in the NI treatment in the root-rhizosphere complex confirm this hypothesis for DMPP under field conditions. Thus, AOA could be able to counteract the inhibiting effect of DMPP on nitrification by maintaining the process, even if possibly on a lower level, underlining the different lifestyle, cellular biochemistry (membrane composition etc.) and hence different susceptibility to inhibitory compounds of bacteria and archaea (Schleper et al., 2005; Valentine, 2007). Yet, the mechanism how DMPP interacts with AOB is poorly described in the literature, so that a speculation why AOA are more tolerant towards this NI is currently not possible. However, as the drop in AOA abundance in bulk soil four weeks after fertilization might have been resulted from the precedent insecticide application, a generalization that AOA are more tolerant against the application of any xenobiotic substance is not justifiable. Anyhow, this observation is not surprising as central metabolism components of archaea are closely related to those of eukaryotes (Cabello et al., 2004; Cavicchioli, 2011; Schleper et al., 2005).

Explicit differences between AOA and AOB also emerged by comparing their abundances in the two soil compartments. While the ratio of *amoA* genes of AOA and AOB ranged between 21 and 150 in the bulk soil, it dropped to 0.5 - 9.3 in the root-rhizosphere complex across the sampling time points, always with wider ratios for the DMPP treatment. This finding (i) points out again the lower susceptibility of AOA compared to AOB in both compartments and (ii) indicates a relative increase of AOB and decrease of AOA in the rhizosphere. As the rhizosphere is known to be a habitat of high microbial activity and higher nutrient status (Hinsinger et al., 2009), it fits to the speculation that AOB could mainly contribute in "nutrient-rich", AOA on the contrary in "nutrient-poor" environments to the nitrification process (Schauss et al., 2009). This assumption might underline the pronounced inhibiting impact of DMPP on AOB when considering that, although the double amount of $NH_4$-N was fertilized with the first application in the NI treatment compared to the pure ASN treatment, constantly lower AOB abundances were assessed in the DMPP treatment. Presumably, the differences in AOB abundance would have been even more prominent if the same amount of ammonium had been applied at the same time point in both treatments.

## 14.6 Conclusion

We observed in a field study performed according to agricultural practice that the nitrification inhibitor DMPP only affected the bacterial ammonia oxidizers, whereas their archaeal counterpart, that numerically outcompeted the ammonia-oxidizing bacteria, remained unaffected. The results of this field experiment are considered as first insight into the topic of dissimilar DMPP effectiveness towards AOB and AOA and their functional and ecological differences under the influence of this nitrification inhibitor.

After this observation under field conditions, further studies under controlled conditions in microcosm experiments with synchronized amounts of applied nitrogen and higher temporal resolution are intended. Thus, for future investigations it will be, e.g., of interest (i) to quantify the currently active ammonia-oxidizing bacteria and archaea via mRNA studies and hence to estimate the effectiveness of DMPP on actual archaeal and bacterial ammonia oxidation rates and (ii) to analyze alterations in diversity of AOB and AOA communities in DMPP-stressed compared to untreated soils.

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# The end


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