Effect of soil management practices on N_2O producing and reducing microbial communities

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät

der Eberhard Karls Universität Tübingen

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

vorgelegt von

Diplom Geoökologe Hans-Martin Krause

aus Mühlacker

Tübingen

2017

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	29.03.2017
Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Andreas Kappler
2. Berichterstatter:	PD Andreas Gattinger

Table of Contents

Abstract

Nitrous oxide (N_2O) is a major greenhouse gas that also contributes to stratospheric ozone depletion. In the last decades atmospheric N_2O concentrations were constantly raising, mainly due to human interventions in the nitrogen cycle. In 2015 atmospheric N_2O concentration accounted for 121% of preindustrial periods. Since the biggest share of anthropogenic N_2O emissions is emitted from agriculturally managed soils, developing soil management practices that reduce N_2O emissions is a key challenge for the agricultural sector.

Nitrogen transformation processes in soils are mainly controlled by microbes and a variety of processes within the microbial nitrogen cycle can produce N_2O. Among those processes denitrification and nitrification are considered as the most important source processes for N_2O. While nitrification occurs mainly under oxic conditions, denitrification is favored when the availability of oxygen is limited. The last step within denitrification, the reduction of N_2O to N_2, is the only know biological sink for N_2O. Developing a detailed understanding how soil management practices affect microbial N_2O production and reduction processes is a prerequisite for the establishment of climate smart agriculture.

Several soil management practices were proposed as possible N_2O mitigation strategies. Among those reduced tillage systems, organic agriculture and the addition of biochar were investigated within this thesis. In order to assess the effect of different soil management practices on N_2O emissions and N_2O producing and reducing microbial communities, incubation experiments under controlled conditions and field experiments were conducted. Functional microbial communities involved in N cycling were investigated using quantitative polymerase chain reaction (qPCR) and next generation sequencing approach. Furthermore, ^{15}N tracing techniques were used to determine source process of N_2O and $N_2O/(N_2O+N_2)$ product ratios in organically and conventionally managed soils.

Under reduced tillage systems the lower soil layer was depleted in organic carbon stocks and showed enhanced potential of N_2O production via nitrification. In contrast, in the upper soil layer under recued tillage soil organic carbon accumulated and an increased abundance of N_2O reducing bacteria which correlated negatively with N_2O emissions was observed. Organic farming systems revealed higher potential for N_2O emissions compared to conventional systems but sustainably enabled functionality of the N_2O reductase by maintaining a stable soil pH without the need for

further liming treatments. Biochar amendment resulted in an increase of free living N fixing and N_2O reducing bacteria along with reduced N_2O emissions. In a field experiment across a whole vegetation period a shift in community structure of N_2O reducing bacteria was observed. Biochar addition favored N_2O reducers which lack the genetic capability to produce N_2O and thus most likely act as sink for N_2O.

Among all observed soil management practices the addition of biochar was the most effective strategy to reduced N_2O emissions. Activity, abundance or community structure of N_2O reducing bacteria could be linked to N_2O emissions in all experiments conducted. This highlights that the functioning of the N_2O reducing community is susceptible to management induced changes in soil properties and strengthens the viability of N_2O mitigation strategies focusing on biological N_2O reduction.

Zusammenfassung

Lachgas (N_2O) ist ein wichtiges Treibhausgas das auch zum Abbau von stratosphärischem Ozon beiträgt. Hauptsächlich durch menschliches Einwirken auf den globalen Stickstoffzyklus stieg im letzten Jahrzehnt die atmosphärische N_2O Konzentrationen kontinuierlich an. Im Jahr 2015 betrug die atmosphärische N_2O Konzentration 121% im Vergleich zum vorindustriellen Zeitalter. Da der Grossteil der menschlich verursachten N_2O Emissionen aus landwirtschaftlich genutzten Böden stammen ist die Entwicklung von Bodenbearbeitungsmethoden die N_2O Emissionen mindern können eine wichtige Herausforderung für den gesamten landwirtschaftlichen Sektor.

Stickstoff wird um Boden vor allem mikrobiell umgesetzt. Innerhalb des mikrobiellen Stickstoffzyklus kann N_2O durch mehrere Prozesse entstehen. Dabei werden Nitrifikation und Denitrifikation als die wichtigsten N_2O bildenden Prozesse angesehen. Während Nitrifikation hauptsächlich unter oxischen Bedingungen stattfindet, wird die Denitrifikation durch sauerstofflimitierende Bedingungen begünstigt. Der letzte Schritt der Denitrifikation, die Reduktion von N_2O zu N_2 stellt die einzige bekannte biologische N_2O Senke dar. Um eine klimafreundliche Landwirtschaft entwickeln zu können ist es daher unabdinglich ein grundlegendes Verständnis über die Auswirkung von Bodenbearbeitungsmethoden auf N_2O produzierende und reduzierende Bodenbakterien zu bekommen.

Mehrere Bodenbearbeitungsmethoden wurden als mögliche Strategien vorgeschlagen um N_2O Emissionen zu verringern. Gegenstand diese Arbeit waren die reduzierte Bodenbearbeitung, der biologische Landbau und die Ausbringung von Biokohle. Um den Einfluss der verschiedenen Bodenbearbeitungsmethoden auf N_2O Emissionen und N_2O produzierende und reduzierende Bodenbakterien zu untersuchen wurden sowohl Inkubationsexperimente unter kontrollierten Bedingungen als auch Experimente im Feld durchgeführt. Funktionelle mikrobielle Gemeinschaften, die an Stickstoffumsetzungen beteiligt sind, wurden mit Hilfe von molekularbiologischen Methoden wie der quantitativen Polymerase-Kettenreaktion (qPCR) und modernen Sequenzierungstechniken untersucht. Weiterhin wurden ^{15}N Markierungstechniken angewandt um die Herkunft von N_2O und das $N_2O/(N_2O+N_2)$ Verhältnis in Böden unter biologischem und konventionellen Landbau zu bestimmen.

Unter Reduzierter Bodenbearbeitung konnte in den tieferen Bodenschichten ein höheres Potenzial zur N_2O Bildung durch Nitrifikation festgestellt werden. In Gegensatz dazu war eine erhöhte

Abundanz von N_2O reduzierenden Bakterien in der oberen Bodenschicht zu beobachten welche auch mit reduzierten N_2O Emissionen in Verbindung gebracht werden konnten. Boden der unter biologischen Anbaumethoden bewirtschaftet wurde zeigte ein höheres Potenzial N_2O zu emittieren, aber im Gegensatz zum konventionellen Landbau konnte die Funktionalität der N_2O-Reduktase langfristig und ohne zusätzliche Kalkung durch einen stabilen Boden pH-Wert gewährleistet werden. Neben einer deutlichen Minderung von N_2O Emissionen bewirkte die Zugabe von Biokohle einen Anstieg an stickstofffixierenden und N_2O reduzierenden Bakterien. Im Feld konnte eine Veränderung der Gemeinschaftsstruktur von N_2O reduzierenden Bakterien über eine gesamte Vegetationsperiode festgestellt werden. Durch die Zugabe von Biokohle wurden jene N_2O reduzierenden Bakterien gefördert denen die genetische Fähigkeit N_2O zu produzieren fehlte und somit als N_2O Senke wirksam werden können.

Unter allen untersuchten Bodenbearbeitungsmethoden war die Zugabe von Biokohle die wirksamste Strategie N_2O Emissionen zu vermindern. Die Aktivität, Abundanz oder Struktur von N_2O reduzierenden Bakteriengemeinschaften konnte in allen Experimenten mit den N_2O Emissionen in Verbindung gebracht werden. Die deutet darauf hin, dass die Funktionalität dieser Gemeinschaft durch das Einwirken von Bodenbearbeitungsmethoden auf chemische und physikalische Bodeneigenschaften beeinflussbar ist. Daher scheint eine Fokussierung auf Bodenbearbeitungsmethoden welche biologische N_2O Reduktion fördert ein vielversprechender Ansatz zu sein N_2O Emissionen aus landwirtschaftlichen Böden zu verringern.

Introduction

N_2O emissions and nitrogen cycling

Nitrous oxide (N_2O) is a major greenhouse gas that also contributes to stratospheric ozone depletion (Ravishankara *et al.*, 2009). It is a trace gas with a current atmospheric concentration of 328 ppb (IPCC, 2013). In the last decades, concentrations were rising linearly and reached 121% of the preindustrial period. Due to its long atmospheric half life time of 114 years and its specific absorption spectra closing earths radiation window at wavelengths of 4.50 and 2.87 µm, N_2O has a global warming potential 298 times greater than CO_2 (IPCC, 2013). Dominant sources for N_2O emissions are closely related to microbial processes in soils, sediments and water bodies (Singh *et al.*, 2010a). Natural N_2O emissions from soil and oceans were stable over centuries and accounted for ~10.5 Tg N_2O-N $year^{-1}$ in 2006 (Syakila and Kroeze, 2011). In the same year anthropogenic N_2O emissions summed up to 8.3 Tg N_2O-N $year^{-1}$ being the main cause for increasing atmospheric N_2O concentrations (Syakila and Kroeze, 2011). It is estimated that anthropogenic N_2O emissions account for ~6% of the human induced increase in earth radiation (IPCC, 2013). Among anthropogenic sources, the agricultural sector is the biggest single contributor to N_2O emissions accounting for 5.3 Tg N_2O-N $year^{-1}$ (Syakila and Kroeze, 2011). Within the agricultural sector, N_2O from agriculturally managed soils is the most important source for greenhouse gases and closely linked to soil management practices like fertilization and tillage (Smith *et al.*, 2008). The amount and, to a lesser extent, speciation of N fertilization play important roles affecting the magnitude of N_2O emissions (Shcherbak *et al.*, 2014).

Increased N_2O emissions from agricultural soil are induced by human interventions in the nitrogen cycling. Since the invention of the Haber Bosch process the excessive use of mineral fertilizers in order to increase crop yields has raised global budgets of mineral N (Fowler *et al.*, 2013). High rates of N fertilization together with low nitrogen use efficiencies are the main causes for loss of N from agroecosystems via nitrate (NO_3^-) leaching or ammonia (NH_3) volatilization (Fowler *et al.*, 2013). High NO_3^- concentrations in surface waters are a threat to human health and can cause eutrophication. Similarly, dry and wet deposition of atmospheric N oxides (NO_x) and NH_3 leads to eutrophication and the loss of biodiversity in distant ecosystems. In fact, nitrogen cycling is one of the processes for which human interventions were identified to have exceeded planetary boundaries and severe consequences on ecosystem functioning are expected (Rockstrom *et al.*, 2009). Therefore, balancing N cycling and improving N use efficiency in agroecosystems while maintaining

productivity is a key challenge for the whole agricultural sector (Ollivier *et al.*, 2011). Developing strategies for N_2O mitigation is one major aspect within this challenging task (Thomson *et al.*, 2012).

Microbial processes in N cycling

Nitrogen fixation

N cycling in soils almost entirely depends on microbial activity and involves several processes under varying oxygenation conditions. Principally, N enters the microbial N cycle by fixation of atmospheric dinitrogen (N_2). This crucial step is performed by N fixing bacteria which can either occur free living in the soil or form symbiotic relationships with plants (e.g. legumes) (Canfield *et al.*, 2010). N fixation is catalyzed the nitrogenase enzyme. The catalytic subunit of this enzyme is encoded by the functional gene *nifH* (Reed *et al.*, 2011) (Figure 2). Since the functionality of this enzyme is inhibited under the presence of oxygen (O_2) enhanced N fixation by free living bacteria can be expected under O_2 limiting conditions (Vitousek *et al.*, 2002). N becomes available for other soil organisms in the form of ammonium (NH_4^+) by mineralization of plant material from symbiotically N-fixing plants or lysis of free living N fixing bacteria (Francis *et al.*, 2007).

Nitrification

Under oxic conditions, NH_4^+ is oxidized to NO_3^- in the process of nitrification. This process is a stepwise pathway divided in the two sub-processes ammonia oxidation and nitrite oxidation. The first and rate limiting step is the oxidation of NH_4^+ to hydroxylamine (NH_2OH) (Kelly *et al.*, 2011). This reaction is catalyzed by the ammonia monooxygenase and can be performed by ammonia oxidizing bacteria (AOB) and archaea (AOA) (Treusch *et al.*, 2005; Taylor *et al.*, 2012). The catalytic subunit of the ammonia monooxygenase is encoded by the functional gene *amoA*. NH_2OH can be further oxidized abiotically to N_2O which makes nitrification a direct source process for N_2O emissions (Butterbach-Bahl *et al.*, 2013; Otte *et al.*, 1999). NH_2OH can also be oxidized enzymatically to NO_2^- by ammonium oxidizing microorganisms via hydroxylamine oxidoreductase encoded by the functional gene *hao* (Braker and Conrad, 2011). In the successive process of nitrite oxidation NO_3^- is formed by oxidation of NO_2^- via nitrite oxidoreductase encoded by the functional gene *nxrA* (Poly *et al.*, 2008). Until recently is was wide recognized that the process of nitrite oxidation and ammonia oxidation are performed by phylogenetically different guilds (Vlaeminck *et al.*, 2011). However, species of the phylum *Nitrospira*, which is known for their genetic capability for nitrite oxidation, were detected that hosted functional genes for ammonium oxidation. This suggests that all nitrification steps can be

performed within one species in the newly named process of “comammox” (complete ammonium oxidation) (Daims *et al.*, 2015; van Kessel *et al.*, 2015).

Denitrification

Denitrification is a microbial respiratory process in which N oxides are used as alternative electron acceptor when O_2 is not available for aerobic respiration. In this process, NO_3^- is stepwise reduced to dinitrogen (N_2) with the obligatory intermediates NO_2^-, nitric oxide (NO) and N_2O. The first reduction step to NO_2^- is mediated by nitrate reductases encoded by the functional genes *napA* and *narG* (Braker and Conrad, 2011). The reduction of NO_2^- to NO, the key step in denitrification, is either catalyzed by a copper or cytochrome cd1 nitrite reductase encoded by the functional genes *nirK* and *nirS*, respectively (Philippot *et al.*, 2007). Subsequently, N_2O is produced by the reduction of NO catalyzed by a *norB*-encoded nitric oxide reductase (Braker and Conrad, 2011). NO_2^- reduction and NO reduction can also be performed by ammonia oxidizers, which leads to N_2O formation in the process named nitrifier-denitrification (Kool *et al.*, 2011). Most likely, the reduction of NO_2^- and NO is performed as detoxifying mechanism due to nitrosative stress (Philippot *et al.*, 2007). Nevertheless, nitrifier-denitrification is considered as independent N_2O producing process (Kool *et al.*, 2011). The last step in denitrification, the reduction of N_2O to N_2 is mediated by the nitrous oxide reductase encoded by the functional gene *nosZ* and the recently discovered atypical *nosZ-II* gene (Sanford *et al.*, 2012; Jones *et al.*, 2013). Since N_2O is produced as obligatory intermediate, the process of denitrification is widely recognized as one of the most important source process for N_2O (Zumft and Kroneck, 2006; Butterbach-Bahl *et al.*, 2013). It needs to be noted that the nitrous oxide reductase is highly O_2 and pH sensitive. Constricted functionality of the nitrous oxide reductase can lead to incomplete denitrification and N_2O formation (Butterbach-Bahl *et al.*, 2013). Yet, N_2O reduction is also the only known biological sink process for N_2O. Denitrifiers are defined as microbes which produce N_2O or N_2 and whose growth rates are coupled to the reduction of N oxides (Philippot *et al.*, 2007). Importantly, denitrification is a modular pathway and not all microbes involved possess the whole set of enzymatic systems (Graf *et al.*, 2014). Around a third of denitrifiers lack the functional gene *nosZ* and thus act as a source for N_2O (Philippot *et al.*, 2011). The occurrence of the atypical *nosZ* gene is phylogenetically widespread and many atypical *nosZ* bearing microbes lack antecedent denitrification genes for which they cannot be referred to as typical denitrifiers. However, due to their genetic capability to reduce N_2O these microbes can act as a sink for N_2O (Jones *et al.*, 2014).

Dissimilatory Nitrate Reduction to Ammonium (DNRA)

Similar to denitrification the process of DNRA transforms NO_3^- heterotrophically under anoxic conditions. After the initial reduction step to NO_2^-, NH_4^+ is produced by a cytochrome nitrite

reductase encoded by the functional gene *nrfA* (Welsh *et al.*, 2014). DNRA competes with denitrification for NO_3^- and organic C and there are indications that DNRA becomes the dominating NO_3^- reducing process under carbon rich and highly reducing environments (Schmidt *et al.*, 2011). Despite the ecological significance of this process, which maintains N within the ecosystem, the detailed mechanisms of this process are not well understood (Chen *et al.*, 2015). Up to date it is not clear whether N_2O can be produced in the process of DNRA (Butterbach-Bahl *et al.*, 2013).

Anaerobic Ammonium Oxidation (Anammox)

During the anammox process, NH_4^+ is oxidized to N_2 using NO_2^- as electron acceptor. The discovery of the anammox proofed the existence of an ammonium oxidizing process under anoxic conditions and an alternative source for N_2 apart from heterotrophic denitrification (Francis *et al.*, 2007). Although the exact pathway is not completely understood, the hydrazine oxidoreductase, encoded by the functional gene *hzo,* seems to mediate the key reaction within this process (Lam and Kuypers, 2011). There are also indications that N_2O can be formed in the course of this process (Harris *et al.*, 2015).

Factors controlling N_2O emissions from soils

N_2O emissions often occur under changing biogeochemical conditions and show large temporal and spatial variability (Butterbach-Bahl *et al.*, 2013). Factors controlling N_2O emissions can be classified as proximal and distal controls which influence N_2O emissions either directly or indirectly (Wallenstein *et al.*, 2006; Braker and Conrad, 2011).

Proximal controls include soil pH, availability of C and N species as well as the oxygenation status of the soil. The O_2 concentration in soils largely depends on the water content and is often expressed by water filled pore space (WFPS). Since soil aeration status determines the predominant N cycling process it seems plausible that this parameter was identified as main driver for N_2O emissions on a regional scale (Jungkunst *et al.*, 2006). Principally, high water contents favor anoxic processes like denitrification, DNRA and anammox (Butterbach-Bahl *et al.*, 2013). Yet, it needs to be noted that oxic and anoxic processes can occur even within one soil aggregate and denitrification can be a major source for N_2O emissions even at WFPS around 80% (Butterbach-Bahl *et al.*, 2013). At a WFPS below 70% the relative importance of NH_4^+ as source for N_2O increases and nitrification and nitrifier-denitrification becomes the major source for N_2O emissions (Kool *et al.*, 2011). Apart from soil oxygenation status also the availability and speciation of N affects the magnitude of N_2O emissions. Levels of produced N_2O originating from NH_4^+ are several orders of magnitudes lower (10^3-10^6) compared to NO_3^- derived N_2O (Canfield *et al.*, 2010; Braker and Conrad, 2011). In

agroecosystems N_2O emissions often occur after N fertilization which makes fertilization rate the best single predictor for N_2O emissions from agricultural soils (Shcherbak *et al.*, 2014). N_2O emissions increase exponentially with increasing fertilization rates which further highlights the importance of efficient N use in agroecosystems (Shcherbak *et al.*, 2014). Since heterotrophic N cycling processes like denitrification require organic C as electron donors, the availability of C is considered as another proximal control of N_2O emissions (Wallenstein *et al.*, 2006). In fact the amount of available C often limits denitrification and any factor influencing C mineralization rates (e.g. root exudation, incorporation of crop residues, temperature) can have a major impact on denitrification rates (Saggar *et al.*, 2013). Generally, it is assumed that increased availability of C also increases N_2O emissions from denitrification (Bhandral *et al.*, 2007; Saggar *et al.*, 2013). Yet, it needs to be considered that this only holds true when NO_3^- concentrations are not limiting. Under NO_3^- shortage and when C is easily available, N_2O reduction can lower N_2O emissions and N_2O/N_2O+N_2 product ratios (Miller *et al.*, 2008; Senbayram *et al.*, 2012). N_2O reduction is impeded at low soil pH (Saggar *et al.*, 2013; Baggs *et al.*, 2010). A low pH was shown to impede correct folding of the nitrous oxide reductase enzyme in *Paracoccus denitrificans* and high N_2O emissions were thus ascribed to dysfunctional nitrous oxide reductases (Bergaust *et al.*, 2010). I was also suggested that a low soil pH favors fungal denitrification which would increase N_2O emissions since fungi generally lack genetic capability for N_2O reduction (Saggar *et al.*, 2013).

Distal controls are of N_2O emissions are less dynamic and affect N_2O emissions indirectly. For example soil texture is a major soil parameter that influences soil pore space and soil hydrology (Wallenstein *et al.*, 2006). Consequently, clay rich soils tend to exhibit rather reducing conditions compared to sandy soils. Soil texture also affects other proximal controls of N_2O emission since high clay contents relate to increased organic C stocks and N retention (Grüneberg *et al.*, 2013; Gaines and Gaines, 1994). It was also shown that soil texture affects richness and diversity of bacterial communities (Carson *et al.*, 2010). Since the N cycling is mainly mediated by microbial activity the size, metabolic activity and structure of the functional communities involved in N_2O production and reduction can be considered as another distal control for N_2O emissions (Wallenstein *et al.*, 2006). Denitrifier abundance has been related to N_2O emissions in a range of studies (Chen *et al.*, 2015; Tatti *et al.*, 2014; Morales *et al.*, 2010). Yet, it needs to be noted that this relationship is not straightforward since quantifying sole size of a functional community does not comprise a measure for microbial activity and efficiency of enzymatic reactions (Bier *et al.*, 2015). Consequently, other studies found no significant relationship between functional gene abundances and N_2O emissions (Henderson *et al.*, 2010; Dandie *et al.*, 2011). Apart from community size also the structure of

functional guilds can have a great impact on its functionality. It was shown that community composition of nitrous oxide reducing bacteria was a major driver affecting N_2O/N_2O+N_2 product ratios in annual and perennial cropping systems (Domeignoz-Horta *et al.*, 2015). In turn, the size and structure of microbial communities can be influence by soil management practices. This term summarizes all soil related techniques used for crop cultivation like tillage, fertilization, incorporation of crop residues, crop rotation and pest control. Soil management practice has often been described in terms of changes in environmental conditions and availability of nutrients which can affect microbial communities in various ways. For example, land use intensity and fertilization regime can induce a shift in size and structure of the denitrifier community (Hallin *et al.*, 2009; Tatti *et al.*, 2014; Meyer *et al.*, 2013). Managing soil microbiology by adapting soil management practice is currently considered as one of the most promising approaches to mitigate N_2O emissions (Thomson *et al.*, 2012). Yet, currently we lack a detailed understanding of how different soil management practices affect N_2O emissions and functional communities involved in N_2O production and reduction.

Soil management practices

A range of soil management practices are currently debated as possible measures for N_2O mitigation. The major challenge in identifying effective mitigation strategies for N_2O emissions is the complex nature of N_2O producing and reducing processes that are sensitive to a wide range of factors as described above. Spatial and temporal variability of these factors often impede reliable extrapolation of research findings on N_2O emissions to other locations or times (Venterea *et al.*, 2012). Not surprisingly, lowering fertilizer N input is probably the only commonly accepted strategy for N_2O mitigation across all pedoclimatic conditions (Venterea *et al.*, 2012). Yet, from the view of food production decreasing N inputs often result in lower crop yields. In order to tackle this issue, a major demand of future climate smart agriculture is a high nitrogen use efficiency (Thomson *et al.*, 2012). In this thesis reduced tillage, organic farming and the amendment of biochar are in investigated. Organic farming and reduced tillage systems are already widely used in agricultural practice while the amendment of biochar is gaining attention among researchers and farmers. In the following the current knowledge about the impact of these soil management practices on mechanisms of N_2O emission N_2O production and reduction will be summarized.

Reduced Tillage

Reducing tillage intensity is widely discussed as a management tool to prevent soil erosion, increase soil quality and possibly to mitigate N_2O emissions (Derpsch *et al.*, 2010; Reay *et al.*, 2012). In no-till

systems, weed control is mostly achieved by the intense use of pesticides (Gattinger *et al.*, 2014). Since the use of agrochemicals is not an option in organic systems, reduced tillage was developed as method which also tackles the issue of weed control by superficially working the soil in a non-inversive manner (Mäder and Berner, 2012). Reducing tillage intensity leads to the accumulation of organic carbon in the upper few cm of the soil profile, while deeper soil layers are depleted in organic carbon compared to conventional tillage (Luo *et al.*, 2010). Thus, no net effect of reduced tillage on C sequestration can be expected (Powlson *et al.*, 2014; Luo *et al.*, 2010). Reducing tillage intensity was repeatedly suggested as tool to mitigate N_2O emissions (Thomson *et al.*, 2012; Reay *et al.*, 2012). However, a recent meta-analysis reported no clear effects of no-till or reduced tillage systems on N_2O emissions (van Kessel *et al.*, 2013). While several field studies claimed N_2O mitigation due to reduced tillage (Mosier *et al.*, 2006; Gregorich *et al.*, 2008) other studies showed no effect or increased N_2O emissions (Ball *et al.*, 1999; Venterea *et al.*, 2005). It was therefore suggested that reduced tillage becomes an effective tool for N_2O mitigation only when it is applied for at least 10 years (van Kessel *et al.*, 2013). In the long-term, it was proposed that the development of an improved soil structure might prevent local anaerobiosis within the soil profile (Six *et al.*, 2004). Also the type and placement of fertilizers was shown to influence the N_2O mitigation potential under reduced tillage. N fertilizer placed <5 cm soil depth significantly reduced N_2O emissions under reduced tillage (van Kessel *et al.*, 2013). Up to date there is little knowledge how reduced tillage influences functional communities involved in N_2O production and reduction. Studies investigating the impact of reduced tillage intensity on N cycling microbial communities are scare and mainly focus on denitrifiers (Baudoin *et al.*, 2009; Melero *et al.*, 2011). For this thesis the reduced tillage field trial in Frick served as research platform in order to investigate the impact of tillage systems on fertilizer induced N_2O emissions and the underlying functional microbial communities involved in N cycling. The field trial was established in autumn 2002 and compares conventional tillage with reduced tillage under organic agricultural practice (Mäder and Berner, 2012).

Organic Agriculture

In organic agricultural practice the use of synthetic fertilizers and pest control is prohibited. In order to achieve high crop yield organic agriculture aims on closing nutrient cycles and increasing efficiency of food production (Smith *et al.*, 2015). One important issue concerning organic agricultural systems is the so called “yield gap”. Dependent on the crop organic systems produce around 80% of conventional yields, due to lower fertilizer inputs (de Ponti *et al.*, 2012). Yet, energy and nutrient use efficiency of organic systems clearly outcompete their conventional counterparts (Smith *et al.*, 2015). Core practices of organic agricultural systems are incorporation of legumes and cover cops in the

crop rotation and the application of organic fertilizers, such as compost, slurry or stacked manure (Diacono and Montemurro, 2010). These practices impact soils C budgets and a recent global meta-analysis showed organic soil management to enhance topsoil C stocks compared to conventional systems (Gattinger *et al.*, 2012). However, it is not clear whether these differences reflect a net gain or a reduced loss of organic C in organically managed soils (Gattinger *et al.*, 2012). The incorporation of legumes and cover crops within the crop rotation fundamentally affect N cycling in organic systems. While organic N from crop residues of legumes can be considered as net N input for the next cropping season, cover crops in organic systems catch N in fallow periods and thus prevent loss of N through leaching or erosion. Consequently, organically managed systems are designed to catch and keep N within the agroecosystem and are less dependent on N fertilization compared to conventionally managed systems (Smith *et al.*, 2015). Furthermore the use of organic fertilizers results in a slow release of mineralized N and thus lowers the availability of N for plants and microbes (Dawson *et al.*, 2008). A recent meta-analysis compared N inputs with N_2O emissions in agricultural systems and found an exponential increase of N_2O emissions with increasing N fertilization rates within one cropping season (Shcherbak *et al.*, 2014). However, due to the diversified sources of N, the same relationship does not hold true for organically managed systems (Skinner *et al.*, 2014). A global meta-analysis showed that organic systems decreased area scaled N_2O emissions while increasing yield scaled N_2O emissions due to the yield gap in organic systems (Skinner *et al.*, 2014). Since it is generally acknowledged that C availability affects denitrification rates, N_2O emissions in organically managed soil might be altered by enhanced C stocks (Morley and Baggs, 2010). Several studies suggested that N_2O emissions might increase due to enhanced availability of C sources for heterotrophic denitrifiers (Cayuela *et al.*, 2010; Bhandral *et al.*, 2007; Flessa and Beese, 2000). However, also the opposite effect was reported and increased N_2O reduction was hypothesized to mitigate N_2O emissions after addition of organic C (Miller *et al.*, 2009). Regular addition of organic farmyard manure in organic systems was often proposed to induce a shift in bacterial and fungal community composition (Hartmann *et al.*, 2006; Hartmann *et al.*, 2014; Wang *et al.*, 2016). And amount and type of N fertilizers were shown to impact abundance of denitrifiers (Hallin *et al.*, 2009; Tatti *et al.*, 2014). Increased potential denitrification rates after manure addition compared to inorganic N fertilization was observed to correlate with denitrifier abundance (Clark *et al.*, 2012). However, in another field experiments manure addition increased abundance of denitrifiers without affecting N_2O emissions (Tatti *et al.*, 2014). While there are several indications that organic fertilization increases denitrifier abundance a thus soil capacity for denitrification it is not clear whether enhanced N_2O reduction might counterbalance this effect by lowering $N_2O/(N_2O+N_2)$ product ratios. In order to tackle this question the DOK field trial served as research platform to

investigate the impact of organic soil management practice on N_2O producing and reducing microbial communities and $N_2O/(N_2O+N_2)$. This trail was established in 1978 and compares organic and conventional farming systems (Fließbach *et al.*, 2007). Typical for Swiss agricultural practice all farming systems are managed according the same crop rotation and farming systems in this specific field trial mainly differ in pest control and the amount and type of fertilization.

Biochar amendment

Biochar is defined as a carbon rich product of combustion of biomass under oxygen limited conditions for application to soils (Verheijen *et al.*, 2010). Its use as soil amendment is recently discussed due to its proposed beneficial effects on soil quality and as option to mitigate climate change (Woolf *et al.*, 2010; Verheijen *et al.*, 2010). Depended on the feedstock the properties of biochar can vary, but all biochars share some common characteristics like low pH, a high content of aromatic carbon structures and a high surface area (Atkinson *et al.*, 2010). The potential of biochar to mitigate climate change is largely based on its recalcitrant nature, which slows the rate at which photosynthetically fixed C is released in the form of CO_2 (Cheng *et al.*, 2008). However, the use of biochar as soil amendment also holds other co-benefits for soil quality. It was shown that biochar can improve agricultural productivity, particularly in degraded soils with low fertility (Woolf *et al.*, 2010). Furthermore biochar can reduce leaching of nutrients and improve water holding capacity (Singh *et al.*, 2010b; Lehmann and Joseph, 2009). Apart from that, biochars potential to mitigate N_2O emissions was repeatedly demonstrated (Cayuela *et al.*, 2013; Yanai *et al.*, 2007). A recent meta-analysis showed that biochar reduced N_2O emissions across 30 studies by around 50% (Cayuela *et al.*, 2013). Yet, field studies showed a much higher variability and less consistent response to biochar addition compared to laboratory experiments (Cayuela *et al.*, 2013; Verhoeven and Six, 2014; Pereira *et al.*, 2015). Up to date the underlying mechanism of biochar mitigating N_2O emissions is poorly understood. Since biochar was shown to be redox active increased heterotroph activity around biochar particles might stimulate formation of anoxic microsites and thus favor conditions for microbes performing complete denitrification (Van Zwieten *et al.*, 2014; Kappler *et al.*, 2014). Furthermore, the addition of biochar might increase soil pH which was shown to enhance N_2O reduction (Baggs *et al.*, 2010). Also sorption of N_2O onto biochar particles and beneficial effects on soil structure enhancing soil aeration could be possible mechanisms contributing to N_2O mitigation after biochar addition (Cornelissen *et al.*, 2013; Harter *et al.*, 2016a; Hagemann *et al.*, 2016). Recently, it was shown in a laboratory experiment that biochar stimulates a shift in community composition of N_2O reducers, favoring microbes either specialized on N_2O reduction or complete denitrification (Harter *et al.*, 2016b). Yet, it is still unclear whether this observation also holds true

under field conditions and on the long term. A newly established biochar field trial at Agroscope Reckenholz served as research platform to investigate the impact of biochar on community structure of N_2O reducing bacteria across a vegetation period. In order to test the liming effect of the biochar as potential mechanism for reduced N_2O emission, an additional liming treatment was included in this field trial.

Objectives of the thesis

The overarching objective of this thesis was to investigate the underlying mechanisms of N_2O production and reduction in agricultural soils under different soil management practice. For this issue it is crucial to investigate functional guilds involved in N cycling since they ultimately perform redox reactions resulting in N_2O production and reduction. Although the impact of single parameters on microbial N cycling and N_2O emissions is quite well understood there is a fundamental knowledge gap when it comes to complex soil management practices. Therefore, incubation experiments under controlled conditions as well as field experiments were conducted.

The first experiment (Chapter 2) aimed at assessing the impact of reduced tillage on N_2O emissions and nitrifying and denitrifying microbial communities under different fertilization regimes. Since reducing tillage intensity changes soil geochemical parameters in dependency of soil depth soil samples from two different soil depths were incubated. Furthermore, fertilization under field conditions was mimicked by incubating the soil under rather oxic conditions. The main objectives of this experiment were:

I. To quantify the impact of tillage system on N_2O emissions and N transforming processes in dependency of soil depth and fertilization strategy
II. To quantify the microbial potential for nitrification, denitrification and N_2O reduction in soils under reduced and conventional tillage

The second experiment (Chapter 3) aimed at investigating N_2O production and reduction potential of soils under organic and conventional soil management practice. In order to promote denitrification and N_2O reduction soils were incubated under oxygen limited conditions mimicking situations occurring after a heavy rain event. N_2O reduction and the origin of N_2 and N_2O emissions were determined using ^{15}N tracing techniques. The main objectives of this experiment were:

I. To quantify N_2O emission and N_2O reduction potential of soils under organic and conventional farming

II. To assess the functional impact of denitrifier gene abundance and expression on N_2O production and reduction under organic and conventional farming

The third experiment (Chapter 4) was performed in order to assess the impact of biochar amendment on N cycling and aimed at identifying links between mitigation of N_2O emissions and functional microbial communities involved in N fixation, nitrification, and denitrification. Since highest N_2O emissions often occur after heavy rain fall soils were incubated under oxygen limited conditions. The main objectives of this experiment were:

I. To quantify the impact of biochar amendment on N_2O emissions and the dynamics of mineral nitrogen species
II. To quantify the genetic capability of the microbial community to perform nitrogen fixation, nitrification and denitrification in biochar amended soil

The forth experiment (Chapter 5) aimed at assessing community structure of N_2O reducers across a vegetation period after biochar amendment under field conditions. In order to assess the pH effect of biochar addition to soils a limed control was included in this field experiment. The main objectives of this experiment were.

I. To identify biochar and lime induced shifts in community composition of N_2O reducers across a vegetation period
II. To identify indicator species driving N_2O mitigation after biochar amendment under field conditions

References

Atkinson, C. J., Fitzgerald, J. D. & Hipps, N. A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil* 337(1-2): 1-18. http://dx.doi.org/10.1007/s11104-010-0464-5

Baggs, E., Smales, C. & Bateman, E. (2010). Changing pH shifts the microbial sourceas well as the magnitude of N2O emission from soil. *Biology and Fertility of Soils* 46(8): 793-805. http://dx.doi.org/10.1007/s00374-010-0484-6

Ball, B. C., Scott, A. & Parker, J. P. (1999). Field N2O, CO2 and CH4 fluxes in relation to tillage, compaction and soil quality in Scotland. *Soil and Tillage Research* 53(1): 29-39. http://dx.doi.org/10.1016/S0167-1987(99)00074-4

Baudoin, E., Philippot, L., Cheneby, D., Chapuis-Lardy, L., Fromin, N., Bru, D., Rabary, B. & Brauman, A. (2009). Direct seeding mulch-based cropping increases both the activity and the abundance of denitrifier communities in a tropical soil. *Soil Biology and Biochemistry* 41(8): 1703-1709. http://dx.doi.org/10.1016/j.soilbio.2009.05.015

Bergaust, L., Mao, Y., Bakken, L. R. & Frostegard, A. (2010). Denitrification Response Patterns during the Transition to Anoxic Respiration and Posttranscriptional Effects of Suboptimal pH on Nitrogen Oxide Reductase in Paracoccus denitrificans. *Applied and Environmental Microbiology* 76(19): 6387-6396. http://dx.doi.org/10.1128/aem.00608-10

Bhandral, R., Bolan, N., Saggar, S. & Hedley, M. (2007). Nitrogen transformation and nitrous oxide emissions from various types of farm effluents. *Nutrient Cycling in Agroecosystems* 79(2): 193-208. http://dx.doi.org/10.1007/s10705-007-9107-5

Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T., Nemergut, D., Osborne, B. B., Ruiz-González, C., Schimel, J. P., Waldrop, M. P. & Wallenstein, M. D. (2015). Linking microbial community structure and microbial processes: an empirical and conceptual overview. *FEMS Microbiology Ecology*.http://dx.doi.org/10.1093/femsec/fiv113

Braker, G. & Conrad, R. (2011). Diversity, structure, and size of N2O-producing microbial communities in soils-what matters for their functioning? *Advances in Applied Microbiology* 75: 33-70. http://dx.doi.org/10.1016/B978-0-12-387046-9.00002-5

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society* 368(1621). http://dx.doi.org/10.1098/rstb.2013.0122

Canfield, D. E., Glazer, A. N. & Falkowski, P. G. (2010). The Evolution and Future of Earth's Nitrogen Cycle. *Science* 330(6001): 192-196. http://dx.doi.org/10.1126/science.1186120

Carson, J. K., Gonzalez-Quiñones, V., Murphy, D. V., Hinz, C., Shaw, J. A. & Gleeson, D. B. (2010). Low Pore Connectivity Increases Bacterial Diversity in Soil. *Applied and Environmental Microbiology* 76(12): 3936-3942.http://dx.doi.org/10.1128/aem.03085-09

Cayuela, M. L., Oenema, O., Kuikman, P. J., Bakker, R. R. & Van Groenigen, J. W. (2010). Bioenergy by-products as soil amendments? Implications for carbon sequestration and greenhouse gas emissions. *GCB Bioenergy* 2(4): 201-213. http://dx.doi.org/10.1111/j.1757-1707.2010.01055.x

Cayuela, M. L., van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A. & Sánchez-Monedero, M. A. (2013). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agriculture, Ecosystems & Environment* 191: 5-16.http://dx.doi.org/10.1016/j.agee.2013.10.009

Chen, Z., Wang, C., Gschwendtner, S., Willibald, G., Unteregelsbacher, S., Lu, H., Kolar, A., Schloter, M., Butterbach-Bahl, K. & Dannenmann, M. (2015). Relationships between denitrification

gene expression, dissimilatory nitrate reduction to ammonium and nitrous oxide and dinitrogen production in montane grassland soils. *Soil Biology and Biochemistry* 87: 67-77. http://dx.doi.org/10.1016/j.soilbio.2015.03.030

Cheng, C. H., Lehmann, J., Thies, J. E. & Burton, S. D. (2008). Stability of black carbon in soils across a climatic gradient. *Journal of Geophysical Research-Biogeosciences* 113(G02027). http://dx.doi.org/10.1029/2007jg000642

Clark, I. M., Buchkina, N., Jhurreea, D., Goulding, K. W. T. & Hirsch, P. R. (2012). Impacts of nitrogen application rates on the activity and diversity of denitrifying bacteria in the Broadbalk Wheat Experiment. *Philosophical Transactions of the Royal Society* 367(1593): 1235-1244.http://dx.doi.org/10.1098/rstb.2011.0314

Cornelissen, G., Rutherford, D. W., Arp, H. P. H., Dörsch, P., Kelly, C. N. & Rostad, C. E. (2013). Sorption of Pure N2O to Biochars and Other Organic and Inorganic Materials under Anhydrous Conditions. *Environmental Science & Technology* 47(14): 7704-7712. http://dx.doi.org/10.1021/es400676q

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H. & Wagner, M. (2015). Complete nitrification by Nitrospira bacteria. *Nature* 528(7583): 504-509. http://dx.doi.org/10.1038/nature16461

Dandie, C. E., Wertz, S., Leclair, C. L., Goyer, C., Burton, D. L., Patten, C. L., Zebarth, B. J. & Trevors, J. T. (2011). Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. *FEMS Microbiology Ecology* 77(1): 69-82. http://dx.doi.org/10.1111/j.1574-6941.2011.01084.x

Dawson, J. C., Huggins, D. R. & Jones, S. S. (2008). Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. *Field Crops Research* 107(2): 89-101.http://dx.doi.org/10.1016/j.fcr.2008.01.001

de Ponti, T., Rijk, B. & van Ittersum, M. K. (2012). The crop yield gap between organic and conventional agriculture. *Agricultural Systems* 108: 1-9. http://dx.doi.org/10.1016/j.agsy.2011.12.004

Derpsch, R., Friedrich, T., Kassam, A. & Li, H. (2010). Current Status of Adoption of No-till Farming in the World and Some of its Main Benefits. *International Journal of Agricultural and Biological Engineering* 3: 1-26. http://dx.doi.org/10.3965/j.issn.1934-6344.2010.01.0-0

Diacono, M. & Montemurro, F. (2010). Long-term effects of organic amendments on soil fertility. A review. *Agronomy for Sustainable Development* 30(2): 401-422.http://dx.doi.org/10.1051/agro/2009040

Domeignoz-Horta, L. A., Spor, A., Bru, D., Breuil, M.-C., Bizouard, F., Léonard, J. & Philippot, L. (2015). The diversity of the N2O reducers matters for the N2O:N2 denitrification end-product ratio across an annual and a perennial cropping system. *Frontiers in Microbiology* 6: 971. http://dx.doi.org/10.3389/fmicb.2015.00971

Flessa, H. & Beese, F. (2000). Laboratory estimates of trace gas emissions following surface application and injection of cattle slurry. *Journal of Environmental Quality* 29(1): 262-268. http://dx.doi.org/10.2134/jeq2000.00472425002900010033x

Fließbach, A., Oberholzer, H.-R., Gunst, L. & Mäder, P. (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. *Agriculture, Ecosystems & Environment* 118(1–4): 273-284. http://dx.doi.org/10.1016/j.agee.2006.05.022

Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., Sheppard, L. J., Jenkins, A., Grizzetti, B., Galloway, J. N., Vitousek, P., Leach, A., Bouwman, A. F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M. & Voss, M. (2013). The global nitrogen cycle in the twenty-first century. *Philosophical Transactions of the Royal Society* 368(1621). http://dx.doi.org/10.1098/rstb.2013.0164

Francis, C. A., Beman, J. M. & Kuypers, M. M. M. (2007). New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. *The ISME Journal* 1(1): 19-27.http://dx.doi.org/10.1038/ismej.2007.8

Gaines, T. P. & Gaines, S. T. (1994). Soil texture effect on nitrate leaching in soil percolates. *Communications in Soil Science and Plant Analysis* 25(13-14): 2561-2570.http://dx.doi.org/10.1080/00103629409369207

Gattinger, A., Jawtusch, J., Muller, A. & Mäder, P. (2014).Climate change and agriculture: No-till agriculture - a climate smart solution. Vol. Report No.2, pp. 24 Mozartstraße 9, 52064 Aachen, Germany: Bischöfliches Hilfswerk, MISEREORe.V.

Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N. E.-H. & Niggli, U. (2012). Enhanced top soil carbon stocks under organic farming. *Proceedings of the National Academy of Sciences* 109(44): 18226-18231. http://dx.doi.org/10.1073/pnas.1209429109

Graf, D. R. H., Jones, C. M. & Hallin, S. (2014). Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N2O Emissions. *Plos One* 9(12): e114118. http://dx.doi.org/10.1371/journal.pone.0114118

Gregorich, E. G., Rochette, P., St-Georges, P., McKim, U. F. & Chan, C. (2008). Tillage effects on N2O emission from soils under corn and soybeans in Eastern Canada. *Canadian Journal of Soil Science* 88(2): 153-161. http://dx.doi.org/10.4141/CJSS06041

Grüneberg, E., Schöning, I., Hessenmöller, D., Schulze, E. D. & Weisser, W. W. (2013). Organic layer and clay content control soil organic carbon stocks in density fractions of differently managed German beech forests. *Forest Ecology and Management* 303: 1-10.http://dx.doi.org/10.1016/j.foreco.2013.03.014

Hagemann, N., Harter, J., Kaldamukova, R., Guzman-Bustamante, I., Ruser, R., Graeff, S., Kappler, A. & Behrens, S. (2016). Does soil aging affect the N2O mitigation potential of biochar? A combined microcosm and field study. *GCB Bioenergy*: n/a-n/a.10.1111/gcbb.12390

Hallin, S., Jones, C. M., Schloter, M. & Philippot, L. (2009). Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *ISME J* 3(5): 597-605.http://dx.doi.org/10.1038/ismej.2008.128

Harris, E., Joss, A., Emmenegger, L., Kipf, M., Wolf, B., Mohn, J. & Wunderlin, P. (2015). Isotopic evidence for nitrous oxide production pathways in a partial nitritation-anammox reactor. *Water Research* 83: 258-270.http://dx.doi.org/10.1016/j.watres.2015.06.040

Harter, J., Guzman-Bustamante, I., Kuehfuss, S., Ruser, R., Well, R., Spott, O., Kappler, A. & Behrens, S. (2016a). Gas entrapment and microbial N2O reduction reduce N2O emissions from a biochar-amended sandy clay loam soil. *Scientific Reports* 6: 39574. http://dx.doi.org/10.1038/srep39574

Harter, J., Weigold, P., El-Hadidi, M., Huson, D. H., Kappler, A. & Behrens, S. (2016b). Soil biochar amendment shapes the composition of N2O-reducing microbial communities. *Science of The Total Environment* 562: 379-390.http://dx.doi.org/10.1016/j.scitotenv.2016.03.220

Hartmann, M., Fliessbach, A., Oberholzer, H.-R. & Widmer, F. (2006). Ranking the magnitude of crop and farming system effects on soil microbial biomass and genetic structure of bacterial communities. *FEMS Microbiology Ecology* 57(3): 378-388. http://dx.doi.org/10.1111/j.1574-6941.2006.00132.x

Hartmann, M., Frey, B., Mayer, J., Mader, P. & Widmer, F. (2014). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal* 9: 1177-1194. http://dx.doi.org/10.1038/ismej.2014.210

Henderson, S. L., Dandie, C. E., Patten, C. L., Zebarth, B. J., Burton, D. L., Trevors, J. T. & Goyer, C. (2010). Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions and denitrification in anoxic soil microcosms amended with glucose and plant residues. *Applied and Environmental Microbiology*: AEM.02993-02909. http://dx.doi.org/10.1128/aem.02993-09

IPCC (2013). *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Jones, C., Graf, D., Bru, D., Philippot, L. & Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *The ISME Journal* 7(2): 417-426. http://dx.doi.org/10.1038/ismej.2012.125

Jones, C. M., Spor, A., Brennan, F. P., Breuil, M.-C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S. & Philippot, L. (2014). Recently identified microbial guild mediates soil N2O sink capacity. *Nature Climate Change* 4: 801-805. http://dx.doi.org/10.1038/nclimate2301

Jungkunst, H. F., Freibauer, A., Neufeldt, H. & Bareth, G. (2006). Nitrous oxide emissions from agricultural land use in Germany— a synthesis of available annual field data. *Journal of Plant Nutrition and Soil Science* 169(3): 341-351. http://dx.doi.org/10.1002/jpln.200521954

Kappler, A., Wuestner, M. L., Ruecker, A., Harter, J., Halama, M. & Behrens, S. (2014). Biochar as an Electron Shuttle between Bacteria and Fe(III) Minerals. *Environmental Science & Technology Letters* 1(8): 339-344. http://dx.doi.org/10.1021/ez5002209

Kelly, J. J., Policht, K., Grancharova, T. & Hundal, L. S. (2011). Distinct Responses in Ammonia-Oxidizing Archaea and Bacteria after Addition of Biosolids to an Agricultural Soil. *Applied and Environmental Microbiology* 77(18): 6551-6558.http://dx.doi.org/10.1128/aem.02608-10

Kool, D. M., Dolfing, J., Wrage, N. & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry* 43(1): 174-178. http://dx.doi.org/10.1016/j.soilbio.2010.09.030

Lam, P. & Kuypers, M. M. M. (2011). Microbial Nitrogen Cycling Processes in Oxygen Minimum Zones. *Annual Review of Marine Science* 3(1): 317-345. http://dx.doi.org/10.1146/annurev-marine-120709-142814

Lehmann, J. & Joseph, S. (2009).Biochar for environmental management: An introduction. In *Biochar for environmental management, science and technology*, 1-12 (Eds J. Lehmann and S. Joseph). London: Earthscan.

Luo, Z. K., Wang, E. L. & Sun, O. J. (2010). Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. *Agriculture, Ecosystems and Environment* 139(1-2): 224-231. http://dx.doi.org/10.1016/j.agee.2010.08.006

Mäder, P. & Berner, A. (2012). Development of reduced tillage systems in organic farming in Europe. *Renewable Agriculture and Food Systems* 27(1): 7-11. http://dx.doi.org/10.1017/S1742170511000470

Melero, S., Perez-de-Mora, A., Manuel Murillo, J., Buegger, F., Kleinedam, K., Kublik, S., Vanderlinden, K., Moreno, F. & Schloter, M. (2011). Denitrification in a vertisol under long-term tillage and no-tillage management in dryland agricultural systems: Key genes and potential rates. *Applied Soil Ecology* 47(3): 221-225. http://dx.doi.org/10.1016/j.apsoil.2010.12.003

Meyer, A., Focks, A., Radl, V., Keil, D., Welzl, G., Schöning, I., Boch, S., Marhan, S., Kandeler, E. & Schloter, M. (2013). Different Land Use Intensities in Grassland Ecosystems Drive Ecology of Microbial Communities Involved in Nitrogen Turnover in Soil. *Plos One* 8(9): e73536.http://dx.doi.org/10.1371/journal.pone.0073536

Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C. & Trevors, J. T. (2008). Crop residue influence on denitrification, N2O emissions and denitrifier community abundance in soil. *Soil Biology and Biochemistry* 40(10): 2553-2562. http://dx.doi.org/10.1016/j.soilbio.2008.06.024

Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C. & Trevors, J. T. (2009). Influence of Liquid Manure on Soil Denitrifier Abundance, Denitrification, and Nitrous Oxide Emissions. *Soil Science Society of America Journal* 73(3): 760-768. http://dx.doi.org/10.2136/sssaj2008.0059

Morales, S. E., Cosart, T. & Holben, W. E. (2010). Bacterial gene abundances as indicators of greenhouse gas emission in soils. *The ISME Journal* 4(6): 799-808. http://dx.doi.org/10.1038/ismej.2010.8

Morley, N. & Baggs, E. M. (2010). Carbon and oxygen controls on N2O and N2 production during nitrate reduction. *Soil Biology and Biochemistry* 42(10): 1864-1871.http://dx.doi.org/10.1016/j.soilbio.2010.07.008

Mosier, A. R., Halvorson, A. D., Reule, C. A. & Liu, X. J. (2006). Net global warming potential and greenhouse gas intensity in irrigated cropping systems in northeastern Colorado. *Journal of Environmental Quality* 35(4): 1584-1598. http://dx.doi.org/10.2134/jeq2005.0232

Ollivier, J., Töwe, S., Bannert, A., Hai, B., Kastl, E.-M., Meyer, A., Su, M. X., Kleineidam, K. & Schloter, M. (2011). Nitrogen turnover in soil and global change. *FEMS Microbiology Ecology* 78(1): 3-16. http://dx.doi.org/10.1111/j.1574-6941.2011.01165.x

Otte, S., Schalk, J., Kuenen, J. G. & Jetten, M. S. M. (1999). Hydroxylamine oxidation and subsequent nitrous oxide production by the heterotrophic ammonia oxidizer Alcaligenes faecalis. *Applied Microbiology and Biotechnology* 51(2): 255-261. http://dx.doi.org/10.1007/s002530051390

Pereira, E. I. P., Suddick, E. C., Mansour, I., Mukome, F. N. D., Parikh, S. J., Scow, K. & Six, J. (2015). Biochar alters nitrogen transformations but has minimal effects on nitrous oxide emissions in an organically managed lettuce mesocosm. *Biology and Fertility of Soils* 51(5): 573-582.10.1007/s00374-015-1004-5

Philippot, L., Andert, J., Jones, C. M., Bru, D. & Hallin, S. (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. *Global Change Biology* 17(3): 1497-1504. http://dx.doi.org/10.1111/j.1365-2486.2010.02334.x

Philippot, L., Hallin, S. & Schloter, M. (2007).Ecology of Denitrifying Prokaryotes in Agricultural Soil. In *Advances in Agronomy*, Vol. Volume 96, 249-305 (Ed L. S. Donald). http://dx.doi.org/10.1016/S0065-2113(07)96003-4.

Poly, F., Wertz, S., Brothier, E. & Degrange, V. (2008). First exploration of Nitrobacter diversity in soils by a PCR cloning-sequencing approach targeting functional gene nxrA. *FEMS Microbiology Ecology* 63(1): 132-140. http://dx.doi.org/10.1111/j.1574-6941.2007.00404.x

Powlson, D. S., Stirling, C. M., Jat, M. L., Gerard, B. G., Palm, C. A., Sanchez, P. A. & Cassman, K. G. (2014). Limited potential of no-till agriculture for climate change mitigation. *Nature Climate Change* 4(8): 678-683. http://dx.doi.org/10.1038/nclimate2292

Ravishankara, A. R., Daniel, J. S. & Portmann, R. W. (2009). Nitrous Oxide (N2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949): 123-125. http://dx.doi.org/10.1126/science.1176985

Reay, D. S., Davidson, E. A., Smith, K. A., Smith, P., Melillo, J. M., Dentener, F. & Crutzen, P. J. (2012). Global agriculture and nitrous oxide emissions. *Nature Climate Change* 2(6): 410-416.http://dx.doi.org/10.1038/nclimate1458

Reed, S. C., Cleveland, C. C. & Townsend, A. R. (2011). Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. *Annual Review of Ecology, Evolution, and Systematics* 42(1): 489-512. http://dx.doi.org/10.1146/annurev-ecolsys-102710-145034

Rockstrom, J., Steffen, W., Noone, K., Persson, A., Chapin, F. S., Lambin, E. F., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H. J., Nykvist, B., de Wit, C. A., Hughes, T., van der Leeuw, S., Rodhe, H., Sorlin, S., Snyder, P. K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R. W., Fabry, V. J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P. & Foley, J. A. (2009). A safe operating space for humanity. *Nature* 461(7263): 472-475.http://dx.doi.org/10.1038/461472a

Saggar, S., Jha, N., Deslippe, J., Bolan, N. S., Luo, J., Giltrap, D. L., Kim, D. G., Zaman, M. & Tillman, R. W. (2013). Denitrification and N2O:N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of The Total Environment* 465(0): 173-195. http://dx.doi.org/10.1016/j.scitotenv.2012.11.050

Sanford, R. A., Wagner, D. D., Wu, Q., Chee-Sanford, J. C., Thomas, S. H., Cruz-García, C., Rodríguez, G., Massol-Deyá, A., Krishnani, K. K., Ritalahti, K. M., Nissen, S., Konstantinidis, K. T. & Löffler, F. E. (2012). Unexpected nondenitrifier nitrous oxide reductase gene diversity and

abundance in soils. *Proceedings of the National Academy of Sciences* 109(48): 19709-19714. http://dx.doi.org/10.1073/pnas.1211238109

Schmidt, C. S., Richardson, D. J. & Baggs, E. M. (2011). Constraining the conditions conducive to dissimilatory nitrate reduction to ammonium in temperate arable soils. *Soil Biology and Biochemistry* 43(7): 1607-1611.http://dx.doi.org/10.1016/j.soilbio.2011.02.015

Senbayram, M., Chen, R., Budai, A., Bakken, L. & Dittert, K. (2012). N2O emission and the N2O/(N2O;N2) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. *Agriculture, Ecosystems & Environment* 147(0): 4-12.http://dx.doi.org/10.1016/j.agee.2011.06.022

Shcherbak, I., Millar, N. & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences*. http://dx.doi.org/10.1073/pnas.1322434111

Singh, B. K., Bardgett, R. D., Smith, P. & Reay, D. S. (2010a). Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology* 8(11): 779-790.http://dx.doi.org/10.1038/nrmicro2439

Singh, B. P., Hatton, B. J., Balwant, S., Cowie, A. L. & Kathuria, A. (2010b). Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of Environmental Quality* 39(4): 1224-1235. http://dx.doi.org/10.2134/jeq2009.0138

Six, J., Ogle, S. M., Jay breidt, F., Conant, R. T., Mosier, A. R. & Paustian, K. (2004). The potential to mitigate global warming with no-tillage management is only realized when practised in the long term. *Global Change Biology* 10(2): 155-160. http://dx.doi.org/10.1111/j.1529-8817.2003.00730.x

Skinner, C., Gattinger, A., Muller, A., Mäder, P., Flieβbach, A., Stolze, M., Ruser, R. & Niggli, U. (2014). Greenhouse gas fluxes from agricultural soils under organic and non-organic management — A global meta-analysis. *Science of The Total Environment* 468–469(0): 553-563.http://dx.doi.org/10.1016/j.scitotenv.2013.08.098

Smith, L. G., Williams, A. G. & Pearce, B. D. (2015). The energy efficiency of organic agriculture: A review. *Renewable Agriculture and Food Systems* 30(3): 280-301. http:/dx.doi.org/10.1017/S1742170513000471

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M. & Smith, J. (2008). Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society* 363(1492): 789-813. http://dx.doi.org/10.1098/rstb.2007.2184

Syakila, A. & Kroeze, C. (2011). The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management* 1(1): 17-26. http://dx.doi.org/10.3763/ghgmm.2010.0007

Tatti, E., Goyer, C., Chantigny, M., Wertz, S., Zebarth, B. J., Burton, D. L. & Filion, M. (2014). Influences of over winter conditions on denitrification and nitrous oxide-producing microorganism abundance and structure in an agricultural soil amended with different nitrogen sources. *Agriculture, Ecosystems & Environment* 183(0): 47-59.http://dx.doi.org/10.1016/j.agee.2013.10.021

Taylor, A. E., Zeglin, L. H., Wanzek, T. A., Myrold, D. D. & Bottomley, P. J. (2012). Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. *The ISME Journal* 6(11): 2024-2032.http://dx.doi.org/10.1038/ismej.2012.51

Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M. & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society* 367(1593): 1157-1168. http://dx.doi.org/10.1098/rstb.2011.0415

Treusch, A. H., Leininger, S., Kletzin, A., Schuster, S. C., Klenk, H. P. & Schleper, C. (2005). Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. *Environmental Microbiology* 7(12): 1985-1995. http://dx.doi.org/10.1111/j.1462-2920.2005.00906.x

van Kessel, C., Venterea, R., Six, J., Adviento-Borbe, M. A., Linquist, B. & van Groenigen, K. J. (2013). Climate, duration, and N placement determine N2O emissions in reduced tillage systems: a meta-analysis. *Global Change Biology* 19(1): 33-44. http://dx.doi.org/10.1111/j.1365-2486.2012.02779.x

van Kessel, M. A. H. J., Speth, D. R., Albertsen, M., Nielsen, P. H., Op den Camp, H. J. M., Kartal, B., Jetten, M. S. M. & Lücker, S. (2015). Complete nitrification by a single microorganism. *Nature* 528(7583): 555-559. http://dx.doi.org/10.1038/nature16459

Van Zwieten, L., Singh, B. P., Kimber, S. W. L., Murphy, D. V., Macdonald, L. M., Rust, J. & Morris, S. (2014). An incubation study investigating the mechanisms that impact N2O flux from soil following biochar application. *Agriculture, Ecosystems & Environment* 191: 53-63.http://dx.doi.org/10.1016/j.agee.2014.02.030

Venterea, R. T., Burger, M. & Spokas, K. A. (2005). Nitrogen oxide and methane emissions under varying tillage and fertilizer management. *Journal of Environmental Quality* 34(5): 1467-1477. http://dx.doi.org/10.2134/jeq2005.0018

Venterea, R. T., Halvorson, A. D., Kitchen, N., Liebig, M. A., Cavigelli, M. A., Grosso, S. J. D., Motavalli, P. P., Nelson, K. A., Spokas, K. A., Singh, B. P., Stewart, C. E., Ranaivoson, A., Strock, J. & Collins, H. (2012). Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. *Frontiers in Ecology and the Environment* 10(10): 562-570.http://dx.doi.org/10.1890/120062

Verheijen, F., Jeffery, S., Bastos, A., van der Velde, M. & Diafas, I. (2010). Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg, 149pp.

Verhoeven, E. & Six, J. (2014). Biochar does not mitigate field-scale N2O emissions in a Northern California vineyard: An assessment across two years. *Agriculture, Ecosystems & Environment* 191: 27-38.http://dx.doi.org/10.1016/j.agee.2014.03.008

Vitousek, P. M., Cassman, K., Cleveland, C., Crews, T., Field, C. B., Grimm, N. B., Howarth, R. W., Marino, R., Martinelli, L., Rastetter, E. B. & Sprent, J. I. (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57(1): 1-45. http://dx.doi.org/10.1023/a:1015798428743

Vlaeminck, S. E., Hay, A. G., Maignien, L. & Verstraete, W. (2011). In quest of the nitrogen oxidizing prokaryotes of the early Earth. *Environmental Microbiology* 13(2): 283-295.10.1111/j.1462-2920.2010.02345.x

Wallenstein, M. D., Myrold, D. D., Firestone, M. & Voytek, M. (2006). Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. *Ecological Applications* 16(6): 2143-2152. http://dx.doi.org/10.1890/1051-0761(2006)016[2143:ecodca]2.0.co;2

Wang, W., Wang, H., Feng, Y., Wang, L., Xiao, X., Xi, Y., Luo, X., Sun, R., Ye, X., Huang, Y., Zhang, Z. & Cui, Z. (2016). Consistent responses of the microbial community structure to organic farming along the middle and lower reaches of the Yangtze River. *Scientific Reports* 6: 35046. http://dx.doi.org/10.1038/srep35046

Welsh, A., Chee-Sanford, J. C., Connor, L. M., Löffler, F. E. & Sanford, R. A. (2014). Refined NrfA Phylogeny Improves PCR-Based nrfA Gene Detection. *Applied and Environmental Microbiology* 80(7): 2110-2119. http://dx.doi.org/10.1128/AEM.03443-13

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J. & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications* 1(5): 1-9. http://dx.doi.org/10.1038/ncomms1053

Yanai, Y., Toyota, K. & Okazaki, M. (2007). Effects of charcoal addition on N2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Science and Plant Nutrition* 53(2): 181-188. http://dx.doi.org/10.1111/j.1747-0765.2007.00123.x

Zumft, W. G. & Kroneck, P. M. H. (2006). Respiratory Transformation of Nitrous Oxide (N2O) to Dinitrogen by Bacteria and Archaea. *Advances in Microbial Physiology* Volume 52: 107-227.http://dx.doi.org/10.1016/S0065-2911(06)52003-X

Chapter 1

Tillage system affects fertilizer-induced nitrous oxide emissions

Maike Krauss[1#], Hans-Martin Krause[1#], Simone Spangler[1,2], Ellen Kandeler[2], Sebastian Behrens[3], Andreas Kappler[4], Paul Mäder[1], Andreas Gattinger[1]

[1] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland

[2] Institute of Soil Science and Land Evaluation, Department of Soil Biology, University of Hohenheim, 70599 Stuttgart, Germany

[3] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA

[4] Geomicrobiology and Microbial Ecology, Center for Applied Geoscience, University of Tübingen, 72076 Tübingen, Germany

[#] These authors contributed equally

Published in Biology and Fertility of Soils, 2016, 53(1), pp. 49-59

Biol Fertil Soils
DOI 10.1007/s00374-016-1152-2

ORIGINAL PAPER

Tillage system affects fertilizer-induced nitrous oxide emissions

Maike Krauss[1] · Hans-Martin Krause[1] · Simone Spangler[1,2] · Ellen Kandeler[2] · Sebastian Behrens[3] · Andreas Kappler[4] · Paul Mäder[1] · Andreas Gattinger[1]

Received: 14 June 2016 / Revised: 22 September 2016 / Accepted: 25 September 2016

Abstract Since the development of effective N_2O mitigation options is a key challenge for future agricultural practice, we studied the interactive effect of tillage systems on fertilizer-derived N_2O emissions and the abundance of microbial communities involved in N_2O production and reduction. Soil samples from 0–10 cm and 10–20 cm depth of reduced tillage and ploughed plots were incubated with dairy slurry (SL) and manure compost (MC) in comparison with calcium ammonium nitrate (CAN) and an unfertilized control (ZERO) for 42 days. N_2O and CO_2 fluxes, ammonium, nitrate, dissolved organic C, and functional gene abundances (16S rRNA gene, *nirK*, *nirS*, *nosZ*, bacterial and archaeal *amoA*) were regularly monitored. Averaged across all soil samples, N_2O emissions decreased in the order CAN and SL (CAN = 748.8 ± 206.3, SL = 489.4 ± 107.2 μg kg^{-1}) followed by MC (284.2 ± 67.3 μg kg^{-1}) and ZERO (29.1 ± 5.9 μg kg^{-1}). Highest cumulative N_2O emissions were found in 10–20 cm of the reduced tilled soil in CAN and SL. N_2O fluxes were assigned to ammonium as source in CAN and SL and correlated positively to bacterial *amoA* abundances. Additionally, *nosZ* abundances correlated negatively to N_2O fluxes in the organic fertilizer treatments. Soils showed a gradient in soil organic C, 16S rRNA, *nirK*, and *nosZ* with greater amounts in the 0–10 than 10–20 cm layer. Abundances of bacterial and archaeal *amoA* were higher in reduced tilled soil compared to ploughed soils. The study highlights that tillage system induced biophysicochemical stratification impacts net N_2O emissions within the soil profile according to N and C species added during fertilization.

Keywords Nitrous oxide · Nitrification · Denitrification · Fertilization · Reduced tillage · Soil organic carbon

Maike Krauss and Hans-Martin Krause contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00374-016-1152-2) contains supplementary material, which is available to authorized users.

✉ Hans-Martin Krause
hans-martin.krause@fibl.org

[1] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland

[2] Institute of Soil Science and Land Evaluation, Department of Soil Biology, University of Hohenheim, 70599 Stuttgart, Germany

[3] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA

[4] Geocmicrobiology, Center for Applied Geoscience, Eberhard-Karls-Universität Tübingen, 72076 Tübingen, Germany

Introduction

Nitrous oxide (N_2O) is a major greenhouse gas and the predominant ozone depleting substance in the stratosphere, estimated to account for 6 % of global warming (Montzka et al. 2011; Ravishankara et al. 2009). Concentration of atmospheric N_2O has risen by 20 % since the preindustrial period mostly due to anthropogenic interventions in the N cycle (Davidson 2009). Around 60 % of anthropogenic N_2O emissions originate from microbial processes in agriculturally managed soils (Syakila and Kroeze 2011). To develop effective mitigation strategies for N_2O emissions, a detailed understanding of microbial responses on agricultural management practices is needed (Venterea et al. 2012). Reduced tillage (RT) or no tillage (NT) practices are widely used in cereal-based cropping systems due to their beneficial effects regarding the prevention of soil erosion and water conservation (Derpsch et al. 2010; Powlson et al. 2014). Stratification of soil organic matter increases with reduced tillage intensity (Luo et al. 2010) which

Published online: 25 October 2016

impacts soil physicochemical properties like soil aeration and C availability within the soil profile. This can affect microbial community composition and functioning (Wallenstein et al. 2006). For reduced tillage, changes in PLFA profiles were already reported for bacteria, archaea, and fungi (Kuntz et al. 2013). Studies investigating the impact of tillage systems on N cycling microbial communities in relation to N_2O emissions are however scarce and mainly focus on denitrifiers solely (Baudoin et al. 2009; Melero et al. 2011). In relation to fertilizer-induced N_2O emissions, knowledge is lacking about nitrifiers affected by tillage system, but is important as many fertilizers are ammonium based.

Fertilizer types can influence N_2O emissions due to different N species (NH_4^+, NO_3^-, and N_{org}) and amounts of available C added (Butterbach-Bahl et al. 2013). In a simplified view, ammonium (NH_4^+) is the major source for N_2O emissions via nitrification under oxic conditions while under suboxic conditions N_2O is mostly produced by reduction of nitrate (NO_3^-) in the process of denitrification. Under moderate moisture conditions, both denitrification and nitrification appear simultaneously in different microsites (Butterbach-Bahl et al. 2013). Denitrification thereby shows a higher potential for N_2O production as N_2O is an obligate intermediate during this process. Furthermore, the addition of C during organic fertilization was repeatedly shown to increase denitrification and N_2O emissions (Flessa and Beese 2000). In contrast, C addition under nitrate shortage was also shown to promote N_2O reduction to dinitrogen (N_2), thereby lowering N_2O emissions (Miller et al. 2009; Senbayram et al. 2012; van Groenigen et al. 2004).

To assess the abundance of functional communities involved in nitrification and denitrification, functional gene quantification via qPCR presents the most widely used approach. Bacterial and archaeal *amoA* are used as marker genes for nitrification, while *nirK* and *nirS* genes are often used to assess denitrifier abundance (Philippot et al. 2011). The *nosZ* gene serves as a marker for nitrous oxide reduction, the only known process that acts as a sink for N_2O (Butterbach-Bahl et al. 2013). As not all denitrifier possess the complete set of denitrifying enzymatic systems, the genetic potential to reduce N_2O and the N_2O/N_2 product ratio also depends on denitrifier community composition (Domeignoz-Horta et al. 2015; Graf et al. 2014). Especially the recently discovered clade of bacteria bearing atypical *nosZ* genes were found to lack antecedent denitrifying enzymatic systems (Jones et al. 2014).

Although the overall impact of reduced tillage systems on N_2O emissions based on annual budgets is reported to be similar to plowing systems and emissions even tend to decrease when RT is applied in the long run (Rochette 2008; Six et al. 2004; van Kessel et al. 2013), tillage systems may still respond differently to mitigation options. For example, fertilization methods offer an opportunity for system optimization. Banded placement of mineral fertilizers at depths >5 cm lowered N_2O emissions significantly under NT/RT (van Kessel et al. 2013). However, few studies exist regarding the impact of organic fertilizer types and their placement on N_2O emissions under contrasting tillage strategies. As various techniques exist for the application of organic fertilizers, stratification of soil microbial communities as a result of tillage system change could affect N_2O emissions.

The objective of this study was therefore to gain basic knowledge about N_2O processes for fertilizers with different composition of N and C species in two tillage systems and soil depths. We therefore used laboratory experiments to simulate a fertilizer application to a clayey soil from the long-term organic tillage trial in Frick, Switzerland (Berner et al. 2008; Gadermaier et al. 2012). We hypothesized that (1) tillage systems affect soil properties and abundance of N cycling microbial communities within the soil profile, (2) fertilizer types determine N_2O emissions and predominant N cycling processes, and (3) tillage system–induced changes in biophysicochemical soil properties affect N_2O emissions in dependency of fertilization strategy.

Materials and methods

Site conditions, and soil and fertilizer sampling

Soil samples were taken from the long-term organic tillage trial in Frick, Switzerland (47°30′N, 8°1′E, 350 m a.s.l.). Tillage treatments include plowing to a depth of 15–18 cm (CT) and reduced tillage with a skim and chisel plough (RT) to 5–10 cm. The soil was classified as Vertic Cambisol with a texture of 45 % clay, 33 % silt, and 22 % sand. Samples from the upper (0–10 cm) and lower (10–20 cm) topsoil were taken across all four field replicates in March 2013, homogenized, air-dried to a gravimetric water content of 17.9 (±0.9) %, and sieved to 5-mm aggregates before storage at 4 °C. In order to assess the impact of fertilizer type on N_2O emissions and N cycling microbial communities, two organic fertilizers also used in the field trial, liquid dairy slurry (SL) and dairy manure compost (MC, stable manure composted for 18 weeks), were compared with calcium ammonium nitrate (CAN, 27 % N) and an unfertilized control (ZERO). Basic physicochemical properties and nutrient contents of soils and fertilizers are given in Tables 1 and 2.

Experimental setup

After preincubation for 1 week at room temperature, the equivalent of 100 g dry soil was filled in 250 ml DURAN wide neck glass bottles (Schott AG, Mainz, Germany) which served as microcosms. The soil aggregates were evenly compacted to a bulk density of 1.25 g cm^{-3}. In order to mimic a moderate fertilization event, fertilizer N addition was

Table 1 Means and standard deviations of C and N contents of fertilizers ($n = 4$)

	Dry matter		Total C	Dissolved organic C	Total N	Nitrate	Ammonium	Organic N	C/N	pH (H_2O)
Fertilizer	%	mg applied	mg applied		mg applied					
Calcium ammonium nitrate (CAN)	100	41.0	–	–	11.07[a]	5.54[a]	5.54[a]	–	–	7.81 (0.01)
Slurry (SL)	2.43	198.9	63.92 (1.31)	7.95 (0.54)	11.02[b]	–	3.33 (0.02)	7.69 (0.18)	5.8	7.34 (0.01)
Manure compost (MC)	20.02	284.3	89.52 (3.66)	3.63 (0.41)	11.05[b]	3.00 (0.07)	0.01 (0.01)	8.04 (0.07)	8.1	8.09 (0.01)

Inputs refer to the amount of fertilizer applied to each microcosm

[a] Total N refers to the manufacture specifications of 27 % N as ammonium nitrate

[b] Total N was calculated as the sum of nitrate, ammonium, and N_{org}

normalized to 35 kg N ha^{-1} (11 mg N_t per microcosm). Slurry (SL), CAN solution, and H_2O_{demin} (ZERO) were evenly spread superficially in its liquid form, while particles of fresh manure compost were homogenized with the dry soil before compaction. This procedure assured homogeneous physicochemical soil conditions in all treatments to focus on the reaction of microbial communities under simulated conditions. Water-filled pore space (WFPS) was adjusted to 60 % to account for moisture conditions during fertilization in the field. Microcosms were incubated at constantly 20 °C in the dark in a completely randomized order. Swelling of soil samples due to the high clay content and loss of water during incubation were compensated by added H_2O_{demin} on a daily base. Analysis of greenhouse gases (N_2O, CO_2) was carried out daily within the first week after fertilizer application and weekly thereafter for 42 days. Parallel microcosm sets were set up and stored in the same way and destructively sampled for soil analysis after 1, 3, 7 and 42 days of incubation.

Physicochemical analysis

Soil pH was determined in a 1:2.5 (*w*/*v*) dilution with H_2O_{demin}. Soil organic C (SOC) and fertilizer C_t (60 °C dried samples) were analyzed by dry combustion (multi N/C2100S + HT1300; Analytik Jena AG, Jena, Germany). SOC was determined by the subtraction of 105 °C (C_t) by 500 °C (inorganic C) treated and then at 1000 °C combusted samples. Total soil N (N_t) was also determined by combustion (CN Vario Max; Elementar Analysensysteme GmbH, Hanau, Germany). Microbial biomass C and N (C_{mic}, N_{mic}) were assessed with the chloroform fumigation extraction method with 0.5 M K_2SO_4 as described in Fließbach et al. (2007). Dissolved organic C (DOC) was extracted using 0.01 M $CaCl_2$ filtered through a 0.45-µm membrane filter (Porafil® CM; Macherey-Nagel, Düren, Germany) with a vacuum device (SM; Sartorius AG, Göttingen, Germany). Extracts were determined with a TOC/TNb analyzer (DIMA-TOC 100; Dimatec Analysentechnik GmbH, Essen, Germany).

Kjehldahl wet digestion (2020 Digestor; Foss Tecator AB, Höganäs, Sweden) was employed to quantify organically bound N (N_{org}) in both organic fertilizers. Ammonium contents in liquid slurry were analyzed by direct distillation (Büchi 315; Büchi AG, Flawil, Switzerland) whereas ammonium and nitrate of fresh manure compost and soil samples were determined by $CaCl_2$ (0.01 M $CaCl_2$ at 1:4 *w*/*v*) extraction. After filtration (MN 619EH; Macherey-Nagel, Düren, Germany), ammonium and nitrate contents were determined spectrophotometrically (SAN-plus Segmented Flow Analyzer; Skalar Analytical B.V., Breda, Netherlands).

Table 2 Means and standard deviations of physicochemical properties for soil samples from conventional (CT) and reduced tillage (RT) and two soil depths (0–10, 10–20 cm) before incubation ($n = 4$)

	Soil organic C (g kg^{-1})			Microbial biomass C (mg kg^{-1})			Total N (g kg^{-1})			Microbial biomass N (mg kg^{-1})			pH (H_2O)		
Treatment	Tillage[ns], depth***			Tillage†, depth***			Tillage***, depth***			Tillage**, depth**			Tillage*, depth***		
CT, 0–10 cm	26.82	b	(0.10)	667.9	b	(12.7)	2.87	b	(0.05)	62.65	bc	(3.42)	7.22	ab	(0.09)
CT, 10–20 cm	24.04	c	(0.32)	581.8	c	(22.2)	2.67	c	(0.05)	58.95	c	(2.90)	7.24	a	(0.03)
RT, 0–10 cm	28.56	a	(0.39)	892.0	a	(52.7)	3.30	a	(0.04)	76.57	a	(4.23)	7.10	b	(0.02)
RT, 10–20 cm	22.22	d	(0.14)	655.5	b	(7.6)	2.65	c	(0.06)	65.58	b	(1.78)	7.22	a	(0.01)

Significant differences (ANOVA) within tillage and depth factors are indicated in the headline. Values with different letters are statistically different at $p < 0.05$ (Tukey test). Level of significance for tillage and depth factors: †$p < 0.1$, *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, *ns* not significant

Greenhouse gas analysis

Constant temperature conditions during GHG sampling were assured by a temperature-controlled tray (20 °C) directly placed at an autosampler (MPS 2XL; Gerstel AG, Sursee, Switzerland). Microcosm headspaces were gently fanned and sealed with a lid containing a rubber septum before sampling. Three gas samples of 5 ml were directly taken every 20 min and analyzed by gas chromatography (7890A; Agilent Technologies, Santa Clara, CA). To avoid a vacuum effect, 5 ml of helium gas was injected and mixed in the microcosm headspace prior to sampling. CO_2 concentrations were determined with a flame ionization detector (FID) and N_2O with an electron capture detector (μECD).

Molecular analysis

DNA extraction of soil samples was performed using Fast DNA® Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the instructions given by the manufacturer. Quality and quantity of DNA extractions were determined spectrophotometrically (NanoDrop 2000 UV–vis Spectrometer; Thermo Fisher Scientific, Wilmington, DE, USA). Yields of extracted DNA ranged from 73.0 to 138.4 ng/μl, and no treatment specific bias was detected. Functional genes were quantified using SYBR green approach (Kapa SYBR® Fast qPCR Kit Master Mix (2×) Universal; Kapa Biosystems, Wilmington, MA) on a Rotor-Gene Q platform (Rotor-Gene Q; QIAGEN, Venlo, Netherlands). Master Mix compositions, temperature profiles, and gene specific primers are listed in Supplement Table S1 and S2. For qPCR analysis, biological triplicates were used, of which each sample was analyzed twice. Measurement of a sample was repeated when C_t values differed by more than 0.5. In each qPCR run, negative controls as well as a serial dilution of plasmids containing a fragment of the respective target gene were included. Concentration of standard plasmids was determined spectrophotometrically (NanoDrop 2000 UV–vis Spectrometer; Thermo Fisher Scientific, Wilmington, DE, USA) and gene copy numbers of standard curves (ranging from 10^1 to 10^8 gene copies/μl) were calculated using molecular weight of the standard plasmids according to Behrens et al. (2008). For each gene, a joined standard curve was constructed with C_t values from the serial dilution of standard plasmid from six independent qPCR runs. Efficiencies of qPCR reactions ranged from 88 to 96 % for bacterial *amoA* (AOB), 91–99 % for archaeal *amoA* (AOA), 92–99 % for *nosZ*, 88–90 % for *nir*K, 92–95 % for *nirS*, and 92–97 % for 16S rRNA gene. R^2 was above 0.999 for all qPCR runs.

Data transformation and statistics

All data preparation and statistical analyses were performed in R (R Core Team 2013). Gas fluxes were calculated using a linear model considering the He dilution. Cumulative gas emissions (μg kg^{-1}) were integrated according to the trapezoidal integration method Eq. 1:

$$\text{cumulative } flux = \Sigma_i^n (t_{i+1} - t_i) * \left(f_i + f_{i+1}\right)/2 \quad (1)$$

with t = sampling time (h) and f = gas flux (μg kg^{-1} h^{-1}) and n = number of sampling dates.

Treatment effects on initial soil and gene data (ANOVA) as well as linear regressions were assessed with a linear model. Log-transformed cumulative gas data were assessed with a linear mixed effect model using the nlme package with microcosm replicates as random effect (Pinheiro et al. 2014). Post hoc pairwise comparisons (Tukey test) were calculated with the multcomp package (Hothorn et al. 2008). Linear regressions of physicochemical and gene time series data with N_2O-N fluxes were calculated with generalized least square models considering the temporal autocorrelation in a compound symmetry correlation structure. Normality and homoscedasticity of residuals were assessed graphically.

Results and discussion

Effects of tillage system on soil biophysicochemical parameters

Stratification of soil organic C and N was more pronounced in RT compared to CT (Table 2) in line with results of a recent meta-analysis (Luo et al. 2010). In RT, soil organic C and total N contents in the upper soil layers were 28.5 and 24.5 % higher, respectively, compared to the lower soil layers. In CT, the increase accounted only for 11.5 and 7.5 %, respectively (Table 2). The overall effect of tillage system was significant for total N but not for soil organic C content as soil organic C was 6.5 % higher in the upper soil layer but 7.6 % lower in the lower soil layer in RT compared to CT. Microbial biomass showed a significant effect of soil depth and tillage system with higher concentrations in the upper soil layer and the RT system. Microbial C and N showed highest concentrations in the upper soil layer under RT (892.0 and 76.57 mg kg^{-1}) and lowest concentrations in the lower soil layer under CT (581.8 and 58.95 mg kg^{-1}) (Table 2). While an increase of microbial biomass in the upper soil layers due to reduced tillage intensity was regularly reported (Heinze et al. 2010; Kaurin et al. 2015), a generally increased microbial biomass under RT, although occasionally observed (Jacobs et al. 2009), seems not to be a normal case (van Capelle et al. 2012).

Soil depth and tillage system affected the abundance of functional gene markers for nitrification and denitrification differently. While ammonium oxidizing archaea (AOA) and ammonium oxidizing bacteria (AOB) were significantly more abundant under RT and hardly affected by soil depth, the opposite was true for most functional gene markers involved in denitrification. Here, significant effects of soil depth were found for *nirK* and typical *nosZ* but not for *nirS* abundances (Table 3). However, it should be noted that the primer pairs we used for *nirK* and *nirS* quantification are limited to alpha-, beta-, and gamma-proteobacteria and do not cover all phylogenetic groups detected by recently established primers (Wei et al. 2015). Similar to our study, declining abundance of denitrifiers with the increase of soil depth had been observed across a variety of agroecosystems (Boz et al. 2013; Marhan et al. 2011; Melero et al. 2011; Regan et al. 2011). Only abundance of *nirK* bearing denitrifiers was affected by tillage system with 148 and 143 % increased gene copy numbers under RT in the upper and lower soil layer. This confirms increased denitrifier abundances as observed elsewhere for no-till (Baudoin et al. 2009; Melero et al. 2011; Tellez-Rio et al. 2015) and minimum tillage systems (Tellez-Rio et al. 2015). 16S rRNA gene copy numbers were significantly increased under RT and in the upper soil layers. 16S rRNA gene copy numbers declined in the order RT 0–10 cm (9.1×10^9) and CT 0–10 cm (7.4×10^9), RT 10–20 cm (6.4×10^9) and CT 10–20 cm (5.8×10^9) confirming results from microbial biomass data. Abundances of denitrifiers were highly collinear to 16S rRNA gene copy numbers and also correlated to soil organic C contents (Supplementary Table S3). It was shown that AOA dominate in agriculturally managed soils with AOA/AOB ratios of up to 232 (Leininger et al. 2006). In our study, AOA also exceeded AOB abundances by more than one order of magnitude, with mean AOA/AOB ratios of 39 and 8 for CT and RT, respectively. RT thereby enhanced abundances of AOA (+65 % in 0–10 cm and +55 % in 10–20 cm) and AOB (+60 % in 0–10 cm and +38 % in 10–20 cm) compared to CT. In line with our data, enhanced AOA and AOB abundances in no-till had been observed in a paddy rice system (Li et al. 2015). We have found no studies on the long-term impact of reduced tillage systems on nitrifying guilds in aerobically managed agricultural soils. Yet, higher contents of mineralizable N in topsoils was frequently reported for NT in comparison with ploughing systems (Balesdent et al. 2000) which suggests an enhanced NH_4^+ supply and the promotion of nitrifier abundances.

Impact of fertilizer type on N_2O emissions and abundance of N cycling microbial communities

In our setup, fertilization showed a greater impact on cumulative N_2O emissions and N_2O fluxes compared to tillage system which, however, showed an interactive effect. Discussing

Table 3 Means and standard deviations of the general bacterial marker gene 16S rRNA and the functional genes *amoA* (bacterial and archaeal), *nirK*, *nirS*, and *nosZ* for soil samples from conventional (CT) and reduced tillage (RT) and two soil depths (0–10, 10–20 cm) before incubation ($n = 3$)

	16S rRNA (copies g^{-1})			Archaeal *amoA* (AOA) (copies g^{-1})			Bacterial *amoA* (AOB) (copies g^{-1})			*nirK* (copies g^{-1})			*nirS* (copies g^{-1})			*nosZ* (copies g^{-1})		
Treatment	Tillage*, depth*			Tillage*, depth^{ns}			Tillage***, depth^{ns}			Tillage*, depth*			Tillage^{ns}, depth^{ns}			Tillage^{ns}, depth**		
CT, 0–10 cm	7.4×10^9	ab	(3.6×10^8)	2.8×10^8	b	(1.1×10^8)	7.4×10^6	b	(5.8×10^5)	3.0×10^7	b	(1.4×10^7)	3.0×10^8	a	(1.9×10^8)	1.9×10^8	a	(8.9×10^6)
CT, 10–20 cm	5.8×10^9	b	(5.1×10^8)	3.0×10^8	ab	(1.3×10^8)	7.3×10^6	b	(2.9×10^5)	1.4×10^7	b	(1.3×10^6)	3.2×10^8	a	(1.3×10^8)	1.5×10^8	ab	(5.4×10^6)
RT, 0–10 cm	9.1×10^9	a	(1.4×10^9)	4.3×10^8	ab	(7.0×10^7)	1.3×10^7	a	(1.5×10^6)	7.2×10^7	a	(2.9×10^7)	4.7×10^8	a	(3.9×10^8)	1.8×10^8	a	(2.1×10^7)
RT, 10–20 cm	6.4×10^9	b	(8.3×10^8)	5.4×10^8	a	(5.8×10^7)	1.6×10^7	a	(2.3×10^6)	3.4×10^7	ab	(7.7×10^6)	2.4×10^8	a	(2.6×10^7)	1.2×10^8	b	(2.3×10^7)

Significant differences (ANOVA) within tillage and depth factors are indicated in the headline. Values with different letters are statistically different at $p < 0.05$ (Tukey test). Level of significance for tillage and depth factors: $^{\dagger}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$, *ns* not significant

fertilizer impacts on N_2O emissions and predominant N-transforming processes first is therefore a prerequisite to evaluate implications of tillage systems later on.

Averaged per fertilizer treatment and highest cumulative N_2O emissions over the 42-day period were observed in CAN and SL (CAN = 748.8 ± 206.3, SL = 489.4 ± 107.2 μg kg^{-1}) followed by MC (284.2 ± 67.3 μg kg^{-1}) in contrast to ZERO (29.1 ± 5.9 μg kg^{-1}) (Fig. 1). In a similar incubation study with 65 % WFPS, the same trend in cumulative N_2O emissions (CAN = 2.7, organic cattle slurry = 2.4, and ZERO = 0.6 mg N_2O-N kg^{-1} soil, 98 days) on a sandy soil has been found (Velthof et al. 2003). Thus, ammonium addition induced more climate-relevant N_2O emissions than nitrate application under oxic conditions. Additionally, increasing nitrate concentrations in all fertilization treatments suggest nitrification to be the predominant N transforming process under the chosen conditions.

In SL and CAN, N_2O fluxes correlated positively to decreasing ammonium concentrations and AOB abundances (Table 4). This highlights ammonium oxidation as controlling

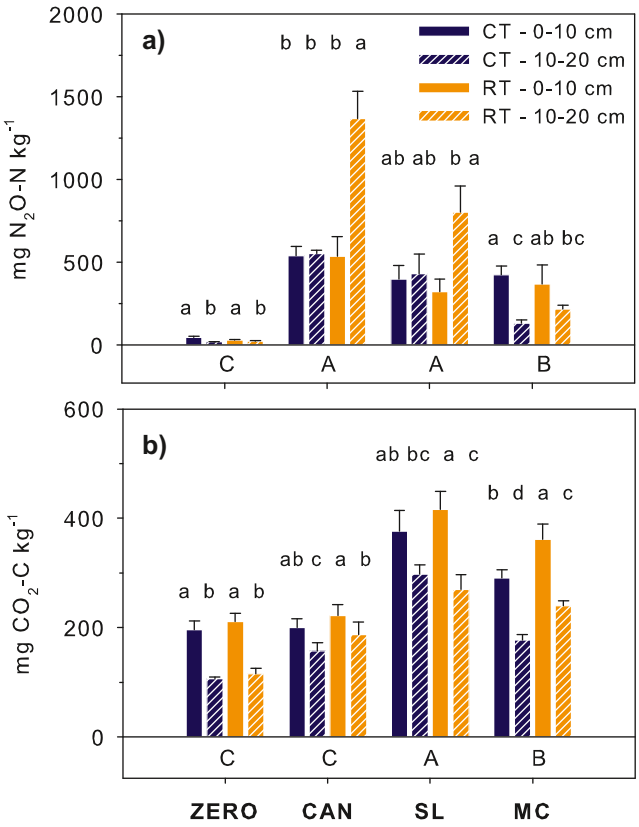


Fig. 1 Cumulative emissions of (**a**) N_2O-N and (**b**) CO_2-C of soil samples from conventional tillage (*CT*) and reduced tillage (*RT*) systems and two soil depths (0–10, 10–20 cm) after application of demineralized water (*ZERO*), calcium ammonium nitrate (*CAN*), slurry (*SL*), and manure compost (*MC*) during 42 days of incubation. *Bars* represent means and standard errors (n = 4). *Capital letters* indicate significant differences between and *small letters* within fertilizer treatments (ANOVA, Tukey test, $p < 0.05$)

factor for N_2O fluxes after addition of ammonium and confirms the findings of Di et al. (2009) who suggested AOB rather than AOA to drive ammonium oxidation rates under ammonium excess. In accordance, Kool et al. (2011) demonstrated ammonium to be the major source of N_2O emissions at 50 and 70 % WFPS through the processes of nitrification and nitrifier-denitrification. Both processes likely occurred also in our case although we could not distinguish them in our setup. In contrast to AOB, AOA abundances correlated positively to N_2O fluxes in ZERO but negatively in SL and CAN (Table 4). Furthermore, an increased growth of AOA in SL was detected compared with CAN (Supplement Fig. S1). This further suggests AOA growth rather to be attributable to N_{org} addition, as already proposed by Taylor et al. (2012). For the abundances of *nirK* and *nirS* bearing bacteria, we could observe few significant relationships with N_2O fluxes. Miller et al. (2009) investigated denitrifier abundances after application and could not find significant relationships with N_2O fluxes. Similar to our study, missing relationships between N_2O fluxes and the abundance of denitrifying communities might be caused by the limited phylogenetic diversity covered by the used primers (Wei et al. 2015).

After organic fertilization in SL and MC, we observed an increase in typical *nosZ*-bearing bacteria that significantly negatively correlated to N_2O fluxes (Table 4). This correlation indicates that denitrifiers with the genetic potential to reduce N_2O played a major role in determining net N_2O fluxes after organic fertilization. This is most likely linked to increased availability of C substrates and the formation of anoxic microsites after organic fertilizer addition by increased soil respiration (Butterbach-Bahl et al. 2013; Miller et al. 2009). Significantly elevated CO_2 emissions in SL and MC compared with ZERO and CAN add to this interpretation (Fig. 1). Also, faster declining N_2O fluxes in SL compared to CAN support the hypothesis of increased N_2O reduction due to organic fertilization.

In MC, about 30 mg nitrate-N kg^{-1} were added to each microcosm (Table 2). Still, nitrate contents after 1 day of incubation did not significantly rise above initial background concentrations (28.4 ± 2.4 mg NO_3-N kg^{-1} across all soil samples, Fig. 2). This suggests instant denitrification or immobilization of manure-derived nitrate in MC despite prevailing nitrifying conditions. The massive but short-lived N_2O peaks directly after fertilization further hint towards denitrification as a major N_2O-producing process in MC (Fig. 2). Significant negative correlation of N_2O fluxes with changes in *nirS* and typical *nosZ* abundances in MC (Table 5) may represent growth of respective heterotrophic microorganisms after N_2O emissions terminated and may be associated with the addition of C-rich material. Generally, N_2O produced in the course of denitrification can exceed those of nitrification by some orders of magnitude (Braker and Conrad 2011; Canfield et al. 2010). This might explain highest N_2O flux rates in MC

Table 4 Regressions of gas fluxes with soil biophysicochemical properties during the 42 days of incubation after application of demineralized water (ZERO), calcium ammonium nitrate (CAN), slurry (SL), and manure compost (MC)

	Coefficients (β) and significance levels (F test)													
	CO_2-C		N_2O-N											
Treatment	16S rRNA		Ammonium		Archaeal *amoA* (AOA)		Bacterial *amoA* (AOB)		*nirK*		*nirS*		*nosZ*	
ZERO	3.9×10^{-8}	***	0.004	ns	1.3×10^{-10}	**	1.5×10^{-9}	ns	1.4×10^{-11}	ns	6.7×10^{-10}	ns	4.2×10^{-11}	ns
CAN	2.0×10^{-8}	*	1.26	**	-1.9×10^{-8}	†	1.9×10^{-7}	†	-1.0×10^{-8}	†	-1.8×10^{-7}	†	-2.4×10^{-8}	ns
SL	-1.2×10^{-7}	***	1.73	***	-4.0×10^{-9}	*	2.6×10^{-7}	*	-3.2×10^{-9}	ns	-9.8×10^{-8}	ns	-3.5×10^{-8}	*
MC	-4.6×10^{-9}	ns	1.58	ns	-2.1×10^{-9}	ns	-2.2×10^{-8}	ns	5.6×10^{-9}	ns	-4.9×10^{-7}	**	-9.9×10^{-8}	*

Timelines of CO_2-C fluxes were correlated with 16S rRNA gene abundances and N_2O-N fluxes with soil ammonium concentrations and functional gene abundances across all soil samples and for each fertilization treatment. The temporal correlation was considered in the generalized least square model. Level of significance: †$p < 0.1$, *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, *ns* not significant

compared to the other fertilization treatments reaching up to 23.8 μg N_2O-N kg^{-1} h^{-1} in the first hours of incubation.

Whether functional gene quantification can be linked to process rates is currently debated (Bier et al. 2015; Rocca et al. 2015). Although relationships between functional gene abundances and process rates are not straightforward, a recent meta-study showed that provision of nutrients by fertilization increased reliability of functional gene analysis as an indicator for process rates in the agricultural context (Rocca et al. 2015). By time series regression, we could link N_2O fluxes to changes in abundances of AOB after addition of ammonium, while abundances of typical *nosZ*-bearing bacteria were significantly correlated to N_2O fluxes after the addition of organic fertilizers. This shows that fertilizer type not only affects N_2O fluxes but also the abundance of N-transforming microbial communities.

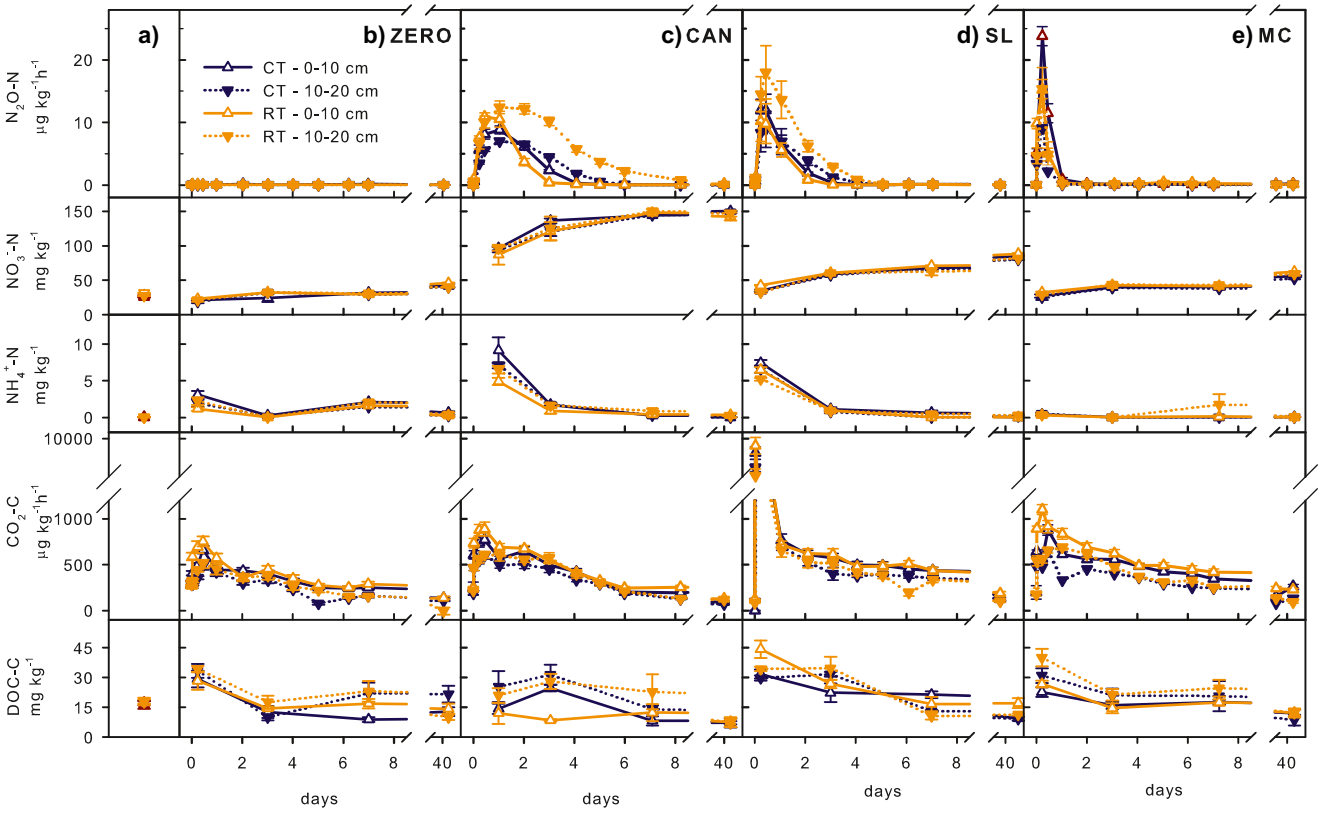


Fig. 2 Fluxes of N_2O-N, soil nitrate (NO_3^--N) and ammonium (NH_4^+-N) contents, CO_2-C fluxes, and dissolved organic C (DOC) contents of soil samples from conventional tillage (CT) and reduced tillage (RT) systems and two soil depths (0–10, 10–20 cm). Panel (**a**) shows soil physicochemical parameters before incubation. Panels (**b**)–(**e**) show soil physicochemical parameters, N_2O-N and CO_2-C emissions after application of demineralized water (*ZERO*), calcium ammonium nitrate (*CAN*), slurry (*SL*), and manure compost (*MC*) during 42 days of incubation. *Error bars* show the standard error of the mean of each treatment ($n = 4$)

Table 5 Linear regression of cumulative CO_2-C (mg kg^{-1} soil) and N_2O-N (μg kg^{-1} soil) emissions with initial soil organic C contents (g kg^{-1} soil) for each fertilization treatment

	Coefficients (β), significance levels (*F* test) and R^2					
Treatment	CO_2-C			N_2O-N		
ZERO	17.9	***	0.73	2.3	ns	0.17
CAN	7.0	†	0.18	−111.7	*	0.46
SL	23.9	**	0.56	−65.9	*	0.36
MC	22.5	**	0.56	36.9	*	0.30

Treatments include the application of demineralized water (ZERO), calcium ammonium nitrate (CAN), slurry (SL), and manure compost (MC). Level of significance: †$p < 0.1$, *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, *ns* not significant

Tillage system–induced stratification in biophysicochemical soil properties affects N_2O emissions in dependency of fertilization strategy

Enhanced soil respiration was observed in the upper compared to the lower soil layers regardless of fertilizer treatment. An effect of tillage system was only detected in MC with 24.6–35.4 % higher cumulated CO_2 emissions in RT compared with CT in the upper and lower soil layers, respectively (Fig. 1). Cumulative soil respiration was therefore positively correlated to soil organic C contents (Table 5). Across all fertilization treatments, dynamics of nitrate concentration as an indicator of ongoing net nitrification did not differ between soils during incubation (Fig. 2). Increased nitrifier abundances in RT were thus not directly translated into a higher net nitrification. Yet, average cumulative N_2O emissions were 8 % lower in the 0–10 cm layer and 81 % higher in the 10–20 cm layer of RT compared with CT. This effect was far greatest in CAN and SL with significantly higher cumulative N_2O emissions in the lower soil layer in RT compared to all other soils (Fig. 1). In addition, cumulative N_2O emissions correlated significantly with soil organic C contents in the fertilized treatments (Table 5). This correlation was positive for MC and negative for CAN and SL. N_2O emissions in ZERO were too low to show a distinct effect. These observations suggest that the long-term effect of tillage systems on C distribution and microbial communities within the profile influenced fertilizer-induced soil respiration and related heterotrophic processes more than nitrification. Ammonium-derived N_2O emissions were therefore conversely affected rather than nitrate-derived N_2O emissions in our experimental setup.

Positive correlation in MC can be explained by heterotrophic activity and denitrification due to fertilizer C addition besides soil organic C availability and the affiliated higher abundance of denitrifiers in the respective soil layers. As cumulative N_2O emissions cannot be explained by differing response of nitrification between soil layers in CAN and SL, negative correlation with soil organic C is a hint towards an increased N_2O reduction in C- and denitrifier-rich layers. The prolonged phase of N_2O fluxes after ammonium addition in the lower soil layer in RT also suggests lower N_2O reduction. N_2O fluxes thereby lasted 3 days longer in CAN compared with SL (Fig. 1). Provision of labile C in SL seemed to enhance N_2O reduction in addition. The fact that the tillage-induced soil organic C effect on N_2O emissions was not entirely masked by the addition of labile fertilizer C emphasizes the important role of soil organic C on N_2O formation. This was not reported yet for tillage systems but for long-term fertilization experiments. For sandy and C-poor soils, no soil organic C impact on N_2O emissions was reported (Jaeger et al. 2013). In contrast, a long-term fertilization effect was found for silt loam soils where increased soil organic C contents enhanced denitrification rates, such as in our case (Dambreville et al. 2006; Tatti et al. 2013). The marked C effect in our study could therefore be associated with the high clay content and associated high soil organic C concentrations (22–28 g kg^{-1}). Clay soils are known to have a high potential of binding carbon (von Luetzow et al. 2006) which was mirrored by a fast soil organic C accumulation in this soil already after some years of management change (Gadermaier et al. 2012). Besides the impact of tillage systems on soil organic C stratification, other specifically tillage-related biochemical effects could explain the marked differences between tilled and untilled soil layers. It was shown that tillage operations disrupt soil aggregates, increasing soil organic matter accessibility for microorganisms and creating new surfaces for microbial colonization (von Luetzow et al. 2006; Wiesmeier et al. 2014). Vogel et al. (2014) found hotspots for microbial activity to be located at existing colonized organic-mineral complexes. Tilled soil layers may have therefore provided better conditions for denitrifiers and N_2O reduction than the untilled lower soil layer in RT. Our experimental setup therefore offered the opportunity to track the influence of tillage systems on fertilizer-induced N_2O emissions with regard to a range of microbial and physicochemical soil properties. Under real field conditions, soil physical conditions like, e.g., constraints in diffusion (Petersen et al. 2008) will additionally regulate microbial N_2O production and reduction.

Conclusions

Our study showed the interactive effect of tillage system impact on soil properties on fertilizer-induced N_2O emissions. It is one of the first studies that detected higher nitrifier abundances in reduced compared to ploughed soils. Soil organic C and fertilizer C and N species helped explain net N_2O emissions within the soil profile, while functional gene abundances partly explained microbial processes. Nitrification was shown to be an important driver of N_2O emissions in conditions close to fertilizer field applications. Additionally, indications for

increased N_2O reduction after organic fertilization and in soil layers with high soil organic C contents were found. The role of N_2O reduction after organic fertilization requires further investigation by addressing atypical *nosZ*-bearing denitrifiers and quantifying N_2 emissions in a stable isotope approach.

Increased N_2O emissions in lower soil layers may be compensated through higher N_2O reduction in the topsoil of reduced tilled systems. Yet, our results suggest that placing ammonium in lower soil depths may increase N_2O production considerably. Higher N_2O emissions have already been observed after injection of slurry into deeper soil layers in the field (Montes et al. 2013), and there is a need to clarify if increased availability of N due to decreased NH_3 loss or microbial responses to C availability as seen in our study are the main drivers.

Acknowledgments We kindly thank our laboratory technicians Anton Kuhn and Adolphe Munyangabe for assistance. For external laboratory analysis, we acknowledge Hans Ruedi Oberholzer and his co-workers from Agroscope Reckenholz. We gratefully acknowledge the financial support for this project provided by the COOP Sustainability Fund and the CORE Organic II funding bodies, being partners of the FP7 ERA-Net project TILMAN-ORG (www.coreorganic2.org). We also thank for the financial support of the Swiss National Science Foundation in frame of the National Research Program "Soil as a Resource" (NRP 68). We thank the Swiss Federal Office for the Environment for financing the gas chromatograph and the Software AG-Stiftung, Stiftung zur Pflege von Mensch, Mitwelt und Erde and Swiss Federal Office for Agriculture for financing the Frick tillage trial. We thank Simon Moakes for his help with English editing.

References

Balesdent J, Chenu C, Balabane M (2000) Relationship of soil organic matter dynamics to physical protection and tillage. Soil Tillage Res 53:215–230. doi:10.1016/s0167-1987(99)00107-5

Baudoin E, Philippot L, Cheneby D, Chapuis-Lardy L, Fromin N, Bru D, Rabary B, Brauman A (2009) Direct seeding mulch-based cropping increases both the activity and the abundance of denitrifier communities in a tropical soil. Soil Biol Biochem 41:1703–1709. doi:10.1016/j.soilbio.2009.05.015

Behrens S, Azizian MF, McMurdie PJ, Sabalowsky A, Dolan ME, Semprini L, Spormann AM (2008) Monitoring abundance and expression of "dehalococcoides" species chloroethene-reductive dehalogenases in a tetrachloroethene-dechlorinating flow column. Appl Environ Microbiol 74:5695–5703. doi:10.1128/aem.00926-08

Berner A, Hildermann I, Fließbach A, Pfiffner L, Niggli U, Mäder P (2008) Crop yield and soil fertility response to reduced tillage under organic management. Soil Tillage Res 101:89–96. doi:10.1016/j.still.2008.07.012

Bier RL, Bernhardt ES, Boot CM, Graham EB, Hall EK, Lennon JT, Nemergut D, Osborne BB, Ruiz-González C, Schimel JP, Waldrop MP, Wallenstein MD (2015) Linking microbial community structure and microbial processes: an empirical and conceptual overview. FEMS Microbiol Ecol. doi:10.1093/femsec/fiv113

Boz B, Mizanur Rahman M, Bottegal M, Basaglia M, Squartini A, Gumiero B, Casella S (2013) Vegetation, soil and hydrology management influence denitrification activity and the composition of nirK-type denitrifier communities in a newly afforested riparian buffer. New Biotechnol 30:675–684. doi:10.1016/j.nbt.2013.03.009

Braker G, Conrad R (2011) Diversity, structure, and size of N_2O-producing microbial communities in soils—what matters for their functioning? In: Laskin AI, Sariaslani S, Gadd GM (Eds) Adv Appl Microbiol, vol 75. pp 33–70. doi:10.1016/b978-0-12-387046-9.00002-5

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos Trans R Soc Lond B Biol Sci 368:20130122. doi:10.1098/rstb.2013.0122

Canfield DE, Glazer AN, Falkowski PG (2010) The evolution and future of Earth's nitrogen cycle. Science 330:192–196. doi:10.1126/science.1186120

Dambreville C, Henault C, Bizouard F, Morvan T, Chaussod R, Germon J-C (2006) Compared effects of long-term pig slurry applications and mineral fertilization on soil denitrification and its end products (N_2O, N_2). Biol Fertil Soils 42:490–500. doi:10.1007/s00374-005-0040-y

Davidson EA (2009) The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. Nat Geosci 2:659–662. doi:10.1038/ngeo608

Derpsch R, Friedrich T, Kassam A, Li H (2010) Current status of adoption of no-till farming in the world and some of its main benefits. Int J Agric Biol Eng 3:1–25. doi:10.3965/j.issn.1934-6344.2010.01.0-0

Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S, He JZ (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat Geosci 2:621–624. doi:10.1038/ngeo613

Domeignoz-Horta LA, Spor A, Bru D, Breuil M-C, Bizouard F, Léonard J, Philippot L (2015) The diversity of the N_2O reducers matters for the N_2O:N_2 denitrification end-product ratio across an annual and a perennial cropping system. Front Microbiol 6:971. doi:10.3389/fmicb.2015.00971

Flessa H, Beese F (2000) Laboratory estimates of trace gas emissions following surface application and injection of cattle slurry. J Environ Qual 29:262–268. doi:10.2134/jeq2000.00472425002900010033x

Fließbach A, Oberholzer H-R, Gunst L, Mäder P (2007) Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agr Ecosyst Environ 118:273–284. doi:10.1016/j.agee.2006.05.022

Gadermaier F, Berner A, Fließbach A, Friedel JK, Mäder P (2012) Impact of reduced tillage on soil organic carbon and nutrient budgets under organic farming. Renewable Agric Food Syst 27:1–13. doi:10.1017/S1742170510000554

Graf DRH, Jones CM, Hallin S (2014) Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. Plos One 9:e114118. doi:10.1371/journal.pone.0114118

Heinze S, Rauber R, Joergensen RG (2010) Influence of mouldboard plough and rotary harrow tillage on microbial biomass and nutrient stocks in two long-term experiments on loess derived Luvisols. Appl Soil Ecol 46:405–412. doi:10.1016/j.apsoil.2010.09.011

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

Jacobs A, Rauber R, Ludwig B (2009) Impact of reduced tillage on carbon and nitrogen storage of two Haplic Luvisols after 40 years. Soil Tillage Res 102:158–164. doi:10.1016/j.still.2008.08.012

Jaeger N, Duffner A, Ludwig B, Flessa H (2013) Effect of fertilization history on short-term emission of CO_2 and N_2O after the application of different N fertilizers—a laboratory study. Arch Agron Soil Sci 59:161–171. doi:10.1080/03650340.2011.621420

Jones CM, Spor A, Brennan FP, Breuil M-C, Bru D, Lemanceau P, Griffiths B, Hallin S, Philippot L (2014) Recently identified microbial guild mediates soil N_2O sink capacity. Nature Clim Change advance online publication doi:10.1038/nclimate2301

Kaurin A, Mihelič R, Kastelec D, Schloter M, Suhadolc M, Grčman H (2015) Consequences of minimum soil tillage on abiotic soil properties and composition of microbial communities in a shallow Cambisol originated from fluvioglacial deposits. Biol Fertil Soils 51:923–933. doi:10.1007/s00374-015-1037-9

Kool DM, Dolfing J, Wrage N, Van Groenigen JW (2011) Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. Soil Biol Biochem 43:174–178. doi:10.1016/j.soilbio.2010.09.030

Kuntz M, Berner A, Gattinger A, Scholberg JM, Mäder P, Pfiffner L (2013) Influence of reduced tillage on earthworm and microbial communities under organic arable farming. Pedobiologia 56:251–260. doi:10.1016/j.pedobi.2013.08.005

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809. doi:10.1038/nature04983

Li S, Jiang XJ, Wang XL, Wright AL (2015) Tillage effects on soil nitrification and the dynamic changes in nitrifying microorganisms in a subtropical rice-based ecosystem: a long-term field study. Soil Tillage Res 150:132–138. doi:10.1016/j.still.2015.02.005

Luo ZK, Wang EL, Sun OJ (2010) Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agr Ecosyst Environ 139:224–231. doi:10.1016/j.agee.2010.08.006

Marhan S, Philippot L, Bru D, Rudolph S, Franzaring J, Högy P, Fangmeier A, Kandeler E (2011) Abundance and activity of nitrate reducers in an arable soil are more affected by temporal variation and soil depth than by elevated atmospheric CO_2. FEMS Microbiol Ecol 76:209–219. doi:10.1111/j.1574-6941.2011.01048.x

Melero S, Perez-de-Mora A, Manuel Murillo J, Buegger F, Kleinedam K, Kublik S, Vanderlinden K, Moreno F, Schloter M (2011) Denitrification in a vertisol under long-term tillage and no-tillage management in dryland agricultural systems: key genes and potential rates. Appl Soil Ecol 47:221–225. doi:10.1016/j.apsoil.2010.12.003

Miller MN, Zebarth BJ, Dandie CE, Burton DL, Goyer C, Trevors JT (2009) Influence of liquid manure on soil denitrifier abundance, denitrification, and nitrous oxide emissions. Soil Sci Soc Am J 73:760–768. doi:10.2136/sssaj2008.0059

Montes F, Meinen R, Dell C, Rotz A, Hristov AN, Oh J, Waghorn G, Gerber PJ, Henderson B, Makkar HPS, Dijkstra J (2013) SPECIAL TOPICS—Mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. J Anim Sci 91:5070–5094. doi:10.2527/jas.2013-6584

Montzka SA, Dlugokencky EJ, Butler JH (2011) Non-CO_2 greenhouse gases and climate change. Nature 476:43–50. doi:10.1038/nature10322

Petersen SO, Schjonning P, Thomsen IK, Christensen BT (2008) Nitrous oxide evolution from structurally intact soil as influenced by tillage and soil water content. Soil Biol Biochem 40:967–977. doi:10.1016/j.soilbio.2007.11.017

Philippot L, Andert J, Jones CM, Bru D, Hallin S (2011) Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. Glob Chang Biol 17:1497–1504. doi:10.1111/j.1365-2486.2010.02334.x

Pinheiro J., Bates D., DebRoy S., Sarkar D., R Core Team (2014) nlme: Linear and Nonlinear Mixed Effects Models, R package version 3.1-118 edn

Powlson DS, Stirling CM, Jat ML, Gerard BG, Palm CA, Sanchez PA, Cassman KG (2014) Limited potential of no-till agriculture for climate change mitigation. Nat Clim Chang 4:678–683. doi:10.1038/nclimate2292

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125. doi:10.1126/science.1176985

Regan K, Kammann C, Hartung K, Lenhart K, Müller C, Philippot L, Kandeler E, Marhan S (2011) Can differences in microbial abundances help explain enhanced N_2O emissions in a permanent grassland under elevated atmospheric CO_2? Glob Chang Biol 17:3176–3186

Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, Nemergut DR, Graham EB, Wallenstein MD (2015) Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. ISME J 9:1693–1699. doi:10.1038/ismej.2014.252

Rochette P (2008) No-till only increases N_2O emissions in poorly-aerated soils. Soil Tillage Res 101:97–100. doi:10.1016/j.still.2008.07.011

Senbayram M, Chen R, Budai A, Bakken L, Dittert K (2012) N_2O emission and the $N_2O/(N_2O + N_2)$ product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agr Ecosyst Environ 147:4–12. doi:10.1016/j.agee.2011.06.022

Six J, Ogle SM, Jay Breidt F, Conant RT, Mosier AR, Paustian K (2004) The potential to mitigate global warming with no-tillage management is only realized when practised in the long term. Glob Chang Biol 10:155–160. doi:10.1111/j.1529-8817.2003.00730.x

Syakila A, Kroeze C (2011) The global nitrous oxide budget revisited. Greenhouse Gas Meas Manage 1:17–26. doi:10.3763/ghgmm.2010.0007

Tatti E, Goyer C, Zebarth BJ, Burton DL, Giovannetti L, Viti C (2013) Short-term effects of mineral and organic fertilizer on denitrifiers, nitrous oxide emissions and denitrification in long-term amended vineyard soils. Soil Sci Soc Am J 77:113–122. doi:10.2136/sssaj2012.0096

Taylor AE, Zeglin LH, Wanzek TA, Myrold DD, Bottomley PJ (2012) Dynamics of ammonia-oxidizing archaea and bacteria populations and contributions to soil nitrification potentials. ISME J 6:2024–2032. doi:10.1038/ismej.2012.51

Tellez-Rio A, Garcia-Marco S, Navas M, Lopez-Solanilla E, Rees RM, Luis Tenorio J, Vallejo A (2015) Nitrous oxide and methane emissions from a vetch cropping season are changed by long-term tillage practices in a Mediterranean agroecosystem. Biol Fertil Soils 51:77–88. doi:10.1007/s00374-014-0952-5

van Capelle C, Schrader S, Brunotte J (2012) Tillage-induced changes in the functional diversity of soil biota—a review with a focus on German data. Eur J Soil Biol 50:165–181. doi:10.1016/j.ejsobi.2012.02.005

van Groenigen JW, Kasper GJ, Velthof GL, van den Pol-van Dasselaar A, Kuikman PJ (2004) Nitrous oxide emissions from silage maize fields under different mineral nitrogen fertilizer and slurry applications. Plant Soil 263:101–111. doi:10.1023/B:PLSO.0000047729.43185.46

van Kessel C, Venterea R, Six J, Adviento-Borbe MA, Linquist B, van Groenigen KJ (2013) Climate, duration, and N placement determine N_2O emissions in reduced tillage systems: a meta-analysis. Glob Chang Biol 19:33–44. doi:10.1111/j.1365-2486.2012.02779.x

Velthof GL, Kuikman PJ, Oenema O (2003) Nitrous oxide emission from animal manures applied to soil under controlled conditions. Biol Fertil Soils 37:221–230. doi:10.1007/s00374-003-0589-2

Venterea RT, Halvorson AD, Kitchen N, Liebig MA, Cavigelli MA, Del Grosso SJ, Motavalli PP, Nelson KA, Spokas KA, Singh BP, Stewart CE, Ranaivoson A, Strock J, Collins H (2012) Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. Front Ecol Environ 10:562–570. doi:10.1890/120062

Vogel C, Mueller CW, Höschen C, Buegger F, Heister K, Schulz S, Schloter M, Kögel-Knabner I (2014) Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils. Nat Commun 5:2947. doi:10.1038/ncomms3947

von Luetzow M, Koegel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance

under different soil conditions—a review. Eur J Soil Sci 57:426–445. doi:10.1111/j.1365-2389.2006.00809.x

Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecol Appl 16:2143–2152. doi:10.1890/1051-0761(2006)016[2143:ECODCA]2.0.CO;2

Wei W, Isobe K, Nishizawa T, Zhu L, Shiratori Y, Ohte N, Koba K, Otsuka S, Senoo K (2015) Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. ISME J 9:1954–1965. doi:10.1038/ismej.2015.9

Wiesmeier M, Schad P, von Lutzow M, Poeplau C, Sporlein P, Geuss U, Hangen E, Reischl A, Schilling B, Kogel-Knabner I (2014) Quantification of functional soil organic carbon pools for major soil units and land uses in southeast Germany (Bavaria). Agr Ecosyst Environ 185:208–220. doi:10.1016/j.agee.2013.12.028

Supplementary Information

Tillage system affects fertilizer-induced nitrous oxide emissions

Maike Krauss[1#], Hans-Martin Krause[1#], Simone Spangler[1,2], Ellen Kandeler[2], Sebastian Behrens[3], Andreas Kappler[4], Paul Mäder[1], Andreas Gattinger[1]

[1] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland

[2] Institute of Soil Science and Land Evaluation, Department of Soil Biology, University of Hohenheim, 70599 Stuttgart, Germany

[3] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA

[4] Geomicrobiology and Microbial Ecology, Center for Applied Geoscience, University of Tübingen, 72076 Tübingen, Germany

[#] These authors contributed equally

Supplement Table S1 Quantitative PCR master mix compositions and thermal profiles for the different functional genes

Target gene	Reaction mixture	Volume [µl]	Thermal profile		Reference
16S rRNA	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 10 sec 61.5 – 20 sec 72°C – 20 sec	35 cycles	modified after Nadkarni et al. (2002)
	341F (2 µM)	0.4			
	797R (2 µM)	1.2			
	PCR water	2.4			
	DNA - template	1			
archaeal *amoA* (AOA)	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 15 sec 55°C – 30 sec 72°C – 30 sec	40 cycles	modified after Towe et al. (2010)
	amo19F (5 µM)	1			
	CrenamoA616r48x (5 µM)	1			
	PCR water	2			
	DNA - template	1			
bacterial *amoA* (AOB)	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 15 sec 59.5°C – 30 sec 72°C – 30 sec	40 cycles	modified after Towe et al. (2010)
	amoA1F (5 µM)	0.75			
	amoA2R (5 µM)	0.75			
	BSA (10% w/v)	0.4			
	PCR water	2.1			
	DNA - template	1			
nirK	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 10 sec 62-57°C – 20 sec 72°C – 10 sec 95°C – 15 sec 57°C – 20 sec 72°C – 10 sec	6 cycles 35 cycles	modified after Babić et al. (2008)
	nirK876 (2 µM)	1			
	nirK1040 (2 µM)	1			
	PCR water	2			
	DNA - template	1			
nirS	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 15 sec 58°C – 30 sec 72°C – 15 sec	40 cycles	modified after Babić et al. (2008)
	nirScd3af (5 µM)	1			
	R3cd (5 µM)	1			
	PCR water	1.6			
	BSA	0.4			
	DNA - template	1			
nosZ	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 10 sec 65-60°C – 20 sec 72°C – 15 sec 95°C – 15 sec 60°C – 15 sec 72°C – 15 sec	6 cycles 35 cycles	modified after Babić et al. (2008)
	nosZ2R (5 µM)	1			
	nosZ2F (5 µM)	1			
	PCR water	2			
	DNA - template	1			

Supplement Table S2 Primers used for quantitative PCR

Target gene	Primer	Sequence 5`- 3`	Amplicon size (bp)	Reference
16S rRNA	341F 797R	CCT ACG GGA GGC AGC AG GGA CTA CCA GGG TAT CTA ATC CTG TT	466	(Muyzer et al. 1993) (Nadkarni et al. 2002)
archaeal *amoA*	amo19F CrenamoA616r48x	ATG GTC TGG CTW AGA CG GCC ATC CAB CKR TAN GTC CA	628	(Leininger et al. 2006) (Schauss et al. 2009)
bacterial *amoA*	amoA1F amoA2R	GGG GTT TCT ACT GGT GGT CCC CTC KGS AAA GCC TTC TTC	491	(Rotthauwe et al. 1997)
nirK	nirK876C nirK1040	ATY GGC GGV CAY GGC GA GCC TCG ATC AGR TTR TGG TT	162	(Henry et al. 2006)
nirS	nirScd3af R3cd	AAC GYS AAG GAR ACS GG GAS TTC GGR TGS GTC TTG A	413	(Kandeler et al. 2006; Throbäck et al. 2004)
nosZ	nosZ2F nosZ2R	CGC RAC GGC AAS AAG GTS MSS GT CAK RTG CAK SGC RTG GCA GAA	267	(Henry et al. 2006)

Supplement Table S3 Pearson correlation coefficients of soil organic C (SOC) contents and functional gene abundances across all soil samples before incubation

	SOC	16S rRNA	archaeal *amoA*	bacterial *amoA*	*nirK*	*nirS*
16S	0.88					
archaeal *amoA*	-0.37	0.10				
bacterial *amoA*	-0.20	0.29	0.98			
nirK	0.68	0.94	0.42	0.58		
nirS	0.88	0.78	-0.26	-0.11	0.68	
nosZ	0.95	0.73	-0.60	-0.44	0.45	0.68

References

Babić KH, Schauss K, Hai B, Sikora S, Redžepović S, Radl V, Schloter M (2008) Influence of different Sinorhizobium meliloti inocula on abundance of genes involved in nitrogen transformations in the rhizosphere of alfalfa (Medicago sativa L.) Environmental Microbiology 10:2922-2930 doi:10.1111/j.1462-2920.2008.01762.x

Henry S, Bru D, Stres B, Hallet S, Philippot L (2006) Quantitative Detection of the nosZ Gene, Encoding Nitrous Oxide Reductase, and Comparison of the Abundances of 16S rRNA, narG, nirK, and nosZ Genes in Soils Appl Environ Microbiol 72:5181-5189 doi:10.1128/aem.00231-06

Kandeler E, Deiglmayr K, Tscherko D, Bru D, Philippot L (2006) Abundance of narG, nirS, nirK, and nosZ Genes of Denitrifying Bacteria during Primary Successions of a Glacier Foreland Appl Environ Microbiol 72:5957-5962 doi:10.1128/aem.00439-06

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils Nature 442:806-809 doi:10.1038/nature04983

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA Appl Environ Microbiol 59:695-700

Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set Microbiology 148:257-266 doi:10.1099/00221287-148-1-257

Rotthauwe J, Witzel K, Liesack W (1997) The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations Appl Environ Microbiol 63:4704-4712

Schauss K et al. (2009) Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils Environmental Microbiology 11:446-456 doi:10.1111/j.1462-2920.2008.01783.x

Throbäck IN, Enwall K, Jarvis Å, Hallin S (2004) Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE FEMS Microbiology Ecology 49:401-417 doi:10.1016/j.femsec.2004.04.011

Towe S, Albert A, Kleineidam K, Brankatschk R, Dumig A, Welzl G, Munch JC, Zeyer J, Schloter M (2010) Abundance of Microbes Involved in Nitrogen Transformation in the Rhizosphere of Leucanthemopsis alpina (L.) Heywood Grown in Soils from Different Sites of the Damma Glacier Forefield Microbial Ecology 60:762-770 doi:10.1007/s00248-010-9695-5

Chapter 2

Nitrous oxide production and reduction processes in soils as influenced by long-term farming systems

Hans-Martin Krause[1], Cecile Thonar[1], Wolfram Eschenbach[2], Reinhard Well[2], Paul Mäder[1], Sebastian Behrens[3], Andreas Kappler[4], Andreas Gattinger[1]

[1] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland

[2] Thünen Institute of Climate-Smart Agriculture, Federal Research Institute for Rural Areas Forestry and Fisheries, 38116 Braunschweig, Germany

[3] Institute of Soil Science and Land Evaluation, Department of Soil Biology, [3] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA

[4] Geomicrobiology and Microbial Ecology, Center for Applied Geoscience, University of Tübingen, 72076 Tübingen, Germany

Under revision for publication at Soil Biology and Fertility

Abstract

N_2O is a potent greenhouse gas with an atmospheric lifetime of 114 years which also contributes to ozone layer destruction. Mitigating N_2O emissions especially challenges the agricultural sector that is responsible for the gross of anthropogenic N_2O release. In order to develop effective mitigation strategies a detailed understanding of drivers for N_2O production and reduction in agriculturally managed soils is needed. Denitrification is recognized as one of the most important source processes for N_2O emissions from soils. However, the last step in denitrification, the reduction of N_2O to N_2 is the only known sink for N_2O in soil. Although the impact of single parameters on denitrification is quite well documented, there is still a knowledge gap when it comes to the impact of complex farming systems on N_2O production and reduction. In this experiment, we incubated soil samples from the DOK long term field trial in Therwil (CH) comparing organic (BIOORG) and conventional (CONMIN) farming systems with an a non-fertilized control (NOFERT). Soil samples were incubated under 90%WFPS after fertilization with $NH_4{}^{15}NO_3$ equivalent to a moderate fertilization event in the field with 40 kg-N ha^{-1}. In order to assess soil's potential for N_2O production and reduction we combined direct measurements of denitrification end products N_2O and N_2 with molecular analysis of functional denitrifying communities involved in NO_2^- and N_2O reduction on DNA and mRNA levels. In order to monitor N cycling processes under the chosen conditions stable isotope tracing was employed to quantify nitrification and NO_3^- consumption rates. Results revealed increased NO_3^- consumption and highest potential for N_2O emissions in BIOORG as a result of increased soil organic carbon contents. N_2 production was similar in BIOORG and CONMIN and significantly lower in NOFERT, most likely due to significantly decreased pH inhibiting N_2O reduction. This caused highest $N_2O/(N_2O+N_2)$ ratios in NOFERT (0.88 ±0.02) followed by BIOORG (0.790 ±0.01) and CONMIN (0.68 ±0.02). Lowest $N_2O/(N_2O+N_2)$ ratios in CONMIN were reflected by lowest N_2O emissions and coincided with elevated *nosZ* transcript copies in the beginning of incubation. Although highest N_2O emissions in BIOORG were detected the incubation setup cannot directly be translated to field condition. Nevertheless, our results emphasize that farming system induced changes on soil geochemical parameters like soil pH and soil organic carbon affect microbial N_2O production and reduction processes during denitrification.

Introduction

Nitrous oxide (N_2O) is a major greenhouse gas contributing to radiative forcing of Earth`s climate. It is also the most important anthropogenic compound involved in the catalytic breakdown of stratospheric ozone (Kanter *et al.*, 2013). Its current atmospheric concentration is 328 ppb and mostly due to anthropogenic interventions in the nitrogen cycle, it rises linearly by 0.25% $year^{-1}$. The global warming potential of N_2O exceeds that of CO_2 298-fold and because of its long atmospheric lifetime of 114 years reducing atmospheric N_2O concentrations will be a long term issue (Forster *et al.*, 2007). Mitigation of N_2O emissions especially challenges the agricultural sector which accounts for ~60% of anthropogenic N_2O emissions (Syakila and Kroeze, 2011). Within the agricultural sector, fertilization is the most important source of N_2O emissions (Stern, 2006). The amount and speciation of N, added during fertilization directly affects N availability and N_2O emissions (Shcherbak *et al.*, 2014; Stehfest and Bouwman, 2006) and large N_2O fluxes often occur directly after fertilization events (Gregorich *et al.*, 2005; Thangarajan *et al.*, 2013).

Different N species can serve as substrates for N_2O formation from a variety of N transforming processes depending on soil aeration status. Under oxic conditions, NH_4^+ serves as substrate for nitrification. In this process N_2O can be formed as a byproduct via NH_2OH oxidation (Schreiber *et al.*, 2012). Under oxygen limiting conditions NO_3^- reduction leads to N_2O formation via denitrification. Yet, the separation of nitrification and denitrification as sources for N_2O is a simplification as a variety of microbial metabolic pathways (e.g. nitrifier-denitrification, co-denitrification) and abiotic reactions (chemodenitrification, chemical decomposition of NH_2OH) can form N_2O and might even occur within the same soil aggregate (Kool *et al.*, 2011; Butterbach-Bahl *et al.*, 2013). The process of denitrification is of special importance since it is generally considered as one of the most important processes for N_2O production and the last step in denitrification, the reduction of N_2O to N_2, is the only known biological sink for N_2O (Thomson *et al.*, 2012).

As most N transformation processes in soils are carried out by microbes, a detailed understanding of the drivers for the functioning of denitrifying and especially N_2O reducing microbial communities in agriculturally managed soils is a prerequisite in order to develop effective mitigation strategies. It is important to note that denitrification is a modular pathway which involves four enzymatic systems in the subsequent reduction of NO_3^-, NO_2^-, NO and N_2O (Tiedje *et al.*, 1982). The most widely used genetic marker system targeting denitrification are the functional genes *nirK* and *nirS* encoding for copper- and heme-bearing NO_2^- reductases (Jones *et al.*, 2008). The last step of denitrification, the reduction of N_2O to N_2, is catalyzed by the nitrous oxide reductases encoded by

the functional genes *nosZ* and *nosZ-II* (Jones *et al.*, 2013). Not all microbes involved in denitrification necessarily possess the whole set of denitrifying genes (Graf *et al.*, 2014). Around one third of bacteria involved in NO_2^- reduction lack the genetic capability for N_2O reduction and thus are likely to produce N_2O as denitrification end product (Philippot *et al.*, 2011). On the contrary, it is reported that other microbes bearing the functional genes *nosZ* and especially *nosZ-II* lack antecedent enzymatic system and thus might act as a sink for N_2O (Graf *et al.*, 2014). In the last years there had been several studies investigating mechanisms of N_2O emissions via gene abundance and/or expression with variable results (Chen *et al.*, 2015; Morales *et al.*, 2010; Harter *et al.*, 2014; Miller *et al.*, 2008; Dandie *et al.*, 2011; Henderson *et al.*, 2010; Németh *et al.*, 2014). Yet, it needs to be noted that most studies lacked quantification of N_2 and the process of N_2O consumption was not directly assessed.

Due to its high atmospheric background concentrations, quantification of N_2 as a denitrification end product is extremely challenging (Groffman *et al.*, 2006) and all approaches to measure N_2 have to deal with inherent drawbacks (Saggar *et al.*, 2013). Recent development of simultaneous tracing of ^{15}N in N_2O and N_2 from the same gas sample has significantly increased the efficiency and reliability of ^{15}N tracing experiments (Lewicka-Szczebak *et al.*, 2013). This method is especially valuable in the agricultural context, since provision of additional N is an intrinsic part of fertilization research. However, ensuring homogenous distribution of added ^{15}N remains challenging in ^{15}N tracing experiments (Spott *et al.*, 2006). Quantification of N_2 provides valuable information about N_2O consumption and $N_2O/(N_2O+N_2)$ product ratios are an important measure for soils performance as sink for N_2O. The $N_2O/(N_2O+N_2)$ product ratio can be affected by C and N availability (Senbayram *et al.*, 2012) as well as soil pH (Cuhel *et al.*, 2010) and the denitrifying community composition (Jones *et al.*, 2014; Philippot *et al.*, 2009). While the impact of single parameters on denitrification processes is quite well studied, still there is a knowledge gap when it comes to complex farming systems.

In the last decades organic farming systems gained attention due to positive effects on numerous soil quality indicators (Maeder *et al.*, 2002), soil organic carbon stocks (Gattinger *et al.*, 2012) and N_2O and CH_4 fluxes (Skinner et al., 2014). It was also shown that organic farming systems increased richness and diversity of microbial community (Hartmann *et al.*, 2014). However, it is unclear how the legacy of farming systems affects denitrification and especially N_2O reduction. In order to assess the impact of farming system on denitrification and $N_2O/(N_2O+N_2)$ product ratio, we performed an incubation trial with soil samples originating from the DOK long term field trial (D: bio-

dynamic, O: bio-organic, K: german “konventionell” integrated). This field trial compares farming systems in place since 1978.

The object of this study was to assess the effect of farming system induced changes in soil geochemical parameters on N cycling under oxygen limited conditions with a special emphasis on N_2O and N_2 emissions. We also aimed at identifying the functional impact of denitrifier gene abundance and expression on N_2O production and reduction processes.

Material and Methods

Sampling site

Soils were collected from the DOK system comparison trial in Therwil/BL, Switzerland. Soil sampling took place in autumn 2013. Soil type was classified as Haplic Luvisol on deposits of alluvial loess. Composite soil samples originated from 4 replicate parcels of BIOORG, CONMIN and NOFERT treatments and were collected to a depth of 20 cm. Samples were sieved to 2 mm and stored at 4°C. Sampling was done during the 6th crop rotation after cultivation of *Zea mays*. The previous culture consisted of two years cultivation of grass clover. All farming systems are subjected to the same 7 year crop rotation. Details on the experimental setup of the field trial are described in Fließbach *et al.* (2007). Briefly, BIOORG represents a livestock-based organic farming system with farmyard manure fertilization without pesticides and did not receive liming treatment. CONMIN is characterized by a livestock-free system comprising mineral fertilization and chemical pest control. This treatment received 4.7 t ha $CaCO_3$ in the 4th crop rotation from 1999 to 2005 (Oberholzer *et al.*, 2009). NOFERT did not receive any fertilization, pest control or liming treatment. The 5th crop rotation started in 2006 with maize, followed by winter wheat, soja, potato, winter wheat and two years of grass clover. Soil samples were taken after harvest of the first crop in the 6th crop rotation which was maize. In the vegetation period before sampling CONMIN received 170 kg N ha^{-1} in the form of calcium ammonium nitrate, while BIOORG received 136 kg N ha^{-1} as rotted manure and 46 kg N ha^{-1} in the form of cattle slurry.

Incubation setup

For each microcosm, the equivalent of 150 g dry soil was placed in 250 ml DURAN wide neck glass bottles (Schott AG, Mainz, Germany) and compacted to a bulk density of 1.20±0.02 g/cm^3 by tapping the glass bottles on a soft surface. After pre-incubation of 7 days at a water filled pore space (WFPS) of 50%, an N containing solution equivalent to a moderate fertilization event (40 kg N ha^{-1} or 11 mg N per microcosm) was added in the form of 60at% enriched $NH_4{}^{15}NO_3$. Deionized and autoclaved water was added to adjust to 90% WFPS in order to stimulate denitrifying conditions and enhance distribution of added N solution. Swelling properties of the soil samples resulted in a WFPS of 87.5 ±1.5 % with no farming system specific bias. To assure constant conditions, water content was checked gravimetrically and corrected every second day by adding evaporated water to each microcosm. Microcosms were incubated with open bottles in order to mimic conditions after a heavy rain event in the field Each treatment was incubated in triplicates at 20±1.2°C in the dark. In total 63 microcosms (three treatments times three reps times seven sampling dates) were prepared enabling

destructive sampling for geochemical and molecular biological analysis after 0, 2, 5, 8, 11, 14 and 17 days. After destructive sampling, soil was homogenized and divided into subsamples for subsequent analysis.

Geochemical analyses

pH, soil organic carbon and total N

Directly after beginning of the incubation key soil parameters were assessed in triplicates. Soil pH was determined in a 1:2.5 (w/v) dilution with demineralized H_2O Soil organic carbon (SOC) was analyzed by dry combustion (multi N/C2100S + HT1300, Analytik Jena AG, Jena, Germany) and total N in soils was determined by combustion (CN Vario Max, Elementar Analysensysteme GmbH, Hanau, Germany).

Mineral nitrogen (N_{min}) and water extractable organic carbon (WEOC)

For determination of NH_4^+, NO_3^- and WEOC 80 ml of 0.01 M $CaCl_2$ was added to 20 g soil sample and shaken for 1 h at 130 rpm (SM-30, Edmund Bühler GmbH, Hechingen Germany). The soil solution was filtered through a folded filter (MN619EH, Machery-Nagel, Düren, Germany) and stored at -20°C until analysis. Concentrations of NH_4^+ and NO_3^- were quantified by continuous flow analyses (San Plus, Skalar Analytical B.V., Breda, Netherland) while WEOC was determined via TOC Analyzer (multi N/C 2100S, analyticJena, Jena, Germany). All parameters were quantified at each time point but changes in WEOC over time were negligible. Therefore only initial WEOC contents are reported.

Greenhouse gas fluxes

Before destructive soil sampling, production of CO_2 and N_2O were measured after closing each microcosm with a gas-tight lid equipped with a rubber septum for up to 40 min. A cooling tray with circulatory water flow assured constant temperature (20±0.8°C) of the microcosms during gas sampling. Gas samples of 5ml were taken from the headspace of the microcosms and directly injected into a gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA) after 0,20 and 40 minutes by using an autosampler (MPS 2XL, Gerstel, Baltimore, MD). In order to avoid underpressure, 5 ml He was injected to the headspace of the microcosm prior to gas sampling. CO_2 and N_2O concentrations in gas samples were determined via flame ionization detector (FID) linked to a methanizer and electron capture detector (ECD), respectively. Calibration curves for N_2O (r^2>0.99) and CO_2 (r^2>0.999) were obtained by a threefold analysis of 3 standard gases with 0.308, 2.94 and 90

ppm N_2O and 300, 2960 and 9000 ppm CO_2 before and after each sampling. For flux calculation, a linear enrichment of gases in the headspace was assumed. Gas samples for $^{15}N_2$ and $^{15}N_2O$ analysis were taken with a syringe directly before destructive sampling after a prolonged enrichment period of 1h and stored in 12.5 ml gas tight vials. $^{15}N_2$ and $^{15}N_2O$ concentrations in the gas samples were quantified via isotope ratio mass spectrometry, as described in Lewicka-Szczebak *et al.* (2013). Prior to analysis, in a part of the sample N_2O is frozen in a liquid N trap and which enables the quantification of ^{29}R ($^{29}N_2/^{28}N_2$) and ^{30}R ($^{30}N_2/^{29}N_2$) of N_2, N_2O and N_2O+N_2 from the same sample (Lewicka-Szczebak *et al.*, 2013). Fraction of NO_3^- derived N_2O and/or N_2 (f_p)were calculated according to Spott *et al.* (2006) using eq 1.

$$f_p = \frac{a_m - a_{bgd}}{a_p - a_{bgd}} \qquad (eq.1)$$

were a_{bgd} is the 15N abundance of the atmospheric background, a_p is the ^{15}N abundance of the active NO_3^- pool and a_m is the ^{15}N abundance of N_2 and/or N_2O. a_m and a_p were calculated using eq 2 and 3

$$a_m = \frac{29_R + 2 * 30_R}{2\,(1 + 29_R + 30_R)} \qquad (eq.2)$$

$$a_p = \frac{30_{x_m} - a_{bgd} * a_m}{a_m - a_{bgd}} \qquad (eq.3)$$

In which $^{39}x_m$ is the measure fraction of m/z 30 in N_2 and/or converted N_2O calculated as ^{30}R /(1+^{29}R+^{30}R). ^{15}N enrichment of the active NO_3^- pools for N_2O and N_2 formation are shown in Figure S1. The denitrification product ratio was calculated according to eq 4.

$$pr = \frac{f_{N2O}}{f_{N2O+N2}} \qquad (eq.4)$$

NO_3^- derived N_2O and N_2 fluxes were assessed according to eq 4 and 5.

$$N2O_{NO3}flux = f_{N2O} * N2O_{total}flux \qquad (eq.5)$$

$$N2_{NO3}flux = \left(\left(\frac{1}{pr}\right) * N2O_{NO3}flux\right) - N2O_{NO3}\,flux \qquad (eq.)6$$

In which N_2O_{total} flux was obtained by flux calculation from GC measurements. Hybrid N_2O and N_2 was determined as described in Spott and Stange (2011) , but found irrelevant.

Isotopic analysis of $^{15}NH_4^+$ and $^{15}NO_3^-$ and calculation of N transforming processes

The ^{15}N abundance in NH_4^+ and NO_3^- was determined according to the procedure described in Stange et al. (2007), whereby NO_3 was reduced to NO by vanadium chloride ($V^{III}Cl_3$) and NH_4^+ was oxidized to N_2 by sodium hypobromite (BrNaO). The NO and N_2 obtained were then analyzed using a quadrupole mass spectrometer (GAM 200, InProcess Instruments, Bremen, Germany). The analytical precision was determined by repeated measurements of standards (1 at%, 5 at%, 50 at%, 75 at%) and was consistently around 1.2%. Gross nitrification and NO_3^- consumption rates were assessed using the pool dilution approach according to eq.1 and eq.2 provided by Davidson *et al.* (1991) and Stark (2000).

$$p = ((M_0 - M_1) / t) * \frac{\log(H_0 M_1 / H_1 M_0)}{\log(M_0 / M_1)} \quad (eq.1)$$

$$c = p - ((M_0 - M_1) / t) \quad (eq.2)$$

where p and c are the nitrification and NO_3^- consumption rates (mg N kg^{-1} day^{-1})rate M is the amount of NO_3^--N (mg N kg^{-1} dry soil), H the ^{15}N atom fraction of NO_3^-, subscripts 0 and 1 mark first and second time point, respectively. t represents the incubation interval between first and second time point (days). For all calculations a homogeneous distribution of the labeled pool and negligible immobilization of the ^{15}N tracer into the organic N pool within incubation intervals were assumed. Cumulative values had been quantified by summing the amount of N transformed during all incubation intervals.

Molecular biological analyses

Subsamples for DNA and RNA extraction were collected and stored at -80°C. DNA and RNA were co-extracted from 0.5 g soil samples via phenol chloroform extraction as described in Griffiths *et al.* (2010). In order to assess for individual DNA and RNA recovery rates 2.50^{10} copies of a linearized plasmid (pJET1.2, CloneJET PCR Cloning Kit, Thermo Scientific, Waltham, MA) carrying a fragment of cassava mosaic virus (APA9, gene accession Nr. AJ427910) (Thonar *et al.*, 2012) and 2.85^{10} transcripts (MEGAscript® T7 transcription KIT, Invitrogen, Carlsbad, CA) were added to the soil samples before bead beating. DNA and RNA concentrations were assessed fluorimetrically with Qubit 2.0 (Qubit dsDNA HS Assay Kit and Qubit RNA HS Assay Kit, Invitrogen, Carlsbad, CA, USA) directly after extraction. Absolute yield for DNA and RNA ranged from 108.4 to 325.2 ng g dry $soil^{-1}$ and 22.4 to

78.8 ng g dry soil^{-1}, respectively, without treatment specific bias. Reverse transcription was conducted using QuantiTect Reverse Trancription Kit (Qiagen, Venlo, Netherlands) with an integrated removal step for genomic DNA. Successful removal of genomic DNA was assured by the performance of negative controls without addition of reverse transcriptase. Quantitative PCR of functional genes was performed using KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, Wilmington, MA) and a Rotor-Gene-Q (QIAGEN, Venlo, Netherlands). Each 10 µl reaction volume included ~1 ng of template DNA or cDNA. Primers and thermal protocols used for functional gene quantification are listed in Table S1 and S2. Standard curves were constructed by running a serial dilution with concentrations ranging from ~10^8 to ~10^2 gene copy numbers per reaction of a plasmid bearing a copy of the respective gene. Specifications of vector plasmids and host genes are given in Table S3. Concentrations of standards were measured fluorimetrically with Qubit 2.0 (Qubit dsDNA HS Assay Kit, Invitrogen, Carlsbad, CA). Efficiencies of qPCR yielded 94-96% for APA9 gene fragment, 86-92% for *nirK*, 89-92% for *nirS*, 77-82% for *nosZ* and 82-86 % for *nosZ-II* assays and specificity of the amplification was tested via melt curve and agarose gel analysis. Each reaction was performed in analytical duplicates and repeated if Ct values differed more than 0.5. Raw data was analyzed via LinReg PCR by assessing enzyme kinetics for each reaction individually (Ramakers *et al.*, 2003). Additional to normalization of functional gene abundances per soil g dry weight, DNA and RNA recovery rates obtained by APA9 quantification were used to correct functional gene and transcript copy numbers. Recovery rates averaged 51.4±12.5% for DNA and 2.3±0.8% for RNA. It needs to be noted that the absolute values of gene and especially transcript numbers exceeded reported values from other studies, as a consequence of calculation integrating the recovery rate of the internal standard. (Snider *et al.*, 2015; Chen *et al.*, 2015; Tatti *et al.*, 2013).

Statistical analysis

For N_2O, N_2 and CO_2 fluxes as well as functional gene and transcript abundances the effects of farming system at a specific time point were examined by one way analysis of variance (ANOVA) with a post hoc Tukey test using JMP 5.0.1. In the same way time integrated N transformation rates, and cumulated N_2O and N_2 production as well as $N_2O/(N_2O+N_2)$ product ratios were tested for effects of farming system. Differences were considered as significant at $p<0.05$.

Results

Basic soil properties

Quantification of basic geochemical parameters in soils from different farming systems prior to incubation revealed significantly increased SOC content in BIOORG, followed by CONMIN and then NOFERT. Initial WEOC did not show any effect of farming system, while pH and total N contents were significantly decreased in NOFERT (Table 1). Soil C/N ratio was significantly increased in BIOORG compared to NOFERT, while CONMIN did not differ significantly from the other treatments.

Table 1: Basic soil properties before incubation. Data shows means and standard errors (n=3). Values not connected by the same letter differ significantly at $p<0.05$

treatment	pH (H_2O)		WEOC (mg -C kg^{-1})		SOC (g kg^{-1})		Total N (g kg^{-1})		C/N	
BIOORG	6.13 ±0.02	a	36.00 ±1.60	a	14.15 ±0.05	a	1.70 ±0.01	a	8.33 ±0.01	a
CONMIN	6.15 ±0.03	a	34.09 ±1.75	a	13.66 ±0.05	b	1.72 ±0.06	a	7.94 ±0.25	ab
NOFERT	5.54 ±0.04	b	32.14 ±2.17	a	11.74 ±0.08	c	1.65 ±0.03	b	7.11 ±0.13	b

Abbreviations: WEOC – water extractable organic carbon; SOC- soil organic carbon.

CO_2, N_2O and N_2 fluxes

Across the incubation period cumulated CO_2 emissions were significantly enhanced in BIOORG compared to CONMIN. Cumulated CO_2 emissions in NOFERT did not differ significantly from the other farming systems. After addition of $NH_4{}^{15}NO_3$, the greatest N_2O fluxes appeared at 5 days with mean N_2O emissions of 74.01 ±23.47, 56.91 ±2.90 and 42.79 ±2.84 µg N_2O-N kg dry $soil^{-1}$ h^{-1} for BIOORG, NOFERT and CONMIN, respectively (Figure 1A). Thereafter, N_2O emission declined and reached stable levels after 11 days. In the last phase of incubation CONMIN showed significantly decreased N_2O emissions at days 14 and 17 as compared to BIOORG and NOFERT. N_2O emissions in NOFERT soil emerged slowly and after 2 days of incubation N_2O emissions in NOFERT were significantly lower compared to CONMIN and BIOORG. From day 8 on, N_2O emissions reached similar high levels like BIOORG. N_2 emissions in NOFERT were detectable after 5 days of incubation but remained at constantly low levels throughout the incubation (Figure 1B). In BIOORG and CONMIN, N_2 emissions increased till day 8 and slightly declined thereafter. Highest N_2 emissions occurred at the end of incubation reaching 24.02±6.40, 24.25±3.35 and 7.44±1.07 µg N_2-N kg dry $soil^{-1}$ h^{-1} in BIOORG, CONMIN and NOFERT, respectively (Figure 1B). $N_2O/(N_2O+N_2)$ product ratio constantly declined in BIOORG and CONMIN but remained at high levels throughout the incubation time in NOFERT (Figure

1C). At days 2, 8, 11 and 17 the $N_2O/(N_2O+N_2)$ product ratios were significantly higher in BIOORG compared to CONMIN meaning that significantly lower portion of N_2O was further reduced to N_2. This resulted in distinct $N_2O/(N_2O+N_2)$ product ratios of cumulated fluxes for the different soils that decreased in the order NOFERT, BIOORG to CONMIN (Table 2). Yet, cumulated NO_3^- derived N_2O emissions were the highest for BIOORG (16.18±1.66 mg N_2O-N kg^{-1}) followed by NOFERT (13.87±0.46 mg N_2O-N kg^{-1}) and the lowest in CONMIN (9.59±0.15 mg N_2O-N kg^{-1}) (Table 2). Interestingly, cumulated N_2 emissions did not differ between BIOORG and CONMIN but were significantly lower in NOFERT (Table 2).

Table 2: Cumulative gas fluxes, measures of denitrification and cumulative N-transforming processes for soil samples from conventional (CONMIN) and organic (BIOORG) farming system in comparison with an unfertilized control (NOFERT) after 17 days of incubation and fertilization with $NH_4^{15}NO_3$. Data shows means and SE (n=3). Values not connected by the same letter differ significantly at $p<0.05$.

	BIOORG		CONMIN		NOFERT	
	gaseous emissions					
CO_2-C mg kg^{-1}	61.49 ±4.97	a	49.26 ±3.12	b	53.18 ±5.30	ab
NO_3^- derived N_2O-N mg kg^{-1}	16.18 ±1.66	a	9.59 ±0.15	b	13.87 ±0.46	a
NO_3^- derived N_2-N mg kg^{-1}	4.41 ±0.41	a	4.81 ±0.41	a	1.95 ±0.33	b
	measures of denitrification					
$N_2O/(N_2O+N_2)$	0.79 ±0.01	b	0.67 ±0.02	c	0.88 ±0.02	a
NO_3^- derived N_2O emissions (%)	95.01		94.29		95.52	
	N transforming processes					
Gross NO_3^- consumption mg-N kg^{-1}	21.00 ±0.24	a	18.44 ±0.24	b	15.33 ±0.37	c
Nitrification mg-N kg^{-1}	11.31 ±0.07	b	12.02 ±0.06	a	6.22 ±0.02	c

Cumulative data was obtained by subsequently cumulating means of time weighted process rates calculated for each time point. Abbreviations: DNRA – dissimilatory nitrate reduction to ammonium.

N transforming processes

Generally, NO_3^- consumption was the dominating N transforming process in all soils. Across the whole incubation period 21.00±0.24 mg of NO_3^--N kg^{-1} was consumed in BIOORG followed by CONMIN (18.44±0.24 mg NO_3^--N kg^{-1}) and NOFERT (15.33±0.37 mg NO_3^--N kg^{-1}) (Table 2). Cumulated NO_3^- consumption was significantly increased in BIOORG from day 11 on, while cumulated NO_3^- consumption in NOFERT was significantly lower at day 17 (Figure 2A). For BIOORG and NOFERT the sum of cumulated N_2 and N_2O fluxes (20.59 and 15.82 mg N kg^{-1}, respectively) was in good agreement with gross NO_3^- consumption, whereas in CONMIN N_2+N_2O fluxes were significantly lower

(14.40 mg N) compared to gross NO_3^- consumption (Table 2). Nitrification in CONMIN started slow and cumulated nitrification was significantly decreased after 2 days of incubation compared to NOFERT and BIOORG. Yet, from day 14 on cumulative nitrification was the highest in CONMIN. Significantly decreased cumulated nitrification was observed in NOFERT from day 8 on (Figure 2B).

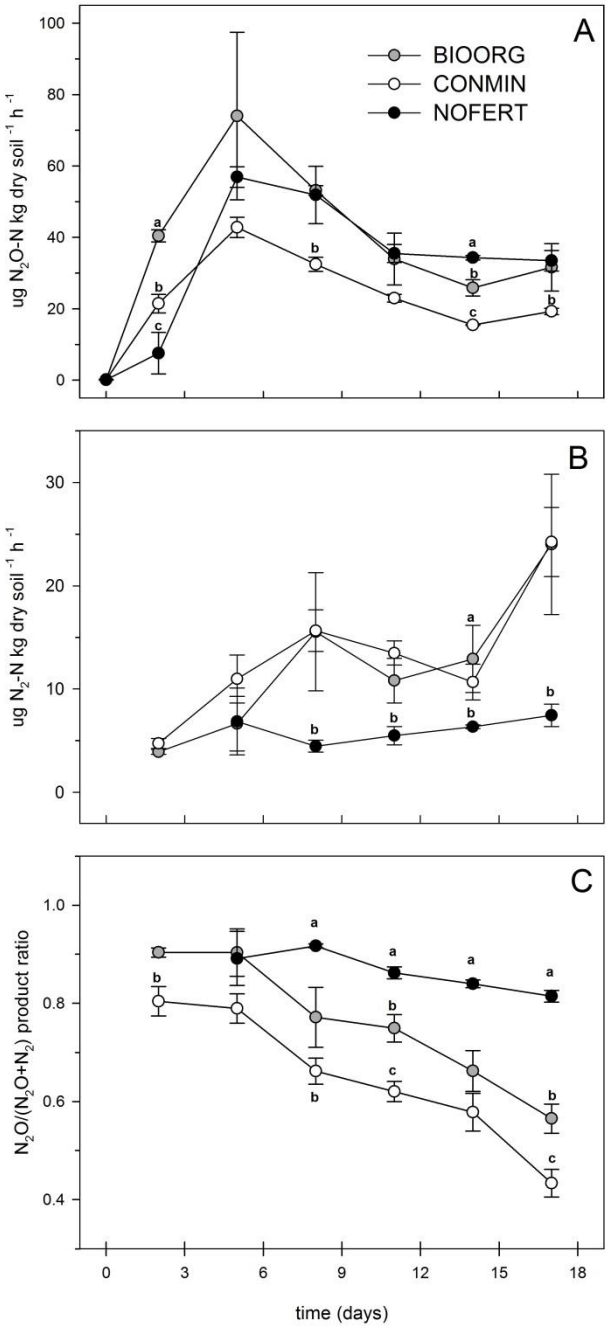


Figure 1: Panel **A** shows N_2O emissions from incubated soil samples originating from different soil management practices (CONMIN- conventional, BIOORG – organic, NOFERT – unfertilized) after fertilization with $NH_4^{15}NO_3$. Panel **B** shows temporal patterns of N_2 emission and panel **C** displays $N_2O/(N_2O+N_2)$ product ratio. Data is only shown if all replications resulted in measurable amounts of N_2. Small letters indicated significant differences in between treatments at a specific time point at $p< 0.05$. Data points are means ± SD (n=3).

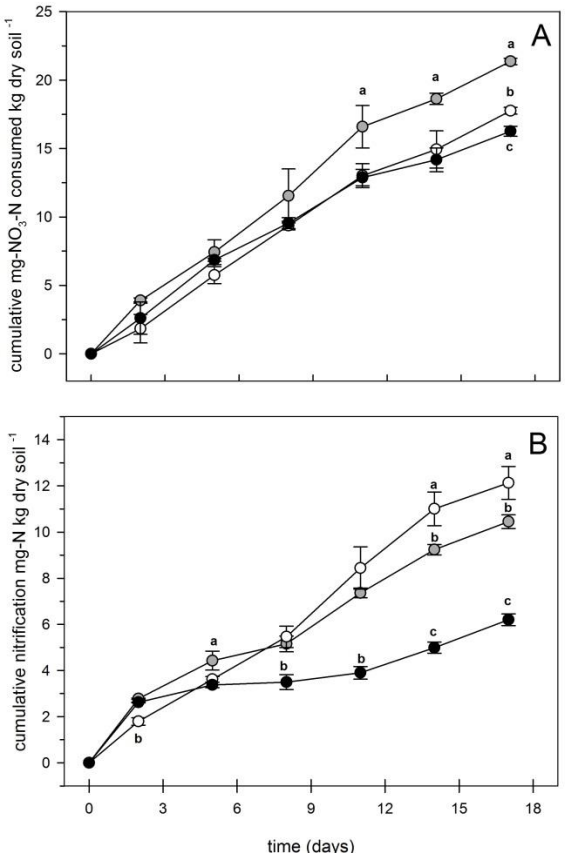


Figure 2: Panels **A-C** show cumulative gross NO_3^- consumption, DNRA and nitrification from incubated soil samples originating from different soil management practices (CONMIN- conventional, BIOORG – organic, NOFERT – unfertilized) after fertilization with $NH_4^{15}NO_3$. Small letters indicated significant differences in between treatments at a specific time point at $p< 0.05$. Data points are means ± SD (n=3).

Gene and transcript abundances of denitrifying genes

Abundances of functional genes involved in NO_2^- reduction (*nirK*, *nirS*) did not show strong variations within the incubation period and were not influenced by the farming system (Figure 3A and C). Only NOFERT showed significantly reduced abundances for *nirS* gene at day 11 and *nirK* gene at day 8 and 11. Generally *nirS* gene abundance exceeded *nirK* gene abundance by around one order of magnitude. In terms of *nirS* and *nirK* gene expression little differences were observed between farming systems (Figure 4A and C). Gene expression of both nitrite reductases increased after fertilization in the beginning of the incubation. While *nirK* transcripts increased by almost one order of magnitude in CONMIN and NOFERT, increase in *nirS* transcripts was only 2-3 fold. Gene copy numbers of *nosZ* and *nosZ-II* were stable during incubation (Figure 3B and D). Significant differences between soils were detected for *nosZ* abundance at day 8 and 11 with the lowest gene abundances in NOFERT. *NosZ-II* gene abundance was the lowest in CONMIN at all sampling points, although this

was not significant. Transcription of *nosZ-II* and especially *nosZ* fluctuated strongly during incubation (Figure 4B and D). Directly after fertilization the transcripts of *nosZ* increased by almost one order of magnitude in all soils and reached a first peak after 2 days of incubation. Thereafter, expression declined in all farming systems at day 5 and stayed at stable levels in CONMIN. Significantly increased *nosZ* transcripts in CONMIN could be observed during increasing N_2O emissions till day 5. In that period N_2O emissions and *nosZ* transcripts correlated negatively with N_2O emissions across all soils (r^2=0.81) (Supplementary Figure S2). *NosZ* transcripts in NOFERT and BIOORG peaked a second time at day 11 and were higher compared to CONMIN although this was significant for BIOORG only. For *nosZ-II* no transcripts were detectable till day 2 in CONMIN and BIOORG and day 5 in NOFERT. Afterwards transcripts increased and peaked at day 8 in CONMIN and at day 11 in BIOORG and NOFERT (Figure 4B and D). However, no farming system effects were detected for transcripts of *nosZ-II*.

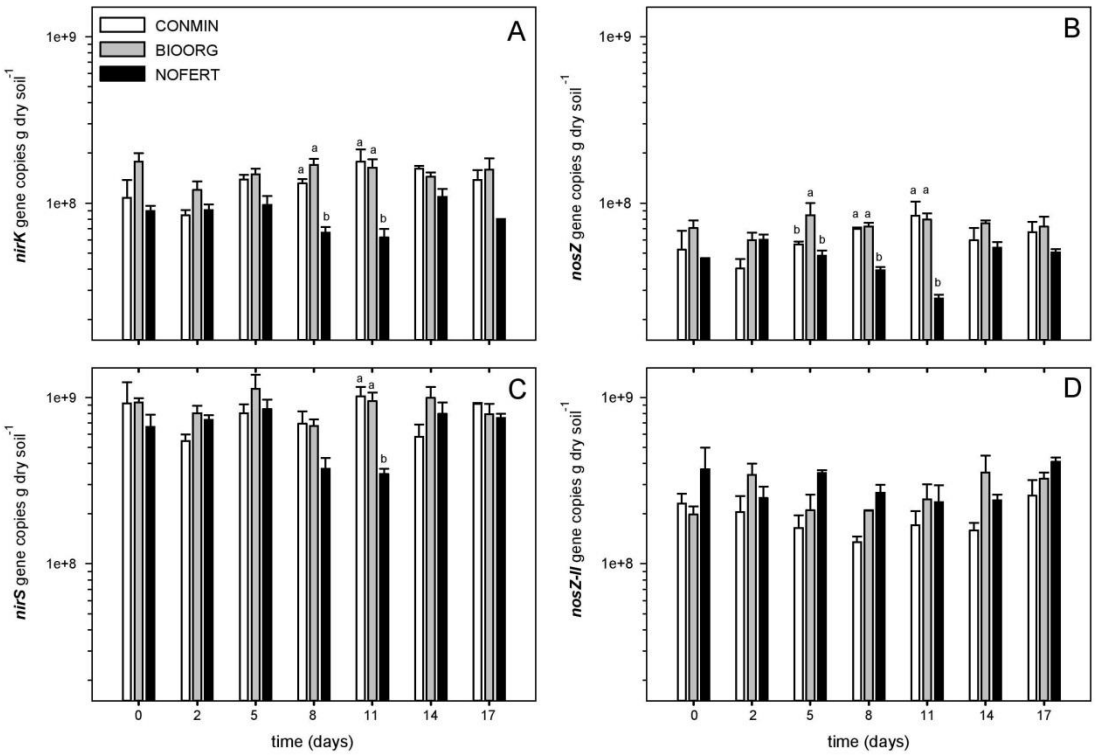


Figure 3: Gene copies of nitrite reductases (*nirK* and *nirS*, **A** and **C**) and nitrous oxide reductases (*nosZ* and *nosZ-II*, **B** and **D**) communities in DNA of soil samples originating from different farming system (CONMIN-conventional, BIOORG – organic, NOFERT – unfertilized) after fertilization with $NH_4{}^{15}NO_3$. Letters indicated significant differences at a specific time point in between treatments at p< 0.05. Data points are means ± SE (n=3).

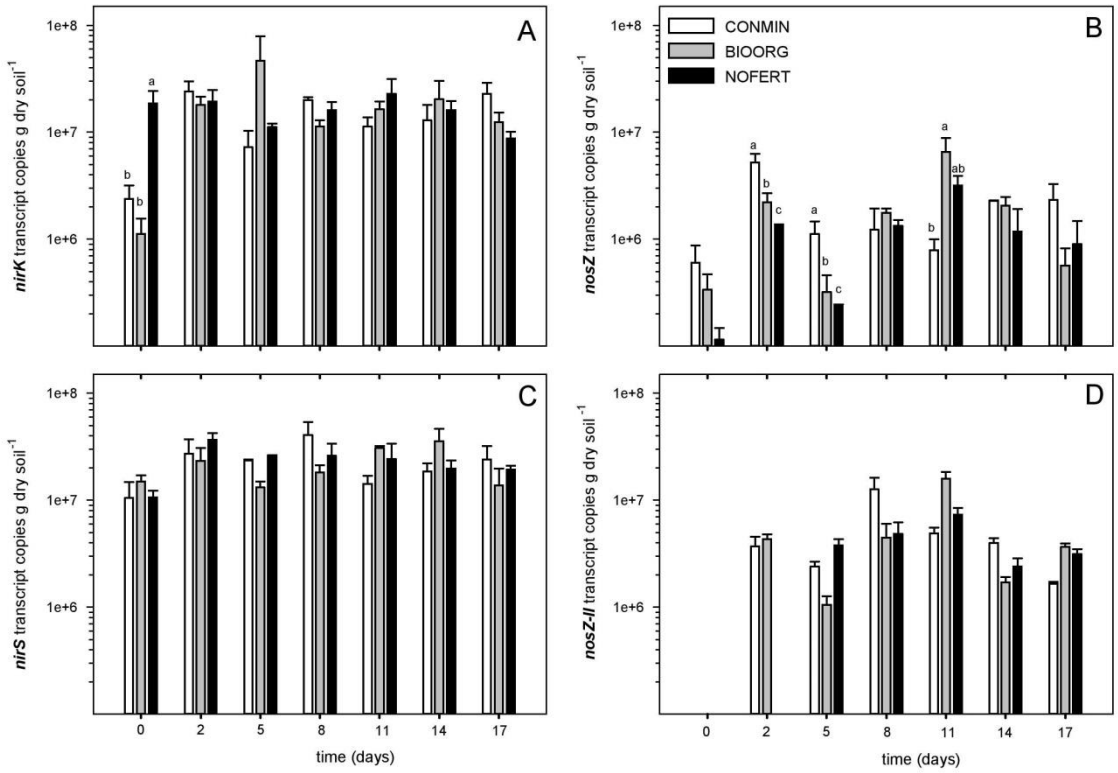


Figure 4: Transcript copies of nitrite reductases (*nirK* and *nirS*, **A** and **C**) and nitrous oxide reductases (*nosZ* and *nosZ-II*, **B** and **D**) communities in soil samples originating from different farming systems (CONMIN-conventional, BIOORG – organic, NOFERT – unfertilized) after fertilization with $NH_4^{15}NO_3$. Letters indicated significant differences at a specific time point in between treatments at $p < 0.05$. Data points are means ± SE (n=3). Lacking data point indicate transcript copies below detection limit in two or three replicates.

Discussion

Geochemistry of the microcosms and N transforming processes

At the beginning of the experiment microcosms were fertilized with 11 mg N as NH_4NO_3 in correspondence to a moderate fertilization event in the field of 40 kg N ha^{-1}. Despite differing fertilization history, all soils were treated equally in order to assess long-term impact of farming systems on N transformations. Chosen incubation conditions (WFPS of 90%) aimed at favoring denitrification, but occurrence of nitrification proved partial availability of oxygen. This was expected as incubated soil samples were open to the atmosphere and soil pores were not completely water saturated, a situation which can occur in the field after a raining event. During most times of the year we would expect soil conditions to be rather oxic and thus nitrification related processes to be the major source for N_2O emissions. Yet, it was frequently reported that highest N_2O emissions in the field occur under conditions when low oxygen concentrations in the soil favor reducing processes like denitrification (Groffman *et al.*, 2009). Not surprisingly the oxygenation status of the soil was reported to be the major control of N_2O emission on a regional scale (Jungkunst *et al.*, 2006). As distinct SOC contents presented a major distinguishing factor for soils from the different farming systems, no additional C was added during fertilization. Constant bacterial abundances as indicated by stable 16S rRNA gene numbers indicated limited bacterial growth and suggest C-limiting conditions in our setup (Supplementary Figure S3). Nevertheless, significantly increased CO_2 emissions in BIOORG compared to CONMIN demonstrate enhanced heterotrophic activity as a consequence of long term organic fertilization and elevated SOC levels (Table 2). This is in agreement with the studies of Hartmann *et al.* (2014) and Carpenter-Boggs *et al.* (2000) where increased soil microbial activity and/or abundance in organically managed soil had been described.

Nitrifying bacteria can contribute to N_2O emission directly via NH_2OH oxidation and nitrifier denitrification and indirectly through NO_3^- provision for denitrification (Wrage *et al.*, 2001). The occurrence of nitrification in our setup was not surprising as NH_4^+ oxidation and NO_3^- reduction processes can co-occur over a wide range of soil moisture conditions (Kool *et al.*, 2011). Although the occurrence of nitrification (and possibly nitrifier denitrification) was proven, the contribution of this pathway to N_2O emission seems negligible as NO_3^- derived N_2O emissions accounted for ~95% of N_2O emission in all soils. Similarly, Kool *et al.* (2011) ascribed ~92% of N_2O emissions to denitrification at a WFPS of 90%.

For the calculation of nitrification and NO_3^- consumption rates and NO_3^- derived N_2O and N_2 emissions with the stable isotope approach homogeneous distribution of the added tracer and

negligible N recycling within the incubation time must be assumed (Stark, 2000). The latter point was addressed by limiting incubation intervals to 72 hours. Nevertheless, our approach might have overestimated N transformation rates since we could not account for N immobilization within an incubation interval. Although in our case a high WFPS assisted in uniform distribution of the added tracer assuring homogenous labeling can only be approximated (Stark, 2000; Stange *et al.*, 2007). Good agreement of $^{15}NO_3^-$ and the ^{15}N enrichments of the active NO_3^- pool for N_2O and N_2 formation is an indication for homogeneity in ^{15}N labeling (Stevens *et al.*, 1997). While, ^{15}N enrichments in the NO_3^- and the active NO_3^- pool for N_2O formation was almost identical, the active NO_3^- pool for N_2 formation showed higher ^{15}N signatures (Supplementary Figure S1). This indicates homogeneous conditions for N_2O formation but increased N_2 formation at isolated microsites, e.g. NO_3^- derived N_2O from lower soil was more likely to be further reduced to N_2 compared to NO_3^- derived N_2O from shallow soil within our soil microcosm. Similarly, distinct active pools for nitrification were already reported during an oxic incubation experiment (Deppe *et al.*, 2017).

Impact of farming systems on N_2O emissions and N_2O/N_2O+N_2 product ratio

The main goal of this incubation study was to assess the long term impact of farming system on soil's potential to perform N_2O production and reduction within the process of denitrification. Generally, N_2 production was low compared to other studies focusing on denitrification end products. In-situ measurements of $N_2O/(N_20+N_2)$ product ratios in a grassland agroecosystems were reported not to exceed 0.45 (Baily *et al.*, 2012; McGeough *et al.*, 2012; Friedl *et al.*, 2016). Nevertheless, $N_2O/(N_2O+N_2)$ product ratios are known to be largely dependent on the relative availabilities of C and NO_3^- (Senbayram *et al.*, 2012; Morley and Baggs, 2010; Senbayram *et al.*, 2009) and $N_2O/(N_2O+N_2)$ product ratios of up to 0.94 were reported in an incubation experiment under C limiting conditions with NO_3^- excess (Senbayram *et al.*, 2012). Most likely this was the case in our study where high NO_3^- concentrations reduced the relative importance of N_2O reduction and shifted dominating denitrification end product towards N_2O (Supplementary Figure S1). It might also be that O_2 was allowed to penetrate in the soil column inhibiting functionality of the nitrous oxide reductase. However, this seems unlikely since addition a recent incubation study observed that N_2O reduction was not inhibited after addition of low molecular C sources at a WFPS of 90% (Giles *et al.*, 2017)

In accordance with enhanced NO_3^- consumption in BIOORG, other studies demonstrated long term addition of farmyard manure to increase denitrification rates (Tatti *et al.*, 2013) as well as N_2O emissions after NH_4NO_3 application (Jäger *et al.*, 2013). Yet, quantification of N_2 additionally revealed N_2O reduction to play a key role affecting total N_2O budgets. Lowest N_2O emissions in CONMIN can`t solely be assigned to decreased NO_3^- consumption but also to high N_2 production. This is reflected by

the significantly lowest $N_2O/(N_2O+N_2)$ product ratio at most time points of sampling and proved most efficient reduction of N oxides during the subsequent denitrification steps in CONMIN. There are two possible explanations how legacy of farming systems might have affected $N_2O/(N_2O+N_2)$ ratios in CONMIN and BIOORG. First, lower SOC levels in CONMIN compared to BIOORG might have reinforced C limitation and shifted denitrification end product towards N_2. Additionally, it needs to be considered that fertilization history of CONMIN is characterized by repeated fertilization with NH_4NO_3 without C addition. Therefore, adaptation of the denitrifying community to reoccurring conditions might have led to most efficient use of provided N oxides under C-limiting conditions. Latter hypothesis is supported by significantly increased *nosZ* expression in CONMIN in the beginning of incubation.

It is also known that pH can greatly impact $N_2O/(N_2O+N_2)$ ratios (Cuhel *et al.*, 2010). Most likely low pH was the main driver for high $N_2O/(N_2O+N_2)$ ratios in NOFERT. In this farming system constant low N_2 production was observed, although expression of *nosZ* and *nosZ-II* genes was similar to BIOORG. The study of Bergaust *et al.* (2010) showed that acidic conditions below a pH of 6 impedes correct folding and thus functioning of the nitrous oxide reductase as a posttranscriptional effect in *Paracoccus denitrificans*. Liu *et al.* (2014) demonstrated that pH-induced dysfunctional assembly of the nitrous oxide reductase can affect whole communities. Since NOFERT did not receive any liming treatment throughout management history since 1978 pH was significantly lower and most likely dropped under the functional threshold that enables correct folding of the N_2O reductase. Agricultural practice maintaining pH above 6 should therefore be considered as important part of climate friendly farming systems. Unlike CONMIN, also BIOORG did not receive liming treatment throughout the management of the field trial. Nevertheless the pH stayed constant and the functionality of the nitrous oxide reductase was not inhibited. This suggests organic farming systems to maintain functionality of N_2O reductase more effectively on the long run compared to conventional farming systems. Although NO_3^- consumption and N_2O emissions were enhanced due to increased SOC levels in BIOORG in our setup increased N_2O fluxes in the field in organic farming systems seem unlikely considering the anomalous addition of NO_3^- in our setup. In fact, a recent meta study showed organic farming systems to decrease area scaled N_2O emissions due to lower inputs of available N (Skinner *et al.*, 2014).

Influence of denitrifier abundance and activity on dynamics of N_2O emissions

The approach of predicting microbial processes with functional gene or transcript abundance is currently under debate as correlations between process rates and functional gene or transcript abundances are often missing (Bier *et al.*, 2015; Rocca *et al.*, 2015). In terms of denitrification,

functional gene and transcript abundances had been studied extensively with variable results. While there are examples for studies that could successfully link denitrifier gene and/or transcript copies with N_2O emissions (Harter *et al.*, 2014; Chen *et al.*, 2015; Morales *et al.*, 2010; Tatti *et al.*, 2013), in other studies significant relations were missing (Miller *et al.*, 2009; Henderson *et al.*, 2010; Dandie *et al.*, 2011). Therefore, experimental conditions need to be evaluated carefully for each experiment before comparing molecular data with process rates. In our case, C limitation most likely hampered growth of heterotrophic denitrifiers and thus differentiation of functional gene abundance in between farming systems was negligible. Consequently, mRNA analysis as a measure for functional activity becomes more important when comparing the impact of farming systems on denitrifying communities. Expression of *nirK* and *nirS* genes was rather stable, while *nosZ* and *nosZ-II* transcription levels fluctuated throughout the incubation time. This suggests increased susceptibility of *nosZ* and *nosZ-II* regulation to environmental factors compared to genes involved in nitrite reduction. Nevertheless, it needs to be noted that the primers used to quantify *nirK* and *nirS* genes only detect alpha-, beta and gammaproteobacteria and other phylogenetic groups involved in nitrite reduction that were not accounted for in our analysis can significantly contribute to N_2O emissions (Wei *et al.*, 2015). Still, correlation between *nosZ* transcripts and N_2O emissions before day 5 (r^2=0.81) shows the potential use of mRNA analysis as predictor for N_2O emissions during increasing N_2O emission rates. In our case peak expression of *nosZ* genes in CONMIN and BIOORG were followed by increased N_2 emissions indicating functional impact of increased *nosZ* transcription levels in these soils. This is in line with the study of Chen *et al.* (2015) who found transcript of *nosZ* to be a correlate with N_2O reduction in an incubation trial after NO_3^- and glucose addition. Similar to other studies abundance and/or expression of functional genes involved in N_2O reduction showed higher explanatory power for N_2O emission compared to genes involved in nitrite reduction (Chen *et al.*, 2015; Krauss *et al.*, 2016). Still, *nosZ* transcripts and N_2O emissions in our case did not correlate throughout the incubation period which emphasizes limited applicability of this approach and shows that gene expression can`t necessarily be translated to enzyme activity. Also the fact that soil pH can affect N_2O reduction at a posttranscriptional level further limits the explanatory power of this analysis. Nevertheless, understanding environmental and regulatory factors involved in N_2O reduction is crucial for the development of effective N_2O mitigation strategies. While the expression of the denitrification genes is known to be regulated by O_2 and the concentrations of NO, NO_3^- and NO_2^- via a variety of different regulatory proteins (Spiro, 2012; Zumft and Kroneck, 2007) the regulation of *nosZ* seems to be decoupled from antecedent denitrifying enzymatic systems (van Spanning *et al.*, 2007). For the denitrifying model organism *Paracoccus denitrificans* a combined upregulation of *nosZ* due to oxygen depletion (via NNR regulatory protein) and NO concentrations

(via FnrP regulatory protein) was demonstrated (Bergaust *et al.*, 2012) which could explain distinct *nosZ* transcription peaks in BIOORG and NOFERT. Yet, non-detectable expression of *nosZ-II* at the beginning of incubation further illustrates distinct evolution of nitrous oxide reductases as described by Sanford *et al.* (2012) is also reflected in regulation mechanics. There are several indications that these functionally equivalent enzymes are ecologically not redundant. The *nosZ-II* bearing and nitrate-ammonifying microbe *Wollinella succinogenes* was shown to mediate upregulation of *Nap*, *Nrf* and *Nos* genes via nitrosative stress regulator, while lacking the O_2 sensing regulatory proteins (Torres *et al.*, 2016; Kern and Simon, 2015). A population response to high NO concentrations could explain delayed transcription of *nosZ-II* in our setup and would further stress the importance of *nosZ-II* bearing bacteria as sink for N_2O as consequence of nitrosative stress regulation. On a DNA level it was already shown that n*osZ-II* dominated microbial communities increase soils N_2O sink capacity (Jones *et al.*, 2014). In our case, however, farming systems significantly only affected gene expression of the typical *nosZ* gene suggesting differences in N_2O reduction to be mainly driven by this functional group.

Conclusions

In conclusion, we showed that at 90% WFPS increased SOC levels due to organic farming increased NO_3^- consumption and susceptibility for N_2O emissions when NO_3^- is available in excess. Furthermore, pH seemed to the major determining factor for N_2O reduction since a low pH impedes the functionality of the nitrous oxide reductase. Therefore maintenance of a high pH seems to be a crucial part of climate friendly farming systems. Despite significant differences in between farming systems in *nosZ* transcripts during emerging N_2O emissions transcript or gene copy numbers of denitrifying genes seem to be a weak predictor for N_2O emissions under C limiting conditions. Nevertheless, temporal dynamics of *nosZ* expression stressed the importance of the N_2O reducing functional communities as a regulator of the N_2O sink.

Acknowledgements

We gratefully acknowledge the financial support for this project provided by the Swiss National Science Foundation in frame of the National Research Program “Soil as a Resource” (NRP 68, Grant Nr. 406840_143137). We would like to thanks Simon Moakes for English correction.

References

Baily, A., Watson, C., Laughlin, R., Matthews, D., McGeough, K. & Jordan, P. (2012). Use of the 15 N gas flux method to measure the source and level of N2O and N2 emissions from grazed grassland. *Nutrient Cycling in Agroecosystems* 94(2-3): 287-298.http://dx.doi.org/10.1007/s10705-012-9541-x

Bergaust, L., Mao, Y., Bakken, L. R. & Frostegard, A. (2010). Denitrification Response Patterns during the Transition to Anoxic Respiration and Posttranscriptional Effects of Suboptimal pH on Nitrogen Oxide Reductase in Paracoccus denitrificans. *Applied Environmental Microbiology* 76(19): 6387-6396.http://dx.doi.org/10.1128/aem.00608-10

Bergaust, L., van Spanning, R. J. M., Frostegård, Å. & Bakken, L. R. (2012). Expression of nitrous oxide reductase in Paracoccus denitrificans is regulated by oxygen and nitric oxide through FnrP and NNR. *Microbiology* 158(Pt 3): 826-834.http://dx.doi.org/10.1099/mic.0.054148-0

Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T., Nemergut, D., Osborne, B. B., Ruiz-González, C., Schimel, J. P., Waldrop, M. P. & Wallenstein, M. D. (2015). Linking microbial community structure and microbial processes: an empirical and conceptual overview. *FEMS Microbiology Ecology*.http://dx.doi.org/10.1093/femsec/fiv113

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society* 368(1621). http://dx.doi.org/10.1098/rstb.2013.0122

Carpenter-Boggs, L., Kennedy, A. C. & Reganold, J. P. (2000). Organic and Biodynamic Management Effects on Soil Biology 1 Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the product by the USDA. L. Carpenter-Boggs currently at USDA-ARS, North Central Soil Conservation Research Lab., 803 Iowa Ave., Morris, MN 56267. *Soil Science Society of America Journal* 64(5).http://dx.doi.org/10.2136/sssaj2000.6451651x

Chen, Z., Wang, C., Gschwendtner, S., Willibald, G., Unteregelsbacher, S., Lu, H., Kolar, A., Schloter, M., Butterbach-Bahl, K. & Dannenmann, M. (2015). Relationships between denitrification gene expression, dissimilatory nitrate reduction to ammonium and nitrous oxide and dinitrogen production in montane grassland soils. *Soil Biology and Biochemistry* 87: 67-77. http://dx.doi.org/10.1016/j.soilbio.2015.03.030

Cuhel, J., Simek, M., Laughlin, R. J., Bru, D., Cheneby, D., Watson, C. J. & Philippot, L. (2010). Insights into the Effect of Soil pH on N(2)O and N(2) Emissions and Denitrifier Community Size and Activity. *Applied and Environmental Microbiology* 76(6): 1870-1878.http://dx.doi.org/10.1128/Aem.02484-09

Dandie, C. E., Wertz, S., Leclair, C. L., Goyer, C., Burton, D. L., Patten, C. L., Zebarth, B. J. & Trevors, J. T. (2011). Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. *FEMS Microbiology Ecology* 77(1): 69-82. http://dx.doi.org/10.1111/j.1574-6941.2011.01084.x

Davidson, E. A., Hart, S. C., Shanks, C. A. & Firestone, M. K. (1991). Measuring gross nitrogen mineralization, and nitrification by 15 N isotopic pool dilution in intact soil cores. *Journal of Soil Science* 42(3): 335-349.http://dx.doi.org/10.1111/j.1365-2389.1991.tb00413.x

Deppe, M., Well, R., Giesemann, A., Spott, O. & Flessa, H. (2017). Soil N2O fluxes and related processes in laboratory incubations simulating ammonium fertilizer depots. *Soil Biology and Biochemistry* 104: 68-80.http://dx.doi.org/10.1016/j.soilbio.2016.10.005

Fließbach, A., Oberholzer, H.-R., Gunst, L. & Mäder, P. (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. *Agriculture, Ecosystems & Environment* 118(1–4): 273-284. http://dx.doi.org/10.1016/j.agee.2006.05.022

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., Haywood, J., Lean, J., Lowe, D. C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M. & Dorland, R. v. (2007).Changes in Atmospheric Constituents and in Radiative Forcing. In *Climate Change 2007: The physical science basis.Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, 105 (Eds S. Solomon, D. Qin, M. Manning, M. Marquis, K. Averyt, M. M. B. Tignor, H. LeRoy Miller Jr. and Z. Chen). Cambridge, United Kingdomand New York, NY, USA: Cambridge University Press.

Friedl, J., Scheer, C., Rowlings, D. W., McIntosh, H. V., Strazzabosco, A., Warner, D. I. & Grace, P. R. (2016). Denitrification losses from an intensively managed sub-tropical pasture – Impact of soil moisture on the partitioning of N2 and N2O emissions. *Soil Biology and Biochemistry* 92: 58-66.http://dx.doi.org/10.1016/j.soilbio.2015.09.016

Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N. E.-H. & Niggli, U. (2012). Enhanced top soil carbon stocks under organic farming. *Proceedings of the National Academy of Sciences* 109(44): 18226-18231. http://dx.doi.org/10.1073/pnas.1209429109

Giles, M. E., Daniell, T. J. & Baggs, E. M. (2017). Compound driven differences in N2 and N2O emission from soil; the role of substrate use efficiency and the microbial community. *Soil Biology and Biochemistry* 106: 90-98.http://dx.doi.org/10.1016/j.soilbio.2016.11.028

Graf, D. R. H., Jones, C. M. & Hallin, S. (2014). Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N2O Emissions. *Plos One* 9(12): e114118. http://dx.doi.org/10.1371/journal.pone.0114118

Gregorich, E. G., Rochette, P., VandenBygaart, A. J. & Angers, D. A. (2005). Greenhouse gas contributions of agricultural soils and potential mitigation practices in Eastern Canada. *Soil and Tillage Research* 83(1): 53-72.http://dx.doi.org/10.1016/j.still.2005.02.009

Griffiths, R. I., Whiteley As Fau - O'Donnell, A. G., O'Donnell Ag Fau - Bailey, M. J. & Bailey, M. J. (2010). Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. (0099-2240 (Print))

Groffman, P. M., Altabet, M. A., Bohlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., Kana, T. M., Nielsen, L. P. & Voytek, M. A. (2006). Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications* 16(6): 2091-2122.http://dx.doi.org/10.1890/1051-0761(2006)016[2091:MFMDDA]2.0.CO;2

Groffman, P. M., Butterbach-Bahl, K., Fulweiler, R. W., Gold, A. J., Morse, J. L., Stander, E. K., Tague, C., Tonitto, C. & Vidon, P. (2009). Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* 93(1): 49-77.10.1007/s10533-008-9277-5

Harter, J., Krause, H.-M., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., Kappler, A. & Behrens, S. (2014). Linking N2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. *The ISME Journal*.http://dx.doi.org/10.1038/ismej.2013.160

Hartmann, M., Frey, B., Mayer, J., Mader, P. & Widmer, F. (2014). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME Journal*.http://dx.doi.org/10.1038/ismej.2014.210

Henderson, S. L., Dandie, C. E., Patten, C. L., Zebarth, B. J., Burton, D. L., Trevors, J. T. & Goyer, C. (2010). Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions and denitrification in anoxic soil microcosms amended with glucose and plant residues. *Applied Environmental Microbiology*: AEM.02993-02909.http://dx.doi.org/10.1128/aem.02993-09

Jäger, N., Duffner, A., Ludwig, B. & Flessa, H. (2013). Effect of fertilization history on short-term emission of CO2 and N2O after the application of different N fertilizers – a laboratory study.

Archives of Agronomy and Soil Science 59(2): 161-171.http://dx,doi.org/10.1080/03650340.2011.621420

Jones, C., Graf, D., Bru, D., Philippot, L. & Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *The ISME Journal* 7(2): 417-426. http://dx.doi.org/10.1038/ismej.2012.125

Jones, C. M., Spor, A., Brennan, F. P., Breuil, M.-C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S. & Philippot, L. (2014). Recently identified microbial guild mediates soil N2O sink capacity. *Nature Climate Change* advance online publication.http://dx.doi.org/10.1038/nclimate2301

Jones, C. M., Stres, B., Rosenquist, M. & Hallin, S. (2008). Phylogenetic Analysis of Nitrite, Nitric Oxide, and Nitrous Oxide Respiratory Enzymes Reveal a Complex Evolutionary History for Denitrification. *Molecular Biology and Evolution* 25(9): 1955-1966.http://dx.doi.org/10.1093/molbev/msn146

Jungkunst, H. F., Freibauer, A., Neufeldt, H. & Bareth, G. (2006). Nitrous oxide emissions from agricultural land use in Germany— a synthesis of available annual field data. *Journal of Plant Nutrition and Soil Science* 169(3): 341-351. http://dx.doi.org/10.1002/jpln.200521954

Kanter, D., Mauzerall, D. L., Ravishankara, A. R., Daniel, J. S., Portmann, R. W., Grabiel, P. M., Moomaw, W. R. & Galloway, J. N. (2013). A post-Kyoto partner: Considering the stratospheric ozone regime as a tool to manage nitrous oxide. *Proceedings of the National Academy of Sciences* 110(12): 4451-4457.http://dx.doi.org/10.1073/pnas.1222231110

Kern, M. & Simon, J. (2015). Three transcription regulators of the Nss family mediate the adaptive response induced by nitrate, nitric oxide or nitrous oxide in Wolinella succinogenes. *Environmental Microbiology*: n/a-n/a.http://dx.doi.org/10.1111/1462-2920.13060

Kool, D. M., Dolfing, J., Wrage, N. & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry* 43(1): 174-178. http://dx.doi.org/10.1016/j.soilbio.2010.09.030

Krauss, M., Krause, H.-M., Spangler, S., Kandeler, E., Behrens, S., Kappler, A., Mäder, P. & Gattinger, A. (2016). Tillage system affects fertilizer-induced nitrous oxide emissions. *Biology and Fertility of Soils*: 1-11.http://dx.doi.org/10.1007/s00374-016-1152-2

Lewicka-Szczebak, D., Well, R., Giesemann, A., Rohe, L. & Wolf, U. (2013). An enhanced technique for automated determination of 15N signatures of N2, (N2 +N2O) and N2O in gas samples. *Rapid Communications in Mass Spectrometry* 27(13): 1548-1558.http://dx.doi.org/10.1002/rcm.6605

Liu, B., Frostegård, Å. & Bakken, L. R. (2014). Impaired Reduction of N2O to N2 in Acid Soils Is Due to a Posttranscriptional Interference with the Expression of nosZ. *MBio* 5(3).10.1128/mBio.01383-14

Maeder, P., Fliessbach, A., Dubois, D., Gunst, L., Fried, P. & Niggli, U. (2002). Soil Fertility and Biodiversity in Organic Farming. *Science* 296(5573): 1694-1697.http://dx,doi.org/10.1126/science.1071148

McGeough, K. L., Laughlin, R. J., Watson, C. J., Müller, C., Ernfors, M., Cahalan, E. & Richards, K. G. (2012). The effect of cattle slurry in combination with nitrate and the nitrification inhibitor dicyandiamide on in situ nitrous oxide and dinitrogen emissions. *Biogeosciences* 9(12): 4909-4919.http://dx.doi.org/10.5194/bg-9-4909-2012

Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C. & Trevors, J. T. (2008). Crop residue influence on denitrification, N2O emissions and denitrifier community abundance in soil. *Soil Biology and Biochemistry* 40(10): 2553-2562. http://dx.doi.org/10.1016/j.soilbio.2008.06.024

Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C. & Trevors, J. T. (2009). Influence of Liquid Manure on Soil Denitrifier Abundance, Denitrification, and Nitrous Oxide Emissions *Soil Science Society of America Journal* 73(3).http://dx.doi.org/10.2136/sssaj2008.0059

Morales, S. E., Cosart, T. & Holben, W. E. (2010). Bacterial gene abundances as indicators of greenhouse gas emission in soils. *The ISME Journal* 4(6): 799-808. http://dx.doi.org/10.1038/ismej.2010.8

Morley, N. & Baggs, E. M. (2010). Carbon and oxygen controls on N2O and N2 production during nitrate reduction. *Soil Biology and Biochemistry* 42(10): 1864-1871.http://dx.doi.org/10.1016/j.soilbio.2010.07.008

Németh, D. D., Wagner-Riddle, C. & Dunfield, K. E. (2014). Abundance and gene expression in nitrifier and denitrifier communities associated with a field scale spring thaw N2O flux event. *Soil Biology and Biochemistry* 73(0): 1-9.http://dx.doi.org/10.1016/j.soilbio.2014.02.007

Oberholzer, H.-R., Fließbach, A., Mäder, P. & Mayer, J. (2009). Einfluss von biologischer und konventioneller Bewirtschaftung auf biologische Bodenqualitätsparameter: Entwicklungen im DOK Langzeitversuch nach pH-Regulierung. *Beiträge zur 10. Wissenschaftstagung Ökologischer Landbau.*

Philippot, L., Andert, J., Jones, C. M., Bru, D. & Hallin, S. (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. *Global Change Biology* 17(3): 1497-1504. http://dx.doi.org/10.1111/j.1365-2486.2010.02334.x

Philippot, L., Čuhel, J., Saby, N. P. A., Chèneby, D., Chroňáková, A., Bru, D., Arrouays, D., Martin-Laurent, F. & Šimek, M. (2009). Mapping field-scale spatial patterns of size and activity of the denitrifier community. *Environmental Microbiology* 11(6): 1518-1526.http://dx.doi.org/10.1111/j.1462-2920.2009.01879.x

Ramakers, C., Ruijter, J. M., Deprez, R. H. L. & Moorman, A. F. M. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* 339(1): 62-66.http://dx.doi.org/10.1016/S0304-3940(02)01423-4

Rocca, J. D., Hall, E. K., Lennon, J. T., Evans, S. E., Waldrop, M. P., Cotner, J. B., Nemergut, D. R., Graham, E. B. & Wallenstein, M. D. (2015). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. *The ISME Journal* 9(8): 1693-1699.http://dx.doi.org/10.1038/ismej.2014.252

Saggar, S., Jha, N., Deslippe, J., Bolan, N. S., Luo, J., Giltrap, D. L., Kim, D. G., Zaman, M. & Tillman, R. W. (2013). Denitrification and N2O:N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of The Total Environment* 465(0): 173-195. http://dx.doi.org/10.1016/j.scitotenv.2012.11.050

Sanford, R. A., Wagner, D. D., Wu, Q., Chee-Sanford, J. C., Thomas, S. H., Cruz-García, C., Rodríguez, G., Massol-Deyá, A., Krishnani, K. K., Ritalahti, K. M., Nissen, S., Konstantinidis, K. T. & Löffler, F. E. (2012). Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *Proceedings of the National Academy of Sciences* 109(48): 19709-19714. http://dx.doi.org/10.1073/pnas.1211238109

Schreiber, F., Pascal, W., Kai, M. U. & George, F. W. (2012). Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Frontiers in Microbiology* 3.http://dx.doi.org/10.3389/fmicb.2012.00372

Senbayram, M., Chen, R., Budai, A., Bakken, L. & Dittert, K. (2012). N2O emission and the N2O/(N2O+N2) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. *Agriculture, Ecosystems & Environment* 147(0): 4-12.http://dx.doi.org/10.1016/j.agee.2011.06.022

Senbayram, M., Chen, R., Mühling, K. H. & Dittert, K. (2009). Contribution of nitrification and denitrification to nitrous oxide emissions from soils after application of biogas waste and other fertilizers. *Rapid Communications in Mass Spectrometry* 23(16): 2489-2498.10.1002/rcm.4067

Shcherbak, I., Millar, N. & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences*. http://dx.doi.org/10.1073/pnas.1322434111

Skinner, C., Gattinger, A., Muller, A., Mäder, P., Flieβbach, A., Stolze, M., Ruser, R. & Niggli, U. (2014). Greenhouse gas fluxes from agricultural soils under organic and non-organic management — A global meta-analysis. *Science of The Total Environment* 468–469(0): 553-563.http://dx.doi.org/10.1016/j.scitotenv.2013.08.098

Snider, D., Thompson, K., Wagner-Riddle, C., Spoelstra, J. & Dunfield, K. (2015). Molecular techniques and stable isotope ratios at natural abundance give complementary inferences about N2O production pathways in an agricultural soil following a rainfall event. *Soil Biology and Biochemistry* 88: 197-213.http://dx.doi.org/10.1016/j.soilbio.2015.05.021

Spiro, S. (2012). Nitrous oxide production and consumption: regulation of gene expression by gas-sensitive transcription factors. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 367(1593): 1213-1225.http://dx.doi.org/10.1098/rstb.2011.0309

Spott, O., Russow, R., Apelt, B. & Stange, C. F. (2006). A 15N-aided artificial atmosphere gas flow technique for online determination of soil N2 release using the zeolite Köstrolith SX6®. *Rapid Communications in Mass Spectrometry* 20(22): 3267-3274.http://dx.doi.org/10.1002/rcm.2722

Spott, O. & Stange, F. (2011). Formation of hybrid N2O in a suspended soil due to co-denitrification of NH2OH. *Journal of Plant Nutrition and Soil Science* 174(4): 554-567 http://dx,doi.org/10.1002/jpln.201000200

Stange, Spott, O., Apelt, B. & Russow, R. W. B. (2007). Automated and rapid online determination of 15N abundance and concentration of ammonium, nitrite, or nitrate in aqueous samples by the SPINMAS technique. *Isotopes in Environmental and Health Studies* 43(3): 227-236.http://dx.doi.org/10.1080/10256010701550658

Stark, J. (2000).Nutrient Transformations. In *Methods in Ecosystem Science*, 215-234 (Eds O. Sala, R. Jackson, H. Mooney and R. Howarth). Springer New York.

Stehfest, E. & Bouwman, L. (2006). N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. *Nutrient Cycling in Agroecosystems* 74(3): 207-228.http://dx.doi.org/10.1007/s10705-006-9000-7

Stern (2006). The Stern Review on the Economic Effects of Climate Change. *Population and Development Review* 32(4): 793-798.http://dx.doi.org/10.1111/j.1728-4457.2006.00153.x

Stevens, R. J., Laughlin, R. J., Burns, L. C., Arah, J. R. M. & Hood, R. C. (1997). Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry* 29(2): 139-151.http://dx.doi.org/10.1016/S0038-0717(96)00303-3

Syakila, A. & Kroeze, C. (2011). The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management* 1(1): 17-26. http://dx.doi.org/10.3763/ghgmm.2010.0007

Tatti, E., Goyer, C., Zebarth, B. J., Burton, D. L., Giovannetti, L. & Viti, C. (2013). Short-Term Effects of Mineral and Organic Fertilizer on Denitrifiers, Nitrous Oxide Emissions and Denitrification in Long-Term Amended Vineyard Soils. *Soil Science Society of America Journal* 77(1).http://dx,doi.org/10.2136/sssaj2012.0096

Thangarajan, R., Bolan, N. S., Tian, G., Naidu, R. & Kunhikrishnan, A. (2013). Role of organic amendment application on greenhouse gas emission from soil. *Science of The Total Environment* 465: 72-96.http://dx.doi.org/10.1016/j.scitotenv.2013.01.031

Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M. & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society* 367(1593): 1157-1168. http://dx.doi.org/10.1098/rstb.2011.0415

Thonar, C., Erb, A. & Jansa, J. (2012). Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources* 12(2): 219-232.http://dx.doi.org/10.1111/j.1755-0998.2011.03086.x

Tiedje, J. M., Sexstone, A. J., Myrold, D. D. & Robinson, J. A. (1982). Denitrification: ecological niches, competition and survival. *Antonie Van Leeuwenhoek* 48(6): 569-583

Torres, M. J., Simon, J., Rowley, G., Bedmar, E. J., Richardson, D. J., Gates, A. J. & Delgado, M. J. (2016).Nitrous Oxide Metabolism in Nitrate-Reducing Bacteria: Physiology and Regulatory Mechanisms. In *Advances in Microbial Physiology*.

van Spanning, R. J. M., Richardson, D. J. & Ferguson, S. J. (2007).Chapter 1 - Introduction to the Biochemistry and Molecular Biology of Denitrification. In *Biology of the Nitrogen Cycle*, 3-20. http://dx.doi.org/10.1016/B1978-044452857-044452855.044450002-044452853 (Ed H. B. J. F. E. Newton). Amsterdam: Elsevier.

Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S. & Senoo, K. (2015). Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. *The ISME Journal*.http://dx.doi.org/10.1038/ismej.2015.9

Wrage, N., Velthof, G. L., van Beusichem, M. L. & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry* 33(12–13): 1723-1732.http://dx.doi.org/10.1016/S0038-0717(01)00096-7

Zumft, W. G. & Kroneck, P. M. H. (2007).Respiratory Transformation of Nitrous Oxide (N2O) to Dinitrogen by Bacteria and Archaea. In *Advances in Microbial Physiology*, Vol. Volume 52, 107-227. http://dx.doi.org/110.1016/S0065-2911(1006)52003-X (Ed K. P. Robert).

Supplementary Information

Nitrous oxide production and reduction processes in soils as influenced by long-term farming systems

Hans-Martin Krause[1], Cecile Thonar[1], Wolfram Eschenbach[2], Reinhard Well[2], Paul Mäder[1], Sebastian Behrens[3], Andreas Kappler[4], Andreas Gattinger[1]

[1] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland

[2] Thünen Institute of Climate-Smart Agriculture, Federal Research Institute for Rural Areas Forestry and Fisheries, 38116 Braunschweig, Germany

[3] Institute of Soil Science and Land Evaluation, Department of Soil Biology, [3] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA

[4] Geomicrobiology and Microbial Ecology, Center for Applied Geoscience, University of Tübingen, 72076 Tübingen, Germany

Supplement Table S1: Master mixes and temperature protocols used for functional gene quantification

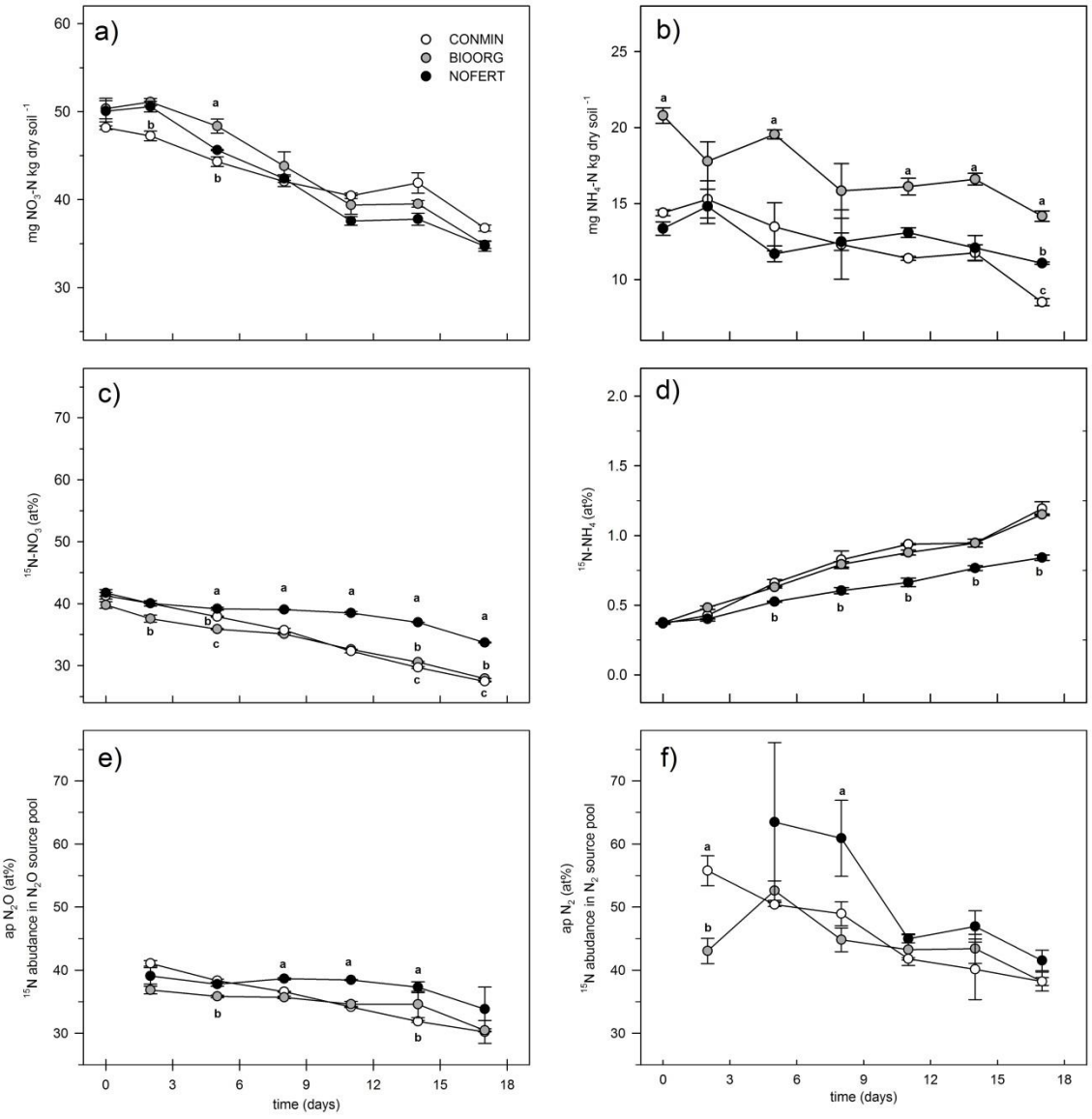
Target gene	Reaction mixture	Volumes [µl]	Thermal profile		Reference
APA9	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5			
	APA9_F (2 µM)	2	95°C – 10`	35	Modified after(Thonar *et al.*, 2012)
	APA9_R (2 µM)	2	52°C - 30 `	cycles	
	DNA - template	1			
nosZ-II	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5			
	nosZ-II-F (10 µM)	1	95°C – 15`		
	nosZ-II-R (10 µM)	1	54°C – 30`	40	Modified after(Jones *et al.*, 2013)
	DMSO	0.4	72°C – 30`	cycles	
	PCR water	1.6	80°C – 10`		
	DNA - template	1			
nosZ	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 10`		
			65-60°C – 20`	6 cycles	
	nosZ2R (5 µM)	1	80°C – 15`		Modified after (Babić *et al.*, 2008)
	nosZ2F (5 µM)	1			
	PCR water	2	95°C – 15`		
			60°C – 15`	35 cycles	
	DNA - template	1	80°C – 15`		
nirS	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5			
	nirScd3af (5 µM)	1	95°C – 15`	40 cycles	Modified after (Babić *et al.*, 2008)
	R3cd (5 µM)	1	58°C – 30`		
	PCR water	1.6	80°C – 15`		
	BSA	0.4			
	DNA - template	1			
nirK	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 10`		
			62-57°C – 20`	6 cycles	
	nirK876 (2 µM)	1	80°C – 10`		Modified after(Babić *et al.*, 2008)
	nirK1040 (2 µM)	1			
	PCR water	2	95°C – 15`		
			57°C – 20`	35 cycles	
	DNA - template	1	80°C – 10`		

Supplement Table S2. Primers used for quantitative PCR

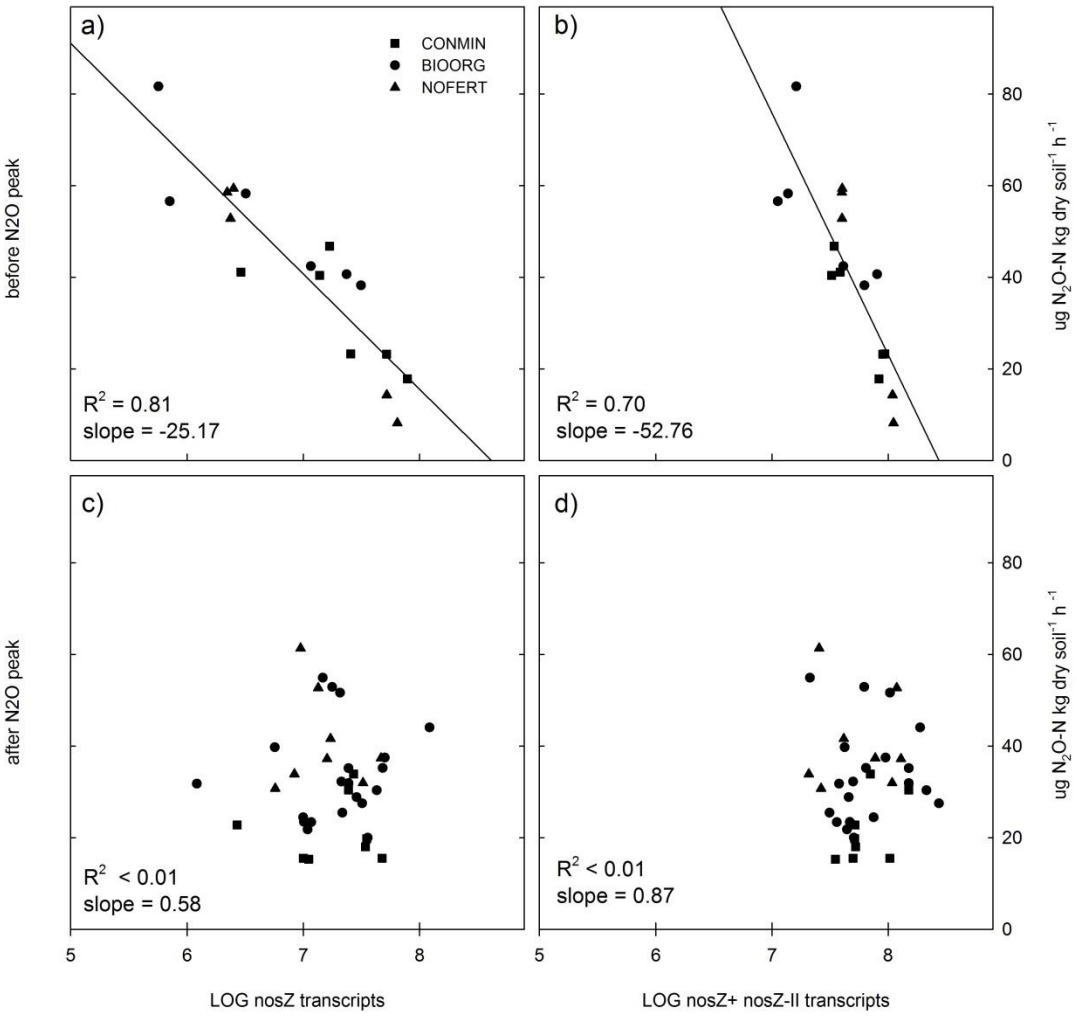
Target gene	Primer	Sequence 5`- 3`	Amplicon size (bp)	Reference
APA9	APA9_F	GGG GTT TCT ACT GGT GGT	80	(Thonar *et al.*, 2012)
	APA9_R	CCC CTC KGS AAA GCC TTC TTC		
nosZ-II	nosZ-II-F	CTI GGI CCI YTK CAY A	690-720	(Jones *et al.*, 2013))
	nosZ-II-R	GCI GAR CAR AAI TCB GTR C		
nosZ	nosZ2F	CGC RAC GGC AAS AAG GTS MSS GT	267	(Henry *et al.*, 2006)
	nosZ2R	CAK RTG CAK SGC RTG GCA GAA		
nirS	nirScd3af	AAC GYS AAG GAR ACS GG	413	(Kandeler *et al.*, 2006; Throbäck *et al.*, 2004)
	R3cd	GAS TTC GGR TGS GTC TTG A		
nirK	nirK876C	ATY GGC GGV CAY GGC GA	162	(Henry *et al.*, 2006)
	nirK1040	GCC TCG ATC AGR TTR TGG TT		

Supplement Table S3. Standard plasmid and genes

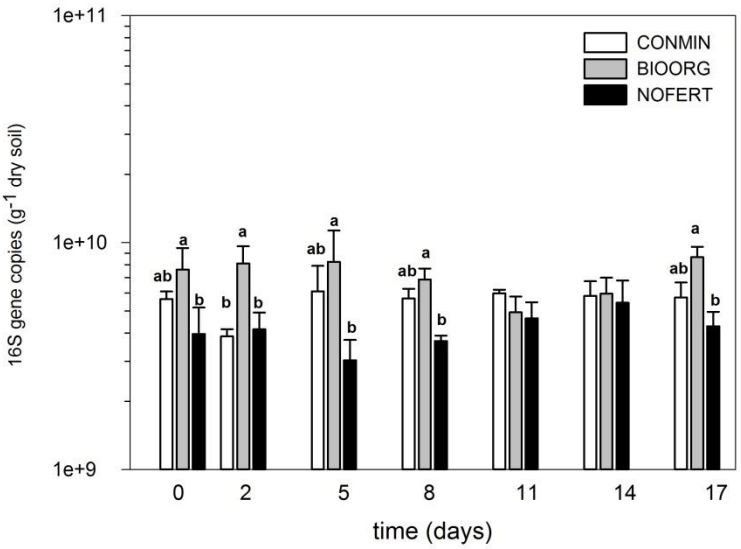
Target gene	Plasmid	Source of standard	Size of insert
APA9	Pjet 1.2	*African cassava mosaic virus*	945
nosZ-II	pEX-A	*Gemmatimonas aurantiaca*	800
nosZ	pCR4-TOPO	*Ensifer meliloti 1021*	1884
nirS	pCR4-TOPO	*Ralstonia eutropha H16*	1607
nirK	pCR4-TOPO	*Ensifer meliloti 1021*	542



Supplement Figure S1 shows changes of N species in concentration and ^{15}N enrichment after fertilization with $NH_4{}^{15}NO_3$. Dynamics in NO_3^- and NH_4^+ concentrations are shown in panels **C** and **D**, while development of ^{15}N enrichment is shown in panels **E** and **F.** Small letters indicated significant differences in between treatments at a specific time point at $p< 0.05$. Data points are means ± SD (n=3).



Supplement Figure S2 Linear regression of *nosZ* (a and c) and *nosZ+nosZ-II* (b and d) transcripts with N_2O emissions before (a and b) and after (c and d) the N_2O emission peak at day 5.



Supplement Figure S3: 16S rRNA gene copy numbers in soil samples originating from different farming systems (CONMIN- conventional, BIOORG – organic, NOFERT – unfertilized) after fertilization with NH_4NO_3. Letters indicated significant differences at a specific time point in between treatments at $p < 0.05$. Data points are means ± SE (n=3).

Contribution of hybrid N_2O to total N_2O flux was calculated using *eq. S1* according to (Spott and Florian Stange, 2011)

$$eq.S1 \qquad C = \frac{a_{NH_4} * \left(2 * 30_{x_m} + 29_{x_m} - 2 * a_{NO_3}\right) + a_{NO_3} * \left(-2 * 30_{x_m} + 29_{x_m}\right) - 2 * 30_{x_m}}{(a_{NH_4} - a_{NO_3})^2}$$

a_{NO_3}*: measured ^{15}N atom fraction of NO_3^- pool*

a_{NH_4}*: measured ^{15}N atom fraction of NH_4^+ pool*

29_{x_m}: *fraction of m/z 29 in N_2 and converted N_2O* as calculated for 30_{x_m} *in eq.S5*

References

Babić, K. H., Schauss, K., Hai, B., Sikora, S., Redžepović, S., Radl, V. & Schloter, M. (2008). Influence of different Sinorhizobium meliloti inocula on abundance of genes involved in nitrogen transformations in the rhizosphere of alfalfa (Medicago sativa L.). *Environmental Microbiology* 10(11): 2922-2930.http://dx.doi.org/10.1111/j.1462-2920.2008.01762.x

Henry, S., Bru, D., Stres, B., Hallet, S. & Philippot, L. (2006). Quantitative Detection of the nosZ Gene, Encoding Nitrous Oxide Reductase, and Comparison of the Abundances of 16S rRNA, narG, nirK, and nosZ Genes in Soils. *Applied Environmental Microbiology* 72(8): 5181-5189.http://dx.doi.org/10.1128/aem.00231-06

Jones, C., Graf, D., Bru, D., Philippot, L. & Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *The ISME Journal* 7(2): 417-426. http://dx.doi.org/10.1038/ismej.2012.125

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D. & Philippot, L. (2006). Abundance of narG, nirS, nirK, and nosZ Genes of Denitrifying Bacteria during Primary Successions of a Glacier Foreland. *Appl. Environ. Microbiol.* 72(9): 5957-5962.http://dx.doi.org/10.1128/aem.00439-06

Spott, O. & Florian Stange, C. (2011). Formation of hybrid N2O in a suspended soil due to co-denitrification of NH2OH. *Journal of Plant Nutrition and Soil Science* 174(4): 554-567.http://dx.doi.org/10.1002/jpln.201000200

Thonar, C., Erb, A. & Jansa, J. (2012). Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources* 12(2): 219-232.http://dx.doi.org/10.1111/j.1755-0998.2011.03086.x

Throbäck, I. N., Enwall, K., Jarvis, Å. & Hallin, S. (2004). Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiology Ecology* 49(3): 401-417.http://dx.doi.org/10.1016/j.femsec.2004.04.011

Chapter 3

Linking nitrous oxide emissions from biochar-amended soil to the structure and function of the N-cycling microbial community

Johannes Harter[1#], Hans-Martin Krause[1#], Stefanie Schüttler[1], Reiner Ruser[2], Markus Fromme[3], Thomas Scholten[3], Andreas Kappler[1], Sebastian Behrens[1]

[1] Geomicrobiology and Microbial Ecology, Center for Applied Geoscience, University of Tübingen, 72076 Tübingen, Germany

[2] Fertilization and Soil Matter Dynamics, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany

[3] Soil Science and Geomorphology, Institute of Geography, University of Tübingen, 72076 Tübingen, Germany

[#] These authors contributed equally

Published in The ISME Journal, 2014, 8, pp. 660-674

The ISME Journal (2013), 1–15

www.nature.com/ismej

ORIGINAL ARTICLE

Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community

Johannes Harter[1,4], Hans-Martin Krause[1,4], Stefanie Schuettler[1], Reiner Ruser[2], Markus Fromme[3], Thomas Scholten[3], Andreas Kappler[1] and Sebastian Behrens[1]
[1]*Geomicrobiology and Microbial Ecology, Center for Applied Geosciences, University of Tuebingen, Tuebingen, Germany;* [2]*Fertilisation and Soil Matter Dynamics, Institute of Crop Science, University of Hohenheim, Stuttgart, Germany and* [3]*Soil Science and Geomorphology, Department of Geography, University of Tuebingen, Tuebingen, Germany*

Nitrous oxide (N_2O) contributes 8% to global greenhouse gas emissions. Agricultural sources represent about 60% of anthropogenic N_2O emissions. Most agricultural N_2O emissions are due to increased fertilizer application. A considerable fraction of nitrogen fertilizers are converted to N_2O by microbiological processes (that is, nitrification and denitrification). Soil amended with biochar (charcoal created by pyrolysis of biomass) has been demonstrated to increase crop yield, improve soil quality and affect greenhouse gas emissions, for example, reduce N_2O emissions. Despite several studies on variations in the general microbial community structure due to soil biochar amendment, hitherto the specific role of the nitrogen cycling microbial community in mitigating soil N_2O emissions has not been subject of systematic investigation. We performed a microcosm study with a water-saturated soil amended with different amounts (0%, 2% and 10% (w/w)) of high-temperature biochar. By quantifying the abundance and activity of functional marker genes of microbial nitrogen fixation (*nifH*), nitrification (*amoA*) and denitrification (*nirK*, *nirS* and *nosZ*) using quantitative PCR we found that biochar addition enhanced microbial nitrous oxide reduction and increased the abundance of microorganisms capable of N_2-fixation. Soil biochar amendment increased the relative gene and transcript copy numbers of the *nosZ*-encoded bacterial N_2O reductase, suggesting a mechanistic link to the observed reduction in N_2O emissions. Our findings contribute to a better understanding of the impact of biochar on the nitrogen cycling microbial community and the consequences of soil biochar amendment for microbial nitrogen transformation processes and N_2O emissions from soil.
The ISME Journal advance online publication, 26 September 2013; doi:10.1038/ismej.2013.160
Subject Category: Geomicrobiology and microbial contributions to geochemical cycles
Keywords: nitrogen cycle; biochar; denitrification; nitrification; nitrous oxide; *nosZ*; N_2O emission; greenhouse gas; soil microbial community

Introduction

Mankind's increased combustion of fossil fuels and demand for nitrogen in agriculture and industry continuous to impact the global biogeochemical cycling of nitrogen (Galloway *et al.,* 2008). The loss of anthropogenic nitrogen to the environment causes many problems from increasing freshwater nitrate concentrations to raising nitrous oxide (N_2O) emissions that accelerate global climate change (Duce *et al.,* 2008). A better understanding of the structure and functioning of microbial communities involved in nitrogen transformations (such as nitrification, denitrification and nitrogen fixation) is a prerequisite to potentially counteract effects of nitrogen pollutions (Jetten, 2008).

Biochar is a carbon-rich solid produced by pyrolysis of biomass. Pyrolysis is the thermal decomposition of biomass under limited oxygen supply (Atkinson *et al.,* 2010). Biochars have a broad variety of specific physicochemical properties, which highly depend on feedstock and production temperature (Sohi *et al.,* 2010; Singh *et al.,* 2010a). Biochar produced by high-temperature pyrolysis ($>550\,^{\circ}C$) possesses a high surface area ($>400\,m^2\,g^{-1}$) and a highly aromatic carbon structure, which leads to a high sorption capacity and elevated recalcitrance toward biodegradation (Joseph *et al.,* 2010; Keiluweit *et al.,* 2010; Uchimiya *et al.,* 2010). It has been shown

Correspondence: S Behrens, Geomicrobiology and Microbial Ecology, Center for Applied Geosciences, University of Tuebingen, Sigwartstraße 10, D-72076, Tuebingen, Germany.
E-mail: sebastian.behrens@ifg.uni-tuebingen.de
[4]These authors contributed equally to this work.
Received 5 April 2013; revised 9 August 2013; accepted 14 August 2013

in several studies that biochar incorporation into soil can have diverse effects on soil quality, plant growth and greenhouse gas (GHG) emissions (Chan *et al.,* 2008; Major *et al.,* 2010; Singh *et al.,* 2010b; van Zwieten *et al.,* 2010). Biochar application to arable soil is currently heavily debated in context of soil carbon sequestration and mitigation of atmospheric CO_2 emissions but also as one potential strategy to reduce the release of other potent GHGs such as methane and nitrous oxide.

Nitrous oxide acts as a potent greenhouse agent in the atmosphere and represents a particular environmental problem due to its long atmospheric lifetime of 114 years. N_2O is a key player in atmospheric chemical processes and represents the major source of stratospheric NO_x, which acts as an ozone-depleting catalyst (Ravishankara *et al.,* 2009). Soils and oceans represent the largest sources of N_2O emissions, with anthropogenic sources, such as agriculture or fossil fuel combustion, accounting for almost two-thirds of the total emissions (Denman *et al.,* 2007). The atmospheric N_2O concentration of currently 319 ppb has increased by 49 ppb since the beginning of the industrial era (Forster *et al.,* 2007). The expansion of farm lands and enhanced fertilizer application are thought to increase emissions by 35–60% by 2030 (Smith *et al.,* 2007). Different microbial nitrogen-transforming processes contribute to the formation of N_2O. Major sources in soils are microbial nitrification, nitrifier denitrification and heterotrophic denitrification (Wrage *et al.,* 2005). Other microbial processes that can lead to the formation of N_2O are heterotrophic nitrification (Papen *et al.,* 1989; Blagodatsky *et al.,* 2006), codenitrification (Tanimoto *et al.,* 1992; Kumon *et al.,* 2002) and dissimilatory nitrate reduction to ammonia (Smith and Zimmerman, 1981; Bleakley and Tiedje, 1982; Smith, 1982, 1983). Which microbial N_2O formation process dominates is largely controlled by soil geochemical conditions (Braker and Conrad, 2011). In temperate, arable soils major determinants of microbial N_2O formation are oxygen partial pressure, pH, H_2S concentration and the availability and speciation of nitrogen and organic carbon (Blackmer and Bremner, 1978; Sorensen *et al.,* 1980; Stevens *et al.,* 1998; Senga *et al.,* 2006; Wallenstein *et al.,* 2006; Baggs *et al.,* 2010; Cuhel *et al.,* 2010; Braker and Conrad, 2011; Philippot *et al.,* 2013).

Nitrification is the two-step oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) via nitrite (NO_2^-). The process is carried out by chemolithoautotrophic ammonia oxidizers and nitrite oxidizers. Ammonia-oxidizing bacteria (AOB) or archaea (AOA) oxidize NH_4^+/NH_3 via the intermediate hydroxylamine (NH_2OH) to NO_2^-. The key enzyme of this process is the ammonia monooxygenase encoded by the gene *amoA*. During ammonia oxidation, N_2O can be formed by chemical decomposition of NH_2OH. However, levels of produced N_2O are usually orders of magnitude lower (10^3–10^6) than those of nitrite (Arp and Stein, 2003; Treusch *et al.,* 2005; Robertson, 2007; Canfield *et al.,* 2010; Braker and Conrad, 2011).

Denitrification is the stepwise reduction of nitrate or nitrite to N_2 via the intermediates NO and N_2O. In contrast to nitrification, N_2O is an obligate intermediate of denitrification. During denitrification, nitrate-reducers reduce nitrate to nitrite, which is further reduced by nitrite-reducing bacteria to nitric oxide (NO). The later step is catalyzed by the key enzyme nitrite reductase encoded by the genes *nirS* or *nirK*. Nitric oxide reducers convert NO to N_2O, which can be the end product of denitrification or be further reduced to N_2 under conditions of complete denitrification. N_2O reduction to N_2 is catalyzed by the enzyme nitrous oxide reductase encoded by the gene *nosZ* in N_2O-reducing bacteria (Canfield *et al.,* 2010; Braker and Conrad, 2011). In contrast to the multiplicity of mechanisms by which N_2O can be formed, N_2O reduction to N_2 by nitrous oxide-reducing microorganisms is the only microbial sink for N_2O (Thomson *et al.,* 2012).

Another important process essential to the biogeochemical cycling of nitrogen in soils is nitrogen fixation. Nitrogen fixation counteracts the loss of gaseous nitrogen to the atmosphere through microbial nitrification and denitrification by constantly replenishing the bioavailable nitrogen pool through the fixation of atmospheric N_2 into organic nitrogen (Jetten, 2008). The key enzyme of microbial nitrogen fixation is the highly oxygen sensitive nitrogenase encoded by the gene *nifH*.

Several studies have documented that biochar induces shifts in the microbial community composition (Rondon *et al.,* 2007; Steinbeiss *et al.,* 2009; Anderson *et al.,* 2011; Khodadad *et al.,* 2011; Ducey *et al.,* 2013), whereas other studies described that the addition of biochar to soils does affect soil N_2O emissions (Yanai *et al.,* 2007; Singh *et al.,* 2010b; Taghizadeh-Toosi *et al.,* 2011; Felber *et al.,* 2012; Cayuela *et al.,* 2013). However, a potential link between the observed shifts in microbial community composition and the decreased soil N_2O emissions has not been subject of systematic investigation so far.

We set up water-saturated soil microcosms with different amounts (0%, 2% and 10% w/w) of high-temperature biochar (700 °C). During a 3-month incubation experiment, we quantified N_2O and CO_2 emissions from the soil microcosms and followed the geochemical parameters NO_3^-, NO_2^-, NH_4^+, dissolved organic carbon (DOC) and pH. Besides, we determined the abundance of key functional marker genes involved in microbial nitrification, denitrification and N_2-fixation (*amoA*, *nirS*, *nirK*, *nosZ* and *nifH*) by real-time PCR. The main objectives of this study were to quantify the responses of the different nitrogen-transforming functional microbial groups on soil biochar amendment and to evaluate whether alterations in the abundance and activity among the different

N-cycling functional groups might explain the reduced N_2O formation and release from soil.

Materials and methods

Soil sampling and biochar production

Soil samples from the top 10 cm were collected at the vineyard ''Mythopia'' of the Delinat Institute in Ayent (Switzerland) (46°16′4.08″N and 7°24′28.48″E). The soil is characterized as loamy sand (calcaric leptosol) with ~50% (w/w) gravel. The field moist soil was passed through a 2 mm mesh-size sieve, homogenized using a drill with a mixing blade and then stored at 4 °C in tightly closed plastic bags in the dark for less than 5 months. The biochar used in this study was produced from green waste via high-temperature pyrolysis (700 °C) by Swiss Biochar. The biochar was dried at 40 °C and only the particle size fractions between 1 mm and 2 mm were used. Soil and biochar physicochemical properties and elemental composition are summarized in Table 1 and Table S1 in the Supplementary Information.

Experimental setup

Soil microcosms were set up in 500 ml DURAN wide neck glass bottles (Schott AG, Mainz, Germany) (Figure S1 in the Supplementary Information). Each bottle contained 202 g of field-wet soil (dry weight 180 g) or soil-biochar mixture. Three treatments with different amounts of biochar (0% (control), 2% and 10% (w/w)) were prepared. Two percent (w/w) biochar was chosen because it represents a common field application rate of 24 t ha^{-1}. Ten percent (w/w) biochar was chosen in order to exaggerate biochar effects on soil geochemistry and microbiology. Ten percent (w/w) biochar also resembles the amounts of char found in terra preta patches (Atkinson *et al.,* 2010).

Table 1 Physicochemical properties of the soil (calcaric leptosol) and the biochar used in this study

Parameters	*Soil*	*Biochar*
Sand (%)	44.94	ND
Silt (%)	35.37	ND
Clay (%)	19.69	ND
pH (H_2O)	8.4	9.8
C_{tot} (%)	1.87	51.90
C_{org} (%)	0.91	48.87
N_{total} (%)	0.17	0.59
S (%)	0.04	0.15
C:N	11	88
Particle density (g cm^{-3})	ND	2.0
Ash content (%)	ND	45.7
CEC ($mmol_c$ kg^{-1})	ND	103.4
EC (mS m^{-1})	ND	33.7
Total surface area (m^2 g^{-1})	ND	303

Abbreviations: CEC, cation exchange capacity; EC, electrical conductivity; ND, not determined.

The soil-biochar mixture was homogenized using a spatula and then carefully compacted by tapping the microcosms on a soft surface. All treatments were set up in duplicates. The soil microcosms were incubated open to ambient atmosphere at 28 °C in a daylight incubator. For soil geochemical and molecular analyses, duplicate soil microcosms of each treatment (a total of six bottles) were sampled destructively. Samples were taken right after microcosm setup (day 0) and after 1, 8, 15, 22, 29, 57 and 85 days of incubation. The water-filled pore space (WFPS) in the soil microcosms was adjusted to 95% in order to create water-saturated conditions similar to soil water contents in winter/spring or after a heavy rainfall. The WFPS of the microcosms was calculated according to Yanai *et al.* (2007) using a particle density of 2.00 g cm^{-3} for the biochar and 2.65 g cm^{-3} for the soil (Yanai *et al.,* 2007). During incubation the water content was controlled gravimetrically each week and adjusted to the initial WFPS by adding deionized water with a spray bottle. At the beginning of the experiment, the soil microcosms were fertilized with a nutrient solution containing carbon (555 mg kg^{-1} as molasses), nitrogen (250 mg kg^{-1} as NH_4NO_3), phosphorus and potassium (150 mg kg^{-1} and 188 mg kg^{-1} as KH_2PO_4). The bulk density of all three soil-biochar mixtures was determined experimentally after drying the soil for 72 h at 105 °C. The 10% (w/w) biochar microcosms had the lowest bulk density (0.99 g cm^{-3}), followed by bottles with 2% biochar (1.10 g cm^{-3}) and 0% biochar (1.21 g cm^{-3}). Before subsampling for geochemical and molecular biological analyses the soil of each microcosm was transferred into a separate, clean container and thoroughly homogenized with a spatula.

Geochemical analyses

Soil and biochar elemental composition, particle size distribution, particle density, surface area, ash and moisture content, cation exchange capacity, electrical conductivity and pH were determined according to protocols of the International Organization for Standardization. For details please refer to the Supplementary Information.

During the microcosm experiment, soil pH was determined in a 1:5 dilution with deionized water according to International Organization for Standardization 10390. For the determination of NH_4^+ and NO_3^-, the equivalent of 5 g dry soil was mixed with 20 ml of 0.5 M K_2SO_4 and shaken for 1 h at 130 r.p.m. (HS501, IKA, Staufen, Germany) (Singh *et al.,* 2010b). The soil solution was filtered through a 150 μm pore size cellulose filter (Whatman, Maidstone, UK) and the filtrate was again filtered through a 0.45 μm pore-size syringe filter (Millex-HA, Merck Millipore, Billerica, MA, USA). The obtained filtrate was frozen until analysis. The concentrations of NH_4^+ and NO_3^- in the filtered solution were quantified by continuous flow

analysis (3-QuAAtro, Bran & Lübbe, Nordersted, Germany). On the basis of the determined NH_4^+ and NO_3^- concentrations in the soil extract NH_4^+ and NO_3^- concentrations were converted to mg NO_3^-/ NH_4^+ per kg dry soil according to equation 1 (Eq. 1), in which V is the volume of extracting agent in L, m is the amount of dry soil in g and c is the measured concentration of NH_4^+ or NO_3^- mg l^{-1}.

$$C\left[\frac{mg}{kg\,dry\,soil}\right]=\frac{c\left[\frac{mg}{L}\right]V[L]}{m[g]}\times 1000 \qquad (1)$$

Sample preparation for NO_2^- quantification was carried out as described above for NO_3^-/NH_4^+, but without the second filtration step. In order to obviate changes in nitrite concentration owing to freezing and storage, NO_2^- concentrations were analyzed spectrophotometrically directly after the extraction using a Nitrite-Test Kit (1.14776.0001, Merck, Darmstadt, Germany) according to manufacturer's instructions. For DOC, quantification sample preparation was carried out as described for NO_3^-/ NH_4^+ but with 40 ml 0.5 M K_2SO_4 instead of 20 ml. The filtered solution was analyzed using a HighTOC analyzer (Elementar, Hanau, Germany). Both NO_2^- and DOC concentrations were also converted to mg per kg dry soil according to Equation 1.

For the determination of trace gas fluxes, the soil microcosm bottles were closed with a butyl rubber stopper before sampling. Four headspace gas samples of 25 ml were taken every hour and transferred into 22.5 ml evacuated sample vials using a gas-tight syringe (1100TLL 100.0 ml Gastight, Hamilton, Reno, NV, USA). To avoid negative pressure in the soil microcosms, a gasbag filled with N_2 was connected after each sampling, which ensured a consistent ambient atmospheric pressure. The trace gas concentrations in the vials were measured using a gas chromatograph equipped with an electron capture detector (^{63}Ni-ECD) for N_2O and CO_2 (Hewlett Packard, 5890 Series II). The gas chromatograph setup and configuration have been described in detail previously (Loftfield *et al.,* 1997). Gas fluxes were calculated using the slope of the temporal change in concentration of the closed bottle according to the equations published in Ruser *et al.* (1998).

Molecular biological analyses

In order to quantify the abundance and expression (reverse transcription) of microbial nitrogen-cycling functional marker genes, quantitative polymerase chain reaction (qPCR) was performed. Soil samples were homogenized and aliquots were stored at −20 °C for DNA extraction and at −80 °C for RNA extraction. DNA and RNA extractions were carried out in duplicates for each sample. Total DNA was extracted from 0.25 g of soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) with the following modifications: Bead Tubes were placed in a 70 °C water bath for 10 min, cooling steps were performed on ice, and before the elution step, filter tubes were incubated at room temperature for 5 min. DNA concentration and quality were determined spectrophotometrically (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA), fluorometrically (Qubit 2.0 Fluorometer, Life Technologies, Carlsbad, CA, USA), and by agarose gel electrophoresis. Total RNA was isolated from 1.5 g of soil using the RNA PowerSoil Total RNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration of the extracted RNA was determined using the Qubit 2.0 Fluorometer (Life Technologies). DNA extraction efficiencies varied only slightly between different soil samples (mean DNA yield 5.4 ± 1.7 μg per g dry soil) and did not show any biochar-related bias. However, total RNA extraction efficiencies varied significantly between the different soil samples (mean RNA yield 0.77 ± 0.6 μg RNA per g dry soil). Therefore, gene transcript copy numbers were normalized to nanogram extracted RNA instead of soil dry weight. DNA digestion was performed with the Ambion TURBO DNA-free Kit (Life Technologies) with an extended incubation time of 45 min at 37 °C. In order to assess RNA integrity, the RNA quality indicator was determined with the Experion Automated Electrophoresis Station (Bio-Rad Laboratories, Hercules, CA, USA). RNA was transcribed into complementary DNA using the SuperScript III Reverse Transcriptase (Life Technologies) according to the manufacturer's protocol. To test for the absence of residual DNA contamination in the complementary DNA preparations, we performed reverse transcription control reactions lacking reverse transcriptase enzyme. No PCR amplicons could be obtained from any sample when reverse transcriptase was omitted from the reactions.

Quantification of phylogenetic and functional marker genes (16S rRNA gene (Bacteria), *amoA* (Bacteria and Archaea), *nifH*, *nirK*, *nirS* and *nosZ*) was carried out using the SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Hercules, CA, USA) and gene-specific primers. For details on plasmid standards, gene-specific qPCR primers, reaction mixtures and thermal programs, please refer to Tables S2–S4 in the Supplementary Information. Each sample was quantified in duplicates using the iCycler iQ Real-Time PCR Detection System and the iQ 5 Optical System software, version 2.0 (Bio-Rad laboratories). During qPCR setup, evaluation and data analysis, we followed the MIQE guidelines (Bustin *et al.,* 2009). For qPCR data analysis, the background subtracted raw data were exported from the iCycler system and analyzed using the Real-Time PCR Miner software (Zhao and Fernald, 2005). The algorithm calculates the efficiency (E) and threshold cycle (CT) based on the kinetics of each individual reaction. The initial template concentration N (gene copy numbers per qPCR reaction volume) was then calculated with the

following equation (Eq. (2)).

$$N = (1 + E)^{CT} \quad (2)$$

Calibration curves (log gene copy number per reaction volume versus log N) were obtained using serial dilutions of standard plasmids according to Behrens *et al.* (2008) (further details on qPCR assay validation and data analyses are given in Table S5 in the Supplementary Information). Plasmid DNA concentrations were quantified using the Qubit 2.0 Fluorometer (Life Technologies). To verify the amplification of individual PCR products and the correct amplicon size, melting curve analysis and agarose gel electrophoresis were performed. Gene copy numbers per g dry soil were calculated according to Behrens *et al.* (2008). Total bacterial cell numbers per g dry soil were calculated from the qPCR 16S rRNA gene copy numbers considering the average bacterial rRNA operon number (4.2) as derived from the Ribosomal RNA Operon Copy Number Database (http://rrndb.mmg.msu.edu/index.php) (Klappenbach *et al.,* 2001). Transcript copy numbers were normalized to nanogram RNA.

Statistical analyses

In order to identify statistically significant biochar effects, a univariate analysis of variance (ANOVA) with the 'least significant difference' *post hoc* test ($P<0.05$) was performed using the IBM SPSS Statistics 20 software package (IBM, Armonk, NY, USA). The statistical analysis was performed for each time point of sampling and for each measured parameter (geochemical and molecular). Using a univariate ANOVA, all concentration or copy number values from the control (no biochar) were individually compared with the two biochar-containing soil microcosms (2% (w/w) biochar and 10% biochar) in order to reveal differences between the control and the biochar microcosms that were statistically significant. In the provided graphs, significant differences between biochar-amended and control microcosms are indicated by lower case characters above the corresponding data points as specified in the legend of each figure.

Results

In order to provide a better overview of the data, we only show the data of the control microcosms without biochar and the 10% (w/w) biochar microcosms here. Results of the 2% (w/w) biochar-amended microcosms in comparison with the control microcosms are given in the Supplementary Information (Supplementary Figures S2, S3). In general, the 2% and 10% biochar microcosms behaved similarly with sometimes slightly more pronounced variances and trends in comparison with the control microcosm observable for the 10% biochar microcosms. The 2% biochar data will explicitly be mentioned when the data with respect to a 'biochar effect' were significantly different from the 10% biochar microcosms. *P*-values, otherwise explicitly stated, are given for the comparison of the control vs the 10% biochar microcosms.

Soil microcosm geochemical parameters

pH values were close to neutral in all microcosms and slightly increased during incubation. In the control microcosms, the pH increased from 7.2 to 7.9 and in the biochar-containing microcosms from pH 7.5 to 8.2. Overall soil pH values were significantly higher in microcosms amended with biochar than in the control microcosms over the whole course of the experiment.

We added 250 mg kg^{-1} dry soil NH_4NO_3 to each microcosm at the beginning of the experiment. The amount of added NH_4NO_3 corresponds to 90 kg nitrogen ha^{-1}, which is a common agricultural field application rate (Singh *et al.,* 2010b). Within the first 8 days, NO_3^- concentrations decreased rapidly from 127.4 ± 2.6 mg kg^{-1} dry soil (control) and 113.6 ± 15.8 mg kg^{-1} dry soil (10% (w/w) biochar) to 1.2 ± 0.06 mg kg^{-1} dry soil and 2.7 ± 0.02 mg kg^{-1} dry soil, respectively (Figure 1a). From day 8 to day 85, nitrate concentrations stayed below 3.7 ± 0.3 mg kg^{-1} dry soil in all treatments (Figure 1a). Only at day 1, nitrate concentrations were significantly lower ($P=0.002$) in the 10% biochar microcosms compared with the control microcosm (Figure 1a), whereas from day 8 to day 85, nitrate concentrations were always slightly higher ($P<0.038$) in the 10% biochar microcosms than in the control microcosms.

Compared with the nitrate concentrations, ammonia concentrations decreased more slowly but constantly with time reaching concentrations of 7.7 ± 1.0 (control) and 12.5 ± 0.3 (10% biochar) mg kg^{-1} dry soil at day 85 (Figure 1a). Only at day 8, ammonia concentrations were significantly lower in the 10% biochar microcosms ($P=0.014$), whereas from day 29 to day 85 they were significantly higher in the 10% biochar microcosms compared with the control microcosms ($P<0.029$) (Figure 1a).

Nitrite concentrations were highest at day 1 in the biochar and in the control microcosms (37.7 ± 2.7 mg kg^{-1} dry soil and 52.4 ± 2.1 mg kg^{-1} dry soil, respectively) (Figure 1b). ANOVA revealed that the higher nitrite concentrations in the control compared with the biochar microcosms at day 1 were statistically significant ($P=0.016$) (Figure 1b). Corresponding to the nitrate and the nitrite data, the highest N_2O fluxes were recorded during the first week (until day 8) in all three treatments (control, 2%, and 10% biochar). At day 1, N_2O fluxes were significantly higher in the control microcosms without biochar (5631 ± 766 µg N_2O-N m^{-2} h^{-1}) compared with the biochar-containing microcosms (175 ± 116 µg N_2O-N m^{-2} h^{-1} in the 10%

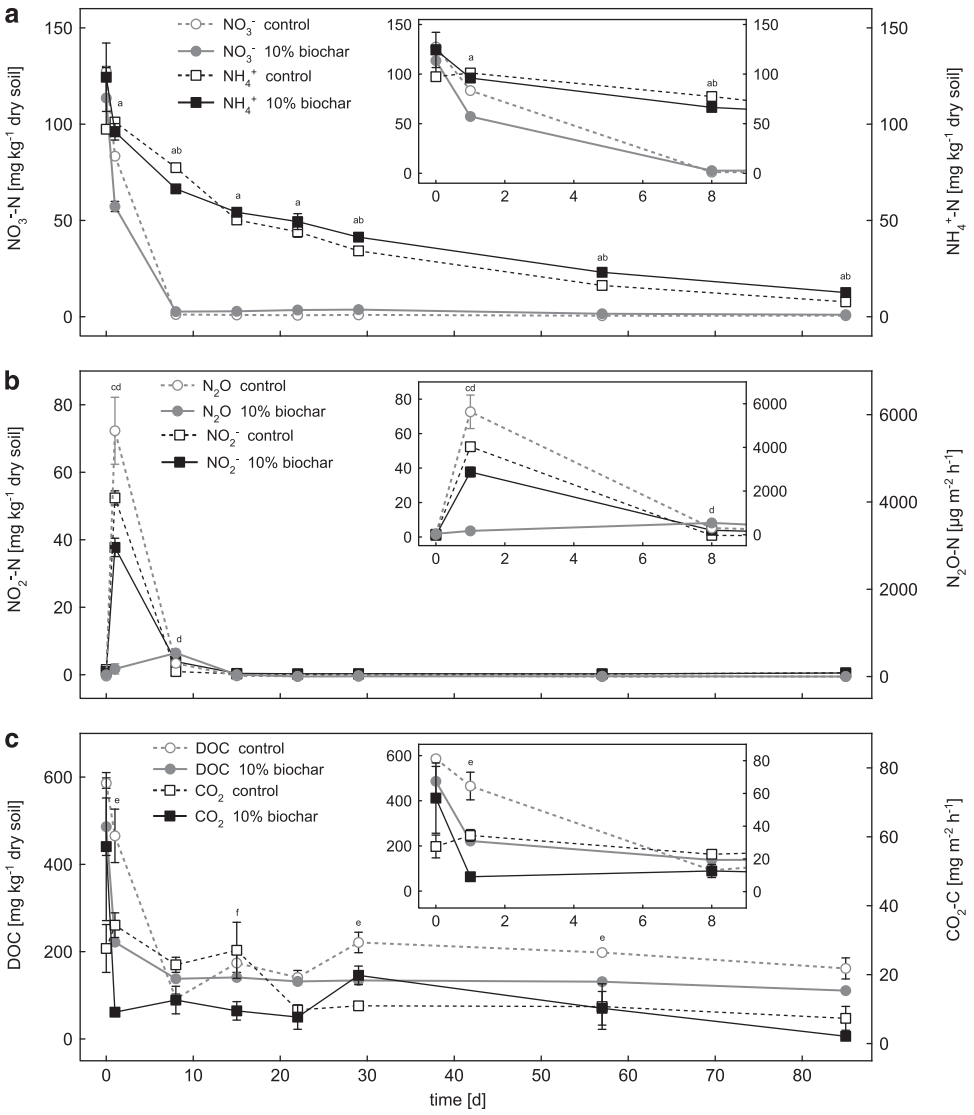


Figure 1 Change in nitrogen (**a**, **b**) and carbon (**c**) geochemical parameters in the control and 10% (w/w) biochar-containing soil microcosms over time. Panels **a**, **b** show changes in the concentrations of nitrate, nitrite, ammonium and nitrous oxide, whereas panel c shows the DOC and carbon dioxide data. The small inserted graphs show a magnified view of the data for the first 8 days. Open symbols with dashed lines represent data of the control microcosms without biochar. Filled symbols with solid lines represent data of the soil microcosms with 10% (w/w) biochar. Statistically significant differences (univariate ANOVA, *post hoc*: least significant difference) between control and 10% (w/w) biochar microcosms at a certain time point are indicated by lower-case letters above the individual data points (a = NO_3^-, b = NH_4^+, c = N_2O, d = NO_2^-, e = DOC, f = CO_2).

biochar-containing and $2969 \pm 554\,\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$ in the 2% biochar-containing microcosms) ($P = 0.002$ and 0.017, respectively) (Figure 1b and Supplementary Figure S2b). After day 1, N_2O fluxes decreased strongly to $< 500\,\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$ at day 8 and $< 50\,\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$ from day 15 to day 85 in all three treatments (Figure 1b and Supplementary Figure S2b).

Initial DOC concentrations resembled the amount of DOC added in form of molasses at the beginning of the experiment ($555\,mg\ kg^{-1}$ dry soil). DOC concentrations decreased rapidly within the first week in all setups leveling off at an average concentration of $119.6 \pm 22.0\,mg\ kg^{-1}$ dry soil at day 8 (Figure 1c). As can be seen in Figure 1c, the 10% biochar-containing microcosms showed significantly lower DOC concentrations compared with the control at day 1 ($P = 0.018$) and between day 29 and day 57 ($P < 0.023$).

CO_2 fluxes decreased from $57.2 \pm 21.5\,mg\ m^{-2}\ h^{-1}$ to $12.6 \pm 4.0\,mg\ m^{-2}\ h^{-1}$ during the first day of incubation in the 10% biochar microcosms (Figure 1c). Initial CO_2 fluxes in the control microcosm were lower than in the biochar microcosms ($27.6 \pm 7.0\,mg\ m^{-2}\ h^{-1}$) and further decreased to $9.8 \pm 0.5\,mg\ m^{-2}\ h^{-1}$ after day 22 (Figure 1c). However, according to the ANOVA, CO_2 fluxes showed no significant differences between biochar-containing and control microcosms throughout the whole course of the experiment (Figure 1c)

except for day 15 when significantly higher CO_2 emissions from the control microcosms were measured ($P = 0.039$).

Abundance of 16S rRNA and N-cycling functional marker genes

As shown in Figure 2a, total bacterial abundance increased temporarily during the beginning of the experiment reaching a maximum of 2.8×10^{10} gene copies per g dry soil at day 8. Afterwards, bacterial 16S rRNA gene copy numbers slowly returned to initial values of 5.5×10^9 gene copies per g dry soil. The statistical analysis revealed no significant differences between control and biochar-containing soil microcosms with respect to total bacterial 16S rRNA gene copy numbers.

The abundance of bacteria capable of fixing nitrogen was determined by quantification of *nifH* gene copy numbers. In accordance to 16S rRNA gene copy numbers, *nifH* gene copy numbers increased rapidly within the first 8 days reaching a maximum of 1.7×10^{10} gene copies per g dry soil (Figure 2a) at day 8. Between day 8 and day 29, *nifH* gene copies slightly fluctuated before they remained quite constant from day 29 to 85 reaching final gene

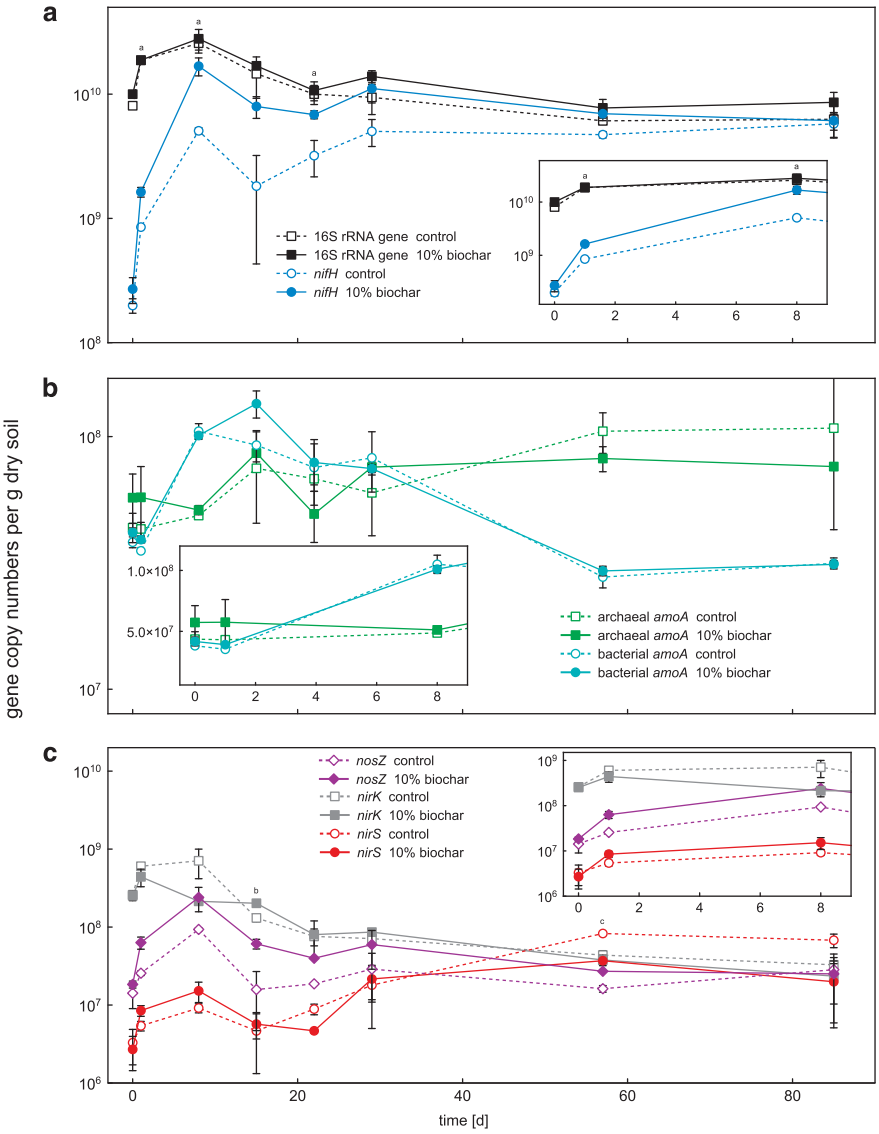


Figure 2 Gene copy numbers per gram dry soil over time for various key genes of microbial nitrogen transformation processes in the control and 10% (w/w) biochar-containing microcosms. Panel **a** shows changes in total bacterial 16S rRNA and *nifH* gene copy numbers. In panel **b**, archaeal and bacterial *amoA* gene copy numbers are shown. Panel **c** summarizes the gene copy data for *nirS*, *nirK* and *nosZ*. The small inserted graphs show a magnified view of the data for the first 8 days. Note that the *y* axes of the inserted graphs in panels **a**, **b** and **c** have a slightly different scale from the corresponding overview graphs. Open symbols with dashed lines represent data measured in the control microcosms without biochar. Filled symbols with solid lines represent data of the soil microcosms with 10% (w/w) biochar. Statistically significant differences (univariate ANOVA, *post hoc*: least significant difference) between control and 10% (w/w) biochar microcosms at a certain time point are indicated by lower-case letters above the individual data points (a = *nifH*, b = *nosZ* and c = *nirS*).

counts close to the total copy number of bacterial 16S rRNA genes (control microcosms: 5.8×10^9 gene copies per g dry soil; 10% (w/w) biochar-containing microcosms: 6.1×10^9 gene copies per g dry soil; 2% biochar-containing microcosms: 6.8×10^9 gene copies per g dry soil) (Figure 2a and Supplementary Figure S3a). Over the whole incubation period, *nifH* gene copy numbers were consistently higher in the biochar-containing microcosms compared with the control microcosms with significantly higher values at day 1 ($P = 0.031$), 8 ($P = 0.018$) and 22 ($P = 0.031$) (Figure 2a).

The abundance of AOA and AOB was quantified by determining archaeal and bacterial *amoA* gene copy numbers. Archaeal *amoA* gene copies fluctuated within the first month between 4.3×10^7 and 8.6×10^7 gene copies per g dry soil in the control and the 10% biochar-containing microcosms. From day 22 to day 85, archaeal *amoA* gene copies increased from 6.8×10^7 to 1.1×10^8 gene copies per g dry soil in the control microcosms and from 5.0×10^7 to 7.6×10^7 gene copies per g dry soil in the 10% biochar microcosms (Figure 2b). Bacterial *amoA* gene copies increased from 3.8×10^7 to 1.1×10^8 gene copies per g dry soil at day 8 in the control microcosms and from 4.2×10^7 to 1.4×10^8 gene copies per g dry soil at day 15 in the 10% biochar microcosms. After the initial increase, bacterial *amoA* gene copies decreased toward the end of the incubation period (day 85) in both control and biochar microcosms reaching 3.2×10^7 and 3.1×10^7 gene copies per g dry soil, respectively (Figure 2b). ANOVA revealed no significant differences between control and biochar-amended microcosms (2% and 10% biochar) for the archaeal and bacterial *amoA* gene data.

Nitrite-reducing bacteria were quantified by determining the copy numbers of *nirS* and *nirK* per g dry soil in each microcosm. As shown in Figure 2c, initial *nirS* gene copy numbers were two orders of magnitude lower (3.0×10^6) than *nirK* gene copy numbers (2.0×10^8); however, over the course of the experiment, *nirS* and *nirK* gene copy numbers approximated. *nirK* gene copy numbers decreased, whereas *nirS* gene copy numbers increased. After 85 days, *nirS* gene copy numbers even outnumbered *nirK* gene copy numbers, as *nirS* increased to 4.4×10^7 gene copies per g dry soil and *nirK* decreased to 2.8×10^7 gene copies per g dry soil (Figure 2c).

Differences between the biochar and the control microcosms with respect to nitrite-reductase gene copy numbers were most of the time not significant based on ANOVA. Only at day 57, *nirS* gene copy numbers were significantly higher in the control microcosms than in the 10% (w/w) biochar-containing microcosms ($P = 0.0009$) (Figure 2c).

The abundance of nitrous oxide-reducing bacteria was followed by quantifying *nosZ* gene copy numbers. *nosZ* gene copy numbers initially increased from 1.4×10^7 to 9.3×10^7 gene copies per g dry soil (control microcosms) and from 1.8×10^7 to 2.4×10^8 gene copies per g dry soil (10% biochar) toward day 8. Thereafter, *nosZ* gene copy numbers decreased and reached 2.8×10^7 gene copies per g dry soil in the control microcosms and 2.5×10^7 gene copies per g dry soil in the 10% biochar-containing microcosms at the end of the experiment (Figure 2c). Significantly higher *nosZ* gene copy numbers in biochar-containing compared to control microcosms were quantified at day 15 ($P = 0.042$) (Figure 2c).

Functional gene ratios and nosZ gene transcript abundance

As shown in Figure 3a, the ratio of *nosZ* gene copies over the sum of *nirS* and *nirK* gene copies (*nosZ*/(*nirS* + *nirK*)) was strongly affected by biochar addition and was always higher in the biochar-containing microcosms compared with the control microcosms throughout the entire experiment. A *nosZ*/(*nirS* + *nirK*) ratio of 1 means equal copy numbers of nitrite and nitrous oxide reductase genes per g dry soil. In the biochar-containing microcosms, the ratio reached 1 at day 8 shortly after the greatest differences in N_2O emissions between the control and biochar-containing microcosms have been quantified ($P = 0.002$) (Figure 3a). Statistical analysis confirmed significantly higher *nosZ*/(*nirS* + *nirK*) ratios in the 10% (w/w) biochar-containing microcosms compared with the control microcosms at day 1, ($P = 0.023$), 8 ($P = 0.044$) and at day 57 ($P = 0.013$) (Figure 3a).

As shown in Figure 3b, the ratio of archaeal *amoA* gene copies over bacterial *amoA* gene copies only slightly changed between day 1 and day 29 (values from 0.5 to 1.5). After day 29, the AOA/AOB ratio increased to 2.8 and 3.8 in biochar-containing and control microcosms, respectively, independent of biochar addition (Figure 3b).

The relative abundance of *nifH* gene copies (*nifH* gene copies over bacterial 16S rRNA gene copies) increased in the control microcosms from $2.5 \pm 0.4\%$ to $94.2 \pm 7.5\%$ at day 85 (Figure 3c). In the 10% biochar-containing microcosms, the relative abundance of *nifH* gene copies increased from $2.7 \pm 0.6\%$ to $90.8 \pm 4.8\%$ at day 57 (Figure 3c). Until day 85 the relative abundance of *nifH* gene copies then decreased to $71.8 \pm 2.9\%$ in the 10% biochar-containing microcosms. Significantly higher *nifH*/16S rRNA gene ratios in biochar-containing microcosms compared with the control microcosms were statistically confirmed for day 1, 8, 15, 22 and day 85 ($P < 0.043$) (Figure 3c).

As shown in Figure 4, the number of *nosZ* gene transcripts was about sixfold higher in the 10% biochar microcosms compared with the control microcosms at day 1. Gene transcript copy numbers were strongly affected by biochar addition and increased from 1.1×10^4 to 1.8×10^4 *nosZ* gene transcripts per ng RNA in the 10%

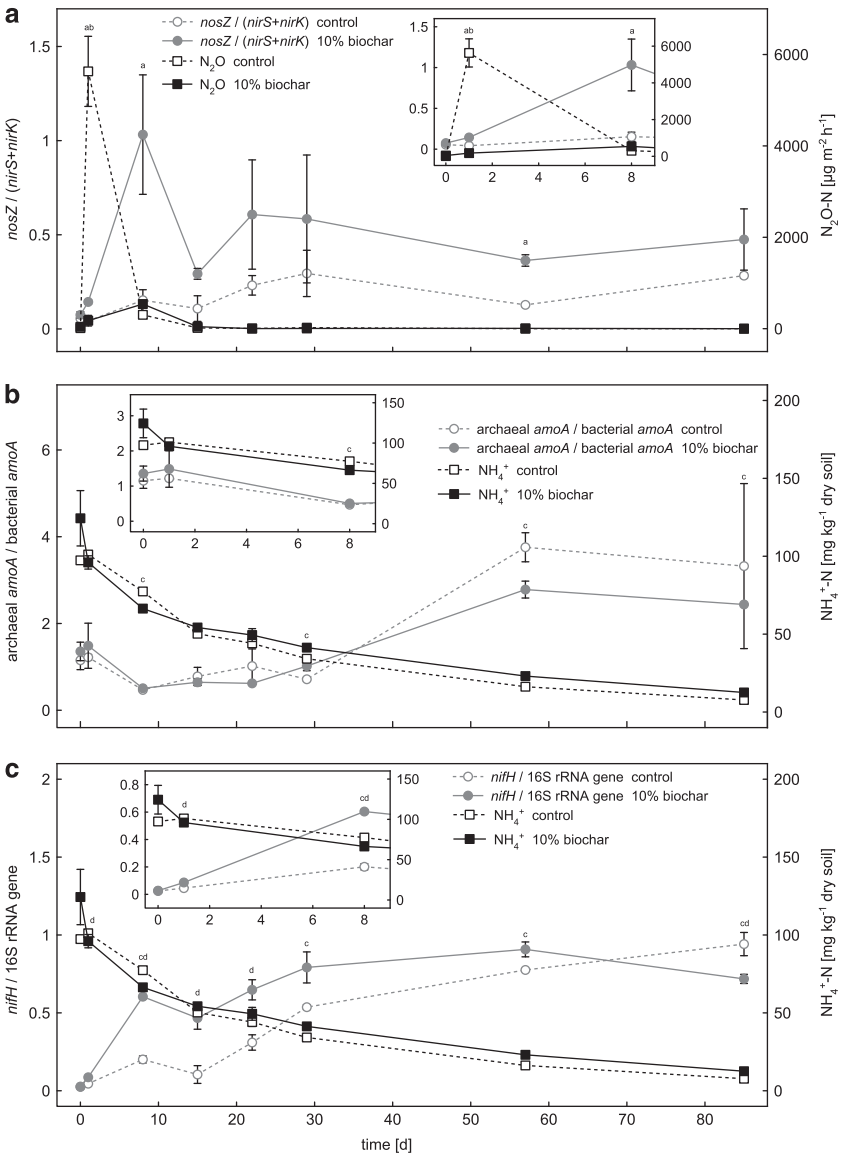


Figure 3 Changes in gene ratios or relative gene abundances plotted together with selected geochemical parameters of the control and 10% (w/w) biochar-containing soil microcosms over time. Panel **a** shows N_2O emissions in comparison with the ratio of *nosZ* over the sum of *nirS* and *nirK* gene copy numbers (*nosZ*/(*nirS* + *nirK*)). In panel **b**, ammonium concentrations are plotted together with the ratio of AOA over AOB (AOA/AOB ratio) as calculated from the bacterial and archaeal *amoA* gene copies numbers. Panel **c** shows ammonium concentrations and the relative abundance of *nifH* genes over total bacterial 16S rRNA genes. The small inserted graphs show a magnified view of the data for the first 8 days. Note that the *y* axes of the inserted graphs in panels **b** and **c** have a slightly different scale from the corresponding overview graphs. Open symbols with dashed lines represent data measured in the control microcosms without biochar. Filled symbols with solid lines represent data of the soil microcosms with 10% (w/w) biochar. Statistically significant differences (univariate ANOVA, *post hoc*: least significant difference) between control and 10% (w/w) biochar microcosms at a certain time point are indicated by lower-case characters above the individual data points (a = *nosZ*/(*nirS* + *nirK*), b = N_2O, c = NH_4^+ and d = *nifH*/bacterial 16S rRNA genes).

biochar-containing microcosms between day 0 and day 1 (Figure 4). In contrast, in the control microcosms without biochar *nosZ* gene transcripts per ng RNA decreased from 0.7×10^4 to 0.3×10^4 in the same period, what resulted in significantly higher *nosZ* transcript copy numbers in the 10% biochar-containing microcosms compared with the control microcosms at day 1 ($P = 0.03$). Notably, N_2O emissions were inversely correlated to the *nosZ* transcript copy numbers (highest in the control microcosms and significantly lower in the biochar-containing microcosms ($P = 0.002$)) at day 1 (Figure 1b, Supplementary Figure S2b).

Discussion

Geochemistry of the microcosms

At the beginning of the experiments all soil microcosms were amended with NH_4NO_3, KH_2PO_4, and molasses at typical field application rates (N: 90 kg ha^{-1}, P: 50 kg ha^{-1}, K: 63 kg ha^{-1}) in order

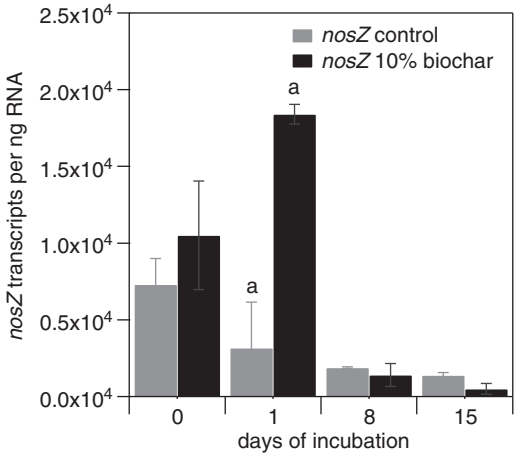


Figure 4 *nosZ* transcript copy numbers per ng RNA in the control microcosms and in the soil microcosms with 10% (w/w) biochar during the initial 15 days of incubation. Gray bars represent data measured in the control microcosms without biochar. Black bars represent data of the soil microcosms with 10% (w/w) biochar. Statistically significant differences between 10% (w/w) biochar-containing and control microcosms are indicated by the letter 'a' above the respective time points.

to simulate a fertilization event and prevent carbon limitation (Singh *et al.,* 2010b). Because our soil microcosms were plant-free and the soil contained little plant material, we added molasses as a carbon source to create a situation when larger quantities of plant-derived carbon become available, for example, after a cut or heavy rainfall (Felber *et al.,* 2012).

The observed rapid decrease in DOC in all microcosms corresponded to the quantified CO_2 formation rates at the beginning of incubation suggesting that in general biochar addition did not impair carbon-based microbial respiration (Figure 1c). Furthermore, the decrease in DOC within the first week until day 8 correlated well to the increase in total bacterial 16S rRNA gene copy numbers in all microcosms ($R^2 = 0.79$) (Figures 1c, 2a), indicating that the oxidation of readily available organic carbon stimulated microbial growth in the soil microcosms.

The succession of NH_4^+ and NO_3^- concentrations in the soil microcosms confirmed the expected predominance of anoxic conditions in the water-saturated soil microcosms (WFPS 95%). Rapidly decreasing NO_3^- concentrations suggests that denitrification prevailed under these conditions (Figure 1a). Evidence for the occurrence of NO_3^- reduction was also provided by the intermittent accumulation of NO_2^- within the first week (Figure 1b) (Lam and Kuypers, 2011). Slowly decreasing NH_4^+ concentrations could either be due to NH_4^+ assimilation or due to low levels of either aerobic or anaerobic ammonia oxidation caused by oxygen diffusion into the top layers of the soil microcosms or by oxidation of NH_4^+ with NO_2^- or an alternative electron acceptor such as iron (Lam and Kuypers, 2011; Yang *et al.,* 2012). N_2O emissions from soil were highest at day 1 and hence most likely a direct consequence of the initial fertilizer application and soil moisture adjustment (Figure 1b). As NH_4^+, NO_3^- and DOC concentrations decreased N_2O fluxes declined in all microcosms because electron donors and acceptors for microbial N_2O formation became limiting. The significantly lower N_2O emissions from biochar-containing microcosms observed within the first week (Figure 1b, Supplementary Figure S2b) agree with the findings of several recently published field- and laboratory-based studies using different biochars and soils (Yanai *et al.,* 2007; Singh *et al.,* 2010b; van Zwieten *et al.,* 2010; Taghizadeh-Toosi *et al.,* 2011; Wang *et al.,* 2011b; Augustenborg *et al.,* 2012; Wang *et al.,* 2012; Zheng *et al.,* 2012; Zhang *et al.,* 2012a, b). According to these studies, the most important environmental factors responsible for the reduced N_2O emissions from biochar-amended soil were: (i) limited bioavailability of electron donors and acceptors (DOC, NO_3^- and NH_4^+) for microbial nitrification and denitrification due to sorption/immobilization onto biochar particles (Singh *et al.,* 2010b; Taghizadeh-Toosi *et al.,* 2011; Wang *et al.,* 2011a); (ii) improved soil aeration through biochar addition and consequently reduced denitrification (Yanai *et al.,* 2007; van Zwieten *et al.,* 2010; Augustenborg *et al.,* 2012; Zhang *et al.,* 2012b); and (iii) increased activity of N_2O-reducing bacteria due to an elevated soil pH caused by biochar addition (van Zwieten *et al.,* 2010; Zheng *et al.,* 2012).

16S rRNA and N-cycling functional marker genes

Ammonia oxidation. In accordance with Ducey *et al.* (2013), no significant correlation between the abundance of AOA and AOB and soil biochar amendment was found in this study (Figure 2b). However, independent of the amount of biochar added the AOA/AOB gene ratio increased over time in all microcosms (Figure 3b).

N_2 fixation. Soil biochar amendment alters several environmental parameters known to affect the abundance and activity of N_2-fixing bacteria, such as oxygen availability, pH, C:N ratio and nitrogen availability (Reed *et al.,* 2007; Hsu and Buckley, 2008; Atkinson *et al.,* 2010; Singh *et al.,* 2010a). It is therefore most likely that the interplay of multiple of these parameters might be responsible for the elevated *nifH* gene copy numbers in the biochar-containing microcosms (Figure 2a). The biochar-containing microcosms had a slightly elevated pH ($\leqslant$ 0.3 pH units) and slightly lower concentrations of K_2SO_4-extractable NO_3^- and NH_4^+ (statistically significant only at individual time points) compared with the control microcosms.

Cusack *et al.* (2009) found a positive correlation between biological nitrogen fixation and forest soil

C:N ratio in tropical and lower montane rainforests. Even though high-temperature pyrolysis biochar is highly stable and mostly recalcitrant toward microbial degradation (Joseph *et al.,* 2010) many soil microorganisms are capable of degrading aromatic carbon structures when other more readily available carbon sources become limiting. The biochar used in this study had a C:N ratio of 88, whereas the C:N ratio of the soil was 11 (Table 1). Assimilation and biomass synthesis from biochar-carbon therefore required an additional source of nitrogen what might favor microorganisms capable of nitrogen fixation when alternative organic and inorganic nitrogen sources became limiting or non-bioavailable with time.

Denitrification. Net N_2O formation and release from soils have been shown to be strongly linked to the abundance and activity of N_2O-reducing bacteria as the only biotic sink of N_2O in the environment (Thomson *et al.,* 2012). Philippot *et al.* (2011) showed that one-third of all denitrifiers, defined as *nirS*- or *nirK*-containing microorganisms (Jones *et al.,* 2008), lack the genetic potential for N_2O reduction and thus are major contributors to microbial N_2O production (Philippot *et al.,* 2011). Our data suggest that the addition of biochar changed the denitrifier microbial community composition by promoting the growth (Figure 3a) and activity of N_2O-reducing bacteria (containing a *nosZ* gene) (Figure 4) relative to *nirS*- and *nirK*-containing denitrifiers. By this our findings support the hypothesis of Anderson *et al.* (2011) who suggested that decreased N_2O emissions from biochar-amended soil might be caused by an enhanced growth and activity of microorganisms capable of complete denitrification (Anderson *et al.,* 2011).

The incorporation of biochar into soil alters various geochemical soil parameters which are known to affect the diversity, abundance and functioning of N_2O-producing microbial communities in soils and thereby soil N_2O emissions, such as nitrogen speciation (NO_3^-/NH_4^+) and availability, pH and oxygen saturation (Richardson *et al.,* 2009; Braker and Conrad, 2011). Singh *et al.* (2010b) argued that over time the addition of biochar to soils increases sorption of inorganic nitrogen compounds such as NH_4^+ and NO_3^- to the soil matrix which decreases their availability for microbial N_2O production. We cannot exclude that biochar aging and associated changes in its cation exchange capacity might have affected NO_3^- sorption during the 3 months of incubation (Singh *et al.,* 2010b), but the observed reduction of N_2O emissions occurred within the first week of incubation (Figure 3a) and the fresh biochar used in this study showed little to no NO_3^- and NH_4^+ sorption in preliminary experiments (Supplementary Figure S4).

Bergaust *et al.* (2010) reported that soil pH exerts a strong control on the N_2O/N_2 product ratio in soils (high ratios at low pH), because at a pH below 7 N_2O reductase synthesis and assembly are inhibited. As in our experiments the pH in the presence of biochar increased $\leqslant 0.3$ pH units and the soil pH was rather alkaline (pH 8.4), the observed decrease in N_2O emission are unlikely to be caused by post-translational effects on N_2O reductase folding and inhibition.

Van Zwieten *et al.* (2009) postulated that biochar amendment can create anoxic microsites within soil particles and aggregates, for example, through the promotion of heterotrophic microbial respiration and growth on the surface of biochar particles which leads to local anaerobiosis. The formation of anoxic microsites would enhance complete versus incomplete denitrification by stimulating growth and activity of N_2O-reducing microorganisms, because N_2O reductases have been reported to be more sensitive to O_2 than enzymes involved in N_2O formation (Betlach and Tiedje, 1981; Jungkunst *et al.,* 2006). This might in particular be relevant for well-aerated soils and would generally not apply to water-saturated conditions as present in our microcosm experiment. However, as oxygen diffusion into the top soil layers of our microcosms was possible because the microcosms were incubated under ambient atmosphere, biochar addition might have contributed to the formation of more anoxic microsites in the top layers of the soil microcosms. Further evidence for a potentially lower oxygen availability in the biochar-containing microcosms also comes from the elevated *nifH* gene copy numbers in the microcosms because a low oxygen partial pressure is also considered to be one of the controlling factors of microbial N_2-fixation (Vitousek *et al.,* 2002; Reed *et al.,* 2011) (Figure 3c).

A recent study by Cayuela *et al.* (2013) using 15 agricultural soils showed that biochar consistently reduced the $N_2O/(N_2+N_2O)$ ratio, which demonstrated that soil biochar amendment promoted the last step of denitrification. According to Cayuela *et al.* (2013) biochar can function as an '*electron shuttle*' facilitating the transfer of electrons to soil denitrifying microorganisms. Taken together with its acid buffer capacity and its high surface area, the electron shuttling properties of biochar would promote the reduction of N_2O to N_2. The increased abundance and gene expression activity of *nosZ*-containing microorganisms observed in this study might be one explanation for the decreased ratio of $N_2O/(N_2+N_2O)$ observed by Cayuela *et al.* (2013).

Conclusions and implications. The N_2O fluxes quantified in this study agree with the N_2O fluxes previously quantified in water-saturated (WFPS $>70\%$) soil microcosms, flow-through columns or field sites after the application of high doses of fertilizers (Flessa *et al.,* 1995; Clayton *et al.,* 1997; Flessa *et al.,* 1998; Flechard *et al.,* 2005; Ruser *et al.,* 2006; Yanai *et al.,* 2007; van Zwieten *et al.,* 2010;

Singh *et al.,* 2010b). The added carbon in form of molasses thereby created a situation with high microbial activity, comparable to field situations when larger quantities of residues become available such as after a cut, during the winter/spring season when freeze-thaw cycles occur or after heavy rainfalls (Felber *et al.,* 2012). According to our data, N_2O emission peaks in water-saturated soils after fertilizer application may be reduced by up to 96% in the presence of 120 t ha^{-1} biochar (10% (w/w) biochar) and up to 47% in the presence of 24 t ha^{-1} biochar (2% w/w biochar) if the magnitude of the biochar effect in the lab is similar in the field. However, one needs to take into account that under field conditions emissions are usually less pronounced because most of the soil organic matter or plant residues are not readily biodegradable and first need to be broken down into monomers in order to effectively stimulate microbial N_2O production activity. Furthermore, typical biochar application rates are in the range of 5 to 50 t ha^{-1}. So assuming a potential N_2O emission reduction of about 47% as observed for the 2% (w/w) biochar-containing microcosms seems to be a more realistic and economic scenario and is in good agreement with results from other laboratory and field studies that reported reduction of N_2O emissions by 50–80% (Lehmann and Joseph, 2009; Singh *et al.,* 2010b; van Zwieten *et al.,* 2010; Taghizadeh-Toosi *et al.,* 2011; Zhang *et al.,* 2012a). However, the general impact of our findings needs additional evaluation and it would be a far stretch to extrapolate our results directly to field emissions because (i) only one soil and one biochar have been used, (ii) the impact of biochar on the microbial community of nitrogen-transforming microorganisms might vary considerably depending on soil and biochar type, (iii) N_2 formation has not been quantified and (iv) only a relative short time period of 3 months has been considered in the experiments presented here. Nonetheless, the documented changes in the relative abundance of N_2O-forming and reducing microorganisms and the changes in *nosZ* gene expression provide (i) new mechanistic insights into the effect of biochar on the structure and functioning of the denitrifying soil microbial community; and (ii) offer a tentative explanation for the observed reduction of N_2O emissions caused by soil biochar amendment as an increased abundance and gene expression activity of *nosZ*-containing microorganisms might enhance the direct microbial reduction of N_2O to N_2 thereby decreasing net soil N_2O release.

In order to confirm the findings of this study and further advance our understanding on the impact of biochar on the nitrogen cycling microbial community and soil N_2O emissions, field studies with different biochars over longer time periods are needed. Furthermore, two recent studies revealed a physiological dichotomy in the diversity of N_2O-reducing microorganisms (Sanford *et al.,* 2012; Jones *et al.,* 2013). These recent findings might also be of importance for understanding the relationship between N_2O reduction and the activity and diversity of N_2O-reducing microorganisms in biochar-amended soils and should be taken into account in future studies.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We thank Hans-Peter Schmidt of the Delinat Institute for Ecology und Climate Farming (Ayent, Switzerland) for providing soil and biochar, Elizaveta Krol (Center for Biological Systems Analysis, University of Freiburg, Germany) for providing the strain *Ensifer melilotii* 1021, Stefanie Töwe (Helmholtz Zentrum München: Research Unit Environmental Genomics, Germany) for providing archaeal and bacterial *amoA* standard plasmids for qPCR and Sven Marhan (Institute of Soil Science and Land Evaluation, University of Hohenheim, Germany) for helpful discussions. We are also grateful for the technical support of Ellen Struve, Karin Stögerer and Bernice Nisch.

References

Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA *et al.* (2011). Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* **54**: 309–320.

Arp DJ, Stein LY. (2003). Metabolism of inorganic N compounds by ammonia-oxidizing bacteria. *Crit Rev Biochem Mol Biol* **38**: 471–495.

Atkinson CJ, Fitzgerald JD, Hipps NA. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil* **337**: 1–18.

Augustenborg CA, Hepp S, Kammann C, Hagan D, Schmidt O, Muller C. (2012). Biochar and earthworm effects on soil nitrous oxide and carbon dioxide emissions. *J Environ Qual* **41**: 1203–1209.

Baggs EM, Smales CL, Bateman EJ. (2010). Changing pH shifts the microbial source as well as the magnitude of N_2O emission from soil. *Biol Fert Soils* **46**: 793–805.

Behrens S, Azizian MF, McMurdie PJ, Sabalowsky A, Dolan ME, Semprini L *et al.* (2008). Monitoring abundance and expression of "*Dehalococcoides*" species chloroethene-reductive dehalogenases in a tetrachloroethene-dechlorinating flow column. *Appl Environ Microbiol* **74**: 5695–5703.

Bergaust L, Mao Y, Bakken LR, Frostegard A. (2010). Denitrification response patterns during the transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrogen oxide reductase in *Paracoccus denitrificans*. *Appl Environ Microbiol* **76**: 6387–6396.

Betlach MR, Tiedje JM. (1981). Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. *Appl Environ Microbiol* **42**: 1074–1084.

Blackmer AM, Bremner JM. (1978). Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. *Soil Biol Biochem* **10**: 187–191.

Blagodatsky SA, Kesik M, Papen H, Butterbach-Bahl K. (2006). Production of NO and N_2O by the heterotrophic nitrifier *Alcaligenes faecalis* parafaecalis under varying conditions of oxygen saturation *Geomicrobiol J* **23**: 165–176.

Bleakley BH, Tiedje JM. (1982). Nitrous oxide production by organisms other than nitrifiers and denitrifiers. *Appl Environ Microbiol* **44**: 1342–1348.

Braker G, Conrad R. (2011). Diversity, structure, and size of N_2O-producing microbial communities in soils-What matters for their functioning? *Adv Appl Microbiol* **75**: 33–70.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M *et al.* (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**: 611–622.

Canfield DE, Glazer AN, Falkowski PG. (2010). The evolution and future of Earth's nitrogen cycle. *Science* **330**: 192–196.

Cayuela ML, Sanchez-Monedero MA, Roig A, Hanley K, Enders A, Lehmann J. (2013). Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions? *Sci Rep* **3**: 1732.

Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S. (2008). Using poultry litter biochars as soil amendments. *Aust J Soil Res* **46**: 437–444.

Clayton H, McTaggart IP, Parker J, Swan L, Smith KA. (1997). Nitrous oxide emissions from fertilised grassland: A 2-year study of the effects of N fertiliser form and environmental conditions. *Biol Fert Soils* **25**: 252–260.

Cuhel J, Simek M, Laughlin RJ, Bru D, Cheneby D, Watson CJ *et al.* (2010). Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity. *Appl Environ Microbiol* **76**: 1870–1878.

Cusack DF, Silver W, McDowell WH. (2009). Biological nitrogen fixation in two tropical forests: Ecosystem-level patterns and effects of nitrogen fertilization. *Ecosystems* **12**: 1299–1315.

Denman KL, Brasseur G, Chidthaisong A, Ciais P, Cox PM, Dickinson RE *et al.* (2007). Coupling between changes in the climate system and biogeochemistry. In: Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor MMB *et al.* (eds) *Climate Change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA, pp 499–587.

Duce RA, LaRoche J, Altieri K, Arrigo KR, Baker AR, Capone DG *et al.* (2008). Impacts of atmospheric anthropogenic nitrogen on the open ocean. *Science* **320**: 893–897.

Ducey TF, Ippolito JA, Cantrell KB, Novak JM, Lentz RD. (2013). Addition of activated switchgrass biochar to an aridic subsoil increases microbial nitrogen cycling gene abundances. *Appl Soil Ecol* **65**: 65–72.

Felber R, Hüppi R, Leifeld J, Neftel A. (2012). Nitrous oxide emission reduction in temperate biochar-amended soils. *Biogeosciences Discuss* **9**: 151–189.

Flechard CR, Neftel A, Jocher M, Ammann C, Fuhrer J. (2005). Bi-directional soil/atmosphere N_2O exchange over two mown grassland systems with contrasting management practices. *Glob Change Biol* **11**: 2114–2127.

Flessa H, Dorsch P, Beese F. (1995). Seasonal variation of N_2O and CH_4 fluxes in differently managed arable soils in Southern Germany. *J Geophys Res-Atmos* **100**: 23115–23124.

Flessa H, Wild U, Klemisch M, Pfadenhauer J. (1998). Nitrous oxide and methane fluxes from organic soils under agriculture. *Eur J Soil Sci* **49**: 327–335.

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW *et al.* (2007). Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor MMB *et al.* (eds) *Climate Change 2007: The physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA, p 105.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, Freney JR *et al.* (2008). Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* **320**: 889–892.

Hsu S-F, Buckley DH. (2008). Evidence for the functional significance of diazotroph community structure in soil. *ISME J* **3**: 124–136.

Jetten MSM. (2008). The microbial nitrogen cycle. *Environ Microbiol* **10**: 2903–2909.

Jones CM, Graf DR, Bru D, Philippot L, Hallin S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *ISME J* **7**: 417–426.

Jones CM, Stres B, Rosenquist M, Hallin S. (2008). Phylogenetic analysis of nitrite, nitric oxide, and nitrous oxide respiratory enzymes reveal a complex evolutionary history for denitrification. *Mol Biol Evol* **25**: 1955–1966.

Joseph SD, Camps-Arbestain M, Lin Y, Munroe P, Chia CH, Hook J *et al.* (2010). An investigation into the reactions of biochar in soil. *Aust J Soil Res* **48**: 501–515.

Jungkunst HF, Freibauer A, Neufeldt H, Bareth G. (2006). Nitrous oxide emissions from agricultural land use in Germany - a synthesis of available annual field data *J Plant Nutr Soil Sc* **169**: 341–351.

Keiluweit M, Nico PS, Johnson MG, Kleber M. (2010). Dynamic molecular structure of plant biomass-derived black carbon (biochar). *Environ Sci Technol* **44**: 1247–1253.

Khodadad CLM, Zimmerman AR, Green SJ, Uthandi S, Foster JS. (2011). Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol Biochem* **43**: 385–392.

Klappenbach JA, Saxman PR, Cole JR, Schmidt TM. (2001). rrndb: the ribosomal RNA operon copy number database. *Nucleic Acids Res* **29**: 181–184.

Kumon Y, Sasaki Y, Kato I, Takaya N, Shoun H, Beppu T. (2002). Codenitrification and denitrification are dual metabolic pathways through which dinitrogen evolves from nitrate in *Streptomyces antibioticus*. *J Bacteriol* **184**: 2963–2968.

Lam P, Kuypers MMM. (2011). Microbial nitrogen cycling processes in oxygen minimum zones. *Annu Rev Mar Sci* **3**: 317–345.

Lehmann J, Joseph S. (2009). *Biochar for Environmental Management: Science and Technology*. Earthscan: London; Sterling, VA, USA.

Loftfield N, Flessa H, Augustin J, Beese F. (1997). Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane,

carbon dioxide, and nitrous oxide. *J Environ Qual* **26**: 560.

Major J, Rondon M, Molina D, Riha SJ, Lehmann J. (2010). Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil* **333**: 117–128.

Papen H, Vonberg R, Hinkel I, Thoene B, Rennenberg H. (1989). Heterotrophic nitrification by *Alcaligenes faecalis*—NO_2^-, NO_3^-, N_2O, and NO production in exponentially growing cultures. *Appl Environ Microbiol* **55**: 2068–2072.

Philippot L, Andert J, Jones CM, Bru D, Hallin S. (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. *Glob Change Biol* **17**: 1497–1504.

Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones CM *et al.* (2013). Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* **7**: 1609–1619.

Ravishankara AR, Daniel JS, Portmann RW. (2009). Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science* **326**: 123–125.

Reed SC, Cleveland CC, Townsend AR. (2007). Controls over leaf litter and soil nitrogen fixation in two lowland tropical rain forests. *Biotropica* **39**: 585–592.

Reed SC, Cleveland CC, Townsend AR. (2011). Functional ecology of free-living nitrogen fixation: A contemporary perspective. In: Futuyma DJ, Shaffer HB, Simberloff D (eds) *Annual Review of Ecology, Evolution and Systematics*. Annual Reviews: Palo Alto, CA, USA, pp 489–512.

Richardson D, Felgate H, Watmough N, Thomson A, Baggs E. (2009). Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle—could enzymic regulation hold the key? *Trends Biotechnol* **27**: 388–397.

Robertson GP. (2007). Nitrogen transformation. In: Paul EA (ed) *Soil Microbiology, Biochemistry and Ecology*. Springer: New York, NY, USA, pp 341–364.

Rondon MA, Lehmann J, Ramirez J, Hurtado M. (2007). Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol Fert Soils* **43**: 699–708.

Ruser R, Flessa H, Schilling R, Steindl H, Beese F. (1998). Soil compaction and fertilization effects on nitrous oxide and methane fluxes in potato fields. *Soil Sci Soc Am J* **62**: 1587–1595.

Ruser R, Flessa H, Russow R, Schmidt G, Buegger F, Munch JC. (2006). Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. *Soil Biol Biochem* **38**: 263–274.

Sanford RA, Wagner DD, Wu QZ, Chee-Sanford JC, Thomas SH, Cruz-Garcia C *et al.* (2012). Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. *P Natl Acad Sci USA* **109**: 19709–19714.

Senga Y, Mochida K, Fukumori R, Okamoto N, Seike Y. (2006). N_2O accumulation in estuarine and coastal sediments: The influence of H_2S on dissimilatory nitrate reduction. *Estuar Coast Shelf S* **67**: 231–238.

Singh B, Singh BP, Cowie AL. (2010a). Characterisation and evaluation of biochars for their application as a soil amendment. *Aust J Soil Res* **48**: 516–525.

Singh BP, Hatton BJ, Singh B, Cowie AL, Kathuria A. (2010b). Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *J Environ Qual* **39**: 1224–1235.

Smith MS. (1982). Dissimilatory reduction of NO_2^- to NH_4^+ and N_2O by a soil *Citrobacter* sp. *Appl Environ Microbiol* **43**: 854–860.

Smith MS. (1983). Nitrous oxide production by *Escherichia coli* is correltaed with nitrate reductase activity. *Appl Environ Microbiol* **45**: 1545–1547.

Smith MS, Zimmerman K. (1981). Nitrous oxide production by non-denitrifying soil nitrate reducers. *Soil Sci Soc Am J* **45**: 865–871.

Smith P, Martino D, Cai Z, Gwary D, Janzen H, Kumar P *et al.* (2007). Agriculture. In: Metz B, Davidson OR, Bosch PR, Dave R, Meyer LA (eds) *In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press: Cambridge, United Kingdom and New York, NY, USA.

Sohi SP, Krull E, Lopez-Capel E, Bol R. (2010). A review of biochar and its use and function in soil. *Adv Agron* **105**: 47–82.

Sorensen J, Tiedje JM, Firestone RB. (1980). Inhibition by sulfide of nitric and nitrous-oxide reduction by denitrifying *Pseudomonas fluorescens*. *Appl Environ Microbiol* **39**: 105–108.

Steinbeiss S, Gleixner G, Antonietti M. (2009). Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol Biochem* **41**: 1301–1310.

Stevens RJ, Laughlin RJ, Malone JP. (1998). Measuring the mole fraction and source of nitrous oxide in the field. *Soil Biol Biochem* **30**: 541–543.

Taghizadeh-Toosi A, Clough TJ, Condron LM, Sherlock RR, Anderson CR, Craigie RA. (2011). Biochar incorporation into pasture soil suppresses *in situ* nitrous oxide emissions from ruminant urine patches. *J Environ Qual* **40**: 468–476.

Tanimoto T, Hatano K, Kim DH, Uchiyama H, Shoun H. (1992). Co-denitrification by the denitrifying system of the fungus *Fusarium oxysporum*. *FEMS Microbiol Lett* **93**: 177–180.

Thomson AJ, Giannopoulos G, Pretty J, Baggs EM, Richardson DJ. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philos T R Soc B* **367**: 1157–1168.

Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk HP, Schleper C. (2005). Novel genes for nitrite reductase and *Amo*-related proteins indicate a role of uncultivated mesophilic Crenarchaeota in nitrogen cycling. *Environ Microbiol* **7**: 1985–1995.

Uchimiya M, Wartelle LH, Lima IM, Klasson KT. (2010). Sorption of deisopropylatrazine on broiler litter biochars. *J Agr Food Chem* **58**: 12350–12356.

van Zwieten L, Kimber S, Morris S, Downie A, Berger E, Rust J *et al.* (2010). Influence of biochars on flux of N_2O and CO_2 from Ferrosol. *Aust J Soil Res* **48**: 555–568.

Van Zwieten L, Singh B, Joseph S, Kimber S, Cowie A, Chan K. (2009). Biochar and emissions of Non-CO_2 greenhouse gases from soil. In: Lehmann J, Joseph S (eds) *Biochar for Environmental Management Science and Technology*. Earthscan: London, UK, pp 227–249.

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB *et al.* (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* **57**: 1–45.

Wallenstein MD, Myrold DD, Firestone M, Voytek M. (2006). Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. *Ecol Appl* **16**: 2143–2152.

Wang J, Zhang M, Xiong Z, Liu P, Pan G. (2011a). Effects of biochar addition on N_2O and CO_2 emissions from two paddy soils. *Biol Fert Soils* **47**: 887–896.

Wang JY, Pan XJ, Liu YL, Zhang XL, Xiong ZQ. (2012). Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. *Plant Soil* **360**: 287–298.

Wang S, Wang Y, Feng X, Zhai L, Zhu G. (2011b). Quantitative analyses of ammonia-oxidizing Archaea and Bacteria in the sediments of four nitrogen-rich wetlands in China. *Appl Microbiol Biotechnol* **90**: 779–787.

Wrage N, van Groenigen JW, Oenema O, Baggs EM. (2005). A novel dual-isotope labelling method for distinguishing between soil sources of N_2O. *Rapid Commun Mass Sp* **19**: 3298–3306.

Yanai Y, Toyota K, Okazaki M. (2007). Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Sci Plant Nutr* **53**: 181–188.

Yang WH, Weber KA, Silver WL. (2012). Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. *Nature Geosci* **5**: 538–541.

Zhang AF, Bian RJ, Pan GX, Cui LQ, Hussain Q, Li LQ *et al.* (2012a). Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crop Res* **127**: 153–160.

Zhang AF, Liu YM, Pan GX, Hussain Q, Li LQ, Zheng JW *et al.* (2012b). Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. *Plant Soil* **351**: 263–275.

Zhao S, Fernald RD. (2005). Comprehensive algorithm for quantitative real-time polymerase chain reaction *J Comput Biol* **12**: 1047–1064.

Zheng JY, Stewart CE, Cotrufo MF. (2012). Biochar and nitrogen fertilizer alters soil nitrogen dynamics and greenhouse gas fluxes from two temperate soils. *J Environ Qual* **41**: 1361–1370.

Supplementary Information accompanies this paper on The ISME Journal website (http://www.nature.com/ismej)

Supplementary Information

Linking nitrous oxide emissions from biochar-amended soil to the structure and function of the N-cycling microbial community

Johannes Harter[1#], Hans-Martin Krause[1#], Stefanie Schüttler[1], Reiner Ruser[2], Markus Fromme[3], Thomas Scholten[3], Andreas Kappler[1], Sebastian Behrens[1]

[1] Geomicrobiology and Microbial Ecology, Center for Applied Geoscience, University of Tübingen, 72076 Tübingen, Germany

[2] Fertilization and Soil Matter Dynamics, Institute of Crop Science, University of Hohenheim, 70599 Stuttgart, Germany

[3] Soil Science and Geomorphology, Institute of Geography, University of Tübingen, 72076 Tübingen, Germany

[#] These authors contributed equally

Geochemical analyses

Total and organic carbon, sulfur and nitrogen were quantified using a Vario EL elemental analyzer (Elementar, Hanau, Germany) according to ISO 10694, 13878 and 15178. Particle size distribution of the soil was determined according to ISO 11277 by sieving and sedimentation. CEC was determined according to ISO 13536. Surface area of the biochar was determined after Brunauer, Emmett and Teller (BET) according to ISO 9277 with a ASAP 2000 (Micromeritics, Norcross, GA). Ash and moisture content were determined according to ASTM D1762. EC and pH for characterization of the biochar were determined in a 1:20 biochar/water solution according to Rajkovich et al (2012) with a TetraCon® 925 conductivity meter (WTW, Weilheim, Germany) and a SenTix® S940 pH meter (WTW, Weilheim, Germany), respectively (Rajkovich et al 2012). Soil pH was determined in a 1:5 solution (deionized water) according to ISO 10390. Particle density of the biochar was determined with a pycnometer according to DIN 18124.

Table S1: Elemental composition of the soil (calcaric leptosol) and the biochar as determined by X-Ray Fluorescence (XRF). Values are given in % of total mass or in mg kg^{-1}.

	Soil	**Biochar**
SiO_2 [%]	56.06	25.95
TiO_2 [%]	1.06	0.16
Al_2O_3 [%]	18.29	4.02
Fe_2O_3 [%]	5.44	1.32
MnO [%]	0.05	0.05
MgO [%]	1.15	1.28
CaO [%]	4.83	11.64
Na_2O [%]	0.88	0.67
K_2O [%]	2.90	1.65
P_2O_5 [%]	0.36	0.47
Co [mg kg^{-1}]	21	0
Cr [mg kg^{-1}]	148	34
Ni [mg kg^{-1}]	116	0
V [mg kg^{-1}]	167	23
Zn [mg kg^{-1}]	53	0

Fig. S1: Soil microcosms. From left to right: biochar-free control, 2% (w/w) biochar, and 10% (w/w) biochar-containing microcosms. The water-filled pore space (WFPS) was adjusted to 95%.

Table S2: Bacterial *strains and primers used for the construction of qPCR-standards.*

Gene	Strain	Primer	Sequence	Amplicon size [bp]	Cloning vector[a]
16S rRNA	*Thiomonas* sp.	GM3F	AGAGTTTGATCMTGGCTCAG	1384	pCR2.1®
		1392R	ACGGGCGGTGTGTRC		
nifH	*Acidithiobacillus*	nifH_AbFo_F	GAGTGACAAATTAAGGCAAA	820	pCR4®
	ferrooxidans	nifH_AbFo_R	GAACTCCATGAGCATTTCTT		
nirK	*Sinorhizobium*	nirK_Sm_F	TCTGAGCAATTCCAGATGAC	1097	pCR4®
	meliloti 1021	nirK_Sm_R	ATCAGATCGTCGTTCCAGT		
nirS	*Ralstonia*	nirS_Re_F	CATTGCCGCTCTCACTCT	1607	pCR4®
	eutropha H16	nirS_Re_R	GTTATAGGCGTTGAACTTGC		
nosZ	*Sinorhizobium*	nosZ_Sm_F	TCAAACGAAGAAACCAAGAT	1884	pCR4®
	meliloti 1021	nosZ_Sm_R	CTTCATCTCCATGTGCATC		

qPCR-standards for archaeal and bacterial *amoA* genes have been commercially synthesized.

[a] vectors obtained from Invitrogen™, Life Technologies Ltd, Paisley, UK.

Table S3: Quantitative PCR reaction mixtures and thermal profiles for the different target genes.

Target gene	Reaction mixture	Volumes [µl]	Thermal profile	Reference
16S rRNA gene	SsoFast™ EvaGreen® Supermix	10	98 °C – 10 s	Modified after Nadkarni et al (2002)
	341f (5 µM)	0.3	61.5 °C – 45 s	
	797R (5 µM)	0.9		
	BSA (10% w/v)	0.5	40 cycles	
	PCR water	6.3		
	template (5-50 ng μL^{-1})	2		
nifH	SsoFast™ EvaGreen® Supermix	10	98 °C – 45 s	Modified after Towe et al (2010)
	*nifH*F (5 µM)	1	55 °C – 45 s	
	*nifH*R (5 µM)	1	72 °C – 45 s	
	BSA (3% w/v)	0.5		
	PCR water	5.5	45 cycles	
	template (5-50 ng μL^{-1})	2		
archael *amoA*	SsoFast™ EvaGreen® Supermix	10	98 °C – 45 s	Modified after Towe et al (2010)
	amo19F (5 µM)	1	55 °C – 45 s	
	cren*amoA*616r48x (5 µM)	1	72 °C – 45 s	
	BSA (10% w/v)	0.5		
	PCR water	5.5	40 cycles	
	Template (5-50 ng μL^{-1})	2		
bacterial *amoA*	SsoFast™ EvaGreen® Supermix	10	98 °C – 45 s	Modified after Towe et al (2010)
	*amoA*1F (5 µM)	1.5	60 °C – 45 s	
	*amoA*2R (5 µM)	1.5	72 °C – 45 s	
	BSA (10% w/v)	0.5		
	PCR water	4.5	40 cycles	
	Template (5-50 ng μL^{-1})	2		
nirK	iQ™ SYBR® Green Supermix	10	98 °C – 15 s	Modified after Ollivier et al (2010)
	nirK876C (5 µM)	2	63-58 °C – 30 s	
	nirK1040 (5 µM)	2	72 °C – 30 s	
	BSA (10% w/v)	0.5	6 cycles	
	DMSO (≥ 99.5%)	0.4	98 °C – 15 s	
	PCR water	3.1	58 °C – 30 s	
	Template (5-50 ng μL^{-1})	2	72 °C – 30 s	
			40 cycles	
nirS	SsoFast™ EvaGreen® Supermix	10	98 °C – 60 s	Modified after Ollivier et al (2010)
	cd3af (5 µM)	2	57 °C – 60 s	
	R3cd (5 µM)	2	72 °C – 60 s	
	BSA (10% w/v)	0.5		
	DMSO (≥ 99.5%)	0.4	40 cycles	
	PCR water	3.1		
	Template (5-50 ng μL^{-1})	2		
nosZ	SsoFast™ EvaGreen® Supermix	10	98 °C – 30 s	Modified after Babić et al (2008)
	nosZ2F (5 µM)	1	65-60 °C – 30 s	
	nosZ2R (5 µM)	1	72 °C – 30 s	
	BSA (10% w/v)	0.5	6 cycles	
	DMSO (≥ 99.5%)	0.4	98 °C – 15 s	
	PCR water	5.1	60 °C – 15 s	
	Template (5-50 ng μL^{-1})	2	72 °C – 30 s	
			40 cycles	

Table S4: Primers used for quantitative PCR.

Gene	Primer	Sequence	Amplicon size [bp]	Reference
16S	341f	CCTACGGGAGGCAGCAG	466	Muyzer et al (1993)
rRNA	797r	GGACTACCAGGGTATCTAATCCTGTT		Nadkarni et al (2002)
nifH	nifHF	AAAGGYGGWATCGGYAARTCCACCAC	458	Rosch et al (2002)
	nifHR	TTGTTSGCSGCRTACATSGCCATCAT		
archaeal	amo19F	ATGGTCTGGCTWAGACG	624	Leininger et al (2006)
amoA	CrenamoA616r48x	GCCATCCABCKRTANGTCCA		Schauss et al (2009)
bacterial	amoA1F	GGGGTTTCTACTGGTGGT	500	Rotthauwe et al (1997)
amoA	amoA2R	CCCCTCKGSAAAGCCTTCTTC		
nirK	nirK876C	ATYGGCGGV<u>C</u>AYGGCGA[a]	164	Modified after Henry et al (2004)
	nirK1040	GCCTCGATCAGRTTRTGG		
nirS	cd3af	GTNAAYGTNAARGARACNGG	413	Michotey et al (2000)
	R3cd	GASTTCGGRTGSGTCTTGA		Throback et al (2004)
nosZ	nosZ2F	CGCRACGGCAASAAGGTSMSSGT	267	Henry et al (2006)
	nosZ2R	CAKRTGCAKSGCRTGGCAGAA		

[a] Insertion of a cytosine (underlined) in order to increase target gene coverage.

Table S5: qPCR parameters (efficiency, slope and R^2) for evaluation of the different target gene assays.

	efficiency [%]	slope	R^2
16S rRNA gene	94.6	-3.46	0.99
nifH	94.8	-3.46	0.99
archaeal *amoA*	92.5	-3.51	0.99
bacterial *amoA*	95.3	-3.44	0.99
nirK	96.6	-3.41	0.99
nirS	79.5	-3.94	0.99
nosZ	75.8	-4.17	0.99

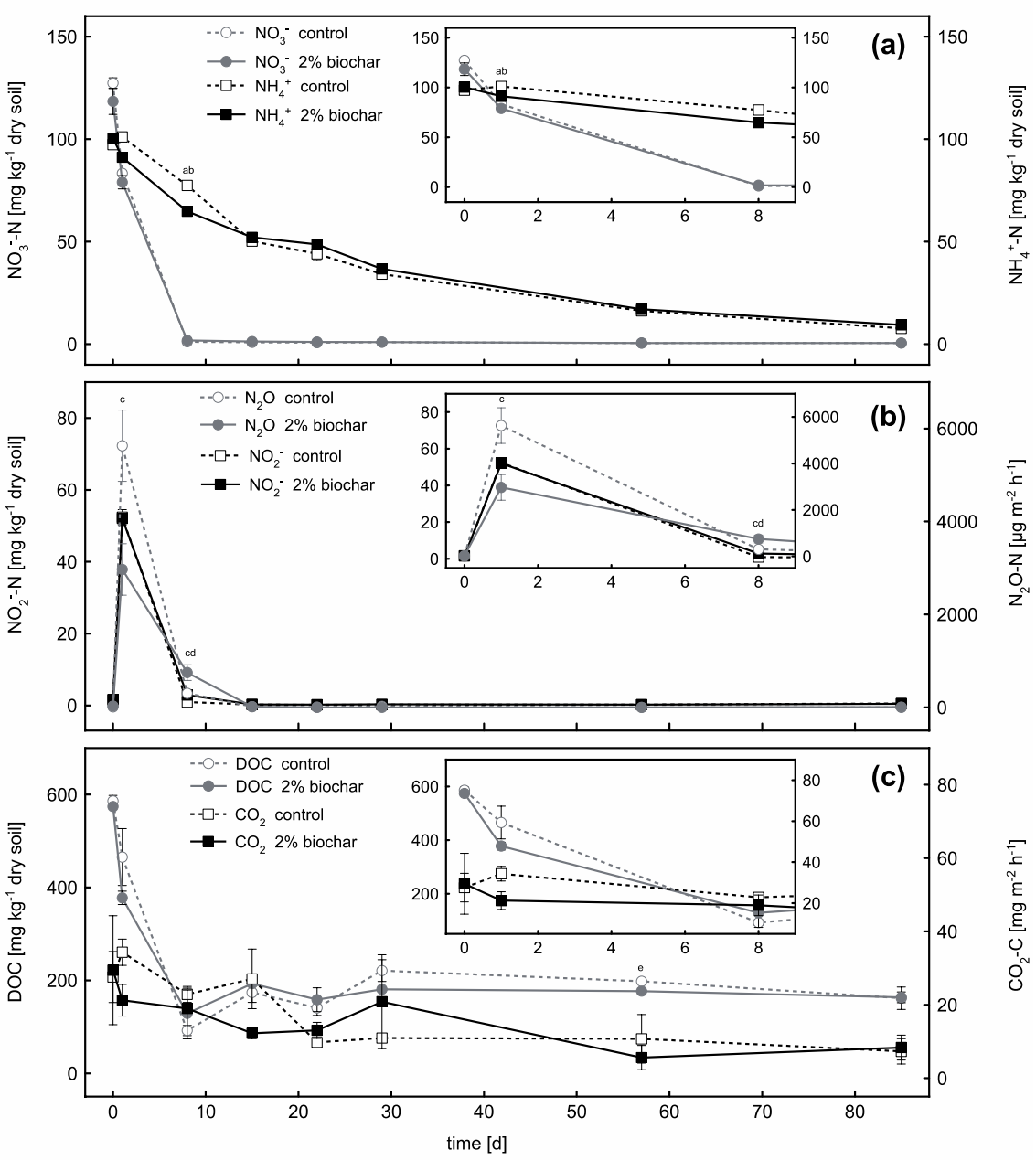


Fig. S2: Change in nitrogen (a, b) and carbon (c) geochemical parameters in the 2% (w/w) biochar-containing and control soil microcosms over time. Panel a and b show changes in the concentrations of nitrate, nitrite, ammonium and nitrous oxide, while panel c shows the DOC and carbon dioxide data. The small inserted graphs show a magnified view of the data for the first 8 days. Open symbols with dashed lines represent data of the control microcosms without biochar. Filled symbols with solid lines represent data of the soil microcosms with 2% (w/w) biochar. Statistically significant differences (univariate ANOVA, post-hoc: LSD) between control and 2% (w/w) biochar microcosms at a certain time point are indicated by lower-case letters above the individual data points (a = NO_3^-, b = NH_4^+, c = N_2O, d = NO_2^-, e = DOC, f = CO_2).

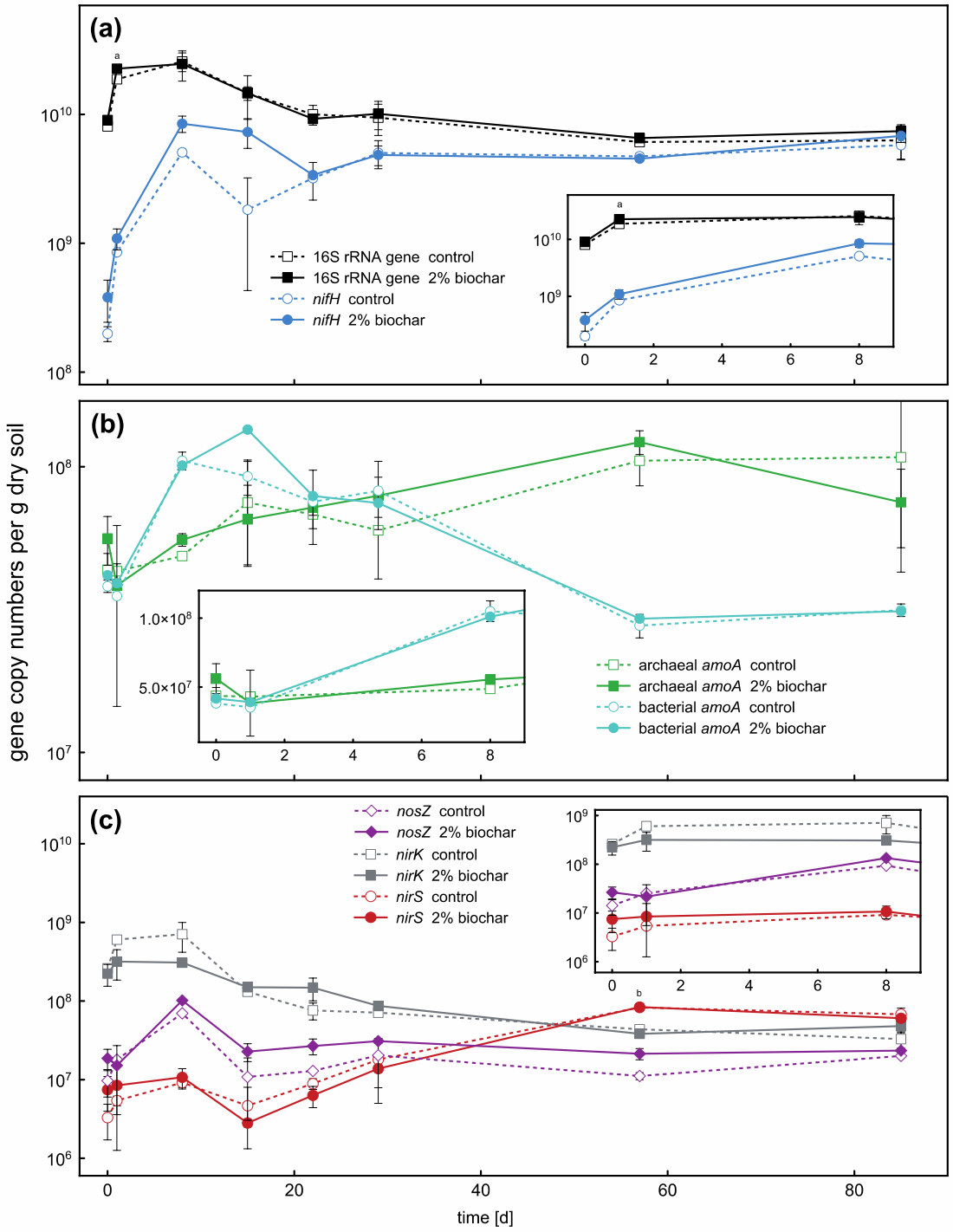


Fig. S3: Gene copy numbers per gram dry soil over time for various key genes of microbial nitrogen transformation processes in the 2% (w/w) biochar-containing and control microcosms. Panel a shows changes in total bacterial 16S rRNA and *nifH* genes copy numbers. In panel b archaeal and bacterial *amoA* gene copy numbers are shown. Panel c summarizes the gene copy number data for *nirS*, *nirK*, and *nosZ*. The small inserted graphs show a magnified view of the data for the first 8 days. Open symbols with dashed lines represent data measured in the control microcosms without biochar. Filled symbols with solid lines represent data of the soil microcosms with 2% (w/w) biochar. Statistically significant differences (univariate ANOVA, post-hoc: LSD) between control and 2% (w/w) biochar microcosms at a certain time point are indicated by lower-case letters above the individual data points (a = *nifH*, b = *nosZ*, c = *nirS*).

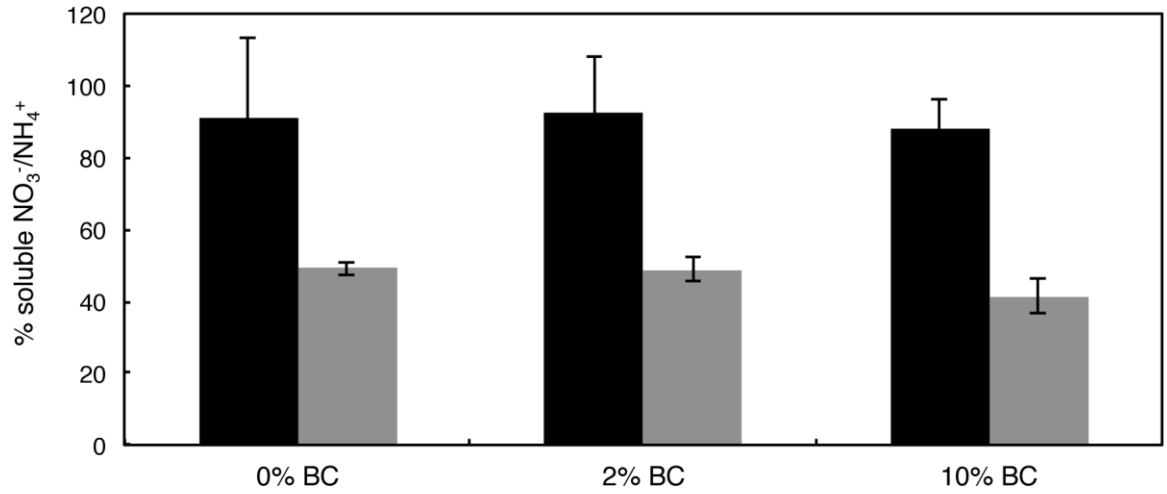


Fig. S4: Ratio of water- to K_2SO_4-extractable (0.5 N) NO_3^- (black bars) and NH_4^+ (gray bars) in the water-saturated (95% water-filled pore space) soil microcosms with different amounts (w/w) of biochar after 24h of incubation at room temperature. Data has been normalized to the K_2SO_4-extractable NO_3^- and NH_4^+. The amount of water-extractable NO_3^- and NH_4^+ is expressed as fraction of the K_2SO_4-extractable NO_3^- and NH_4^+. % BC refers to the amount (w/w) of biochar added to the individual soil microcosms.

References

Babić KH, Schauss K, Hai B, Sikora S, Redžepović S, Radl V *et al* (2008). Influence of different Sinorhizobium meliloti inocula on abundance of genes involved in nitrogen transformations in the rhizosphere of alfalfa (Medicago sativa L.). *Environ Microbiol* **10:** 2922-2930.

Henry S, Baudoin E, Lopez-Gutierrez JC, Martin-Laurent F, Baumann A, Philippot L (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *J Microbiol Meth* **59:** 327-335.

Henry S, Bru D, Stres B, Hallet S, Philippot L (2006). Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. *Appl Environ Microbiol* **72:** 5181-5189.

Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW *et al* (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442:** 806-809.

Michotey V, Mejean V, Bonin P (2000). Comparison of methods for quantification of cytochrome cd(1)-denitrifying bacteria in environmental marine samples. *Appl Environ Microbiol* **66:** 1564-1571.

Muyzer G, Dewaal EC, Uitterlinden AG (1993). Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA. *Appl Environ Microbiol* **59:** 695-700.

Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology-(UK)* **148:** 257-266.

Ollivier J, Kleineidam K, Reichel R, Thiele-Bruhn S, Kotzerke A, Kindler R *et al* (2010). Effect of Sulfadiazine-Contaminated Pig Manure on the Abundances of Genes and Transcripts Involved in Nitrogen Transformation in the Root-Rhizosphere Complexes of Maize and Clover. *Appl Environ Microbiol* **76:** 7903-7909.

Rajkovich S, Enders A, Hanley K, Hyland C, Zimmerman AR, Lehmann J (2012). Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biol Fert Soils* **48:** 271-284.

Rosch C, Mergel A, Bothe H (2002). Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. *Appl Environ Microbiol* **68:** 3818-3829.

Rotthauwe JH, Witzel KP, Liesack W (1997). The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Appl Environ Microbiol* **63:** 4704-4712.

Schauss K, Focks A, Leininger S, Kotzerke A, Heuer H, Thiele-Bruhn S *et al* (2009). Dynamics and functional relevance of ammonia-oxidizing archaea in two agricultural soils. *Environ Microbiol* **11:** 446-456.

Throback IN, Enwall K, Jarvis A, Hallin S (2004). Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE. *FEMS Microbiol Ecol* **49:** 401-417.

Towe S, Albert A, Kleineidam K, Brankatschk R, Dumig A, Welzl G *et al* (2010). Abundance of Microbes Involved in Nitrogen Transformation in the Rhizosphere of Leucanthemopsis alpina (L.) Heywood Grown in Soils from Different Sites of the Damma Glacier Forefield. *Microb Ecol* **60:** 762-770.

Chapter 4

Biochar and lime addition affect community composition of nitrous oxide reducers under the cultivation of *Zea mays*

Hans-Martin Krause[1], Roman Hüppi[2], Jens Leifeld[2], Mohamed El-Hadidi[3], Johannes Harter[4], Andreas Kappler[4], Martin Hartmann[5], Sebastian Behrens[6] Paul Mäder[1], Andreas Gattinger[1]

[1]Soil Sciences, Research Institute of Organic Agriculture (Fibl), Frick, Switzerland

[2]Climate and Air Pollution Group, Agroscope Institute for Sustainability Sciences, Zurich, Switzerland

[3]Algorithms in Bioinformatics, Center for Bioinformatics, University of Tübingen, Tübingen, Germany

[4]Geocmicrobiology, Center for Applied Geoscience, University of Tübingen, Tübingen, Germany

[5]Forest Soils and Biogeochemistry, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

[6] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA

Submitted to Environment Microbiology

Abstract

N_2O is a major greenhouse gas and the gross of anthropogenic N_2O emission originates from agriculturally managed soils. Therefore the development of N_2O mitigation strategies is a key challenge for agriculture. Biochar as soil amendment has been discussed as promising tool to improve soil quality, sequester carbon and mitigate N_2O emissions. Yet, the mechanism how biochar addition can affect N_2O emissions is still poorly understood. While the liming effect of biochar is discussed to enhance N_2O reduction, other studies related increased size and/or taxonomic composition of N_2O reducing bacteria after biochar addition to decreased N_2O emissions. Yet, most studies focusing on the mechanistic impact of biochar on N_2O emissions were restricted to short term changes during incubation experiments. In order to test whether the influence of N_2O reducers on N_2O emissions after biochar addition also holds under field conditions we performed a field experiment investigating impact of biochar under the cultivation of *Zea mays* across a whole vegetation period. The liming effect of biochar was assessed by including a separate lime treatment. Size and taxonomic composition of typical and atypical *nosZ* bearing bacteria were assessed after harrowing, fertilization and harvest using qPCR and Illumina sequencing approaches. In the biochar amended soil N_2O emission decreased by 52% compared to the control across the whole vegetation period. The size of typical and atypical *nosZ* bearing communities increased after fertilization and taxonomic composition of the atypical nosZ gene differentiated in between treatments after fertilization. The bacterial taxa associated with the biochar treatment lacked antecedent denitrification genes indicating specialized N_2O reducers to play an important role in mitigating N_2O emissions after biochar addition under field conditions. The liming effect of biochar seemed not to be the sole driver for differentiation of atypical *nosZ* bearing community. Our results broaden our understanding of biochar`s impact on N_2O emissions on a field scale and might prove valuable for the development of N_2O mitigation strategies.

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas that also catalyzes stratospheric ozone depletion (Ravishankara *et al.*, 2009). Atmospheric N_2O concentrations are constantly rising and reached 328 ppb in 2016 (121% of preindustrial levels) mainly due to anthropogenic intervention in the nitrogen cycle (WMO, 2016; Davidson, 2009). Since agriculturally managed soils emit 4.3-5.8 Tg N_2O yr^{-1}, developing effective mitigation strategies is a key challenge for the agricultural sector (Butterbach-Bahl *et al.*, 2013). As N_2O is a long lived greenhouse gas with an atmospheric half life time of 114 years elevated atmospheric N_2O concentrations will be a long term issue (IPCC, 2013). N_2O in soils is mainly produced by microbially mediated nitrogen transformations and the process of denitrification is widely recognized as the single most important N_2O producing process. Denitrification describes the stepwise reduction of nitrate (NO_3^-) to dinitrogen (N_2) and occurs under oxygen limited conditions especially after fertilization increased the bioavailability of nitrogen compounds (Davidson, 2009). Within denitrification N_2O is produced as an obligatory intermediate. The last step in denitrification, i.e. the reduction of N_2O to N_2, presents the only known biological sink for N_2O. The enzyme performing this last reduction step is encoded by the functional gene *nosZ* (Philippot et al., 2007). The functionality of this enzyme is highly sensitive to oxygen and pH (Liu *et al.*, 2014; Zumft and Kroneck, 2006). Therefore the last step in denitrification is often impaired and N_2O is produced as the dominating denitrification end product in agricultural soils.

Since denitrification is a modular process and not all microbes involved possess the whole set of denitrification genes (Graf *et al.*, 2014), the community composition of denitrifyers and especially N_2O reducers is an important factor which can influence N_2O emissions (Jones *et al.*, 2014; Philippot *et al.*, 2011). While some denitrifyers lack *nosZ* und thus the genetic capability to reduce N_2O (Philippot *et al.*, 2011), other N_2O reducers lack antecedent denitrification genes and thus act as sink for N_2O. This especially applies for the recently described atypical *nosZ* gene in which ~50% of theaffiliated taxa lack the genetic capability to produce N_2O (Graf *et al.*, 2014; Jones *et al.*, 2013). Recently it was shown that agricultural practice can change the diversity of N_2O reducers and affect dominating denitrification end product (Domeignoz-Horta *et al.*, 2015). Identifying and developing agricultural practice that fosters exclusive N_2O reducers presents an important step towards climate smart agriculture.

Biochar is a carbon-rich product of incomplete combustion that is added to the soil to improve soil quality and enhance carbon sequestration (Verheijen *et al.*, 2010). Although the physicochemical properties of biochar largely depend on the feedstock and production temperature

the majority of biochar share some common characteristics like alkaline pH, high surface area and stable aromatic carbon structures (Atkinson *et al.*, 2010; Mandal *et al.*, 2016). Due to these physicochemical properties the amendment of biochar to agriculturally managed soils is discussed as management tool to address environmental issues associated with current agricultural practice (Singh *et al.*, 2010; Sohi *et al.*, 2010; Lehmann and Joseph, 2009; Woolf *et al.*, 2010; Mandal *et al.*, 2016). Biochar amendment is also discussed as management option to mitigate N_2O emission since decreased N_2O emissions were reported from a range of field and incubation experiments (Cayuela *et al.*, 2013a). Several parameters like biochar feedstock, soil texture and the chemical form of N fertilizer were found to influence the extend of N_2O mitigation due to biochar addition, but the key mechanism causing decreased N_2O emissions is still poorly understood (Cayuela *et al.*, 2013a; Cayuela *et al.*, 2013b; Clough *et al.*, 2013). Some authors suggest increased pH due to biochar addition to be the main driver in lowering soil derived N_2O emissions (van Zwieten *et al.*, 2010; Zheng *et al.*, 2012) as there is evidence that elevated soil pH fosters N_2O reduction (Cuhel *et al.*, 2010; Simek *et al.*, 2002). Furthermore, it was suggested that reduced N_2O emission after biochar addition was caused by increased activity of *nosZ* bearing denitrifyers and a shift in denitrifier community composition (Harter *et al.*, 2014; Van Zwieten *et al.*, 2014).

There is evidence that biochar addition can affect the microbial community composition as assessed by molecular fingerprint techniques (Anderson *et al.*, 2011; Chen *et al.*, 2013). Recent studies using next generation sequencing approaches targeting the 16SrRNa gene also revealed an increase of bacterial taxa involved in nitrogen transformation and degradation of complex organic structures (Kolton *et al.*, 2011; Chen *et al.*, 2015). While the potential of biochar amendment to shape the general microbial community composition seems to be evident, to our knowledge there is only one study that investigated the impact of biochar on the community composition of N_2O reducing bacteria. Harter *et al.* (2016) reported increased relative abundances of typical *nosZ* bearing bacteria capable of complete denitrification and atypical *nosZ* bearing bacteria restricted to N_2O reduction due to biochar addition in a short term incubation experiment that showed reduced N_2O emissions after biochar amendment (Harter *et al.*, 2014). Biochar mediating microbial electron transfer (Kappler *et al.*, 2014) might increase microsites suitable for complete denitrification by local anoxia. But also increased sorption of N_2O (Cornelissen *et al.*, 2013) and NO_3^- or pH induced enhancement of N_2O reduction cannot be excluded as undelaying mechanisms driving changes in community composition of N_2O reducers (Clough *et al.*, 2013; Hagemann *et al.*, 2016). The effect of biochar amendment on N_2O emissions under field condition generally seems less pronounced compared to incubation experiments (Cayuela *et al.*, 2013b). We therefore wanted to investigate

whether biochar induced a shift in community composition N_2O reducers under field conditions and link this to biochar's potential to reduce N_2O emissions. We assessed size and taxonomic composition of N_2O reducers over a whole vegetation period on a biochar field trial that was connected to a N_2O measuring campaign (Hüppi *et al.*, 2015).By including a liming treatment we aimed at assessing the liming effect of the biochar independently.

Material and Methods

Sampling site

The experiment was established on a cropland at the Agroscope research station in Zürich (Switzerland) on Eutric Mollic Gleysol in January 2014. The type of soil is a clay loam with a particle size distribution of 37% sand, 27% silt and 36% clay. In the year before the experiment the field was under conventional management with *Zea mays*. Mean annual rainfall and air temperature from 1981-2010 was 1054 mm and 9.4°C.

Experimental setup

In January 2014 20 t ha^{-1} biochar were added to the respective microplots. Freshly applied biochar was worked into the top 1-3 cm of the soil. Basic properties of the biochar are listed in Table 1. In order to investigate the liming effect of biochar an additional treatment was established by adding 5 t ha^{-1} limestone. Greenhouse gas measurement with an automated closed chamber system started at 14th of February and lasted till harvest of *Zea mays* in October. Details of N_2O quantification and pH adjustment in the lime and biochar treatments are described elsewhere (Hüppi *et al.*, 2015). Harrowing of the field plots was done at 31th of March. For the first and third N fertilization of *Zea mays* N-fertilizer (LONZA-Ammonsalpeter; 27.5% N) at a rate of 40 kg ha^{-1} was used on May 26th and July 16th. The second N fertilization was carried out on June 16th using NH_4NO_3 at a rate of 80 kg ha^{-1}. The harvest took place on 13th of October. In order to assess community structure of N_2O reducers the first set of soil samples from each microplot was taken after “harrowing”. A N_2O emission event occurred 9 days after the second fertilization straight after a rain event. Therefore the second sampling date labeled as “fertilization” refers to soil sampling at the 25th of June 2014 (Supplementary Figure S1). The third soil sampling was performed at the end of the cropping period after “harvest”. For DNA analysis a homogenized composite soil sample was taken from each microplot, immediately frozen in liquid nitrogen and stored at -80 °C till further processing.

Molecular biological methods

DNA extraction and quantification of functional genes

DNA was extracted via phenol chloroform extraction as described in Griffiths *et al.* (2010). Before beat beating 9.1^9 copies of a linearized plasmid (pJET1.2, CloneJET PCR Cloning Kit, Thermo Scientific, Waltham, MA) carrying a fragment of cassava mosaic virus (APA9, gene accession Nr. AJ427910) were added to the soil samples in order to assess DNA recovery rates for each sample (Thonar *et al.*, 2012). DNA yield were assessed fluorometrically with Qubit 2.0 (Qubit dsDNA HS Assay Kit and Qubit

RNA HS Assay Kit, Invitrogen, Carlsbad, CA, USA) directly after extraction and ranged from 21.7 to 60 ng ul^{-1}. DNA extracts were further purified using OneStep ™ PCR Inhibitor Removal Kit (D6030, Zymo Research, Irvine, USA). Analysis of functional genes on a RNA level was attempted but failed due to extremely inconsistent yields which were most likely caused by field heterogeneity and the influence of daily temperature fluctuations. Quantitative PCR of functional genes was performed using KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, Wilmington, MA) and a Rotor-Gene-Q (QIAGEN, Venlo, Netherlands). Each 10 µl reaction volume included ~1 ng of template DNA. Primers and thermal protocols used for functional gene quantification are listed in Table S1 and S2. Standard curves were constructed by running a serial dilution with concentration ranging from ~10^8 to ~10^2 gene copy numbers per reaction of a plasmid bearing a copy of the respective gene. Specifications of vector plasmids and host genes are given in Table S3. Concentrations of standards were measured fluorometrically with Qubit 2.0 (Qubit dsDNA HS Assay Kit, Invitrogen, Carlsbad, CA). Each reaction was performed in analytical duplicates and repeated if Ct values differed more than 0.5. Efficiencies of qPCR yielded 86-90% for APA9 gene fragment, 79-84% for typical *nosZ* and 77-78 % for atypical *nosZ* assays. Specificity of the amplification was checked by meltcurve and agarose gel analysis. Raw data was analyzed via LinReg PCR by assessing enzyme kinetics for each reaction individually (Ramakers *et al.*, 2003). Additional to normalization of functional gene abundances per soil g dry weight, DNA extraction efficiencies obtained by APA9 quantification were used to correct functional gene copy numbers. Calculated extraction efficiencies ranged 34.03 % to 95.5% without treatment specific bias. Details on primers, master mixes and source of qCPR standards can be found in Supplementary Tables S1-3.

Illumina amplicon sequencing

DNA extracts were amplified for Illumina sequencing using a two-step PCR procedure. In the first step the products of three independent PCRs were pooled and purified using DNA Clean & Concentrator ™ (D4033, Zymo Research, Irvine, USA). Primer, master mixes and thermal cycling conditions for typical *nosZ* and atypical *nosZ* are shown in Supplementary Table S4. Obtained purified products were checked via Agarose Gel analysis. Subsequent library preparation and sequencing were performed at Genome Quebec Innovation Center (Montreal, Canada) according to the amplicon sequencing guidelines given by Illumina (San Diego, CA, USA). Sequencing was performed on an Illumina MiSeq® sequencing system using the 2x 250 bp providing MiSeq® Reagent Kit v2 (Illumina, San Diego, CA, USA). Primer sequences of the obtained raw sequences were removed using Cutadapt v1.9 (Martin, 2011). Given the length of the fragments of ca 700bp reads could not be merged and forward reads

were used for further processing due to higher phred scores. Reads were quality filtered using USEARCH fastq_filter function with a maximum error threshold of 1%. Chimeric sequences were removed using the uchime_denovo algorithm implemented in USEARCH (Edgar *et al.*, 2011). Quality filtering yielded in a mean of 57206 ±9594 and 27473±6997 high quality reads per sample for typical and atypical *nosZ* genes, respectively. No treatment specific bias could be detected. Quality filtered sequences for typical and atypical *nosZ* were translated to protein sequences and mapped against the NCBI Reference Sequence protein database (RefSeq) using DIAMOND (Buchfink *et al.*, 2015) in blastx mode with a minimum protein sequence identity cutoff of 70% and an e-value cutoff of 10^{-10}. 98.7 ±0.4% and 97.9 ±0.7% of the reads matched KEGG database entries assigned to the typical and atypical nitrous oxide reductases, respectively. Database hits were used for further analysis using the Lowest Common Ancestor (LCA) algorithm implemented in MEGAN6 Ultimate Edition (Huson *et al.*, 2016). The LCA analysis parameters "Top percent" and "Min Support" were set to 0.5% and 15. This implies that all hits within the top 0.5% of the best bit score are used for further analysis and a minimum of 15 reads is needed for each taxa to be considered. Given the basic principle of the LCA algorithm sequences that are conserved among different species were assigned to taxa of higher rank (Huson *et al.*, 2007). On average 50527 ±8515 and 22346 ±5771 typical and atypical *nosZ* sequences were assigned on a species level and used for further statistical analysis. It needs to be noted that a direct prove that a given sequence is associated to a specific microbial taxon is hard to obtain. Therefore specific species names mentioned need to be treated with caution and whenever species names are mentioned we refer to microbes that contain typical and atypical *nosZ* genes that are closely related to the respective gene of the named species.

Statistical analysis

Community composition of typical and atypical *nosZ* genes was analyzed using R version 3.3.1 (R Core Team, 2013) (Supplementary Figure S2). Permutational multivariate analysis of variance (Permanova) was performed using the adonis command in the "vegan" package in order to assess significance of treatment and sampling date on community composition of typical and atypical N_2O reducers. Additionally, the strictly non-parametric analysis of similarities (Anosim) which can only test for one factor was used as a measure of group separation using the anosim function. Anosim and Permanova were performed with 10^4 permutations. Community composition of typical and atypical *nosZ* gene sequences was visualized via Principal Coordinate Analysis (PCoA) using Bray Curtis distances. Indicator species associated with one or two specific sampling dates were assessed using the

multipatt function of the “indicspecies” package. The same command was used to assess indicator species associated with one or two treatments at specific sampling dates.

Results

N_2O emissions and basic soil parameters

Basic biochar and soil properties are listed in Table 1. As described in detail in (Hüppi *et al.*, 2015), liming treatment and biochar addition resulted in an elevated soil pH of 0.4 pH units on average. The pH fluctuated across the vegetation period but was lowest in the control treatment through the whole vegetation period. Bulk density and soil mineral N content did not statistically differ between treatments throughout the experimental period. Cumulative N_2O emissions across the vegetation period in the biochar treatment were 52% lower compared the control. Due to large variabilities in the lime treatment a significant effect on N_2O emissions was missing (Figure 1).

Table 1: Basic biochar and soil properties as described in Hüppi *et al.* (2015)

parameter	biochar	soil
pH	9.8	6.3
C/N ratio	94.0	9.0
C_{org} (%)	62.1	-
BET surface (m^2 g^{-1})	148.0	-
Ash content (%)	20.0	-
O/C ratio	0.11	-
H/C ratio	0.33	-
Bulk density (g cm^3)	-	1.3

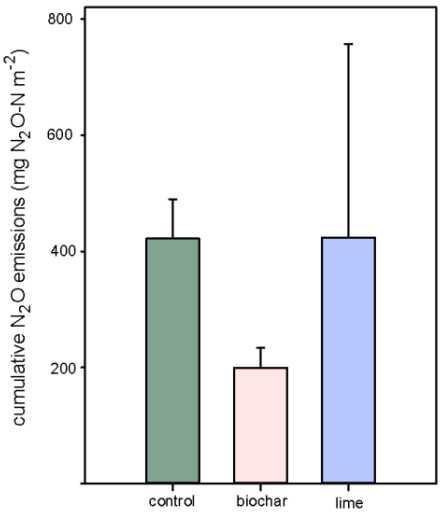


Figure 1: Cumulated N_2O emissions in control, biochar and lime treatment for the whole vegetation period (data from Hüppi *et al.* (2015))

Community size of typical and atypical *nosZ* bearing bacteria

Typical *nosZ* gene abundances did not show any significant treatment effects at the beginning of the vegetation period after harrowing. One week after fertilization typical *nosZ* gene abundances in the control and especially biochar treatment increased yielding significantly highest *nosZ* abundances in the biochar treatment (biochar: $3.09*10^8 \pm 3.51*10^7$, control $1.84*10^8 \pm 3.17*10^7$ and lime $1.03*10^8 \pm 3.54*10^7$). Before harvest typical *nosZ* gene abundances in all treatments declined almost below initial levels and no treatment effects could be detected (Figure 2a). Also abundances of the atypical *nosZ* gene did not show any treatment effect after harrowing. Significantly increased gene copy numbers of atypical *nosZ* gene were found in the biochar treatment compared to the lime treatment after fertilization (biochar: $8.82*10^8 \pm 1.53*10^8$, control $7.06*10^8 \pm 1.14*10^7$ and lime $4.79*10^8 \pm 7.21*10^7$). In comparison to harrowing only the addition of biochar yielded in increased atypical *nosZ* gene copy numbers after fertilization. At the end of the vegetation period lowest levels of atypical *nosZ* gene abundances were found with treatments not showing any significant effect on the size of atypical *nosZ* bearing bacterial community (Figure 2b).

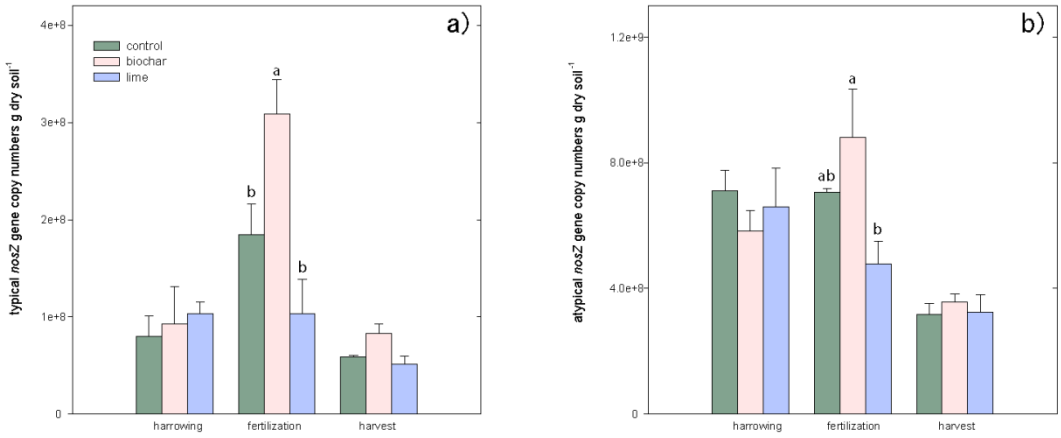


Figure 2: Gene copy numbers per g dry soil of the typical (a) and atypical (b) nitrous oxide reductases after sowing, fertilization and harvest of *Zea mays*.

Community composition of typical and atypical *nosZ* bearing bacteria

Permanova and Anosim showed a significant effect of sampling date on community composition of typical and atypical *nosZ* bearing bacteria (Table 2). Also the factor parcel, as measure of spatial heterogeneity, significantly introduced variance in community composition of atypical N_2O reducers. For typical *nosZ* gene sequences this effect was only significant according for Permanova while

according to Anosim this relationship was a trend (Table 2). An effect of lime and biochar amendment across all sampling dates was observed for atypical *nosZ* bearing only (Table 2).

Table 2: Significant levels for factors explaining the variance in dissimilarity matrices of typical and atypical nosZ bearing bacterial community as analyzed by Permanova and Anosim.

	typical *nosZ*		atypical *nosZ*	
Factors	Permanova	Anosim	Permanova	Anosim
Sampling date	<0.001***	<0.001***	0.012*	0.039*
Treatment	0.114^{ns}	0.677^{ns}	0.003**	0.008**
Parcel	0.002**	0.059^{ns}	<0.00.1***	<0.001***
Sampling date * treatment	0.319^{ns}		0.690^{ns}	

Taxa indicative for one or two sampling dates and showing a relative share above 0.5% are displayed in Figure 3. For the typical *nosZ* gene relative abundance of *Paracoccus denitrificans* increased while *Sinorhizobium fredii*, *Methylobacterium sp. 4-46* and *Hyphomicrobium denitrificans* decreased in harvest compared to harrowing and fertilization. The abundance of *Thiobacillus denitrificans* and *Pseudogulbenkiania sp. NH8B* was significantly highest after fertilization compared to harrowing and harvest. The abundance of *Bradyrhizobium diazoefficiens* decreased from harrowing to fertilization but recovered again and showed highest relative abundance at harvest (Figure 3a). For atypical *nosZ* the relative share of *Opitutus terrae and Ignavibacterium album* was significantly decreased at harvest compared to harrowing and fertilization while the opposite effect was observed for *Niastella koreensis, Haliscomenobacter hydrossis, Gemantimonas aurantica* and *Caldinella aerophila* (Figure 3b).

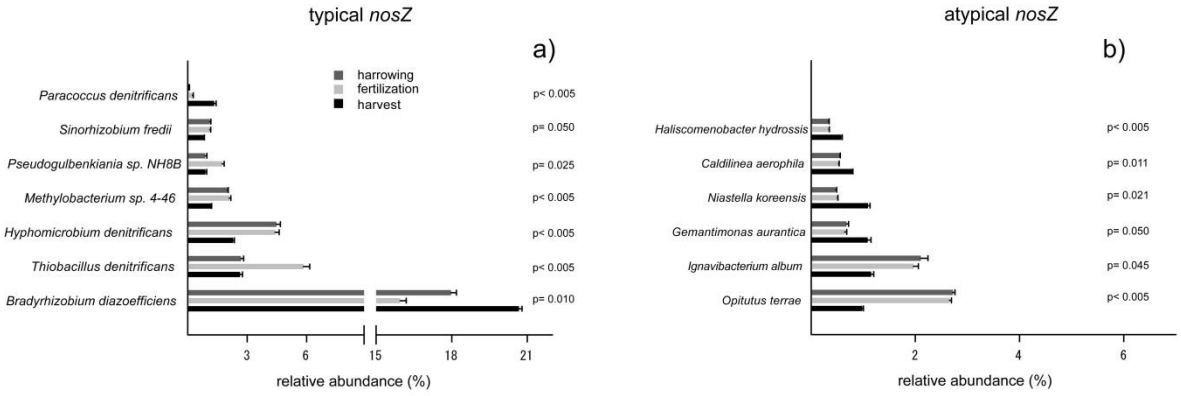


Figure 3: Relative abundances of read affiliated taxa at the soil sampling dates “harrowing”, “fertilization” and “harvest” across all treatments. Taxa were identified using the multipatt function in the indicspecies package of R. Only taxa with a mean relative share above 0.5 % that differ significantly in between sampling dates are displayed.

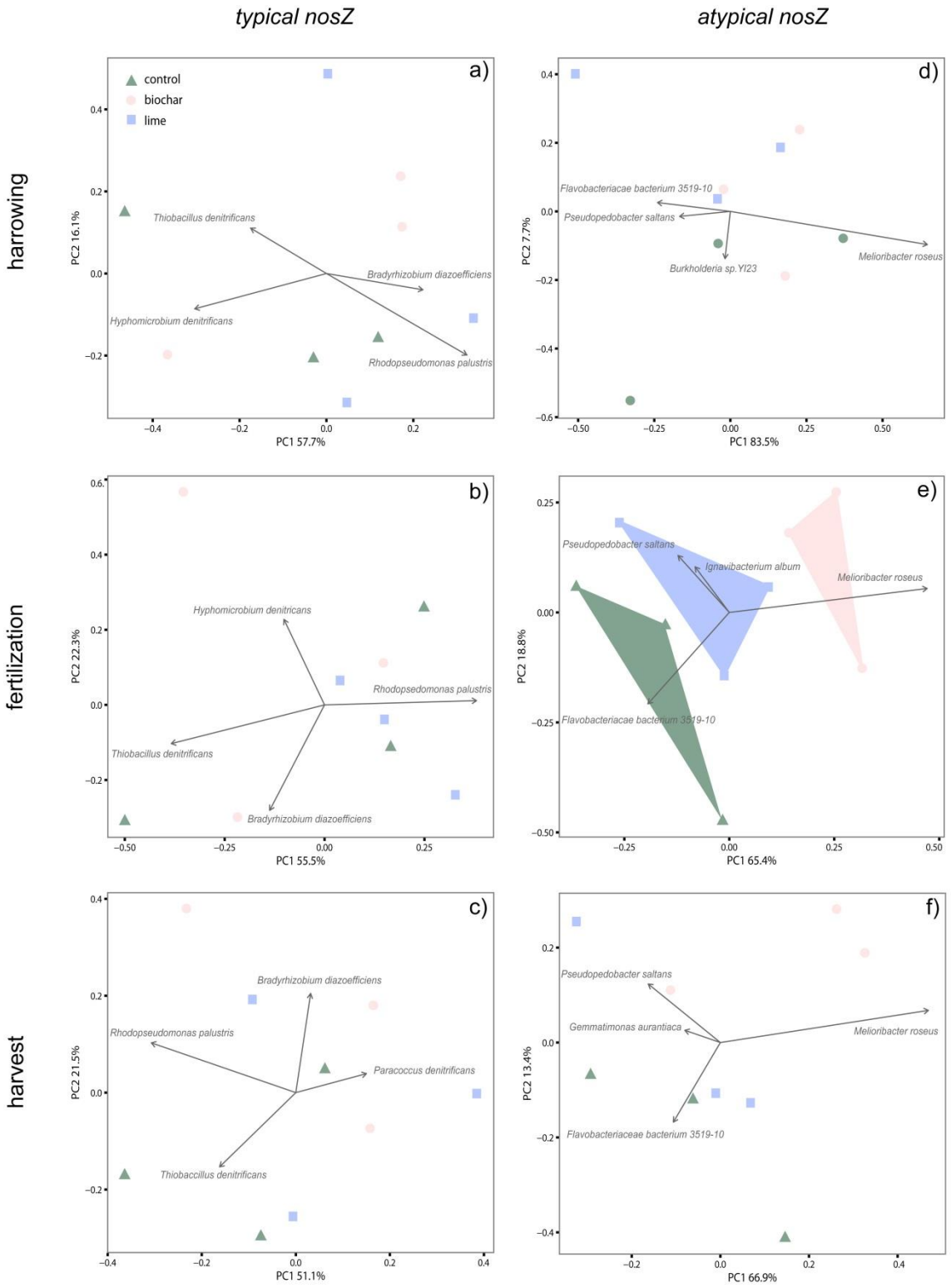


Figure 4: Principal coordinate analysis (PCoA) plot based on the genetic diversity of typical (a)-c)) and atypical (d)-f)) nitrous oxide reductase after sowing (a) and d)), fertilization (b) and e)) and harvest (c) and f)) of *Zea mays* in the control, biochar and lime treatment. The PCoA was calculated using Bray-Curtis dissimilarity matrix based on the relative share of assigned gene sequences in the respective soil samples. Biplots also show the four most abundant gene sequences affecting ordination and the assigned taxa. Data points of a treatment were connected when Permanova showed a significant treatment effect.

Assessing the treatment effect on community composition of typical and atypical *nosZ* gene sequences at specific sampling dates revealed no clear clustering of typical *nosZ* bearing bacteria throughout the vegetation period (Figure 4). The same was true for the atypical *nosZ* gene sequences after harrowing. After fertilization atypical *nosZ* gene sequences showed treatment specific clusters and analysis of variance via permanova revealed a significant treatment effect (p=0.04). At harvest a similar clustering was observed but lacked statistical significance. At fertilization and harvest the samples of the lime treatment clustered in between the control and biochar treatment (Figure 4e and 4f). At fertilization, when a significant treatment effect was observed, atypical *nosZ* sequences assigned to *Anaeromyxobater sp. Fw109-5* and *Flavobacteriaceae bacterium 3519-10* were indicative for the control and lime treatment. On the contrary, a significant increase of the atypical *nosZ* gene affiliated with *Melioribacter roseus* was detected in the biochar treatment (Figure 5).

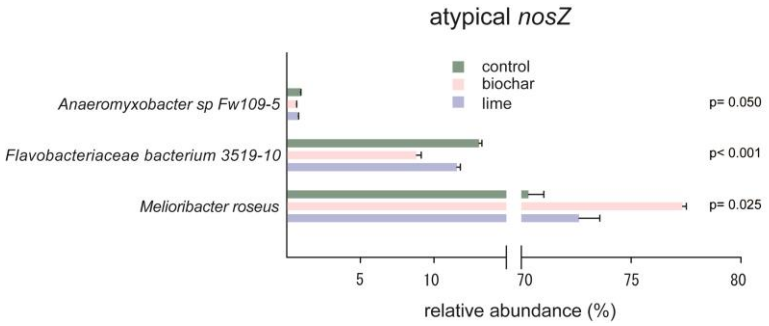


Figure 5: Relative abundances of atypical *nosZ* read affiliated taxa in the control, biochar and lime treatments at fertilization. Taxa were identified using the multipatt function in the indicspecies package of R. Only taxa with a mean relative share above 0.5 % are displayed.

Discussion

As described in detail in Hüppi *et al* (2015), biochar addition decreased N_2O emission by 52% compared to the control. This coincides very well with the meta-analysis conducted by Cayuela *et al.* (2013b) that showed biochar addition to lower N_2O emissions by an average of 54% across 30 incubation and field studies. Yet, it should not be neglected that field date generally showed a less pronounced impact on N_2O emissions (Cayuela *et al.*, 2013b) and there are other field studies lacking significant reductions in N_2O emissions after biochar addition (Scheer *et al.*, 2011; Verhoeven and Six, 2014). There is a growing body of studies that showed biochar's potential to decrease N_2O emissions by influencing abundance and/or activity of N_2O reducing bacteria (Harter *et al.*, 2014; Van Zwieten *et al.*, 2014; Liu *et al.*, 2017; Xu *et al.*, 2014). Yet, the only study investigating N_2O fluxes and abundances of typical *nosZ* bearing bacteria at the beginning and the end of a on an arable field experiment over a whole vegetation period did not find an influence of biochar amendment neither on N_2O emissions nor on the size of the N_2O reducing microbial community (Dicke *et al.*, 2015). In our case, biochar amendment increased abundance of typical and partly atypical *nosZ* bearing bacteria after fertilization when high N availability and water contents favored denitrification (Hüppi *et al.,* 2015). In the study of Dicke *et al.* (2015) generally low N_2O emissions together with low water contents favoring nitrification as major N_2O producing pathway might have hampered differentiation of N_2O emissions and *nosZ* gene abundances in between treatments.

In contrast to the atypical N_2O reducers, typical *nosZ* bearing bacteria are more likely to possess antecedent denitrification genes (Graf *et al.*, 2014) and thus have the genetic capability to process NO_3^- and other intermediate products of denitrification. Therefore it seems plausible that abundance of typical *nosZ* bearing bacteria showed a greater response to N-fertilization compared to atypical *nosZ* bearing bacteria (Figure 2). Biochar amendment significantly increases the abundance of this functional guild which further indicates enhanced denitrification after N addition in this treatment as already suggested by Xu *et al.* (2014) and Castaldi *et al.* (2011) .

Yet, not only size and activity of the N_2O reducing microbial community but also community structure was reported to affect N_2O reduction (Domeignoz-Horta *et al.*, 2015). There are strong indications that agricultural practice has the potential to shape diversity of N_2O reducers as it was shown for residue management in a long term field trial (Bent *et al.*, 2016). Especially the diversity of the atypical *nosZ* bearing bacteria was shown to have a strong influence on denitrification end products and determine N_2O sink capacity of soils (Jones *et al.*, 2014). Furthermore, inoculation with the atypical *nosZ* bearing and non-denitrifying strain *Dyadobacter fermentans* was shown to

significantly reducing N_2O emissions in 4 out of 11 soil as assessed by an incubation experiment (Domeignoz-Horta *et al.*, 2016). There is growing evidence that managing community structure of N_2O reducers might be a useful tool in mitigating soil derived N_2O emissions.

In our case sampling time points had a greater impact on typical and atypical nosZ bearing bacteria compared to the treatment effect. This seems plausible considering variations in N-availability, soil temperature and water contents in between sampling dates (Hüppi *et al.,* 2015).The fact that the only significant treatment effect was observed after fertilization seems plausible since provision of N lead to an increased formation of N_2O (Supplementary Figure S1*).* Anderson *et al.* (2014) found seasonal changes to be the major driver of the general bacterial community composition in a biochar field trial. The authors reported that the addition of biochar did not have any significant impact on bacterial community composition. Still, *nosZ* abundance increased through the course of the experiment (Anderson *et al.*, 2014).

Increased relative abundance of *Thiobacillus denitrificans* after fertilization can be explained by high N availability and water contents since this facultative anaerobic bacterium is known for removing excess NO_3^- coupled to sulfide oxidation under anoxic conditions (Shao *et al.*, 2010). Also *Pseudogulbenkiania sp. NH8B* significantly increased in relative abundance. This strain was first isolated from rice paddy soil, is known for its strong denitrifying activity and its ability to perform N_2O reduction (Ishii *et al.*, 2011). In contrast, the slow growing and effective N fixing bacteria *Bradyrhizobium diazoefficiens* (Delamuta *et al.*, 2013) decreased in relative abundance at fertilization when N was easily available. Decreased abundance of *Methylobacterium sp4-46* and *Sinorhizobium fredii* after harvest might be due to removed host plants since both taxa are known for their ability to form symbiosis with legumes which have been present in form of weeds before harvest. After harvest the metabolic versatile taxa of *Paracoccus denitrificans* increased in relative abundance (Baker *et al.*, 1998). A differentiation of taxa at harvest was also seen for the atypical *nosZ* bearing taxa which seemed to driven by soil aeration status. The obligatory anaerobe, slow growing taxa *Opitutus terrae* decreased in relative abundance possibly due to low water contents (Chin *et al.*, 2001). Similarly, relative abundance of the anaerobic bacteria *Ignavibacterium album* also significantly decreased at harvest (Iino *et al.*, 2010). The strictly aerobic bacteria *Niastella korensis*, *Haliscomenobacter hydrossis and* Gemmatimonas aurantiaca as well as the facultative aerobic bacteria *Caldinelinea aerophila* were associated with sampling date at harvest (Zhang *et al.*, 2003; Sekiguchi *et al.*, 2003; Daligault *et al.*, 2011; Weon *et al.*, 2006). Although a range of atypical *nosZ* bearing bacteria was affected by sampling date the relative abundance of each species did not exceed 3%. PCoA analysis showed also other species which were not associated to any sampling date

like *Melioribacter roseus*, Pedobacter saltans and *Flavobacteriaceae bacterium 3519-10* to contribute to ordination of soil samples (Figure 5 b),d) and f)).

Melioribacter roseus which was identified as indicator species for the biochar treatment at fertilization lacks antecedent denitrification genes and might thus act as sink for N_2O (Kadnikov *et al.*, 2013). In contrast *Flavobacteriaceae bacterium 3519-10* and *Anaeromyxobacter sp. Fw 109-5*, which were associated with lime and control treatment, possess the whole set of denitrification genes (Graf *et al.*, 2014). This observation goes in line with the hypothesis of Hagemann *et al.* (2016) who suggested promotion of complete denitrifyers and N_2O reducers relying on external N_2O to be a major driver for N_2O mitigation after biochar addition. Harter et al. (2016) observed this effect in an incubation experiment and similarly to our experiment relative abundance of *Flavobacteriaceae bacterium 3519-10* was significantly decreased in the biochar treatment. Yet, *Pseudopedobacter saltans* which was one of the most abundant atypical nosZ bearing bacteria and associated with the biochar treatment in Harter et al.(2016) was much lower in relative abundance (up to 5%) and did not show any significant treatment effect in our experiment. Unlike Harter et al. (2016) we also could not detect any significant treatment effects in the typical *nosZ* bearing bacteria and less taxa responded to biochar as soil amendment. Given the fact that our experiment was conducted under real field conditions it seems plausible that effects were less pronounced and partly superimposed by spatial heterogeneities. Despite dissimilarities in taxa responding to biochar addition we also found indications for the same functional pattern since *Melioribacter roseus* and *Pseudopedobacter saltans* both rely on extern N_2O. Furthermore the increased abundance of N_2O reducers lacking antecedent denitrification genes due to biochar addition was a community specific effect (Supplementary Figure S3). Response of different taxa to biochar addition seems attributable to the soil inherent difference in community composition of atypical *nosZ* bearing bacteria. Since indicator species at fertilization were either associated with the lime and control treatment or the biochar treatment it seems unlikely that changes in community composition can be attributable to the liming effect of biochar only. Since lime treatment at fertilization was clustered between the control and biochar treatment other mechanism seem to contribute to differentiation of community composition of atypical *nosZ* bearing bacteria. Cornelissen *et al.* (2013) proved the general potential of N_2O sorption onto biochar. Increased retention time of N_2O in soils due to biochar addition seem one possible mechanism which could explain promotion of specialized N_2O reducers.

Although decreased N_2O emissions and a differentiation in atypical *nosZ* bearing bacteria after fertilization in the first year after biochar application was observed we don`t know whether this effect holds true for subsequent vegetation periods. The fading effect on atypical *nosZ* bearing

bacteria at harvest might be due to decreased N turnover. But also the transient nature of the biochar might have decreased functional impact on N_2O reducers and N_2O emission (Quilliam *et al.*, 2012). To solve this open question long term field experiments for more than one year investigating the impact of biochar on N_2O emission are needed.

Acknowledgements

We would like to thank the SNF for the financial support in frame of the National Research Program 68 “Soil as a Resource”. We also acknowledge the contribution of scientists at the McGill University and Génome Québec Innovation Center in Montréal, Canada, for the paired-end sequencing on Illumina MiSeq. The authors declare to have no conflict of interest.

References

Anderson, C. R., Condron, L. M., Clough, T. J., Fiers, M., Stewart, A., Hill, R. A. & Sherlock, R. R. (2011). Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* 54(5-6): 309-320.http://dx.doi.org/10.1016/j.pedobi.2011.07.005

Anderson, C. R., Hamonts, K., Clough, T. J. & Condron, L. M. (2014). Biochar does not affect soil N-transformations or microbial community structure under ruminant urine patches but does alter relative proportions of nitrogen cycling bacteria. *Agriculture, Ecosystems & Environment* (0).http://dx.doi.org/10.1016/j.agee.2014.02.021

Atkinson, C. J., Fitzgerald, J. D. & Hipps, N. A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil* 337(1-2): 1-18. http://dx.doi.org/10.1007/s11104-010-0464-5

Baker, S. C., Ferguson, S. J., Ludwig, B., Page, M. D., Richter, O. M. & van Spanning, R. J. (1998). Molecular genetics of the genus Paracoccus: metabolically versatile bacteria with bioenergetic flexibility. *Microbiol Mol Biol Rev* 62(4): 1046-1078

Bent, E., Németh, D., Wagner-Riddle, C. & Dunfield, K. (2016). Residue management leading to higher field-scale N2O flux is associated with different soil bacterial nitrifier and denitrifier gene community structures. *Applied Soil Ecology* 108: 288-299.http://dx.doi.org/10.1016/j.apsoil.2016.09.008

Buchfink, B., Xie, C. & Huson, D. H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nature Methods* 12(1): 59-60.http://dx.doi.org/10.1038/nmeth.3176

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society* 368(1621). http://dx.doi.org/10.1098/rstb.2013.0122

Castaldi, S., Riondino, M., Baronti, S., Esposito, F. R., Marzaioli, R., Rutigliano, F. A., Vaccari, F. P. & Miglietta, F. (2011). Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere* 85(9): 1464-1471.http://dx.doi.org/10.1016/j.chemosphere.2011.08.031

Cayuela, M. L., Sánchez-Monedero, M. A., Roig, A., Hanley, K., Enders, A. & Lehmann, J. (2013a). Biochar and denitrification in soils: when, how much and why does biochar reduce N2O emissions? *Sci. Rep.* 3.http://dx.doi.org/10.1038/srep01732

Cayuela, M. L., van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A. & Sánchez-Monedero, M. A. (2013b). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agriculture, Ecosystems & Environment* 191: 5-16.http://dx.doi.org/10.1016/j.agee.2013.10.009

Chen, J., Liu, X., Li, L., Zheng, J., Qu, J., Zheng, J., Zhang, X. & Pan, G. (2015). Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China. *Applied Soil Ecology* 91: 68-79.http://dx.doi.org/10.1016/j.apsoil.2015.02.012

Chen, J., Liu, X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J., Zhang, X., Wang, J. & Yu, X. (2013). Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Applied Soil Ecology* 71: 33-44.http://dx.doi.org/10.1016/j.apsoil.2013.05.003

Chin, K. J., Liesack, W. & Janssen, P. H. (2001). Opitutus terrae gen. nov., sp. nov., to accommodate novel strains of the division 'Verrucomicrobia' isolated from rice paddy soil. *International Journal of Systematic and Evolutionary Microbiology* 51(Pt 6): 1965-1968.http://dx.doi.org/10.1099/00207713-51-6-1965

Clough, T., Condron, L., Kammann, C. & Müller, C. (2013). A Review of Biochar and Soil Nitrogen Dynamics. *Agronomy* 3(2): 275.http://dx.doi.org/10.3390/agronomy3020275

Cornelissen, G., Rutherford, D. W., Arp, H. P. H., Dörsch, P., Kelly, C. N. & Rostad, C. E. (2013). Sorption of Pure N2O to Biochars and Other Organic and Inorganic Materials under Anhydrous Conditions. *Environmental Science & Technology* 47(14): 7704-7712. http://dx.doi.org/10.1021/es400676q

Cuhel, J., Simek, M., Laughlin, R. J., Bru, D., Cheneby, D., Watson, C. J. & Philippot, L. (2010). Insights into the Effect of Soil pH on N(2)O and N(2) Emissions and Denitrifier Community Size and Activity. *Applied and Environmental Microbiology* 76(6): 1870-1878.http://dx.doi.org/10.1128/Aem.02484-09

Daligault, H., Lapidus, A., Zeytun, A., Nolan, M., Lucas, S., Del Rio, T. G., Tice, H., Cheng, J.-F., Tapia, R., Han, C., Goodwin, L., Pitluck, S., Liolios, K., Pagani, I., Ivanova, N., Huntemann, M., Mavromatis, K., Mikhailova, N., Pati, A., Chen, A., Palaniappan, K., Land, M., Hauser, L., Brambilla, E.-M., Rohde, M., Verbarg, S., Göker, M., Bristow, J., Eisen, J. A., Markowitz, V., Hugenholtz, P., Kyrpides, N. C., Klenk, H.-P. & Woyke, T. (2011). Complete genome sequence of Haliscomenobacter hydrossis type strain (O(T)). *Standards in Genomic Sciences* 4(3): 352-360.http://dx.doi.org/10.4056/sigs.1964579

Davidson, E. A. (2009). The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geoscience* 2(9): 659-662.http://dx.doi.org/10.1038/ngeo608

Delamuta, J. R. M., Ribeiro, R. A., Ormeño-Orrillo, E., Melo, I. S., Martínez-Romero, E. & Hungria, M. (2013). Polyphasic evidence supporting the reclassification of Bradyrhizobium japonicum group Ia strains as Bradyrhizobiumdiazoefficiens sp. nov. *International Journal of Systematic and Evolutionary Microbiology* 63(9): 3342-3351.http://dx.doi.org/doi:10.1099/ijs.0.049130-0

Dicke, C., Andert, J., Ammon, C., Kern, J., Meyer-Aurich, A. & Kaupenjohann, M. (2015). Effects of different biochars and digestate on N2O fluxes under field conditions. *Science of The Total Environment* 524–525: 310-318.http://dx.doi.org/10.1016/j.scitotenv.2015.04.005

Domeignoz-Horta, L. A., Putz, M., Spor, A., Bru, D., Breuil, M. C., Hallin, S. & Philippot, L. (2016). Non-denitrifying nitrous oxide-reducing bacteria - An effective N2O sink in soil. *Soil Biology and Biochemistry* 103: 376-379.http://dx.doi.org/10.1016/j.soilbio.2016.09.010

Domeignoz-Horta, L. A., Spor, A., Bru, D., Breuil, M.-C., Bizouard, F., Léonard, J. & Philippot, L. (2015). The diversity of the N2O reducers matters for the N2O:N2 denitrification end-product ratio across an annual and a perennial cropping system. *Frontiers in Microbiology* 6: 971. http://dx.doi.org/10.3389/fmicb.2015.00971

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27(16): 2194-2200.http://dx.doi.org/10.1093/bioinformatics/btr381

Graf, D. R. H., Jones, C. M. & Hallin, S. (2014). Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N2O Emissions. *Plos One* 9(12): e114118. http://dx.doi.org/10.1371/journal.pone.0114118

Griffiths, R. I., Whiteley As Fau - O'Donnell, A. G., O'Donnell Ag Fau - Bailey, M. J. & Bailey, M. J. (2010). Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Applied and Environmental Microbiology* 66 (12)(0099-2240): 5488-5491

Hagemann, N., Harter, J. & Behrens, S. (2016).Chapter 7 - Elucidating the Impacts of Biochar Applications on Nitrogen Cycling Microbial Communities. In *Biochar Application*, 163-198: http://dx.doi.org/10.1016/B978-0-12-803433-0.00007-2.

Harter, J., Krause, H.-M., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., Kappler, A. & Behrens, S. (2014). Linking N2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. *ISME J*.http://dx.doi.org/10.1038/ismej.2013.160

Harter, J., Weigold, P., El-Hadidi, M., Huson, D. H., Kappler, A. & Behrens, S. (2016). Soil biochar amendment shapes the composition of N2O-reducing microbial communities. *Science of The Total Environment* 562: 379-390.http://dx.doi.org/10.1016/j.scitotenv.2016.03.220

Hüppi, R., Felber, R., Neftel, A., Six, J. & Leifeld, J. (2015). Effect of biochar and liming on soil nitrous oxide emissions from a temperate maize cropping system. *SOIL* 1(2): 707-717.http://dx.doi.org/10.5194/soil-1-707-2015

Huson, D., Auch, A., Qi, J. & Schuster, S. (2007). MEGAN analysis of metagenomic data. *Genome Res* 17(3): 377 - 386.http://dx.doi.org/10.1101/gr.5969107

Huson, D. H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.-J. & Tappu, R. (2016). MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. *PLoS Comput Biol* 12(6): e1004957.http://dx.doi.org/10.1371/journal.pcbi.1004957

Iino, T., Mori, K., Uchino, Y., Nakagawa, T., Harayama, S. & Suzuki, K.-i. (2010). Ignavibacterium album gen. nov., sp. nov., a moderately thermophilic anaerobic bacterium isolated from microbial mats at a terrestrial hot spring and proposal of Ignavibacteria classis nov., for a novel lineage at the periphery of green sulfur bacteria. *International Journal of Systematic and Evolutionary Microbiology* 60(6): 1376-1382.http://dx.doi.org/10.1099/ijs.0.012484-0

IPCC (2013).The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.

Ishii, S., Tago, K., Nishizawa, T., Oshima, K., Hattori, M. & Senoo, K. (2011). Complete Genome Sequence of the Denitrifying and N(2)O-Reducing Bacterium Pseudogulbenkiania sp. Strain NH8B. *Journal of Bacteriology* 193(22): 6395-6396.http://dx.doi.org/10.1128/JB.06127-11

Jones, C., Graf, D., Bru, D., Philippot, L. & Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *The ISME Journal* 7(2): 417-426. http://dx.doi.org/10.1038/ismej.2012.125

Jones, C. M., Spor, A., Brennan, F. P., Breuil, M.-C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S. & Philippot, L. (2014). Recently identified microbial guild mediates soil N2O sink capacity. *Nature Climate Change* 4: 801-805. http://dx.doi.org/10.1038/nclimate2301

Kadnikov, V. V., Mardanov, A. V., Podosokorskaya, O. A., Gavrilov, S. N., Kublanov, I. V., Beletsky, A. V., Bonch-Osmolovskaya, E. A. & Ravin, N. V. (2013). Genomic Analysis of Melioribacter roseus, Facultatively Anaerobic Organotrophic Bacterium Representing a Novel Deep Lineage within Bacteriodetes/Chlorobi Group. *Plos One* 8(1): e53047.http://dx.doi.org/10.1371/journal.pone.0053047

Kappler, A., Wuestner, M. L., Ruecker, A., Harter, J., Halama, M. & Behrens, S. (2014). Biochar as an Electron Shuttle between Bacteria and Fe(III) Minerals. *Environmental Science & Technology Letters* 1(8): 339-344. http://dx.doi.org/10.1021/ez5002209

Kolton, M., Harel, Y. M., Pasternak, Z., Graber, E. R., Elad, Y. & Cytryn, E. (2011). Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Applied Environmental Microbiology*: AEM.00148-00111.http://dx.doi.org/10.1128/aem.00148-11

Lehmann, J. & Joseph, S. (2009).Biochar for environmental management: An introduction. In *Biochar for environmental management, science and technology*, 1-12 (Eds J. Lehmann and S. Joseph). London: Earthscan.

Liu, B., Frostegard, A. & Bakken, L. R. (2014). Impaired reduction of N2O to N2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. *MBio* 5(3): 01383-01314.http://dx.doi.org/10.1128/mBio.01383-14

Liu, Q., Liu, B., Zhang, Y., Lin, Z., Zhu, T., Sun, R., Wang, X., Ma, J., Bei, Q., Liu, G., Lin, X. & Xie, Z. (2017). Can biochar alleviate soil compaction stress on wheat growth and mitigate soil N2O

emissions? *Soil Biology and Biochemistry* 104: 8-17.http://dx.doi.org/10.1016/j.soilbio.2016.10.006

Mandal, S., Sarkar, B., Bolan, N., Novak, J., Ok, Y. S., Van Zwieten, L., Singh, B. P., Kirkham, M. B., Choppala, G., Spokas, K. & Naidu, R. (2016). Designing advanced biochar products for maximizing greenhouse gas mitigation potential. *Critical Reviews in Environmental Science and Technology*: 1-35.http://dx.doi.org/10.1080/10643389.2016.1239975

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *2011* 17(1).http://dx.doi.org/10.14806/ej.17.1.200

Philippot, L., Andert, J., Jones, C. M., Bru, D. & Hallin, S. (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N2O emissions from soil. *Global Change Biology* 17(3): 1497-1504. http://dx.doi.org/10.1111/j.1365-2486.2010.02334.x

Philippot, L., Hallin, S. & Schloter, M. (2007).Ecology of Denitrifying Prokaryotes in Agricultural Soil. In *Advances in Agronomy*, Vol. Volume 96, 249-305 (Ed L. S. Donald). http://dx.doi.org/10.1016/S0065-2113(07)96003-4.

Quilliam, R. S., Marsden, K. A., Gertler, C., Rousk, J., DeLuca, T. H. & Jones, D. L. (2012). Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. *Agriculture, Ecosystems & Environment* 158(0): 192-199.http://dx.doi.org/10.1016/j.agee.2012.06.011

R Core Team (2013).R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Ramakers, C., Ruijter, J. M., Deprez, R. H. L. & Moorman, A. F. M. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* 339(1): 62-66.http://dx.doi.org/10.1016/S0304-3940(02)01423-4

Ravishankara, A. R., Daniel, J. S. & Portmann, R. W. (2009). Nitrous Oxide (N2O): The Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science* 326(5949): 123-125. http://dx.doi.org/10.1126/science.1176985

Scheer, C., Grace, P., Rowlings, D., Kimber, S. & Van Zwieten, L. (2011). Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. *Plant and Soil* 345(1): 47-58.http://dx.doi.org/10.1007/s11104-011-0759-1

Sekiguchi, Y., Yamada, T., Hanada, S., Ohashi, A., Harada, H. & Kamagata, Y. (2003). Anaerolinea thermophila gen. nov., sp. nov. and Caldilinea aerophila gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain Bacteria at the subphylum level. *International Journal of Systematic and Evolutionary Microbiology* 53(6): 1843-1851.http://dx.doi.org/10.1099/ijs.0.02699-0

Shao, M. F., Zhang, T. & Fang, H. H. (2010). Sulfur-driven autotrophic denitrification: diversity, biochemistry, and engineering applications. *Appl Microbiol Biotechnol* 88(5): 1027-1042.http://dx.doi.org/10.1007/s00253-010-2847-1

Simek, M., Jisova, L. & Hopkins, D. W. (2002). What is the so-called optimum pH for denitrification in soil? *Soil Biology & Biochemistry* 34(9): 1227-1234.http://dx.doi.org/10.1016/S0038-0717(02)00059-7

Singh, B. P., Hatton, B. J., Balwant, S., Cowie, A. L. & Kathuria, A. (2010). Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of Environmental Quality* 39(4): 1224-1235. http://dx.doi.org/10.2134/jeq2009.0138

Sohi, S. P., Krull, E., Lopez-Capel, E. & Bol, R. (2010). A Review of Biochar and Its Use and Function in Soil. *Advances in Agronomy, Vol 105* 105: 47-82.http://dx.doi.org/10.1016/S0065-2113(10)05002-9

Thonar, C., Erb, A. & Jansa, J. (2012). Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources* 12(2): 219-232.http://dx.doi.org/10.1111/j.1755-0998.2011.03086.x

van Zwieten, L., Kimber, S., Morris, S., Downie, A., Berger, E., Rust, J. & Scheer, C. (2010). Influence of biochars on flux of N2O and CO2 from Ferrosol. *Australian Journal of Soil Research* 48(6-7): 555-568.http://dx.doi.org/10.1071/Sr10004

Van Zwieten, L., Singh, B. P., Kimber, S. W. L., Murphy, D. V., Macdonald, L. M., Rust, J. & Morris, S. (2014). An incubation study investigating the mechanisms that impact N2O flux from soil following biochar application. *Agriculture, Ecosystems & Environment* 191: 53-63.http://dx.doi.org/10.1016/j.agee.2014.02.030

Verheijen, F., Jeffery, S., Bastos, A., van der Velde, M. & Diafas, I. (2010). Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg, 149pp.

Verhoeven, E. & Six, J. (2014). Biochar does not mitigate field-scale N2O emissions in a Northern California vineyard: An assessment across two years. *Agriculture, Ecosystems & Environment* 191: 27-38.http://dx.doi.org/10.1016/j.agee.2014.03.008

Weon, H. Y., Kim, B. Y., Yoo, S. H., Lee, S. Y., Kwon, S. W., Go, S. J. & Stackebrandt, E. (2006). Niastella koreensis gen. nov., sp. nov. and Niastella yeongjuensis sp. nov., novel members of the phylum Bacteroidetes, isolated from soil cultivated with Korean ginseng. *International Journal of Systematic and Evolutionary Microbiology* 56(Pt 8): 1777-1782.http://dx.doi.org/10.1099/ijs.0.64242-0

WMO (2016).Greenhouse Gas Bulletin - World Meterological Organization, http://www.wmo.int/gaw/.

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J. & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications* 1(5): 1-9. http://dx.doi.org/10.1038/ncomms1053

Xu, H.-J., Wang, X.-H., Li, H., Yao, H.-Y., Su, J.-Q. & Zhu, Y.-G. (2014). Biochar Impacts Soil Microbial Community Composition and Nitrogen Cycling in an Acidic Soil Planted with Rape. *Environmental Science & Technology* 48(16): 9391-9399.http://dx.doi.org/10.1021/es5021058

Zhang, H., Sekiguchi, Y., Hanada, S., Hugenholtz, P., Kim, H., Kamagata, Y. & Nakamura, K. (2003). Gemmatimonas aurantiaca gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum Gemmatimonadetes phyl. nov. *International Journal of Systematic and Evolutionary Microbiology* 53(4): 1155-1163.http://dx.doi.org/10.1099/ijs.0.02520-0

Zheng, J., Stewart, C. E. & Cotrufo, M. F. (2012). Biochar and nitrogen fertilizer alters soil nitrogen dynamics and greenhouse gas fluxes from two temperate soils. *J Environ Qual* 41(5): 1361-1370.http://dx.doi.org/10.2134/jeq2012.0019

Zumft, W. G. & Kroneck, P. M. H. (2006). Respiratory Transformation of Nitrous Oxide (N2O) to Dinitrogen by Bacteria and Archaea. *Advances in Microbial Physiology* Volume 52: 107-227.http://dx.doi.org/10.1016/S0065-2911(06)52003-X

Supplementary Information

Biochar and lime addition affect community composition of nitrous oxide reducers under the cultivation of *Zea mays*

Hans-Martin Krause[1], Roman Hüppi[2], Jens Leifeld[2], Mohamed El-Hadidi[3], Johannes Harter[4], Andreas Kappler[4], Martin Hartmann[5], Sebastian Behrens[6] Paul Mäder[1], Andreas Gattinger[1]

[1]Soil Sciences, Research Institute of Organic Agriculture (Fibl), Frick, Switzerland

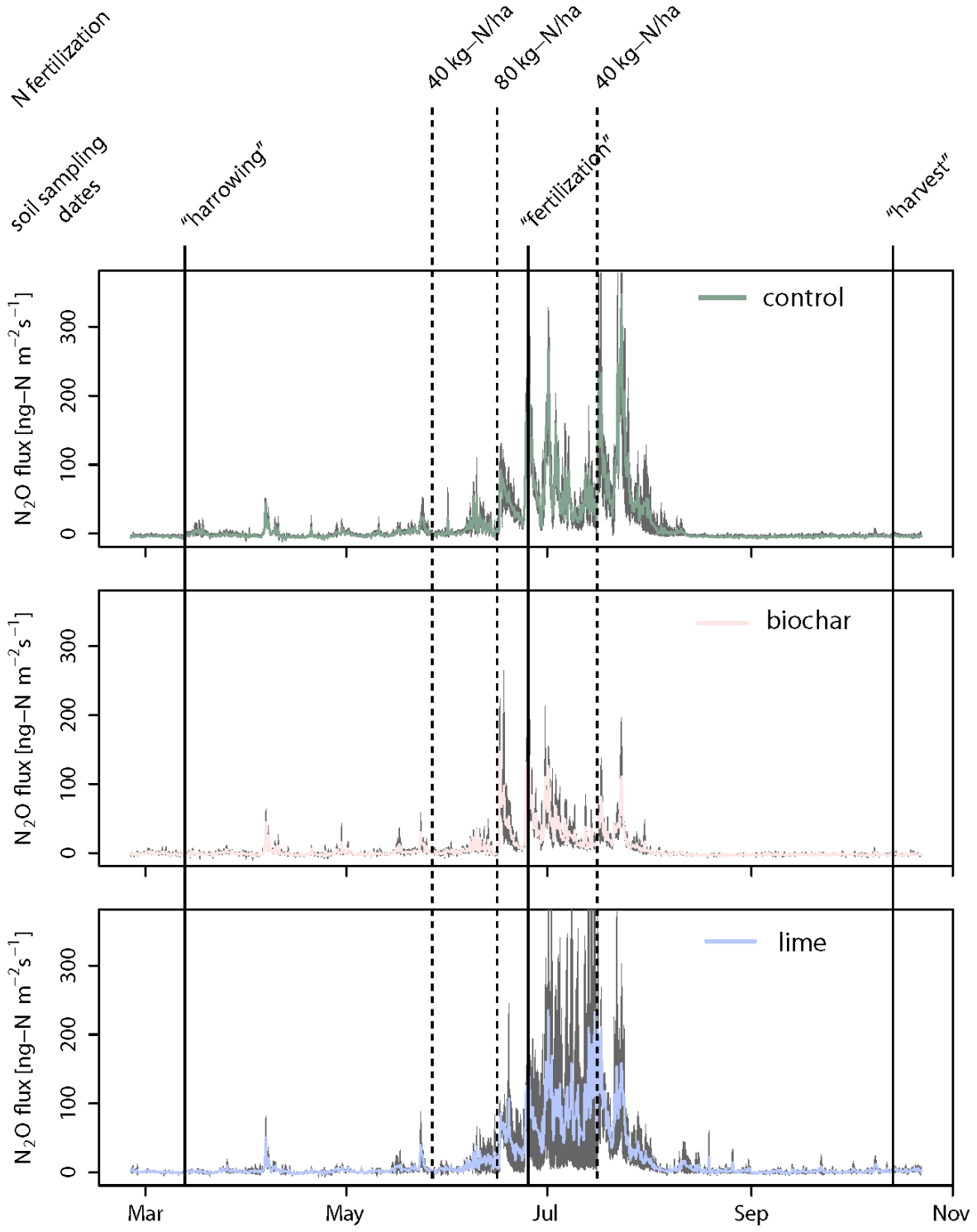
[2]Climate and Air Pollution Group, Agroscope Institute for Sustainability Sciences, Zurich, Switzerland

[3]Algorithms in Bioinformatics, Center for Bioinformatics, University of Tübingen, Tübingen, Germany

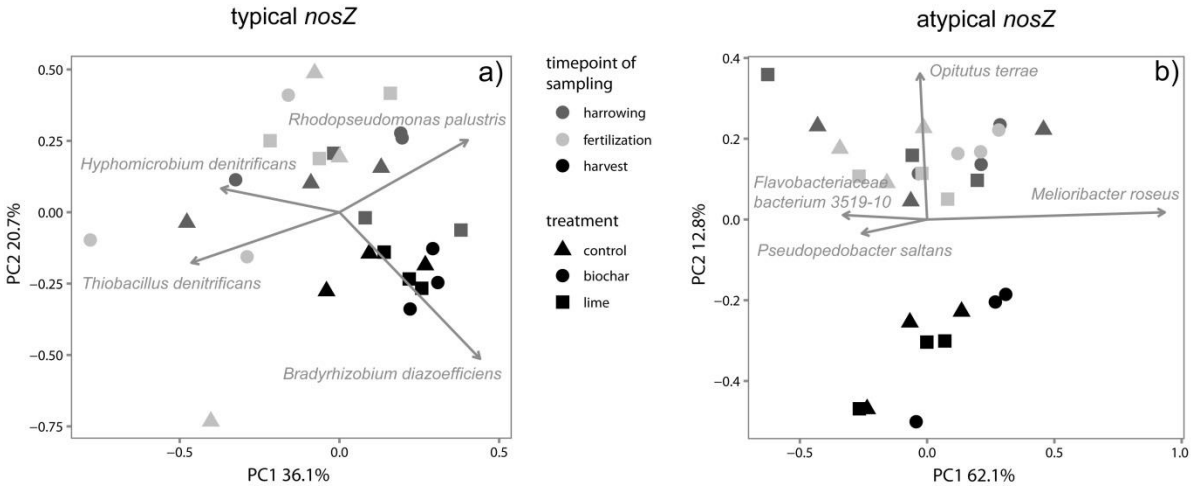
[4]Geocmicrobiology, Center for Applied Geoscience, University of Tübingen, Tübingen, Germany

[5]Forest Soils and Biogeochemistry, Swiss Federal Research Institute WSL, Birmensdorf, Switzerland

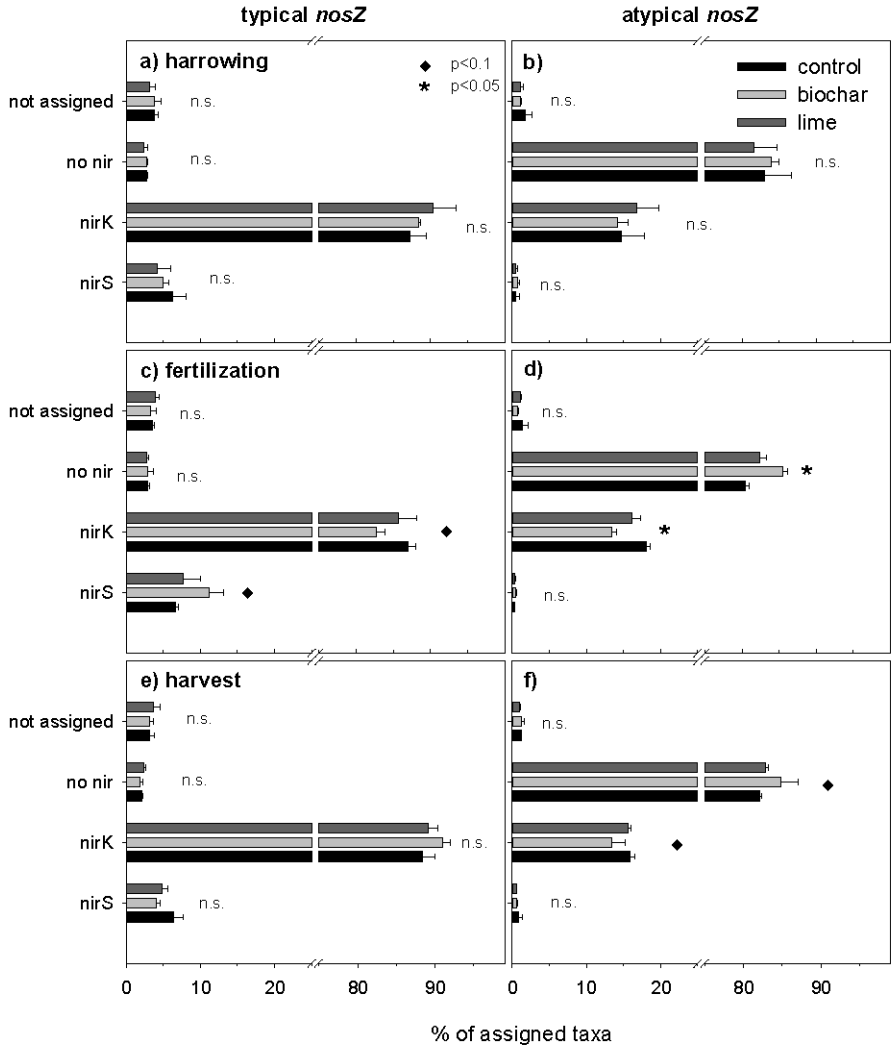
[6] College of Civil, Environmental, and Geo Engineering, University of Minnesota, Minneapolis, MN 55455, USA



Supplementary Figure S1: N_2O emissions during vegetation period of Zea mays after addition of biochar and lime in comparison to the control (modified after Hüppi et al 2015). Colored lines represent means (n=3) and gray area standard errors. Soil sampling and fertilization events are indicated by vertical lines.



Supplementary Figure S2: Principal coordinate analysis (PCoA) plot based on the genetic diversity of the typical and atypical nitrous oxide reductase after sowing, fertilization and harvest of Zea mays. The PCoA was calculated using Bray-Curtis dissimilarity matrix based on the relative share of assigned gene sequences in the respective soil samples. Biplots also show the four most abundant gene sequences affecting ordination and the assigned taxa.



Supplementary Figure S2: Relative abundance of identified typical and atypical *nosZ* bearing taxa possessing *nirS, nirK* or no nitrite reductase (no nir) in control biochar and lime treatment at the different sampling dates. Taxa with unknown genetic capability for nitrite reduction are summarized in "not assigned". Data was obtained by checking presence of *nirS* and *nirK* sequences in the genomes of the identified taxa on the base on data provided by Graf *et al.* (2014).

Supplement Table S1: Master mixes and temperature protocols used for functional gene quantification

Target gene	Reaction mixture	Volumes [µl]	Thermal profile		Reference
APA9	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5			
	APA9_F (2 µM)	2	95°C – 10`	35 cycles	Modified after (Thonar *et al.*, 2012)
	APA9_R (2 µM)	2	52°C - 30 `		
	DNA - template	1			
atypical *nosZ*	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5			
	nosZ-II-F (10 µM)	1	95°C – 15`	40 cycles	Modified after (Jones *et al.*, 2013)
	nosZ-II-R (10 µM)	1	54°C – 30`		
	DMSO	0.4	72°C – 30`		
	PCR water	1.6	80°C – 10`		
	DNA - template	1			
typical nosZ	Kapa SYBR®Fast qPCR Kit Master Mix (2X) Universal	5	95°C – 10`		
			65-60°C – 20`	6 cycles	
	nosZ2R (5 µM)	1	80°C – 15`		Modified after (Babić *et al.*, 2008)
	nosZ2F (5 µM)	1			
	PCR water	2	95°C – 15`		
			60°C – 15`	35 cycles	
	DNA - template	1	80°C – 15`		

Supplement Table S2. Primers used for quantitative PCR

Target gene	Primer	Sequence 5`- 3`	Amplicon size (bp)	Reference
APA9	APA9_F	GGG GTT TCT ACT GGT GGT	80	Thonar et al. (2011)
	APA9_R	CCC CTC KGS AAA GCC TTC TTC		
atypical *nosZ*	nosZ-II-F	CTI GGI CCI YTK CAY A	690-720	(Jones *et al.*, 2013)
	nosZ-II-R	GCI GAR CAR AAI TCB GTR C		
typical nosZ	nosZ2F	CGC RAC GGC AAS AAG GTS MSS GT	267	(Henry *et al.*, 2006)
	nosZ2R	CAK RTG CAK SGC RTG GCA GAA		

Supplement Table S3. Standard plasmid and source of standard

Target gene	Plasmid	Source of standard	Size of insert
APA9	Pjet 1.2	*African cassava mosaic virus*	945
atypical *nosZ*	pEX-A	*Gemmatimonas aurantiaca*	800
typical nosZ	pCR4-TOPO	*Ensifer meliloti 1021*	1884

Supplementary Table S4. Thermal profiles, master mixes and primer sequences used for amplicon generation from soil DNA extracts for Illumina sequencing

typical nosZ			atypical nosZ		
thermal profile					
95	10 min		95	10 min	
95	15 sec		95	15 sec	
60	20 sec	30x	54	30 sec	30x
72	40 sec		72	30 sec	
72	3 min		72	3 min	
master mix (25µl)					
reagent	volume (µl)		reagent	volume (µl)	
Kapa Sybr Mix 2x	12.5		Kapa Sybr Mix 2x	12.5	
nosZ_1F (5 µM)	2		nosZ-II_F (10 µM)	2	
nosZ_2R (5 µM)	2		nosZ-II_R (10 µM)	2	
T4 gp 32 (0.5 µg/µl)	0.5		T4 gp 32	0.5	
Tris (10mM)	5		Tris (10mM)	5	
Sample (~1ng/ul)	3		Sample (~1ng/ul)	3	
primer sequences					
nosZ_1F CS1tag – CGCTSTTYMTIGAYAGYCAG (Jones *et al.*, 2014)			nosZ-II_F CS1tag – CTIGGICCIYTKCAYAC (Jones *et al.*, 2013)		
nosZ_2R CS2tag – CAKRTGCAISGCRTGGCAGAA modified after (Henry *et al.*, 2006)			nosZ-II_R CS2tag - GCIGARCARAAITCBGTRC (Jones *et al.*, 2013)		

References

Babić, K. H., Schauss, K., Hai, B., Sikora, S., Redžepović, S., Radl, V. & Schloter, M. (2008). Influence of different Sinorhizobium meliloti inocula on abundance of genes involved in nitrogen transformations in the rhizosphere of alfalfa (Medicago sativa L.). *Environmental Microbiology* 10(11): 2922-2930.http://dx.doi.org/10.1111/j.1462-2920.2008.01762.x

Graf, D. R. H., Jones, C. M. & Hallin, S. (2014). Intergenomic Comparisons Highlight Modularity of the Denitrification Pathway and Underpin the Importance of Community Structure for N2O Emissions. *Plos One* 9(12): e114118. http://dx.doi.org/10.1371/journal.pone.0114118

Henry, S., Bru, D., Stres, B., Hallet, S. & Philippot, L. (2006). Quantitative Detection of the nosZ Gene, Encoding Nitrous Oxide Reductase, and Comparison of the Abundances of 16S rRNA, narG, nirK, and nosZ Genes in Soils. *Applied Environmental Microbiology* 72(8): 5181-5189.http://dx.doi.org/10.1128/aem.00231-06

Jones, C., Graf, D., Bru, D., Philippot, L. & Hallin, S. (2013). The unaccounted yet abundant nitrous oxide-reducing microbial community: a potential nitrous oxide sink. *The ISME Journal* 7(2): 417-426. http://dx.doi.org/10.1038/ismej.2012.125

Jones, C. M., Spor, A., Brennan, F. P., Breuil, M.-C., Bru, D., Lemanceau, P., Griffiths, B., Hallin, S. & Philippot, L. (2014). Recently identified microbial guild mediates soil N2O sink capacity. *Nature Climate Change* 4: 801-805. http://dx.doi.org/10.1038/nclimate2301

Thonar, C., Erb, A. & Jansa, J. (2012). Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities—marker design, verification, calibration and field validation. *Molecular Ecology Resources* 12(2): 219-232.http://dx.doi.org/10.1111/j.1755-0998.2011.03086.x

Discussion

Using molecular tools to assess microbial N_2O production and reduction

In order to investigate the functional impact of N cycling bacteria on N_2O emissions different methods where used in the course of this thesis. In all experiments functional genes involved in N cycling had been quantified using the qPCR approach. On a DNA level this approach can determine the genetic potential of a microbial community to perform a specific process. Quantification of functional genes on the mRNA level results in a measure of activity, which is considered as a more precise estimate of functionality (Philippot and Hallin, 2005). In a simplified view functional genes are prerequisites for any enzyme-catalyzed reaction and the abundance of functional genes should therefore correlate to process rates. Yet, a recent meta-analysis showed that in many environments a direct relationship between the size and activity of the functional community and process rates was missing (Rocca *et al.*, 2015; Henderson *et al.*, 2010; Dandie *et al.*, 2008). The relationship can be obscured by various factors including availability of substrates, PCR biases and primer design (Bier *et al.*, 2015). Nevertheless, several studies successfully linked functional gene abundance and/or activity with process rates in N cycling (Chen *et al.*, 2015; Theodorakopoulos *et al.*, 2017; Miller *et al.*, 2009) and the meta-analysis approach revealed significant correlations for N cycling gene with process rates in an agricultural context (Rocca *et al.*, 2015). This is most likely due the inherent need for N fertilization in agricultural systems, which impedes N shortage as limiting factor for microbial growth. The explanatory power of functional gene analysis in relation to process rates also depends on prevailing redox conditions and the availability of a carbon source. Correlating N_2O emissions and functional gene and/or transcript abundance is especially challenging since both parameters are measured on different scales. While measured N_2O fluxes are a result of spatial integration, microbial activity leading to N_2O production and reduction are often restricted to hotspots within the soil profile (Groffman *et al.*, 2009). The kinetics of N_2O efflux from the soil profile can be significantly affected by water content and soil texture and thus further hamper direct comparison to functional gene analysis (Clough *et al.*, 2005). In incubation experiments the kinetic effects of soil heterogeneity on N_2O effluxes is often minimized by prevenient sieving and homogenization of the soil samples.

For comparison of functional gene analysis and N_2O emissions experimental conditions must be carefully evaluated. In Chapter 1 functional gene analysis on DNA level could be successfully linked to N_2O production in an experimental setup in which fertilization with different N sources and rather oxic conditions enabled simultaneous occurrence of nitrification and denitrification processes

at different microsites (Davidson *et al.*, 1986; Kool *et al.*, 2011). In this setup fertilization treatment affecting substrate availability was the major factor controlling N_2O production and reduction processes. While the abundance of, mainly autotrophic, ammonium oxidizing bacteria correlated with N_2O emissions after addition of NH_4^+, the abundance of nitrous oxide reducers negatively correlated with N_2O emission when NO_3^- was present and organic carbon was added during fertilization. The fact that changes in *nirK* and *nirS* bearing communities could not be correlated with N_2O fluxes might be partly explained by methodological issues in primer design. It was shown that the primer pairs commonly used for *nirS* and *nirK* quantification do not cover the total N_2O producing community (Wei *et al.*, 2015). When assessing changes in abundance of heterotrophic communities in short term experiments the availability of a carbon source is of immanent importance. In Chapter 3 a carbon source was added and changes in denitrifier abundances could be linked to N_2O emissions. In contrast, the experimental setup in Chapter 2 did not allow addition of a carbon source in order not to mask farming system induced differences in SOC contents. Consequently, functional gene abundance of denitrifying marker genes was not a suitable measure to predict N_2O emissions. Under C limiting conditions analysis of denitrifying genes on the mRNA level was a better predictor for N_2O emissions, proofing higher sensitivity of this parameter. In line with this several studies showed mRNA analysis to outcompete plain quantification of N cycling genes in predicting process rates (Chen *et al.*, 2015; Dandie *et al.*, 2011; Theodorakopoulos *et al.*, 2017; Henderson *et al.*, 2010). Under controlled conditions in short term experiments functional gene analysis on RNA level seems to be the methods of choice when assessing the functional impact of microbial communities on process rate in N cycling. Under field conditions however, soil heterogeneity might additionally impede meaningful quantification of gene expression. Due to extremely variable mRNA yields across sampling dates and sampling plots quantification of gene expression in the biochar field experiment did not result in robust results (Chapter 4). Across the vegetation period, quantification of functional genes on a DNA base proofed as a more stable and reliable measure to assess the functional impact of N_2O reducing bacteria. Addressing the temporal issue, mRNA might be used to track short term response of functional communities under controlled conditions quantification of gene abundance seems to be more reflective for microbial processes performance across longer time periods (Rocca *et al.*, 2015). It also needs to be noticed that due to sensitivity of mRNA and the complex methodological procedure quantification of functional gene expression is challenging and robust results cannot always be obtained. Despite publication bias there is a number of studies that failed in quantifying functional gene expression (Dandie *et al.*, 2011; Sharma *et al.*, 2006; Liu *et al.*, 2010).

On top of PCR based quantification of functional genes Illumina sequencing was applied in order to identify the effect of biochar addition on community structure of N_2O reducers in a field experiment. Comparing relative abundance of single species enabled a new level of understanding the functional impact of biochar on N_2O reducing microbial communities. However, assessing the structure of functional communities via sequencing approach is prone to the same PCR and primer related biases as the qPCR approach (Schirmer *et al.*, 2015). It also needs to be noticed that when working with functional genes data base quality is a major issue which can significantly bias results of mapping (Petrenko *et al.*, 2015). Furthermore, due to horizontal gene transfer it hard to prove that a gene specific gene fragment actually appears in the assigned species. Although sequencing approaches have to deal with inherent weaknesses the capability of these methods to characterize whole microbial communities clearly outcompete other fingerprinting methods (e.g. PLFA) which are frequently used in agro-ecological research.

Comparing insights from incubation trials with long term N_2O measuring campaigns in the field

In this thesis the functional role of microbes involved in N_2O production and reduction under different soil management strategies was assessed. For all soil management practices incubation experiments under controlled conditions were conducted (Chapter 1-3). These experiments complemented long term field campaigns measuring N_2O emissions in situ and aimed at increasing our understanding of the functional role of microbes under different soil management strategies. Long term field measurements are ultimately needed in order to proof N_2O mitigation under field conditions and recommend specific soil management practices as effective tools for N_2O mitigation. In the following the results of the long term N_2O measurement campaign for the soil management practice investigated in this thesis will be put in context with results obtained by analyzing functional microbial communities involved in N_2O production and reduction.

In the Frick tillage field trial N_2O emissions were quantified during two cropping seasons including the cultivation of grass-clover and winter wheat in 2014 and 2015. During this period reduced tillage did not significantly lower cumulated N_2O emissions (Krauss *et al.*, submitted). In line with this negligible changes in N_2O emission had been observed in other field trials comparing reduced and conventional tillage systems (Parkin and Kaspar, 2006). The results of the Frick campaign further strengthen the findings of a recent meta-analysis that revealed no significant difference of no-till or reduced tillage system on N_2O emission across 293 pairwise comparisons (van

Kessel *et al.*, 2013). Yet, it needs to be noticed that the effect of tillage intensity on cumulated N_2O emissions across cropping seasons might be strongly affected by weather conditions (Gregorich *et al.*, 2005) and type of fertilizer application (Venterea *et al.*, 2005). It was proposed that reduced tillage intensity has the potential for reducing N_2O emissions only when it is applied for more than 10 years (van Kessel *et al.*, 2013). It was hypothesized that within this period soil structure improves by biological stabilization of soil aggregates which in turn lead to increased soil aeration and lower susceptibility for denitrifying conditions (Six *et al.*, 2004). The field campaign in Frick was conducted 10 to 12 years after establishment, but it well might be that stable soil aggregates are still developing. Still increasing SOC contents in the upper soil layer indicate that a new equilibrium hasn`t been reached yet and changes in SOC related soil properties can still be expected (Krauss *et al.*, submitted). Furthermore, extremely high clay content at the field trial in Frick might mask improvements in soil structure and prolong timespan till increased soil aeration can become effective. The N_2O mitigation potential of reduced tillage seems to be limited, especially as it requires long term application to became effective. The benefits of this soil management practice are mainly presented by a lower susceptibility to soil erosion and in maintaining soil quality. Nevertheless, we showed that reducing tillage intensity affects soil geochemical parameters as well as genetic potential of functional N cycling communities in dependency of soil depth. Since increased abundance of *nosZ* bearing bacteria was detected in the upper soil layer under reduced tillage in our incubation experiment we hypothesized that this soil layer holds an increased potential to reduce N_2O emissions (Chapter 1). The results from a recent incubation study observing enhanced N_2 formation in a C enriched upper soil layer compared to lower soil layers provides further support for this hypothesis (Kuntz *et al.*, 2016). Also in line with this, placement of N fertilizers in a depth below 5 cm was shown to reduce N_2O emissions under reduced tillage (van Kessel *et al.*, 2013). Seemingly increased retention time of N_2O in the soil can increases probability of microbial N_2O reduction especially when passing through C enriched upper soil layers. Consequently, the interaction between different mechanisms of N_2O production and reduction in the upper and lower soil layer need to be considered when assessing the functional impact of reduced tillage on N_2O emissions. After addition of NH_4^+ containing fertilizers we observed increased potential of N_2O production in the lower soil layer under reduced tillage (Chapter 1). This is in line with increased abundance of nitrifiers and a positive correlation of bacterial nitrifiers with N_2O emissions indicated nitrification as main source for N_2O emissions. Increased abundance of nitrifiers under reduced tillage intensity was already reported in another studies (Li *et al.*, 2015; Attard *et al.*, 2010), but we were able to link this with increased N_2O emission potential via nitrification. The mechanism behind enhanced nitrification under reduced tillage intensity is still unclear. Soils under reduced tillage were reported to possess

enhanced potential for mineralization of complex organic crop residues (Kainiemi *et al.*, 2013). This might facilitates a steady supply of NH_4^+ and foster the abundance of nitrifiers. In line with this mineralization rates were the best predictor for enhanced abundance of nitrite oxidizing bacteria in an incubation experiment comparing different tillage strategies (Attard *et al.*, 2011). However, under reduced tillage systems mineralization of crop residues would mainly occur in the upper soil layer and mineralization potential of lower soil layers had not been investigated yet (Kainiemi *et al.*, 2013). It is also discussed whether enhanced mineralization potential of soils under reduced tillage translate to field conditions since mineral N concentrations in spring under reduced were often found to be lower compared to conventionally managed soils. This was ascribed to higher bulk densities in reduced tillage system leading to a delayed warming of the soil and thus retarding mineralization process (Mäder and Berner, 2012). However, the agronomic approach of quantifying concentration of mineral N seems not to be the best predictor for quantification of mineralization rates since production and consumption rates cannot be estimated. Summarizing, we found indications that microbial mechanism of N_2O production and reduction change with soil depth due to decreased tillage intensity. Increased N_2O production in the lower soil layer and enhanced N_2O reduction in the upper soil layer under reduced tillage under field conditions might have counteracted, which could explain negligible effect of reduced tillage on N_2O emission. Additionally, N_2O emissions from lower soil layers in the field might not become as effective as suggested by the incubation experiment since lower soil layers did receive direct N fertilization.

In the DOK trial N_2O emissions had been quantified from August 2012 till March 2014. The field trial compares organic and conventional farming systems since 1978. The N_2O measuring campaign included the cultivation of grass-clover, maize and green manure. During this period area scaled N_2O emission from organically managed soils were ~40% lower compared to their conventional counterparts (Gattinger *et al.*, submitted). However, due to the yield gap of organic system the yield scaled N_2O emissions between both systems were comparable (Gattinger *et al.*, submitted). The results from the DOK field campaign match with a recent meta-analysis in which lower area scaled N_2O emissions in organically managed soil compared to conventionally managed soils were found among 19 studies worldwide (Skinner *et al.*, 2014). This global effect was ascribed to lower fertilizer N inputs in organic systems and the use of organic fertilizers which retard the release of inorganic easily available N. In contrast to conventional systems the fraction of fertilizer N emitted as N_2O in organic system is extremely variable and thus N fertilization rates did not correlate with N_2O emissions (Skinner *et al.*, 2014). This highlights that due to crop rotation and organic N fertilization soil N cycling in organic systems is more complex and thus N_2O emissions are harder to

predict. During the vegetation period of the long term N_2O measuring campaign in the DOK trial the organic treatment received 182 kg-N ha^{-1} in the form of slurry and composted manure (Gattinger *et al.*, submitted). This is somewhat extraordinary since it exceeded N addition in the conventional system which received 170 kg –N ha^{-1} in the form of calcium ammonium nitrate. Decreased bioavailability of N in the organic system thus seems to be a major cause for reduced area scaled N_2O emissions in the organic systems. N cycling and thus N_2O emissions in organic systems might also be affected by elevated soil organic C contents (Gattinger *et al.*, 2012). It is often assumed that provision of a C source fosters denitrification rates and thus N_2O emissions (Morley and Baggs, 2010). In line with this increased SOC contents in organically managed soils coincided with elevated NO_3^- consumption and higher N_2O emissions in our incubation experiment (Chapter 2). However, it needs to be noticed that experimental conditions aimed at assessing the potential of organically and conventionally managed soils for which NO_3^- was provided in excess in all systems. Results are therefore not directly comparable to field conditions where NO_3^- addition to organically managed soil is not an option. The situation of high NO_3^- loads coinciding with high SOC contents would only occur after conversion from an organic to a conventional system. The results from our incubation experiment thus suggest that increased N_2O emissions can be expected after such a change in management practice (Chapter 2). Lower N_2O emissions during the field campaign suggest that the increased potential for N_2O emissions in organically managed soils was counterbalanced by decreased bioavailability of N. It was also argued that increased SOC contents might foster N_2O reduction and thus lower N_2O emissions (Miller *et al.*, 2009). Especially under NO_3^- limited conditions high availability of C shifts denitrification end product toward N_2 since the kinetic unfavorable process of N_2O reduction is performed by bacteria capable of complete denitrification in order to compensate shortage of NO_3^- as electron acceptor (Senbayram *et al.*, 2012). $N_2O/(N_2O+N_2)$ product ratios are also affected by the speciation of low molecular weight C sources and greater N_2O reduction was observed after the addition of citric acid compared to glucose or glutamine (Giles *et al.*, 2017). In our incubation experiment organically managed soil showed greater SOC contents but netto N_2 production did not differ compared to conventionally soil (Chapter 2). The relative importance of complete denitrification even was enhanced in conventionally managed soils as indicated by lowest $N_2O/(N_2O+N_2)$ product ratios and significantly increased expression of *nosZ* directly after fertilization. Unfortunately, we could not investigate the impact of farming system induced differences in SOC contents on N_2O/N_2O+N_2 product ratios under field conditions as several attempts to quantify $^{15}N_2$ failed due to concentrations below detection limit. We assume that during the experimental period low water contents in the field prevented denitrifying conditions till ^{15}N label was leached or assimilated by plants and/or microbes. Apart from the complex impact of NO_3^-

and SOC contents on $N_2O/(N_2O+N_2)$ product ratio, in our incubation experiments we also observed long term application of organic soil management practice to ensure the functionality of the nitrous oxide reductase by maintaining stable soil pH. In the unfertilized control which never received any fertilization or liming a pH below 6 was reported and N_2 production was impeded. Most likely this was due to a posttranscriptional effect of low pH on the nitrous oxide reductase impeding correct folding and thus functionality of the enzyme (Liu *et al.*, 2014). Conventional soil management practice relied on addition of calcium ammonium nitrate and repeated liming treatments in order to ensure stable pH and thus active N_2O reduction (Chapter 2). This underlines the potential of organic soil management practice to maintain soils function as sink for N_2O on the long term. Summarizing, despite inducing increased potential for N_2O emissions organic farming systems can be considered as suitable strategy to mitigate N_2O emissions mainly due to provision rather stable N species during fertilization and long term stabilization of soil pH. Additionally, fertilization of organic systems with high loads of mineral N, as it would be the case after reconversion to a conventional system, most likely would result in high N_2O emissions and cannot be recommended in terms of climate smart agricultural practice.

In the Biochar field trial at Agroscope Reckenholz N_2O emissions were monitored across one cropping season under the cultivation of maize in 2015. Biochar addition reduces N_2O emissions by 52%, but due to high spatial variability in the liming treatment the liming effect of biochar on N_2O emissions could not be clearly resolved (Hüppi *et al.*, 2015). Nevertheless, the results of the field campaign further corroborated findings of a meta-analysis emphasizing biochars potential to reduce N_2O emissions by an average of ~50% across 32 studies (Cayuela *et al.*, 2013). In our incubation experiment we could link the abundance and activity of N_2O reducers to decreased N_2O emissions under denitrifying conditions (Chapter 3). Since then a biochar induced increase in size and activity of N_2O reducers has also been confirmed by other studies (Van Zwieten *et al.*, 2014; Xu *et al.*, 2014), while in a long term field study this effect could not be confirmed (Hagemann *et al.*, 2016b). It was hypothesized that specialized N_2O reducers lacking the genetic capability to produce N_2O play a major role in N_2O mitigation after biochar addition by fostering N_2O reduction (Hagemann *et al.*, 2016a). Such a shift in community composition of N_2O reducers was already observed in short term incubation trial (Harter *et al.*, 2016b). Although we could observe a similar functional shift for atypical *nosZ* bearing bacteria the effect was fading toward the end of the cropping season (Chapter 4). This raises the question whether the transient nature of the biochars impedes N_2O reduction in arable soils on the long term (Quilliam *et al.*, 2012). On the contrary, it was shown that biochar has the potential to reduce N_2O emission 3 years after incorporation (Hagemann *et al.*, 2016b). Although

the liming effect of biochar on N_2O emissions could not be resolved in the field campaign the shift in community composition of N_2O reducers after biochar addition was beyond sole effect of liming. This suggests pH not to be the only mechanisms affecting N_2O community composition in biochar amended soil. One of the most promising hypothesis describing the mode of action of N_2O mitigation in biochar amended soils involves physical entrapment of N_2O and N_2 linked to increased activity of N_2O reducers (Harter *et al.*, 2016a). By retarding release of N_2O to the atmosphere biochar assists in increasing the availability of N_2O for specialized N_2O reducers which lowers $N_2O/(N_2O+N_2)$ product ratio. However, it needs to be noticed that not all biochars possess the same potential to reduce N_2O emission and there are field trials in which incorporation of biochar showed negligible effects on N_2O emissions (Verhoeven and Six, 2014; Scheer *et al.*, 2011). Also the issue of N_2O entrapped by biochar raises the question of possibly enhanced pulse emission after soil disturbances like soil tillage. Nevertheless biochars potential to reduce to mitigate N_2O emissions was repeatedly demonstrated (van Zwieten *et al.*, 2010; Singh *et al.*, 2010; Spokas and Reicosky, 2009) and therefore should to be seriously considered as soil management practice for the mitigation of agricultural N_2O emissions.

Soil management practice as tool for N_2O mitigation

A wide range of soil management strategies had been proposed to decrease N_2O emissions (Table 1). Although there are several strategies for N_2O mitigation from agricultural soil effectivity of most of the proposed strategies can be strongly affected by pedoclimatic conditions in a given location and thus exact N_2O mitigation potentials are hard to quantify. Many strategies aim at increasing nitrogen use efficiency of agricultural systems (Reay *et al.*, 2012). For this purpose decreasing the amount of mineral N in agricultural system is a key aspect which can be achieved by adapting timing of fertilization to plant needs, decreasing the total amount of fertilization and the use of organic or slow release N fertilizers (Venterea *et al.*, 2012). Nitrogen use efficiency of agricultural systems can also be enhanced by improving crop rotations, e.g. via incorporation of cover crops and legumes (Smith *et al.*, 2015) or by breading and cultivation of nitrogen efficient crop species (McAllister *et al.*, 2012). As discussed before reducing tillage intensity may provide several benefits for soil quality and physical soil protection, but its effectivity as N_2O mitigation strategy seems rather limited. This is especially true since N_2O mitigation in reduced tillage systems seems to become only effective on the long term (Six *et al.*, 2004). Organic farming systems combine several of the proposed N_2O mitigation strategies like the use of organic N fertilization and incorporation of legumes and cover crops in the crop rotation. Although these practices are not exclusive for organic agricultural practice they are core

principles that should be applied in all organic systems. It therefore seems reasonable to assume that organic agricultural systems provide a valuable contribution for N_2O mitigation in agricultural systems. Nevertheless, high yield scaled N_2O emissions in organic systems emphasize the need for additional management practices enhancing N_2O mitigation from agriculturally managed soils.

Table 2: Potential N_2O mitigation strategies for arable soils

management practice	reference
Adjustment of N fertilization rate to crop needs	(Venterea *et al.*, 2012)
Optimized timing and placement of N fertilization	(Roy *et al.*, 2014)
Slow release or organic fertilization	(Venterea *et al.*, 2012)
Integration of cover crop and legumes in the crop rotation	(Smith *et al.*, 2008)
Cultivation and breading of crops with high nitrogen use efficiency	(McAllister *et al.*, 2012)
Reducing tillage intensity	(van Kessel *et al.*, 2013)
Amendment of biochar	(Cayuela *et al.*, 2013)
Liming treatment	(Baggs *et al.*, 2010)
Use of nitrification inhibitors	(Ruser and Schulz, 2015)
Inoculation with N_2O reducers	(Itakura *et al.*, 2013)

Several soil amendments were proposed to strengthen N_2O mitigation potential like nitrification inhibitors, liming treatments, biochar or inoculation with N_2O reducing bacteria (Baggs *et al.*, 2010; Itakura *et al.*, 2013; Ruser and Schulz, 2015; Woolf *et al.*, 2010). The use of chemical nitrification inhibitors, like DMPP, aims at preventing formation of NO_3^- and thus reduces leaching and formation of N_2O via denitrification (Ruser and Schulz, 2015). Although the impact of these products on soil microbiology is poorly understood, since September 2016 the use of a nitrification inhibitor containing DMPP is part of the Swiss national strategy for climate protection (www.bafu.admin.ch/klima/). It needs to be noted that biological nitrification inhibitors were already identified e.g. in the form of root exudates from sorghum and rice (Zakir *et al.*, 2008; Sun *et al.*, 2016). Breading for crop releasing biological nitrification inhibitors would allow indirect manipulations of soil NO_3^- concentrations. Up to date research on this topic is scarce but biological inhibition of nitrification could be an interesting option to lower N_2O emissions also for organic agricultural systems. This is especially interesting for reduced tillage systems were the effect of enhanced nitrification potential in the lower soil layer might be diminished by root exudates inhibiting nitrification. Since nitrification is a major process contributing to soil acidification nitrification inhibitors might indirectly lower $N_2O/(N_2O+N_2)$ product ratios emissions by preserving a

stable soil pH (Ruser and Schulz, 2015). Commonly for this purpose liming treatments in agricultural soils are employed to maintain a soil pH of ~6.5. Under slightly acidic conditions availability of different nutrients is considered to be best balanced which positively affects crop performance (Goulding, 2016). However, due to economic constrains liming treatment in agricultural practices are not always conducted and often soils are managed at suboptimal pH levels (Goulding, 2016). Due to dysfunctionality of the nitrous oxide reductase acidic soil are more susceptible to high N_2O fluxes (Liu *et al.*, 2014; Baggs *et al.*, 2010). In Chapter 2 it was shown that effect can be caused by missing soil management practice. Consequently, maintaining a stable soil pH not only enhances crop performance but should be also considered as key aspect for a climate smart agricultural practice. However managing soil pH by addition of CaCO3 was sometimes argued to result in dissolution of CO_2 and thus increase total GHG emissions from soils (West and McBride, 2005). However this effect is not straightforward and liming action as sink for CO_2 due to enhanced autotrophic activity has also been suggested (Hamilton *et al.*, 2007). Similar to liming treatments biochar as soil amendment can also increase soil pH. A recent study suggests that the liming effect of biochar plays a major role in lowering $N_2O/(N_2O+N_2)$ product ratios (Obia *et al.*, 2015). In addition biochar was shown to retard release of N_2O and N_2 emissions by physical entrapment and stimulate activity of specialized N_2O reducers (Harter *et al.*, 2016a), indicating increased soil pH seems not to be the only mechanisms involved in reduction of N_2O emissions from soils. Among the three soil management practices the amendment of biochar resulted in greatest reduction of N_2O emissions. Although certified biochars are allowed in conventional agricultural practice at least in Switzerland biochar was not included in the permitted resources which can be used in organic agricultural practice in 2017 (www.betriebsmittelliste.ch). For the further development of effective N_2O mitigation strategies the use of biochar should be combined with other proposed strategies. Biochar ability to enhance N_2O reduction also goes in line with the review of Thompson et al. (2012) who suggested that promotion of biological N_2O reduction is the most promising approach to mitigate N_2O emissions from agricultural systems. It was argued that due to the inherent need for N fertilization in agricultural system soil management practices which completely eliminate N_2O production might not be possible to develop (Thomson *et al.*, 2012). The fact that N_2O can be produced by several biotic and abiotic processes and the phylogenetically widespread ability among microbes to produce N_2O adds further complexity to the task of reducing N_2O emissions by minimizing N_2O production (Butterbach-Bahl *et al.*, 2013; Wei *et al.*, 2015; Philippot *et al.*, 2007). In all experiments conducted within this thesis the abundance, activity or community structure of N_2O reducing bacteria could be related to the magnitude of N_2O emission under different soil management practices. This highlights that the functioning of N_2O reducers is highly susceptibility to management induced changes in soil physics

and chemistry and therefore strengthens viability of the N_2O mitigation approach focusing on biological N_2O reduction. Direct inoculation of N_2O reducers might also be an option for N_2O mitigation since the *nosZ* overexpressing bacterium *Bradyrhizobium japonicum* and the non-denitrifying but N_2O reducing bacterium *Dyadobacter fermentans* were already shown to decrease N_2O emissions (Itakura *et al.*, 2013; Domeignoz-Horta *et al.*, 2016). However, inoculation of microbial strains or populations often show only transient effects (Trabelsi and Mhamdi, 2013). Competition for substrate and habitat were identified as major challenges preventing long term functioning of the inoculated microbes (Trabelsi and Mhamdi, 2013). The use of biochar as carrier material for N_2O reducing microbes might to some extend overcome this issue by providing additional habitat and provision of N_2O by physical entrapment. Biochar has been already used as carrier material for *Enterobacter cloacae* and prolonged survival of this strain significantly (Hale *et al.*, 2014). So far no research has been published on biochar inoculation with bacterial strains involved N_2O reduction. By minimizing competition during the establishment of an active N_2O reducing community this approach might further enhance N_2O reducing capacity of biochar amendments.

Summary

In this thesis the effect of reduced tillage, organic farming systems and biochar amendment on the functioning of N_2O producing and reducing microbial communities had been investigated. The key insight in microbial mechanism of N_2O production and reduction can be summarized as follows:

- Tillage system affects fertilizer induced N_2O emission in dependency of soil depth. Increased potential for N_2O production via nitrification in lower soil layers under reduced tillage and indication for enhanced N_2O reduction in the upper soil layer under reduced tillage had been observed.
- Organically managed soils possess enhanced N_2O emission potential but ensured sustainable functionality of the nitrous oxide reductase.
- For climate smart agricultural practice maintaining a soil pH above ~6 is a key aspect in order to enable active N_2O reduction.
- The ability of biochar to reduce N_2O emission is linked to the activity and structure of the denitrifying microbial community.
- A community shift of N_2O reducing bacteria towards specialized N_2O reducers along with reduced N_2O emissions could be observed under field conditions across one vegetation period.
- Across all experiments the functioning of N_2O reducers was susceptible to changes of soil management practice. This strengthens the theory that promotion of complete denitrification is one of the most promising approaches to reduce N_2O emissions from agriculturally managed soils.

Outlook

For the further development of climate smart agricultural practice combinations of different N_2O mitigation strategies seem promising but were rarely investigated. In the following a list of possible combinations is presented along with a brief discussion on possible experimental setups.

- Biochar as carrier for the inoculation with N_2O reducing strains or communities

In order to test the N_2O mitigation potential of inoculation of N_2O reducers combined with biochar amendment the first step would be to perform a literature review to identify suitable bacterial

strains which efficiently reduce N_2O. In a next step pure culture studies combined with quantification of N_2O and N_2 emissions would be needed in order to quantify N_2O reduction potential. Since inoculation of communities were proven to enhance resilience of functionality in microbial inoculation a second step could be to test N_2O reduction of more complex communities under laboratory conditions. The most promising strains or communities could be tested for functionality and longevity in pot trials under controlled conditions. Therefore a method of establishing the desired community on the biochar surface as well as the development of strain specific qPCR assays would be needed. The last step would be test the method of inoculation under field conditions in a field experiment which monitors greenhouse gas emissions across longer periods.

- The impact of soil tillage on N_2O pulse emissions in biochar amended soils

The fact that biochar was shown to physically retain N_2O within the soil raises the question of N_2O pulse emissions after tillage operations. Quantifying such pulse emissions would be crucial in order to calculate N_2O mitigation potential in biochar amended soil across several cropping seasons. The most common approach to measure N_2O emissions in the field is the static chamber approach which requires fixed installation of equipment in the field. For tillage operation such an approach is not suitable since disturbance of the soil structure almost certainly would yield in direct release of entrapped N_2O. One possible approach could be N_2O quantification via eddy-covariance measurements. This laser-based method is designed to measure greenhouse gas fluxes on an ecosystems scale but cannot differentiate in between different treatments in the commonly used plot designs. This problem could be overcome by performing soil tillage in the different treatments at different days. The fact that reduced tillage systems only superficially work the soil surface in a non-turning manner might proof synergistic for N_2O release after tillage in biochar amended soils. By reducing tillage intensity release of N_2O after tillage operation might be minimized.

- Biochar amendments in organic agricultural systems

Amendment of biochar in organically managed soils might further reduce N_2O emissions in these systems. However in order to be officially considered as soil amendment in organic agricultural practice in Switzerland further research on possible negative side effects on soil quality like accumulation of PAH and heavy metals is needed. Incubation trials under controlled conditions might be useful to determine to which extend biochar amendment can reduce N_2O emissions in organically managed soil. These experiments could also provide further information about risk assessment of biochar amendment in organic farming systems and thus contribute to the approval of biochar in organic agricultural systems

References

Attard, E., Poly, F., Commeaux, C., Laurent, F., Terada, A., Smets, B. F., Recous, S. & Roux, X. L. (2010). Shifts between Nitrospira- and Nitrobacter-like nitrite oxidizers underlie the response of soil potential nitrite oxidation to changes in tillage practices. *Environmental Microbiology* 12(2): 315-326.http://dx.doi.org/10.1111/j.1462-2920.2009.02070.x

Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., Philippot, L., Schmid, B. & Le Roux, X. (2011). Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. *Global Change Biology* 17(5): 1975-1989.10.1111/j.1365-2486.2010.02340.x

Baggs, E., Smales, C. & Bateman, E. (2010). Changing pH shifts the microbial sourceas well as the magnitude of N2O emission from soil. *Biology and Fertility of Soils* 46(8): 793-805. http://dx.doi.org/10.1007/s00374-010-0484-6

Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T., Nemergut, D., Osborne, B. B., Ruiz-González, C., Schimel, J. P., Waldrop, M. P. & Wallenstein, M. D. (2015). Linking microbial community structure and microbial processes: an empirical and conceptual overview. *FEMS Microbiology Ecology*.http://dx.doi.org/10.1093/femsec/fiv113

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society* 368(1621). http://dx.doi.org/10.1098/rstb.2013.0122

Cayuela, M. L., van Zwieten, L., Singh, B. P., Jeffery, S., Roig, A. & Sánchez-Monedero, M. A. (2013). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agriculture, Ecosystems & Environment* 191: 5-16.http://dx.doi.org/10.1016/j.agee.2013.10.009

Chen, Z., Wang, C., Gschwendtner, S., Willibald, G., Unteregelsbacher, S., Lu, H., Kolar, A., Schloter, M., Butterbach-Bahl, K. & Dannenmann, M. (2015). Relationships between denitrification gene expression, dissimilatory nitrate reduction to ammonium and nitrous oxide and dinitrogen production in montane grassland soils. *Soil Biology and Biochemistry* 87: 67-77. http://dx.doi.org/10.1016/j.soilbio.2015.03.030

Clough, T. J., Sherlock, R. R. & Rolston, D. E. (2005). A Review of the Movement and Fate of N2O in the Subsoil. *Nutrient Cycling in Agroecosystems* 72(1): 3-11.http://dx.doi.org/10.1007/s10705-004-7349-z

Dandie, C. E., Burton, D. L., Zebarth, B. J., Henderson, S. L., Trevors, J. T. & Goyer, C. (2008). Changes in bacterial denitrifier community abundance over time in an agricultural field and their relationship with denitrification activity. *Applied and Environmental Microbiology* 74(19): 5997-6005.http://dx.doi.org/10.1128/Aem.00441-08

Dandie, C. E., Wertz, S., Leclair, C. L., Goyer, C., Burton, D. L., Patten, C. L., Zebarth, B. J. & Trevors, J. T. (2011). Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. *FEMS Microbiology Ecology* 77(1): 69-82. http://dx.doi.org/10.1111/j.1574-6941.2011.01084.x

Davidson, E. A., Swank, W. T. & Perry, T. O. (1986). Distinguishing between Nitrification and Denitrification as Sources of Gaseous Nitrogen Production in Soil. *Applied and Environmental Microbiology* 52(6): 1280-1286

Domeignoz-Horta, L. A., Putz, M., Spor, A., Bru, D., Breuil, M. C., Hallin, S. & Philippot, L. (2016). Non-denitrifying nitrous oxide-reducing bacteria - An effective N2O sink in soil. *Soil Biology and Biochemistry* 103: 376-379.http://dx.doi.org/10.1016/j.soilbio.2016.09.010

Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fliessbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., Scialabba, N. E.-H. & Niggli, U. (2012). Enhanced top soil carbon stocks under

organic farming. *Proceedings of the National Academy of Sciences* 109(44): 18226-18231. http://dx.doi.org/10.1073/pnas.1209429109

Gattinger, A., Skinner, C., Krauss, M., Van der Heijden, M. G., Seitz, B. & Mäder, P. (submitted). Equal yield-scaled and lower area scaled nitrous oxide emissions in organically managed soil *Proceedings of the National Academy of Sciences*

Giles, M. E., Daniell, T. J. & Baggs, E. M. (2017). Compound driven differences in N2 and N2O emission from soil; the role of substrate use efficiency and the microbial community. *Soil Biology and Biochemistry* 106: 90-98.http://dx.doi.org/10.1016/j.soilbio.2016.11.028

Goulding, K. W. T. (2016). Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use and Management* 32(3): 390-399.http://dx.doi.org/10.1111/sum.12270

Gregorich, E. G., Rochette, P., VandenBygaart, A. J. & Angers, D. A. (2005). Greenhouse gas contributions of agricultural soils and potential mitigation practices in Eastern Canada. *Soil and Tillage Research* 83(1): 53-72.http://dx.doi.org/10.1016/j.still.2005.02.009

Groffman, P. M., Butterbach-Bahl, K., Fulweiler, R. W., Gold, A. J., Morse, J. L., Stander, E. K., Tague, C., Tonitto, C. & Vidon, P. (2009). Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* 93(1): 49-77.http://dx.doi.org/10.1007/s10533-008-9277-5

Hagemann, N., Harter, J. & Behrens, S. (2016a).Chapter 7 - Elucidating the Impacts of Biochar Applications on Nitrogen Cycling Microbial Communities. In *Biochar Application*, 163-198: http://dx.doi.org/10.1016/B978-0-12-803433-0.00007-2.

Hagemann, N., Harter, J., Kaldamukova, R., Guzman-Bustamante, I., Ruser, R., Graeff, S., Kappler, A. & Behrens, S. (2016b). Does soil aging affect the N2O mitigation potential of biochar? A combined microcosm and field study. *GCB Bioenergy*: n/a-n/a.http://dx.doi.org/10.1111/gcbb.12390

Hale, L., Luth, M., Kenney, R. & Crowley, D. (2014). Evaluation of pinewood biochar as a carrier of bacterial strain Enterobacter cloacae UW5 for soil inoculation. *Applied Soil Ecology* 84: 192-199.http://dx.doi.org/10.1016/j.apsoil.2014.08.001

Hamilton, S. K., Kurzman, A. L., Arango, C., Jin, L. & Robertson, G. P. (2007). Evidence for carbon sequestration by agricultural liming. *Global Biogeochemical Cycles* 21(2): n/a-n/a.http://dx.doi.org/10.1029/2006GB002738

Harter, J., Guzman-Bustamante, I., Kuehfuss, S., Ruser, R., Well, R., Spott, O., Kappler, A. & Behrens, S. (2016a). Gas entrapment and microbial N2O reduction reduce N2O emissions from a biochar-amended sandy clay loam soil. *Scientific Reports* 6: 39574. http://dx.doi.org/10.1038/srep39574

Harter, J., Weigold, P., El-Hadidi, M., Huson, D. H., Kappler, A. & Behrens, S. (2016b). Soil biochar amendment shapes the composition of N2O-reducing microbial communities. *Science of The Total Environment* 562: 379-390.http://dx.doi.org/10.1016/j.scitotenv.2016.03.220

Henderson, S. L., Dandie, C. E., Patten, C. L., Zebarth, B. J., Burton, D. L., Trevors, J. T. & Goyer, C. (2010). Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions and denitrification in anoxic soil microcosms amended with glucose and plant residues. *Applied and Environmental Microbiology*: AEM.02993-02909. http://dx.doi.org/10.1128/aem.02993-09

Hüppi, R., Felber, R., Neftel, A., Six, J. & Leifeld, J. (2015). Effect of biochar and liming on soil nitrous oxide emissions from a temperate maize cropping system. *SOIL* 1(2): 707-717.http://dx.doi.org/10.5194/soil-1-707-2015

Itakura, M., Uchida, Y., Akiyama, H., Hoshino, Y. T., Shimomura, Y., Morimoto, S., Tago, K., Wang, Y., Hayakawa, C., Uetake, Y., Sanchez, C., Eda, S., Hayatsu, M. & Minamisawa, K. (2013). Mitigation of nitrous oxide emissions from soils by Bradyrhizobium japonicum inoculation. *Nature Climate Change* 3(3): 208-212.http://dx.doi.org/10.1038/nclimate1734

Kainiemi, V., Arvidsson, J. & Kätterer, T. (2013). Short-term organic matter mineralisation following different types of tillage on a Swedish clay soil. *Biology and Fertility of Soils* 49(5): 495-504.http://dx.doi.org/10.1007/s00374-013-0786-6

Kool, D. M., Dolfing, J., Wrage, N. & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry* 43(1): 174-178. http://dx.doi.org/10.1016/j.soilbio.2010.09.030

Krauss, M., Ruser, R., Müller, T., Hansen, S., Mäder, P. & Gattinger, A. (submitted). Impact of reduced tillage on greenhouse gas emissions and soil organic carbon stocks in an organic grass-clover ley-winterwheat cropping sequence. *Agriculture, Ecosystems & Environment*

Kuntz, M., Morley, N. J., Hallett, P. D., Watson, C. & Baggs, E. M. (2016). Residue-C effects on denitrification vary with soil depth. *Soil Biology and Biochemistry* 103: 365-375.http://dx.doi.org/10.1016/j.soilbio.2016.09.012

Li, S., Jiang, X., Wang, X. & Wright, A. L. (2015). Tillage effects on soil nitrification and the dynamic changes in nitrifying microorganisms in a subtropical rice-based ecosystem: A long-term field study. *Soil and Tillage Research* 150: 132-138.http://dx.doi.org/10.1016/j.still.2015.02.005

Liu, B., Frostegard, A. & Bakken, L. R. (2014). Impaired reduction of N2O to N2 in acid soils is due to a posttranscriptional interference with the expression of nosZ. *MBio* 5(3): 01383-01314.http://dx.doi.org/10.1128/mBio.01383-14

Liu, B., Mørkved, P. T., Frostegård, Å. & Bakken, L. R. (2010). Denitrification gene pools, transcription and kinetics of NO, N2O and N2 production as affected by soil pH. *FEMS Microbiology Ecology* 72(3): 407-417.10.1111/j.1574-6941.2010.00856.x

Mäder, P. & Berner, A. (2012). Development of reduced tillage systems in organic farming in Europe. *Renewable Agriculture and Food Systems* 27(1): 7-11. http://dx.doi.org/10.1017/S1742170511000470

McAllister, C. H., Beatty, P. H. & Good, A. G. (2012). Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnology Journal* 10(9): 1011-1025.http://dx.doi.org/10.1111/j.1467-7652.2012.00700.x

Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C. & Trevors, J. T. (2009). Influence of Liquid Manure on Soil Denitrifier Abundance, Denitrification, and Nitrous Oxide Emissions. *Soil Science Society of America Journal* 73(3): 760-768. http://dx.doi.org/10.2136/sssaj2008.0059

Morley, N. & Baggs, E. M. (2010). Carbon and oxygen controls on N2O and N2 production during nitrate reduction. *Soil Biology and Biochemistry* 42(10): 1864-1871.http://dx.doi.org/10.1016/j.soilbio.2010.07.008

Obia, A., Cornelissen, G., Mulder, J. & Dörsch, P. (2015). Effect of Soil pH Increase by Biochar on NO, N2O and N2 Production during Denitrification in Acid Soils. *Plos One* 10(9): e0138781.http://dx.doi.org/10.1371/journal.pone.0138781

Parkin, T. B. & Kaspar, T. C. (2006). Nitrous oxide emissions from corn-soybean systems in the midwest. *J Environ Qual* 35(4): 1496-1506.http://dx.doi.org/10.2134/jeq2005.0183

Petrenko, P., Lobb, B., Kurtz, D. A., Neufeld, J. D. & Doxey, A. C. (2015). MetAnnotate: function-specific taxonomic profiling and comparison of metagenomes. *BMC Biology* 13: 92.http://dx.doi.org/10.1186/s12915-015-0195-4

Philippot, L. & Hallin, S. (2005). Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. *Current Opinion in Microbiology* 8(3): 234-239.http://dx.doi.org/10.1016/j.mib.2005.04.003

Philippot, L., Hallin, S. & Schloter, M. (2007).Ecology of Denitrifying Prokaryotes in Agricultural Soil. In *Advances in Agronomy*, Vol. Volume 96, 249-305 (Ed L. S. Donald). http://dx.doi.org/10.1016/S0065-2113(07)96003-4.

Quilliam, R. S., Marsden, K. A., Gertler, C., Rousk, J., DeLuca, T. H. & Jones, D. L. (2012). Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by

time since application and reapplication rate. *Agriculture, Ecosystems & Environment* 158(0): 192-199.http://dx.doi.org/10.1016/j.agee.2012.06.011

Reay, D. S., Davidson, E. A., Smith, K. A., Smith, P., Melillo, J. M., Dentener, F. & Crutzen, P. J. (2012). Global agriculture and nitrous oxide emissions. *Nature Climate Change* 2(6): 410-416.http://dx.doi.org/10.1038/nclimate1458

Rocca, J. D., Hall, E. K., Lennon, J. T., Evans, S. E., Waldrop, M. P., Cotner, J. B., Nemergut, D. R., Graham, E. B. & Wallenstein, M. D. (2015). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. *The ISME Journal* 9(8): 1693-1699.http://dx.doi.org/10.1038/ismej.2014.252

Roy, A. K., Wagner-Riddle, C., Deen, B., Lauzon, J. & Bruulsema, T. (2014). Nitrogen application rate, timing and history effects on nitrous oxide emissions from corn (Zea mays L.). *Canadian Journal of Soil Science* 94(4): 563-573.http://dx.doi.org/10.4141/cjss2013-118

Ruser, R. & Schulz, R. (2015). The effect of nitrification inhibitors on the nitrous oxide (N2O) release from agricultural soils—a review. *Journal of Plant Nutrition and Soil Science* 178(2): 171-188.http://dx.doi.org/10.1002/jpln.201400251

Scheer, C., Grace, P., Rowlings, D., Kimber, S. & Van Zwieten, L. (2011). Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. *Plant and Soil* 345(1): 47-58.http://dx.doi.org/10.1007/s11104-011-0759-1

Schirmer, M., Ijaz, U. Z., D'Amore, R., Hall, N., Sloan, W. T. & Quince, C. (2015). Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform. *Nucleic Acids Research* 43(6): e37-e37.http://dx.doi.org/10.1093/nar/gku1341

Senbayram, M., Chen, R., Budai, A., Bakken, L. & Dittert, K. (2012). N2O emission and the N2O/(N2O;N2) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. *Agriculture, Ecosystems & Environment* 147(0): 4-12.http://dx.doi.org/10.1016/j.agee.2011.06.022

Sharma, S., Szele, Z., Schilling, R., Munch, J. C. & Schloter, M. (2006). Influence of Freeze-Thaw Stress on the Structure and Function of Microbial Communities and Denitrifying Populations in Soil. *Applied and Environmental Microbiology* 72(3): 2148-2154.http://dx.doi.org/10.1128/aem.72.3.2148-2154.2006

Singh, B. P., Hatton, B. J., Balwant, S., Cowie, A. L. & Kathuria, A. (2010). Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of Environmental Quality* 39(4): 1224-1235. http://dx.doi.org/10.2134/jeq2009.0138

Six, J., Ogle, S. M., Jay breidt, F., Conant, R. T., Mosier, A. R. & Paustian, K. (2004). The potential to mitigate global warming with no-tillage management is only realized when practised in the long term. *Global Change Biology* 10(2): 155-160. http://dx.doi.org/10.1111/j.1529-8817.2003.00730.x

Skinner, C., Gattinger, A., Muller, A., Mäder, P., Flieβbach, A., Stolze, M., Ruser, R. & Niggli, U. (2014). Greenhouse gas fluxes from agricultural soils under organic and non-organic management — A global meta-analysis. *Science of The Total Environment* 468–469(0): 553-563.http://dx.doi.org/10.1016/j.scitotenv.2013.08.098

Smith, L. G., Williams, A. G. & Pearce, B. D. (2015). The energy efficiency of organic agriculture: A review. *Renewable Agriculture and Food Systems* 30(3): 280-301. http:/dx.doi.org/10.1017/S1742170513000471

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M. & Smith, J. (2008). Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society* 363(1492): 789-813. http://dx.doi.org/10.1098/rstb.2007.2184

Spokas, K. A. & Reicosky, D. C. (2009). Impacts of sixteen different biochars on soil greenhouse gas production. *Annals of Environmental Science* 3(1): 4

Sun, L., Lu, Y., Yu, F., Kronzucker, H. J. & Shi, W. (2016). Biological nitrification inhibition by rice root exudates and its relationship with nitrogen-use efficiency. *New Phytologist* 212(3): 646-656.http://dx.doi.org/10.1111/nph.14057

Theodorakopoulos, N., Lognoul, M., Degrune, F., Broux, F., Regaert, D., Muys, C., Heinesch, B., Bodson, B., Aubinet, M. & Vandenbol, M. (2017). Increased expression of bacterial amoA during an N2O emission peak in an agricultural field. *Agriculture, Ecosystems & Environment* 236: 212-220. http://dx.doi.org/10.1016/j.agee.2016.12.002

Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M. & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society* 367(1593): 1157-1168. http://dx.doi.org/10.1098/rstb.2011.0415

Trabelsi, D. & Mhamdi, R. (2013). Microbial Inoculants and Their Impact on Soil Microbial Communities: A Review. *BioMed Research International* 2013: 863240.http://dx.doi.org/10.1155/2013/863240

van Kessel, C., Venterea, R., Six, J., Adviento-Borbe, M. A., Linquist, B. & van Groenigen, K. J. (2013). Climate, duration, and N placement determine N2O emissions in reduced tillage systems: a meta-analysis. *Global Change Biology* 19(1): 33-44. http://dx.doi.org/10.1111/j.1365-2486.2012.02779.x

van Zwieten, L., Kimber, S., Morris, S., Downie, A., Berger, E., Rust, J. & Scheer, C. (2010). Influence of biochars on flux of N2O and CO2 from Ferrosol. *Australian Journal of Soil Research* 48(6-7): 555-568.http://dx.doi.org/10.1071/Sr10004

Van Zwieten, L., Singh, B. P., Kimber, S. W. L., Murphy, D. V., Macdonald, L. M., Rust, J. & Morris, S. (2014). An incubation study investigating the mechanisms that impact N2O flux from soil following biochar application. *Agriculture, Ecosystems & Environment* 191: 53-63.http://dx.doi.org/10.1016/j.agee.2014.02.030

Venterea, R. T., Burger, M. & Spokas, K. A. (2005). Nitrogen oxide and methane emissions under varying tillage and fertilizer management. *Journal of Environmental Quality* 34(5): 1467-1477. http://dx.doi.org/10.2134/jeq2005.0018

Venterea, R. T., Halvorson, A. D., Kitchen, N., Liebig, M. A., Cavigelli, M. A., Grosso, S. J. D., Motavalli, P. P., Nelson, K. A., Spokas, K. A., Singh, B. P., Stewart, C. E., Ranaivoson, A., Strock, J. & Collins, H. (2012). Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. *Frontiers in Ecology and the Environment* 10(10): 562-570.http://dx.doi.org/10.1890/120062

Verhoeven, E. & Six, J. (2014). Biochar does not mitigate field-scale N2O emissions in a Northern California vineyard: An assessment across two years. *Agriculture, Ecosystems & Environment* 191: 27-38.http://dx.doi.org/10.1016/j.agee.2014.03.008

Wei, W., Isobe, K., Nishizawa, T., Zhu, L., Shiratori, Y., Ohte, N., Koba, K., Otsuka, S. & Senoo, K. (2015). Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. *ISME J* 9(9): 1954-1965.http://dx.doi.org/10.1038/ismej.2015.9

West, T. O. & McBride, A. C. (2005). The contribution of agricultural lime to carbon dioxide emissions in the United States: dissolution, transport, and net emissions. *Agriculture, Ecosystems & Environment* 108(2): 145-154.http://dx.doi.org/10.1016/j.agee.2005.01.002

Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J. & Joseph, S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications* 1(5): 1-9. http://dx.doi.org/10.1038/ncomms1053

Xu, H.-J., Wang, X.-H., Li, H., Yao, H.-Y., Su, J.-Q. & Zhu, Y.-G. (2014). Biochar Impacts Soil Microbial Community Composition and Nitrogen Cycling in an Acidic Soil Planted with Rape. *Environmental Science & Technology* 48(16): 9391-9399.http://dx.doi.org/10.1021/es5021058

Zakir, H. A. K. M., Subbarao, G. V., Pearse, S. J., Gopalakrishnan, S., Ito, O., Ishikawa, T., Kawano, N., Nakahara, K., Yoshihashi, T., Ono, H. & Yoshida, M. (2008). Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate,

responsible for biological nitrification inhibition by sorghum (Sorghum bicolor). *New Phytologist* 180(2): 442-451.http://dx.doi.org/10.1111/j.1469-8137.2008.02576.x

Acknowledgements

The work presented in this thesis was performed as part of the Swiss National Research Program 68 “Soil as a Resource” and financed by the Swiss National Science Foundation. I`m grateful for the opportunity to work in a broader scientific community and the participation at various conferences and meetings in the frame of the NRP 68 has always been inspiring and motivating.

First of all I would like to thank my two main supervisors for giving me the opportunity to work on this topic. I want to thank Andreas Kappler for his trust and his nature of supporting young scientists. He enabled a group of interested students to perform experiments on the topic of biochar in at the University of Tübingen which paved my way into science and agriculture. This trust in the young generation cannot be underestimated.

Next I would like to thank my second supervisor Andreas Gattinger for his support throughout the thesis. He enabled me to work at the Research Institute of Organic Agriculture in Frick and could help me with whatever question emerged regarding agricultural practice. His amicable and trusting nature made it a pleasure to work with him and the numerous discussions about the future of agriculture motivated me to further work in this field.

Also a special thanks goes to Sebastian Behrens for his supervision and sharing his knowledge in molecular biology and microbial ecology. His expertise was crucial for the development of the young biochar research group in Tübingen.

I would like to thank Paul Mäder valuable inputs as well as excellent guidance of the soil science department at FIBL. Also great thanks goes to Andreas Fliessbach for countless discussions and valuable inputs

Big thanks also to Cecile Thonar and Thomas Oberhänsli for numerous discussions and their help in establishing methods in the molecular biology lab at FiBL.

Peter de Ruiter and Annette Freibauer supervised the progress of the project in the frame of the NRP 68. Both impressed me with the expertise and personalities and I`m very thankful for their inputs regarding design of the experiments and statistical analysis

In the course of this thesis a number of people contributed to this work by performing specialized measurements. I would like to thank all of them. In particular this refers to Reinhard Well and Dominik Lewicka-Szczebak who performed ^{15}N measurements and provided invaluable help in analyzing and interpreting ^{15}N data. From the laboratory at FiBL I would like to thank Anton Kuhn and Adolf Munyangabe for conducting countless geochemical measurement. The same was true for Ellen Struve and Karin Stögerer from Geomicrobiology group at University of Tübingen. Here I would like to emphasize the great help of Karin Stögerer who was always motivated to solve whatever problem occurred. From the University of Hohenheim Reiner Ruser was great help in measuring N_2O emissions for the first biochar incubation experiment. Many thanks also to Hans-Peter Schmidt for providing biochar for the first incubation experiment. His persistency raised biochar research in

Germany and Switzerland to a new level. Also I would like to thank Martin Hartmann from WSL for sharing his expertise in sequencing and statistical analysis.

I would like to thank all my fellow PhD colleagues from FiBL, the biochar group at the University of Tübingen and from the Agroscope at Reckenholz. In this regard I especially like to thank Johannes Harter for a great time in the lab and countless discussions. Also I would like to thank Maike Krauss for sharing her knowledge regarding gas chromatography, great times in the lab and numerous discussions. Also thanks to Roman Hüppi, Colin Skinner, Michael Scheifele and Nikolas Hagemann for sharing projects, sampling campaign, office space, thoughts and ideas.

With Anton Govednik, a master student from Ljubljana, I shared the ups and downs of a field sampling campaign. Thanks for your great work and being such a brilliant guy.

With Sarah Symanczik I shared countless coffee and cigarette breaks and her personality of spreading good mood wherever she goes is priceless. Also thanks to Timo Stadtlander for his great open nature and countless discussions on scientific and non-scientific issues. Also I would like to thank Jens Wohlfahrt for his great support during the last phase of the thesis.

Warm thanks goes to Lin and David Bautze for sharing office space and life in rural Switzerland

Great thanks to all my great teammates of Union Basel who share my passion for soccer and helped me to clear my mind every Tuesday.

Also I would like to thank my family Ruth Krause, Uta Süsse-Krause and Hatto Zeidler for their support whenever needed

Last but not least I would like to thank Sarah Daum for great times of support and being such a wonderful person.

Statement of personal contribution

The experimental setup of **Chapter 1** was developed by Maike Krauss, Simone Spangler and me. Analysis of Greenhous gas fluxes was mainly performed by Simone Spangler and Maike Krauss, while Molecular biological analysis was conducted by Simone Spangler and me. Geochemical soil analysis was done by Maike Kraus, Simone Spangler and me. Data analysis and interpretation was done by Maike Krauss, Andreas Gattinger, Sebastian Behrens, Ellen Kandeler, Andreas Kappler and me. The paper was written by Maike Krauss and me while all other authors greatly helped in improving the manuscript

The experimental setup for **Chapter 2** was developed by me with important inputs from Andreas Gattinger, Sebastian Behrens and Reinhard Well. Greenhouse gas measurements and soil geochemical analysis was done by me. Quantification of functional gene on DNA and RNA level was done by me. ^{15}N quantification in N_2O and N_2 were carried out by Dominika Lewicka-Szczebak. ^{15}N signal in NO_3^- and NH_4^+ was done by Wolfram Eschenbach. Interpretation of ^{15}N data was done by Reinhard well and me. Interpretation of molecular data was performed by Cecil Thonar, Sebastian Behrens and me. The paper was written by me great help from all authors.

The experimental setup for **Chapter 3** was designed by Sebastian Behrens, Johannes Harter, Andreas Kappler and me with support from Reiner Ruser and Thomas Scholten. The experiment was conducted by Johannes Harter and me. This includes all sample collection and samples preparation steps. Soil geochemical analyses were done by Ellen Struve, Markus Fromme, Johannes Harter and me. Greenhouse gas measurements as well as N_{min} analysis was performed by Reiner Ruser. DNA extraction as well as functional gene analysis on DNA level was performed by Johannes Harter and me. RNA extraction and functional gene analysis on RNA level was done by Stefanie Schüttler. Data analysis and interpretation was carried out by Johannes Harter and me. The manuscript was written by Johannes Harter, Sebastian Behrens, Andreas Kappler and me. All authors helped improving the manuscript

The experimental setup for **Chapter 4** was designed by Andreas Gattinger and me. The field trial was designed by Roman Hüppi and Jens Leifeld. Greenhouse gas emissions were quantified by Roman Hüppi and Jens Leifeld. Sampling campaign was conducted by Roman Hüppi and me. Soil geochemical analysis was performed by Roman Hüppi and me. Quantification of functional gene was conducted by me. Sample preparation for sequencing was performed by me with great support from Martin Hartmann. Sequencing was performed at Mc Gill University in Quebec by Pierre Lapage. Quality control of sequencing data was performed by me with help of Martin Hartmann and Johannes Harter. Mapping of gene sequences was done by Mohamed El-Hadidi. Statistical Analysis was done by me with great support from Martin Hartmann. Paper was written by me with great help of all authors

Curriculum vitae

Personal data

	Hans-Martin Krause 16.04.1984, Mühlacker, Germany
Contact:	Hildastrasse 50, 79102 Freiburg, Germany Weizacker 2, 5079 Zeihen, Switzerland hans.martin.krause@googlemail.com hans-martin.krause@fibl.org +49-17664302583 +0041-791206860

Education

Since 2013	PhD Candidate at Eberhard Karls University of Tübingen. Employed at the Research Institute of Organic Agriculture in the frame of the Swiss National Research Program 68: “Soil as a Resource” Title of the thesis: “Structure and function of N_2O producing and reducing microbial communities as influenced by soil management”
2006-2012	Studies of Geo-ecology at Eberhard Karls University Tübingen, Germany (grade 1.3) Title of the Diploma thesis: “Impact of biochar on microbial denitrification” Awarded with the Sustainability price of the University in Tübingen
2009-2010	Semester Abroad at Universidad de los Andes in Merida, Venezuela Title of the final project report: “Regeneration potential of agriculturally managed soils in the Paramo”
Till 2006	Abitur at Edith Stein Gymnasium in Bretten, Germany

Conferences and Meetings

21-22.11. 2014	Oral presentation at the Swiss Geoscience Meeting in Fribourg Title: "Impact of soil management on N_2O producing and reducing microbial communities"
5-10.9. 2015	Oral presentation at the Meeting of the German Soil Science Society in Munich Title: "Influence of organic and conventional long term fertilization in microbial N_2O production and reduction"
	Oral presentations at the DOK Colloquium in Zürich
14.2.2014	Title: "Influence of reduced tillage on N_2O emission potential and N cycling microbial communities"
11.3.2015	Title: "Impact of soil management on N_2O producing and reducing microbial communities"
11-13.12 2013	Poster presentation at the Soil Metagenomics Symposium in Braunschweig Title: "Influence of reduced tillage on N_2O emission potential and N cycling microbial communities"
29.11.-3.12. 2015	Poster presentation at the Ecology of Soil Microorganisms Conference in Prague Title: "Impact of farming systems on microbial denitrification and N_2O reduction"
20-21.112014 and 12-13.11. 2015	Poster presentation at the second and third NFP 68 program conference in Fribourg and Montreux Title: "Structure and function of N_2O producing and reducing microbial communities as influenced by soil management"

Supervision and educational activities

2013	Master thesis of Simone Spangler
	Supervision of the molecular biological part of the thesis
	Title: "Influence of soil tillage and fertilization on soil microorganisms linked with nitrous oxide production in different soil depths" (University of Hohenheim)
2015	Master thesis of Anton Govednik
	Supervision of the field experiment and molecular analysis

	Title: “Dynamics of denitrifying soil microbial community and greenhouse gas emissions under conventional and organic system of winter wheat production in dependence of pre-crop and fertilization” (University of Ljubljana)
Since 2013	Annual conduction of the seminar “ Introduction to Permaculture design” in the frame of the Studium Oecologicum at Eberhard Karls University of Tübingen with Sarah Daum
Since 2014	Annual conduction of the seminar “ Sustainable Soils” in the frame of the Studium Oecologicum at Eberhard Karls University of Tübingen with Sarah Daum
12.12.2013 and 15.1.2014	Conduction of Workshops for farmers on “Climate friendly agriculture” in collaboration with Bio Suisse

Publications

Harter J. [1], Krause H-M[1], Schuettler S., Ruser R., Fromme M., Scholten T., Kappler A. Behrens S.[2] (2014): Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. The ISME Journal, 8: 660-670,

Krauss M[1], Krause H-M[1,2], Spangler S., Kandeler E., Behrens S., Kappler A., Mäder P., Gattinger A. (2016): Tillage system affects fertilizer-induced nitrous oxide emissions. Biology and Fertility of Soils,

Krause H-M[2], Thonar C., Eschenbach W., Well R., Mäder P., Behrens S., Kappler A., Gattinger A. (Under Revision): N_2O production and reduction processes in soils as influenced by long term farming systems. Soil Biology and Biochemistry

Krause H-M[2], Hüppi R., Leifeld J., El-Hadidi M., Harter J., Kappler A., Hartmann M., Gattinger A. (submitted): Biochar and lime addition affects community composition of nitrous oxide reducing bacteria under cultivation of *Zea mays*. Environmental Microbiology

[1]both authors contributed equally, [2]corresponding author