Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2020:18

Nitrous oxide emission and turnover in arable cropping in South East Norway

Lystgassutslipp og -omsetning innen jordbruket i Sørøst-Norge

Aina Lundon Russenes

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Acknowledgements

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Fureneset, January 2020

Aina Lundon Russenes

Summary

Nitrogen is essential for all life on Earth. With an increasing global population, the use of reactive nitrogen has increased over the last century, resulting in increasing nitrogen pollution. Leaching of nitrate (NO_3^-) causing eutrophication has long been addressed, but it is only over the last three decades that problems with nitrous oxide (N_2O) have come into focus. N_2O is the third most abundant anthropogenic greenhouse gas (GHG), and atmospheric mixing ratios have increased from about 270 ppm in preindustrial times to 329 ppm in 2018. Nitrous oxide has a global warming potential almost 300 times higher than that of carbon dioxide (CO_2) in a 100-year perspective. It is also the dominant ozone depleting substance in the stratosphere. Globally, 6-8% of the anthropogenic greenhouse gas effect can be attributed to N_2O, of which 60% originates from crop production. Reducing N_2O emissions from crop production would thus be a major contribution to stabilizing atmospheric mixing ratios and reducing the GHG footprint of agro-food systems. N_2O is formed in naturally occurring microbial processes in soils, where nitrification (the microbial oxidation of NH_4^+ to NO_3^-) and denitrification (the microbial reduction of NO_3^- to N_2) are the quantitatively most important processes. Since N_2O formation is inevitable in these processes, it is of key importance to understand how external factors control the relative share of N_2O emitted in these processes. This is particularly important for denitrification, as it is the only known process that consumes N_2O by reducing it to harmless dinitrogen (N_2).

The research work of this thesis addressed knowledge gaps concerning the understanding and potential mitigation of N_2O emissions in Norwegian crop production with the specified objectives: i) Does the well-known negative relationship between soil pH and the N_2O product ratio of denitrification affect N2O emissions on the field level, where pH differences are relatively small? ii) Does split application of N to spring wheat instead of one-time fertilization at sowing reduce N_2O emissions relative to crop yield? iii) What controls the buildup of N_2O in the soil during winter, and does the fertilization rate from previous cropping affect winter emissions? iv) Do differences in arable cropping systems affect the soil's potential to nitrify and denitrify and to produce and consume N_2O? All field trials were performed at the NIBIO Apelsvoll research station, located in South-East Norway. Soils used for incubation experiments were also sampled there. The incubation experiments were carried out at NMBU.

Average N_2O emissions correlated negatively with pH in the field, when conditions favored denitrification, giving field-evidence that even small pH differences in the soil affect N_2O

emissions during off season. Addressing the pH dependency of N_2O in denitrification by optimizing soil pH management can thus be an effective way to mitigate N_2O emissions from arable cropping. Nitrogen fertilization given as split application to spring wheat increased yield and yield quality substantially relative to one-time fertilization at sowing, without affecting yield scaled N_2O emissions, thus indicating that optimized N application according to crop demand could be another strategy to reduce N_2O emissions in arable crop production. Field measurements confirmed that off-season emissions are quantitatively important in Norway. Fertilization from previous cereal cropping did not affect winter emissions in a one-year field trial, but a considerable buildup of N_2O in the soil during winter was observed. This buildup seemed to be driven by subnivean mineralization (i.e. N-release) of crop residues and/or soil organic matter in unfrozen soil under continuous snow pack. In addition to release of accumulated N_2O during spring thaw, *de novo* production of N_2O in thawing topsoil was found to be another important N_2O source. These processes are difficult to control, and the focus here must be to ensure conditions enabling complete denitrification, i.e. reduction of N_2O to N_2, a process closely correlated with pH, to lower the N_2O ratio. Long-term differences in arable cropping strategies affected the soil's potential to nitrify and denitrify and to produce and consume N_2O. Repeated application of organic matter as manure or crop residues and inclusion of catch crops or leys in the crop rotation all increased microbial activity in the soil, resulting in higher N_2O production potentials by denitrification, but not so much by nitrification. High nitrification potential and rapid conversion of mineralization-released NH_4^+ to NO_3^- in mixed systems with inclusion of grass-clover ley seem to result in a large N_2O emission potential.

As the rate of N_2O emitted from denitrification clearly is higher than that from nitrification (per unit N converted), the main focus should be to minimize denitrification in agricultural soil. If N_2O first is formed, it is essential to reduce as much as possible to N_2 to minimize the emissions of N_2O to the atmosphere.

Sammendrag

Nitrogen er essensielt for alt liv på jorda. Bruken av reaktivt nitrogen har økt med den økende globale befolkningen, særlig de siste hundre årene. Dette har ført til en større nitrogenbasert forurensing. Avrenning av nitrat (NO_3^-), som blant annet fører til eutrofiering, har fått stor oppmerksomhet i lang tid, men det er først i de siste tre tiårene at problemene med lystgass (N_2O) har kommet i fokus. N_2O er den tredje viktigste klimagassen, og konsentrasjonen av N_2O i atmosfæren har økt fra ca. 270 ppm i førindustriell tid til 329 ppm i 2018. Lystgass har et globalt oppvarmingspotensial omlag 300 ganger høyere enn karbondioksid (CO_2) sett i et hundreårsperspektiv. N_2O er også hovedårsaken til nedbrytning av ozon i stratosfæren. Globalt kan 6-8% av den antropogene drivhusgasseffekten tilskrives N_2O, og av dette kommer 60% fra planteproduksjon i jordbruket. En reduksjon av N_2O utslippet fra jordbruket kan derfor være et viktig tiltak for å stabilisere N_2O andelen i atmosfæren, og ikke minst for å redusere klimagassavtrykket fra matproduksjonen.

N_2O dannes gjennom mikrobielle prosesser i jord. Nitrifikasjon (mikrobiell oksidasjon av NH_4^+ til NO_3^-) og denitrifikasjon (mikrobiell reduksjon av NO_3^- til N_2), regnes som de viktigste prosessene som forårsaker økte N_2O-utslipp. Det er derfor viktig å forstå hvordan eksterne faktorer kontrollerer andelen av N_2O som slippes ut i atmosfæren ved omdannelsen av nitrogen gjennom nitrifikasjon og denitrifikasjon. Særlig gjelder dette for denitrifikasjon, som er den eneste kjente prosessen som kan omdanne N_2O til harmløst di-nitrogen (N_2).

Arbeidet i denne avhandlingen er ment å øke forståelsen av prosessene som leder fram til N_2O-utslipp fra jordbruket i Norge, og hvordan disse utslippene potensielt kan reduseres. Følgende hovedspørsmål adresseres: i) Påvirker den velkjente negative sammenhengen mellom pH og andelen N_2O fra denitrifikasjon også N_2O-utlippi felt når pH varierer relativt lite? ii) Kan delgjødsling av N til vårhvete som et alternativ til å gi all gjødsla om våren ved såing redusere avlingsrelaterte N_2O-utslipp? iii) Hva kontrollerer økte N_2O-konsentrasjoner i jorda under snødekke, og er utslippene påvirket av N-gjødslingsmengdene gitt i vekstsesongen? iv) Blir jordas potensiale for nitrifikasjon og denitrifikasjon påvirket av ulike dyrkingssystem? For å besvare disse spørsmålene ble det gjennomført både feltforsøk og inkubasjonsstudier i laboratorium. Alle feltforsøk ble gjennomført ved NIBIO Apelsvoll forskningsstasjon på Østlandet, der også jordprøver til inkubasjonsstudiene ble tatt. Inkubasjonsstudiene ble gjennomført ved NMBU.

Utslippene av N_2O korrelerte negativt med pH målt i felt i perioder der forholdene favoriserte denitrifikasjon. Dette beviser at sammenhengen mellom pH og N_2O-utslipp også gjelder for vanlig jordbruksjord, særlig utenfor vekstsesongen, selv ved liten variasjon i pH. Optimalisering av jordas pH kan derfor være en effektiv måte å redusere N_2O-utslipp fra jordbruket. Delgjødsling med nitrogen i vårhvete ga betydelig høyere avlinger og bedre avlingskvalitet enn ledd som ikke ble gjødslet eller som bare ble gjødslet ved såing (mindre totalmengde N enn ved delgjødsling), men det var ingen forskjell i avlingsrelaterte N_2O-utslipp mellom ulike mengder N tilført ved delgjødsling. Dette viser at en optimal gjødsling kan bedre kvaliteten uten økte utslipp av N_2O. Målinger i felt viste at N_2O-utslipp utenfor vekstsesongen er av stor betydning i Norge. Mengden N-gjødsel gitt i veksesongen i et ettårig feltforsøk påvirket ikke utslippene av N_2O gjennom vinteren, men det ble observert en betydelig økning i N_2O-konsentrasjonen i det øvre jordlaget under snødekke gjennom vinteren. Denne økningen hadde trolig opphav i mineralisering (frigjøring av N) fra planterester og/eller annet organisk materiale i det øvre jordlaget under et snødekke som virket isolerende på det øvre jordlaget og forhindret frost i jorda. Ved snøsmelting ble N_2O som var blitt akkumulert i jorda gjennom vinteren frigjort, men i tillegg ble en betydelig andel N_2O dessuten dannet i forbindelse med tining/snøsmelting. Dette er prosesser som er vanskelig å kontrollere. For å minimere utslippene er det viktig å sørge for at forholdene for fullstendig denitrifikasjon (reduksjon av N_2O til N_2) er til stede. Denne prosessen er korrelert med pH, og optimalisering av pH kan også redusere andelen av N_2O som slippes ut i perioden omkring snøsmelting. Forsøkene viste også at langtidseffektene av ulike dyrkingssystemer påvirket jordas potensielle nitrifikasjon og denitrifikasjon, samt produksjon og konsumering av N_2O. Gjentatt tilførsel av organisk materiale som husdyrgjødsel eller planterester og bruk av fangvekster eller eng i omløpet økte den mikrobielle aktiviteten i jorda. Dette førte til økt potensiell N_2O-produksjon fra denitrifikasjon, men påvirket ikke potensiell nitrifikasjon tilsvarende. I systemene med kløverrik eng ble det observert høyere nitrifikasjonspotensial, og rask omdanning av mineralisert NH_4^+ til NO_3^- i disse systemene kan føre til økte utslipp av N_2O.

Denitrifikasjon bidrar med en betydelig høyere andel av N_2O-utslippene enn nitrifikasjon. Hovedfokuset for jordbruket bør derfor være å minimere denitrifikasjonen, men der N_2O først har blitt dannet i jorda, er det viktig å sørge for en så effektiv reduksjon av N_2O til N_2 som mulig, for å minimere N_2O utslippene til atmosfæren.

Abbreviations

AOA – ammonia oxidizing archaea
AOB – ammonia oxidizing bacteria
C – carbon
CH_4 – methane
CO_2 – carbon dioxide
DNRA –dissimilatory nitrate reduction to ammonia
DOC – dissolved organic carbon
DON – dissolved organic nitrogen
GHG – greenhouse gas
GWP – global warming potential
H_2O – water
He - helium
IPCC – Intergovernmental Panel on Climate Change
Kg ha^{-1} – kilogram per hectare
ml – milliliter
mM – millimolar
N_2 – nitrogen
N_2O – nitrous oxide
N_2OR – nitrous oxide reductase enzyme
NAR – nitrate reductase enzyme
NH_2OH – hydroxylamine
NH_3 – ammonia
NH_4^+ – ammonium
NIR – nitrite reductase enzyme
NO – nitric oxide
NO_2^- – nitrite
NO_3^- – nitrate
NOB – nitrite oxidizing bacteria
NOR – nitric oxide reductase enzyme
N_r – reactive nitrogen
O_2 – oxygen
ppm – parts per million
ppmv – parts per million volume
PCA - principal component analysis
WFPS – water filled pore space
μL – microliter

List of papers

Paper I

Russenes AL, Korsaeth A, Bakken LR, Dörsch P (2016) Spatial variation in soil pH controls off-season N_2O emission in an agricultural soil. Soil Biology and Biochemistry 99:36-46 DOI: 10.1016/j.soilbio.2016.04.019. Reprinted by permission.

Paper II

Russenes AL, Korsaeth A, Bakken LR, Dörsch P. Effects of nitrogen split application on seasonal N_2O emissions in southeast Norway. Nutrient Cycling in Agroecosystems 115, 41-56. DOI: 10.1007/s10705-019-10009-0. Reprinted by permission.

Paper III

Russenes AL, Korsaeth A, Bakken LR, Dörsch P. Long-term effects of cropping system on potential N_2O production by nitrification and denitrification. Manuscript. To be submitted to Agriculture, Ecosystems & Environment after a final refinement.

Synopsis

1. Introduction

1.1 Background

Molecular nitrogen (N_2) is the most abundant molecule in Earth's atmosphere, yet it is a limiting key element for life on Earth. To become accessible, the relatively inert N_2 molecule has to be converted to reactive N (N_r), i.e. transferred to its reduced or oxidized species. The only natural processes creating N_r are NO_x formation by lightning and the incorporation of atmospheric N into organic N by nitrogen fixing bacteria. In terrestrial N-cycling, nitrogen-fixing bacteria, either free-living or in symbiosis with leguminous plants, are the quantitatively most important source of N_r. Industrial synthetization of nitrogen in the 20th century by the Haber-Bosh process and intense farming of legumes have more than doubled the amount of N_r input to the biosphere (Galloway *et al.*, 2004). Ever increasing amounts of reactive N are added as fertilizers and biologically fixed N to the biosphere to sustain a rapidly increasing global population. This leads to a massive alteration of the global N cycle with severe side effects such as eutrophication and associated loss of biodiversity, atmospheric pollution (NH_3, NO_x) and accumulation of the radiative active trace gas nitrous oxide (N_2O) in the atmosphere. Nitrous oxide is the third most abundant anthropogenic greenhouse gas (GHG) and atmospheric mixing ratios have increased from about 270 ppm in preindustrial time (WMO, 2013) to 329 ppmv in 2018 (Prinn *et al.*, 2018). Nitrous oxide has a global warming potential 263 times higher than that of carbon dioxide (CO_2) in a 100 year perspective (Neubauer and Megonigal, 2015). It is also the dominant ozone depleting substance in the stratosphere (Ravishankara *et al.*, 2009).

Globally, nitrous oxide contributes 6 - 8 % to the anthropogenic greenhouse gas effect (Montzka *et al.*, 2011). Modern agriculture is the single largest source of rising atmospheric N_2O concentrations, about 60 % of which are attributed to the use of N_r in crop production (IPCC, 2007; Syakila and Kroeze, 2011). In Norway, the equivalent N_2O contribution is 5%, and 74% of this origins from agriculture (Statistisk sentralbyrå [SSB], 2019). Mineral fertilizers and manure added to cropping systems produce N_2O as a side product of soil nitrification and as an intermediate of soil denitrification (Erisman *et al.*, 2008). The concurrence of increasing global N-use and atmospheric N_2O concentrations over the last 120 years is illustrated in Fig. 1 and suggests that the increase in atmospheric N_2O is tightly linked to the increasing input of reactive N into the biosphere.

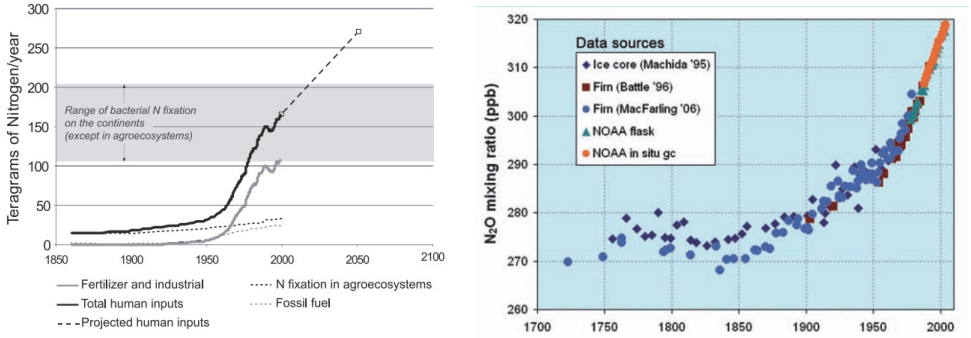


Figure 1. Trends in estimated anthropogenic N inputs since 1850 (left) and measured changes in atmospheric concentrations of N_2O over the last 250 years (right). From (Howarth et al., 2006) and (Lassey and Harvey, 2007), respectively.

As shown in Fig. 2, an estimated share of 32 % of the global N_2O emissions come from anthropogenic sources and of this 77% is of agricultural origin (Davidson *et al.*, 2013). Reducing N_2O emissions from crop production would therefore be a major contribution to stabilizing atmospheric mixing ratios and reducing the GHG footprint of agro-food systems as a whole.

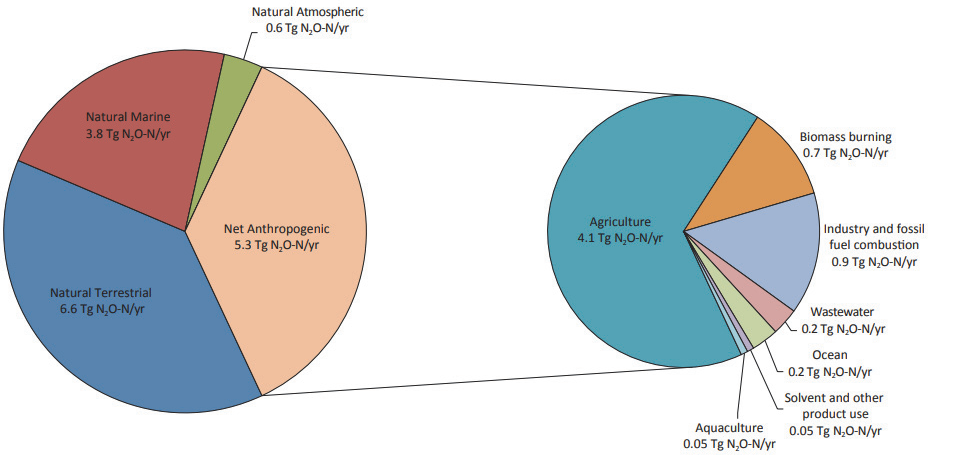


Figure 2: Natural vs. anthropogenic N_2O emissions in 2005 Davidson et al. (2013).

1.2 The quest for sustainable use of reactive nitrogen

Population growth and changing diets as predicted for 2050 will increase the demand for food and feed by 50% compared to 2010 (Godfray *et al.*, 2010; FAO, 2017). Given that soil resources are limited and in many regions under pressure due to overuse and climate change (FAO and ITPS, 2015), continued intensive crop production in industrialized countries will be necessary. Moreover, according to international standards, every country has to provide food security for its own population (FAO *et al.*, 2018) and is hence obliged to facilitate agronomic growth. Taken together, this makes it likely that the use of reactive N will increase rather than decrease in the nearest future, unless agri-food systems are transformed to more nitrogen efficient systems. As long as this is not the case, ways have to be sought to minimize the emission of N_2O per unit nitrogen used for crop production.

Among the three major anthropogenic greenhouse gases (CO_2, CH_4 and N_2O), N_2O is the least understood. The IPCC special report "Global warming of 1.5ºC" from 2018 concludes:

> *"N_2O emissions decline to a much lesser extent than CO_2 in currently available 1.5°C-consistent pathways, … reflecting the difficulty of eliminating N_2O emission from agriculture … As a result, sizeable residual N_2O emissions are currently projected to continue throughout the century, and measures to effectively mitigate them will be of continued relevance for 1.5°C societies…."*.

Alluding to possible mitigation pathways in agriculture, the same report states:

> *"Finally, several mitigation measures that could affect these agricultural non-CO_2 emissions are not, or only to a limited degree, considered in the current integrated pathway literature. Such measures … are very diverse and differ in their development or deployment stages." (IPCC, 2018)*

This assessment of the status of N_2O mitigation in crop production by an internationally recognized scientific body is devastating and reflects the fact that despite more than 30 years of N_2O emission research, no valid mitigation strategies have emerged, other than "good agronomic practice" which tries to limit the use of reactive N. It also highlights that research into mitigation of direct N_2O emissions, next to CH_4 emissions from ruminants, is high on the agenda, also in a carbon-neutral or carbon-negative world. To develop country-specific strategies for N_2O mitigation requires detailed knowledge about how crop and soil

management practices affect soil N-transformation. The current thesis aims to widen the knowledge base needed for mitigating N_2O emissions in Norway.

1.3. Soil microbial processes involved in N_2O turnover

The main reason for the unresolved mitigation of N_2O emissions in crop production is its biological origin and control in multiple, partly interlinked microbial processes, naturally occurring in soils. Nitrification (the microbial oxidation of NH_4^+ to NO_3^-) and denitrification (the microbial reduction of NO_3^- to N_2) are considered to be the quantitatively most important processes for N_2O emissions. Both processes form N_2O, whereas denitrification is the only known process that can consume N_2O by reducing it to harmless dinitrogen (N_2). Firestone and Davidson (1989) presented a "hole in the pipe" model (Fig. 3) to illustrate how both processes interlink in the production of nitic oxide (NO) and nitrous oxide (N_2O). Indeed, nitrification produces the substrate of denitrification by oxidizing NH_3 (which stands in a chemical equilibrium with NH_4^+) to NO_3^-/NO_2^-.

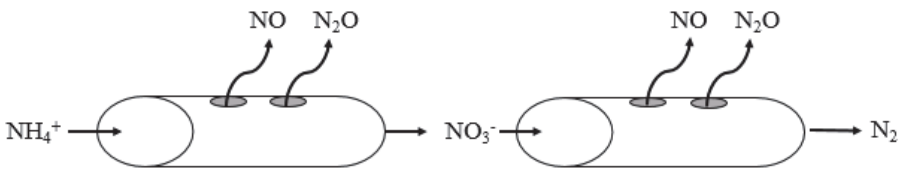
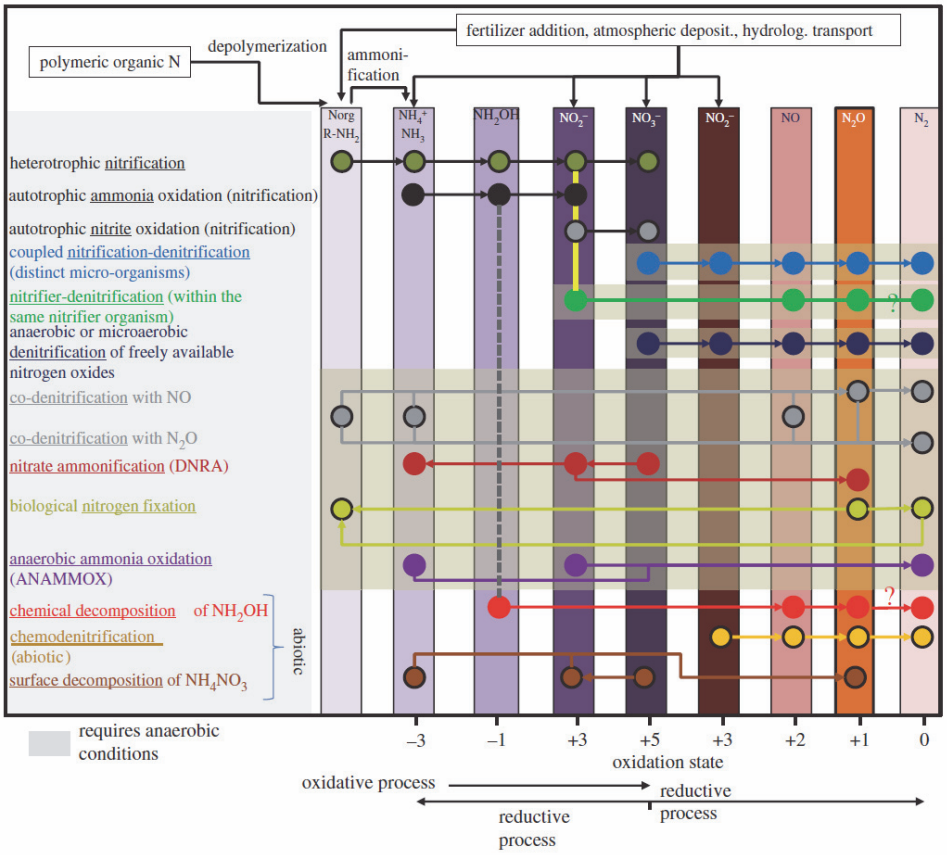


Figure 3. The "hole in the pipe" model describing regulation of NO and N_2O flux. Adapted from Firestone and Davidson (1989)

Besides nitrification and denitrification, there are multiple microbial processes contributing to N_2O formation. Recent research shows that the microbial communities and biochemical pathways involved in N_2O production by these processes are not fully understood (Morley *et al.*, 2008; Baggs, 2011; Schreiber *et al.*, 2012; Thomson *et al.*, 2012; Butterbach-Bahl *et al.*, 2013; Graf *et al.*, 2014; Stieglmeier *et al.*, 2014). A more modern view of microbial and chemical processes involved in N_2O production and consumption is given by Butterbach-Bahl *et al.* (2013) and presented in Fig. 4. After this review was published, a novel microbial N-transformation process was discovered for certain ammonia oxidizing bacteria (*Nitrospira* spec.): COMMAMOX - complete ammonium oxidization (Daims *et al.*, 2015; van Kessel *et al.*, 2015), which oxidizes NH_3 directly to NO_3^- , in contrast to dividing this process among two separate groups of ammonia oxidizing bacteria and archaea (AOB + AOA) and nitrite

oxidizing bacteria (NOB). This adds one more player to the N cycle potentially producing N_2O. However, a recent study by Kits *et al.* (2019) suggests that the N_2O yield of Commamox is rather small.



Abbreviations: Norg/R-NH_2, monomeric organically bound N forms; NH_4^+, ammonium; NH_3, ammonia; NH_2OH, hydroxylamine; NO_2^-, nitrite; NO_3^- nitrate; NO, nitric oxide; N_2O, nitrous oxide; N_2, molecular dinitrogen. DNRA, Dissimilatory Nitrate Reduction to Ammonium

Figure 4. Biotic and abiotic processes directly or indirectly involved in N_2O formation and consumption. Processes requiring limitations of O_2 are underlined by grey segments. Processes predominantly requiring anaerobic (or micro-aerobic) conditions are underlined by grey illuminated segments (Butterbach-Bahl et al., 2013)

1.4 Effects of crop and soil management on N_2O emissions

Direct N_2O emissions from soil are known to exhibit large spatial and temporal variability (Parkin, 1987; Röver *et al.*, 1999; Mathieu *et al.*, 2006) complicating the monitoring of annual N_2O emissions even on a sub-hectare basis. This variability is commonly attributed to the

heterogeneity in conditions known to affect major N turnover processes in the soil matrix. Particularly the distribution of O_2 and the diffusion of NH_4^+ and NO_3^- between oxic and anoxic compartments are important in controlling nitrification and denitrification (Fig. 4) and hence the magnitude of N_2O emissions (Smith, 1980; Schlüter *et al.*, 2019). Denitrification produces significantly more N_2O from reducing NO_3^- than nitrification from oxidizing NH_4^+ (see chapter 1.5 and 1.6). The distribution and bioavailability of organic carbon is another important factor (Megonigal *et al.*, 2003). It fuels heterotrophic activity in soil and may create anoxic hotspots which support denitrification. Oxygen is the preferred terminal electron acceptor of most denitrifying organisms as it generates more energy than the respiratory reduction of NO_3^- (ibid). Soil organic matter is also the source for NH_4^+, the substrate of nitrification when no fertilizer NH_4^+ is available. Soil water content relative to soil porosity strongly affects O_2 transport and the diffusion of substrates to and from reducing or oxidizing zones. Within the soil matrix different physicochemical conditions can co-occur in close proximity as illustrated in Fig. 5 (Strong and Fillery, 2002). This creates niches for metabolically distinct organisms such as nitrifiers and denitrifiers, which can be active at the same time and connected through diffusion of substrates.

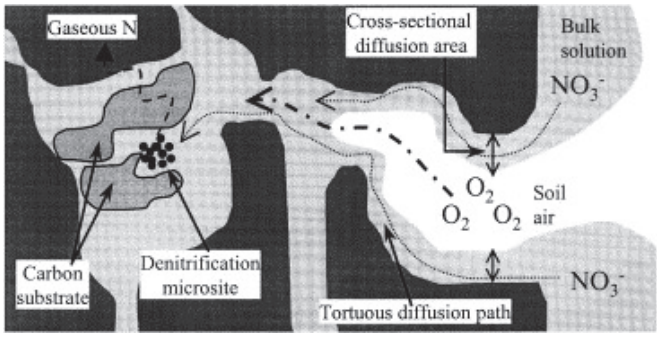


Figure 5 Physical/chemical environment of a denitrification microsite (Strong and Fillery, 2002)

New insights into the dynamics of microbial N-transformations support the importance of soil moisture, pO_2, pH, substrates and microbial taxonomic composition as proximal and distal controllers for N_2O emissions (Bakken *et al.*, 2012; Hallin *et al.*, 2018). Yet, translating novel knowledge about the regulation biology of N_2O into effective mitigation strategies in complex plant-soil systems is not trivial. For instance, little is known about how crop and soil

management affect microscale conditions for nitrifiers and denitrifiers on the long run. While bulk soil properties like water filled pore space (WFPS) and pH can be directly controlled by tillage and liming, small-scale heterogeneity of moisture, O_2, and effective H^+ and base cation concentrations may remain unaffected or alter only slowly over decades. In general, management practices increasing soil aeration by reducing compaction or disturbance of macro-pores created by roots and other biological activity are considered to lower N_2O emissions. Better aeration reduces the anoxic volume of the soil and hence the magnitude of denitrification. On the other hand, absence of soil tillage leads to a denser soil within the first years with less surface infiltration and more organic carbon supporting O_2 consumption which would support denitrification. Accordingly, largely inconsistent effects of tillage regime on N_2O emissions have been reported (van Kessel *et al.*, 2013). Another example is soil drainage. All cultivated soils in Norway have a positive water balance with more precipitation than evapotranspiration on a yearly basis, even though early-summer droughts occur regularly. Combined with the frequent occurrence of marine clays and stagnant conditions in organic-rich soils, many soils have to be drained artificially to reach potential yields. A common notion is that improving drainage systems should mitigate N_2O emissions by improving soil aeration (Grossel *et al.*, 2016). A recent field experiment on a molic-umbric gleysol in Western Norway shows, however, that new tile drainage can have the opposite effect, with N_2O emissions increasing along with the number of field drains, probably because tiling disturbs the naturally organic-rich soil releasing carbon and nitrogen, as well as lowering the WFPS levels, which may have resulted in higher occurrence of periods with WFPS between 60 and 80% (Hansen *et al.*, In prep.).

Among the more promising management practices to reduce N_2O emissions from cropped soils are soil pH management and customizing N supply to plant requirements by split-application. Most soils in Norway are naturally acidic and acidity has been shown to unequivocally suppress N_2O reduction to N_2 in denitrification (Bakken *et al.*, 2012). Likewise, decreasing the availability of N substrates for microorganism during crop production by better timing and dosage of N fertilizers has been shown to have a N_2O mitigation potential (van Groenigen *et al.*, 2010).

In the following, a more detailed account of the ecology of nitrifiers and denitrifiers and their N_2O producing (and reducing) pathways is given.

1.5 Nitrification

Nitrification is the microbial oxidation of ammonia (NH_3) to nitrite (NO_2^-) and further to nitrate (NO_3^-). This process was discovered in the late 19th century and up to 2005 was believed to be mediated by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) only. In 2005, ammonia oxidizing archaea (AOA) were discovered (Könneke *et al.*, 2005). Leininger *et al.* (2006) found that AOA actually outnumber AOB in soils. Recently, certain members of the phylum *Nitrospirae* were found to oxidize NH_3 all the way to NO_3^- (Daims *et al.*, 2015; van Kessel *et al.*, 2015). Including "complete ammonia oxidizing organisms (Comammox)", there are now three prokaryotic groups mediating the conversion of NH_4^+ to NO_3^- in soils.

Common to all three groups is that their activity is strongly controlled by NH_3 availability and pH. The NH_3 availability depends on the magnitude and source of NH_4^+ supply, the soil's clay content and the soil pH. Clay may transiently fix NH_4^+ while pH controls the partitioning between NH_4^+ and NH_3. Nitrifiers in general are poor competitors for NH_4^+ in the soil, as nitrification usually only increases when NH_4^+ supply exceeds the demand of plants and heterotrophic prokaryotes (Robertson and Groffman, 2007). Plant requirements for mineral nitrogen are usually high and return of plant residues with high C:N ratios favors heterotroph microbial activity and microbial immobilization of inorganic N. Autotrophic nitrifiers are not directly stimulated by organic matter return as they assimilate C from CO_2 (AOB and Comammox) or bicarbonate (AOA), but the mineralization of organic matter ultimately provides the NH_4^+ needed for their energy metabolism. All nitrifiers are obligate aerobes as oxygen is required for the oxidation of NH_3 to NO_2^- and further to NO_3^-. Access to oxygen (molecular and dissolved in water) is therefore the second most important factor regulating nitrification in soils (Firestone and Davidson, 1989; Schuster and Conrad, 1992; Robertson and Groffman, 2007). Soil pH is another important factor. It controls the chemical equilibrium between NH_4^+ and NH_3, the substrate of ammonium monooxygenase (*amo*A), catalyzing the first and rate-limiting oxidation step in nitrification. AOB and NOB have a pH optimum at 7.5 – 8.0 (Prosser, 1990), but nitrification was always known to occur in acidic soils (Robertson, 1989). The discovery of ammonia oxidizing archaea (AOA) has largely solved the pH conundrum of nitrification by showing that nitrifying archaea outnumber AOB under acidic conditions (Gubry-Rangin *et al.*, 2011). In general, AOA seem to be more robust in coping with adverse conditions such as acidity and low NH_3 availability. They also have been shown to be less sensitive than AOB to certain nitrification inhibitors (Lehtovirta-Morley *et al.*,

2013). Since AOA seem to thrive at low NH_3 concentrations (Lehtovirta-Morley, 2018), there has been some controversy about whether AOA can play a significant role in fertilized soils (Di *et al.*, 2009). There is ample evidence, however, that AOA account for a significant part of the nitrifying activity in moderately acid soils even if fertilized regularly (Gubry-Rangin *et al.*, 2010; Hink *et al.*, 2017b; Nadeem *et al.*, *in revision*). The relative abundance of AOB and AOA is important as AOA produce inherently less N_2O per unit oxidized NH_3 than AOB (Hink *et al.*, 2017b; Tzanakakis *et al.*, 2019). Hence, agronomic practices increasing the relative abundance of AOA over AOB may potentially reduce N_2O emissions from nitrification (Hink *et al.*, 2018).

Autotrophic nitrification can form N_2O in two ways: i) during the oxidation of hydroxylamine, an obligate intermediate of NH_3-oxidation to NO_2^- and ii) by enzymatic reduction of NO_2^- to NO and further to N_2O under partially anaerobic conditions. Since all known AOB possess homologues of the denitrification genes *nir* and *nos*, encoding for nitrite and nitric oxide reduction, respectively, the latter process was termed "nitrifier denitrification". Nitrifier denitrification has long been considered to be the dominant pathway of N_2O production in nitrification and was thought to sustain respiratory metabolism during partial anoxia (Prosser, 1990; Kool *et al.*, 2011; Wrage-Mönnig *et al.*, 2018). Recent work with AOB pure cultures, however, has shown that the contribution of "nitrifier denitrification" to energy yielding cellular respiration is miniscule (Hink *et al.*, 2017a). This casts some doubt on the dominance of "nitrifier denitrification" as the main source of N_2O from nitrification.

Irrespective of the biochemical pathway of N_2O production in nitrification, autotrophic ammonia oxidation plays a major role for N_2O emissions by i) being a source of N_2O, ii) providing NO_2^- and NO_3^- as substrates for denitrification and iii) being a major oxygen sink in the soil during periods of high nitrifier activity. The oxidation of 1 mol of NH_3^+ to NO_3^- consumes 2 moles of O_2 and if stimulated by NH_4^+ fertilization or liming, may easily induce local anoxia and induce heterotrophic denitrification (see below). Ammonium-induced heterotrophic denitrification is termed coupled nitrification-denitrification (Nadeem *et al.*, *in revision*). In summary, the abundance and activity of NH_3 oxidizing bacteria and archaea depend on NH_4^+/NH_3 availability and soil pH. Vigorous nitrification activity produces N_2O, oxidative by hydroxylamine decomposition, reductive by reduction of nitrite, and probably most importantly, by consuming oxygen, which induces heterotrophic denitrification. Hence, fertilizer formulation and timing as well as chemical inhibitors should have a certain potential to mitigate N_2O emissions associated with nitrification.

1.6 Denitrification

Denitrification is an anaerobic respiratory process, reducing NO_3^- stepwise to N_2 in the absence of oxygen with NO_2^-, nitric oxide (NO) and N_2O as obligate intermediates. The ability to denitrify is found among numerous, phylogenetically unrelated bacteria, some archaea and few fungi. Heterotrophy is the dominant life form among denitrifiers, though lithotrophy (use of reduced inorganic compounds as energy source) and phototrophy (use of light as energy source) are known. In the context of soil, denitrification is generally described as a process requiring an organic carbon source and hence considered to be heterotrophic (Firestone and Davidson, 1989; Davidson *et al.*, 2000). Even though denitrifying activity is constrained by physical factors (anoxia) and substrate availability (carbon, NO_2^-, NO_3^-), the abundance of bacterial denitrifiers seems to be highly stable (Butterbach-Bahl *et al.*, 2013; Chen *et al.*, 2019; Roco *et al.*, 2019).

Denitrification is triggered by oxygen shortage in the presence of N-oxides (Zumft, 1997). Thus, carbon availability, N-oxides and absence of O_2 are the most important controllers for denitrification activity in soil. Both, O_2 availability and NO_3^- availability are highly controlled by soil moisture. Oxygen diffusion is 10000 times lower in water than in air (Megonigal *et al.*, 2003) and NO_3^- has to diffuse to the site in soil at which denitrification is active, which requires water. Denitrification activity is therefore believed to be localized in soil in so-called "hot-spots", i.e. saturated microsites of high respiratory activity that become anoxic but still have access to N oxyanions (Strong and Fillery, 2002; Schlüter *et al.*, 2019). These conditions occur during so-called "hot-moments", i.e. when respiratory activity in hot spots surpasses O_2 diffusion from the atmosphere to the hotspot. The former explains why seemingly well aerated soils denitrify, whereas the latter is used to explain the high temporal variability of N_2O emissions with N_2O emission peaks exceeding background emissions by three orders of magnitude (e.g. Flessa *et al.*, 1995). The episodic nature of N_2O emissions due to vigorous denitrification during "hot moments" makes it difficult to scale up measured fluxes to annual emissions (Groffman *et al.*, 2000) and to derive statistically significant mitigation measures in field experiments.

As N_2O is an obligate intermediate in denitrification, the rate of denitrification will influence the amount of N_2O emitted to the atmosphere (Firestone and Davidson, 1989; Butterbach-Bahl *et al.*, 2013). A second important controller is the inherent stoichiometry of the overall

process, i.e. the product ratio of N_2O relative to N_2, also written as $N_2O/(N_2O+N_2)$. The overall process of reducing nitrate to N_2 is mediated by the key enzymes (in the order of the reaction sequence) nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR) and nitrous oxide reductase (N_2OR) and can be written as:

$$2\,NO_3^- \xrightarrow{NAR} 2NO_2^- \xrightarrow{NIR} 2NO(g) \xrightarrow{NOR} N_2O(g) \xrightarrow{N2OR} N_2(g) \qquad (1)$$

The complete reduction of 1 mol of NO_3^- to 1 mol of N_2 consumes 10 moles of electrons which are used for energy generation, only 20% of which is generated in the last denitrification step (Zumft, 1997). Whereas many denitrifying organisms have genes coding for all four key enzymes, some denitrifiers lack the gen for N_2O reduction, thus making N_2O the final product of denitrification (Graf *et al.*, 2014). Hence, diversity and abundance of denitrifying bacteria may affect the product stoichiometry of denitrification with consequences for N_2O emissions.

Another way to affect the product ratio of denitrification is by cellular regulation of the expression or activity of the enzymes involved. Upon encountering anoxia in soil, denitrifiers have to switch from oxic to anoxic respiration, which requires coordinated *de novo* expression of denitrification enzymes if NO_3^- is to be reduced completely to N_2. Not all denitrifiers are able to do so and cultured denitrifiers vary widely in the way they coordinate the induction of denitrification enzymes when facing anoxia (e.g. Liu *et al.*, 2013), which has led to the term "denitrification regulatory phenotype" (Bergaust *et al.*, 2011). Therefore, the inherent N_2O product ratio of a soil denitrifier community depends, among others, on its taxonomic composition (Braker *et al.*, 2012). Next to community composition, external factors play an important role in regulating the N_2O product ratio of denitrification. For instance, the reductant to oxidant ratio, i.e. the availability of readily decomposable carbon as an electron donor relative to the availability of NO_3^- as an electron acceptor has been reported to affect the N_2O product ratio (Wu *et al.*, 2018). The most pervasive external factor to affect the N_2O product ratio appears, however, to be soil pH with increasing $N_2O/(N_2O+N_2)$ ratios with decreasing soil pH. This relationship was already reported in the 1950ties by Wijler and Delwiche (1954) and Nommik (1956) who observed this phenomenon long before N_2O emissions from soils became an environmental issue. Since then, the principal effect of pH on the N_2O product ratio has been confirmed in many laboratory studies (Simek and Cooper,

2002; Liu *et al.*, 2010; Raut *et al.*, 2012; Qu *et al.*, 2014; Obia *et al.*, 2015). More recently, the underlying mechanisms have been discovered. It was found that low pH interferes with the making of the N_2OR enzyme in the periplasm of gram-negative denitrifying bacteria, apparently inhibiting the maturation of the enzyme (Bergaust *et al.*, 2010; Liu *et al.*, 2014; Samad *et al.*, 2016). Based on this relationship, it was concluded that liming of acidic soils could be a promising way to reduce N_2O emissions from denitrification (Bakken and Frostegård, 2017), but field evidence for this was missing.

Soil pH is a master variable (Brady and Weil, 1999) affecting virtually every aspect of soil chemistry and microbial activity in soil, and is thus easily confounded with other factors when exploring direct causal relationships (e.g. Bakken *et al.* (2015)). Accordingly, Bandibas *et al.* (1994) found no positive correlation between N_2O emissions and soil acidity and variable relationships have been reported from liming experiments (Goodroad and Keeney, 1984; Stevens *et al.*, 1998). Rapid increase of soil pH by liming may even increase N_2O emissions by transiently enhancing microbial respiration and nitrogen mineralization, as shown by Curtin *et al.* (1998) or by increasing nitrification through shifting the NH_4^+/NH_3 equilibrium towards NH_3 and increasing the AOB/AOA abundance ratio (Nadeem *et al.*, *in revision*). Soil pH also influences C availability by controlling sorption and desorption of dissolved organic matter (DOM) and may thus affect denitrification (Gmach *et al.*, 2020). Whereas direct effects of soil management on soil processes are well studied, little is known about how long-term differences in soil management as given by different cropping systems affect nitrification and denitrification and their N_2O product stoichiometries.

1.7 Nitrogen turnover in Norwegian arable cropping

In Norway, only 3% of the total land area is cultivated, and only one third of this area is suitable for grain or other arable cropping. Norway hence relies on import of e.g. fruit, vegetables and grain. Actually less than 50% of the food required to feed the Norwegian population is produced in Norway, and only 37% of it is produced without imported concentrates (Arnoldussen *et al.*, 2014). Since the 1970s, agricultural production has been geographically divided, with husbandry at large being located in marginal regions where soil conditions are less suited for arable cropping. Only 32 % of the total farmed area in Norway is arable (Arnoldussen *et al.*, 2014) and over 80 % of this area is located in the South-Eastern parts of Norway (Stabbetorp, 2019). One important implication of this regional division of agricultural management within Norway is that arable cropping receives little or no input of

animal manures. Arable cropping in Southeast-Norway thus relies largely on the input of mineral N and P in the form of commercial fertilizers, which are known to lower the soil pH (Malhi *et al.*, 1998). In addition to the natural acidity of the mostly siliceous Norwegian soils, soils under arable cropping have relatively low soil pH, as input of acidifying mineral fertilizers are high while liming has been neglected over the last decades (Nesheim, 2014). Growing seasons in Norway are short and the use of winter varieties or cover crops is uncommon. Fields are often left bare during winter, prone to leaching of base cations. Microbial processes are left with little or no competition by plants during off-season and likely dominate nitrogen processes in winter. Depending on snow pack conditions, Norwegian arable soils can be exposed to pronounced freezing/thawing cycles which have been shown to fuel off-season mineralization and N_2O emissions (Sehy *et al.*, 2004; Mørkved *et al.*, 2006; Risk *et al.*, 2014; Song *et al.*, 2017). This makes off-season processes an important aspect of N_2O emission research in Norway as well as on-season processes. All these factors may have implications for N-turnover in general and N_2O -turnover in particular and form the backdrop for the research objectives of this thesis.

2. Objectives

The research work of this thesis addressed knowledge gaps concerning the understanding and potential mitigation of N_2O emissions in Norwegian crop production. Specifically the thesis addressed following questions:

i) Can the well-known negative relationship between soil pH and the N_2O product ratio of denitrification be found on a field level with relatively small differences in soil pH and does soil pH affect *in situ* emissions during off-season? (Paper I)

ii) Does split application of N to summer wheat instead of one-time fertilization at sowing reduce N_2O emissions relative to crop yield? (Paper II)

iii) What controls the buildup of N_2O in the soil during winter and does fertilization rate from previous cropping affect winter emissions? (Paper II)

iv) Do differences in arable cropping systems affect the soils potential to nitrify and denitrify and to produce and consume N_2O? (Paper III)

3. Materials and methods

3.1 Location of field trials and sampled soil

All field trials (Paper I and II) were performed at the NIBIO Apelsvoll research station, and all soils used for incubation experiments (Paper I and III) were sampled there. The NIBIO Apelsvoll research station is located in Southeast Norway (60°42′ N, 10°51′ E, 250 m above sea level), and the fields involved in this work are classified as imperfectly drained brown earth (Gleyed melanic brunisoils, Canadian System of Soil Classification) with dominantly loam and silty sand textures. For the period 2000-2014, mean annual precipitation was 693 mm and mean annual air temperature at 2 m was 5.1 °C.

Meteorological data were obtained from the meteorological station at Apelsvoll, located approx. 150 m from the experimental site described in Paper I, 400 m from the experimental site described in Paper II, and 300 m from the soil sampling site described in Paper III. Soil temperature and volumetric moisture content were measured continuously by sensors (5TE, Decagon Devices, Inc.) permanently installed at depths of 5, 20 and 35 cm in selected plots (Paper I and II).

3.2 Static chamber measurements

To measure N_2O emissions in the field (Paper I and II), vented aluminum chambers sized 50 × 50 × 20 cm were deployed on permanently installed aluminum frames. The frames had a 3 cm grove on top, which was filled with water before deployment to serve as an air tight seal between the frame and the chamber. The chamber was equipped with a 1/8’ PTFE sampling tube and a 3-way stop cock for gas sampling by disposable PE syringes. Another 1 meter 1/8’ PTFE tube formed as a “pigtail” was used as an artificial leak connecting the chamber and atmosphere. This leak allows for air pressure equilibration when placing the chamber on the frames and transfers air pressure fluctuations to the chamber (Hutchinson and Mosier, 1981). No electrical fan in the chamber was used. Temperature was measured both inside and outside selected chambers during each measurement campaign.

The use of manual static chambers represents a low-tech method to monitor N_2O fluxes in the field, and is still the method of choice when comparing effects of in-field treatments on a plot level. Microbial turnover of N_2O (i.e. production and consumption) is highly controlled by variable soil conditions and therefore intra-treatment variability of N_2O emissions is often

high. In order to account for possible small-scale variation in N_2O emissions, four frames were placed in each of the three selected 2 x 8 m plots used in the off-season study (supplementary Fig. S1; Paper I). To explore the potential effect of diurnal temperature variation around the freezing point, measurements were performed twice a day (at approximately 9 am and 3 pm) in periods when diurnal freezing and thawing was expected. Additional measurements were carried out on selected dates late in the evening and at night time, providing a dataset consisting of 6-hourly measurement for two 24-hour periods (Paper I). Manual static chambers were also used for gas sampling in the field experiment designed for studying the effects of various levels of split N application on the N_2O-emissions (Paper II). Here, pairs of chambers were placed 10 cm apart in each plot to increase the measurement area and to account for the potential effect of soil variation. As the crop grew, extensions of the frames were installed to minimize the influence on the crop during gas sampling. Chamber measurements are illustrated in Fig. 6.

All gas sampling followed the same procedure. Immediately after placing the chamber, a first sample was taken. Then, with 15 minutes intervals, another three samples were taken, so all together four gas samples were taken for each deployment. The exact timing of gas sampling was monitored by stopwatches, so that possible deviations from the intended measurement scheme could be taken account for in the calculations. To estimate the rate of change, either a linear (default) or a quadratic regression was fitted to the observed change of N_2O concentration with time. A quadratic fit was only used in cases where CO_2 and N_2O accumulation in the chamber showed a convex downwards trend (i.e. decreasing accumulation rates with time). The function yielding the least sum of squares (i.e. of the distances between each set of estimated and measured concentrations) was selected for the further calculations. All fluxes were inspected visually by plotting N_2O over time. Measured accumulation of CO_2 was used to infer faulty values due to contamination or leaky chambers, which were excluded. No fluxes were discarded or set to zero, even if the coefficient of determination of the regression was low (e.g. $R^2 < 0.7$). Low R^2 values occurred predominately with low fluxes, close to zero.

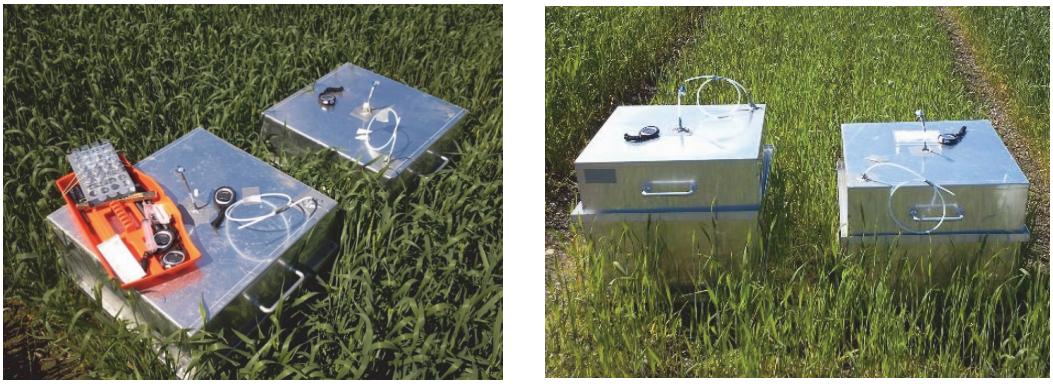


Figure 6. Flux sampling by static chambers during growing season 2011, extensions included on the right.

N_2O fluxes (Paper I and II) were calculated from the concentration change over time according to the equation

$$F_{N_2O} = \frac{d_{N_2O}}{d_t} * \frac{V_C}{A} * \frac{M_n}{V_m} \quad (2)$$

where F_{N2O} is the emission flux (µg N_2O-N m^{-2} h^{-1}), d_{N2O}/dt the rate of N_2O accumulation in the chamber (ppmv h^{-1}), V_c the chamber volume (L), M_n the molecular mass of N in N_2O (g mol^{-1}), V_m the molecular volume (L mol^{-1}) at chamber temperature, and A the area covered by the chamber (m^2).

Cumulative fluxes were calculated plotwise for selected periods by linear interpolation between dates.

3.3 Soil sampling

Mineral N content was measured in each plot at each flux measurement date (Paper I and II), by taking a soil sample (soil auger with 15 mm diameter, sampling at 0-15 cm depth, 5-8 probes per sample) from the area outside the chamber, avoiding the area closest to the frames.

At the end of the measurement campaign (Paper I), soil was sampled from within each of the frames for an incubation experiment. In each frame, 9 samples were taken by a 15 mm diameter soil auger across a diagonal and each three adjacent samples were pooled, yielding a total of 3 samples per frame. The samples were stored at 4°C until start of the incubation experiment.

To study long term effects of different management practices on potential nitrification and denitrification (Paper III), soil was sampled in December from each block of a long-term management experiment - the Apelsvoll Cropping System. Here combinations of arable cropping in monoculture are compared to mixed ley cultivations managed conventionally or organically. To avoid immediate effects of management from the preceding growing season, plots with barley as main crop were chosen, as this was the one common crop present in every block. Soil samples consisted of 10 pooled sub-samples taken with a soil auger (18 mm diameter) from 0 - 20 cm depth along a transect through each plot.

3.4. Soil air probes and flux measurements over winter

To evaluate whether split N application rates affect overwinter GHG turnover (Paper II), soil air probes were installed after the growing season to monitor O_2, CO_2, N_2O and CH_4 concentrations in the soil air throughout winter as illustrated in Fig. 7. Flux measurements were continued monthly for all chambers measured in the growing season to monitor emissions through the snow pack. Extension collars were included as snow depth increased. Relative N_2O concentrations measured belowground ($\mu L\ L^{-1}$) were converted to $\mu g\ N_2O\text{-}N\ m^{-2}$ and 0.24 m depth to estimate the amount of N_2O accumulating under the snow pack. For this, temperature and air-filled porosity along the profile was interpolated to estimate molar volume and total air-filled pore volume, respectively. Maximum measured N_2O concentrations shortly before spring thaw were compared with emission fluxes during spring thaw.

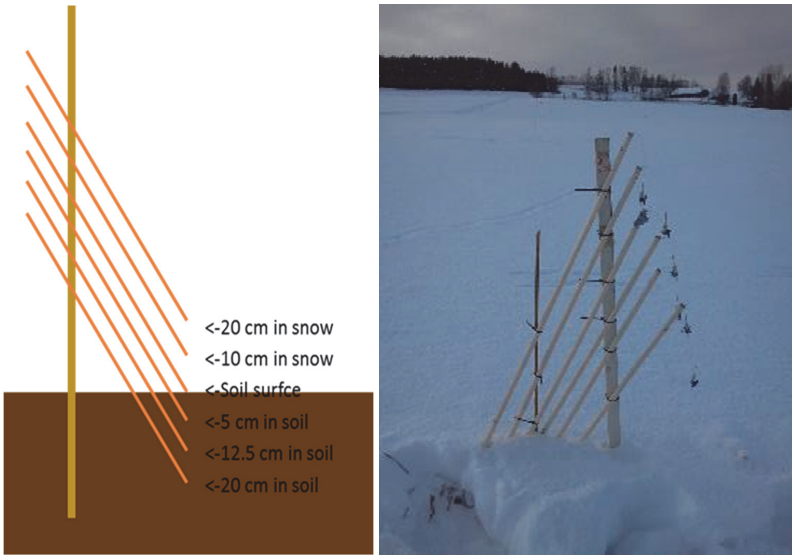


Figure 7. Setup of air probes for measurement of concentration of O_2, CO_2, N_2O and CH_4 concentrations in the soil and snow air over winter.

3.5. Soil incubations

Soil incubations were performed using the protocols and calculations developed by the NMBU Nitrogen group. The incubation experiment presented in Paper I was set up to be directly comparable with previous laboratory studies showing pH dependency of the $N_2O/(N_2O + N_2)$ ratio (e.g. Raut *et al.*, (2012)). Prior to incubation, soil samples were flooded and drained twice with a 2 mM KNO_3 or a 2 mM KNO_3 + 10 mM sodium glutamate solution to ensure equally distributed substrates in the samples. For this, soils were placed on filter paper in Buchner funnels to which a suction was applied (ca. 100 cm water column, ~pF 2). After final drainage, soils were transferred to 120 ml serum bottles and a subsample was used to determine the moisture content.

Three independent experiments were carried out to evaluate respiration and denitrification in the samples retrieved from inside the frames (Paper 1). In experiment 1, samples were incubated oxically for 15 hours to determine respiration by O_2 depletion. In experiment 2, soil samples were flushed with helium (He) prior to a 20 hour incubation to asses anoxic respiration (denitrification) and $NO/N_2O/N_2$ product stoichiometries. In a third experiment, soil samples were incubated anoxically for 90 hours in the presence of 10 mM glutamic acid

to ensure ample supply of carbon in all samples, and to measure denitrification unconstrained by energy limitation.

For the experiment comparing the effect of different management systems on potential nitrification and denitrification (Paper 3), agitated soil slurries were chosen to eliminate potential diffusion constraints within the soils, thus allowing the active organisms to fully access substrates and express their process potentials. For the denitrification assay, soils were suspended in 40 ml of a 1mM KNO_3 solution in 120 ml serum bottles equipped with magnetic stirring bars and crimp-sealed with butyl rubber septa. The bottles were He-washed by repeatedly evacuating and He-filling while stirring the bottles vigorously. For the nitrification assay, soils were suspended in 40 ml of a 1mM NH_4Cl solution in 120 ml serum bottles capped with silicone lined butyl septa to avoid inhibition of nitrification from substances leaking from the butyl septa. Denitrification bottles were kept for 122 h permanently stirred (300 rpm) at 20°C in the water bath under the incubation robot while nitrification bottles were incubated in air at room temperature (~20°C) shaken horizontally (200 rpm) to ensure fully oxic conditions for a 70 h period. For measuring N_2O accumulation and O_2 levels in the nitrification assay, the bottles were placed intermittently (6 times) in the water bath of the robotic incubator while stirring them with magnetic stirrers, before setting them back to horizontal shaking.

To evaluate the product stoichiometry of denitrification from the measured N gas kinetics, we calculated an N_2O index (I_{N2O}) for experiment 3 (Paper I) and the denitrification experiment in Paper III. The index is a measure for the relative amount of N_2O accumulating transiently during anaerobic incubation:

$$I_{\mathrm{N_2O}} = \int_0^T \mathrm{N_2O}(t)\mathrm{d}t \; / \int_0^T [\mathrm{N_2O}(t) + N_2(t)]\,\mathrm{d}t \qquad (3)$$

where $\int N_2O(0 - T)$ is the cumulated N_2O production/reduction until time T, estimated by trapezoid integration, whereas $\int N_2(0 - T)$ is the cumulated production of N_2 during the same time interval. The value of I_{N2O} depends on the cut-off time T. Time T was chosen as the time when the NO concentration in each bottle fell below 5 nmol, which coincided with the (N_2 + N_2O) concentration curve reaching a stable plateau (Paper I), as illustrated in figure 8, or when a minimum of 7 µmol N was denitrified (Paper III).

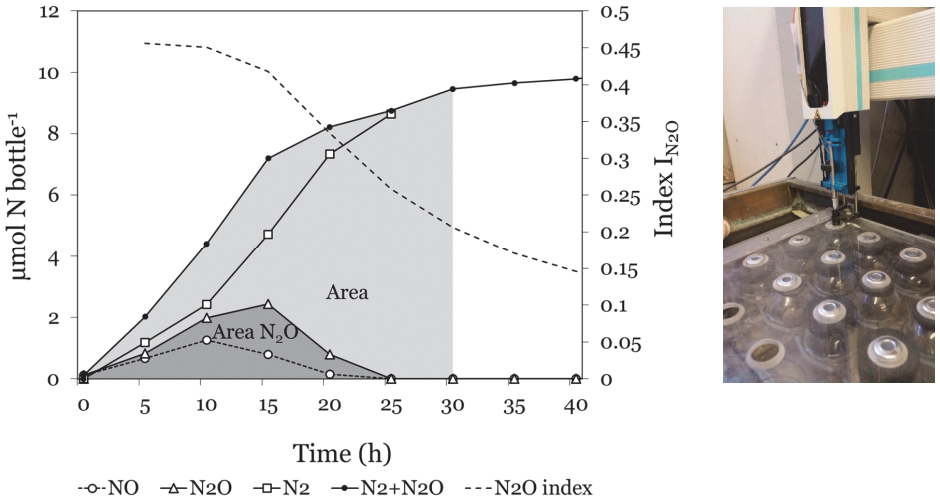


Figure 8: Left panel: measured N gas kinetics during anoxic incubation of a glutamate amended soil from frame 1 in plot 1 (Paper 1). Shown are the areas under the N_2O and the N_2 curves (shaded) used to calculate the $N_2O/(N_2O+N_2)$ product index (I_{N2O}, Eq. 3). The shaded areas also indicate the cut-off time (T, Eq. (3)) given by a NO-concentration < 5nmol bottle^{-1}. Right panel: robotic gas sampling during the incubation experiment.

3.6 Soil analyses

Mineral N, Loss on ignition (LOI) and gravimetric soil moisture were measured by standard methods (Paper I, II and III). Soil pH was measured by dispersion in 1 mM $CaCl_2$ (Paper I and II) or in 1 mM KNO_3 (Paper III).

3.7 Statistics

One-way ANOVA was used to identify differences in respiration rates measured in the oxic soil incubations (Paper I), effects of N-fertilizer levels on yield, yield quality, cumulative N_2O emissions, yield-scaled N_2O emissions and N_2O soil air concentrations (Paper II) and differences between N-transformation variables (Paper III). N_2O emissions throughout autumn (Paper I) were analyzed by a general linear model to evaluate differences between the factors date, frames and plots. Fischer's LSD-method was used for multiple comparisons (Paper II and III). Pearson correlation coefficient was calculated for all pairs of variables (Paper I and III) followed by multiple linear regression (MLR) analysis. All analyses were

performed at the 0.05 probability level, using the software package Minitab® (release 17.2 (Paper I), version 17.2.1 (Paper II) or version 18.1 (Paper III)).

To calculate an optimal N rate, an N response function for yield (Paper II) was fitted to data using the least square method (Excel® 2013). In the experiment comparing different management strategies (Paper III), principal component analysis (PCA) was used to see if score plots would separate cropping systems on the basis of X- and Y-variables by use of the software package Unscrambler (version 10.5).

4. Schematic overview

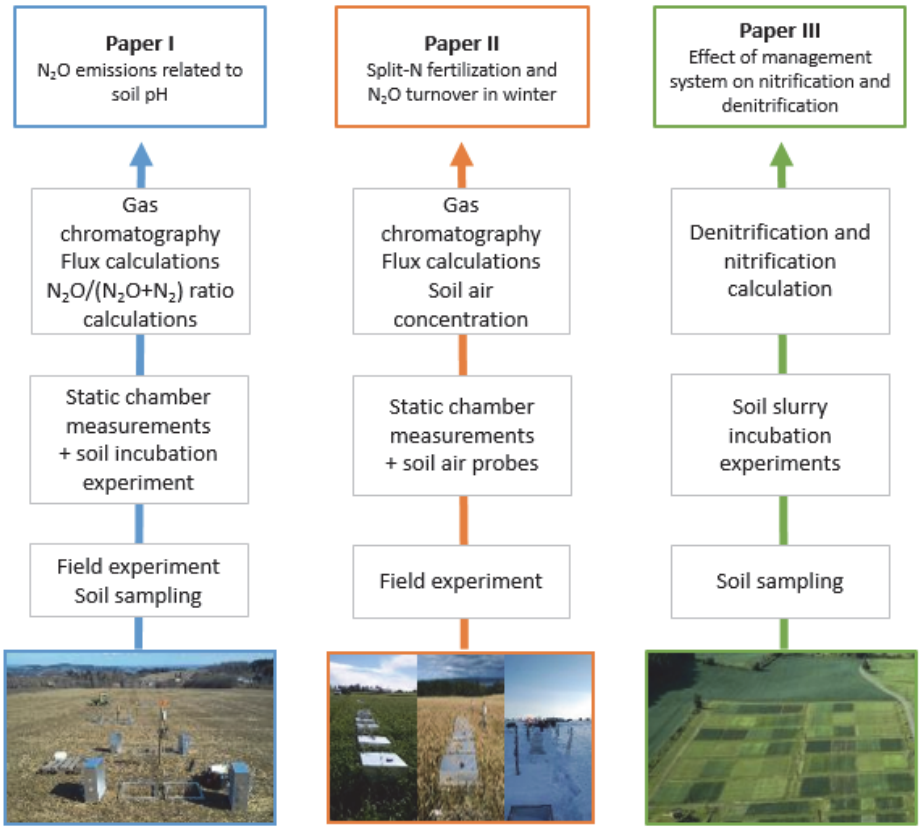


Figure 9: Overview of the approaches and experiments conducted to meet the specific objectives of this thesis.

5. Main results

5.1 Effect of small scale pH variation on N_2O emissions under field conditions (Paper I)

Soil pH has long been regarded as a master variable directly or indirectly controlling N_2O emissions from soil. An almost linear decrease of the denitrification N_2O ratio with increasing pH has been observed in numerous laboratory experiments. Meta studies evaluating field experiments found a similar relationship based on studies covering a wide range of soil pH, i.e. comparing acidic soils around pH 4-5 with soils having a pH of 7 or above. Liming experiments with uniformly limed soils disregard natural small-scale pH variation. Also the duration of liming experiments is of importance, as all soil processes require time to reach equilibrium in response to the pH change. As described in chapter 1.4, variability in soil conditions occurs on a small scale and the relationship between N_2O emissions and soil pH should therefore be addressed on as scale as small as possible. When studying pH effects on N_2O emissions within cereal crop production, it is also important to focus on the pH range normal for cereal crop production (pH 5.5 -7), as essential nutrients and micronutrients become gradually less plant available if the pH goes below or above this range (Truog, 1946). The field study on pH effects in off-season was performed in a wheat stubble field (Paper I) and soil pH was at the lower end of the optimal pH-range for crop production, with only small spatial pH differences; three plots with contrasting soil pH were selected with average pH_{H2O} values ranging from 5.6 to 6.3 (measured at the start of a long term field experiment on the site in 2001). The off-season was chosen to avoid confounding effects of fertilization, root activity and strong fluctuations in soil moisture content, all of which may modify soil pH locally, as well as directly influencing N_2O emissions. Off-season periods also often experience wet conditions and/or freeze-thaw cycles, causing denitrifying conditions and hence increasing risk for N_2O-emissions.

Average N_2O emissions rates correlated negatively with plot pH. When analyzing the soil from within each frame, pH differences between the plots were smaller what was measured in 2001, with pH_{CaCl2} being 5.48, 5.54 and 5.8. Average N_2O emissions in autumn were markedly higher from the area with lowest soil pH (Fig. 10A). During autumn, average emission rates in the three plots changed with time, with higher rates after rainfall and elevated WFPS (Fig. 10B). At snowmelt in early spring, WFPS again increased (Fig. 10B). A positive correlation between N_2O emission rates and WFPS may be explained by the effects of WFPS on the denitrifying conditions in soil. As WFPS increases, the air filled pore space

(AFPS) and thus O_2 availability decreases, which stimulates denitrification. This effect was most evident in autumn and at snowmelt (thawing in early spring), as WFPS increased above 60%.

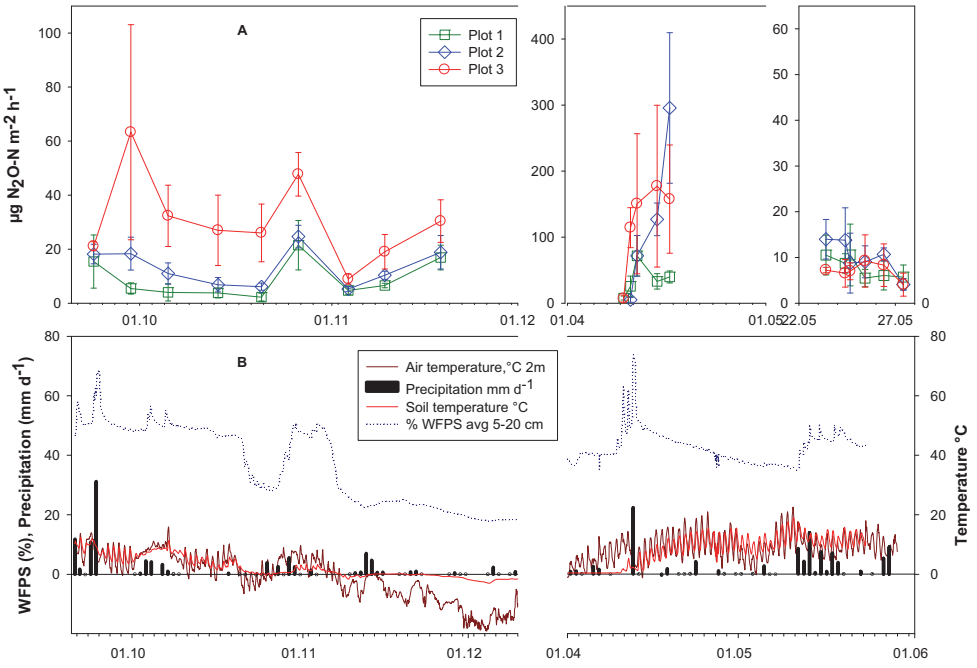


Figure 10 A: Measured N_2O emissions in autumn, during spring thaw and after spring thaw for individual plots with comparable soil texture but differing in soil pH; shown are plot averages (n=4), error bars are standard deviation. B: water filled pore space (WFPS; average for all treatments) and weather data.

The positive effect of precipitation on WFPS appeared to be weaker in late spring, and no correlation was observed between N_2O emissions and WFPS. At this time, soil temperatures were higher and there was substantial growth of weeds within the frames, which may have reduced the effect of increased precipitation by evapotranspiration. Thus, the lower level of WFPS was less favorable for denitrification, which was reflected by the reduced N_2O emissions measured in late spring.

Figure 11 demonstrates that soil pH could explain the observed variation in N_2O emissions between plots in periods dominated by denitrifying conditions (high WFPS). Emission rates were calculated as averages of four chambers placed within each plot. When cumulating

fluxes for each chamber for selected periods (Paper I, as shown in Fig. 11), emission rates were negatively related to soil pH during autumn and spring thaw (Fig. 11A, B), but not during late spring (Fig. 11 C).

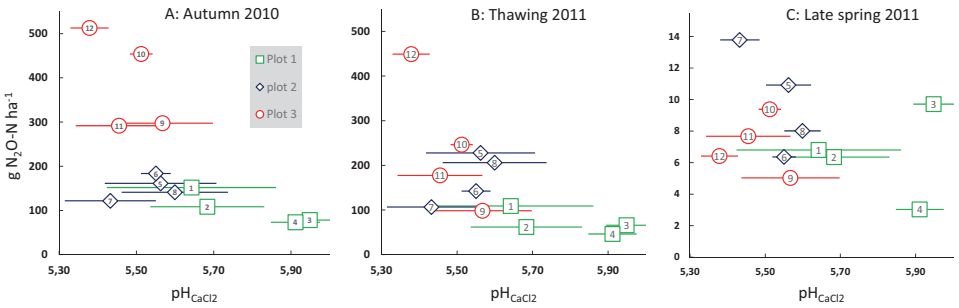


Figure 11. Cumulative N_2O emission in autumn 2010 (A), during spring thaw 2011 (B) and in late spring 2011 (C). Shown are single frame cumulative emissions plotted against the measured pH within frames (average of 3 samples). The length of the measurements periods were 56, 7 and 4 days for autumn, thawing and late spring, respectively.

5.2 The $N_2O/(N_2O+N_2)$ ratio of denitrification and its relations with soil pH and N_2O-emissions (Paper I and III)

Measuring N_2 emission from denitrification *in situ* is impossible due to the high atmospheric concentration of N_2 and requires ^{15}N-labelling of the nitrate pool followed by analysis of ^{15}N in N_2 in the chamber air over time (Bergsma *et al.*, 2001; Well *et al.*, 2019). To measure N_2 release in the present study, NO_3^- amended soils from the field plots were incubated in a He atmosphere and N_2 and N_2O were measured directly to estimate the $N_2O/(N_2O+N_2)$ ratio (I_{N2O}) of denitrification as a function of soil pH. It was hypothesized that the negative relationship between cumulative N_2O emissions and soil pH found *in situ* during periods with high denitrification would correspond to higher I_{N2O} values of denitrification in soil samples with lower pH. A negative relationship between I_{N2O} and pH (r = -0.248; Paper I) was observed in the anoxic experiment without glutamate amendment, though not significant. Further analysis indicated that the results also were affected by variation in metabolic activity. Therefore, glutamate was added to the soil in a third experiment to ensure ample availability of carbon, and indeed, there was a highly significant negative correlation between I_{N2O} and pH

(r = -0.754; Paper I) when amended with glutamate (Fig. 12). The same negative correlation with pH was found for I_{N2O} estimated for soils sampled in the long-term field experiment (Paper III), although the correlation was somewhat weaker (r = -0.593).

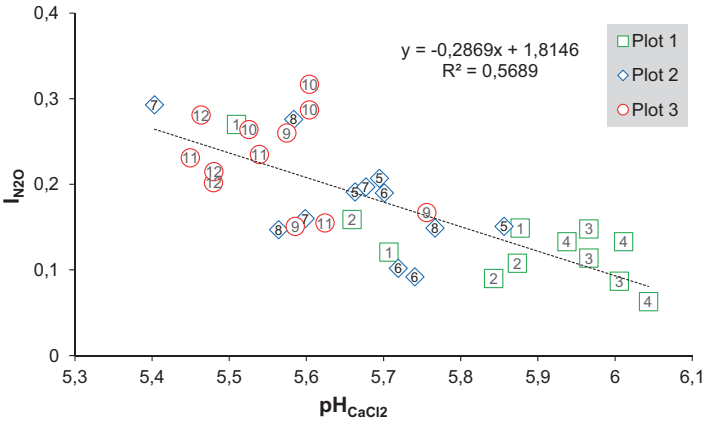


Figure 12. N_2O index for the anoxic incubation of glutamate amended soil versus soil pH (measured after incubation). Data are shown for individual soil samples. Different symbols represent the plots, and numbers in symbols identify the frame from which the soil samples were taken (three soil samples from each frame).

To test if I_{N2O} and its pH dependency could be a measure for the propensity of a soil to emit N_2O under non-limiting denitrifying conditions, the cumulative field N_2O emissions for each measurement period were plotted against the average I_{N2O} (Fig. 13). In the autumn and thawing periods, the correlations were positive and high (r=0,844 and r=0.753 respectively), supporting the conclusion that soil pH is an important factor regulating N_2O emissions from denitrification in agricultural soils during the off-season.

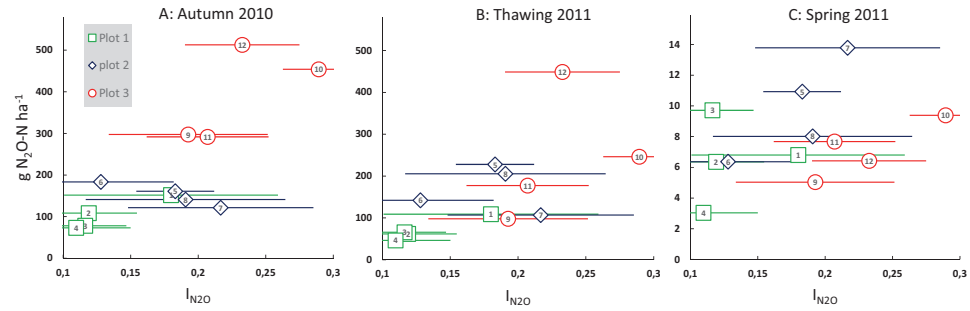


Figure 13. Cumulative N_2O emissions versus N_2O index for autumn, spring thaw and spring. The data show cumulated N_2O emissions from each frame and period plotted against the average N_2O index (I_{N2O}) of soils sampled from within the frames in spring (n=3).

5.3 Effect of N split application rates on N_2O emissions in spring wheat production (Paper II)

The field experiment, in which effects of various amounts of N applied as split-fertilization on the N_2O emissions were measured (Paper II), showed that the emissions were positively linked with the amount of N applied. This effect was, however, limited to the first 15-21 days after split N application. A large proportion of the total N_2O emissions occurred after the cropping season (underlining the findings in Paper I), and during this period the amounts of N applied as split fertilization appeared to not affect the emissions.

Total growing-season N_2O emissions were highest in the treatments receiving 180 kg ha^{-1} (180N) and 220 kg ha^{-1} (220N) fertilizer N (of which 100 kg N ha^{-1} was given at sowing), but when scaling the emissions with yields, largest yield scaled emissions were measured in an unfertilized treatment (0N) (Fig. 14). Of the plots receiving split fertilization, the 180N-treatment tended to have slightly lower yield scaled emissions than the other treatments receiving N-fertilization.

The economic optimal fertilizer rate was calculated to be 190 kg N ha^{-1}, which would imply a split fertilizer rate of 90 kg N ha^{-1}. Nitrogen yield was found to increase by 23% when adding 80 kg N ha^{-1} compared to 40 kg N ha^{-1} as split application, while the corresponding N_2O emissions were reduced by 16%. Optimizing N fertilization by appropriate split N application thus appears to reduce N_2O intensity.

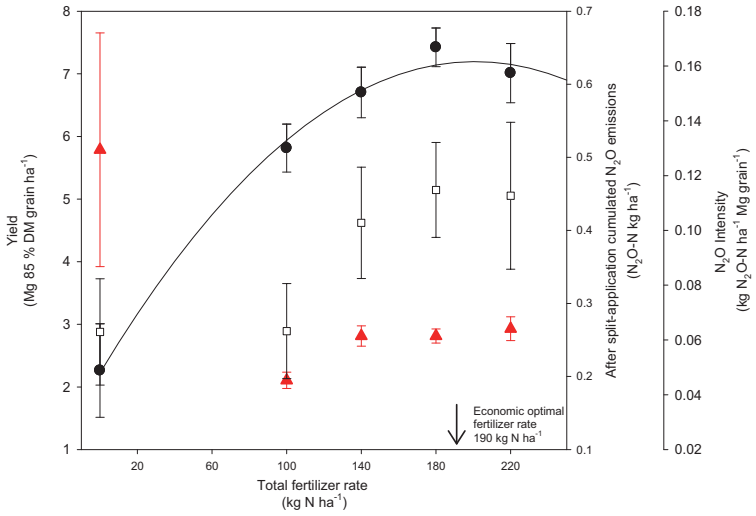


Figure 14. N-level response of grain yield (●), season cumulated N_2O emissions (□) and yield-scaled cumulative N_2O emissions (N_2O intensities, ▲) for the period from split fertilization to harvest (season). Given are average values (N_2O: n=4, Yield: n=2), error bars are SD for N_2O and min/max for yield. The line represents a quadratic yield response curve fitted to the data.

5.4 N_2O dynamics under snow cover and during spring thaw (Paper II)

N_2O fluxes were measured during spring thaw in both field experiments (Paper I and II). Very low fluxes were measured at the onset of spring thaw, while the ground was still covered with snow. As the snow melted in the fields, N_2O emissions increased rapidly for the first days but declined again after 7 days (Paper I). Peak measurements during thawing reached a level, which was up to 15 times higher than the highest emissions measured in the autumn period. This led to an interest in further analysis of N_2O emissions at spring thaw as well as N_2O dynamics in soil air under the snowpack (Paper II).

N_2O production in the top soil during continuous snow cover was monitored in the split-N experiment (Paper II), both by measuring soil air concentrations over time under the snowpack and by flux measurements on the snow pack and during spring thaw. The objective was to look closer into whether N_2O flux measured at spring thaw/snow melt originated from N_2O accumulated in the top soil under snow cover before snow melt, or if the changes in soil conditions at spring thaw induced *de novo* production of N_2O.

When measuring soil air concentrations of N_2O during winter (snow cover), substantial N_2O accumulation in the top soil was observed (Fig. 15). The degree of accumulation was not affected by the amounts of split-N fertilization during the previous growing season. The accumulated N_2O was rapidly released upon spring thaw, with fluxes reaching levels twice as high as those measured after split-N application. These findings indicate that at least some of the high N_2O fluxes observed during thawing may be explained by release of N_2O accumulated in the soil during the period of snow cover. It is, however, likely that there was a significant contribution from spring thaw induced *de novo* production of N_2O, considering the favorable conditions for denitrification during snow melt. In order to estimate the size of order for this contribution, we performed a rough calculation by comparing the maximum amount of N_2O accumulated in the soil with the cumulative N_2O emissions measured from onset of spring thaw. The estimates suggested that not more than 7 to 28% of the N_2O emitted during spring thaw could be explained by physical release of N_2O formed and accumulated throughout winter and that a substantial share of the emitted N_2O must have originated from *de novo* production.

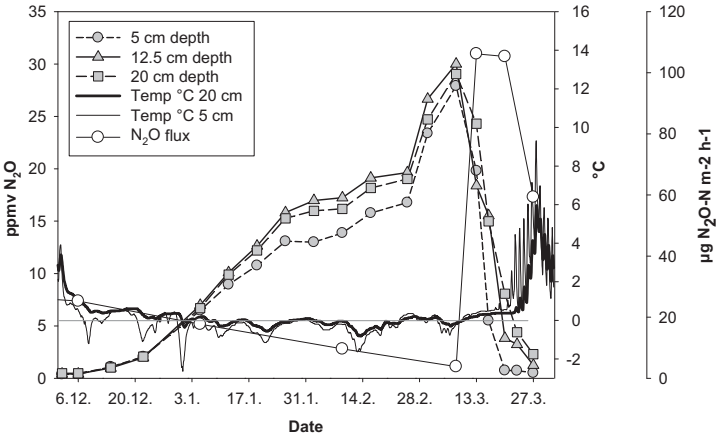


Figure 15. Accumulation of N_2O (n=10) throughout winter in three soil depths, soil temperatures at 5 cm depth (n=5) and 20 cm depth (n=5), and average N_2O emissions (n=20) for all plots.

5.5 Effects of cropping system on soil nitrification and denitrification (Paper III)

Comparing the potential for N_2O production by denitrification and nitrification, clear differences were observed between soils from six long-term cropping system with different crop rotations and management (Paper III). In the conventional arable system (CA1) both

potential nitrification and denitrification were very small, indicating low microbial activity. Highest potential N_2O production from nitrification was observed in the conventional mixed dairy farming system (CM), while highest potential N_2O production from denitrification was observed in the organic mixed dairy farming with 75% ley (OM2) and in the Control (Fig. 16).

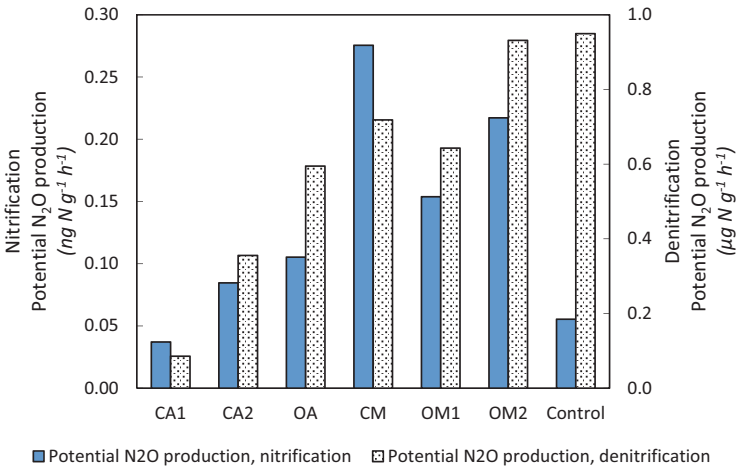


Figure 16. Potential N_2O production from denitrification (right y-axis) and nitrification (left y-axis) from the cropping systems CA1: Conventional arable, CA2: Conventional arable w/ catch crop, OA: Organic arable, CM: Conventional mixed dairy farming, OM1: Organic mixed dairy farming with 50% ley, OM2: Organic mixed dairy farming with 75% ley, and the Control: Boarder area with perennial grass clover mix.

In order to understand the observed system differences in terms of potential nitrification and denitrification and their inherent N_2O stoichiometries, the data were compared with a range of soil properties, including data on soil nutrients and microbiology previously measured in the same soils. Many of the soil properties were significantly correlated with the measured potentials and N_2O stoichiometries. Since long-term differences in crop rotations and management may affect many soil properties simultaneously, and these properties are often inter-correlated, principal component regression (PCR) was used to further analyze the data. First, an initial PCA was run, providing estimates for the unknown regression coefficients for the PCR-models. The first PC (PC1), which explained 33.2 % of the total variation, was mainly related to microbial abundance (16S rRNA and ITS) and activity (invertase and urease), along with the amount of SOM (ignition loss, total C) (Fig. 17A). PC2, explaining

20.8% of the variation, was mainly dominated by the plant available macronutrients P and K (P-AL and K-AL) (Fig. 17A). The enzyme Catalase, bacterial diversity and pH dominated PC3, which explained 16.4% of the variation (Fig. 17C). Principal components number 4 and 5 explained 9.2 and 4.8 % of the variation, respectively. PC4 was dominated by the C/N-ratio of the soil, whereas PC5 by the enzyme Phosphatase. The resulting PCR model explained between 61 and 73% of the observed differences in potential nitrification and denitrification along with their inherent N_2O stoichiometries by soil properties, and PC1 was the most important variable in all models. The results show that cropping systems with a crop rotation and management stimulating microbial growth, increases the potential for both nitrification, denitrification and related N_2O-production. Whether a system is managed organically or conventionally, did not affect the results.

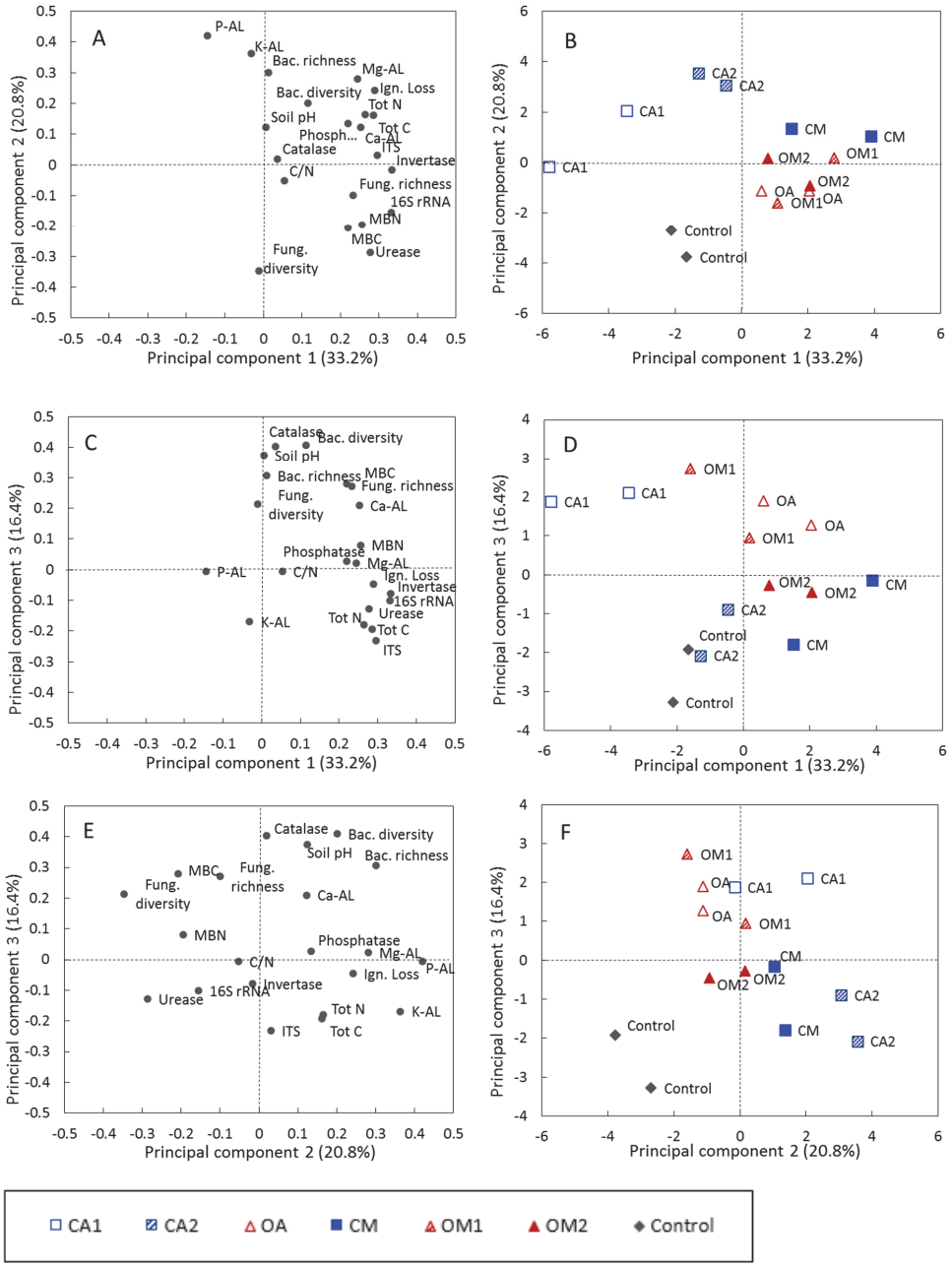


Figure 17. Loading (left side sub-plots) and score plots (right side sub-plots) of the three first principal components.

6. Discussion

With a diversity of organisms and metabolic pathways involved in N_2O production and consumption, the exact mechanisms of N_2O turnover in soil are difficult to study and have therefore been traditionally treated as a "black box" (Robertson, 1989). Even though research within this field has steadily progressed throughout the last thirty years, no clear-cut mitigation strategies for N_2O emissions from cultivated soils have emerged. This may partly be because interactions of known factors show large regional differences depending on natural geographic and climatic conditions as well as soil and crop management regimes. In South-East Norway, only few studies have investigated N_2O emissions from cereal cropping; more field experiments are needed to disclose seasonal and management dependent emission patterns of N_2O in Norwegian arable soils and to identify potential mitigation strategies.

6.1 Soil pH effects on N_2O production and emissions

There is well-established evidence that soil pH plays an important role for the relative amount of N_2O produced by nitrification and denitrification *ex situ*, but this effect may be overridden by fertilization and root activity affecting mineral N and C availability and pO_2 during the growing season (Hinsinger *et al.*, 2003; Wang *et al.*, 2017). To see whether soil pH can have a direct effect on N_2O emissions, an off-season approach was chosen, i.e. N_2O emissions were studied *in situ* in a wheat stubble field with no further input of mineral N or crop residues (Paper I). Off-season emissions dominate N_2O budgets in cool-temperate regions (Flessa *et al.*, 1995; Tatti *et al.*, 2014), but most of the research on N_2O emissions has been carried out during in the growing season. The off-season study clearly showed that soil pH plays an important role in controlling N_2O emissions from denitrification on the field level, with lower soil pH favoring higher N_2O emission. Incubation studies with soils from the stubble field (natural pH variation; Paper I) and the Apelsvoll system experiment (management induced pH variation; Paper III) revealed significant negative correlations between soil pH and the N_2O product ratio of denitrification, despite overall small differences in soil pH. This suggests that higher N_2O emissions from acidic soils are due to incomplete denitrification. This was confirmed by the off-season field measurements (Paper I), which showed a clear link between emission peaks and periods of denitrifying conditions; N_2O emissions followed differences in soil pH most clearly when WFPS increased as a result of heavy rain in autumn or during snow melt in spring, causing anoxia in the soil. C and N required for denitrification were likely provided by decomposition of decaying wheat roots and/or soil organic matter released by

freezing-thawing during autumn and early winter. Interestingly, the pH effect on field emissions was absent in spring when the soil was dry and emission rates were small.

Denitrification *per se* did not seem to be affected by small-scale variation in soil pH. For instance, when analyzing the effects of cropping systems (Paper III) on denitrification, small differences in soil pH between the cropping systems had no significant effect on the denitrification potential, whereas they explained 59% of the variation in I_{N2O}, the N_2O product ratio of denitrification. How may these observations be explained?

Collectively, the results presented in Paper I and III confirm the well-established impairment of denitrifier N_2O reductase by acidity (Bergaust *et al.*, 2010; Bakken *et al.*, 2012; Liu *et al.*, 2014). Since acidity affects N_2O reductase post-transcriptionally, by preventing or delaying the maturation of the fully functional enzyme (Bakken and Frostegård, 2017), soil pH management, probably targeting pH values above those necessary for crop production, emerges as an important mitigation option. N_2O reductase is the only enzyme known to reduce N_2O to N_2 and thus an important factor to reduce losses of N_2O to the atmosphere. The ever increasing input of reactive N to soils, as a consequence of agricultural intensification, will cause increased N_2O emissions to the atmosphere, as it must be assumed that N_2 fixed from the atmosphere ultimately will be returned to the atmosphere as gaseous N (Schlesinger, 2009). Increased use of reactive N is inevitable for future food production, and as long as the nitrogen use efficiency cannot be improved substantially, the goal must be to minimize the amount of N_2O emitted relatively to the amount of N_2 returned to the atmosphere. The findings of this study support the idea that the N_2O product ratio of denitrification can be improved by soil pH management towards more complete denitrification, reducing excess nitrogen all the way to N_2. However, there remains uncertainty as to how pH management affects N_2O emissions from nitrification.

Nitrification may be the main source of N_2O under oxic conditions (Smith, 1997), and pH is an important factor influencing nitrification as it controls the chemical equilibrium between NH_4^+ and NH_3, as stated in the introduction. The N_2O emissions observed during late spring in Paper I could be ascribed entirely to nitrification. In this period, WFPS fluctuated less and weeds covered the soil in the frames, competing with microbiological processes for available N. The average emissions observed in late spring were 3 - 20 µg N_2O-N m^{-2} h^{-1}, which is in the lower range of emission rates observed by others on similar soils and conditions (Mørkved *et al.*, 2007; Wu *et al.*, 2008). There was, however, no correlation between pH and the N_2O-emissions in late spring. Moreover, when investigating the effect of cropping system on

microbial N transformations (Paper III), no correlation between soil pH and potential nitrification or potential N_2O production by nitrification was observed. Mørkved *et al.* (2007) reported that the N_2O yield of nitrification (N_2O/NO_3^-) was only marginally affected by soil pH in soils with pH within the normal range of agricultural soils. By contrast, Nadeem *et al.* (*in revision*) observed both increasing nitrification potentials and increasing N_2O yields (N_2O-N per unit $NO_2^- + NO_3^-$ produced) of nitrification in higher pH soils in a liming experiment. These observations may be explained by looking closer at the microorganisms involved in the nitrification process. Ammonia-oxidizing bacteria (AOB) and archaea (AOA) are the key organisms oxidizing ammonia in agricultural soils, but occupy different niches in the soil. The optimum pH for AOB is 7.5 – 8.0 (Prosser, 1990), but nitrification is also observed in acidic soils (Robertson, 1989). Under acidic conditions, AOA outnumber AOB (Gubry-Rangin *et al.*, 2011). AOA have an inherently lower N_2O yield than AOB, and the overall effect of pH on N_2O emissions from nitrification thus depends on the niche speciation of AOB and AOA (Hink *et al.*, 2017b; Tzanakakis *et al.*, 2019). Together, this makes pH effects on N_2O production by nitrification less straight forward than by denitrification, and may explain the lack of correlation between soil pH and N_2O emissions during spring.

The clear correlation between N_2O emissions (attributed to denitrification) and pH observed in this study may be used as an argument for liming of acidic agricultural soils. A challenge with liming may, however, be that when applying currently available, carbonaceous liming agents, geochemically fixed CO_2 is released to the atmosphere, perhaps offsetting the beneficial reduction of N_2O emissions (Goulding, 2016). In fact, the Tier 1 emission factor for CO_2 from lime (IPCC, 2006) assumes that all carbon contained in calcite or dolomite applied to soil is emitted as CO_2. This is not necessarily true as carbonaceous lime in moderately acid soils forms bicarbonate, which when leached, can act as a net carbon sink (Hamilton *et al.*, 2007). The latter reinforces the importance of proper pH management, preventing soils from acidifying beyond carbonic acid weathering.

Another approach to sustain soil pH in a favorable range could be site-specific application of lime (i.e. precision liming) to better match typical, within-field variation in lime requirement. This approach would require more detailed mapping of soil pH than what is currently standard practice, by average one sample per hectare. It should be noted, that precision liming based on commercially available pH monitoring with a spatial resolution of 10-20 m would still be too coarse to address the small-scale variability in microbial hotspots for N_2O. As argued by Goulding (2016), change in bulk pH by root uptake and exudation is small, but

acidification in near proximity of the roots may be significant, potentially creating hotspots for incomplete denitrification and N_2O release.

In Norway, the growing season is short and winter varieties, such as winter wheat, are grown on a small share of the arable land only (<15%). This implies that there is an extended period with bare soils, leaving nutrients made available in the soil matrix by microbiological or physical processes exposed for losses. Moreover, off-season periods often experience wet conditions and/or freeze-thaw cycles, creating denitrifying conditions and hence risk for N_2O-emissions. Although liming is no silver bullet, an increased focus on adjusting pH at high spatial resolution could be an efficient mitigation potential for arable cropping in Norway.

6.2 Effect of split-N application rate on wheat yields and N_2O intensities

A common method to optimize N fertilization in wheat cropping is to apply only a part of the N fertilizer at sowing in spring (and all the necessary P and K), and then apply the rest in one or more split applications during the cropping season. This reduces the risk of N losses caused by e.g. heavy rain after sowing and before the applied N is fully taken up by the crop. Moreover, the approach allows for both for in-season and within-field adjustments of N application rates, based on an evaluation of the crop status (i.e. biomass and N concentration) at time of split fertilization. Split fertilization adjusted to within-field variation in N-demand is called precision fertilization, and this practice further reduces the risk for N losses, as the site-specific approach normally increases the N use efficiency.

The effect of split N-fertilization on N_2O emissions was tested in a field experiment (Paper II), in which the levels of split-N application were set at 40, 80 and 120 kg N ha^{-1}, representing normal to elevated N levels for Norwegian spring wheat production. No in-season (or site-specific) adjustments were applied, but the set-up allowed for *a posteriori* calculations of optimum fertilizer rates. All plots received 100 kg N ha^{-1} at sowing, and plots with only spring fertilization and without any N addition were included in the experiment. A total fertilizer N rate of 180 kg ha^{-1} (180N) resulted in highest yield and best grain quality, with lowest yield scaled N_2O emissions, and it was closest to the calculated economical optimum N rate of 185-190 kg N.

The lowest (area-scaled) N_2O emissions were measured in the treatments not receiving any split fertilization (i.e. 0N and 100N), but here the yield and yield quality were substantially lower than in the 180N treatment. In light of the expected increase in food demand linked to

forecasted increased global population (United Nations, 2017), yield level and quality should be considered together with the environmental impact. This is in line with others emphasizing the importance of focusing on yield-scaled emissions rather than area-scaled emissions when evaluating agricultural N_2O emissions (van Groenigen *et al.*, 2010; Cui *et al.*, 2014; Giweta *et al.*, 2017).

The IPCC (2007) Tier1 approach, used by Norwegian authorities for agricultural emission accounting, assumes a linear increase in N_2O emissions with increasing N-fertilization level, which is in line with e.g. Mosier *et al.* (2006). Both a metastudy by van Groenigen *et al.* (2010) and a field experiment by Lebender *et al.* (2014) presented results indicating an exponential increase in yield scaled N_2O emissions when N-input exceeded 200 kg N ha^{-1}. By contrast, no increase in N_2O emissions was observed when increasing the total fertilizer application from 180 to 220 kg N ha^{-1} in the present study (Paper II). This was attributed to having missed a significant incident of N_2O emissions after a heavy rainfall a month after split fertilization. The rainfall increased measured WFPS to above 70%, which according to e.g. Ruser *et al.* (2006) could cause high N_2O emissions, but emissions were not measured during this critical period. In hindsight, more frequent measurements of N_2O emissions would have increased the sensitivity of the experiment, and so would an additional treatment with a higher N-level, e.g. 260 kg N ha^{-1}. Regardless, the 180N treatment was close to the calculated economical optimum N rate of 190 kg N ha^{-1} (based on Norwegian prices) or 185 kg N ha^{-1} (EU prices).

6.3 Effect of continuous snow cover on emissions of N_2O in winter and under spring-thaw

N_2O dynamics under continuous snow cover are understudied, and show variable results. Snow cover, especially when associated with the formation of ice layers in the snow-pack, are thought to hinder N_2O flux through the snow (Kim and Tanaka, 2002), which would result in a transient accumulation of N_2O in the soil, if not reduced to N_2. On the other hand, e.g. Maljanen *et al.* (2007) observed no significant production and accumulation of N_2O under snow cover. Given the elevated N_2O emissions observed in the *in situ* pH study during freeze-thaw episodes in autumn and during spring thaw (Paper I), winter-time N_2O accumulation and emission were investigated in more detail. For this, the split-application study (Paper II) was extended by off-season monitoring, including subsoil measurements of N_2O, CO_2 and O_2 in

the soil air. This also opened for the possibility to test whether different fertilization levels would affect belowground accumulation of N_2O and hence off-season emissions.

N_2O emissions were measured on top of the snowpack on a monthly basis (Paper II). Though a continuous snow pack covered the field, the emissions were similar in magnitude to rates reported by others (Maljanen *et al.*, 2007; Risk *et al.*, 2013; Németh *et al.*, 2014). While N_2O was partly emitted through the snow-pack, there was also a substantial accumulation of N_2O in soil under the snow cover, with maximum concentrations measured just before snowmelt of about 34, 36 and 34 $\mu L\ L^{-1}$ at soil depths of 5, 12.5 and 20 cm, respectively. Subnivean soil temperatures fluctuated around 0°C and caused freeze-thaw cycles within the top 5 cm of the soil. Such cycles may have provided liquid water and anaerobic conditions, which both may induce microbial denitrification (Congreves *et al.*, 2018). N_2O production at temperatures as low as -4°C has been observed by Öquist *et al.* (2004), who related this to the occurrence of non-frozen anoxic microsites. Temperatures fluctuating around 0°C provide microsites sustaining relative high N-turnover, with conditions triggering N_2O emission pulses from denitrification Dörsch *et al.* (2004). Repeated freezing and thawing may also liberate physically protected soil organic matter, fueling nitrification and denitrification. Although soil mineral N levels measured in the experiments presented in Paper I and II were very low in spring, both spring thaw measurement campaigns detected relative high N_2O emissions. This indicates that freeze-thaw driven substrate-release (i.e. from roots, aboveground crop residue and soil organic matter) under the snow pack may be sufficient to sustain high denitrification rates. A metastudy by Song *et al.* (2017) concluded that freeze-thaw significantly increases dissolved organic C (DOC) and N (DON) as well as dissolved inorganic nitrogen (DIN), providing ample substrates for denitrification. Mineralization of N below the topsoil must also be considered, as N_2O may also be produced in deeper soil layers (Burton and Beauchamp, 1994). In the experiment presented in Paper II, this effect was probably negligible as the impact of different fertilization rates of only a single year was studied. With prolonged differences in fertilization rates over multiple years, feedbacks from increased root biomass or NO_3^- leached to deeper soil layers may be expected, probably resulting in a larger contribution of sub-surface denitrification to subnivean N_2O production.

Substantial subnivean N_2O accumulation was observed (Paper II) pointing at active N_2O production below the snow. As discussed by Risk *et al.* (2013), the amounts of N_2O dissolved in soil water may be of about the same magnitude as the amounts measured in soil air, if assuming perfect equilibrium. This would roughly double the estimated amount of N_2O

accumulated under the snow pack (Paper II). However, N_2O dissolved in anoxic water may be oversaturated and undergo reduction to N_2. In the present study, only gaseous N_2O in the soil air was analyzed.

Cumulative N_2O emissions observed upon spring thaw were higher than what could be explained by release of N_2O accumulated under the snow cover, suggesting substantial *de novo* production of N_2O during spring-thaw. This implies that spring-thaw emissions only in part depend on off-season conditions and that soil conditions at the time of spring thaw must be taken into account , e.g. by assuring efficient N_2O reduction through sufficiently high pH.

6.4 Effects of cropping systems on potential N_2O production by nitrification and denitrification

Both nitrification and denitrification are governed by soil properties, which in turn are shaped by long-term effects of crop rotation and soil management. From an environmental point of view, it is of special interest to identify which factors associated with different cropping systems affect soil microbial N-turnover and its N_2O emission potential. *In situ* studies of selected management factors such as tillage regime, soil compaction, etc. on N_2O emissions are often inconclusive, and the literature is inconsistent when ranking management practices in terms of their potential to promote or reduce N_2O emissions. In the experiment presented in Paper III, a long-term cropping system experiment in SE Norway was used, comprising six systems representing conventional and organic crop rotations, with differences in crops, residue retention, soil management and fertilization regime, to study contrasts between systems in terms of potential N_2O production by nitrification and denitrification.

Potential denitrification was measured *ex situ* in stirred anoxic soil slurries, analyzing soils from all six cropping systems and from an undisturbed grass-clover lay bordering the cropping system experiment, which served as a control. There were clear differences between a system with conventional arable cropping, with straw removal and without any organic fertilizer inputs (CA1), and all systems with inputs of organic matter (either as organic fertilizers and/or as crop residues). The differences observed between the cropping systems in terms of potential denitrification could largely be explained by differences in SOM content, caused by the long-term differences in fertilizer regime and residue handling. This may partly be explained by the relatively large share of readily decomposable organic matter in organic fertilizers, which may result in transient O_2 depletion, favoring denitrifying organisms. Moreover, there also was a positive correlation between potential denitrification and both

microbial abundance and enzyme activity, and between SOM and the same microbial parameters. Numerous studies have shown that SOM affects microbial abundance (e.g. Martínez-García *et al.* (2018), and it has been documented for various long-term cropping systems that microbial community composition has consequences for the functional potential of N cycling processes (Chen *et al.*, 2018; Wang *et al.*, 2018; Babin *et al.*, 2019).

Potential N_2O-production by denitrification scaled positively with the same microbial properties that explained potential denitrification, but here the effect of cropping system was more diverse. As for denitrification, CA1 had the lowest potential of all six systems, but here an arable cereal-dominated system with plant residues and catch crops incorporated into the soil by spring tillage (CA2) was intermediate, with significantly lower potential N_2O production than systems with ley in the rotation. The findings showed that SOM affected the potential N_2O-production by denitrification mainly indirectly, by providing conditions stimulating microbial growth, indicating that other variables were more important. A principal component model, could explain 66% of the observed variation in potential N_2O-production in denitrification by the three first principal components. PC1 was dominated by microbial abundance and enzyme activity, along with the amount of SOM. PC2 was dominated by plant available macronutrients P and K, and the enzyme catalase, bacterial diversity and pH dominated PC3.

When analyzing the N_2O ratio of denitrification, a clear negative correlation with soil pH was observed as previously mentioned. As reported by Chen *et al.* (2019) in a study on microbial richness and enzyme activity in the same cropping system, limitations in available carbon had the highest impact. Denitrification seems thus to be largely controlled by inclusion of organic matter in the cropping systems.

Potential nitrification was also studied in incubated soil sampled from the cropping systems (Paper III). The highest nitrification potential was observed in the organic mixed cropping system with the highest fraction of ley in the rotation (OM2, 75% ley), the system receiving the largest amounts of manure. The input of available N as well as P is known to stimulate nitrification (Chu *et al.*, 2007). The conventional mixed cropping system (CM) received, however, more available N and P than OM2, since inorganic fertilizers was applied in addition to the manure, but neither input of organic N, inorganic N or P was correlated with potential nitrification. The primary factors controlling nitrification activity and nitrifier growth are ammonium and pH, by controlling the equilibrium of NH_4^+ and NH_3 and by regulating the AOA and AOB activity and/or abundance. Input of inorganic fertilizers clearly

affects soil pH and AOA have been found to increase in inorganically fertilized soils by (Keil *et al.*, 2011; Wertz *et al.*, 2012). The effect of organic *versus* inorganic fertilizer input is, however, contradictory. High nitrification potentials have been implicated with long-term inorganic rather than organic N fertilization, and Chu *et al.* (2007) found that long term inorganic fertilizers increased nitrification potentials, while Kong *et al.* (2019) reported higher nitrification rates in long-term organically fertilized soil. The latter was attributed to AOA, but Fan *et al.* (2011) found manure addition to restore AOB nitrification as the acidifying effect of inorganic fertilizers would be alleviated. Yet, other studies suggest AOA to be stimulated by organic amendments (Schauss *et al.*, 2009; Hink *et al.*, 2018), thus suggesting nitrification to be dominated by AOA. This seemed to be the fact for the Control and the cropping system with 75% grass clover ley, and this may explain lower observed N_2O yields of oxic nitrification in these soils, which are characteristic for AOA (Hink *et al.*, 2017b). One must also consider the type of organic fertilizer used, as e.g. a study by Delin and Engström (2010) showed clear differences in N availability relative to time of inclusion, thus causing different competitive conditions for microorganisms relative to crop demand. The N_2O yield of nitrification measured in the incubation experiment (Paper III) was not correlated with any of the soil or microbial variables, which suggests that the relative potential to form N_2O by nitrification may be decoupled from soil management.

7. Conclusions

This study analyzed N turnover in arable cropping in South-East Norway with focus on N_2O production and emission. Regarding the specific research questions, the following can be concluded:

i) It was found for the first time that the well-known negative relationship between pH and the N_2O product ratio of denitrification controls N_2O emissions *in situ* (Paper I), even with small differences in soil pH within the optimal range for arable crop production. Small-scale differences in soil pH affected *in situ* emissions during off-season, which in turn were correlated with the pH-dependent N_2O product ratio of denitrification assessed *ex situ*. Accordingly, this correlation was only found during periods of denitrifying conditions. However, denitrification has a much higher inherent N_2O yield than nitrification (per unit N converted) and is likely the source of high N_2O emissions after rainfalls and during freeze-thaw episodes. Recent evidence also points towards denitrification as an important N_2O source after ammonium fertilization, as vigorous nitrification may induce local anoxia and directly provide NO_2^- and NO_3^- to denitrification ("coupled nitrification-denitrification"). Addressing the pH dependency of N_2O in denitrification by soil pH management thus appears as an effective way to mitigate N_2O emissions from arable cropping.

ii) Additional N given as split application to spring wheat increased yield and yield quality substantially relative to one-time fertilization at sowing, without affecting yield scaled N_2O emissions (Paper II). N_2O emissions per unit crop produced were clearly smaller with optimized N fertilization than those in a zero N control. In light of the need to feed a growing global population, optimized N application according to crop demand could be another strategy to reduce N_2O emissions in arable crop production.

iii) Field measurements confirmed that off-season emissions are quantitatively important in Norway, and with a changing winter climate, it is crucial to understand factors controlling those emissions. Fertilization from previous cereal cropping did not affect winter emissions in a one-year field trial (Paper II). The buildup of N_2O in the soil during winter seemed to be driven by subnivean mineralization (i.e. N-release) of crop residues and/or soil organic matter in unfrozen soil under the continuous snow pack. Despite the continuous snow cover, repeated freeze-thaw transitions were recorded in 5 cm soil depth, which may have stimulated mineralization and N_2O formation by nitrification and denitrification. Together, this suggests

that the management of crop residues/soil organic matter may be a key for controlling off-season N_2O emissions under cool-temperate conditions, like in Norway. Next to release of accumulated N_2O during spring thaw, *de novo* production of N_2O in thawing topsoil was found to be another important N_2O source, and this process is difficult to control.

iv) Long-term differences in arable cropping strategies clearly affected the soil's potential to nitrify and denitrify and to produce and consume N_2O (Paper III). Repeated application of organic matter as manure or crop residues and inclusion of catch crops or leys in the crop rotation all increased microbial activity in the soil, resulting in higher N_2O production potentials by denitrification, but not so much by nitrification. Potential N_2O emission by nitrification and denitrification was smallest in a conventional cropping system with no retention of crop residues or other organic inputs. However, this system is known to have the highest NO_3-leakage, implying that the risk for indirect N_2O emissions outside the system is high. Given the high nitrification potential and rapid conversion of mineralization-released NH_4^+ to NO_3^-, mixed systems with inclusion of grass-clover ley seem to have the largest N_2O emission potential.

8. Outlook

The regulation of N-transformations in soil is complex, as are the responses to agricultural management. The results presented in this thesis are from studies on well-aerated mineral soils in SE Norway, where cropping seasons are short and off-season processes have a substantial effect on the total N-turnover. The latter is valid for all agricultural production systems in the boreal region, and the results from this thesis should therefore be of interest for N_2O emission research beyond the geographical location of the experiments performed.

The research presented in this thesis gives compelling evidence that increasing soil pH towards optimum levels (i.e. for crop production) of pH 6.0 - 7.5 limit N_2O emissions. As IPCC in 2019 upheld that 100% of the CO_3 in calcareous limes are emitted as CO_2 (IPCC, 2019), the use of liming agents will be included as a GHG source in the calculations of climate gas emissions from agriculture, and thereby counteract the positive effects of reduced N_2O ratio by increased soil pH. This decision (IPCC 2019) is in clear contradiction to earlier reports (e.g. Hamilton *et al.* (2007), showing that carbonate C is not released as CO_2 in moderately acidic soils, but much to the contrary even may sequester CO_2 due to the formation of 2 mol of HCO_3^- per mol lime-derived C. Ongoing research may lead to future adjustments of the calculation schemes for liming-related emissions. Regardless of these details, the aim should be to ensure optimal soil pH for plant production at as high spatial resolution as possible, in which case pH could be corrected by precision liming, saving on the amount of carbonates applied. Automated soil sampling is in development, but mapping of entire fields is still underexploited. Using alternate siliceous liming agents such as rapidly weathering mafic minerals could also be another interesting option. A crucial factor may be the occurrence of microbial hotspots with low pH. It is difficult to control soil pH on the scale relevant for plant roots and microorganisms, and new methods of placing fertilizers and liming agents have to be sought.

By implementing precision farming techniques, yield scaled N_2O emissions can be reduced while maintaining yield levels and quality. However, N_2O emission during off-season are substantial and management practices must be developed to minimize these emissions too. Focus should be on limiting the occurrence of denitrifying conditions, under which N_2O production is known to be most substantial, thus emphasizing methods aimed at preservation and increase of soil aeration, structure, drainage capacity and microbial activity. Increased microbial activity may, however, increase N_2O emissions, but there will always be tradeoffs,

and soils with lower microbial activity may over time lose fertility, compromising the aim of highest possible food produced per area.

Regardless the factors controlling nitrification and denitrification activity in arable cropping systems, excess N_r causes N pollution. Denitrification is the main pathway converting N_r to N_2 and thereby closing the global N cycle. Hence denitrification may be beneficial, as long as the share of emitted N_2O is kept low. It is questionable, however, whether this really is a sustainable solution for the N_r problem as the fixation of N_2 from the atmosphere is a rather energy consuming process. The focus should therefore be on utilizing N_r as efficiently as possible and keeping the N cycle as closed as possible, with a minimum of dissolved and gaseous N-loss. This includes to look for best possible synchronization between N fertilization and plant N demand.

One of the major constraints limiting research on soil N-turnover in agricultural systems is the lack of efficient and inexpensive measurement methodologies. To truly disclose the fate of N_r input into agricultural systems, all N-pools and transformations must be monitored simultaneously, whether gaseous, aqueous or solid (i.e. organically incorporated).

Whatever the focus, crop production needs to be maintained and the aim must be to keep the yield-scaled emissions as low as possible by ensuring best possible conditions for crop production while minimizing the environmental impact. To reach this goal, good agronomy is more important than ever.

9. References

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Spatial variation in soil pH controls off-season N_2O emission in an agricultural soil

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ABSTRACT

Experiments with soils have provided ample evidence that soil pH controls the $N_2O/(N_2O + N_2)$ ratio of denitrification, which increases with decreasing pH, most probably because low pH interferes with the expression of N_2O reductase in denitrifying bacteria. In contrast, the N_2O/NO_3^- product ratio of nitrification appears to be unaffected by soil pH within the range relevant for agricultural soils (pH 5.5–7.0). We hypothesized that local pH variations in cultivated soil may control *in situ* N_2O emissions during periods of active denitrification. To test this hypothesis, we identified three plots with slightly different soil pH (5.4–5.9) within an agricultural field under spring ploughed cereal cropping, and placed four frames within each plot for measuring N_2O emissions throughout autumn and spring. Soil samples were taken from each frame after the experiment to characterize the kinetics of NO, N_2O and N_2 production by anoxic incubation. The data were used to calculate an N_2O index, I_{N2O}, which is an inverse measure of the capacity of the denitrifying community to effectively express N_2O reductase under anoxia and hence a proxy for the soil's propensity to emit N_2O under denitrifying conditions. N_2O emissions were greatest during spring thaw, intermediate in autumn and low in late spring. Emissions during autumn and spring thaw were inversely related to soil pH, supporting the hypothesis that soil pH influences N_2O emissions when denitrification is the main source of N_2O. During these periods, emissions were positively correlated with I_{N2O}, further substantiating the idea that soil pH affects denitrification product ratios *in situ*. Total organic carbon and nitrate content were negatively correlated with soil pH, thus co-varying with N_2O emissions. However, the relationship of N_2O emission to TOC and nitrate appeared weaker than to pH. Off-season emissions dominate N_2O budgets in many regions. If the pH relationship holds at greater scales, careful soil pH management by precision liming could be a viable tool to reduce N_2O emissions.

1. Introduction

Long before N_2O emissions from soils became an environmental issue, Wijler and Delwiche (1954) and Nommik (1956) observed that the production of nitrous oxide (N_2O) relative to dinitrogen (N_2) during denitrification is higher in acid than in neutral soil. This phenomenon was rediscovered several times throughout the next five decades (Simek and Cooper, 2002). The reason for the higher $N_2O/(N_2O + N_2)$ product ratio in acid soils remained obscure, however, and the functional relationship between the product ratio of denitrification, soil pH and N_2O emissions has not been assessed systematically for the more narrow pH range of cultivated soils or across different soil types.

We recently conducted a series of studies in which a variety of soils from long term agronomic experiments were screened for denitrification product ratios using a robotized incubation system for high-resolution measurement of NO, N_2O and N_2 production in batch incubations (Molstad et al., 2007; Liu et al., 2010; Raut et al., 2012; Qu et al., 2014; Obia et al., 2015). These investigations demonstrated that the $N_2O/(N_2O + N_2)$ product ratio is strongly controlled by soil pH, decreasing linearly with increasing pH within the normal pH range of temperate agricultural soils (4.0–7.0), irrespective of soil type. The underlying mechanisms were investigated by studying gene transcription and enzyme activities during transition from oxic to anoxic respiration in the model organism *Paracoccus denitrificans* (Bergaust et al., 2010) and in suspensions of bacteria extracted from soils (Liu et al., 2014; Brenzinger et al., 2015). These studies showed that the making of functional N_2O reductase was increasingly difficult with declining pH within the

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range of pH 6.0–7.5, while enzymes expressed at pH 7.0 were fully functional at low pH. These results suggest that soil pH controls the product ratio at the cellular level by obstructing or delaying the expression of N_2O reductase. An alternative explanation is that pH affects the product ratio indirectly by controlling the species composition of the denitrifying soil community, as suggested by Jones et al. (2014), based on a screening of agricultural soils. Their interpretation has been questioned, however (Bakken et al., 2015), and the observation that the N_2O product ratio responds immediately to manipulation of the soil pH suggests that the direct effect of pH is more important than community composition (Cuhel and Simek, 2011; Qu et al., 2014).

Whatever mechanism being at work, we may expect that soil pH affects N_2O emission rates, increasing with decreasing pH, all other factors held constant. Even though there is circumstantial evidence for this in meta-studies summarizing field flux observations (Stehfest and Bouwman, 2006; Shcherbak et al., 2014), it is not trivial to test this hypothesis, since N_2O emissions under field conditions vary grossly in response to fluctuating soil moisture, temperature, mineral nitrogen and carbon substrate availability. Another factor which may blur the effect of pH on N_2O emission rates is nitrification. Nitrification is the main source of N_2O under oxic conditions (Smith, 1997), but there is no straightforward relationship between nitrification rate and soil pH (Booth et al., 2005). The N_2O yield (N_2O/NO_3^-) of nitrification is only marginally affected by soil pH within the normal pH range of agricultural soils (Mørkved et al., 2007). Therefore, it is unlikely that N_2O emissions deriving primarily from nitrification correlate with soil pH. Despite the shortcomings and pitfalls of field experiments, studies of N_2O emissions within natural ecosystems with large spatial variations in soil pH have demonstrated declining emission with increasing soil pH, both for a riparian ecosystem (Van den Heuvel et al., 2011) and a forest on drained peat (Weslien et al., 2009; Rütting et al., 2013). To our knowledge, no such study has been carried out within agricultural fields, in which the soil pH is expected to vary within a more narrow range.

The objective of the present study was to explore N_2O emissions along marginal pH gradients in a cereal cropping field outside the vegetation period. Off-season was chosen to avoid confounding effects of fertilization, root activity and strong fluctuations in soil moisture content, all of which may influence N_2O emissions directly, or indirectly via modifying soil pH locally. As a test location, we chose a spring wheat field in Southeast Norway, previously used in a four-year fertilizer trial (Øvergaard et al., 2010, 2013a, b), in which we identified three plots from the same fertilization treatment and with similar soil properties but with marginally different soil pH (5.4–5.8). We installed four permanent frames in each plot for N_2O chamber measurements and monitored N_2O emissions in all 12 frames during autumn (post-harvest until snow cover) and during two periods in the spring (snowmelt and late spring prior to tillage). At the end of the field experiment, we took soil samples from each frame and determined potential oxic and anoxic respiration, along with the kinetics of NO, N_2O and N_2 production during denitrification in laboratory assays.

2. Materials and methods

2.1. Field trial

2.1.1. Experimental site and soil pH measurements

Measurements were conducted between September 2010 and May 2011 in the stubble of a spring wheat (*Triticum aestivum* L.) field, previously used in a four-year (2007–2010) experiment aimed at estimating yields by proximal and remote sensing (Øvergaard et al., 2010, 2013a, b). The field is located at NIBIO Apelsvoll (60°42′ N, 10°51′ E, 250 m above sea level) in Southeast Norway, on an imperfectly drained brown earth (Gleyed melanic brunisoils, Canadian System of Soil Classification) with dominantly loam and silty sand textures. For the period 2000–2014, mean annual precipitation was 693 mm and mean annual temperature 5.1 °C. Based on a soil survey performed in 2001 (Øvergaard et al., 2013a), we selected three 2 m × 8 m large plots with comparable texture but with differences in soil pH (Table 1). Measurements of pH were repeated with higher spatial resolution at the end of the field experiment, as described below. In each of the three plots, four micro plots for flux measurements were established in September 2010 by pressing 50 × 50 × 20 cm aluminium frames a minimum of 7 cm into the soil. Each two frames were placed next to each other (about 20 cm apart), one pair in each end of the plots, about 50 cm from the edge of the plot (Fig. S1). The frames served as bases for chamber measurements of N_2O emissions (see below).

At the end of the field experiment in spring 2011, three equally spaced soil samples (0–20 cm) were taken from the inside of each frame along a diagonal transect. Soil sample were taken with a soil auger (18 mm diameter). For every position along the transect, three cores were taken and mixed by hand. pH was measured after dispersing 10 g of soil from each sample in 0.01 M $CaCl_2$ (Seven Multi, Mettler-Toledo). As expected, pH_{CaCl2} values measured in 2011 were lower than pH_{H2O} values measured in 2001, but when averaging the values for each of the three plots ($n = 12$), the ranking of plots for pH remained: pH_{CaCl2} in frames of plot 1 was higher than in plot 2 and 3, despite the high variability between frames within each plot, particularly in plot 1 (Table 2). All selected plots had been fertilised with 200 kg N ha^{-1} during the cropping season in 2010 (Øvergaard et al., 2013a); half of the dose was given at sowing as compound fertilizer (9.6% NO_3–N, 11.0% NH_4–N, 9.6% K and 3.6% P) and the reminder as calcium nitrate (14.5% NO_3–N, 1.1% NH_4–N and 18.8% Ca) top dressed at the beginning of stem elongation (BBCH-stage 31, Lancashire et al., 2008). Meteorological data were obtained from the meteorological station at Apelsvoll, located approx. 150 m from the experimental site. Soil temperature and volumetric moisture content were measured continuously by sensors (5TE, Decagon Devices, Inc.) permanently installed at depths of 5, 20 and 35 cm in each plot, one set for each pair of frames (Fig. S1).

2.1.2. N_2O flux measurements

N_2O emissions were measured by a static chamber method (Rochette and Bertrand, 2008), placing 51 × 51 × 20 cm large aluminium chambers equipped with a 3-way sampling port and a 3 mm diameter pressure equilibration tube (15 cm long) on the preinstalled frames. The frames had a 3 × 3 cm open groove on top, which was filled with water prior to deployment to secure airtight connection. Samples (~15 ml) were taken from the chambers 0, 15, 30 and 45 min after deployment with a 20 ml polypropylene syringe. Before taking a sample, the air in the chamber was mixed by pulling and pushing the plunger of the syringe three to four times. The samples were transferred to pre-evacuated 12.5 ml glass vials (Chromacol) top crimped with butyl rubber septa. Temperature outside and inside one chamber in each plot was recorded by a handheld digital thermometer after the last sampling. Measurements were carried out once a week from end of September (after harvest) until the field was covered with snow in mid of November. In order to explore the potential effect of diurnal temperature variation around the freezing point,

Table 1
Soil pH (H_2O), texture fractions and total carbon in g 100 g^{-1} fine earth (<2 mm) in the selected plots as measured in 2001, averaged over two samples per plot, taken with 8 m spacing at each end of the plot.

		pH_{H2O} (2001)	Coarse sand	Medium sand	Fine sand	Silt	Clay	C_{tot}
Plot 1	Mean	**6.3**	**13.5**	**19.5**	**19.7**	**32.5**	**14.9**	**1.6**
	Min-max	*6.3–6.3*	*12.3–14.7*	*19.2–19.8*	*19.4–19.9*	*32.1–32.9*	*14.7–15.1*	*1.4–1.8*
Plot2	Mean	**5.8**	**13.2**	**20.9**	**20**	**30.9**	**15.1**	**2.3**
	Min-max	*5.7–5.8*	*11.5–14.9*	*20.1–21.7*	*18.7–21.2*	*30–31.8*	*13.9–16.3*	*2.0–2.5*
Plot 3	Mean	**5.6**	**13.9**	**20.9**	**20.3**	**30.4**	**14.6**	**2.1**
	Min-max	*5.5–5.7*	*12.5–15.3*	*19.4–22.3*	*18.5–22.1*	*29.7–31.1*	*13.5–15.6*	*1.8–2.3*

Table 2
Soil pH measured in soil samples taken from inside each frame (0–20 cm) after terminating the N_2O flux measurements. pH values were measured in 0.01 M $CaCl_2$.

Plot 1				Plot 2				Plot 3			
Frame	pH avg.	Soil sample	pH ($CaCl_2$)	Frame	pH avg.	Soil sample	pH ($CaCl_2$)	Frame	pH avg.	Soil sample	pH ($CaCl_2$)
1	5.64	1	5.704	5	5.56	1	5.426	9	5.57	1	5.752
		2	5.349			2	5.499			2	5.469
		3	5.874			3	5.762			3	5.481
2	5.68	1	5.478	6	5.55	1	5.508	10	5.51	1	5.528
		2	5.817			2	5.542			2	5.538
		3	5.755			3	5.601			3	5.471
3	5.95	1	5.992	7	5.43	1	5.504	11	5.46	1	5.558
		2	5.981			2	5.266			2	5.299
		3	5.872			3	5.527			3	5.508
4	5.91	1	5.977	8	5.60	1	5.517	12	5.38	1	5.319
		2	5.825			2	5.488			2	5.377
		3	5.932			3	5.793			3	5.44
Avg. all frames			**5.80**				**5.54**				**5.48**

two measurements per day (at approximately 9 am and 3 pm) were carried out during periods with soil freezing. To further account for diurnal variation outside the freezing period, additional measurements were carried out on selected dates late in the evening and at night time, resulting in 6-hourly measurement for two 24-h periods. No measurements were carried out during the period of uninterrupted snow pack. Measurements were resumed on April 9, the first day of snow melt. Here the measurements were carried out at mid-day throughout an 8-day period with measurements on day 1, 2, 3, 6 and 8 after the onset of thawing. In late spring, a fertigation experiment was carried out in an attempt to provoke high N_2O emissions from denitrification. 50 l of a 1.6 mM KNO_3 solution (corresponding to 192 mm rain) was added to each frame in four 12.5 L portions (43 kg NO_3^--N ha^{-1}). Application was within 1 h. TDR probes were placed inside the frames to monitor volumetric soil moisture and compare it to soil outside the frames not affected by fertigation. Probes were removed during chamber measurements which were carried out periodically throughout one week. The measurements showed that fertigation did not lead to enduring increase in soil moisture within the frames (Fig. S2), due to fast drainage, possibly enhanced by lateral transport beneath the frames.

2.1.3. Gas analysis and flux calculation

N_2O and CO_2 concentrations were analysed by gas chromatography (GC), as outlined by Nadeem et al. (2012). In short, the gas was drawn from the vials by a hypodermic needle mounted on the robotic arm of an autosampler (Gilson) via a peristaltic pump to the 0.25 ml injection loop of a CG (Model 7890A, Agilent, Santa Clara, CA, USA) with back-flushing. N_2O and CO_2 were separated from bulk air by a 30 m wide bore (0.53 mm) Poraplot Q column connected to an ECD and a TCD detector via a 6-port valve. The electron capture detector (ECD) conditions were 375 °C with 17 ml min^{-1} $ArCH_4$ (90/10 vol %) as makeup gas. He 6.0 was used as a carrier gas. N_2O fluxes were calculated from the concentration change over time according to the equation

$$F_{N_2O} = \frac{d_{N_2O}}{d_t} * \frac{V_C}{A} * \frac{M_n}{V_m} \quad (1)$$

where F_{N2O} is the emission flux (μg N_2O–N m^{-2} h^{-1}), d_{N2O}/dt the rate of N_2O accumulation in the chamber (ppmv h^{-1}), V_c the chamber volume (L), M_n the molecular mass of N in N_2O (g mol^{-1}), V_m the molecular volume (L mol^{-1}) at chamber temperature, and A the area covered by the chamber (m^2). Chamber temperature was measured at the end of each deployment. To estimate d_{N2O}/d_t, we fitted either a linear (default) or a quadratic regression to the concentration change against time. A quadratic fit was only used in cases where CO_2 and N_2O accumulation in the chamber showed a convex downwards trend (i.e. decreasing accumulation rates with time) to estimate time-zero rates for d_{N2O}/d_t. Cumulative fluxes were calculated for selected periods by linear interpolation between dates.

2.1.4. Soil sampling and chemical analyses

Soil samples were taken immediately after flux measurements on every sampling date. Five samples were taken by soil auger next to the frames (as indicated in Fig. S1) and pooled into one composite sample for each flux chamber. All samples were stored at −18 °C until analysis. NH_4^+ and NO_3^- were determined by Flow Injection Analysis (FIAstar5000, SoFIA) after extraction of 40 g soil with 200 ml 2 M KCl for 1 h in a reciprocal shaker. All mineral N concentrations are reported on a gram soil dry weight basis. To assess within-plot variation of soil organic carbon, loss on ignition (LOI) was measured in all samples taken during flux measurements in autumn and spring and converted to TOC (g C 100 g^{-1}) using equation (2) calibrated specifically for the experimental site (Riley, 1996):

$$Soil\ C = (0.81 * LOI - 0.038 * clay\ content - 0.70)/1.72 \quad (2)$$

2.2. Gas kinetics

2.2.1. Soil incubations

Soil samples taken from inside the frames in May 2011 (see 2.1.1) were transferred to the laboratory and stored at 4 °C before using them in incubation experiments within 14 days after sampling. Visible stones were removed. In total, 36 distinct soil samples (3 per frame) were processed.

Three separate incubation experiments were performed. In experiment 1, samples were incubated oxically for 15 h to determine respiration (by O_2 depletion). In experiment 2, soil samples were flushed with He prior to a 20 h incubation to asses anoxic respiration (potential denitrification) and $NO/N_2O/N_2$ product stoichiometries. In a third experiment, soil samples were incubated anoxically for 90 h in the presence of 10 mM glutamic acid. Prior to each experiment, soil samples were flooded and drained twice with a 2 mM KNO_3 solution (with additional 10 mM sodium glutamate in exp. 3) to ensure equally distributed NO_3^- (and glutamate) concentrations. This was done by placing soils in Buchner funnels with filter papers, where they were flooded and then drained by applying vacuum. For draining after the first flooding we applied vacuum for only 2–3 min; for draining after the second flooding we applied vacuum for 20 min (reaching equilibrium at a matrix potential of app. −100 kPa). The samples were then used immediately for oxic and anoxic incubations at 15 °C by transferring 30 g fresh weight soil to 120 ml serum bottles. Flooding, drainage and subsequent incubation was done in one sequence of operation for each experiment, to minimize the time between final drainage and incubation. The soil samples were kept on ice after drainage (during weighing and preparation of the incubation bottles).

All incubation experiments were carried out in a robotized incubation system, which measures headspace concentrations of O_2, CO_2, NO, N_2O and N_2 at high temporal resolution (here 5 hourly). The basic setup of the system has been described by Molstad et al. (2007), and the improved version used here is described by Raut et al. (2012). In short, the system consists of a thermostatic water bath which hosts up to forty-four 120 ml serum bottles crimp-sealed with butyl septa. The system monitors concentrations of gasses by frequent sampling of the bottle headspaces by a hypodermic needle connected to a fully programmable autosampler (CTC, GC Pal). The sample is transported via a peristaltic pump (Gilson Minipuls 3) to multiple sampling loops serving a GC (Model 7890A, Agilent, Santa Clara, CA, USA) equipped with a Poraplot and a Molesieve column (for separation of air) and a chemoluminescence detector (Model 200A; Advanced Pollution Instrumentation, San Diego) for NO analysis. After sampling, the peristaltic pump is reversed, replacing the sample with helium, thus sustaining ~1 atm pressure in the bottles throughout the entire incubation. For each time increment, the rates of production and consumption of each gas were estimated, correcting for the loss by sampling (1% of the headspace) and for leakage of O_2 and N_2 into the incubation bottles during the sampling operation (app. 25 nmol N_2 per sampling).

After each incubation, the pH was measured by dispersing the soil in 75 mL 0.01 M $CaCl_2$ (Table S1). Thereafter, the suspensions were wet-sieved through a 2 mm screen to determine the amount of soil <2 mm diameter. Soil moisture was determined in parallel samples of the original soil after drying (105 °C). All biological activities (respiration and N gas production) are expressed per g dry weight of soil particles < 2 mm.

2.2.2. Gas kinetics

The rates of gas production and consumption in the bottles were calculated for each individual time increment between two measurements, taking the solubility of the gases in soil moisture into account (see Molstad et al., 2007 for details). For the oxic incubation (experiment 1), we used CO_2 as an estimator of oxic respiration rather than the O_2 consumption because the change in O_2 headspace concentration in the fully oxic bottles was too small to reliably estimate biological O_2 consumption. We found, however, that the average ratio between O_2 consumption and CO_2 evolution during the oxic incubations was close to 1 (mol/mol). The assessment of denitrification kinetics and product stoichiometries from the anoxic incubations (experiments 2 and 3) is identical to that described by Qu et al. (2014). Initial denitrification rate was estimated from the initial accumulation of all N-gases ($NO + N_2O + N_2$) as shown in Fig. 1. To characterize each soil's capacity to emit N_2O, we calculated an N_2O index (I_{N2O}) from experiment 3 similar to that in Liu et al. (2010) and Qu et al. (2014). The index is a measure for the relative amount of N_2O accumulating transiently during anaerobic incubation (Fig. 1):

$$I_{N_2O} = \int_0^T N_2O(t)dt \Big/ \int_0^T [N_2O(t) + N_2(t)]dt \quad (3)$$

where $\int N_2O(0–T)$ is the cumulated N_2O production/reduction until time T, estimated by trapezoid integration, whereas $\int N_2(0-T)$ is the cumulated production of N_2 during the same time interval. The value of I_{N2O} depends on the cut-off time T, as illustrated in Fig. 1. Time T was chosen as the time when the NO concentration in the bottle fell below 5 nmol, which coincided with the (N_2 + N_2O) concentration curve reaching a stable level.

2.3. Statistics

To investigate the relationship between cumulative N_2O emission, soil pH, N_2O index (I_{N2O}) and soil nitrate and TOC content in the three measurement periods, we calculated the Pearson correlation coefficient for all variable pairs. Seasonal N_2O emissions were calculated as the cumulative N_2O emission flux for individual frames in the periods "autumn" (23. September–18. November 2010), "thawing" (9.–16. April 2011) and "spring" (23.–27. May 2011). For soil pH we used the average value for each frame based on the soil sampling performed in spring 2011 (three distinct soil samples per frame; Table S1). For the N_2O index (I_{N2O}) we used the frame averages resulting from the incubations in experiment 3, whereas for NO_3^-, NH_4^+ and TOC, we used the seasonal averages measured next to each frame.

In order to evaluate whether a combination of variables provides a better model for cumulative seasonal N_2O emission than any single variable, we used multiple linear regression (MLR) analysis (stepwise, forward selection, $\alpha = 0.15$). As candidate variables we tested soil pH, N_2O index (I_{N2O}), soil nitrate (both for autumn and spring thaw) and TOC content. The stepwise procedure was run repeatedly so that each of the variables was used once as the first variable in the model. Only models in which all variables contributed significantly to the regression were considered. N_2O emissions throughout autumn were analysed by a general linear model to evaluate differences between the factors date, frames and plots. One-way ANOVA was used to identify differences in respiration rates measured in the oxic soil incubations (Exp. 1). Minitab (MINITAB inc. 2000, Release 17.2) was used for all statistical analyses. All tests were performed at the 0.05 probability level.

3. Results

3.1. Field emissions

Fig. 2 shows average N_2O emissions for the three plots differing in pH (Tables 1 and 2), together with mineral N content, soil water filled pore space (in 0–20 cm depth) and weather. Soil moisture dynamics within and across the three plots were similar during the autumn period until the second soil frost as shown in Fig. S2. N_2O emission rates in autumn were small and declined gradually with declining soil temperature. High emissions were observed during snow melt and soil thawing mid of April 2011, whereas emissions during late spring were low despite fertigation.

Emissions throughout autumn differed significantly ($p < 0.005$) between dates, frames and plots. The average emissions for plots 1, 2 and 3 were 8.3, 11.6 and 29 μg N_2O–N m^{-2} h^{-1}, respectively. Thus, the plot with the highest soil pH (plot 1, pH 5.80; Table 2) had the lowest emission and the plot with the lowest pH (plot 3, pH 5.48; Table 2) had the highest emission. However, there were consistent differences in N_2O emissions between the frames within each plot, and even between adjacent frames (Fig. S3). A first approach to explore this variation was to inspect the relationship between cumulative emissions and the pH measured in the individual frames. In Fig. 3, the cumulative N_2O emissions are plotted for each frame and period against the soil pH_{CaCl2} measured in triplicate within the frame in spring 2011 (Table 2). Cumulative emissions were negatively related to pH in the autumn and the thawing period ($R = -0.662$; $p = 0.019$), but not for the late spring period (see correlation matrix Table S2).

Fluctuations in soil temperature and soil moisture did not differ significantly between plots (Fig. S2). Irrigating the frames with 192 mm KNO_3 solution in late spring did not lead to enduring increase in soils moisture (Fig. S2, right panel). WFPS values quickly dropped back to those of the surrounding, none-irrigated soil. The NO_3^- concentrations in plot 1 were lower than in plot 2 and 3 during early autumn, but this difference between the plots diminished gradually throughout autumn (the concentrations increased in plot 1), and early in next spring when the soil had thawed, NO_3^- concentrations were practically the same for the three plots (Fig. 2C). The initially low NO_3^- concentration in plot 1 could be due to somewhat stronger N assimilation by the preceding crop, although this could not be detected as higher grain yield for this plot (Table S5).

The low autumn N_2O emissions in plot 1 could be due to a combination of high pH and low NO_3^- concentrations. However, N_2O emissions were not related to neither NO_3^- nor NH_4^+ in any of the periods. In contrast, N_2O emissions were positively related to TOC in autumn ($r = 0.872$; $p = 0.000$) and during thawing ($r = 0.622$; $p = 0.031$), but not in spring ($r = 0.028$). Both explanatory variables, however, were negatively related to soil pH in autumn (NO_3^-: $r = -0.772$; $p = 0.003$ and TOC: $r = -0.711$; $p = 0.010$), suggesting a strong covariation between all three variables (Table S2).

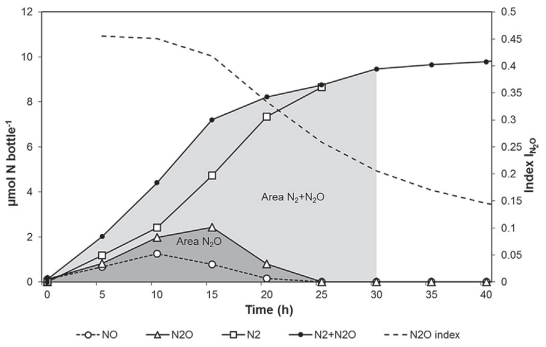

Fig. 1. Calculation of the $N_2O/(N_2O + N_2)$ product index (I_{N2O}, Eq. (3)). The index is the area under the N_2O curve (dark shaded) divided by the area under the N_2+N_2O curve (light + dark shaded area). The areas indicated are for a cut-off time (T, Eq. (2)) of 30 h. The dashed line shows the index as a function of the cut-off time. In the experiment with glutamate, all soils depleted their nitrogen oxyanion pool completely, which is characterized by depletion of NO, coinciding with (N_2+N_2O) reaching a stable level. The data used are for a soil sample from frame 1 in plot 1.

3.2. Soil incubations

3.2.1. Oxic incubation

The oxic incubation lasted 15 h, and the rates of CO_2 production were close to constant for all bottles throughout this period (Fig. S4). The rates differed, however, between individual bottles ranging from 15 to 50 nmol CO_2 g^{-1} dw^{-1} h^{-1}. Replicate soil samples taken from the same frame differed in respiration rates, but no statistically significant differences were found between frames (data not shown). The average for each plot (3 replicate soil samples x 4 frames = 12 samples) suggested slightly smaller respiration rates for plot 1 than for plot 2 and 3 (19.1 versus 25.8 and 27.4 nmol CO_2 g^{-1} dw^{-1} h^{-1}, respectively, Table S3), but this difference was not significant due to the high standard deviations on the plot level. We found substantial pH variation within plots and even within individual frames (Table 2). To explore whether respiration was affected by the variation in pH, we plotted the rates of individual soil samples against their pH measured prior to incubation (Fig. 4). There was a marginal decline in respiration rate with increasing pH ($r = -0.333$; $p = 0.047$).

3.2.2. Anoxic incubation without glutamate

Incubating the soil samples anoxically without glutamate resulted in denitrification rates ranging from 9.2 to 22.3 nmol N g^{-1} dry weight soil and h^{-1} during the first time increment (0–5 h), and the rates declined gradually throughout the 20 h of incubation by 20–50% (Fig. S5A). There was substantial variation between individual samples, whereas the frame averages and plot averages were more similar (Table S4). We suspected that the variation in denitrification rate would be correlated with the respiration during oxic incubation, and this was verified (Fig. 5). We also tested if the denitrification rate was correlated with soil pH and found some declining trend with pH, which, however, was not statistically significant (Fig. S5B). The ratio between measured denitrification rate and the oxic respiration rate is a proxy for the fraction of organisms able to switch from oxic to anoxic respiration (Raut et al., 2012; Samad et al., 2016), and this ratio was not correlated to soil pH (data not shown).

The $N_2O/(N_2+N_2O)$ ratio during the first time increment (0–5 h) was highly variable, ranging from 0.1 to 0.81 (average 0.51) and was negatively related to soil pH ($r = -0.248$), but this relation was not significant ($p = 0.145$). The calculation of an N_2O index (I_{N2O}) for these data was difficult since the rates of denitrification varied grossly; in some samples, ~75% of the nitrate-N was recovered as N-gas at the end of the incubation, in others only 25% of the nitrate N had been reduced to N gases. In principle, the cut off time (*T*, Eq. (1)) should thus be the time when ¼ of the nitrate had been reduced (Qu et al., 2014), but this left us with only one time increment for some of the samples. As an alternative, we tried to calculate I_{N2O} for the entire incubation period. Again, I_{N2O} showed a very weak (although significant) negative correlation with pH ($R^2 = 0.36$, $p = 0.033$). We suspected that I_{N2O} as calculated could be

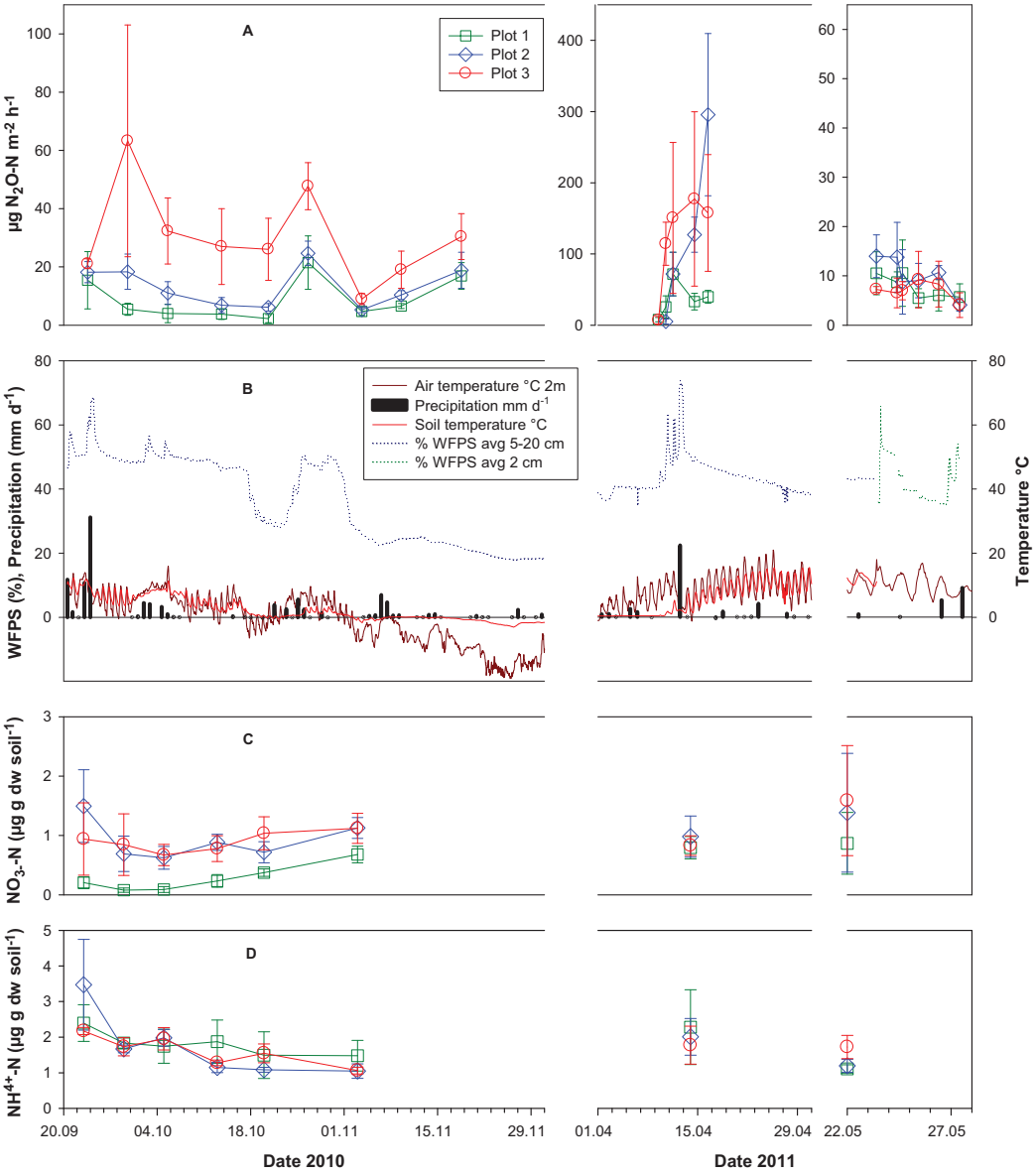

Fig. 2. A: Measured N_2O emissions for individual plots differing in soil texture and pH (see Table 1), B: water filled pore space (WFPS; average for all treatments) and climate data and C: average mineral N content per plot. Note the different scales for N_2O emissions.

influenced by the metabolic activity (as assessed by the oxic incubation), which was confirmed by regression analysis: the index decreased with increasing pH and increasing oxic respiration (see Fig. S6). This shows that I_{N2O} was related to soil pH, but also affected by variation in metabolic activity. To further explore the effect of soil pH on the $N_2O/(N_2+N_2O)$ ratio, we carried out a third experiment, in which we standardized metabolic activity by glutamate addition.

3.2.3. *Anoxic incubation with glutamate*

Anoxic incubation with glutamate resulted in higher denitrification rates than without. During the first time increment (5 h), rates ranged from 17 to 33 nmol g^{-1} h^{-1} (single soil sample values) and showed no correlation with soil pH (Fig. S7A), but a slight positive correlation with oxic respiration (Fig. S7B). The high denitrification rates resulted in a depletion of nitrogen oxyanions within 20–25 h of incubation as seen by a depletion of both NO and

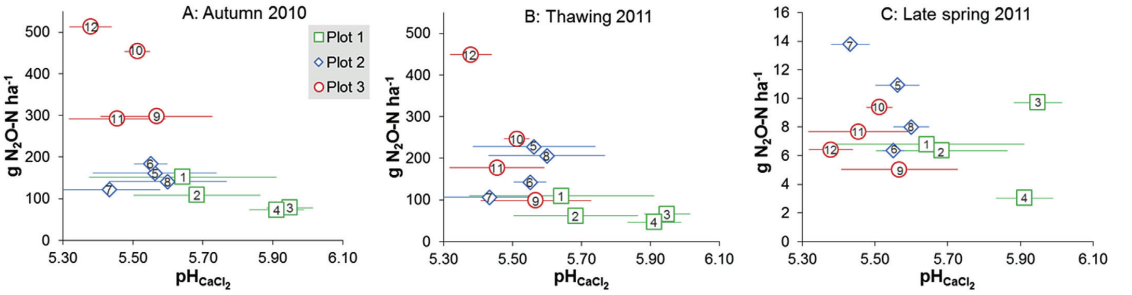

Fig. 3. Cumulative N_2O emission for the autumn 2010 (A), spring thaw 2011 (B) and late spring 2011 (C). Single frame cumulative emissions are plotted against the measured pH within frames (average of 3 samples). The length of the measurements periods were 56, 7 and 4 days for autumn, thawing and late spring, respectively.

N_2O and a stable plateau of N_2 (Fig. 6). The levels of the N_2 plateaus were in reasonable agreement with the intended concentration of NO_3^- in the soil (for details see figure legend in Fig. 6), confirming that all oxyanions were depleted by the end of the incubation.

The pH dependency of the $N_2O/(N_2O + N_2)$ ratio in the glutamate amended soil was inspected by calculating the ratio for the first time increment (0–5 h), and by calculating the N_2O Index, I_{N2O}, as explained in the Materials and Methods section. I_{N2O} varied between 0.06 and 0.32, and showed a strong negative relationship ($r = -0.754$; $p < 0.0001$) to measured pH (Fig. 7). The average pH measured after incubation was 0.2 pH units higher than the average pH of the bulk samples measured prior to the incubation (Table S1). Since we have no independent measure of the pH value during incubation, I_{N2O} in Fig. 7 was plotted against the average pH measured before and after incubation (Table S1). A strong negative relationship with pH was also found for the $N_2O/(N_2+N_2O)$ ratio measured during the first time increment ($r = -0.701$; $p < 0.0001$) (Fig. S8B). Thus, I_{N2O} and the initial product ratio showed essentially the same phenomenon: the relative rate of N_2O reduction (i.e. relative to the rate of N_2 production) increased with increasing soil pH.

3.3. Effects of soil pH, I_{N2O}, nitrate, ammonium and TOC on N_2O field emissions

To unravel the meaning of the N_2O-index, I_{N2O} and its pH dependency as a measure for a soil's propensity to emit N_2O under none-limiting denitrifying conditions, we plotted cumulative field N_2O emissions for each measurement period against the average I_{N2O} ($n = 3$) for individual frames (Fig. 8). The figure shows that these two variables were strongly related in autumn ($r = 0.844$; $p = 0.001$) and during the thawing period ($r = 0.753$; $p = 0.005$), but not in the late spring period ($r = 0.305$; $p = 0.311$). Pearson correlation suggested a high degree of collinearity between soil pH, I_{N2O}, NO_3^-, NH_4^+ and TOC. We therefore tested whether a combination of these explanatory variables could improve the prediction of cumulative N_2O emissions, by applying MLR. None of the tested combinations yielded a significant model with more than one variable included, reflecting the high degree of inter-correlation in the dataset.

4. Discussion

As summarized in the introduction, there is ample experimental evidence that soil pH is a strong controller for the $N_2O/(N_2+N_2O)$ ratio of denitrification in soils as well as in model organisms. Little is known about how this pH-control affects emissions of N_2O from soils *in situ*. The phenomenon is of considerable interest because it may have practical implications: farmers can manipulate soil pH (within the limits determined by the soil characteristics), and thus may have an instrument to reduce N_2O emission during crop production. Hard evidence from field experiments is lacking, however. The relatively few attempts made have given variable results, as

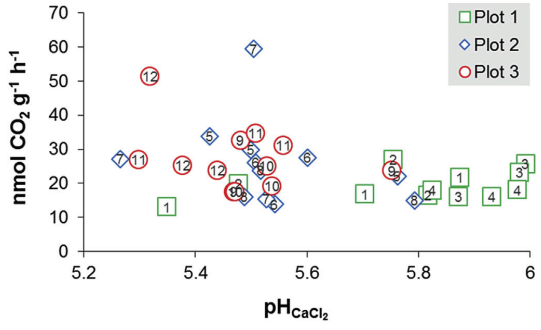

Fig. 4. Oxic respiration rates plotted against soil pH_{CaCl2}. The figure shows the rates (nmol CO_2 g^{-1} soil dry weight h^{-1}) for single soil samples taken at 3 locations within each frame. Different symbols represent the plots, and numbers in symbols identify the frame from which the soil samples were taken (three soil samples from each frame).

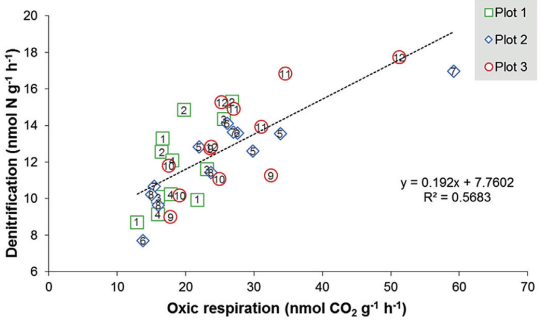

Fig. 5. Average denitrification rate (5–20 h) during anoxic incubation of soil without glutamate amendment. The figure shows the rates (nmol N g^{-1} soil dry weight h^{-1}) for individual soil samples (3 locations within each frame) plotted against the measured respiration during the oxic incubation. Different symbols represent the plots, and numbers in symbols identify the frame from which the soil samples were taken (three soil samples from each frame).

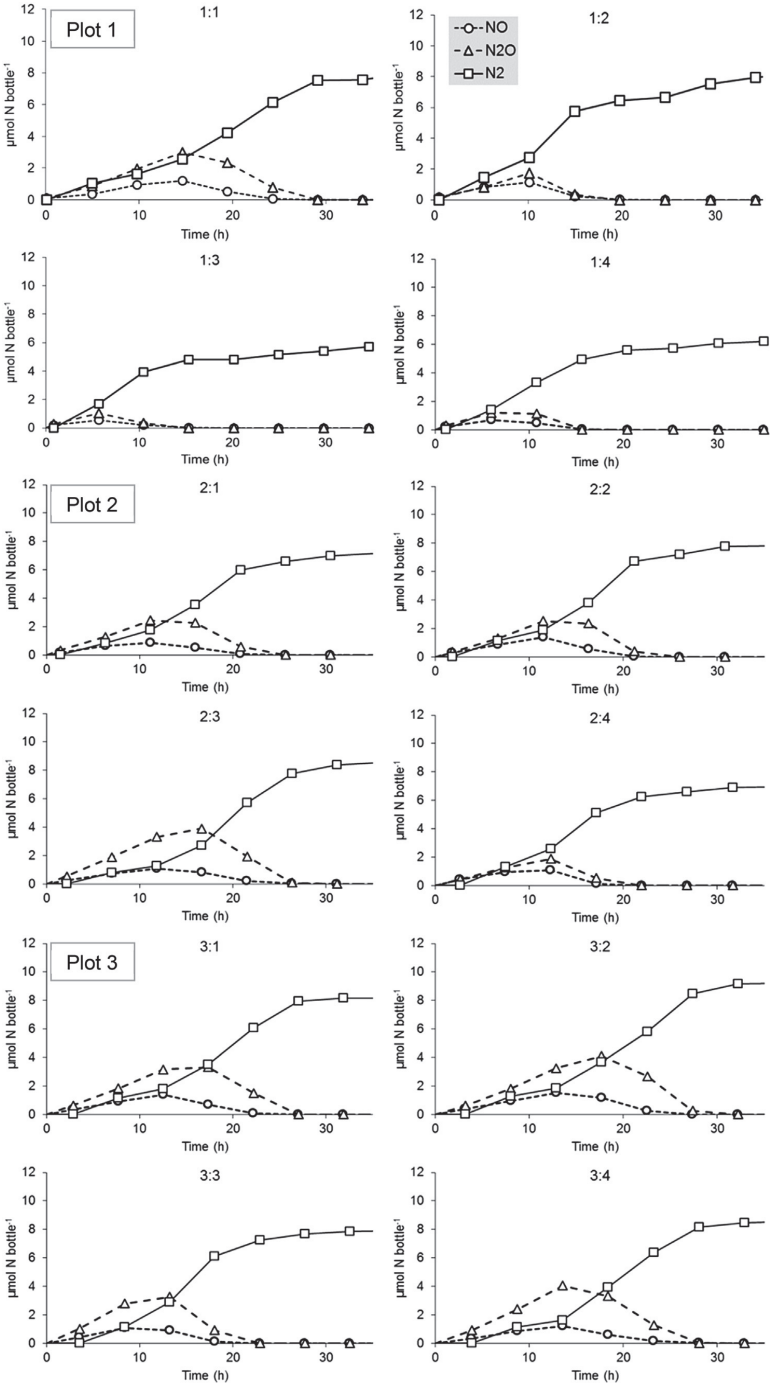

Fig. 6. Gas kinetics during anoxic incubation of soil amended with glutamate. The panels show the measured amounts of NO and N_2O and net N_2 production (cumulative) as µmol N per bottle, plotted against time (h). Each panel is the result from the incubation of the soil sample taken at the middle of each of the four frames within the tree plots (soil pH increase from plot 3 to 1). The N_2 reached plateaus that ranged from 5 to 10 µmol N $bottle^{-1}$, suggesting near 100% recovery of the NO_3 as N-gas: The incubated soil samples

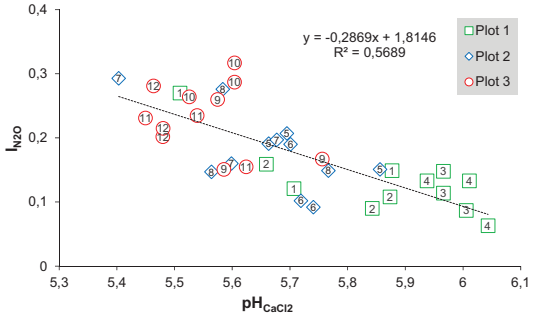

Fig. 7. N_2O index for the anoxic incubation of glutamate amended soil versus soil pH (measured after incubation). Data are shown for individual soil samples. Different symbols represent the plots, and numbers in symbols identify the frame from which the soil samples were taken (three soil samples from each frame).

summarized by Qu et al. (2014), who concluded that we need to conduct more dedicated field experiments that rigorously test the pH effect on soil N_2O emissions. In the present study, we addressed the pH effect by investigating small-scale spatial pH variation together with N_2O emissions in a uniformly cultivated arable field during off-season, while assessing the N_2O product ratio by standardized anoxic laboratory incubations.

When designing the experiment, we expected a relatively even distribution of soil pH within each of the three selected plots, but this was not the case; the frames within each plot differed in soil pH, and even within individual frames there were differences (see standard deviations in Fig. 3 and Table S1). This suggests that there was a rather fine-scaled variability of pH with a range of ±0.1–0.2 pH units, a phenomenon which also has been observed in other soils (Yang et al., 1995, 2001). This means that we should have taken more than three soil samples per frame (each pooled from 3 soil cores) to determine the average soil pH in each frame. Despite this shortcoming, the average pH per frame appeared to have some predictive power regarding N_2O emission: the emissions during the autumn and the thawing period were negatively correlated with the soil pH in the frames (Fig. 3A, B).

It appears likely that emissions during autumn and during thawing were dominated by denitrification, driven by decomposition of fresh plant litter and high soil moisture content during the autumn, and by the frost mediated release of organic substrates during the thawing, as observed by others (Flessa et al., 1995; Mørkved et al., 2006; Wagner-Riddle et al., 2008). Thus, the results for the autumn and the thawing period apparently corroborates our hypothesis that denitrification-driven N_2O emission will increase with decreasing soil pH, because low pH impedes the expression of N_2O reductase (Liu et al., 2014). However, we need to take soil nitrate contents into account: during the autumn, extractable nitrate was very low in the plot with the highest pH (plot1), compared to the two others. Thus the low emissions in plot 1 could be due to nitrate limitation of denitrification, hence lower rates of denitrification with low $N_2O/(N_2+N_2O)$ ratio (Senbayram et al., 2012). The emission of N_2O during autumn was indeed positively correlated with NO_3^- concentration, primarily due to plot 1, which had both low N_2O emissions and low NO_3^- concentrations (Fig. S9). Thus, the low N_2O emissions from plot 1 during the autumn could possibly be ascribed to very low NO_3^- concentrations, rather than to soil pH. However, the emissions during the early spring thaw (Fig. 3B) showed a similar pH relationship between the plots, and in this case, NO_3^- concentrations were practically identical for the three plots (Fig. 2C). This indicates that although soil NO_3^- concentrations may have had an influence on the N_2O emissions during the autumn and early spring, soil pH was the primary factor determining the spatial variation of N_2O emissions.

Soil organic carbon (TOC) content is another factor which could contribute to the spatial variation in N_2O emissions. The average TOC for plots 1, 2 and 3 were 27, 29 and 34 mg C g^{-1}, respectively, and showed a negative relationship with pH and a positive relationship with N_2O emission during autumn and snow melt (Table S2). This could be taken to suggest that the differences in TOC between the plots were the primary drivers for the observed differences in N_2O emission rates. The reasoning would be that higher TOC implies higher availability of C substrates, hence higher denitrification rates and N_2O emission. Although there is evidence for a positive relationship between C-substrate availability and N_2O emission (Robertson and Klemedtsson, 1996; Li et al., 2005), we do not think that this is important in the present experiment. Firstly, differences in TOC content between the plots were marginal. Secondly, the clearly larger N_2O emission from plot 3 compared to plot 2 cannot be explained by greater C availability, as similar oxic respiration rates were measured in both plots (Fig. S10).

In conclusion, while not dismissing the fact that NO_3^- concentrations and organic carbon availability may have an effect on the spatial variability of N_2O emission, our field data show that N_2O flux variation can in fact be related to small differences in soil pH during periods of little plant activity, despite its strong collinearity to other factors.

In late spring, N_2O emissions were very low and showed no relation to soil pH. Our intention was to stimulate denitrification by fertigation at the onset of this period, but this was apparently unsuccessful. Our tentative explanation for this is a combination of low soil respiration (most of the easily available carbon from crop residues had already been consumed) and the very fast drainage (the soil moisture content within the frames showed a very short lived spikes immediately after fertigation, Fig. S2). Thus, the N_2O emission during late spring was probably entirely driven by nitrification. To evaluate the likelihood of this, we need to consider the N_2O–N/NO_3^--N product ratio of nitrification as well as the nitrification rate. The latter was not measured, but a plausible range would be 0.05–0.1 g N m^{-2} d^{-1}, as measured during the growth season in comparable soils and climate conditions (Wu et al., 2008). The N_2O–N/NO_3^--N product ratio in soils with pH > 5 was found to range from 0.002 to 0.009 (Mørkved et al., 2007), which is in good agreement with investigations of both soils and pure cultures of ammonia oxidizing bacteria as cited by Mørkved et al. (2007). The expected emission from nitrification during late spring would thus be 0.1–1.3 mg N_2O–N m^{-2} d^{-1}, or 4–54 μg N_2O–N m^{-2} h^{-1}. The average emissions observed in late spring were in the lower end of this range: 3–20 μg N_2O–N m^{-2} h^{-1} (Fig. 2). This rough calculation suggests that nitrification could indeed account for the N_2O emission measured during late spring. As stated in the introduction, nitrification driven emission is unlikely to be affected by soil pH since the N_2O/NO_3^- product ratio of nitrification in soil has been found to be independent of soil pH for soils with pH > 5 (Mørkved et al., 2007). This would explain why the low N_2O emissions during the late spring showed no relationship with soil pH.

The primary purpose of the soil incubations was to obtain a measure of the soil's propensity to emit N_2O from denitrification.

contained 4.2–5.7 mL soil moisture $bottle^{-1}$, with an intended NO_3^- concentration of 2 mM (by flooding and drainage). Thus, the expected amount of NO_3^- per bottle should be 8.2–11.4 μmol $bottle^{-1}$.

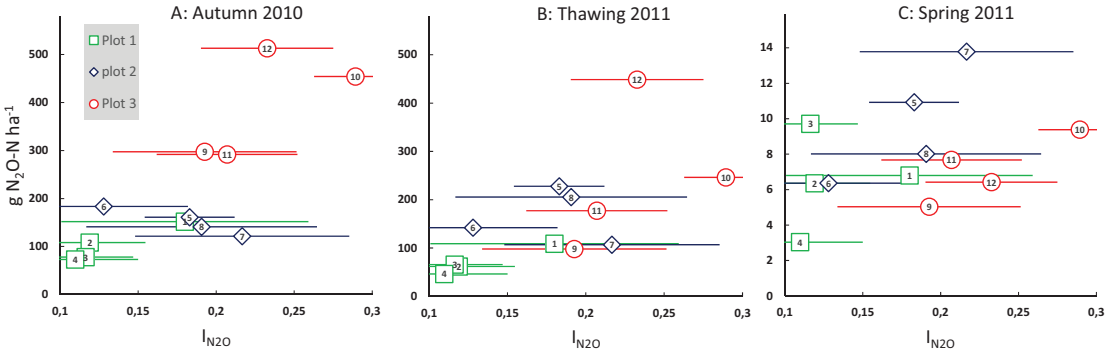

Fig. 8. Cumulative N_2O emissions versus N_2O index for autumn, spring thaw and spring. The data show cumulated N_2O emissions from each frame plotted against the average N_2O index (I_{N2O}) per frame determined by laboratory incubation (n = 3).

Kinetics of N_2O production and reduction to N_2 were measured under standardized conditions regarding temperature, oxygen concentrations, C-substrate availability (in experiment 3) and NO_3^- concentrations (Liu et al., 2010). The results for the incubation of soils without glutamate were problematic for various reasons; denitrification rates were variable, apparently due to variable availability of C substrates (Fig. 5; Table S4), and the incubation period was too short for all the soils to deplete the NO_3^- pool, hence a proper N_2O index as defined by Qu et al. (2014) could not be calculated. The incubation of glutamate-amended soils provided more convincing results (Fig. 6) in the sense that all soils depleted NO_3^- which was recovered as N_2 within the time frame of the incubation, allowing to calculate a unified N_2O index (Eq. (3)). This index is inversely related to the ability of the denitrifying community to express N_2O reductase early and effectively during anoxic spells; a low index means early and effective onset of N_2O reduction, a high index implies N_2O reduction activity lags severely behind the other reduction steps in denitrification. An alternative to calculating the N_2O index is to calculate the initial $N_2O/(N_2+N_2O)$ ratio as done by Jones et al. (2014), and this yields essentially the same information as the N_2O-index, as judged by the close correlation between the two variables (Fig. S8A).

The strong negative relationship between the N_2O index and soil pH (Fig 7; Table S2) shows that pH controls the ability of the denitrifying bacteria to express N_2O reductase early and efficiently, increasingly so with increasing pH. This corroborates previous observations with numerous other soils (Liu et al., 2010; Raut et al., 2012; Qu et al., 2014; Bakken et al., 2015). Our explanation of the phenomenon is that low pH impedes the assembly of the N_2O reductase enzyme. The most sensitive step is possibly the insertion of Cu, which in Gram-negatives takes place in the periplasm, where the organism cannot control the pH adequately (Liu et al., 2014).

As stated earlier, an alternative explanation for a negative correlation between soil pH and the $N_2O/(N_2+N_2O)$ ratio is that low pH selects for organisms which lack the gene for N_2O reductase (Jones et al., 2014). Although we doubt that this could account entirely for the observed pH effect on $N_2O/(N_2+N_2O)$ ratio, we are in no position to claim that the community composition plays no role at all. Neither can we exclude that other soil factors than pH had an effect on the $N_2O/(N_2+N_2O)$ ratio and the N_2O index as measured. In short, it appears naïve to assume that the N_2O index as measured is determined exclusively by soil pH. This would imply that the N_2O index is a better predictor for a soil's propensity to emit N_2O from denitrification than soil pH, which appears to be the case, although an objective evaluation of this would require more data.

We deliberately conducted our field experiment during off-season in a none-ploughed field, as we expected least interference from management related factors such as fertilization, competition for nitrogen between roots and microbiota, and mechanical perturbation by tillage. We found high N_2O emissions after rainfall in September, after a period of night frosts in October and during spring thaw in April. We have no information about growing-season emissions in this field, but other studies have shown that off-season emissions contribute significantly to overall N_2O budgets in agricultural soils of the cool-temperate zone (Flessa et al., 1995; Tatti et al., 2014). Our results indicate that careful pH management of arable soils could mitigate at least some part of the annual N_2O emission. Particularly the negative correlation between soil pH and spring thaw emissions is of interest, because spring thaw emissions can be high (Dörsch et al., 2004). It remains to be tested whether small-scale pH variation also affects N_2O emissions during the cropping period, and how crop management and fertilization strategy interplay with the pH-effect.

In summary, our study demonstrates that spatial variation of soil pH within a narrow range of 5.4–5.9 had an appreciable effect on the N_2O emissions as hypothesized (increasing emissions with decreasing pH), but only for those periods when denitrification was the dominant source of N_2O. Differences in emission strength during such periods followed intrinsic N_2O product ratios (I_{N2O}) of denitrification determined by standardized anoxic incubation. The latter is in accordance with previous experimental findings, pinpointing the role of pH for the functioning of N_2OR in denitrifying bacteria. If this finding can be reproduced at larger scales, moderate liming could indeed be an instrument to reduce N_2O emission from acid soils.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.04.019.

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Supplementary Material

Spatial variation in soil pH controls off-season N_2O emissions in an agricultural soil

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A: Supplementary Tables

Table S1: Measured pH in soil at the end of each incubation experiment (1-3; n=36). The soil in the incubation bottles was dispersed in 0.01 M $CaCl_2$ (1:2.5 w/w). The table shows average pH for each frame and standard deviation. For comparison, the pH measured in bulk soil prior to incubation is included (measured in 0.01 M $CaCl_2$)

Plot	Frame	Experiment 1 $pH_{CaCl2(Oxic)}$	Stdev $_{Exp1}$	Experiment 2 $pH_{CaCl2(Anox)}$	Stdev $_{Exp2}$	Experiment 3 $pH_{CaCl2(Glu)}$	Stdev $_{Exp3}$	Bulk soil $pH_{(CaCl2)}$	Stdev $_{Bulk}$
1	1	5.57	0.09	5.63	0.06	5.75	0.09	5.64	0.22
	2	5.64	0.06	5.70	0.03	5.90	0.04	5.68	0.15
	3	5.73	0.04	5.79	0.02	6.01	0.05	5.95	0.05
	4	5.74	0.03	5.88	0.03	6.08	0.02	5.91	0.06
2	5	5.65	0.03	5.70	0.01	5.91	0.03	5.56	0.14
	6	5.67	0.03	5.65	0.04	5.89	0.03	5.55	0.04
	7	5.56	0.06	5.52	0.07	5.69	0.13	5.43	0.12
	8	5.55	0.02	5.57	0.00	5.68	0.04	5.60	0.14
3	9	5.60	0.07	5.55	0.06	5.71	0.04	5.57	0.13
	10	5.50	0.05	5.48	0.04	5.64	0.04	5.51	0.03
	11	5.45	0.02	5.49	0.03	5.62	0.05	5.46	0.11
	12	5.44	0.07	5.56	0.07	5.57	0.05	5.38	0.05

Table S2: Correlation matrix for soil pH (measured in spring 2011), N_2O index (I_{N2O}) determined in the soil incubation with glutamate addition, seasonal N_2O emissions and mineral N and TOC contents. For pH and I_{N2O}, average single frame values are used whereas nitrate and TOC are averaged from repeated measurements in soil samples taken outside the frames. Shown are Pearson correlation coefficients and their p-values in parenthesis. Significant correlations are in bold ($p<0.05$*; $p<0,01$**)

	pH	I_{N2O}	autumn emission	thaw emission	spring emission	NO_3^- autumn	NO_3^- thawing	NO_3^- spring	NH4+ autumn	NH4+ thawing	NH4+ spring
I_{N2O}	**-0,850**** (0,000)										
Autumn emission	**-0,662*** (0,019)	**0,844**** (0,001)									
Thaw emission	**-0,662*** (0,019)	**0,753**** (0,005)	**0,805**** (0,002)								
Spring emission	-0,319 (0,311)	0,305 (0,334)	-0,074 (0,819)	0,111 (0,732)							
NO_3^- autumn	**-0,772**** (0,003)	**0,652**** (0,022)	0,519 (0,084)	**0,719**** (0,008)	0,221 (0,490)						
NO_3^- thawing	-0,255 (0,423)	0,238 (0,456)	0,128 (0,691)	0,347 (0,269)	0,436 (0,157)	0,002 (0,995)					
NO_3^- spring	-0,508 (0,092)	0,487 (0,108)	0,526 (0,079)	**0,760**** (0,004)	0,189 (0,557)	0,370 (0,236)	**0,747**** (0,005)				
NH4+ autumn	-0,188 (0,559)	-0,161 (0,618)	-0,172 (0,594)	-0,253 (0,427)	-0,073 (0,823)	-0,199 (0,535)	0,096 (0,767)	-0,034 (0,917)			
NH4+ thawing	0,060 (0,853)	-0,177 (0,582)	-0,215 (0,502)	-0,277 (0,383)	0,098 (0,763)	-0,462 (0,131)	0,499 (0,099)	0,158 (0,625)	0,326 (0,301)		
NH4+ spring	**-0,588*** (0,044)	0,523 (0,081)	**0,593*** (0,042)	0,460 (0,132)	-0,076 (0,815)	0,517 (0,085)	-0,002 (0,996)	0,367 (0,240)	-0,123 (0,702)	-0,116 (0,719)	
TOC %	**-0,711*** (0,010)	**0,840**** (0,001)	**0,872**** (0,000)	**0,622*** (0,031)	0,028 (0,931)	**0,630*** (0,028)	-0,011 (0,972)	0,301 (0,342)	-0,286 (0,367)	-0,219 (0,495)	**0,780**** (0,003)

Table S3: Measured CO_2 production during oxic incubation. The rates are given per g dry weight of soil material < 2 mm (nmol CO_2 g^{-1} dw h^{-1}). The table shows the average for each frame (n = 3 replicate bottles), and for plots (n = 3*4 = 12 replicate bottles). ANOVA showed no significant difference between frames and plots.

Plot	Frame	Frame averages	stdev	Plot avg	stdev
		nmol CO_2 g^{-1} dw h^{-1}			
1	1	16,8	4,4		
1	2	21,0	5,2		
1	3	21,5	4,9		
1	4	17,2	1,1	19,1	4,3
2	5	28,5	6,0		
2	6	22,5	7,6		
2	7	33,9	22,7		
2	8	18,3	4,8	25,8	12,4
3	9	24,7	7,4		
3	10	20,6	3,9		
3	11	30,9	3,7		
3	12	33,5	15,5	27,4	9,3

Table S4: Measured denitrification rate during anoxic incubation of soil without glutamate. The rates are per g dry weight of soil material with diameter < 2 mm (nmol N g^{-1} dw h^{-1}). The table shows the average for each frame (n=4 replicate vials), and for plots (n=3*4=12 replicate vials). ANOVA showed no significant difference between frames, nor between plots.

Plot	Frame	Frame averages	stdev	Plot avg	stdev
		nmol N g^{-1} dw h^{-1}			
1	1	10.6	2.4		
1	2	14.2	1.5		
1	3	12.0	2.2		
1	4	10.5	1.5	11.8	2.3
2	5	13.0	0.5		
2	6	11.8	3.6		
2	7	13.8	3.2		
2	8	10.4	0.9	12.2	2.5
3	9	11.0	1.9		
3	10	11.0	0.8		
3	11	15.2	1.5		
3	12	15.3	2.5	12.2	2.7

Table S5: Spring wheat nitrogen yield in plots 1 through 3 in 2010

Plot	Protein (%DM)	Moisture (%)	Starch (% of DM)	Gluten (% of DM)	Moisture at harvest (%)	Yield[1] (Kg ha^{-1})	N in yield (Kg N ha^{-1})
1	14.8	12.5	63.6	36.7	19.0	5750	126
2	12.1	12.0	65.7	26.8	19.0	5925	106
3	13.8	11.7	63.9	32.1	19.3	6301	129

[1]at 15% moisture

B: Supplementary Figures

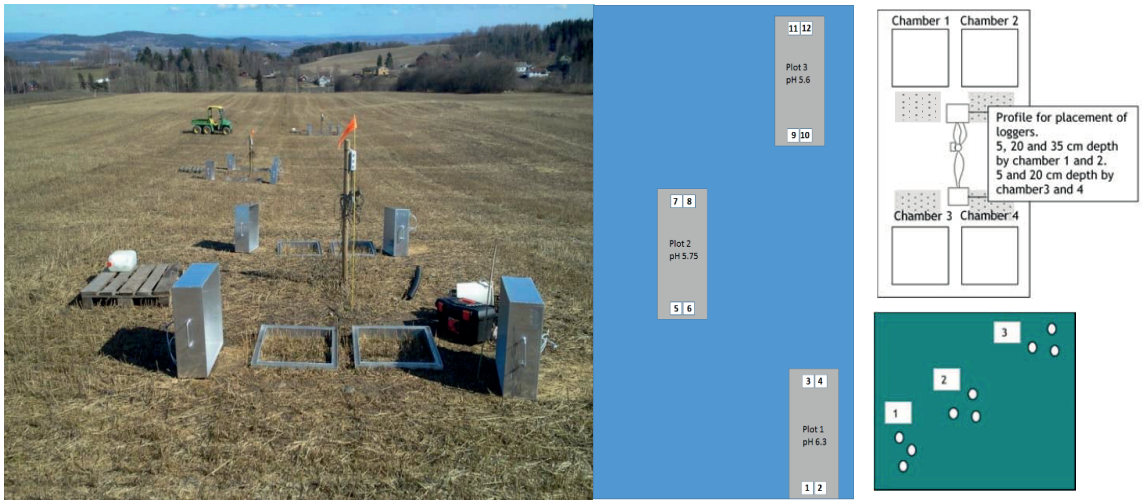


Figure S1: Field plan, sensor placement and sampling scheme. Photo and placement scheme (blue panel right) show the position of the permanent frames used for N_2O emission measurements in the three plots: two adjacent frames were placed at each end of the plot. The pH values shown are the expected values based on grid screening in 2001 (measured by dispersing soil in H_2O, 1:2.5 w/w). The upper right corner (white box) shows the placement of sensors (soil temperature and moisture) within each plot; the shaded areas indicate the area of the plot where soil was sampled for the individual chambers during emission measurement. The lower right corner (green box) exemplifies one permanent frame and shows the locations for final soil sampling along a diagonal line. At each of the three locations, three soil cores were taken (0-20 cm) with an auger, and pooled yielding three distinct samples per frame used for incubation experiments and pH measurements (in 0.01 M $CaCl_2$) in spring 2011.

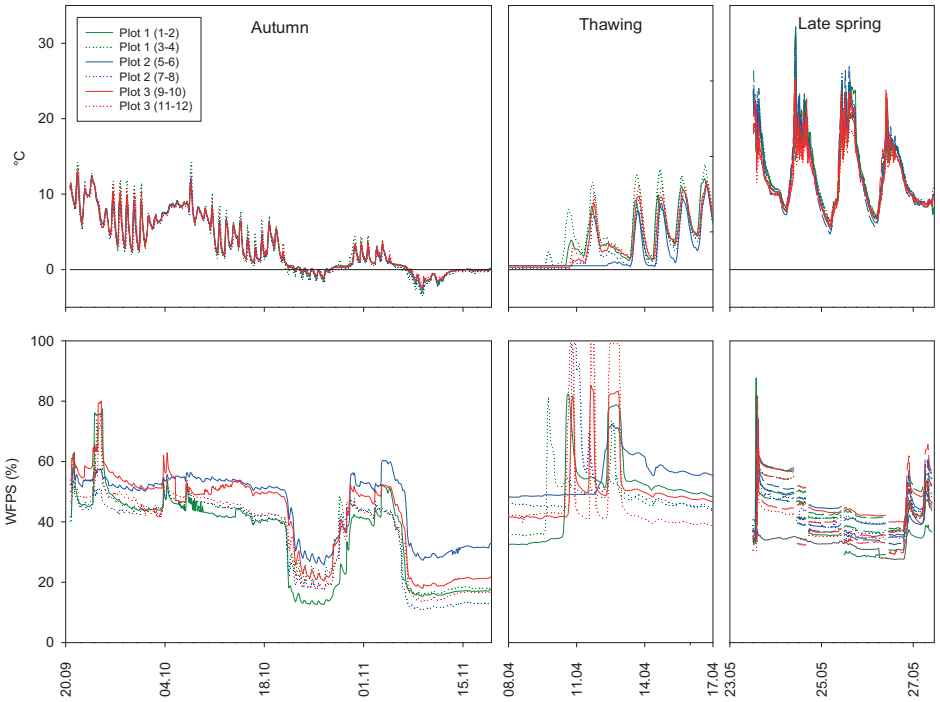


Figure S2: Soil temperature and moisture content in 5 cm soil depth during autumn 2010 (left panel), thawing (middle panel) and late spring 2011 (right panel). The figures show readings for each 2 adjacent frames in each plot (parentheses in figure legend). During late spring 2011 (right panel), sensors were placed in 2 cm depth in each frame and removed during chamber deployment to follow the effect of fertigation on soil moisture. The grey line in right panel indicates average WFPS from one sensor in each of the tree plots placed outside of the frames to monitor uninterrupted soil moisture at 2 cm depth.

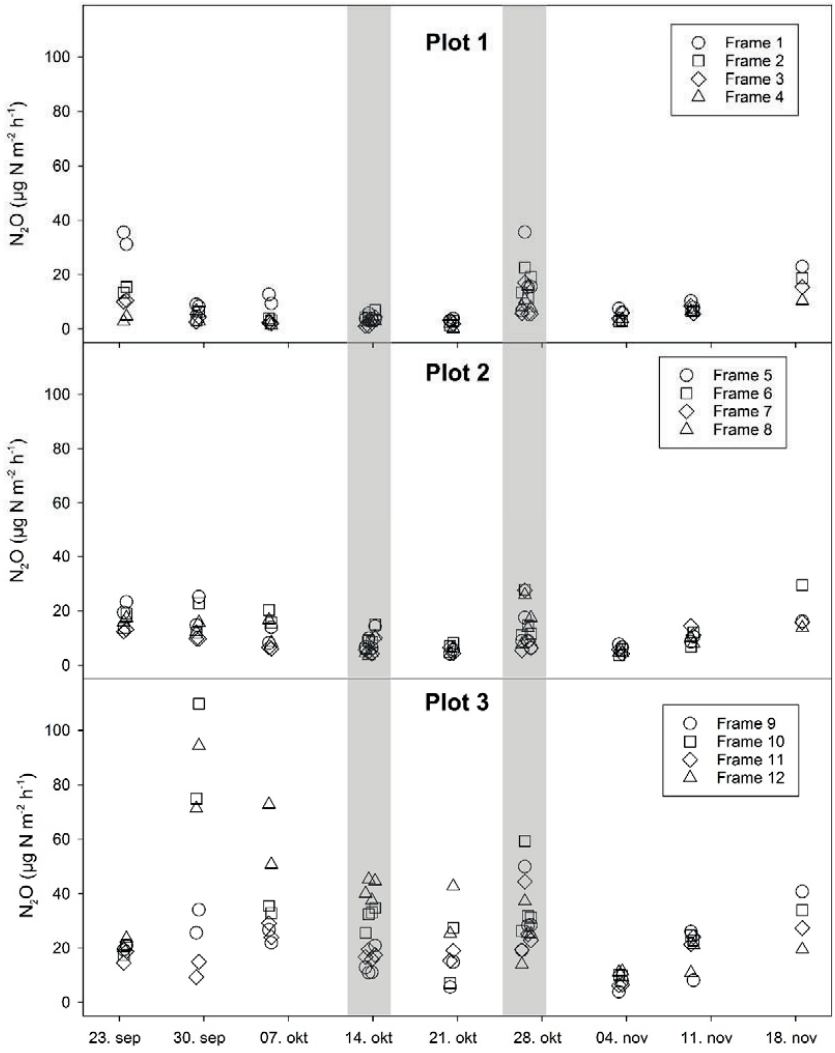
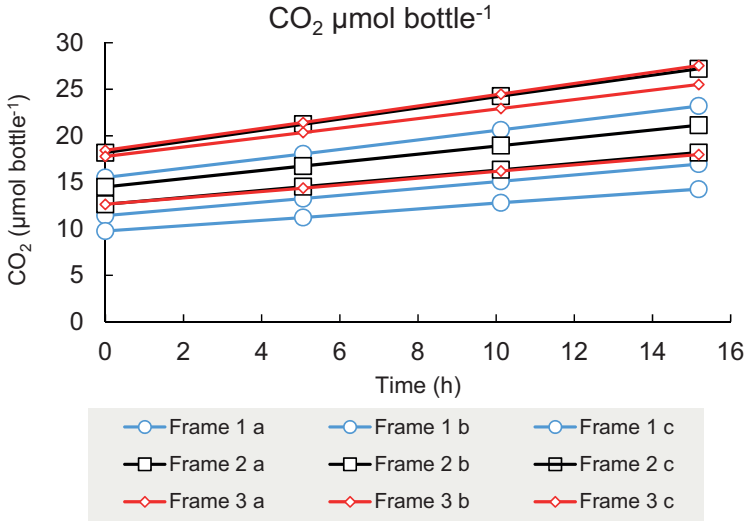


Figure S3: Measured emissions during autumn 2010 in individual frames in plots 1 - 3. The positioning of the frames within the plots is shown in Figure S1 (adjacent frames are 1 and 2, 3 and 4, 5 and 6, etc.). Grey bars denote sampling dates with four measurement per day (diurnal variation).



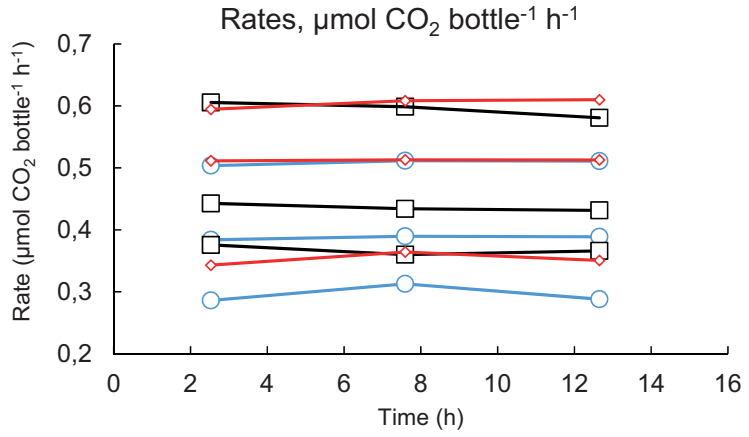
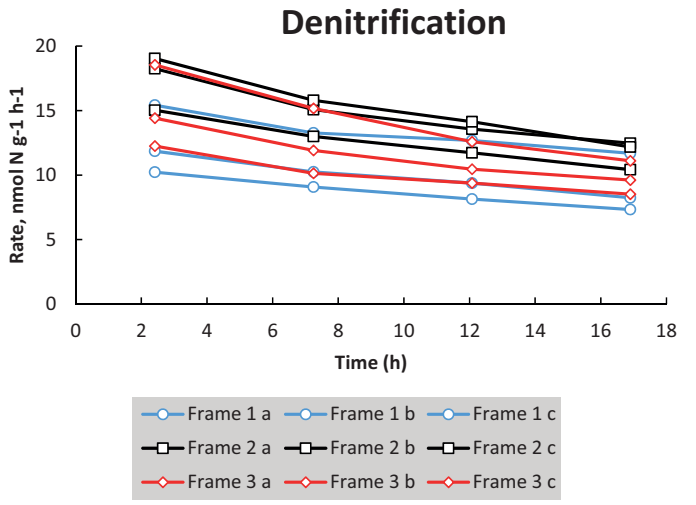


Figure S4: CO_2 production during oxic incubation. The top panel shows the measured amounts of CO_2 bottle^{-1} throughout the 15 h incubation of 9 bottles (from 3 frames, as indicated in the figure legend). The lower panel shows the estimated rate of CO_2 production for three time intervals between two measurements. Very similar results were obtained for the other vials (i.e. a practically constant rate of CO_2 accumulation).

A



B

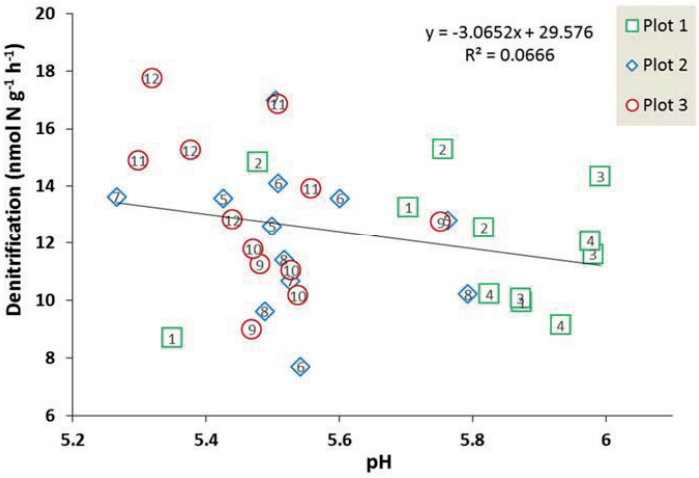


Figure S5: Denitrification rates during anoxic incubation of soil without glutamate. The rates measured for the 4 time intervals throughout 20 hour incubation are plotted against the mid time of each time increment. (A) Results for 9 samples. The rest of the samples showed essentially the same gradual decline in denitrification rates. The lower panel (B) shows the average denitrification rate for each soil sample (bottle) plotted against measured soil pH.

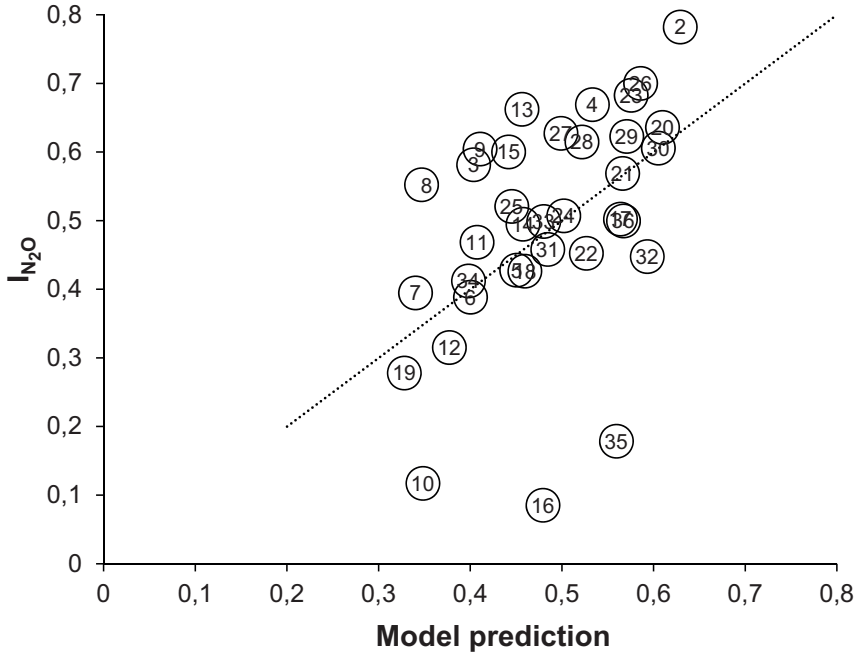


Figure S6: N_2O index for anoxic incubation of soil without glutamate; dependency on pH and oxic respiration. The index (I_{N2O}) is based on the same cutoff time for all bottles (20 h). We suspected that I_{N2O} would depend on the general metabolic activity (or C-avaiability). We tested this by regression analysis with both pH and the oxic respiration rates, and found that both had a significant influence (p=0.011 and 0.033, respectively). The regression model obtained is I_{N2O}=3.542 - 0.52*pH_{CaCl2} - 0.0057*Resp (R^2 = 0.25). The figure shows individual I_{N2O} indices plotted against prediction by the model, the line is 1:1. A similar result was obtained by using pH and average denitrification rate as independent variables (R^2 = 0.35). (Bottle 1 is omitted from this prediction)

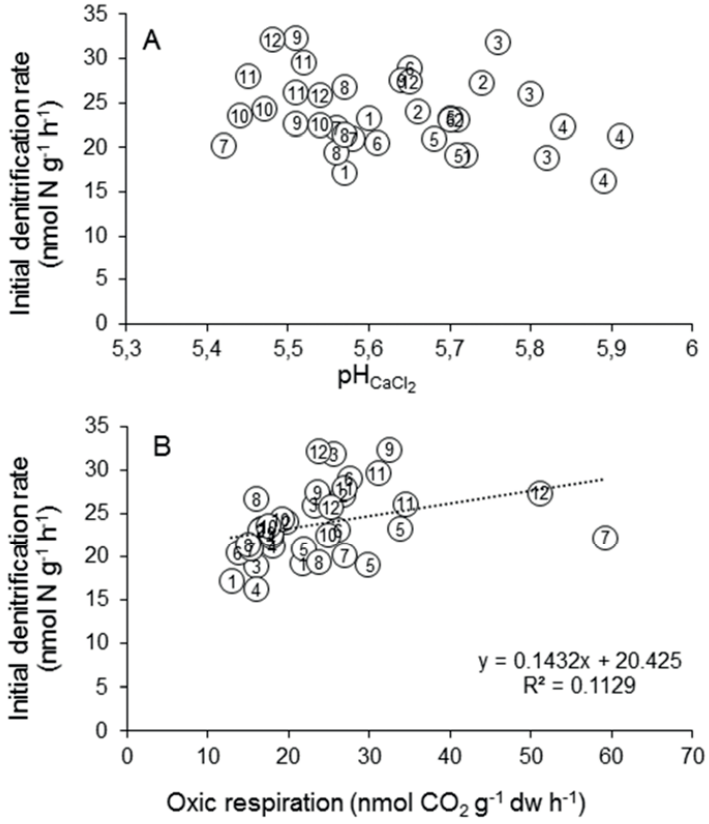


Figure S7: Initial denitrification rates (average rate of the NO + N_2O + N_2 production; n=3) in glutamate amended soil from all frames during the first 5 hours of incubation (frame num bers in symbols). A: correlation with pH measured at the end of the incubation; B: correlation with the respiration measured during the oxic incubation of the same samples. The initial denitrification rate was positively correlated with oxic respiration (p=0.045; regression function shown in the panel), but not with pH (p = 0.47).

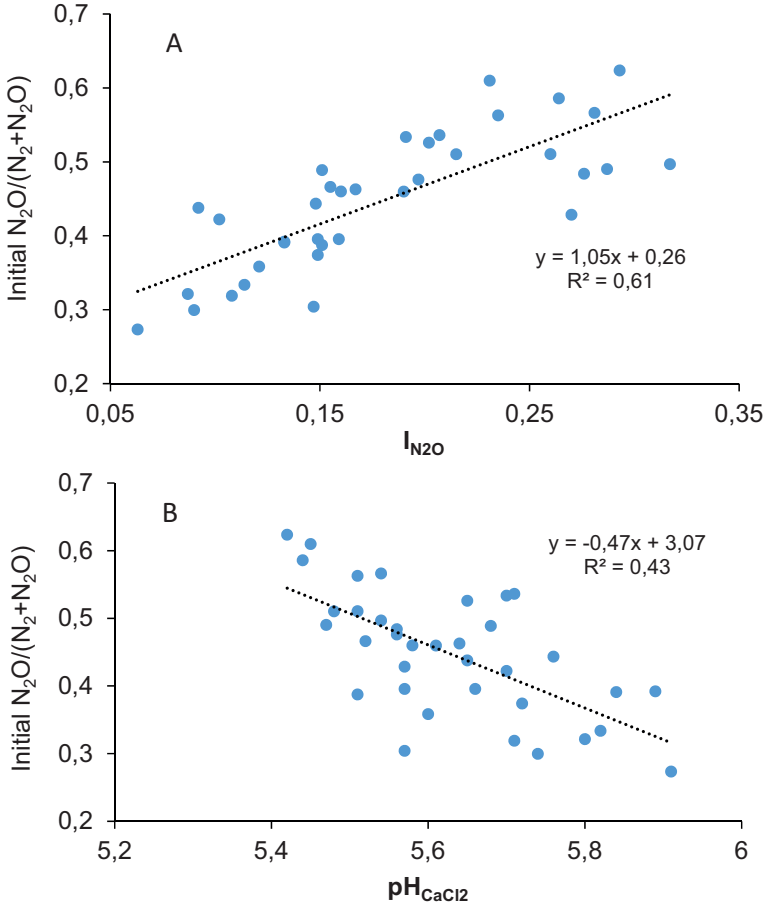


Figure S8: Initial $N_2O/(N_2+N_2O)$ ratio of denitrification during incubation with glutamate. A: correlation with I_{N2O}, B: correlation with measured soil pH. The initial $N_2O/(N_2+N_2O)$ ratio was calculated from the first time increment between two gas samplings (5 h interval) as $V_{N2O}/(V_{N2O}+V_{N2})$, where V_{N2O} is the estimated rate of N_2O production and V_{N2} is the estimated rate of N_2 production.

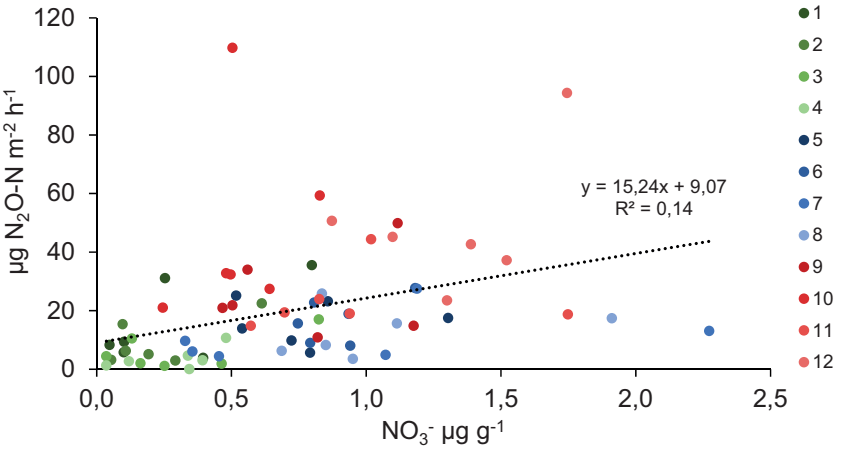


Figure S9: Instantaneous N_2O field emissions (1 – 12; single frame values) and measured soil NO_3^- contents during the autumn period. The soil for NO_3^- extraction was sampled in close perimeter of the frames (cf. Fig. S1). Instantaneous N_2O emissions were positively correlated with soil NO_3^- content at the time of measurement ($p = 0.001$). If excluding the data for plot 1 (frame 1-4), there was no significant correlation ($p = 0.38$)

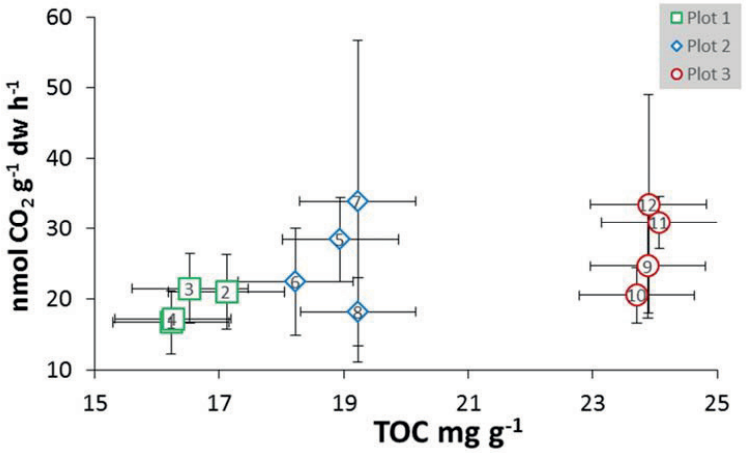


Figure S10: Respiration measured in nmol CO_2 g $soil^{-1}$ during oxic incubation (experiment 1) plotted over TOC in the field soil measured throughout autumn

Paper II

Nutr Cycl Agroecosyst
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ORIGINAL ARTICLE

Effects of nitrogen split application on seasonal N_2O emissions in southeast Norway

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Received: 6 November 2018 / Accepted: 24 June 2019

Abstract Nitrous oxide (N_2O) emissions from cultivated soils correlate positively with the amount of N-fertilizer applied, but a large proportion of the annual N_2O emission occurs outside the cropping season, potentially blurring this correlation. We measured the effect of split-N application (total N addition varying from 0 to 220 kg N ha^{-1}) on N_2O emissions in a spring wheat plot trial in SE Norway from the time of split-N application until harvest, and during the following winter and spring thaw period. N_2O emissions were largest in the two highest N-levels, whereas yield-scaled emission (N_2O intensity) was highest in the 0 N treatment. Nitrogen yield increased by 23% when adding 80 kg N ha^{-1} compared to adding 40 kg N ha^{-1} as split application, while corresponding N_2O emissions were reduced by 16%. No differences in measured emissions between the N-fertilization levels were observed during the winter period or during spring thaw. Measurements of soil air composition below the snow pack revealed that N_2O production continued throughout winter as the concentration in the soil air increased from 0.37 to 30.0 μL L^{-1} N_2O over the 3 months period with continuous snow cover. However, only 7–28% of the N_2O emitted during spring thaw could be ascribed to accumulated N_2O, indicating de novo production of N_2O in the thawing soil. The direct effect of split-N fertilizer rate on N_2O emissions in sub-boreal cereal cropping was limited to the first 15–21 days after N-addition.

Keywords Nitrous oxide · Split-N application rate · Yield-scaled emissions · Spring wheat · Off-season emissions

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10705-019-10009-0) contains supplementary material, which is available to authorized users.

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Introduction

Nitrous oxide (N_2O) is a greenhouse gas with a global warming potential 263 times higher than that of CO_2 in

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a 100 years perspective (Neubauer and Megonigal 2015). N_2O is also a major ozone depleting substance (Ravishankara et al. 2009). The atmospheric mixing ratio of N_2O has increased by approximately 16% since pre-industrial times and 60% of this increase is attributed to the use of reactive nitrogen (N) in crop production (IPCC 2007). Stewart et al. (2005) concluded that at least 30–50% of current global crop yields are realized due to commercial fertilizer N inputs. With a fast growing human population, reactive N use is likely to increase (Tilman et al. 2011). However, the efficiency of commercial N in crop production is low. Erisman et al. (2008) estimated the global nitrogen use efficiency in cereal production to be only about 30% in 2000, a reduction from about 80% in 1960. This means that crop production comes with a high "N cost", including increasing N_2O emissions. Environmentally sound crop management therefore strives to improve N use efficiency and to reduce N_2O emissions in crop production by optimizing rates and timing of N fertilization.

The IPCC Tier 1 emission factor for N_2O assumes that emissions scale linearly with the amount of applied N, irrespective of the form of N fertilizer. Recent meta studies have shown, however, that N_2O emissions expressed as a fraction of N input can increase dramatically when surpassing the N demand of the crop (Van Groenigen et al. 2010). Several studies have identified an exponential increase in N_2O emissions with increasing N surplus (Chantigny et al. 1998; Lebender et al. 2014; McSwiney and Robertson 2005; Van Groenigen et al. 2010; Zebarth et al. 2008), whereas others found moderate responses (Gagnon et al. 2011; Huang et al. 2017; Liu et al. 2012; Mosier et al. 2006).

In the Boreal region, growing seasons are short and winters are long. Therefore, spring wheat (*Triticum aestivum* L.) is one of the preferred cereals in Norway. The profitability of spring wheat depends on the grain quality, in particular the protein content, which is strongly related to nitrogen supply during stem elongation and during the shift from vegetative to generative growth. In Norway, prices for high-quality grain are high, which has resulted in relatively high N-application rates and the use of split-N application [i.e. applying only a part of the N fertilizer at sowing, and the rest in one or more split-N applications later in the season to adjust the N rates to season-dependent N demand (Riley et al. 2012)]. Previous studies indicate that split-N fertilization is an important method ensuring the best protein and bread-making quality (Wieser and Seilmeier 1998; Zebarth et al. 2007).

In cool-temperate climates, a large proportion of the annual N_2O emission occurs off-season, triggered by soil freezing and thaw (Goossens et al. 2001; Kaiser and Ruser 2000). In a recent study in SE Norway, we found low rates of N_2O emissions in spring wheat stubble during autumn before snow cover, but a distinct emission peak during snowmelt (Russenes et al. 2016). Several studies (Christensen and Tiedje 1990; Dörsch et al. 2004; Flessa et al. 1995; Goodroad and Keeney 1984; Wagner-Riddle et al. 2008; Wagner-Riddle and Thurtell 1998) have reported spring-thaw induced N_2O emissions peaks, which they attributed to enhanced denitrification in wet and cold soils during thawing. Other studies (Congreves et al. 2018; Dörsch et al. 2004; Kim and Tanaka 2002; Maljanen et al. 2007; Németh et al. 2014; Risk et al. 2013, 2014) found significant N_2O production also during wintertime, particularly during diurnal freeze–thaw cycles when snow packs were small or absent. Studies involving measurements of N_2O emissions and subnivean N_2O accumulation in snow covered fields are scarce (e.g. Maljanen et al. 2007; Risk et al. 2014), particularly in the Scandinavian region.

The main objective of the present study was to explore the effects of split-N application rate on N_2O emissions in spring wheat cropped in SE Norway during growing season, winter and spring-thaw, considering both area-based emissions and emissions expressed per unit produce. Our hypotheses were that (1) economic optimum N rates result in reduced N_2O losses per unit harvested grain and yield quality during growing season, (2) winter and spring thaw N_2O emissions contribute significantly to overall emissions, and (3) a surplus in growing-season N fertilization would affect subnivean N_2O accumulation and spring emissions. To test this, we set up a field experiment in an ongoing spring-wheat trial with five split-N application rates. N_2O emissions were monitored by closed chambers from split-N application until harvest, throughout winter and during spring thaw. Top-soil dynamics of N_2O (and oxygen) were monitored throughout winter by specially designed soil air probes.

Materials and methods

Experimental site and design

To study the effects of split-N application, we chose plots with different levels of nitrogen fertilization in the first year of a spring wheat (*Triticum aestivum* L.) field trial. The field is located at the Norwegian Institute of Bioeconomy Research (NIBIO)—Apelsvoll research station (60°42′N, 10°51′E, 250 m asl) in southeast Norway on an imperfectly drained brown earth (Gleyed melanic brunisoils, Canada Soil Survey) dominated by loam and silty sand. The content of soil organic material varied between 2.2 and 2.9%. Detailed soil properties are presented in Table 1. Meteorological data were obtained from the meteorological station at Apelsvoll, located approx. 400 m from the experimental site. In the period 2000–2014, annual precipitation was 693 mm and mean annual temperature 5.1 °C.

The field trial was established in spring 2011, by sowing spring wheat (cultivar "Bjarne") on April 19 in ten replicate blocks, each containing seven 2 × 8 m plots (including a border plot on each side). At sowing, all plots except the zero control received 100 kg N ha^{-1} as a compound fertilizer (10% NO_3–N, 11.6% NH_4–N, 9.6% K and 2.6% P). At the beginning of stem elongation (BBCH-stage 31, Lancashire et al. 2008) on June 21, either 0, 40, 80 or 120 kg N ha^{-1} were applied as calcium ammonium nitrate (13.5% NO_3–N and 13.5% NH_4–N) by split-N application, resulting in five N level treatments: 0, 100, 140, 180 and 220 kg N ha^{-1} (hereafter termed 0 N, 100 N, 140 N, 180 N and 220 N, respectively), where 220 N should provide a N-surplus.

Table 1 Soil properties in the two blocks measured 19/4-2011 and texture fractions measured in autumn 2011 in soil samples from 0 to 0.20 m depth

	Block 1	Block 2
pH	6.55 ± 0.15	6.45 ± 0.15
P–AL (mg 100 g^{-1})	6.00 ± 0.50	6.35 ± 0.25
K–AL (mg 100 g^{-1})	7.65 ± 0.85	10.9 ± 2.10
Mg–AL (mg 100 g^{-1})	9.60 ± 0.40	12.5 ± 2.55
Ca–AL (mg 100^{-1})	160 ± 0.00	195 ± 25.0
LOI (%DM)	3.80 ± 0.10	5.05 ± 0.15
K–HNO_3 (mg 100 g^{-1})	33.5 ± 1.50	39.0 ± 3.00
Gravel %	12.3 ± 1.14	12.7 ± 0.24
Total sand	57.8 ± 2.25	51.2 ± 2.45
Total silt	29.6 ± 2.30	30.8 ± 1.40
Clay	12.7 ± 0.10	18.2 ± 1.05

Given are average values and SD

For the N_2O measurements and concentration measurements, we selected two neighboring blocks, in the following designated Blocks 1 and 2. In each of the ten treatment plots (i.e. five in each block), two micro-plots for N_2O flux measurements were established prior to split-N application by pressing two 0.5 × 0.5 × 0.2 m aluminum frames next to each other (about 0.05 m apart) into the soil surface. These permanently installed frames served as bases for N_2O flux chamber (as described below), thus yielding four individual microplots for each N level (2 plots × 2 frames). Average emissions from the two chambers in the same plot were used in the calculations. Soil temperature and volumetric moisture content were measured continuously by sensors (5TE, Decagon Devices, Inc.) for each pair of frames in Block 2. The sensors were permanently installed at depths of 0.05 and 0.20 m. Field setup is illustrated in Online Resource Fig. S1.

Top-soil properties (0–0.20 m) were determined prior to sowing (and fertilization) by sampling soil along a transect through each block, using a 0.025 m soil auger (Table 1). In Norway the ammonium-acetate-lactate method (–AL) by Egner et al. (1960) is used for estimating the content of plant available P, K, Mg and Ca in soil. It is the standard test for agricultural soils in predicting plant availability of these nutrients.

Soil and grain analysis

During the growing season, mineral N content was determined on the date of flux sampling in each plot. Five soil cores were retrieved from 0 to 0.2 m depth and after homogenizing, 0.040 kg of soil was extracted in 0.2 L 2 M KCl by shaking for 1 h in a reciprocal shaker. NO_3^- and NH_4^+ were determined by Flow Injection Analysis (FIAstar5000, SoFIA). All mineral N concentrations are reported on a gram soil dry weight basis.

The mature crop in each microplot used for emission measurements was harvested by hand, dried and threshed manually. All plant material, apart from

the stubble, was removed to avoid interference of N-treatment with soil microclimate and substrate availability as affected by differences in the amounts of plant debris. The rest of the plot was harvested by a plot harvester. A minimum of 0.5 m behind each of the frames were also cut by hand in order to avoid potential disturbance of the gas measurement plots and surrounding area when harvesting. The grain from the plots and within the frames was analyzed by an Infratec^{TM} 1241 Grain Analyser (Foss) for standard cereal quality parameters, including grain moisture and protein. Grain yields were corrected to 15% (w/w) moisture content. Nitrogen yields were calculated from the protein values, assuming 17.4% N in the wheat grain protein.

We fitted a quadratic N response function for yield to our data:

$$Y = a + bN + cN^2 \quad (1)$$

where Y is yield, N is nitrogen fertilizer level, a is the response with no added N-fertilizer (zero control), b represents the slope at origin and c the quadratic component (Korsaeth and Riley 2006). The economic optimum fertilization rate (N_{opt}) was then calculated as the rate at which the marginal cost of N fertilizer equals the marginal revenue (ibid):

$$N_{opt} = \frac{[(P_N/P_Y) - b]}{2c} \quad (2)$$

where P_N is the price of fertilizer N (set to 0.93 € kg N^{-1}) as calcium ammonium nitrate, P_Y is the price of wheat (baking quality, set to 0.34 € kg^{-1}), P_N/P_Y is in the following referred to as the price ratio, and b and c are the same constants as in Eq. 1.

The price level is generally high in Norway, especially for grain (the price for wheat being about 2.2 times that in the EU). To test the effects of changes in the price ratio, we also calculated the economic optimum fertilization rate using EU prices [P_N = 0.65 € kg N^{-1}, P_Y = 0.16 € kg^{-1} (Agrarheute 2018)].

At the end of the measurements in spring 2012, five 0.20 m soil cores were sampled from within each frame. After sieving (2 mm), 0.010 kg of fine soil was dispersed in 0.05 L of 0.01 M $CaCl_2$ solution and shaken for 60 min. The bottles were left to settle for 15 min and pH was measured in the supernatant by a Mettler Toledo pH electrode (Online Resource Table S2).

N_2O flux measurements

N_2O flux measurements took place from June to August 2011 with additional measurements during the time of snow cover in the following winter 2011–2012 including the period of spring thaw in 2012. N_2O emissions were measured by a static chamber method (Rochette and Bertrand 2008), placing 0.51 × 0.51 × 0.20 m aluminum chambers equipped with a 3-way sampling port and a 3 mm diameter pressure equilibration tube (0.15 m long) on the preinstalled frames. The frames had a 0.03 × 0.03 m open groove on the top, which was filled with water prior to deployment to secure airtight connection. As the crop grew higher than 0.2 m, a 0.3 m extension with an identical groove on top was placed on top of each frame in order to prevent crop damage. Air (∼ 15 mL) was sampled from the chambers with a 0.02 L polypropylene syringe 0, 15, 30 and 45 min after deployment. Before each sampling, the air in the chamber was mixed by pulling and pushing the plunger of the syringe three to four times. The samples were transferred to pre-evacuated 12.5 mL glass vials (Chromacol) crimped with butyl rubber septa. Temperature outside and inside the chamber was recorded by a handheld digital thermometer in one replicate chamber per treatment after the last gas sampling. On all sampling dates, fluxes were sampled from Block 1 before noon and Block 2 after noon. All plots within each block were sampled in the same cycle in order to avoid potential differences in emissions caused by diurnal variations. The sampling dates and times are presented in Table S1. The measurements started on the day before split-N application, and continued throughout 3, 6, 10, 17, 30, 49 and 70 days after split-N application. There were no measurements after harvest until snow cover in December 2011. The field was covered by an uninterrupted snow pack from early December until start of snowmelt in March 2012. The extension collars were placed back on the frames in December to allow chamber measurements as snowpack grew in height. During the period of snow coverage, N_2O emissions from the snowpack were measured on a monthly basis. Snowpack higher than the frames (∼ 0.32 m above ground) were removed by hand before chamber deployment. In spring, flux sampling was performed 5, 12 and 19 days after March 8; the date on which the

first bare patches of soil became visible (hereafter defined as onset of snowmelt).

Soil air probes

In autumn 2011, soil air probes (SAP) were installed in each plot in both blocks. The SAPs are described in detail by Nadeem et al. (2012). In brief, they consisted of a sintered polyethylene porous cup with 100 µm pore diameter and a void volume of 6 cm^3 (G1/4-5PU, Bosch Rexroth, Germany), to which a 0.97 mm (inner diameter) Teflon tube was connected via a stop cock. The cup was glued to the end of a 1.5 m long 33 mm diameter PVC tube, while the Teflon tubing was guided to the other end, where it is stopped by a 3-way valve. Six probes were installed on each of the ten plots at 0.20, 0.125 and 0.05 m depth, at soil surface and 0.1 and 0.2 m above soil surface for gas measurements in the snow pack (Fig. S1). The probes were placed in pre-augered holes at an angle of approx. 60° to minimize interruption of vertical soil integrity, and to prevent water from running along the PVC towards the porous cup. Soil air was sampled by first drawing 1.5 mL from the sampling tube with a 20 mL polypropylene syringe to flush the tubing. This volume was discarded. Thereafter 15 mL were drawn slowly from the SAP. After waiting for pressure equilibration, the sample was injected into a pre-evacuated 12.5 mL vial. Concentrations of O_2, CO_2 and N_2O were analyzed by gas chromatography as described below. Soil air observations started on December 2 and continued on a weekly basis until snowmelt, except for the last week in December. Samples were taken twice a week during the first 3 weeks after onset of snowmelt on March 8. Soil air probes located at 0.05 m below the surface in plots with 0 and 140 kg ha^{-1} in Block 1 and in 0.125 m depth in the 140 kg ha^{-1} plot in Block 2 were clogged by ice during the winter, and were therefore omitted.

Gas analysis and flux calculation

N_2O and CO_2 concentrations sampled from the static chambers and the soil air probes were analyzed by use of gas chromatography (GC), as described by Nadeem et al. (2012) and Russenes et al. (2016). An autosampler (Gilson) was used to draw gas from the vials via a peristaltic pump to the injection loops of a He-backflushed GC (Model 7890A, Agilent, Santa Clara, CA, USA) equipped with a 30 m wide-bore (0.53 mm) Poraplot Q column connected to an electron capture detector (ECD) and a 60 m 5Å Molsieve column connected to a thermal conductivity detector (TCD). The conditions for the ECD were 375 °C with 17 mL min^{-1} $ArCH_4$ (90/10 vol%) as makeup gas. He 6.0 was used as a carrier gas. N_2O fluxes were calculated from the concentration change over time by:

$$F_{N_2O} = \frac{d_{N_2O}}{d_t} \times \frac{V_C}{A} \times \frac{M_n}{V_m} \quad (3)$$

where F_{N_2O} is the emission flux (µg N_2O–N m^{-2} h^{-1}), d_{N_2O}/d_t is the rate of N_2O accumulation in the chamber (µL L^{-1} h^{-1}), V_c the chamber volume (L), M_n the molecular mass of N in N_2O (g mol^{-1}), V_m the molecular volume (L mol^{-1}) at chamber temperature, and A is the area covered by the chamber (m^2). Temperature in the chambers were measured at the end of each deployment. To estimate d_{N_2O}/d_t, we fitted either a linear (default) or a quadratic regression to the concentration change against time. A quadratic fit was only used in cases where CO_2 and N_2O accumulation in the chamber showed a convex downwards trend (i.e. decreasing accumulation rates with time) to estimate time-zero rates for d_{N_2O}/d_t. Cumulative fluxes were calculated for selected periods by linear interpolation between dates.

Calculation of N_2O in soil under snow pack

To estimate the amount of N_2O accumulating under the snow pack, we converted relative N_2O concentrations (µL L^{-1}) to µg N_2O–N m^{-2} and 0.24 m depth based on interpolated temperature and air-filled porosity along the profile. Each of the three SAPs placed in the soil at different depths were assumed to represent a 0.08 m layer, with the SAP placed in the middle of the layer. Total pore volume of the soil layers was calculated from soil texture data, using a transfer function calibrated for the same field (Riley 1996). Calculated pore volumes were 43.7% for Block 1 and 45.3% for Block 2, reflecting the differences in texture between the two blocks (Table 1). Since the permanently installed TDR probes did not measure frozen water, we assumed that the soil moisture measured before freezing (December 2) was valid throughout winter. This is justified as snow pack accumulated shortly after soil freezing, but does not

take into account convective redistribution of water in the soil profile during winter. For soil air calculations after spring thaw, we used the values registered by the TDR sensors. As the actual air-filled space will change during winter, only the maximum measured N_2O concentrations shortly before spring thaw were used for comparison with emission fluxes. Total pore space used in the calculations were 43.7 and 45.3 for Block 1 and Block 2 respectively.

Statistics

Effects of N-fertilizer levels on yield, yield quality, cumulative N_2O emissions, yield-scaled N_2O emissions and N_2O soil air concentrations were tested by OneWay Anova, followed by multiple comparisons using Fischer's LSD-method. All statistical analyses were performed at the 0.05 probability level and checked for normality, using the software package Minitab® (version 17.2.1). An N response function for yield was fitted to data using the least square method (Excel® 2013). Data in tables and figures are presented with their standard deviation (SD).

Results

N_2O emission dynamics

Split-N application during stem elongation resulted in increased N_2O emissions in all treatments, but there was also a slight increase in N_2O emissions in treatments which were not top-dressed (N0 and N100) (Fig. 1). Emission rates measured the day after split-N application ranged from 14.6 to 95.8 µg N_2O–N m^{-2} h^{-1}. Flux rates decreased gradually in the weeks after split-N application, but increased again towards the end of the growing season when water filled pore space (WFPS) increased. During the first weeks after split-N fertilization, NO_3^- and NH_4^+ top-soil concentrations (Fig. 1c, d) reflected the different amounts of applied N, before gradually declining towards similarly low and stable levels at crop maturation. Mineral N measured before freezing in early December ranged between 0.21–0.47 µg NO_3–N and 0.70–0.83 µg NH_4–N g dw $soil^{-1}$ (Online Resource Table S3), indicating that the top soil was depleted of mineral N.

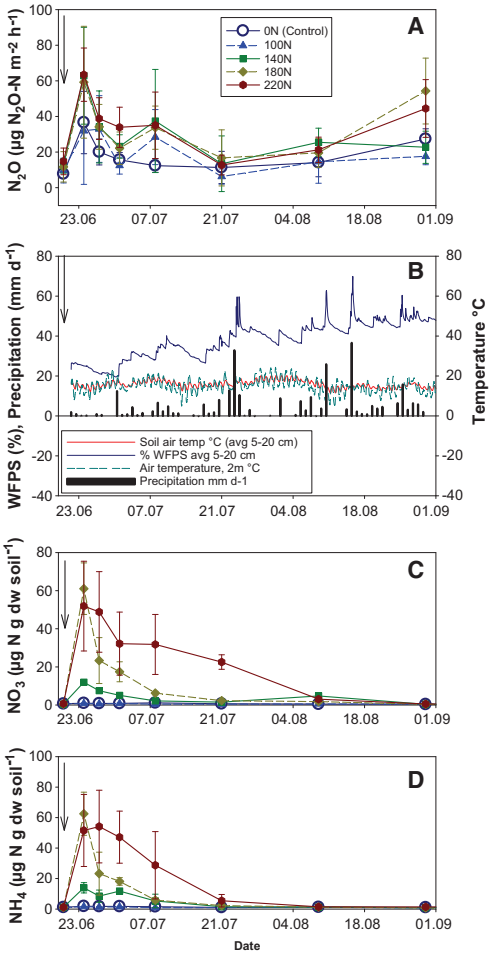

Fig. 1 **a** Mean N_2O emission during the growing season (n = 2; error bars are SD) in five different N treatments after split-N application (arrow), **b** water filled pore space (WFPS; 0.05–0.20 m weighted average for all treatments), daily precipitation, air temperature and soil temperature (average of 0.05–0.20 m depth), **c**, **d** average top-soil (0–0.20 m) mineral N content (n = 2) in plots with different N-fertilizer levels

N_2O emission rates measured during winter (before spring-thaw) were comparable in magnitude to those measured in the growing season. Average emissions were 14.2 µg N_2O–N m^{-2} h^{-1} and maximum emissions were 61.0 µg N_2O–N m^{-2} h^{-1}. At each date with flux measurements, snow depth was measured manually (Fig. 2b). The snow pack peaked on 26. January with 0.35 m and declined throughout

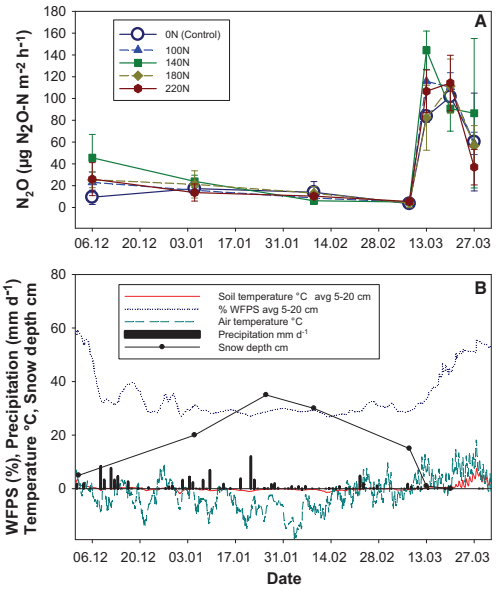

Fig. 2 **a** Mean N_2O emission during winter and spring-thaw in five different N-treatments, **b** non-frozen water filled pore space (WFPS; average for all treatments, 0.05–0.20 m weighted average), soil temperature, daily precipitation and snow depth

February and March. Snow cover was stable throughout winter with little or no melting. The reduction in snow cover from January to February was mainly due to snow compaction and to lesser extent due to snow melting (see climate data in Fig. 2). Air temperatures were mostly below zero °C and formation of ice layers in the snowpack was negligible. In the soil, temperatures fluctuated between − 0.8 and 0.2 °C at 0.05 m and − 2.7 and 0.2 °C at 0.20 m depth until onset of spring thaw on March 8. Thereafter, soil temperature gradually increased, peaking at 6.0 °C at 0.05 m depth and 8.7 °C at 0.20 m depth on March 27. The highest N_2O emission was measured during spring thaw, with rates more than double of those observed during the growing season. Emission rates ranged from 19.5 to 183 µg N_2O–N m^{-1} h^{-1}.

N_2O emissions related to yield, yield quality and optimum N rate

Cumulative N_2O fluxes during the growing season (from split-N application to harvest) increased significantly with increasing N-addition (Table 2,

Table 2 Cumulative N_2O emissions (kg N ha^{-1} $period^{-1}$) for the period after split-N application (in the growing season from the time of split-N fertilization until harvest), spring thawing, winter and spring thaw period and all three periods combined, grain yields (Mg ha^{-1}), nitrogen yield in grain (Mg ha^{-1}) and emission intensity per grain yield (kg N_2O–N emitted per Mg grain ha^{-1}) and N yield (kg N_2O–N emitted per Mg N in grain ha^{-1})

Applied N kg ha^{-1}	Cumulative kg N_2O–N ha^{-1}				Yield Mg grain ha^{-1}	N-yield Mg N ha^{-1}	Grain yield N_2O intensity[1]	N-yield N_2O intensity[2]
	Season 20.06–30.08.11	Winter + spring thaw 02.12.11–27.03.12	Spring thaw 08.03–27.03.12	Season + winter + spring thaw				
0	0.28 ± 0.002[a]	0.61 ± 0.10	0.34 ± 0.24	0.90 ± 0.10[a]	2.26 ± 0.74[a]	0.040 ± 0.013[a]	0.115[c]	7.44[b]
100	0.28 ± 0.013[a]	0.70 ± 0.06	0.31 ± 0.14	0.98 ± 0.05[ab]	5.81 ± 0.54[b]	0.097 ± 0.013[b]	0.045[a]	2.74[a]
140	0.45 ± 0.017[b]	0.83 ± 0.03	0.34 ± 0.14	1.28 ± 0.01[c]	6.70 ± 0.57[bc]	0.136 ± 0.015[bc]	0.061[b]	3.07[a]
180	0.49 ± 0.038[b]	0.72 ± 0.05	0.43 ± 0.08	1.21 ± 0.09[c]	7.42 ± 0.43[c]	0.177 ± 0.007[c]	0.061[b]	2.57[a]
220	0.49 ± 0.034[b]	0.68 ± 0.19	0.34 ± 0.08	1.16 ± 0.15[bc]	7.01 ± 0.67[bc]	0.176 ± 0.011[c]	0.064[b]	2.54[a]

Different letters indicate significant differences between fertilization levels at $p < 0.05$

[1]kg N_2O–N Mg $grain^{-1}$; [2]kg N_2O–N Mg N in $grain^{-1}$

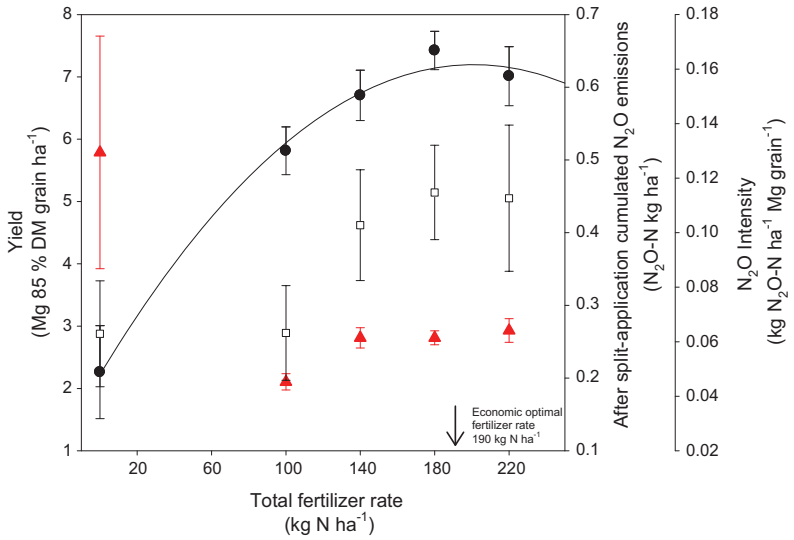

Fig. 3 N-level response of grain yield (filled circle), season cumulated N_2O emissions (open square) and yield-scaled cumulative N_2O emissions (N_2O intensities, filled triangle) for the period from split-N application to harvest (season). Given are average values (N_2O: n = 4, yield: n = 2) error bars are SD for N_2O and min/max for yield. The line represents a quadratic yield response curve fitted to the data (Eq. 1)

$p = 0.001$). Fisher pairwise comparisons indicated significantly larger emissions in 140 N, 180 N and 220 N than in 0 N or 100 N, i.e. additional N-fertilization as split-N application increased N_2O emissions. The cumulative N_2O emissions over the initial 4 weeks after split-N application revealed significant difference between the plots receiving split-N application and the 100 N plots receiving no extra N ($p = 0.001$). The 0 N plots emitted significantly less N_2O than the 100 N plots. Emissions during the initial 4 weeks ranged from 0.12 to 0.24 kg N_2O–N ha^{-1}. Emissions during winter were low, but when cumulated for the 3-month period with snow cover, they summed up to 0.27–0.40 kg N_2O–N ha^{-1}, without revealing any significant differences between N treatments. N_2O emissions cumulated for 20 days of spring thaw (March 8–27 2012; Fig. 2) were substantial, ranging from 0.31 to 0.43 kg N_2O–N ha^{-1}. There were no significant differences between N-treatments (Table 2), nor did the sums of winter and spring thaw emissions show any significant differences between N-treatments.

When summing up growing season and off-season emissions, significant differences between N levels were still present with a p value of 0.039 (Table 2). All fertilized treatments, except 100 N, had significantly larger emissions than 0 N. The treatment 100 N, which was not top-dressed, had significantly smaller emissions than the 140 N treatment (Table 2).

The response of grain yield to applied N was well described by a quadratic response curve (Fig. 3). Grain yields increased with increasing N rates up to 180 kg N ha^{-1}, with a slight decline at the highest N rate (Table 2). We found significantly higher N-yield in 180 N and 220 N than in treatments without split-N fertilization (i.e. 0 N and 100 N, 140 N had intermediate yield). Grain quality evaluated on the basis of protein, wet gluten and Zeleny sedimentation, also responded positively to increased N levels ($p = 0.001$). All measured parameters describing the grain quality in treatments 0 N and 100 N were inferior to treatments receiving split-N application (Online Resource Table S4).

Also the yield-scaled N_2O emissions (N_2O intensities) differed significantly between fertilization levels, both for the emissions cumulated over the growing season ($p < 0.001$) and for the overall cumulated emissions ($p < 0.001$). The season-based N_2O intensity was largest for the unfertilized treatment (0 N), smallest for the treatment with spring fertilization only (100 N), and intermediate for the split-N fertilized treatments. There were no differences between the split-N fertilized treatments (Table 2, Fig. 3). The N-yield-scaled N_2O emissions (Table 2) appeared to be lowest in 180 N and 220 N, although the differences to treatments 100 N and 140 N were not significant. A reduction of N-yield-scaled N_2O emissions of 16% was observed between

the 140 N and 180 N, emissions being lowest in 180 N. The calculated economical optimum fertilization rate was 190 kg N ha^{-1}, using a Norwegian price ratio (Fig. 3), and 185 kg N ha^{-1}, using a price ratio based on EU prices on N fertilizer and grain.

Accumulation of N_2O in soil during winter

The temporal dynamics of N_2O and O_2 concentrations in 0–0.20 m soil depth revealed a clear relationship between increasing N_2O and decreasing O_2 concentrations (Fig. 4). N_2O accumulated gradually under the snow cover during winter (Fig. 5), showing little differences between soil depths. This may be due to severely restricted soil-atmosphere exchange, resulting in equilibration of soil gases across the upper soil profile. Maximum measured concentrations observed just before snowmelt were 33.9, 35.7 and 34.1 μL L^{-1} at the depths of 0.05, 0.125 and 0.20 m, respectively.

Upon spring thaw, N_2O concentrations in the soil air quickly declined along with elevated N_2O emissions (Fig. 5). To evaluate how much of the N_2O emission observed upon spring thaw that could be due to release of accumulated N_2O, we compared the amounts of emitted and accumulated N_2O (Table 3). For this, we calculated the amount of N_2O present at peak concentration per square meter and 0.24 m depth based on the calculated pore volumes of the two blocks and compared it with the cumulative N_2O emission during spring thaw. The fractions amounted to values between 7 and 28%, suggesting that a considerable share of the N_2O emitted during the thawing period was produced de novo. We also measured N_2O concentrations in the snowpack at two heights (0.10 and 0.20 m above soil surface), but found increased concentrations in 220 N plots only (Online Resource Fig. S2).

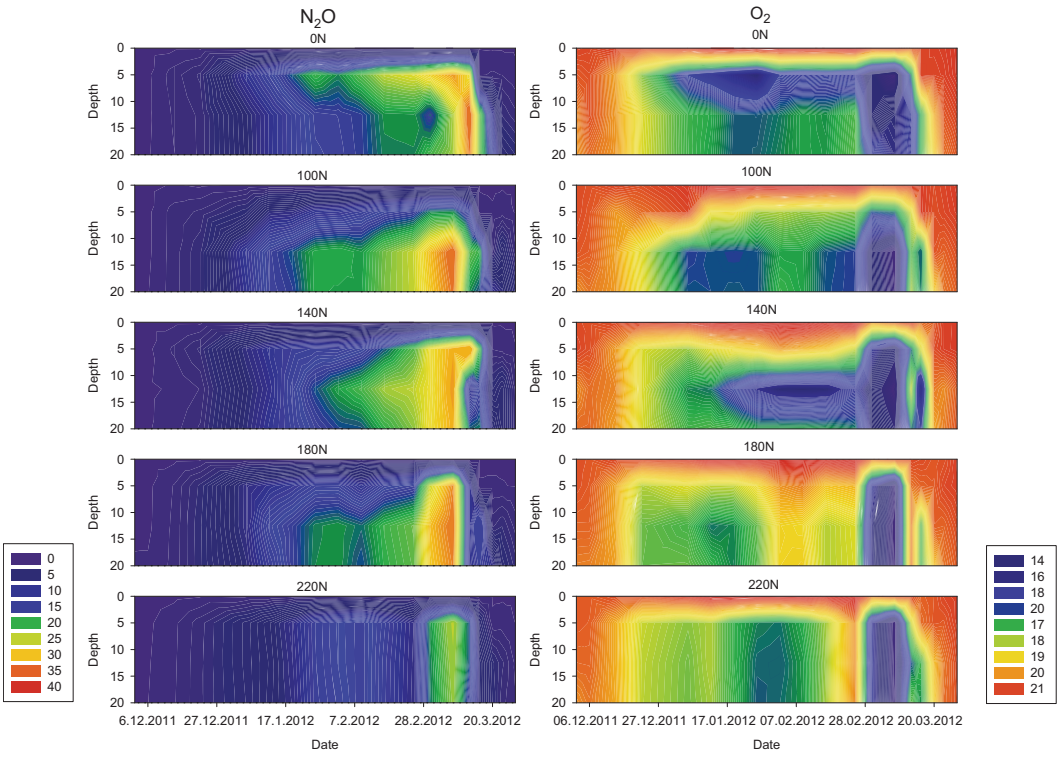

Fig. 4 Mean (n = 2) N_2O concentrations (left, μL L^{-1}) and O_2% concentrations (right, vol%) interpolated between sampling depths and dates for five different N fertilizer treatments. The concentrations were measured during the period of snow cover in winter 2011–2012, and during snowmelt in 2012

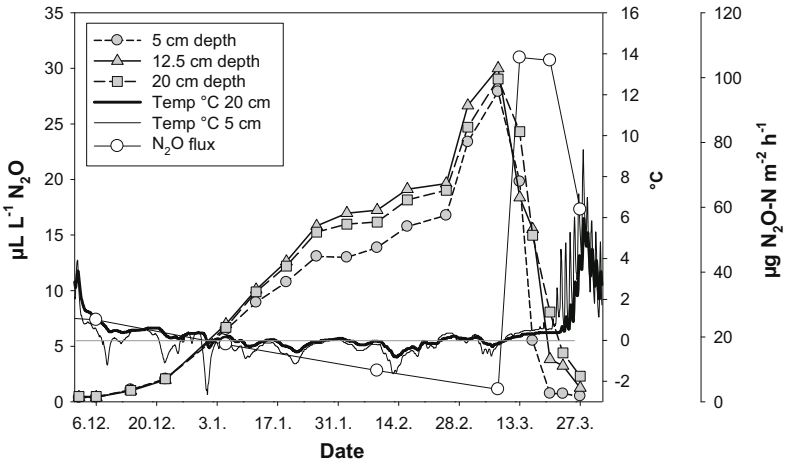

Fig. 5 Accumulation of N_2O (n = 10) throughout winter in three soil depths, soil temperatures at 0.05 m depth (n = 5) and 0.20 m depth (n = 5), and average N_2O emissions (n = 20) for all plots

Discussion

Effect of split-N application rate on yields and N_2O intensities

The treatment with 80 kg split-N application giving a total fertilizer N rate of 180 kg ha^{-1} (180 N) was close to the calculated economical optimum N rate of 190 kg N ha^{-1} (Norwegian price ratio, P_N/P_Y = 2.7) and had largest yields and best grain quality. Further, the grain yield-scaled N_2O emission (N_2O intensity) for the growing season (i.e. from split-N application to harvest) was about half of that measured in the unfertilized treatment (0 N) (Table 2). This supports Van Groenigen et al. (2010), Cui et al. (2014) and Giweta et al. (2017), all emphasizing the importance of focusing on yield-scaled N_2O emissions rather than area-scaled N_2O emissions. The treatment with the smallest grain yield-scaled N_2O emission (season) was the one receiving 100 kg N ha^{-1} in spring only (100 N). This treatment had the lowest N_2O emission per kg produced plant material, but yield and grain quality were significantly poorer in 100 N than in 180 N. In light of the expected increase in food demand, which is closely linked to the forecasted population increase at both global and national levels (United Nations 2017), yield level and grain quality (in particular protein content) should be considered together with environmental impact when evaluating optimum fertilization rates. The optimum N rate is, however, affected by the price ratio between fertilizer N and grain. When using an EU price ratio (P_N/P_Y = 4.1), the economical optimum N rate would be reduced by only 5–185 kg N ha^{-1}. Considering the similar level of calculated grain yield-based N_2O intensities among the split-N fertilized treatments, the use of an EU price ratio would not change our conclusion that a fertilization level close to the economical optimum appears to be sound also from the perspective of reducing N_2O-emissions in crop production. The finding that highest N-yields, observed in the 180 N and 220 N treatments, corresponded with lowest N_2O intensities, supports this conclusion.

In order to omit the matter of price, and focus on the environmental impact, an alternative approach would be to calculate environmentally optimum N rates. Riley et al. (2012) used data from 240 annual N fertilizer trials performed in Norway over the period 1991–2007 to optimize nitrogen fertilizer recommendations in Norway. They calculated the N rate to balance the N removed in grain, and added an amount of N to account for unavoidable losses (assumed to be 25 kg N ha^{-1} covering leaching and denitrification), which they designated as "acceptable balance". Using our best yielding treatment (180 N; 7.42 Mg 85% grain ha^{-1}) as a starting point, this would give an environmentally optimized N rate of 202 kg N ha^{-1} (177 kg N removed by the grain plus 25 kg ha^{-1} unavoidable loss). In comparison, Riley et al. (2012) found that spring wheat, with an expected grain yield level of 7.5 Mg ha^{-1} (close to the 7.42 Mg ha^{-1} in

Table 3 N_2O in the soil atmosphere at the date of peak concentration (μg N_2O–N m^{-2} in the top 0.24 m layer) relative to the cumulative N_2O emission during spring thaw (μg N_2O–N m^{-2} $period^{-1}$ flux)

	0 kg N Block 1	0 kg N Block 2	100 kg N Block 1	100 kg N Block 2	140 kg N Block 1	140 kg N Block 2	180 kg N Block 1	180 kg N Block 2	220 kg N Block 1	220 kg N Block 2
μg N_2O–N m^{-2} in top 0.24 m layer soil	8490	5414	4337	2755	3768	6716	3757	4360	2511	5685
μg N_2O–N m^{-2} $period^{-1}$ flux	36,332	32,641	20,495	40,632	40,451	24,157	49,464	38,829	27,705	39,759
Proportion (% of flux)	23.4	16.6	21.2	6.8	9.3	27.8	7.6	11.2	9.1	14.3

our 180 N), would need an N rate of 176 kg N ha^{-1} to balance the N removed in grain and account for unavoidable losses. We observed a higher protein level in our trial (e.g. 180 N; 16.1%), compared with the average values reported by Riley et al. (2012), which were below 14%, explaining the higher optimum N rate calculated with our data. Nitrogen originating from soil organic matter could also be an N-source for the plants, and in a multi-year trial with different split-N application rates, this effect would have to be taken into account. However, in the first year of a fertilization trial, the impact of N-mineralization on plant N uptake can be assumed to be uniform in all fertilizer treatments (Korsaeth et al. 2002).

As shown in Fig. 3, the treatment with the highest fertilizer N rate (220 N) tended to have lower yields, but similar cumulative N_2O emission (all periods) and N_2O intensity (all periods) as compared with the 180 N treatment. Hence, we did not find any linear increase in N_2O emissions with increasing N-application as predicted by IPCC tier 1 and shown by e.g. Lebender et al. (2014). Regardless of the method used to identify the optimum N rate, it appeared to be somewhere between our two highest N levels (180 N and 220 N). This may partly explain the lack of difference between these two treatments in terms of N_2O intensity. In hindsight, we realize that an extra N level above 220 kg N ha^{-1} (e.g. 260 kg N ha^{-1}) would have been justified in order to improve the sensitivity of our experiment. Another factor, which may partly explain the lack of N-effects at application rates slightly above optimum (i.e. 220 N) on N_2O-emissions (and thus intensity) is an episode with heavy rain occurring at days 31–32 after split-N application (July 22–24). During this period, 56.1 mm of precipitation resulted in a WFPS of more than 70% (Fig. 1b), and it is likely that this could have resulted in large N_2O emissions that were not captured by our measurements. Some of the applied fertilizer N still present in the top-soil of the 220 N treatment may also have been leached into deeper soil layers, or even to the drains. These assumptions are supported by the observed, significant drop in NO_3^- concentration in the 220 N treatment from day 30 to 49 (Fig. 1c). At day 30, the NO_3^- content of the soil was very low in all treatments except for the 220 N treatment, indicating that the plants had taken up most NO_3^- there. Since the N-yield was not higher in 220 N than that in 180 N

and 140 N, it is unlikely that the drop in soil NO_3^- after day 30 was due to plant N uptake. If the concentration of NO_3^- in topsoil of 220 N had remained at a relatively high level, as measured at day 30 prior to the precipitation episode, the measured N_2O emissions on the following days may have been significantly higher in that treatment.

The observed, general increase in N_2O flux rates towards the end of the growing season appeared to be triggered by an increase in WFPS (Davidson et al. 2000). Measured top-soil NO_3^- and NH_4^+ concentrations were, however, at a stable low level during this period (from August 9 onwards), indicating that at least a part of the emitted N_2O was produced in the lower soil layer during this period. We did not measure NO_3^- in deeper soil layers (nor NH_4^+ or any other soil property). From a long term cropping system study located on the same field (Riley and Eltun 1994), we know however, that total N and organic C vary greatly at a small spatial scale in lower soil layers (> 0.20 m depth) of this field. Hence, possible leaching of NO_3^- to the lower soil layer may have led to confounding effects through variability in factors important for N_2O formation.

Effects of N application rate on winter emissions of N_2O

We were not able to identify any clear relationship between N application rate and N_2O emission during winter. This matches the low concentrations of topsoil mineral N measured before freezing in early December, and the possible confounding effects of N_2O formation in lower soil layers. N_2O produced in the lower layers contributes to both, the accumulating N_2O in the top soil and the N_2O emitted through the snow pack (Burton and Beauchamp 1994). However later studies, including ^{15}N tracers, concluded that accumulated N_2O from lower layers does not contribute significantly to the flux measured at spring thaw (Müller et al. 2002; Wagner-Riddle et al. 2008), which is in accordance with the conclusion of the soil profile study by Risk et al. (2014). Regardless the scale of impact on measured flux, the effect of N_2O accumulated in the lower soil may be expected to be uniform across split-N fertilization treatments in our study as a 1 year fertilizing trial most likely would not result in distinct NO_3^- concentrations in lower soil layers in a well-drained field. If some of the excess fertilizer in 220 N reached the drains, it would represent a source for indirect N_2O emissions, but this was beyond the system border of our study and is thus not accounted for. Cumulative off-season N_2O–N losses were 1.5–2.7 times larger than those cumulated for the growing season, but 41–60% of the off-season losses occurred during thawing. This illustrates the importance of including off-season emissions when estimating total N_2O–N loss relative to crop management.

Effect of continuous snow cover on emissions of N_2O in winter and under spring-thaw

Our winter N_2O emissions were similar in magnitude to those reported by Maljanen et al. (2007), Risk et al. (2013) and Németh et al. (2014), the latter using micrometeorological measurements. In contrast to Maljanen et al. (2007), we observed significant N_2O accumulation under the snow cover. Measured soil temperatures fluctuated around 0 °C, causing repeated freezing–thawing cycles, which according to Congreves et al. (2018), may provide liquid water and anaerobicity, both conductive to microbial denitrification. Öquist et al. (2004) found that temperatures as low as − 4 °C allowed for N_2O production by denitrification in non-frozen anoxic microsites. Likewise, diurnal fluctuations in micro-climatic conditions during winter have been found to trigger N_2O emission pulses (Dörsch et al. 2004), likely due to stimulating microbial activity. In our study we observed such fluctuations in the topsoil and at the soil surface despite the continuous snow cover. While a large amount of the produced N_2O clearly was stored in the soil, we also measured N_2O emissions through the snow pack by closed chambers, at rates similar to those measured during summer.

Our N_2O and O_2 soil air observations throughout winter focused on the top soil (0–0.24 m). It is well known that N_2O is also produced in deeper layers (Burton and Beauchamp 1994), but if the different split-N fertilizer applications should be the cause for N_2O build-up in the top soil through N_2O production in deeper soil layers, we would have expected to see more clear concentration gradients of N_2O with soil depths. We did not find any pronounced concentration gradients (Fig. 5), and therefore regard the impact from deeper layers to be independent of irrespective split-N application rate.

Maljanen et al. (2007) reported only minor increase in soil air N_2O concentrations in plots covered with snow. In our study, we found a gradual build-up of N_2O in the soil throughout winter reaching concentrations similar to what Maljanen et al. (2007) found in the plots with bare frost. The build up was followed by a rapid decline during spring thaw from March 8 until 20, during which N_2O concentrations at 0.05 m depth fell below 1 μL L^{-1}. The decline of N_2O in the soil air went along with high emission fluxes of N_2O. As N_2O lingers in the soil, we also must consider the possibility of N_2O reduction to N_2 by denitrifiers which cannot be measured in situ. N_2O reduction could also be active during thawing, which would mean that only a part of the accumulated N_2O reaches the atmosphere as N_2O. The former suggests that continuous snow cover limits overall off-season N_2O emissions, while the latter supports the idea that a considerable part of spring-thaw emissions is due to de novo microbial N_2O production in cold but thawing soil (Röver et al. 1998). Quantitatively, the N_2O measured in soil air right before spring thaw accounted for up to 28% of the emitted N_2O, likely fueling the observed rapid increase in N_2O emission rates upon thawing, similar to what has been reported by Smith et al. (2010) from a corn–soybean–winter wheat rotation field experiment in Canada, indicating de novo production to be the main source of spring thaw N_2O emissions. In our experiment we only measured soil N_2O in the gaseous phase. The aqueous phase (frozen and liquid) in soil may be significantly oversaturated with N_2O (Risk et al. 2013) so that our estimates of the proportion of emitted N_2O originating from physical release are likely underestimated, as the chosen calculation methods does not consider the N_2O in aqueous form. Yet, when including the potential proportion of N_2O dissolved in soil water in a rough calculation for the time point before spring thaw using pre-thaw soil moisture content, and assuming perfect equilibrium, the amount of N_2O stored in the soil upon spring thaw was still less than 40% of the observed emissions. Since we also observed large N_2O emissions after the N_2O soil air concentrations had declined, it is fair to assume that the main source of the observed spring thaw emissions was de novo N_2O production in the top soil.

Method and future scopes

The results presented here are from a 1 year study only. N_2O emissions are highly dependent on weather conditions, and ideally the experiment should have been repeated for consecutive years and preferably in several locations to fully understand the impact of split-N fertilization on N_2O emissions.

It must also be mentioned that the experiment only included flux measurements starting with the split-N applications and does not include N_2O emissions after application of N at sowing. It was beyond the scope of our study to estimate annual emissions from wheat production, but our data show that split-N application appreciably affects N_2O emissions during the growing season, with likely consequences for annual emissions.

Another critical point in our experiment design was the decision to remove plant residues, other than the stubble, after harvest. The practice whether to bale and remove the straw from the field or to leave the plant residue to be incorporated in the soil upon tillage varies in Norway. We chose to remove the plant residues in order to avoid confounding of fertilizer additions with N mineralization. N return to soil through plant residue incorporation could potentially differ between treatments, but in a study by Korsaeth et al. (2002), performed on the same site in the period 1998–2000, the net-mineralization of N from barley straw left on the ground after harvest and incorporated into the soil a few weeks later (which is normal practice when the straw is not removed) was almost negligible until spring.

We found substantial winter emissions. To better understand the effect of crop and soil management on these emissions, we need better understanding of the different sources of N_2O accumulating and being emitted during winter and spring thaw. As noted by Risk et al. (2014), it is impossible to unequivocally assign N_2O to N-cycling in deeper soil layers or de novo production in the top soil during winter, which would be important to know, however, if management strategies were to be sought to minimize these fluxes. Stable isotope approaches (e.g. Müller et al. 2002) could be a way to gain more insight in these processes. Using isotopically labelled N-fertilizer, on the other hand, could help to better understand fertilizer use efficiency in split-N fertilization and its effect on N_2O emissions.

Conclusions

Our findings support the idea that growing-season N_2O emissions in the Boreal region are mainly controlled by the level of fertilizer addition, and that economic optimum N rates result in reduced cumulative N_2O losses per unit harvested product. However our results also show that the effect of split-N application was relatively short-lived (15–21 days) and that factors other than split-N fertilization level (i.e. weather and soil interactions) are important drivers of N_2O emissions.

Effective plant N uptake combined with potential leaching of excess N during autumn resulted in a poor relationship between N fertilizer rates and off-season N_2O emissions. However, off-season N_2O emissions were large compared to growing-season emissions and dominated by spring thaw emissions. We found substantial subnivean N_2O accumulation, which was rapidly released upon spring thaw and apparently contributed to the observed emission peak. Our data suggest that a considerable part of the spring thaw emissions originated from de novo production of N_2O, which raises the question how arable soils in cold seasons should be managed to reduce off-season emissions. Together with the observed diffusive N_2O flux through the snow pack, spring thaw emissions emerge as an important component for the N_2O budget of wheat production in the boreal region. Multi-year studies are however necessary to validate our results.

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Supplementary Material

Effects of nitrogen split application on seasonal N_2O emissions in southeast Norway

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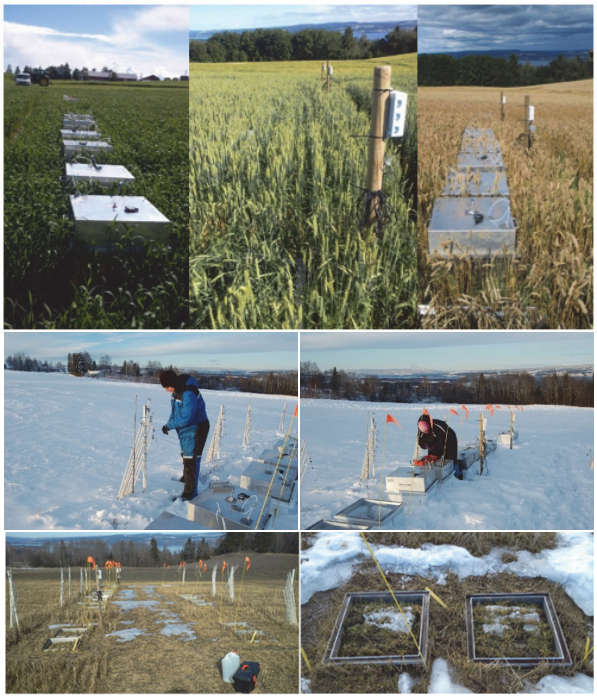
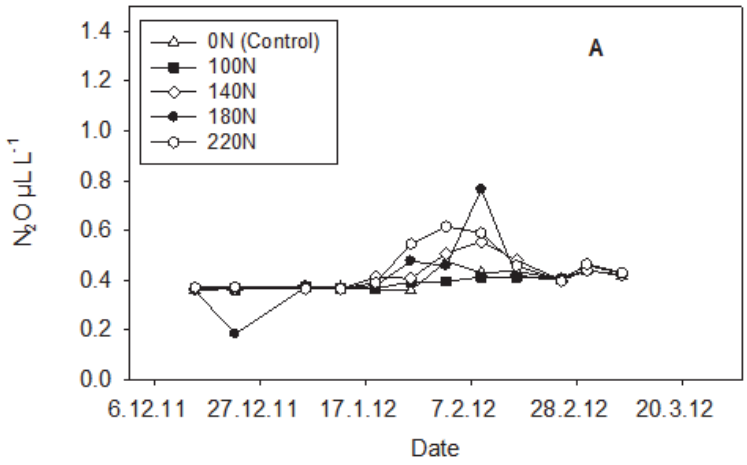


Fig S1: Photos of the field trial showing measurements during the growing season, in winter and the frames at spring thaw, illustrating the field setup.



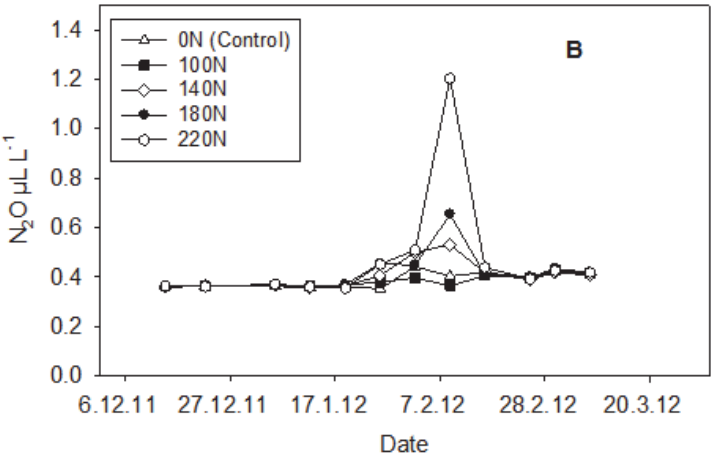


Fig S2: Average N_2O concentrations (avg, n=2) in the snow pack at A) 10cm and B) 20 cm above the soil surface

Table S1: Sampling dates and time

Date of sampling	Block 1	Block 2
20.06.2011	10:50	13:15
24.06.2011	10:20	12:30
27.06.2011	10:10	12:30
01.07.2011	09:55	12:25
08.07.2011	10:00	12:30
21.07.2011	08:45	12:20
09.08.2011	10:10	12:20
30.08.2011	10:05	12:30
06.12.2011	10:50	13:10
05.01.2012	10:30	13:40
09.02.2012	10:30	12:25
08.03.2012	12:20	13:45
13.03.2012	12:20	13:50
20.03.2012	12:25	13:50
27.03.2012	13:30	13:55

Table S2: pH measured in 10 g soil in 50 ml 0,01 M CaCl2 solution.

N-applied	pH CaCl2 Block 1	pH CaCl2 Block 2
0	5.27	5.30
0	5.42	5.57
100	5.37	6.36
100	5.31	5.44
140	5.37	5.60
140	5.55	5.83
180	5.45	5.16
180	5.35	5.41
220	5.88	5.46
220	5.52	5.42

Table S3: Mineral N content measured in December 2011

Date	N-addition Kg N ha^{-1}	NO_{3-} (µg N g dw $soil^{-1}$)	NH_{4+} (µg N g dw $soil^{-1}$)
02.12.11	0	0.32	0.80
	100	0.25	0.79
	140	0.32	0.81
	180	0.47	0.70
	220	0.21	0.83

Table S4: Grain quality parameters measured by FOSS Infratec™ 1241 grain analyzer

N-total	Yield 85% kg ha^{-1}	Protein (DM)	Moisture %	Starch (DM)	Wet Gluten (DM)	Zeleny	N yield (kg ha^{-1})
0	1.517	11.8	14.6	67.6	24.8	39.0	178
0	3.008	12.0	14.8	67.9	25.5	39.1	360
100	5.430	10.5	14.8	68.9	21.3	35.6	572
100	6.197	12.1	14.7	67.3	25.3	39.2	747
140	6.299	13.0	14.6	66.7	27.9	41.3	819
140	7.107	14.4	14.6	65.5	31.5	49.9	1020
180	7.731	16.1	14.6	64.0	37.2	57.8	1242
180	7.115	16.1	14.7	63.6	36.8	57.5	1149
220	6.538	17.1	14.6	62.6	39.6	59.4	1116
220	7.485	17.0	14.7	62.8	39.1	59.6	1271

Paper III

Long-term effects of cropping systems on potential N_2O production by nitrification and denitrification

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Key words: N gas-kinetics, long-term field trials, microbial activity, N_2O product stoichiometry.

Abstract:

Production and consumption of the greenhouse gas nitrous oxide (N_2O) is regulated by nitrifiers and denitrifiers, of which activity depends on soil conditions. Long term cropping shapes soil conditions through crop and soil management. We thus hypothesized that crop management over time affects potential nitrification and denitrification and their inherent N_2O stoichiometries, which are important functional traits for N_2O emissions. Soil was sampled from a 30-year cropping system experiment in SE Norway, representing conventional and organic crop rotations with differences in crops (including cereals, partly potatoes and up to 3-year grass-clover-leys), residue retention, soil management and fertilization regime (including inorganic fertilizer and/or animal manure, or a combination of green manure and biogas digestate). Nitrification and denitrification kinetics were studied by robotized incubation of

agitated soil slurries in the presence of ample N substrates. Potential denitrification showed a clear pattern, with lowest potential in an arable, cereal based system with all straw removed (0.6 µg N g soil^{-1} h^{-1}). All other systems had higher potentials (1.1 - 1.4 µg N g soil^{-1} h^{-1}), which was attributed to higher C returns from plant residue retention, inputs of organic fertilizers, or presence of grass-clover ley in the rotation. Nitrification potentials tended to be higher in mixed systems which combine both arable crops and leys, than in systems with arable crops only. The N_2O ratio of denitrification (N_2O /(N_2O + N_2)) ranged from 0.2 to 0.6, and was strongly negatively correlated with soil pH. By contrast, the N_2O yield of nitrification was not correlated with any of the measured soil variables, but systems receiving organic fertilizers supported higher nitrification potentials than arable systems fertilized with inorganic fertilizers only. Including data on soil nutrients and microbial properties from another study conducted with soils from the same systems (Chen *et al*. 2019), 66 and 73 % of the variation in potential N_2O production by denitrification and nitrification, respectively, could be explained by principal component regressions. Soil pH and factors governing microbial activity, in particular the amounts of organic matter added to the soil in form of organic fertilizers and plant residues, were identified as the main factors determining the risk for high N_2O emissions. The choice of management regime, i.e. conventional versus organic, did not affect the N_2O producing potential of a cropping system.

1. Introduction

The atmospheric concentration of nitrous oxide (N_2O), a greenhouse gas 300 times more potent than CO_2 in a 100 year perspective (IPCC, 2007), has increased rapidly over the last century. This increase is linked to high synthetic nitrogen use in modern agriculture, based on industrially fixing N from atmospheric N_2 (Erisman *et al.*, 2008). Approximately 100 Tg synthetic N are added to the global agricultural N-cycle each year, while biological N fixed by

cultivated legumes adds another 60 Tg N yr^{-1} (Fowler *et al.*, 2013). Excess availability of reactive nitrogen has negative environmental impacts through nitrate (NO_3^-) leaching and N_2O emissions. Nitrous oxide is produced by a number of microbial N transformations, but nitrification and denitrification are commonly regarded to be the most important ones in cultivated soils (Baggs, 2011). Nitrification is the process of oxidizing NH_3 to nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB) and archaea (AOA) (Leininger *et al.*, 2006). Nitrite oxidizing bacteria (NOB) oxidize NO_2^- further to NO_3^-. During autotrophic nitrification, N_2O is formed as a byproduct of hydroxylamine oxidation or through so-called 'nitrifier denitrification', i.e. the respiratory reduction of NO_2^- to NO and N_2O (Wrage-Mönnig *et al.*, 2018). However, recent work with AOB pure cultures questions the role of nitrifier denitrification as a respiratory process and hence the significance of this process for N_2O emissions (Hink *et al.*, 2017a). Irrespective of the biochemical pathway of N_2O production in nitrification, NH_3 oxidation in soils constitutes a major oxygen sink, inducing coupled nitrification-denitrification (Nadeem *et al.*, *in revision*), i.e. the respiratory reduction of NO_2^- and NO_3^- by heterotrophic denitrifying bacteria (Zumft, 1992). Denitrification is a mostly heterotrophic respiratory process, stepwise reducing NO_3^- via the gaseous intermediates nitric oxide (NO) and N_2O to N_2 in the absence of oxygen. The ability to denitrify is shared by about 15% of heterotrophic soil bacteria, but a considerable number of denitrifying genotypes lacks *nos*Z, the gene coding for N_2O reductase, making N_2O the sole end product in denitrification (Graf *et al.*, 2014).

Nitrification and denitrification are governed by a range of environmental factors, which affect both processes simultaneously. Numerous studies have shown that microbial activity is affected by soil temperature, soil moisture, soil organic matter content and pH (Firestone and Davidson, 1989; Beauchamp, 1997; Roos, 2003; Mørkved *et al.*, 2006; Mørkved *et al.*, 2007a; Xun *et al.*, 2015). With ammonium/ammonia or nitrate being the substrates for nitrification

and denitrification, respectively, the dependency of N_2O production and consumption on soil N dynamics is obvious. Accordingly, fertilizer regime (e.g. inorganic vs. organic N) has been shown to play a major role for N_2O emission (Eichner, 1990; McSwiney and Robertson, 2005; Petersen *et al.*, 2006; Gagnon *et al.*, 2011; Battye *et al.*, 2017).

A central factor controlling nitrification and denitrification activity in soils and hence the partitioning of N_2O from these two processes, is pO_2 which varies widely in the soil matrix. Hence, all factors affecting the oxygen availability in soil, such as soil texture, root structure, amount and placement of readily degradable carbon, precipitation and drainage, etc. can be expected to promote or restrain the activity of nitrifiers and denitrifiers (Firestone and Davidson, 1989; Robertson, 1989; Liu *et al.*, 2007). Among the most pervasive factors shaping soil microbial communities is soil pH (Fierer and Jackson, 2006). Soil pH has also been shown to strongly control the $N_2O/(N_2O+N_2)$ product ratio of denitrification, which increases linearly with decreasing pH (Liu *et al.*, 2010; Dörsch *et al.*, 2012; Raut *et al.*, 2012; Qu *et al.*, 2014; Obia *et al.*, 2015). Even though pH differences between cropping systems may be small, a recent field study, examining natural small-scale pH variation in an arable soil in SE Norway showed that this variation controls *in situ* N_2O emissions under denitrifying conditions during off-season (Russenes *et al.*, 2016). By contrast, long-term effects of soil pH on the N_2O yield (Y_{N2O}: N_2O-N/NO_3-N) of nitrification are less clear (Mørkved *et al.*, 2007b). Recent evidence suggests that Y_{N2O} increases with soil pH along with the AOB/AOA abundance ratio (Tzanakakis *et al.*, 2019; Nadeem *et al.*, *in revision*), as ammonium oxidizing bacteria produce inherently more N_2O per unit nitrified N than AOA (Hink *et al.*, 2017b), while being favored by high pH (Nicol *et al.*, 2008). In this way, cropping systems may exert indirect effects on N_2O turnover by modifying soil pH through N fertilizer formulation, root cation exchange and organic acid release. Cropping systems may also affect soil redox conditions through soil aeration by tillage and crop residue management.

A more direct effect of cropping system on nitrification and denitrification may be expected from management factors affecting soil N turnover. Tillage, for instance, is known to disrupt soil structure, which in turn affects substrate availability, aggregate stability and soil microclimate (Young and Ritz, 2000). The use of cover crops increases microbial activity (Cates *et al.*, 2019), whereas inclusion of legumes in crop rotations provides biologically fixed N, which potentially increases N_2O emissions (e.g. Raji and Dörsch, 2019). Inorganic fertilization results in transiently elevated mineral N concentrations in the soil solution, but the microbial availability of this N strongly depends on the crop's performance, i.e. on the competitiveness of the plants to take up fertilizer N. Organic fertilizers provide less readily available nitrogen than inorganic ones, but add readily decomposable organic matter to the top soil which may result in transient O_2 depletion. On the long run, organic amendments increase the organic matter content of the soil and improve the soil structure, thus supporting a more abundant microbial community (Lori *et al.*, 2017). Other external factors affecting potential nitrification and denitrification are phosphorous availability (Mehnaz *et al.*, 2019) and chemical pesticides (Fließbach and Mäder, 2004; Esperschütz *et al.*, 2007; Hussain *et al.*, 2009). Altogether, cropping systems control nitrifiers and denitrifiers in various ways.

In situ studies of long-term management effects on N_2O emissions are elusive. Measuring N_2O emissions in various European crop rotations, Petersen *et al.* (2006) found that N inputs significantly affected N_2O emissions, regardless of whether the management system was organic or conventional, i.e. manure or inorganic N based. N_2O emissions were generally higher from conventional than organic systems, but this was due to lower N-input in the organic systems. In a global meta-analysis, Skinner *et al.* (2014) found higher N_2O emissions under conventional than organic management and attributed this to higher bioavailability of mineral N in conventional management systems. A recent study by Skinner *et al.* (2019) found a clear reduction in N_2O emissions from organic compared to conventional production

on an area based scale, but not for yield scaled emissions (with one exception), which they attributed to higher soil pH in the organic production systems supporting more complete denitrification. Chirinda *et al.* (2010), on the other hand, found no differences in N_2O emissions between cropping systems managed either organically or conventionally, even though the N-input was higher in the conventional system. When comparing long-term (≥10 years) tillage effects, no/reduced tillage has been found to reduce N_2O emissions relative to conventional tillage (Six *et al.*, 2004; Halvorson *et al.*, 2008). A meta-study by van Kessel *et al.* (2013) arrived at the same conclusion, but also showed that conversion of conventional to no till increases N_2O emissions on the short run (<10 years). Overall, the literature is inconsistent when ranking management practices in terms of their potential to promote or reduce N_2O emissions. Therefore, more research is needed to elucidate which factors associated with cropping systems affect the potential of and N_2O emission by nitrification and denitrification.

The 30-year old Apelsvoll cropping system trial consist of six replicated rotation systems comprising differences in crop rotation, soil management and fertilizer regime, including both organic and conventional management practices (Eltun, 1994). Over the years, different management has resulted in clear differences in nutrient dynamics and soil chemical properties (Korsaeth, 2012). We hypothesized that N cycling potentials involved in N_2O formation and reduction have changed over time in dependency of cropping system and relative to adjacent non-cultivated grassland. Instead of quantifying highly dynamic N_2O fluxes (Russenes *et al.*, 2016, 2019), which generally have a low signal-to-noise ratio with respect to agronomic treatment, we incubated soils taken from the different cropping systems and determined nitrification and denitrification kinetics and N_2O accumulation in the presence of ample N substrates. This approach is based on the assumption that, taken all other factors alike, the potential of a soil to form or consume N_2O is ultimately controlled by its long-term

C and N input history and related variables like soil pH and P availability, which, in turn, are controlled by the cropping system. To account for short-term effects of crops present at the time of sampling, soils were taken only from plots with spring barley (*Hordeum vulgare* L.), which are included in each of the Apelsvoll cropping systems, either as monoculture, with a catch crop or undersown with a grass/clover mixture. The overall objective was to test whether long-term differences in cropping system result in distinct nitrification and denitrification potentials and N_2O product stoichiometries and to link these differences to input characteristics and management regimes given by the different cropping systems.

2. Materials and methods

2.1. The cropping system experiment

The long-term Cropping System experiment at Apelsvoll was established in 1989 at NIBIO Apelsvoll Research Centre (60°42′ N, 10°51′ E, 250 m above sea level) in Southeast Norway. The experiment is located on a 3.2 ha, gently sloping field on soil classified as Endostagnic Cambisol (WRB, 1998) with dominantly loam and silty sand textures and consists of 12 pipe-drained blocks (30 x 60 m) which are separated by 7.5 m grass border zones. The climate is humid continental with a mean annual precipitation of 693 mm and a mean annual temperature of 5.1°, measured in the period 2000-2014.

Six cropping systems were established in 1989, by use of randomized complete block design with two replicates (Eltun, 1994). The system was slightly altered in 2000, with a change from eight to four years rotation by merging of two neighboring plots (new plot size: 30x15 m), still keeping each crop present every year. This is described in more detail by Korsaeth (2012). In the Conventional arable (CA1) system, all straw from the cereal plots is removed from the plots, leaving stubble only. In the plots with barley undersown with grass, the straw

is removed after harvest of the cereal crop. In all other plots, plant residues are cut or mulched and left on the ground until soil tillage. The systems are described in more detail in table 1. Annual fertilizer inputs split in inorganic and organic are displayed in table 2.

2.2 Soil sampling and analyses

On December 11th 2015, a soil sample was taken from each rotation plot where barley was grown in the season of 2015 (one plot per block), by pooling 10 sub-samples taken with a soil auger (18 mm diameter) from 0 - 20 cm depth along a transect through the plot. Additionally, two soil samples (0-20 cm depth) were taken from two separate parts of the border area next to the experimental site using the same approach, representing an unfertilized control (Control). The border area surrounding the experimental site has had permanent grass cover since the establishment of the experiment in 1989. The grass is mown regularly in the growing season but not removed. No fertilizer or pesticides are applied, and traffic is limited to mowing with a light-weight tractor.

The soil sampling was performed in early winter before the field was covered in snow, in order to keep the influence of the cropping season (e.g. fertilizer input) as small as possible. The samples were placed in plastic lined cardboard boxes and stored at 4°C until start of the incubation experiments in March 2016. All soil samples were sieved (3.15 mm).

In order to measure moisture content and C and N content, 5 g of each sample was weighed and placed in tin foil cups. After drying at 105°C for 36 h, the samples was weighed and thereafter milled for determination of total C and N content. Soil pH was measured in 3.5 g soil dispersed in 20 ml 1mM KNO_3 solution and placed on shaker for 60 m before measurement.

2.3 Soil incubations

Potential nitrification and denitrification were determined in continuously stirred soil slurries prepared in 120 ml serum bottles equipped with magnetic stirring bars. For each soil sample, two technical replicates were prepared (6 cropping systems + Control × 2 replicates × 2 technical replicates = 28 bottles).

For the denitrification assay, 7 g fresh weight soil were suspended in 40 ml of a 1mM KNO_3 solution. The bottles were crimp-sealed with butyl rubber septa, and shaken horizontally for 15 min to fully disperse the soil. To remove O_2 and N_2 from the headspace, the bottles were He-washed by evacuating and He-filling them six times while stirring at 300 rpm. After temperature equilibrating the bottles in a thermostatic water bath (20°C), equipped with a submersible magnetic stirring board, He-overpressure was released by piercing the septa with a disposable syringe without plunger filled with 4 ml of distilled water (to prevent O_2 and N_2 from entering the bottles). The bottles were incubated in the water bath at 20°C while continuously stirred (300 rpm) for 122 h, which roughly was the time needed to convert the added NO_3-N to N_2. 1 ml aliquots were removed after 1, 28, 46, 75 and 122 h using a syringe with a long hypodermic needle to monitor transient NO_2^- accumulation and NO_3^- depletion.

For the nitrification assay, 7 g fresh weight soil were dispersed in 40 ml of a 1mM NH_4Cl solution. The bottles were crimp-sealed with Teflon-lined butyl-rubbers and incubated in air at room temperature (~20°C) shaken horizontally (200 rpm) to ensure fully oxic conditions. 1-ml aliquots were sampled as described after 0, 24 and 47 h to determine nitrification activity from the accumulation of NO_2^- and NO_3^-. All subsamples were centrifuged immediately after sampling at 10 000g and 4°C to spin down suspended solids, before analyzing NO_2^- and NO_3^- colorimetrically using the VCl3/Griess method (Doane and Horwáth, 2003).

Dry weight of the soil in the bottles in both denitrification and nitrification experiments was determined by drying the bottles after the incubation experiments.

All gas measurements were carried out in a robotized incubation system, which measures O_2, CO_2, NO, N_2O and N_2 concentrations in the headspace of the fully stirred incubation bottles at high temporal resolution (here every 5 hours). The basic setup of the system has been described by Molstad *et al.* (2007), and the improved version used here is described by Raut *et al.* (2012). In short, the system consists of a thermostatic water bath, which holds up to thirty continuously stirred 120 ml serum bottles. The system monitors concentrations of gasses by repeatedly sampling from the bottle headspace with a hypodermic needle connected to the robotic arm of a fully programmable autosampler (CTC, GC Pal). The sample is transported via a peristaltic pump (Gilson Minipuls 3) to multiple sampling loops serving a GC (Model 7890A, Agilent, Santa Clara, CA, USA) equipped with two back-flushed HeySep precolumns, a Poraplot column (for separation of CO_2, CH_4 and N_2O), a Molsieve column (for separation of O_2 and N_2) and a chemoluminescence analyzer (Model 200A; Advanced Pollution Instrumentation, San Diego) for NO. After sampling, the peristaltic pump is reversed, replacing the sample with helium, thus sustaining ~1 atm pressure in the bottles throughout the incubation. Rates of production and consumption for each gas were estimated from concentration changes between time increments and corrected for aqueous dissolution, sampling loss (ca. 1% of the headspace) and leakage of O_2 and N_2 into the bottles (app. 25 nmol N_2 per sampling; for details, see Molstad *et al.*, 2007). Soil slurry pH was measured before and after each incubation. All biological activities (respiration and N gas production) are expressed per g dry weight of soil particles < 3.15 mm.

For the Principal Component Regressions (PCR, see section 2.6 for details), the variables measured for each technical replicate in the incubations were averaged over block (n=2).

2.4. Gas kinetics

Initial denitrification rates were estimated from the initial, linear accumulation of N-gases ($NO + N_2O + N_2$). An example of the gas kinetics is presented in Fig. 1. To characterize each soil's relative capacity to emit N_2O from denitrification, we calculated an N_2O index (I_{N2O}) as described in Liu *et al.* (2010). The index is a measure for the relative amount of N_2O accumulating transiently during anaerobic incubation:

$$I_{\mathrm{N_2O}} = \int_0^T \mathrm{N_2O}(t)\mathrm{d}t \; / \int_0^T [\mathrm{N_2O}(t) + N_2(t)]\,\mathrm{d}t \qquad (1)$$

where $\int N_2O(0 - T)$ is the cumulated N_2O production/reduction until time T, estimated by trapezoid integration, whereas $\int N_2(0 - T)$ is the cumulated production of N_2 during the same time interval. The value of I_{N2O} depends on the cut-off time T. Time T was chosen as the time when total gaseous N accumulation reached 7 μmol bottle^{-1}. The potential denitrification rate was calculated as the average of the rate of total N gas accumulation (NO, N_2O and N_2) and concomitant decrease of NO_3^- and NO_2^- measured colorimetrically.

Potential nitrification was determined from the linear accumulation of NO_2^- and NO_3^- measured at 0, 24 and 48 h in bottles incubated oxically in the presence of NH_4Cl. Upon each subsampling, headspace concentrations of O_2, CO_2, NO, N_2O and N_2 were measured by the incubation robot before moving the bottles back to horizontal shaking. The N_2O production of nitrification was calculated from the linear N_2O increase and divided by the nitrification rate to obtain the N_2O yield of fully oxic nitrification, i.e. $Y_{N2O}(\%) = N_2O\text{-}N/(NO_2^- + NO_3^-\text{-}N)$.

2.5 Additional data

To support the interpretation of the nitrification and denitrification potentials and their N_2O stoichiometries, we included data on soil nutrient levels (P-AL, K-AL, Mg-AL, Ca-AL and ignition loss), soil enzyme activity (catalase, urease, invertase and phosphatase), microbial gene abundance (16s rRNA for bacteria and ITS for fungi), bacterial and fungal richness and diversity, and microbial biomass carbon (MBC) and nitrogen (MBN) from the recent study (Chen *et al.* 2019). In this study, soil was sampled from the same cropping system experiment on October 8, 2014, but in contrast to our study, a mixed sample from each block, compositing soils from all four crops present in each rotation, was used. Despite different sampling date and design, the molecular data should be representative for the long-term effects of cropping systems on microbiota. Control samples were sampled from the same locations, and sampling method (soil auger with 18 mm diameter) and depth (0-20 cm) were identical in both studies.

As documented in numerous publications from the long-term experiment, we have a large quantity of data related to inputs (e.g. nutrients and pesticides) and outputs (e.g. yields, runoff and leaching) for the different cropping systems. Whereas inputs (manure adjusted by feed production and split N application according to crop demand) and more so outputs fluctuate from year to year, soil chemical and microbial properties respond generally slowly to the net-effect of the various flows entering or leaving the system. On this background, we did not expect the input/output data to provide additional insight into the process potentials, but nevertheless included some selected variables: average (2011-2015) inputs of total-N, both in inorganic and organic form, nitrate-N, ammonium-N, P and K, along with average leaching of total-N for the agrohydrological years May 1, 2011 – April 30, 2015 (excluding 2013 due to an upgrading of the drainage sampling system that year). Leaching data were calculated per block (n=12), since there were no such data available for the controls. All soil properties

measured in this study and the values adopted from Chen et al. (2019) and are shown in Table 3.

2.6 Statistics

Differences between N-transformation rates were analyzed by one-way ANOVA followed by multiple comparisons using Fischer's LSD-method. All analyses were performed at the 0.05 probability level, using the software package Minitab® (version 18.1). In order to explain observed differences in the N-transformation variables (forming the Y-matrix), a range of independent variables (forming the X-matrix) were analyzed by Pearson correlation between singular X- and Y-variables, and by principal component regression (PCR). For the PCR, the nutrient input/output-data were excluded from the X-matrix, due to their different number of samples per variable (n=12; one per block) compared with that of the other variables (n=14; i.e. all blocks plus two control plots). Following an initial PCA (i.e. providing estimates for the unknown regression coefficients for the PCR-models, using the software package Unscrambler (version 10.5), we tested a range of linear regressions models, in which the independent variables were defined as the first five principal components (PC 1-5) of the PCA, and the dependent variables were those in the Y-matrix. Considering the low number of data points (n=14), the regression models were restricted to include a maximum of three components (plus the constant). Moreover, all included components had to contribute significantly to the model, as well as the constant (y-intercept). The regressions were run in Minitab® (version 18.1), using the forward selection procedure. The parameter "alfa to enter" was tested in the range 0.05-0.15, in order to obtain models which fulfilled the specified requirements. Best model for each Y-variable was identified by considering the Mallow's C_p, the adjusted coefficient of multiple determination ($R^2_{adj.}$) (Johnson and Wichern, 1992), and professional judgement (Olejnik *et al.*, 2000).

3. Results

3.1 Potential denitrification

The denitrification potential was lowest in CA1, almost 50% lower than in the other systems. The mixed organic system OM1 and the Control had the highest denitrification potentials (Fig. 2A). The organic arable system OA was intermediate, about 30% lower than OM1 and the Control, whereas the other systems did not significantly differ from the two with the highest potentials.

Potential denitrification rates were matched by residual amounts of NO_2^- and NO_3^- measured after the incubation (Fig. 3), which were significantly ($p<0.001$) larger in CA1 than in other cropping systems. There was also a tendency that the organic system OA reduced NO_2^- and NO_3^- at a slower rate, while OM1 appeared to have the most rapid reduction with significantly ($p<0.001$) lower residual amounts of NO_2^- and NO_3^- compared to CA1, CA2, CM and OM2 after 120 h of incubation.

Potential N_2O production from denitrification differed over a range of 0.1 - 0.6 μg N_2O-N g^{-1} h^{-1} (Fig. 2B). The conventional arable cropping system CA1 produced N_2O at the lowest rate while highest N_2O production was observed for the mixed organic system OM2 and the Control. CA2 produced significantly less N_2O than OA, CM, OM2 and the Control, but not OM1, whereas the latter had a significantly lower N_2O production than OM2 and the Control.

The N_2O index (I_{N2O}) ranged from ~ 0.2 to 0.6, with highest values for OM2 and Control, and lowest for CA1 (Fig. 2C). All the other systems (i.e. CA2, OA, CM and OM1) had intermediate values significantly larger than CA1 and smaller than OM2 and Control.

Maximum NO (ng N g dw $soil^{-1}$) accumulation during denitrification was highest ($p<0.001$) in the Control, with 1.03 ng N g dw $soil^{-1}$ (Fig. 2D), followed by OM1, OM2 and OA (0.70,

0.69 and 0.56 ng N g dw soil^{-1}, respectively). The maximum NO accumulation was lowest in the conventional systems, with ~0.35 ng N g dw soil^{-1} in CA2 and CA1, and 0.41 ng N g dw soil^{-1} in CM.

3.2. Potential nitrification

Conventional arable cropping (CA1) had the lowest nitrification potential, roughly half of that measured in CM, OM2 and the Control (Fig. 4A). The highest potential was measured in the mixed organic system OM2, which was significantly higher than in all other systems except for the conventional mixed system (CM) and the Control. N_2O production by nitrification ranged from 0.6 - 2.8 ng N g^{-1} h^{-1} and was lowest in CA1 and the Control, which differed significantly from all other treatments except CA2 (Fig. 4B). Nitrite accumulation during nitrification was negligible (data not shown). Highest N_2O production was observed in CM, significantly higher than in CA1, CA2, OA and the Control. Calculating the N_2O yield of nitrification (Fig. 4C), 0.03 to 0.14 % of the N oxidized by nitrification was converted to N_2O.

3.3 Explanatory variables

Potential denitrification rates were strongly positively correlated with the activity of the enzymes urease and invertase, along with microbial gene abundance (16s rRNA and ITS), but not with bacterial species richness (Table 4). There was also a significantly positive correlation with total C and N in soil, but a negative correlation with P-AL and N input.

Potential N_2O production by denitrification followed a similar pattern with some exceptions. There was no significant correlation with SOM (i.e. total C and N) but bacterial richness correlated negatively with potential N_2O production.

The N_2O product ratio (I_{N2O}) was strongly positively correlated with urease and invertase activity as well as with microbial gene abundance (16s rRNA and ITS) and total N. by contrast, I_{N2O} was negatively correlated with P-AL and soil pH.

Potential denitrification, N_2O production and I_{N2O} correlated all negatively with N runoff and drainage (kg N ha^{-1} $year^{-1}$).

Unlike denitrification, potential nitrification was positively correlated with bacterial richness but negatively with catalase activity (Table 5). None of the other soil enzyme activities showed any correlation with measured nitrification, nor did any of the soil chemical properties.

By contrast, all measured soil properties correlated positively with N_2O production by nitrification. Positive correlation coefficients > 0.7 were found for Mg-AL, Ca-AL, loss of ignition, soil organic N content and phosphatase activity, somewhat lower positive coefficients (> 0.6) for bacterial gene abundance (16s rRNA), whereas N_2O production by nitrification did not correlate with fungal gene abundance (ITS) or invertase activity. The measured N_2O yield correlated with none of the variables listed in Table 3.

3.4 Multivariate analysis

Principal component analysis (PCA) was carried out with available soil chemical and microbial variables (see section 2.5 for details), all with n=14 (Fig. 5). The first five principal components (PCs) of the chosen model (see section 2.6. for details) explained 84.5 % of the variation in the included variables. PC1, which explained 33.2 % of the total variation, was dominated by microbial abundance (16S rRNA and ITS) and enzyme activity (invertase and urease), along with the amount of SOM (ignition loss, total C, total N) (Fig. 5A). PC2, explaining 20.8% of the variation, was dominated by plant available macronutrients P and K (P-AL and K-AL) (Fig. 5A). The enzyme catalase, bacterial diversity and pH dominated PC3, which explained 16.4% of the variation (Fig. 5C). Principal components number 4 and 5 explained 9.2 and 4.8 % of the variation, respectively. PC4 was dominated by the C/N-ratio of the soil, whereas PC5 by the enzyme Phosphatase (data not shown).

When plotting the scores of the first three PCs against each other (Fig. 5B, 5D and 5F), we observed a clustering pattern of the cropping systems. All systems receiving organic fertilizers had a high PC1-score, with CA1 in the opposite end of the scale – receiving inorganic fertilizer only, and with no retention of straw (Fig. 5B). The Control and CA2 were both intermediate. The next component, PC2, clearly separated the Control from the cropping systems, in particular from CA2 (Fig. 5B). There was also a separation between organic and non-organic systems along the PC2-axis. With one exception (one of the two CA1-systems), all the conventional systems had a higher PC2-score than those of the organic systems. The three organic systems clustered together when looking at PC1 and PC2. Considering the scores of PC3, it appeared, however, that OA and OM1 were given a more positive score than that of OM2, which was close to zero (Fig. 5F). A separation between the two former was not possible.

The PCR model explained 61 % of the variation in potential denitrification combining PC1 and PC3 (Table 6). The model best describing the variation in both potential production of N_2O by denitrification and in I_{N2O} included all first three principal components. For Y-variables related to nitrification, it was only possible to find an acceptable model for potential N_2O production. Here, a model combining PC1 and PC5 could explain 73 % of the measured variation.

4. Discussion

Differences in rotations and management between the cropping systems significantly affected the soil's potential to produce N_2O by nitrification and denitrification. The internal ranking between the six systems differed, however, depending on which process was studied.

4.1 Denitrification

Potential denitrification was smallest in the CA1 system. This may be attributed to the low soil organic matter (SOM) content in this system, a factor known to affect soil denitrification

(Burford and Bremner, 1975). CA1 has the lowest SOM content (table 3), as the only input of organic C is via photosynthesis; the straw is removed each year and the soil is left barren after autumn plowing. By contrast, crop residues are retained in all other systems. In addition, OA, CM, OM1 and OM2 receive organic amendments, and systems CM, OM1 and OM2 have perennial crops in rotation. Pervasive effects of increased soil fertility (e.g. organic fertilizer input) on the microbial community composition have been documented for various long-term cropping systems with putative or measured consequences for the functional potential of N cycling processes (Chen *et al.*, 2018; Wang *et al.*, 2018; Babin *et al.*, 2019). In line with these findings, denitrification potentials in our study correlated positively with total C and N contents of the soils and also with bacterial and fungal gene abundances (Table 4). Denitrifiers constitute a sizable, almost constant proportion of the total heterotrophic microbial biomass (Raut *et al.*, 2012), which in turn is a more or less constant fraction of the soil organic matter (Anderson and Domsch, 1989). CA1 had the lowest 16S rRNA and ITS copy numbers (Table 3) and hence the lowest denitrification potential (Fig. 1). Interestingly, microbial activity measured as urease and invertase in the same blocks by Chen *et al*. (2019) was more strongly correlated with denitrification than SOM expressed as soil total C and N (Table 4), confirming that the increased denitrification potential is directly linked to the heterotrophic activity of the soil microbiome. However, denitrification cannot always be directly linked to exoenzyme activity. In our study, with modest pH differences, urease and invertase activity were likely related to general microbial activity and therefore co-varied with denitrification potentials.

Inputs of NO_3-N and leaching losses of total N were negatively correlated with potential denitrification. While excessive leaching of mineral N conceivably limits denitrification over time, a negative relationship between N inputs and denitrification appears counterintuitive. The negative correlation in our study is explained by the fact that CA1 has the highest input

and output fluxes, while having the lowest denitrification potential. The question whether extraneous N stimulates denitrification *per se* is unresolved. However, there is, to the best of our knowledge, no direct evidence that input of reactive N over time supports denitrifier abundance (Enwall *et al.*, 2005). More likely, potential denitrification correlating positively with N input is linked to higher SOM content. Potential denitrification was also negatively correlated to P-AL and P input, which were lowest in OA, OM1, OM2 and Control. High measured denitrification potentials in these systems suggest that denitrification across the cropping systems is not P-limited, despite low P-Al values (Table 3).

Maximum NO accumulation during denitrification showed an interesting pattern, being smallest in the systems with inorganic N fertilization and largest in the Control, which does not receive any N fertilizer. This points towards more stringent regulation of denitrification in a soil with a legacy of regular mineral N input than in a soil with no or little N input which probably is related to the well-known toxicity of NO (Richardson *et al.*, 2009); inorganically fertilized systems are likely more exposed for nitrosative stress than non-fertilized systems, particularly at low pH. NO mitigation by enrichment of the *qnor* gene, coding for quinol-oxidizing nitric oxide reductase has recently been proposed for acid forest soils (Roco *et al.*, 2019). NO is a pollutant involved in tropospheric ozon production (Crutzen, 1981), and high NO production is thus undesired. Our results suggest that organically fertilized systems tend to have higher NO emission potentials than inorganically fertilized systems.

The importance of SOM content, microbial abundance and exoenzyme activities for the denitrification potential in our systems is underlined by the principal component analyses, as these variables dominated the first component (PC1), and PC1 and potential denitrification were highly correlated ($r = 0.68$, data not shown). More than 60% of the variation in potential denitrification could be explained when PC1 and PC3 were combined in a principal component regression PCR (Table 6). The third principal component (PC3) was as PC1

dominated by microbial variables (in this case catalase and bacterial diversity), but pH had a relatively high loading, too. Effects of soil pH on the denitrification capacity reported in the literature are inconsistent. Simek *et al.* (2002) concluded that though denitrification may be most efficient at near neutral pH, denitrifier communities are adapted to prevailing pH, and thus high denitrification may occur also at low soil pH. In our study, pH differences were subtle (pH range: 5.16 – 5.51) and were likely overridden by other factors in their effect on potential denitrification.

Potential N_2O-production by denitrification was positively related to the same microbial properties that explained potential denitrification. Accordingly, there was a positive correlation with PC1 ($r = 0.54$), the loading of which was dominated by microbial variables and SOM (Fig 9A). Potential N_2O-production by denitrification was, however, unrelated to SOM (i.e. total C, total N, ignition loss), meaning that microbial properties likely are the dominating factors. PC1 clearly separated the arable cropping systems (CA1, CA2 and OA) and the Control from the mixed systems, which received manure. PC2 (dominated by plant available P and K and fungal diversity) separated OA and Control from CA2 and CM, but the separation was not reflected in any significant difference in potential denitrification, N_2O production or I_{N2O}. PC3 (mainly bacterial diversity, pH and catalase) separated OM1 and the Control, which corresponded well with observed lower N_2O production and I_{N2O} in OM1 (pH=5.51) than in the Control (pH= 5.16), even though potential denitrification was high in both systems. None of the first three principal components could explain the observed difference in potential denitrification between OA and OM1. However, microbial C and N in the organic arable (OA) system was ~25% lower than in the organic mixed system OM1, probably explaining the lower denitrification potential by less inducible microbial activity. Other factors differing between the cropping systems such as tillage, inorganic fertilizer input or use of pesticides seem to be of no direct effect on denitrification. However, all management

may affect soil carbon mineralization positively or negatively, and limitations in available carbon had the largest impact on microbial richness and activity in our systems (Chen *et al.*, 2019).

As expected, the denitrification product ratio (I_{N2O}) was negatively correlated with soil pH. N_2O reductase is the only known enzyme to reduce N_2O to N_2 and the functioning of this enzyme is highly linked to soil pH (Bergaust *et al.*, 2010; Bakken *et al.*, 2012; Liu *et al.*, 2014). I_{N2O} was also negatively correlated with PC3 (r = -0.635, data not shown) for which pH was one of the dominating factors. Even though soil pH differences between the cropping systems were small, soil pH explained 59% of the variation in I_{N2O}. The pH dependency of I_{N2O} thus largely explains the variation of this important denitrification trait among our systems (Fig. 4), and is in accordance with a combined field and laboratory study in a nearby field, which found a clear effect of small-scale soil pH heterogeneity (pH 5.4 -5.9) on I_{N2O} and off-season N_2O emissions (Russenes *et al.*, 2016).

4.2 Nitrification

The highest nitrification potential was observed in the organic mixed cropping system, which has the highest fraction of ley in the rotation (OM2, 75% ley). This potential was significantly higher than that in all the arable systems along with the organic mixed system with 50% ley (OM1), but indistinguishable from the conventional mixed cropping system (CM) and the permanent unfertilized grass used as Control. OM2 and CM have the largest N inputs of the ley systems, and also the highest total grass production (Korsaeth 2012), and show similarities in microbial diversity and richness. Input of plant available N is known to stimulate nitrification (Chu *et al.*, 2007). This applies for P as well (ibid), but plant available P is higher in CM than that in OM2. This may be explained by the observation that P did not seem to play a role for the nitrification potential in our systems, as it was among the highest in the Control, which has little available P (Table 3). The Control receives neither P nor N other

than from atmospheric depositions, which are small in the region (< 1kg N ha^{-1} y^{-1}), yet Control had a nitrification potential equally to conventional or organic mixed dairy systems. There are, however, considerable amounts of clover in the unfertilized boarder stripes used as Control, which could explain the high nitrification capacity by N supply through biological N fixation.

Ammonium and pH are the primary factors controlling nitrification activity and nitrifier growth, and one would expect higher nitrification potentials with higher N input. Wertz *et al.* (2012) found clear increases in ammonia oxidizer abundance in pine and spruce stands after 6 years of inorganic fertilization, and Keil *et al.* (2011) found more *amoA* gene copies of AOB in fertilized than in non-fertilized grassland. Also, high nitrification potentials have been implicated with long-term inorganic rather than organic N fertilization (Chu *et al.*, 2007). However, Kong *et al.* (2019) reported higher nitrification rates in long-term organically fertilized soil, which they attributed to ammonia oxidizing archaea. Conversely, Fan *et al.* (2011) found that manure addition restored AOB nitrification by alleviating the acidifying effect of long-term inorganic fertilization. It is noteworthy to mention that the long-term fertilization regimes in our cropping systems only had a marginal effect on soil pH, which is generally acidic (pH 5.16 – 5.51). This suggests that nitrification activity is dominated by AOA, which have been shown to be stimulated by application of organic amendments in some studies (Schauss *et al.*, 2009; Hink *et al.*, 2018). Predominance of AOA nitrification in the Control and the OM2 system with 75% grass-clover ley in the rotation is also supported by the low N_2O yields of oxic nitrification (Fig. 4C), which are characteristic for AOA (Hink *et al.*, 2017b). Hence, we attribute elevated nitrification potentials in the mixed systems and the Control to increased AOA abundances. This increase is probably stimulated by long-term input of organic N in form of incorporated grass-clover ley and animal slurry (CM, OM), or as mulched grass (Control).

Potential N_2O production by oxic nitrification (Fig. 4B) followed the nitrification potentials to some extent, and was largest in mixed systems with manure application. Normalized as N_2O yield (Fig. 4C), the formation of N_2O-N per unit nitrified N was largest in the organic arable (OA) and the mixed systems CM and OM1. Interestingly, N_2O yield was not correlated with any of the soil or microbial variables (Table 5), suggesting that other factors control the relative potential to form N_2O by nitrification in our soils. The calculated N_2O yields are well within the range of published values for AOB and AOA nitrification (Hink *et al.*, 2017b; Tzanakakis *et al.*, 2019), and cropping system specific differences in AOA/AOB ratios would be a plausible factor to explain differences in N_2O yield. However, there was no correlation with soil pH, the strongest known driver for the AOA/AOB ratio (Nicol *et al.*, 2008). The second strongest driver for the AOA/AOB ratio is believed to be NH_4^+ availability, with high NH_4^+ concentrations favoring AOB over AOA. Using the N input-output balance in Table 3 as a proxy for system N turnover (assuming that lost N is dominated by nitrified ammonium), soil solution ammonium concentrations are likely highest in OA and OM1, which would suggest that AOB activity dominates N_2O production in soils from these systems. Irrespective of system-specific differences in nitrification N_2O yields, potential N_2O production by nitrification was 2-3 orders of magnitude smaller than that by denitrification (Fig. 7 and 3 respectively).

4.3 N_2O emission potentials

Potential N_2O emission by denitrification and nitrification was lowest in CA1, in line with low microbial abundance (Tab. 3). A conventional arable system with autumn ploughing may thus appear favorable to reduce direct N_2O emissions. However, in absolute terms, most N (in average 44.2 kg N ha^{-1} y^{-1}; Tab. 3) is lost by runoff and drainage from this system, significantly more than from any other system (Korsaeth, 2012). This points to a potential environmental undesired impact of this system; direct N_2O emission potentials by nitrification

and denitrification may be low, but potential indirect emissions from NO_3^- transported out of the system should also be considered.

Higher denitrification potential was observed in systems with leys (Fig. 2A), suggesting that the inclusion of perennial crops in the rotations builds denitrification potential over time and thus facilitates high N_2O emissions during anoxic spells. By contrast, 1-year ley and use of biowaste as organic fertilizer (OA) appears to support less denitrification capacity. Here the denitrification potential was smaller, most likely due to less readily decomposable organic matter in digested biowaste than in animal manure. Petersen *et al.* (2013) concluded that a possible increase in denitrification due to enhanced carbon input by cover crops is probably compensated for by improved soil aeration, reducing the potential for anoxic conditions and hence the potential for denitrification. As we conducted a slurry experiment, such effects could not be captured, and the experimental design thus preventing us from extrapolating our findings to actual field emissions. A recent study by Tenuta *et al.* (2019), measuring N_2O emissions over 11 years in Ontario (Canada) by micrometeorology in fields with and without the inclusion of perennial crops, did not find any conclusive effects on N_2O emissions, partly because of very high spring-thaw associated N_2O emissions following termination of a 4-year, unfertilized grass-legume intercropping period.

There are no N_2O emission measurements for the Apelsvoll field trial available per date, but experience from other field experiments with clover rich leys suggest that perennial cropping under Norwegian conditions is vulnerable to high N_2O emissions during winter and early spring, when freeze-thaw cycles result in considerable mass loss from aboveground biomass (Ievina Sturite, *pers. com.*). Accordingly, Mørkved *et al.* (2006) found in an *ex situ* study that freeze-thaw extracts from clover greatly stimulated potential denitrification. Together with the high nitrification potential, rapidly converting mineralization released NH_4^+ to NO_3^-, we thus

reckon organic mixed systems with inclusion of grass-clover ley to have the largest N_2O emission potential.

5. Conclusion

Our study demonstrates that crop and soil management measurably affect potential denitrification and nitrification and related potential N_2O emissions over time. The potentials were highest in systems using manure and with grass-clover ley in the rotation, which leads to both higher amounts of easily degradable organic matter supporting denitrifiers, and more evenly distributed N availability supporting nitrifiers. Replacing animal manure by biogas digestate seems to restrain the nitrification potential on the level of the inorganically fertilized arable systems. With respect to inherent N_2O stoichiometries, small soil pH differences between systems strongly affected the $N_2O/(N_2+N_2O)$ ratio of denitrification, whereas no clear-cut driver could be identified for variations in the N_2O yield of nitrification. Given the higher potentials in systems with perennial leys and the importance of freeze-thaw driven N_2O emissions in SE Norway, it is concluded that overall N_2O emissions are likely to increase with increasing proportion of perennials in the crop rotation. The results were unaffected of whether the management regime was conventional or organic.

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Figures

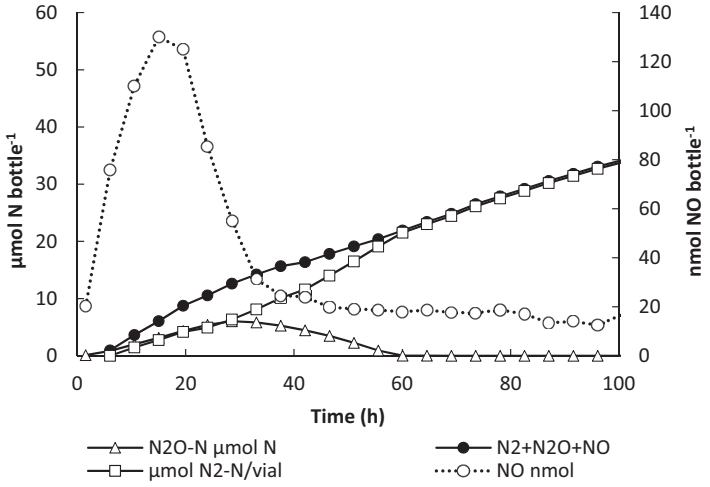

Figure 1. Example of N gas kinetics throughout 100 h of anoxic incubation. Shown are single bottle values for sample 14 (Organic Arable cropping system).

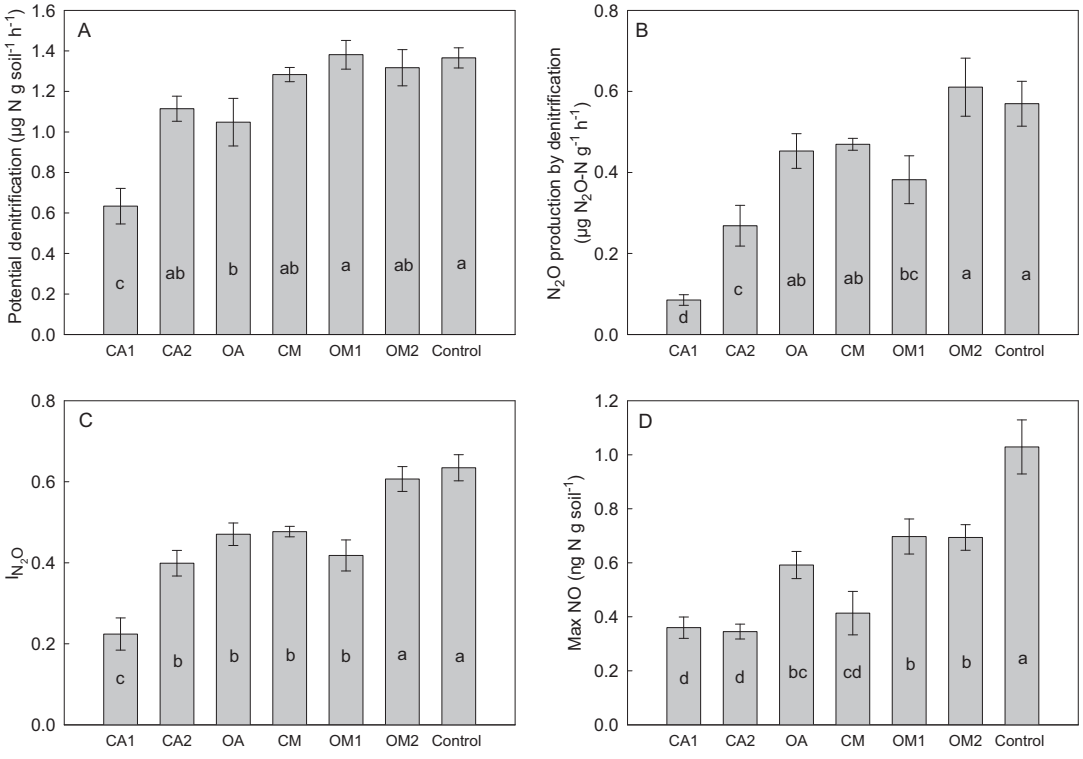

Figure 2. Potential denitrification rates (A), potential N_2O production (B), N_2O index (C) and maximum NO accumulation (D) during anoxic incubation of soils from different cropping systems. Shown are mean values (n=4); error bars are standard error. Different letters indicate significant differences between cropping systems. For treatment names, see Tab.1

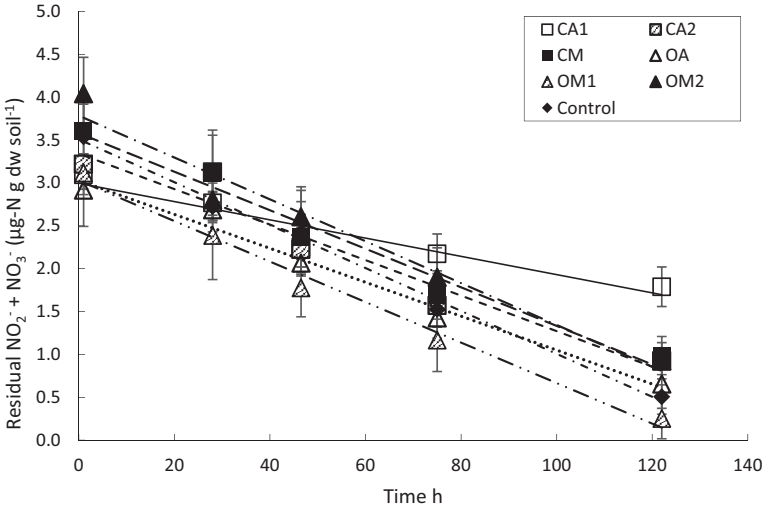

Figure 3. Residual NO_2^- *and* NO_3^- *in the denitrification assay. Shown are mean values (n=4). Error bars are standard deviation.*

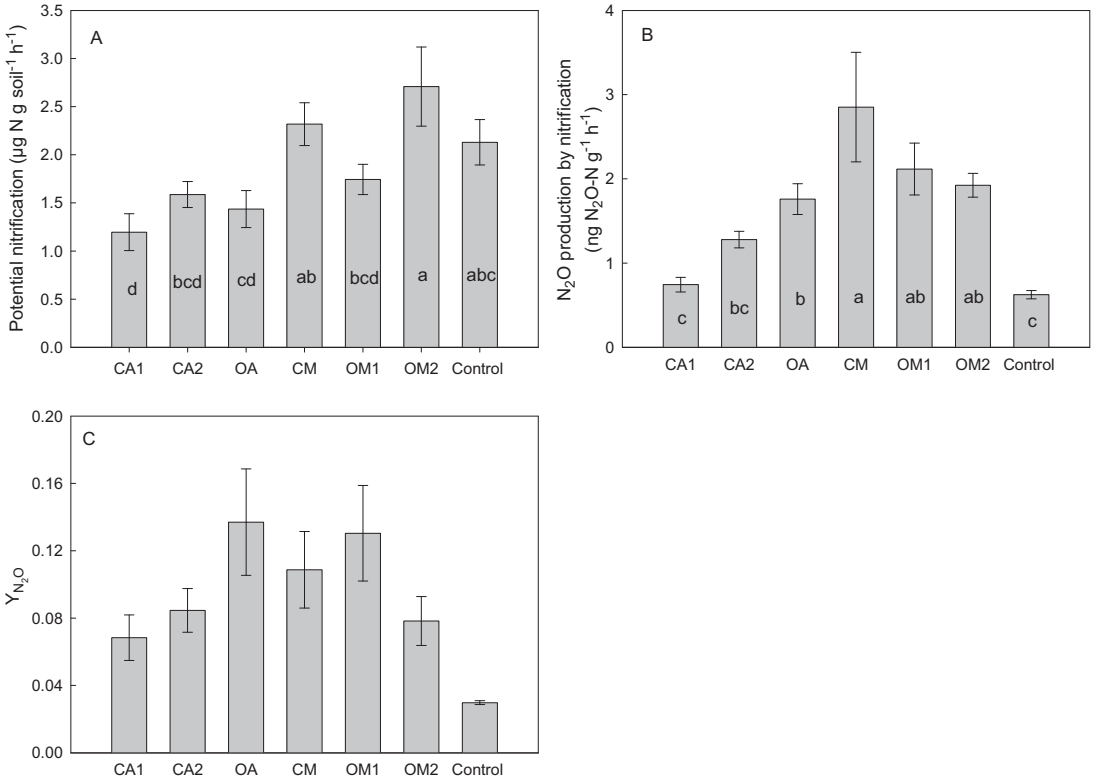

Figure 4 Potential nitrification rates (A), N_2O production rates (B) and N_2O yield (C) of soils from different cropping systems. Shown are mean values (n=4); error bars are standard error and letters indicate significant difference between systems.

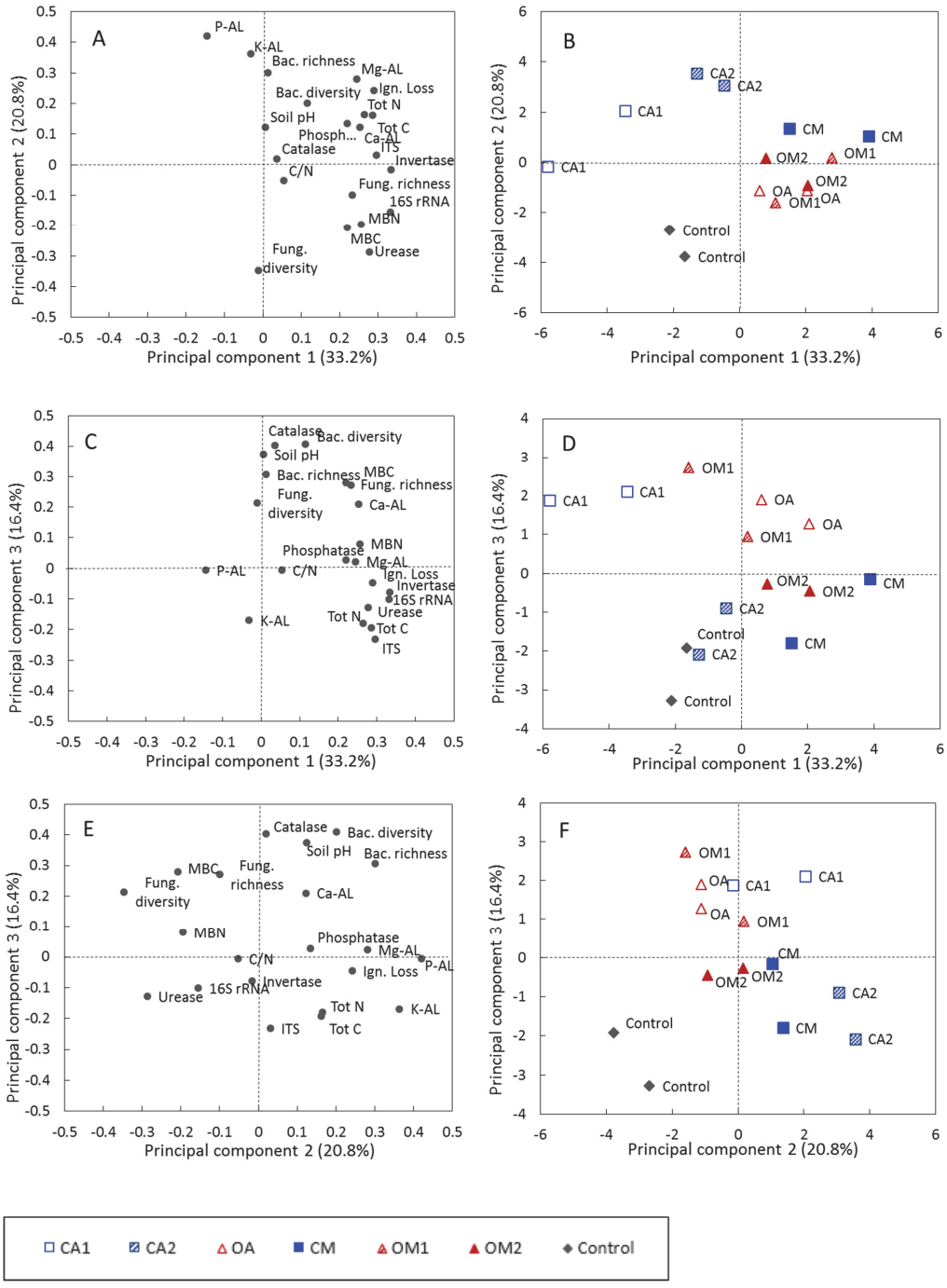

Figure 5. Loading (left panel) and score plots (right panel) of the three first principal components.

Tables

Table 1. Characteristics of the cropping systems at Apelsvoll, 2001-2015. Fertilizer input given as average for the period, as the amounts of manure are adjusted according to fluctuations in the feed production, and the amounts of split fertilization of inorganic N are adjusted to seasonal variations

Crop rotation	Fertilizer, kg ha^{-1}						Plant protection	Soil tillage
	Inorganic			Organic[1]				
	N	P	K	N	P	K		
Conventional arable (CA1)[2]								
Potatoes	123	49	187				Chemical, mechanical	Autumn
Spring wheat	141	23	66				Chemical	ploughing
Spring oats	119	20	56				Chemical	& spring
Spring barley	119	20	56				Chemical	harrowing
Conventional arable (CA2)								
Potatoes	106	43	160				Chemical, mech.	Spring
S. wheat + catch crop[3]	135[4]	24	68				Chemical, mech.[5]	harrowing
Oats + catch crop[3]	115	18	49				Chemical, mech.[5]	only[7]
Barley + catch crop[3]	115	18	49				Chemical, mech.[5]	
Organic arable (OA)								
Barley[8]				80	6	33	Manual weeding	Spring
Grass-clover[9]							Manual w.	ploughing
S. wheat + catch crop[3]				80	6	33	Manual w., mech.[5, 10]	&
Oats + catch crop[6]				80	6	33	Manual w., mech.[5, 10]	harrowing
Conventional mixed dairy farming (CM)								
Barley[11]	49	7	18	64	10	97	Chemical	Spring
1st ley year	104	13	58	52	8	79	-	ploughing
2nd ley year	119	16	66	71	11	107	-	&
S. wheat + catch crop[3]	74	12	31	64	10	98	Chemical, mech.[5]	harrowing
Organic mixed dairy farming (OM1)								
Barley[11]				68	10	91	Manual w.	Spring
1st ley year				31	3	42	Manual w.	ploughing
2nd ley year				50	6	66	Manual w.	&
S. wheat + catch crop[3]				76	11	100	Manual w., mech.[5, 10]	harrowing
Organic mixed dairy farming (OM2)								
Barley[11]				79	11	99	Manual w.	Spring
1st ley year				58	7	76	Manual w.	ploughing
2nd ley year				101	13	131	Manual w.	&
3rd ley year				67	9	86	Manual w.	harrowing

[1.] Digested household biowaste (applied since 2010) in system OA, cattle slurry in CM, OM1 and OM2

[2] Managed as was common for the region in 1985 (tillage and fertilization as in 1985, but for practical reasons, present-day inputs of seeds and chemical plant protection.

[3] Perennial ryegrass (*Lolium perenne* L), sown about one week after the cereals.

[4] Split application of fertilizer with about 75 % given at sowing, and 0-60 kg N ha^{-1} applied at growth stage (GS) 49, according to measured needs.

[5] Weed harrowing performed when the cereals are at GS 11-12.

[6] Italian ryegrass (*Lolium multiflorum* Lam), sown about one week after the oats.

[7] Performed twice with a horizontally rotating harrow.

[8] With undersown grass-clover mixture. Seed mix: 80 % Timothy (*Phleum pratense* L.), 10 % red clover (*Trifolium pratense* L.) and 10 % white clover (*Trifolium repens* L).
[9] Green manure, not harvested but mulched 3-4 times per season.
[10] Harrowed in autumn after harvest some years to reduce the weed pressure
[11] With undersown grass-clover ley. Seed mix: 60 % Timothy (*Phleum pratense* L.), 30% Meadow fescue (*Festuca pratensis* L.) and 10 % red clover (*Trifolium pratense* L.).

Table 2. Annual nitrogen fertilizer input split in inorganic and organic fractions regardless of inorganic or organic fertilizer origin. Values given as kg ha^{-1} averaged for 2011, 2012 and 2014.

System	Inorganic N	Organic N	NO_3^+	NH_4^+
CA1	127	0	59	67
CA2	116	0	54	59
OA	41	21	0	41
CM	140	25	43	99
OM1	35	18	0	35
OM2	41	21	0	41
Control	0	0	0	0

Table 3. Properties of soils used in the incubation experiment, nutrient balances for the cropping systems and soil nutrient levels and microbial properties from Chen et al. (2019). Shown are mean values ± standard deviation

	Management system						
	CA1	**CA2**	**OA**	**CM**	**OM1**	**OM2**	**Control**
Soil chemical properties[1]							
Soil pH	5.42 ±0.04	5.37 ± 0.10	5.34 ± 0.09	5.34 ± 0.01	5.51 ± 0.21	5.32 ± 0.04	5.16 ± 0.04
Total C	2.1 ± 0.54	2.8 ± 0.02	2.7 ± 0.20	3.0 ± 0.06	2.6 ± 0.25	2.8 ± 0.02	2.5 ± 0.01
Total N	0.2 ± 0.06	0.3 ± 0.01	0.3 ± 0.02	0.3 ± 0.00	0.2 ± 0.04	0.3 ± 0.00	0.2 ± 0.01
C/N-ratio	10.2 ± 0.16	10.5 ± 0.33	10.5 ± 0.03	10.7 ± 0.01	10.5 ± 0.57	10.1 ± 0.23	10.4 ± 0.30
P-AL[2]	6.0 ± 0.05	7.2 ± 0.50	2.2 ± 0.70	4.4 ± 0.10	2.2 ± 0.70	2.4 ± 0.90	1.5 ± 0.00
K-AL[2]	5.9 ± 0.50	11.5 ± 0.50	5.4 ± 0.10	7.3 ± 0.45	5.4 ±0.20	4.7 ± 0.30	5.0 ± 0.50
Mg-AL[2]	10.0 ± 0.00	14.0 ± 1.00	11.0 ± 1.00	16.5 ± 0.50	14.5 ±0.50	14.0 ± 0.00	7.5 ± 1.20
Ca-AL[2]	165 ± 25	155 ± 5	185 ± 5	215 ± 35	180 ± 10	185 ± 25	125 ± 5
Ign. Loss[2]	5.8 ± 0.50	6.9 ± 0.05	6.5 ± 0.00	7.3 ± 0.30	6.9 ± 0.35	6.6 ± 0.20	5.8 ± 0.60
Nutrient input and runoff/drainage[3]							
NO_3-N input	58.9	54.0	0	43.2	0	0	0
NH_4-N input	67.1	59.0	41.0	99.5	35.0	40.8	0
P input	23.2	16.9	4.6	15.5	7.8	9.0	0
K input	91.0	67.0	25.6	120.2	75.8	87.0	0
Inorganic N input	126.8	116.3	41.0	139.9	35.0	40.8	0
Organic N input	0	0	20.7	39.2	17.8	20.5	0
Total N input	126.8	116.3	61.7	179.0	52.8	61.4	0
N output Runoff/drainage	44.2±10.21	26.7 ±0.50	29.3 ±3.54	19.8±1.02	25.7 ±1.97	15.9 ±6.81	
Soil microbial properties[4]							
Copy numbers of 16S rRNA gene	0.5 ± 0.04	0.9 ± 0.04	1.1 ± 0.05	1.2 ± 0.10	1.1 ± 0.01	1.1 ± 0.04	1.0 ± 0.11
Copy numbers of ITS gene	0.4 ± 0.00	1.2 ± 0.10	1.1 ± 0.13	1.3 ± 0.02	0.9 ± 0.03	1.3 ± 0.04	0.9 ± 0.07
Catalase	45.8 ± 2.61	36.9 ± 2.51	56.7 ± 0.70	41.6 ± 4.82	46.7 ± 2.31	30.5 ± 5.62	30.5 ± 1.17
Urease	0.2 ± 0.01	0.2 ± 0.00	0.3 ± 0.00	0.3 ± 0.02	0.3 ± 0.00	0.3 ± 0.01	0.3 ± 0.00
Invertase	65.6 ± 1.55	116.5±3.91	128.2±1.30	126.5±2.77	128.8±0.76	125.3±1.12	101.0±2.89
Phosphatase	4.3 ± 0.12	3.9 ± 0.06	4.3 ± 0.58	5.7 ± 0.18	4.5 ± 0.71	4.7 ± 0.31	3.7 ± 0.12
Bac. Richness	1831 ± 76	1826 ± 6	1789 ± 12	1726 ± 26	1821 ± 30	1798 ± 11	1633 ± 36
Bac. Diversity	6.2 ± 0.02	6.2 ± 0.01	6.2 ± 0.04	6.1 ± 0.03	6.2 ± 0.02	6.2 ±0.01	5.9 ± 0.04
Fung. Richness	440 ± 4	452 ± 55	528 ± 15	460 ± 49	507 ± 9	499 ± 6	440 ± 25
Fung. Diversity	3.8 ± 0.09	2.8 ± 0.30	4.0 ± 0.02	3.3 ± 0.20	3.5 ± 0.38	3.9 ± 0.10	3.8 ± 0.00
MBC	248 ± 6	195 ± 6	338 ± 2	309 ± 43	438 ± 2	278 ± 7	275 ± 1
MBN	35.1 ± 1.39	43.3 ± 3.16	63.9 ± 4.01	64.2 ± 3.62	88.3±11.03	61.9±20.77	59.6±17.06

[1]Units; pH as measured in KNO_3, total C, total N and ignition loss: g 100 g DM^{-1}; P-AL, K-AL, Mg_AL, Ca-AL: mg 100 g^{-1}.

[2] From Chen et al. (2019), see section 2.2 for details.

[3] Units: NO_3-N, NH_4-N, P and K input, N output: kg N ha^{-1} y^{-1}.

[4] Units: Copy numbers of 16S rRNA gene 10^{10} g^{-1} soil and ITS gene 10^{8} g^{-1} soil, Catalase: 0.1 M $KMnO_4$ ml g^{-1}, Urease: NH_2-N mg g^{-2}, 24h, Invertase: G mg g^{-1}, 24 h, Phosphatase: Phenol mg g^{-1}, 24 h, Bacterial and fungal richness: Chao 1 index, Bacterial and fungal diversity; Shannons index, MBC and MBN: mg kg^{-1}.

Table 4. Pearson correlation coefficients (r) and significance levels (p) for soil chemical properties, nutrient input/output and soil microbial properties (Chen et al. 2019) which were significantly ($p \leq 0.05$) correlated with potential denitrification, potential N_2O production and/or I_{N2O}

	Potential denitrification		Potential N_2O production by denitrification		I_{N2O}	
Variable	**r**	**p**	**r**	**p**	**r**	**p**
Soil chemical properties						
pH					-0.593	0.025
Tot_C	0.622	0.017				
Tot_N	0.571	0.031			0.549	0.042
P-AL	-0.576	0.031	-0.753	0.002	-0.59	0.026
Nutrient input/output						
NO_3-input	-0.589	0.027	-0.676	0.008	-0.669	0.009
Inorganic N			-0.563	0.036	-0.625	0.017
P_input	-0.633	0.015	-0.702	0.005	-0.753	0.002
N Runoff/Drainage	-0.747	0.005	-0.822	0.001	-0.666	0.018
Microbial properties						
16s rRNA	0.726	0.003	0.683	0.007	0.563	0.036
ITS	0.688	0.007	0.61	0.021	0.627	0.016
Catalase						
Urease	0.777	0.001	0.785	0.001	0.709	0.005
Invertase	0.694	0.006	0.61	0.02	0.541	0.046
Phosphatase						
Bact richness			-0.542	0.045		

Table 5. Pearson correlation coefficients (r) and significance levels (p) for soil chemical properties, nutrient input/output and soil microbial properties (Chen et al. 2019) which were significantly ($p \leq 0.05$) correlated with potential nitrification, potential N_2O production by nitrification or its N_2O yield

	Potential nitrification		**Potential N_2O produced by nitrification**		**Yield% N_2O**	
Variable	**r**	**p**	**r**	**p**	**r**	**p**
Soil chemical properties						
Tot_C			0.539	0.047		
Mg_AL			0.772	0.001		
Ca-AL			0.770	0.001		
Loss on ignition			0.772	0.001		
Nutrient input/output						
Organic N			0.785	0.001		
N Runoff/Drainage	-0.700	0.011				
Microbial properties						
16s rRNA			0.620	0.018		
ITS						
Catalase	-0.680	0.007				
Invertase			0.599	0.024		
Phosphatase			0.702	0.005		
Bact richness	0.537	0.048				

Table 6. Statistics of the principal component regression

Dependent variable	Term	Coefficient	P-value	R^2	R^2(adj)
Potential denitrification					
	Model		0.002	0.668	0.61
	Constant	1.1635	<0.001		
	PC 1	0.0717	0.002		
	PC3	-0.0667	0.026		
Potential N_2O-denitrifcation					
	Model		0.003	0.739	0.66
	Constant	0.4042	<0.001		
	PC1	0.0411	0.007		
	PC2	-0.0496	0.01		
	PC3	-0.0458	0.025		
I_{N2O}					
	Model		0.002	0.754	0.68
	Constant	0.4574	<0.001		
	PC1	0.0258	0.017		
	PC2	-0.0286	0.032		
	PC3	-0.0524	0.002		
Potential N_2O-nitrifcation					
	Model		<0.001	0.775	0.73
	Constant	1.613	<0.001		
	PC1	0.2826	<0.001		
	PC5	-0.31	0.037		

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