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

Philosophiae Doctor (PhD)
Thesis 2023:2

Mitigation of greenhouse gas emissions by pH management of agricultural soils in Norway

Redusering av klimagassutslipp gjennom pH-justering av jord i Norge

Teodora Todorcic Vekic

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Ås (2023)

Thesis number 2023:2
ISSN: 1894-6402
ISBN: 978-82-575-2031-1

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Acknowledgments

This work has been largely financed by the FACCE ERA GAS project, MAGGE pH project, Norwegian Research Council project number 234382 (MIGMIN), Norwegian Institute for Bioeconomy Research (NIBIO), and the Norwegian University of Life Sciences (NMBU)- Faculty for Environmental Sciences and Resource Management (MINA).

I am thankful to my co-supervisor Peter Dörsch, for choosing me and giving me this opportunity, thank you and Lars Bakken for your support. To my main supervisor, Tobias Rütting, I am especially thankful for your constant support, professionalism, kindness, and your timely supervisor engagement. In addition to your inexhaustible scientific support, you infused me with encouragement and faith in my research work. To my co-supervisor, Leif Klemedtsson thank you for your inspiration, encouragement, and selfless support in my pursuit of becoming a scientist and learning from the best.

I would like to thank the dean of MINA Hans-Fredrik Hoen, for his trust and understanding, because at a crucial moment, he made decisions that were important for my further PhD work. I would like to express my gratitude to NIBIO, especially my avdelingssjef Håkon Borch, for his trust and interest in my PhD work, his optimism, and for supporting me with his understanding and suggestions.

Many thanks to the staff of the soil science group at NMBU, particularly Trygve Fredriksen and Øyvind Peder Vartdal for their technical help in the field and the laboratory.

To my friends and colleagues at NIBIO- thank you for your support, and optimism, and for an inspiring and pleasant working environment.

To my friends at Geovetarcentrum (GVC) in Gothenburg: Louise Rütting- thank you for your kindness, guidance, support, and always optimistic outlook on everything concerning my PhD journey and work in science. Åsa Kasimir- thank you for your support and encouragement throughout my PhD journey. Magdalena Wallman, thank you for sharing your wisdom and experiences with me. There are many more of you

whom I have not mentioned but I have learned so much from you all at GVC, just by being in your company.
To my friends Anita and Thomas, and their beautiful, big family- thank you for your strong and noble love, support, trust, and friendship. Every day I learn from you.

I would like to thank my family – for the love, support, faith, and inspiration you provided me just by your example. To my mother, thank you for installing a never-ending curiosity and faith in life in me.

To my mother

Table of Contents

1 Abbreviations and definitions

ANOVA analysis of variance

AOA ammonia-oxidizing archaea

AOB ammonia-oxidizing bacteria

atm atmosphere

C carbon

$CaCl_2$ calcium chloride

Ca^{2+} calcium ion

C_2H_2 acetylene

CO carbon monoxide

CO_2 carbon dioxide

DMPP dimethylpyrazole phosphate

FFR field flux robot

GHG greenhouse gas

GLM general linear model

GWP global warming potential

H_2 hydrogen

H^+ hydrogen ion

He helium

IMU inertial measurement unit system

IPCC International Panel on Climate Change

KNO_3 potassium nitrate

Mg^{2+} magnesium ion

N nitrogen

N_2 dinitrogen

NOB nitrite-oxidizing bacteria

NH_4Cl ammonium chloride

NH_3 ammonia

NH_4^+ ammonium

NH_2OH hydroxylamine

NO_3^- nitrate

NO_2^- nitrite

nar nitrate reductase

nir nitrite reductase

nor nitric oxide reductase

nosZ nitrous oxide reductase

N_2O nitrous oxide

NPK nitrogen, phosphorus and potassium fertilizer

NO nitric oxide

O_2 oxygen

pH negative logarithm of H^+ ions in a solution

ppb parts per billion

ppm parts per million

RTK real-time kinematics

rpm revolutions per minute

TDR time domain reflectometry

WFPS water-filled pore space

2 List of papers

Paper 1

Effect of calcareous and siliceous amendments on N_2O emissions of grassland soil, Soil use and management, under review

Paper 2

Effects of maintenance liming on growing season N_2O emissions in an arable soil of SE Norway, manuscript

Paper 3

3,4-dimethylpyrazole phosphate (DMPP) inhibition of nitrification and its N_2O yield in remolded soil and soil slurries, manuscript

3 Abstract

Liming has been and still is used to neutralize soil acidity, improve soil nutrient availability, increase yields, and recently it has been recognized as a possible nitrous oxide (N_2O) mitigation tool for arable soils. However, studies investigating the pH effect on N_2O emissions have been predominantly laboratory studies. It has been well-known that increased pH reduces the N_2O/N_2 product ratio of denitrification. On the other hand, the increase in pH also may lead to a trade-off by stimulating N_2O emissions stemming from increased NH_3 availability and increased abundance of ammonia-oxidizing bacteria (AOB) relative to ammonia-oxidizing archaea (AOA). Also, the application of traditional calcareous minerals for liming may lead to an increase in carbon dioxide (CO_2) emissions.

This thesis combines a 2.5-year field N_2O measurement campaign after bulk liming (30 t ha^{-1}), a 4-month long N_2O measurement campaign after maintenance liming (1.7-5 t ha^{-1}), and a two-week CO_2 measurement campaign after maintenance liming from clay-loam grassland in south-east Norway. In addition, nitrification increased N_2O emissions have been investigated under laboratory conditions. The field treatments included a control (not limed), calcareous lime, consisting of calcite and dolomite, and siliceous minerals, consisting of larvikite, norite, and olivine. The siliceous materials used were by-products of the mining industry and represent possible carbonate-free liming alternative. In the field study, major emission peaks over the course of 5 years occurred after fertilization and rainfall and during freezing and thawing events. Calcareous minerals were successful at raising soil pH, but siliceous minerals were not. The pH effect-linear relationship between high pH and low N_2O emissions ($p<0.05$) was only visible in an associated 2017 pot experiment under semi-field conditions. None of the liming treatments had an effect on yields. The laboratory study shows that liming almost doubled the net nitrification rates, 5 years after bulk liming, and thus possibly explains the increase in field N_2O emissions. Increased nitrification rates were successfully inhibited with a minimum dose of 3.4 dimethylpyrazole phosphate (DMPP). After maintenance liming, all treatments had a decrease in N_2O emissions and an increase in CO_2 emissions relative to control. By comparing cumulative CO_2 and N_2O emissions relative to control for the period of two weeks after maintenance liming, it was concluded that the decrease in N_2O emissions were not large enough to off-set the increase in CO_2 emissions. This thesis shows that the overall liming or “pH effect” on N_2O emissions under field conditions may be obscured by the sudden increase in microbial activity and fast nutrient turnover rates leading to high N_2O emissions. Liming of grasslands in Norway is not recommended as a management option to mitigate soil greenhouse gas emissions due to increase in CO_2 emissions, which were not off-set by the decrease in N_2O emissions.

Keywords: Nitrous oxide, liming, soil pH, nitrification inhibitor, grassland, Norway

4 Norsk sammendrag

Kalking har blitt, og blir fortsatt brukt, for å nøytralisere pH i jord, forbedre jordens næringsevne, øke avlingene, og nylig har det blitt anerkjent som et mulig nitrogenoksid (N_2O)-reduserende tiltak på dyrket jord. Tidligere studier som undersøker pH-effekten på N_2O-utslipp, har imidlertid hovedsakelig vært laboratoriestudier. Det har vært velkjent at økt pH reduserer N_2O/N_2-forholdet av denitrifikasjon, men økningen i pH kan også føre til å stimulere N_2O-utslipp fra økt NH_3-tilgjengelighet, og økt overflod av ammoniakkoksiderende bakterier (AOB) over ammoniakkoksiderende archaea (AOA). I tillegg, bruk av tradisjonelle kalkholdige mineraler kan føre til økte karbondioksid (CO_2)-utslipp.

Denne oppgaven kombinerer en studie av en 2.5-årig felt-N_2O-overvåkingskampanje etter bulkkalking (30 t ha^{-1}), 4-måneders lang N_2O-overvåkingskampanje etter vedlikeholds kalking (1.7-5 t ha^{-1}), og to ukers CO_2-overvåkingskampanje etter vedlikeholdskalking fra mellomleire eng i Sørøst-Norge. I tillegg har nitrifikasjon økte N_2O-utslipp blitt undersøkt under laboratorieforhold. Felt behandlingene var kontroll (ikke kalket), kalkholdig kalk og dolomitt og silikat mineraler – larvikitt, noritt og olivin, som var biprodukter fra gruveindustrien, som et mulig alternativ til kalkholdige mineraler, siden de ikke inneholder karbonater. I feltstudien oppsto store utslippstopper i løpet av 5 år etter gjødsling, og nedbør, frysing og tining. Kalkholdige mineraler bidro til å øke jordens pH, men silikatmineraler gjorde det ikke. pH-effekt-lineært forhold mellom høy pH og lavt N_2O-utslipp ($p<0.05$) var kun synlig i et assosiert 2017 potteeksperiment under semi-feltforhold. Det var ingen effekt av kalking på avling. Laboratoriestudien viser at kalking nesten doblet nitrifikasjonsratene, 5 år etter bulkkalking, og forklarer dermed muligens økningen i felt N_2O-utslipp. Økte nitrifikasjonshastigheter ble hemmet av en minimumsdose på 3.4 dimetylpyrazolfosfat (DMPP). Etter vedlikeholdskalking hadde alle behandlinger en nedgang i N_2O-utslipp og en økning i CO_2-utslipp i forhold til kontroll. Nedgangen i N_2O-utslipp for alle behandlinger var ikke stor nok til å oppveie økningen i CO_2-utslipp. Dette var beregnet ved sammenligne kumulative CO_2- og N_2O-utslipp i forhold til kontroll for perioden av to uker etter vedlikeholdskalking.

Denne studien viser at total kalking eller "pH-effekt" på N_2O-utslipp under feltforhold kan skjules av den plutselige økningen i mikrobiell aktivitet, og raske næringsomsetningshastigheter som fører til høye N_2O-utslipp. Enger i Norge bør ikke kalkes som metode for å redusere klimagassutslipp fra jord på grunn av økt CO_2-utslipp, som ble ikke oppveid av nedgangen i N_2O-utslipp.

Nøkkelord: Lystgass, kalking, jord pH, nitrifikasjonshemmer, eng, Norge

5 Synopsis

5.1 Introduction

5.1.1 Nitrogen

Nitrogen (N) is an essential element of life on Earth. It is a key component of nucleic acids and deoxyribonucleic acid. Dinitrogen (N_2) makes up 80% of the atmosphere in an "unreactive" stable form in which plants cannot use it. Nitrogen is also a macronutrient in crop production and is often referred to as "the yield element" (Blumenthal et al., 2008). Prior to the invention of up-scaled industrial production of mineral fertilizers via the Haber-Bosch process, N was scarce to farmers. With the invention of the Haber-Bosch process in the early 20th century, industrial fixation of atmospheric N_2 was achieved by using a metal catalyst under high temperatures and pressures, converting it to ammonia (NH_3) by reacting with hydrogen. To this day, the Haber-Bosch process remains a dominant production pathway of mineral N for the use of mineral fertilizers. Besides farming of legumes, the Haber-Bosch process is thought to be responsible for half-world's population today (Sutton et al., 2011) as a consequence of higher yields and increased food availability.

However, due to inefficiencies in the application of N, the amount of reactive nitrogen (Nr) used in the food production is thought to be double the amount required by the crop (Galloway et al., 2003; Galloway et al., 2008; Smil, 1999). These inefficiencies lead to dire environmental consequences such as eutrophication, caused by leaching and run-off of mineral or organic nitrogen, and an increase in climate forcing due to nitrous oxide (N_2O) emissions to the atmosphere.

According to a study by Tian et al. (2020) for the period from 2007 to 2016, the N_2O emissions from additions of organic and synthetic fertilizer applications on agricultural land were responsible for half of the global anthropogenic emissions. The concentration of N_2O in the atmosphere keeps rising and reached in April 2022 value of 335.4 ppb (Figure 1)(Dlugokencky, 2022).

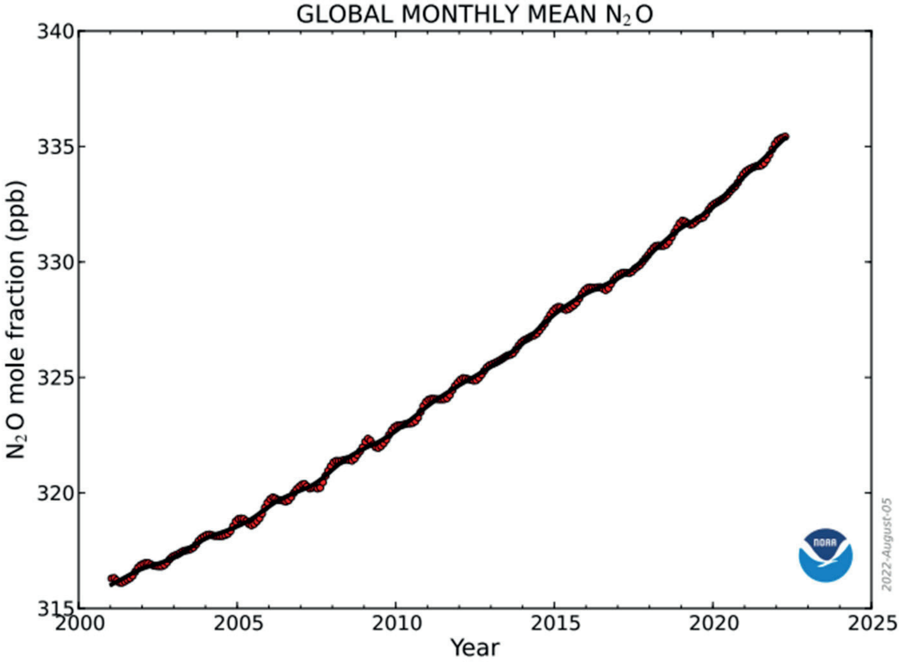

Figure 1. Global monthly mean nitrous oxide (N_2O) emissions from NOAA Global Monitoring Laboratory (Dlugokencky, 2022)

N_2O emissions from anthropogenic activities contribute to 7% of the Earths radiative balance, with 70% being emitted by agriculture (WMO, 2021). As N_2O is more than 265 times more potent at heating the atmosphere than CO_2 (IPCC, 2006), reducing N_2O emissions from Norwegian agriculture would certainly contribute to overall reduction of greenhouse gas effect and reaching aimed emission cuts from agricultural sector.

5.1.2 N_2O production pathways

There is a multitude of processes that lead to N_2O formation in the soil such as autotrophic and heterotrophic nitrification, denitrification, commamox (Daims et al., 2015), and nitrifier-denitrification (Braker & Conrad, 2011; Butterbach-Bahl et al., 2013). However, nitrification and denitrification are the most quantitatively important soil N_2O-producing pathways (Firestone & Davidson, 1989).

5.1.2.1 Nitrification

Nitrification is the microbial oxidation of ammonia/ammonium (NH_3/NH_4^+) to nitrite (NO_2^-) and nitrate (NO_3^-). NH_4^+ is pH and water-dependent in equilibrium with NH_3. Transformations of NH_3 into NO_3^- are done by ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), and nitrite-oxidizing bacteria (NOB). AOA and AOB convert NH_3 to NO_2^-, which is then further oxidized by NOB to NO_3^-. Also, there are AOB (*Nitrospira spec.*) capable of performing a complete NH_3 oxidation (commamox) into NO_3^- without the two-step process of oxidizing NH_3 into NO_2^- and then NO_3^- (Daims et al., 2015). Nitrifying organisms are obligate aerobes, and chemolithoautotrophs as they use CO_2 as their carbon source for biomass growth. During autotrophic nitrification, the conversion of N can be done in two steps by AOB where NH_3 is converted to hydroxylamine (NH_2OH) and then to NO_2^-. This step is mediated by ammonia monooxygenase which can be inhibited by small doses of nitrification inhibitors and are used to increase nitrogen use efficiency (NUE) in agricultural soils (Ruser & Schulz, 2015). AOA oxidize NH_3 to NO_2^- (Schleper & Nicol, 2010) by a different mechanism than proposed for AOB (Kozlowski et al., 2016).

N_2O can be formed during autotrophic nitrification both during the oxidation of NH_2OH and via enzymatic reduction of NO_2^- to NO and subsequently N_2O under partially anaerobic conditions with the latter process termed “nitrifier denitrification”. Autotrophic oxidation of ammonia represents an important role for N_2O emissions by providing substrate for denitrification (another production pathway of N_2O) and depletes O_2 (the oxidation of 1 mol of NH_3 to NO_3^- requires 2 mol O_2) during periods of high nitrifier activity which induces heterotrophic denitrification or coupled nitrification-denitrification (Figure 2).

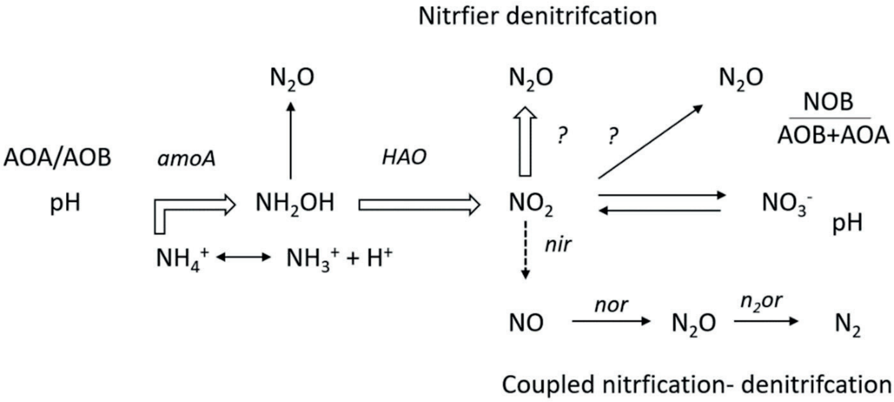

Figure 2. Nitrification process (*Source: Peter Dörsch, lecture*)

In agricultural soils, the rate and extent of the nitrification process is closely linked to the abundance and functional ecotypes of ammonia oxidizers (Norton & Ouyang, 2019), and controlled by environmental factors such as substrate availability NH_4, O_2 concentrations, soil moisture, temperature, and pH.

5.1.2.2 Denitrification

Denitrification is the microbially mediated anaerobic reduction of NO_3^- and NO_2^- to N_2 (Figure 2). Denitrification is thought to be the dominant cause of N_2O emissions from soils (Saggar et al., 2013; Signor & Cerri, 2013) and denitrifiers represent a group of phylogenetically and physiologically heterogeneous microorganisms that can denitrify (Butterbach-Bahl et al., 2013). Heterotrophic denitrification is most common in agricultural soils where denitrifiers use NO_3^- as a terminal electron acceptor during respiration and require organic carbon as their energy source. Denitrification occurs during "hot moments" (rainfall events, freezing and thawing) and in "hot spots"(anoxic microsites in the soil profile) contributing to the high spatial and temporal variability of N_2O emissions in the field making it hard to scale up measured emissions to annual fluxes (Butterbach-Bahl et al., 2013; Groffman, 2012; McClain et al., 2003). Controllers of denitrification activity are anaerobic conditions due to restricted supply of O_2 and active respiration, availability of N oxides: NO_3^-,

NO_2^-, NO or N_2O as terminal electron acceptors, available organic carbon compounds (electron donors) and presence of denitrifiers.

The process of denitrification is controlled by four enzymes in four different stages. The denitrification enzymes that catalyze the sequential reduction of N are: nitrate reductase (*nar*), nitrite reductase (*nir*), nitric oxide reductase (*nor*) and nitrous oxide reductase (*nosZ*). Most denitrifying organisms have all four enzymes but their relative activity may be affected by the community composition and environmental factors (Butterbach-Bahl et al., 2013). Single most pervasive environmental factor to affect the product ratio of denitrification is soil pH, increasing the $N_2O/(N_2O+N_2)$ with decreasing pH, while the denitrification rate increases towards neutral soil pH. This phenomenon was first reported by Wijler and Delwiche (1954), and revisited by many in their laboratory studies (Simek & Cooper, 2002). In addition, soils pH influence on nitrous-oxide reductase (*nosZ*) is thought to be one of factors contributing to soils ability to act as N_2O sink.

5.1.3 pH effect

Soil pH is a master variable affecting physical, chemical and biological activity of the soil (Weil & Brady, 2017) and therefore it is a challenge to explore the direct causal relationship of soil pH under field conditions on N_2O emissions.

Soil pH affects nitrification and denitrification both directly and indirectly. Directly, pH influences nitrification by altering the balance between NH_4^+ and NH_3, pushing the balance towards NH_3 with higher pH. Indirectly, it affects the abundance and phylogenetic diversity of AOBs and AOAs, with AOBs increasing in abundance and activity at higher soil pH (Hink et al., 2018).

During denitrification low soil pH causes the suppression of *nosZ* in the periplasm (Bergaust et al., 2010), lowering the capacity of denitrifying organisms to reduce N_2O (Bakken et al., 2012), soil pH also influences the abundance and phylogenetic diversity of N_2O- reducing microorganisms (Braker et al., 2012; Jones et al., 2014).

Several other factors affect N_2O emissions such as soil texture, soil organic matter content, inorganic N availability, soil moisture and functional microbial community composition (Abdalla et al., 2022; Wessén et al., 2011). Spatial variation of these factors may obscure the pH effect on N_2O emissions. For instance, a central factor for processes producing and consuming N_2O is soil moisture, which greatly depends on soil hydraulic properties influenced by the soils physical structure. Also the rate of pH rise may affect N_2O processes; rapid pH rise by fast-reacting liming agents such as colloidal calcite may boost nitrification and increase available organic C, resulting in a transient increase in N_2O emission (Baggs et al., 2010).

5.1.4 Soil pH management

Liming has been and still is used as a soil conditioner to improve soils biological and chemical properties. In agriculture, liming is used periodically (4-5 years) to counteract excess acidity caused by decomposition of organic matter, leaching of nutrients due to rainfall, fertilization, etc (Yara, 2020). Soil pH is an important soil health indicator because of the importance of pH in governing nutrient availability for crops, it also influences soils ability to transform and decompose certain chemicals and contaminants (Rengel, 2011; Weil & Brady, 2017). Additionally, liming improves the physical structure of the soil due to effects on aggregate formation (Havlin et al., 2014).

By increasing soil pH, nutrient solubility and the availability of macronutrients increases, while plant available heavy metals concentration decrease (Tan, 2011), allowing for optimal plant uptake and generally increased yields (Wang et al., 2021). The influence of liming on yields depends on the type of lime used, crop type, soil type, and fertilization (Holland et al., 2018; Wang et al., 2018). For example, acidic grasslands in Norway, with organic matter content of 0-12% and clay loam texture, have an optimum pH_{H2O} of 5.8 for attaining high yields (Yara, 2020). Liming above this value would not be economically viable and it might even negatively influence the yields (Carran, 1991; Higgins et al., 2012).

Apart from directly influencing soil health and crop productivity, liming was also found to have an effect on reducing N_2O emissions from Swedish forests soil in the 1990s (Klemedtsson et al., 1997). This finding was later applied to agricultural soils where extensive research has continued to this day (Clough et al., 2003; Simek & Cooper, 2002; Zaman et al., 2007). As agricultural production is one of the key contributors to climate forcing it is important that liming effect on N_2O emissions to be scaled to crop yields.

Soils in Scandinavia are predominantly acidic (Fabian et al., 2014) (Figure 3), and liming agricultural soils is a standard practice to mitigate soil acidification. Thus, liming could have as a side-effect, increase the soils capacity to act as a smaller N_2O source by decreasing the product ratio of N_2O/N_2 with increased pH.

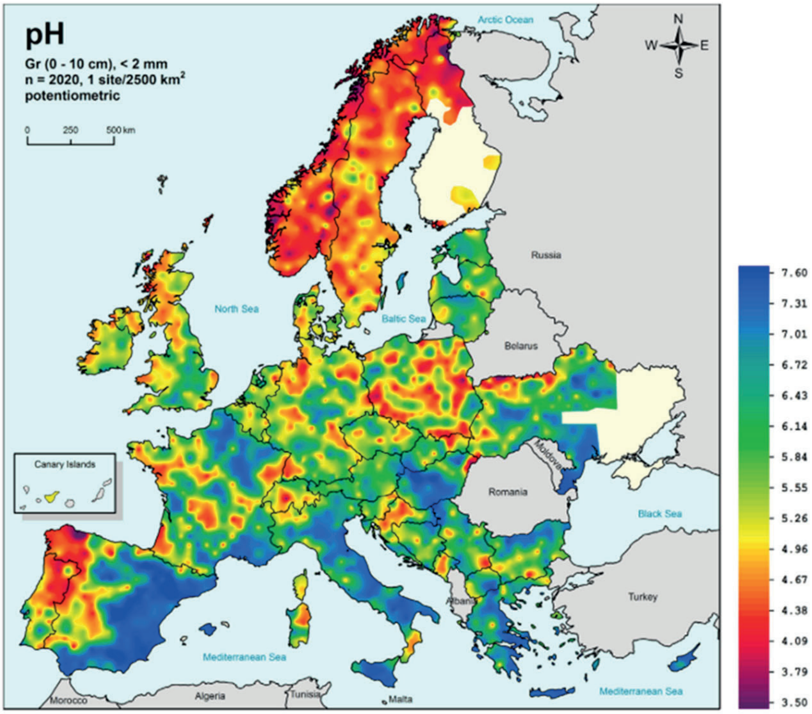

Figure 3. Maps of agricultural and grazing topsoil (0-10 cm) pH ($CaCl_2$) in Europe from GEMAS project (Fabian et al., 2014) using the same color scale. This article was published in Applied Geochemistry, Vol number 48, Fabian C., Reimann C., Fabian K., Birke M., Baritz R. and Haslinger E., GEMAS: Spatial distribution of the pH of European agricultural and grazing land soil, p.207-216, Copyright Elsevier (2014).

Since 2014 the demand for lime has been increasing in Norway (Table 1) and therefore more research is needed to investigate the effect of liming as a management option to mitigate soil N_2O emissions under field conditions. Under the National Program for soil health as proposed by Agricultural Agency in Norway (Landbruksdirektoratet, 2020), liming is referred to as a measure to improve soils physical and biological properties. However, liming overall N_2O mitigation impact might be initially obscured by an initial increase of N_2O (Curtin et al., 1998) and CO_2 emissions after application (IPCC, 2006), due to an initial increase in microbial activity (Han et al., 2011).

In Norway it is common to lime every 5-12 years depending on soil leaching of nitrate (Nesheim, 2014). First application of lime (bulk liming) is usually applied at larger rate, between 30-60 t ha^{-1} of CaO, depending on soil organic matter content and soil texture (Synnes, 2022). Thereafter soil is limed every few years to maintain (maintenance liming) the recommended soil pH_{H2O} (5.8-6, but not above 6.5) for optimal plant growth of a grassland and to ensure soil health. However, to mitigate N_2O emissions, soil target pH might have to be raised above the recommended for optimal plant growth (above pH_{H2O} 6.5). This poses a question how long after bulk liming should maintenance lime be applied if there is a short-term increase in N_2O and CO_2 emissions?

Table 1. Total amount of lime purchased for purpose of agricultural use from 2007 to 2020 by product type in Norway (Mattilsynet, 2021).

Type of product	2007	2009	2010	2011	2014	2017	2020
Ton of product							
Lime powder	22 126	19 491	15 544	12 772	29 555	16 326	20 237
Quicklime	49 498	53 591	52 975	53 359	53 208	48 718	61 267
Coarse dolomite	21 452	18 161	17 250	18 158	26 743	51 076	63 407
Coarse quicklime dolomite	6188	5982	6069	4800	5896	8000	18 468
Burnt lime	1630	1582	6305	1957	1784	3695	2236
Other lime	53 277	56 250	47 045	42 747	37 273	42 701	70 811

Granulated lime	1689	2649	2789	3284	3169	4823	3229
Granulated dolomite					2358	2717	4140
Silicate lime						1390	2161
Sum	**155 860**	**157 706**	**147 977**	**137 077**	**159 986**	**179 446**	**245 955**

Also, if a decrease of N_2O emissions due to decreased N_2O/N_2 product ratio does not off-set the increase in CO_2 emissions from calcareous lime could silicate minerals be a good alternative to the calcareous minerals? Given that the silicate minerals are successful in increasing soils pH. Since 2017 Mattilsynet (2021) reports an increase in demand for silicate lime (Table 1.). Currently, there is a surplus of silicate minerals from the mining industry in Norway which provides an opportunity to test if this surplus could be used as an alternative to calcareous limes. Such silicate materials are reported to counteract acidity (H^+ ions) under laboratory conditions the same way as calcareous limes (Van Noort et al., 2018). However, the effect of siliceous minerals as an alternative to traditional calcareous minerals under the field conditions is still unknown.

While promoting higher pH, liming will increase the AOB/AOA activity and abundance and shift the balance from NH_4^+/NH_3, thus increasing N_2O emissions from nitrification. One way this increase could be mitigated is by recently commercially available nitrification inhibitors that have been becoming more used as a possible solution to inhibit AOBs (Ruser & Schulz, 2015). There have been very few reports on the combined application of lime and nitrification inhibitors. So far this has been studied in acid sugarcane soils (Das et al., 2022) and upland soy-bean Japanese soil (Ikezawa et al., 2022). Application to a grassland soil after liming needs to be tested.
These are the knowledge gaps that this study aims to fill and research objectives of this thesis.

5.1.5 Objectives

The overall objective of this thesis was to test the “pH effect” via liming on N_2O emissions under field conditions and mitigation of N_2O emissions due to stimulated nitrification by using a nitrification inhibitor. The aims of this study are:

1. Field proof of concept of the «pH effect» on N_2O emissions over the course of 2.5 and after 5 years (Paper I and II)

We hypothesised a rapid pH increase from calcareous lime leading to an initial increase in N_2O and CO_2 emissions after application. Later, after the first year, we assumed the increase in N_2O emissions would subside and the pH effect would be visible in all treatments.

2. Test the pH effect of siliceous minerals as an alternative to traditional lime over the course of 2.5 years (Paper I)

We expected that the silicate minerals would slowly weather, gradually increasing soil pH over time. Due to this gradual increase in soils pH, the initial increase in N_2O emissions would not occur.

3. Mitigation of liming-induced N_2O emissions by using 3,4-dimethylpyrazole phosphate (DMPP) nitrification inhibitor (laboratory study) (Paper III)

We hypothesised that the increase in soil pH due to liming, would stimulate N_2O emissions from nitrification. We assumed that the minimum dose of applied 3,4-DMPP would inhibit the increase in N_2O emissions.

To achieve all this, we measured N_2O emissions one year after bulk liming for 2.5 years on a research farm in an intensively managed grassland in south-eastern Norway. We also measured N_2O and CO_2 emissions immediately after maintenance liming, five years after bulk liming application, and conducted a nitrification inhibition laboratory study on soils five years after bulk lime application.

5.2 Materials and Methods

5.2.1 Field site

A field experiment was set up on the research farm of the University of Life Sciences (NMBU) in SE Norway, Ås. The soil is classified as an Albeluvisol (WRB, 2006) (27% clay, 48% silt, 25% sand; pH_{H2O} 5 before liming; total carbon 3%, total nitrogen content 0.28%). This field represents typical farming conditions in Eastern Norway. Albeluvisol is one of the most widespread soil groups on cultivated land in Norway (Sperstad & Nyborg, 2008). From the 1950s until the 1980s the field has been used in crop rotation experiments (Manojlović et al., 2004) and has not been limed since the 1970s, and since the 1990s it has been used for cereal production. Bulk lime was applied in October 2014. The experimental layout consisted of a total of five treatments: calcite, dolomite, olivine, norite, larvikite, and control with four replicates for each treatment distributed in three rows (a total of 24 plots). The application of rock powder was split in two: 15 t ha^{-1} was spread on the surface and then plowed under to 20 cm depth, after which another 15 t ha^{-1} was added and harrowed to 10 cm depth. Rates of application were chosen to achieve soil pH_{H2O} above 6.5. Larvikite, norite, and calcite were byproducts of mining industries and were applied as slurries, while dolomite was granulated, and olivine was applied as a powder (<63 µm). Silicate treatments mineralogy has been described in detail in a study by Van Noort et al. (2018). Soil temperature and volumetric water content were measured by TDR probes (Decagon, ECH2O GS3, 0-10 cm depth, 70-MHz frequency), which were placed in the south and north part of the field. Probes were installed after liming and were removed during fertilization, harvests, and sowing. In late autumn of 2014, winter wheat was sown, but failed to establish and the field was plowed in the spring of 2015. In early June 2015, barley was sown as a cover crop and undersown with a grass mix (20% *Phleum pratense*, 25% *Lolium perenne*, 25% *Festuca pratensis*, 20% *Festuca arundinacea*, 10% *Poa pratense*) and fertilized with 100 kg N ha^{-1} as NPK fertilizer (YaraMila NPK, 22:3:10). There was no harvest in the first year of establishment, and aboveground biomass was mulched in July and October. In spring 2016, urea-N was used, and NPK after the first and second harvest. A third cut occurred in September.

Table 2. Liming materials: composition, producer, grain size and application rate

Treatment	**Dominant mineral***	**Producer**	**Grain size****	**Application**	**CCE (%)*****
Dolomite	$Ca(Mg)CO_3$	Franzenfoss	granulated	24 t ha^{-1****}	109
Calcite	$CaCO_3$	Hustad marmor, Elnesvågen, Norway	Finely dispersed slurry < 0.4-1.5 µm	30 t ha^{-1}	100
Larvikite	32.2% feldspar, ($Na_{0.52}K_{0.29}Ca_{0.19}Al_{1.19}Si_{2.81}O_8$), 24.5% pyroxene ($Mg_{0.24}Fe_{0.30}Ca_{0.46}SiO_3$), 20.7% biotite, 17.5% nepheline(($Na_{0.78}K_{0.22}$)$AlSiO_4$), 2.9% calcite, and 2.2% sphene	Lundhs Real Stone, Larvik, Norway	Slurry < 63 µm	30 t ha^{-1}	47
Norite	65.3% plagioclase ($Na_{0.54}Ca_{0.46}Al_{1.46}Si_{2.54}O_8$), 14.4% pyroxene, 11.4% ilmenite (containing some Mg),7.3% biotite, 1.0% magnetite, and 0.7% periclase	Titania, Hauge i Dalane, Norway	Slurry < 300 µm	30 t ha^{-1}	61
Olivine	95.1% olivine ($Mg_{1.86}Fe_{0.14}SiO_4$), with	Silbeco, Åheim, Norway	Powder < 63 µm	30 t ha^{-1}	65.7

	3.0% orthopyroxene and 1.9% chlorite				

*Minerology and the dissolution rates have been described in a study by Van Noort et al. (2018)

**Particle size as reported by Nadeem et al. (2020)

***CCE Calcium carbonate equivalent or neutralizing value of the material compared to pure (100%) of $CaCO_3$. Calculated as a ratio of molecular weight of $CaCO_3$ and chemical composition of liming treatments (Havlin et al., 2014)

**** Dolomite was applied at a lower rate to adjust for its highest buffering capacity (which is 110% times of $CaCO_3$) to achieve the same targeted pH increase pH($CaCl_2$)= 6.5

In June 2019 original plots of dolomite, calcite and olivine were split into two, with one of each treatment receiving a maintenance lime application of 5 t ha^{-1} and the other none (bulk) (Figure 4). On the same date (24th June 2019) both lime and fertilizer were applied at 100 kg N ha^{-1} (YaraMila NPK, 22:3:10). The same liming products were used in 2019 for maintenance liming as in 2014 for all treatments except calcite, which was applied granulated. For more details see paper II.

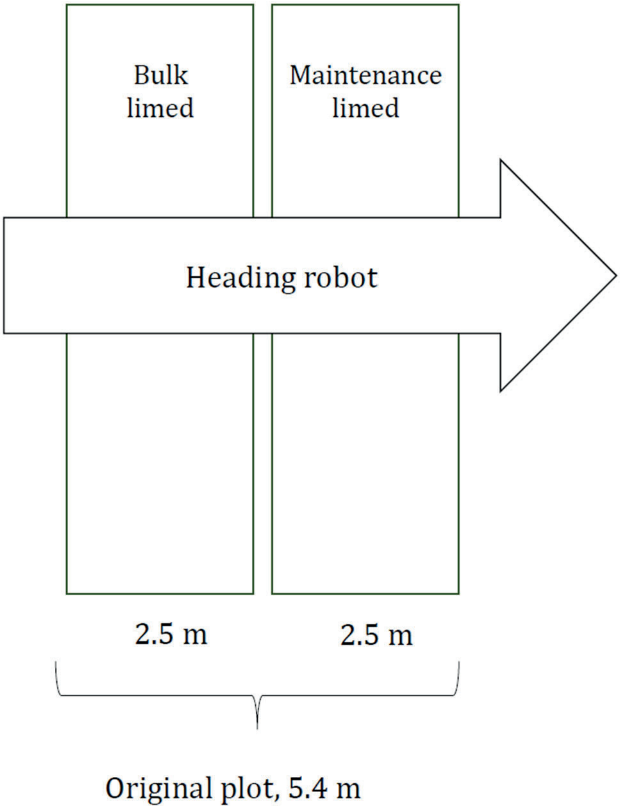

Figure 4. Layout of bulk and maintenance limed plots and orientation of field flux robot's trajectory in the field.

In July 2019, calcite bulk limed, and control (not limed) plots were sampled to 0-15 cm depth by a soil auger, creating a composite sample which was used in the gas kinetics study. For more details see paper III.

5.2.2 Pot experiment

In spring 2017 soil from the field was excavated from 0-25 cm depth, mixed, sieved (6 mm), homogenized, and packed into freely draining pots (10 L, total of 48 pots). To ensure that the bottom soil would be above the drainage hole in the pots, bottom of the pots was filled with a layer of gravel, isolated by a cloth to prevent root ingrowth. Pots were sown with ryegrass and kept outside under ambient conditions, placed on a transparent Plexiglass surface of 1m x 1m size, which was covered underneath with a layer of white plastic material to prevent additional heating. In late October, the soil from the pots was removed and homogenized by hand and fertilized with KNO_3 (equivalent to 50 kg N ha^{-1}) and additional grass (500 g ryegrass m^{-2}) to provoke conditions conducive to denitrification. Thereafter, the pots were measured for N_2O emissions (1st Nov – 15th Dec; 44 days) under natural conditions. On 6th November time domain reflectometry (TDR) soil probes (Decagon, ECH2O GS3) were installed to measure soil water and temperature content in the pots. For more details see paper I.

5.2.3 Soil parameters

Soil temperature and volumetric water content were measured by TDR probes, which were placed in the south and north part of the field. Water filled pore space (WFPS) was calculated as the ratio of volumetric soil water content to total soil porosity, as inferred from the field's average bulk density (Paul, 2015)(Equation 1).

$$\%WFPS = \frac{\text{soil water content x } 100}{1 - \frac{\text{bulk density}}{2.65\ \text{g/cm3}}}$$

Equation 1

The probes were removed during fertilization, harvests and sowing. Precipitation and air temperature data were obtained from the nearby NMBU weather station that is part of the Norwegian meteorological network (MET, 2015-2016; MET, 2019).

Soil pH

Throughout 2015, 2016 and 2019 composite samples for each plot were sampled once or twice a month for measurement of pH both in water and 0.01 M $CaCl_2$ using a Thermo Scientific ROSS Ultra pH/ATC Triode electrode. For more details see paper I and II.

5.2.4 Field N_2O emissions

N_2O and CO_2 emissions were measured using a novel autonomous field flux robot (FFR) designed to record field fluxes using the fast box chamber technique with on-board gas analytics (Fig. 5) (Hensen et al., 2006; Molstad et al., 2014). This method makes it cost effective to measure more frequently and at more points in the field in order to better capture spatial and temporal variability in N_2O fluxes. Measurements were conducted from one to eight times per day. Measurements campaigns are presented in Table 3.

Table 3. Field N_2O and CO_2 campaigns overview. 2019 was the only year in which both N_2O and CO_2 emissions were reported. Other years (2015-2016 field experiment and 2017 pot experiment) only N_2O emissions were presented.

Experiment	Year	Campaign start	Campaign end
Field	2015	20th July	10th Dec
	2016	12th March	12th Dec
	2019	25th June	5th Nov
Pot	2017	3rd Nov	15th Dec

FFR navigates autonomously along predefined waypoints. The robot is powered by a lithium-ion battery pack (2 X 20 V) and consists of: (1) a weatherproof instrument chamber (52 x 140 x 200 cm), (2) high precision real-time kinematic (RTK) GPS achieving centimeter-level precise positioning, (3) an inertial measurement unit (IMU) system, proprietary to Adigo AS.

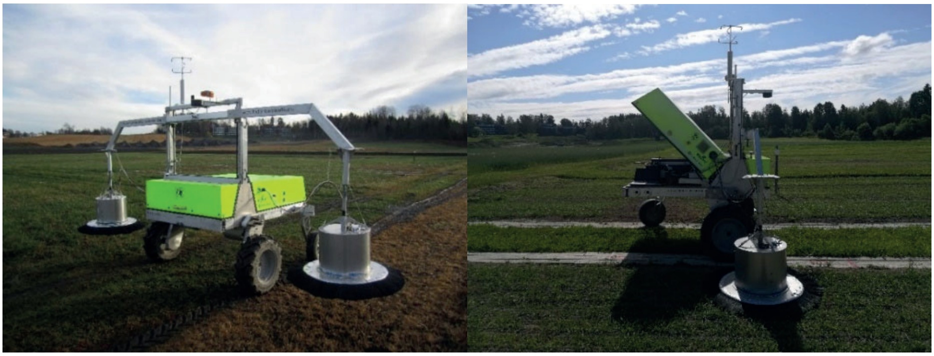

Figure 5. Field flux robot (FFR) (Bakken Lars et al., 2015). This figure shows field flux robot in the field with and without weatherproof instrument chamber being open. A tuneable diode laser spectrometer, CO_2/H_2O infrared gas analyzer and a field laptop are situated in the main body of the robot under a weather proof housing. This picture was borrowed from the Nitrogen groups photos archive.

The weatherproof chamber was equipped with a tuneable diode laser spectrometer (DLT-100, Los Gatos Research, Mountain View, CA, USA) that measures N_2O, CO, and H_2O and a CO_2/H_2O infrared gas analyzer (LI-840A, LI-COR Inc., Lincoln, NE, USA). The robot has an elevator that vertically moves a 0.7 m wide lateral boom at which ends two 100 L aluminium chambers are attached through flexible rubber suspensions. To deploy the chambers for flux measurements, the boom is lowered and presses the chambers on the soil surface. A ring of ribbed foam rubber attached to the base of each chamber ensures close contact with the surface and an additional ring of nylon brush hairs serves as a windbreak. Deployment time is 3 minutes, during which gas circulates through the two chambers and the cuvettes of the two instruments, alternating between the left and right chambers (30 seconds for each), thus providing data for estimation of N_2O and CO_2 fluxes from both chambers. A sonic anemometer fixed to the robot at 2.3 m height delivered continuous wind speed and direction. The minimum concentration increases over the 180-second window to be detected as statistically significant (by linear regression) is approximately 0.2 ppb (equivalent to a flux of 2.3 mg N_2O-N m^2 h^{-1}).

5.2.5 Gas flux calculations

N_2O and CO_2 fluxes were measured at 1 Hz during deployment and were post-processed by a Phyton script to estimate flux rates (Molstad, 2015). CO_2 emissions reported represent instantaneous ecosystem respiration value measured in the dark chambers. The first 6 seconds after each switch between chambers were omitted and 126 seconds were used to find the period of most linear CO_2 accumulation to estimate the N_2O emission rate. Raw data were inspected to identify outliers and altogether 5 % of the measured fluxes were omitted. N_2O and CO_2 fluxes were calculated based on the slope estimates of concentration change over the enclosure time, according to equation 2:

$$N2O\ flux = \frac{dN2O}{dt} * \frac{Vc}{A} * \frac{Mn}{Vm}$$

Equation 2.

where F_{N2O} is the flux (µg N_2O-N m^{-2} h^{-1}), $d[N_2O]/dt$ the rate of N_2O concentration change in the chamber over time (ppmv h^{-1}), V_c the chamber volume (L), A the area covered by the chamber (m^2), M_n the molecular mass of N in N_2O (g mol^{-1}) and V_m the molar volume (L mol^{-1}) at chamber temperature (Tan et al., 2009). V_m was calculated according to the ideal gas law:

$$Vm = R \text{ x } \frac{T_c + 273.15}{P}$$

Equation 3.

Where R is the ideal gas constant, T_c temperature in degrees Celsius, P pressure. Given the short deployment time, chamber temperature was assumed to be equal to air temperature, which was taken from downscaled meteorological data closest in time to the recorded flux estimate and was taken from the Yr database set (https://www.yr.no/nb/historikk/tabell/1-60637/Norge/Viken/Ås/Ås).

Cumulative fluxes were calculated by linear interpolation (i.e., trapezoidal integration) in R studio (gasfluxes package version 0.4-1), R software 4.1.

5.2.6 Yield and yield scaled N_2O emissions

Dry matter yields (g m^{-2}) were reported only for the field experiment in the years where there were harvests.

In the field experiment there were three cuts in 2016: 9th June, 19th July and 8th September. Values presented for the field experiment are sum of each plots replicate (4) average values with standard deviation (±SD) of the three cuts. Surface area of the field experiment was 9.3 m^2.

N_2O-scaled yields were calculated by dividing the cumulative N_2O emissions (mg m-^{2}) with the dry matter yields (g m^{-2}). N_2O emissions were cumulated for each harvest period separately and divided with biomass growth of each corresponding harvest. In the pot experiment, infrequent N_2O measurements did not allow for accurate estimates of cumulative N_2O emissions, or the calculation of yield scaled emissions.

5.2.7 Nitrification gas kinetics

Soil samples of control (not limed) and calcite (bulk limed) plots were sampled in July 2019 to 0-15 cm depth with a soil auger creating a composite sample from four replicates for each of the four plots. Later these samples were used for N_2O production and nitrification inhibition kinetics, in two separate experiments- one in soil slurries and one in packed soils.

In the soil slurries experiment 5 g of fresh-weight soil was suspended in 40 mL NH_4Cl with increasing doses of 3.4-dimethylpyrazole phosphate (DMPP), which were measured twice a day for four days for N_2O production and sampled once a day to assess the effect of DMPP on nitrification. N_2O production was measured by an automated gas kinetics incubation system. Nitrification was measured as a net NO_3^- production, in a nitrification essay form a sub-sample of 1 mL from the soil slurries once per day and were determined by Griess reaction with Vanadium (III) chloride (Doane & Horwáth, 2003) using a microplate reader (Infinite F50, TECAN Austria GmbH) at 540 nm. NH_4^+ was analysed calorimetrically by the sodium salicylate method at 660 nm (Keeney & Nelson, 1982), using the same microplate reader (Infinite F50, TECAN Austria GmbH). Treatments consisted of two controls- one with no DMPP and another with no DMPP + ~1 vol% acetylene (C_2H_2), and a range of four increasing concentrations of DMPP from 0 to 0.27 µg DMPP mL-1. A total of 36 bottles (soil- limed and not limed *3 replicates/treatment (DMPP)+ no DMPP + ~1 vol% C_2H_2). An C_2H_2 control was included to completely inhibit NH_4^+ oxidation by AOB and AOA (Walter et al., 1979). Based on the C_2H_2 controls both nitrification and N_2O production rates were corrected for “heterotrophic nitrification”.

In the packed soils experiment the effective DMPP concentration was higher than in the soil slurries experiment as we assumed the applied DMPP would not evenly diffuse in the entire soil volume. Concentrations of DMPP were in a range from 0 to 6.75 µg DMPP mL^{-1}. The nitrification essay for the packed soil experiment was sampled from 60 mL screw-cap tubes which were sampled 4 times in triplicate in ca. 24 h intervals. All tubes were incubated closed at room temperature (24°C). N_2O production was measured from 120 ml serum bottles, the same as in the soil slurry experiment.

The DMPP concentrations used in these experiments were within the range of maximum inhibitor content legally allowed to be used in agricultural production in Norway (Matdepartementet, 2005).
For more details about this experiment see paper III.

5.2.8 Automated gas kinetics incubation system

Anoxic soil slurries in 120 ml serum flasks were placed in a continuously stirred water bath at 20°C. Prior to gas sampling, slurries were helium washed after which their headspace concentrations were measured for O_2, N_2, N_2O, NO and CO_2 every 2 hours for 4 days using an automated gas kinetics incubation system (Figure 6) as described by Molstad (2007) with modifications.

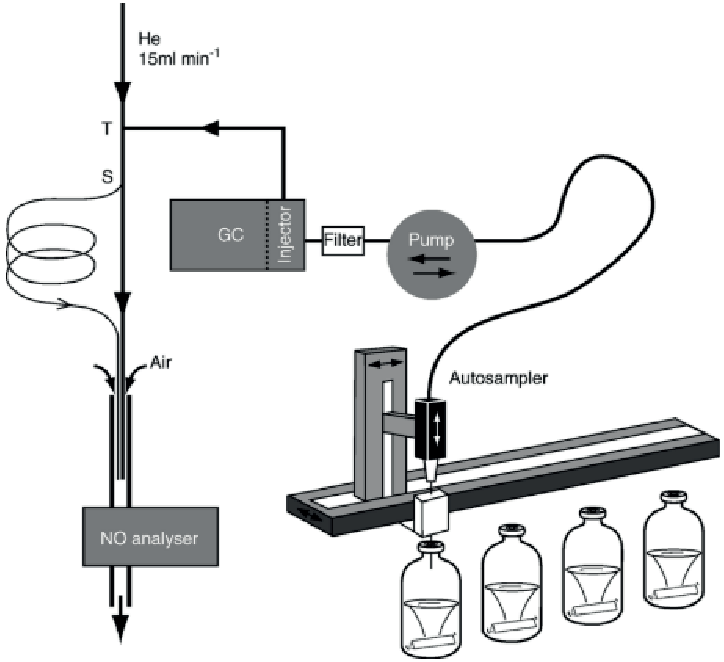

Figure 6. Sampling and gas analysis system (Source: (Molstad, 2007))

The sampling was done by a needle operated by an auto-sampler (CTC PAL). After each sampling, an equivalent of 1 ml of headspace gas is pumped to the gas chromatographer (Model 7890A; Agilent, Santa Clara, CA, USA) by a peristaltic pump (Gilson miniplus3) with Marprene tubing. Headspace was kept constant at ~1 atm by automatically reversing the pump after sampling and backflushing with helium. Dilution by helium and leakage of O_2 and N_2 were evaluated as described by Molstad (2007). After each run, the bottles were placed on a reciprocal shaker (125 rpm).

5.2.9 Statistical analysis

For the field experiment 2015-2016 raw N_2O emissions data were plotted and inspected to identify outliers due to instrument failure. Altogether 5% of the measured fluxes were omitted out of 16 240 flux estimates. All flux rates were ln-transformed and tested for assumptions of a linear relationship and multivariate normal distribution of residuals. An additive linear mixed effect model was used to test for treatments (fixed effects, six levels) on N_2O emissions (Bates et al., 2014).

Variation in N_2O emissions between 24 different plots and between the 209 different sampling days was modelled by means of random effects. Pairwise comparison was used to compare the effects of each treatment to the unlimed control. The same model was used to test differences between liming treatments in the pot experiment. The emission data were adjusted for multiplicity by Bonferroni correction (Bretz et al., 2016; Lenth et al., 2020).

For the 2019 reliming experiment (paper II) a similar additive linear mixed effect model was used to test the effect of maintenance and bulk liming (fixed effects, four and six levels) on N_2O and CO_2 emissions. Variation in N_2O emissions between 36 different plots and between the 41 different sampling days was modelled by means of random effects. Pairwise comparison was used to compare the effects of each treatment to each pair (e.g., calcite bulk relative to calcite maintenance limed, etc) and control (not limed).

The proportion of the random effect variance attributable to each random effect was found by dividing each random effect by the sum of all random effects (i.e., the total variance of the random effects).

Differences in soil pH were tested with a generalised linear model (GLM) with soil pH as a response variable and liming treatments as the predictor. The pH effect on cumulative N_2O emissions was tested by performing an ANOVA.

Differences between treatments in yields and N_2O-scaled yields were also tested using an ANOVA.

To test differences of 3.4-DMPP amended nitrification rates and N_2O emissions between calcite and control a linear regression model was used with nitrification and N_2O being the response variable and liming treatment as a predictor using the lm function in R. All statistics were performed by R software, 4.1.2. R stats package (Hastie & Chambers, 1992), 4.0.2 package MASS (Venables & Ripley, 2002) for GLM and lme4 (Bates et al., 2014) for linear mixed-effects model.

5.3 Results and discussion

5.3.1 Soil pH (Paper I and II)

Liming with calcareous materials (calcite and dolomite) was most successful in raising soil pH (Table 4) compared to the control ($p<0.01$). Silicate minerals did not raise soil pH to the targeted $pH_{(CaCl2)}$ value (6.5) and were more similar in their pH value to that of the non-limed control. Measuring soil pH in $CaCl_2$ is preferred over measurements done in water as it gives a more consistent measurement (Minasny et al., 2011). However, the pH measured in water is a standard for measuring soil pH in Norway (Landbruksdirektoratet, 2020). The differences in values between two methods were significant ($p<0.01$) and were within the range of 0.2-0.5 units lower for $CaCl_2$ (Lake, 2000).

Table 4. Average topsoil (0-15 cm) pH measured in $CaCl_2$ and H_2O from March 2015 to October 2016 for all five treatments (plus control) with SD (n=4), and average for three treatments and control with SD (n=4) from June to September 2019. Calcite and dolomite had significantly increased soils pH ($p<0.01$) relative to the control for all three years. Silicate minerals were not successful in increasing soils pH ($p>0.05$). Samples measured in H_2O were significantly higher in value than samples measured in $CaCl_2$ only for control and olivine ($p<0.01$).

Soil pH ($CaCl_2$)			
Year	2015*	2016**	2019***
Control	5.1 ± 0.2	4.8 ± 0.3	4.9 ± 0.5
Calcite	6.6 ± 0.3	6.3± 0.1	6.5 ± 0.5
Dolomite	5.6 ± 0.3	5.3 ± 0.3	5.8 ± 0.6
Olivine	5.2 ± 0.2	4.9 ± 0.3	5.3 ± 0.6
Larvikite	5.1 ± 0.2	4.9 ± 0.4	
Norite	5.2 ± 0.2	4.9 ± 0.3	
Soil pH (H_2O)			
Year	2015	2016	2019
Control	5.5 ± 0.2	4.9 ± 0.3	5.6 ± 0.5
Calcite	6.9 ± 0.1	6.5 ± 0.2	6.9 ± 0.3
Dolomite	6.1 ± 0.2	5.7 ± 0.4	6.3 ± 0.5

Olivine	5.6 ± 0.2	5.2 ± 0.4	6.1 ± 0.5
Larvikite	5.4 ± 0.2	4.9 ± 0.4	
Norite	5.5 ± 0.3	5.1 ± 0.4	

*Yearly average of nine measurements from March 2015 to October 2015

**Yearly average of four measurements from May 2016 to September 2016

***Yearly average of six measurements from June 2019 to September 2019

We assume this lack of increase in soil pH by silicate minerals is due to their inferior Ca^{2+} and Mg^{2+} base cations content in the material (Table 2), compared to that of calcareous minerals (calcite and dolomite). Even though olivine showed a slightly higher neutralizing capacity than the rest of the siliceous minerals, its wider application as a liming agent might be questioned due to nickel accumulation in the soil, since it has the highest concentration of Ni of all three silicate treatments (Van Noort et al., 2018). Thus, olivine application would have a net negative environmental effect (Cressey, 2014).

5.3.2 Field emissions (Paper I)

The highest field N_2O emission fluxes during 2015-2016 were either fertilization triggered after rainfall or freezing and thawing induced (Figure 7). This finding agrees well with previous studies in intensively managed grasslands (Dobbie & Smith, 2003) and for croplands in boreal climate (Wagner-Riddle et al., 2017; Wallman et al., 2022). Prior to liming, N_2O and CO_2 emissions were not measured in our field experiment. This was a short coming of our field experiment as it did not allow us to compare emissions before and after liming which may have allowed us to identify possible field heterogeneity. Although our approach appears to be standard practice for this type of study.

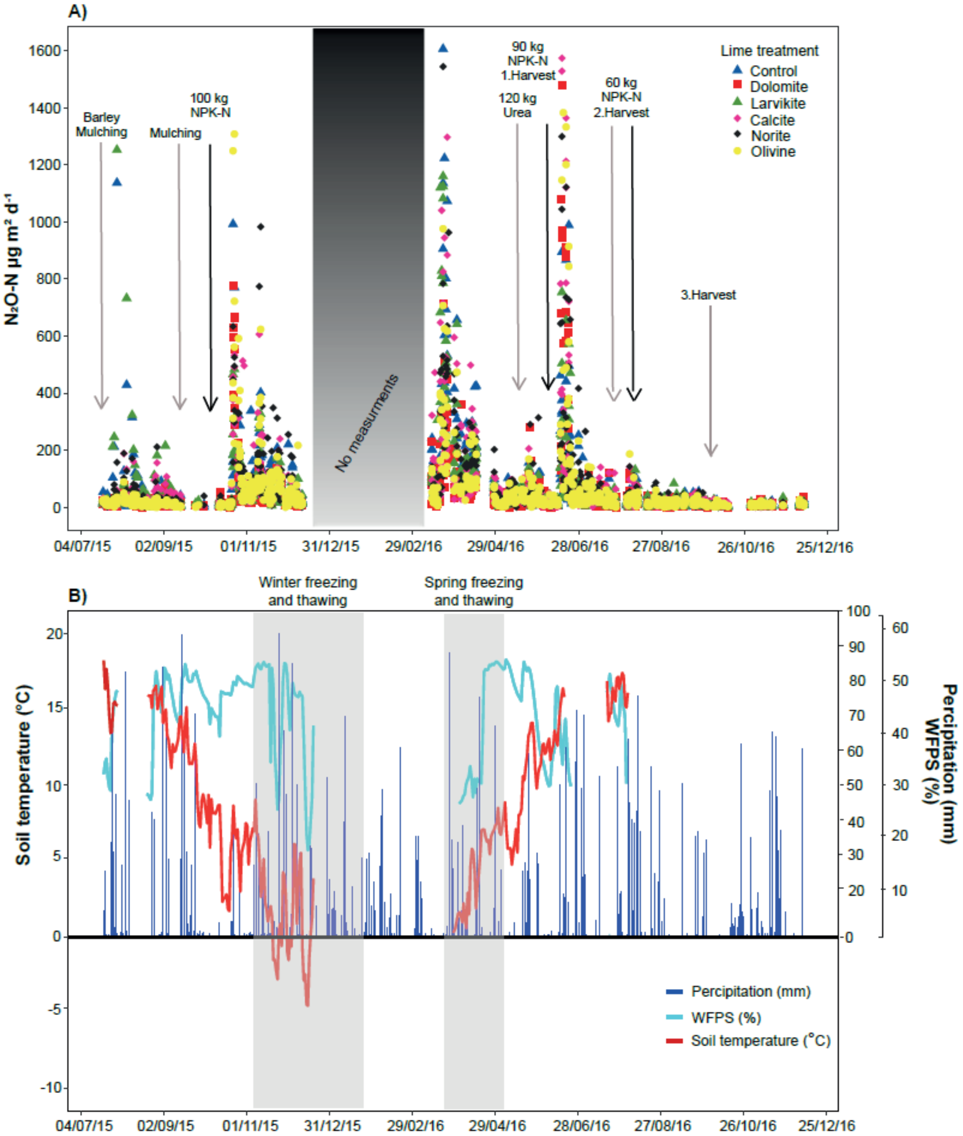

Figure 7. A) Daily average N_2O-N emissions (μg m^2 d^1) from July to December 2015 and March to December 2016. Grey colour represents no measurements from 15th December 2015 to 14th March 2016. During 2016 there were periodic but random gaps in measurement days. Each treatment had four replicates (plots) and measurements were taken daily varying in frequency between one and eight times a day. B) Average daily soil temperature, average daily water filled pore space measured by TDR soil probes and daily precipitation acquired from nearby weather

station. High fluctuation of hourly soil temperatures during spring thaw in 2016 do not depict freezing temperatures when averaged to a daily temperature.

We did not observe the expected linear relationship between soil pH and field annual N_2O emissions, as we assumed we would based on previous studies on pH effects on denitrification and soil N_2O emissions (Simek & Cooper, 2002; Wijler & Delwiche, 1954). Nevertheless, N_2O emissions from dolomite treated plots were significantly lower than that of the control ($p<0.05$; Table 5).

Table 5. Parameter estimates of a linear mixed effects model for ln-transformed mean N_2O emissions ($\mu g\ N_2O\text{-}N\ m^2\ d^{-1}$) in the field experiment for fixed (treatment) and random effects (plots, day number). Each treatment had four replicates (plots). P values indicate differences between treatments and control (not limed). Hourly fluxes were averaged to daily flux estimates for each plot number for the period from 20th July 2015 to 15th December 2016 (total of 13 035 flux estimates). Only dolomite had lower N_2O emissions relative to the control ($p<0.05$).

2015 – 2016				
Fixed effects Treatment	**Estimate ($\mu g\ m^2\ d^{-1}$)**	**N_2O-N emissions P value ($p< 0.05$)**	**Random effects**	**Variance**
Dolomite	-0.28 ± 0.07	0.03	day number	1.28
Larvikite	0.05 ± 0.35	0.85	plot number	0.03
Calcite	-0.04 ± 0.54	0.67	residuals	0.62
Norite	0.10 ± 0.27	0.38		
Olivine	-0.06 ± 0.51	0.64		

Other treatments did not significantly affect N_2O emissions. When examining cumulative N_2O field emissions for different seasons, a similar trend is observed with dolomite treatment having smaller N_2O emissions compared to control (Figure 8), but this difference was not significant. Other treatments did not significantly affect N_2O emissions. Olivine had smaller emissions than the control during the first summer (Figure 8 A), but this difference was not significant. Overall, silicious minerals did not increase soil pH or decrease N_2O emissions sufficiently to be justified as an alternative to traditional limes (dolomite, calcite).

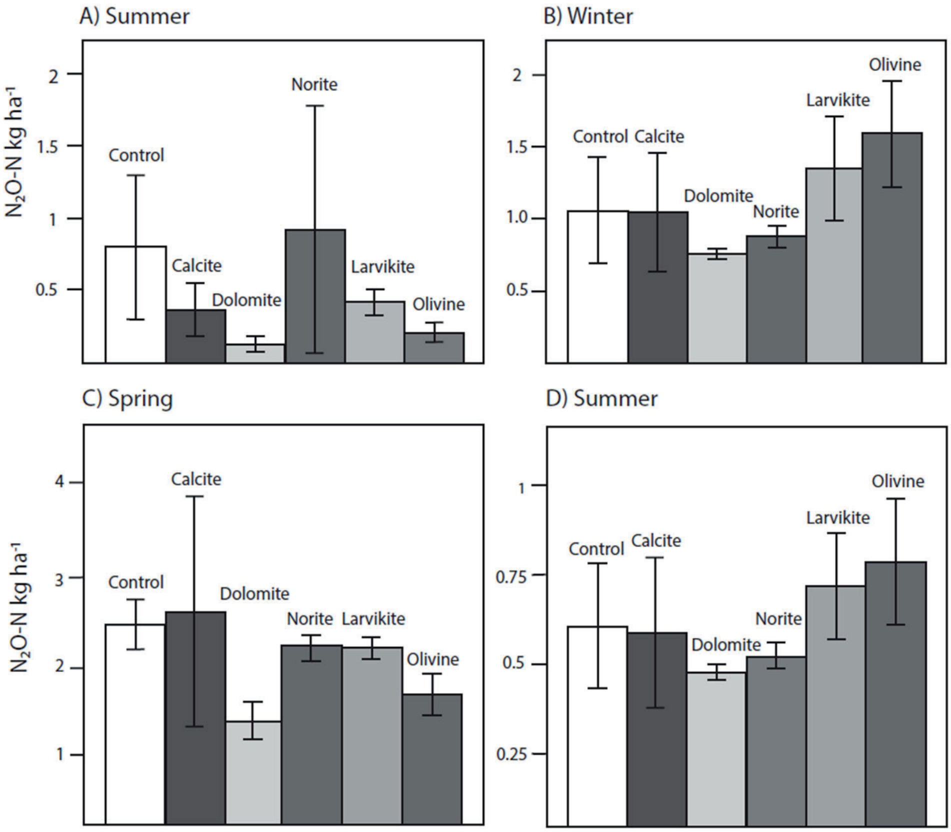

Figure 8. Cumulative N_2O emissions (N kg ha^{-1} $period^{-1}$) in the field experiment: A) growing period 2015 (20th July to 14th September 2015); B) Fall 2015 (20th October to 20th November 2015); C) Spring thaw 2016 (14th March to 15th April 2016); D) Growing-season 2016 (20th July to 15th September 2016). Error bars are standard errors (N=4). Each treatment had four replicates (plots). None of the treatment differences were significant.

We assume that the absence of a clear "pH effect" on field N_2O emissions may be explained by increased microbial activity, priming, and net turnover rates of nitrogen that occurred as a consequence of lime addition to a field that has not been limed for four decades. As the soil pH rises, so does the solubility and availability of nutrients (Tan, 2011), and as soil organic carbon and mineral nitrogen become more available for the microbial population (Simek & Cooper, 2002), both nitrifiers and denitrifiers, have beneficial conditions for growth, their abundance and phylogenetic diversity keeps increasing with the increase in soil pH (Hink et al., 2018; Jones et al., 2014), possibly leading to higher N_2O emissions due to overstimulation of microbial growth. In addition, under field conditions, temporal dynamics of denitrification and nitrification and spatial variation of O_2 availability may have also overridden the pH effect.

We used the pot experiment, under semi-field conditions, to test if by reducing the spatial variability of the field under extremely denitrification conducive conditions the pH effect would occur. In the pot experiment, we did find a clear pH effect on cumulative N_2O emissions (Figure 9), with the highest pH attained by calcite experiencing the lowest N_2O emissions over 44 days. We believe this clear pH effect in the pot experiment could be explained by extremely denitrification-conducive conditions of this experiment and reduced spatial variability of soil moisture, due to free drainage on the bottom of the pots.

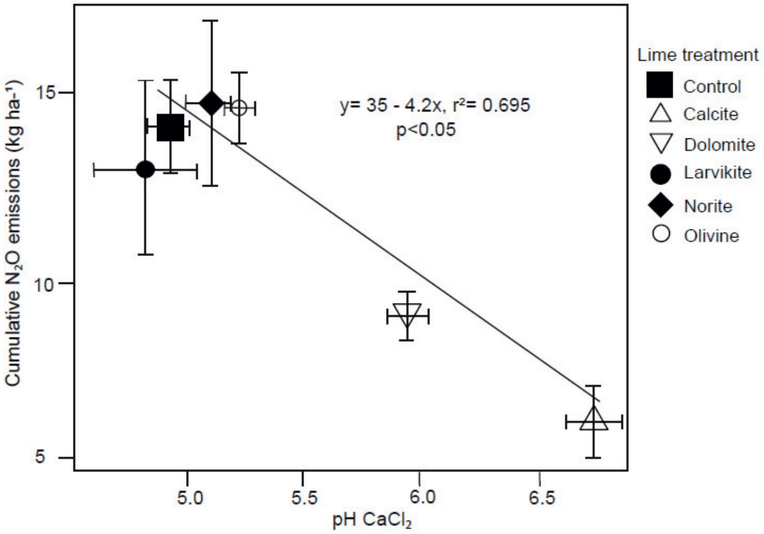

Figure 9. Relationship between cumulative N_2O emissions and measured soil pH in the pot trial. Shown are average cumulative N_2O-N fluxes in kg ha^{-1} over a period of 44 days (1st November to 15th December 2017) plotted over average soil pH in 0.01 M $CaCl_2$ with standard errors for both variables. Error bars are standard errors (N=4). Soil pH was measured on 30th October, and each treatment had 4 replicates. N_2O

emissions were measured from 1st November to 15th December and had total of 2681 flux estimates, each treatment had 4 pots/replicates.

5.3.3 Effect of liming on yields

None of the liming treatments had an effect on the yields relative to control (Table 6). These findings are in accordance with the results from studies by Bleken and Rittl (2022) and Žurovec et al. (2021) done on grasslands. In a study by Bleken and Rittl (2022) done on the same field as ours, they report annual average biomass yields from 2016-2019 with no increase in yields from dolomite limed plots relative to control. Bleken and Rittl (2022) concluded that the positive correlation between N_2O emissions and average annual yields, was due to overall increased microbial activity. In a meta-study by Wang et al. (2021) many studies were found where there was no increase in yield. One possible explanation could be that the field was limed above the recommended rate for optimal plant growth and thus there was no increase in yields. The yield scaled N_2O-emissions for all treatments were lower compared to the control in the field experiment (Table 6), but only the dolomite treatment was significantly different. Similar results were reported in a study by Wang et al. (2021) where they reported a significant reduction in yield scaled N_2O-emissions as the yields increased and N_2O emissions decreased after liming. However, in our study, the lower N_2O emissions were probably the primary cause of the lower yield scaled N_2O-emissions. It is important to report yield scaled N_2O-emissions in terms of liming, because liming as a pH management and N_2O mitigation tool should support food production and crop productivity (Kim et al., 2023).

Table 6. Dry matter yields (g m^{-2}) and yield scaled N_2O emissions. In the field experiment there were three cuts only in 2016: 9th June, 19th July and 8th September. Values presented for dry matter yields are the sum of each plot replicate (4) average values with standard deviation (±SD) of the three cuts. Yield scaled N_2O emissions represent cumulative N_2O-N mg m^2 divided with dry matter yields for each harvests period:8th May- 8th June, 8th June- 18th July and 18th July - 7th September 2016. All treatments had higher yields compared to the control, but none of these differences were significant ($p>0.05$). Dolomite had significantly lower N_2O-yield scaled emissions relative to control ($p<0.01$).

Treatment	Field 2016	
	Dry matter yield g m^{-2}	Yield-scaled N_2O emissions mg N g^{-1}
Control	1590 ± 159	0.5 ±0.6
Calcite	1671 ± 50	0.4 ±0.6
Dolomite	1676 ±26	0.2 ±0.3*
Larvikite	1704 ± 69	0.3 ±0.4
Norite	1686 ± 77	0.3 ±0.4
Olivine	1711 ± 52	0.3 ±0.4

5.3.4 Effect of liming on nitrification potential (Paper III)

We expected that liming would increase nitrification rates (Nadeem et al., 2020), and this hypothesis was confirmed in our laboratory study (Figure 10) where calcite limed packed soil and slurries demonstrated a ~50% increase in nitrification rate compared to control ($p<0.01$) five years after applying bulk lime. In the soil slurries incubation experiment nitrification rates were higher than in packed soils ($p<0.01$).

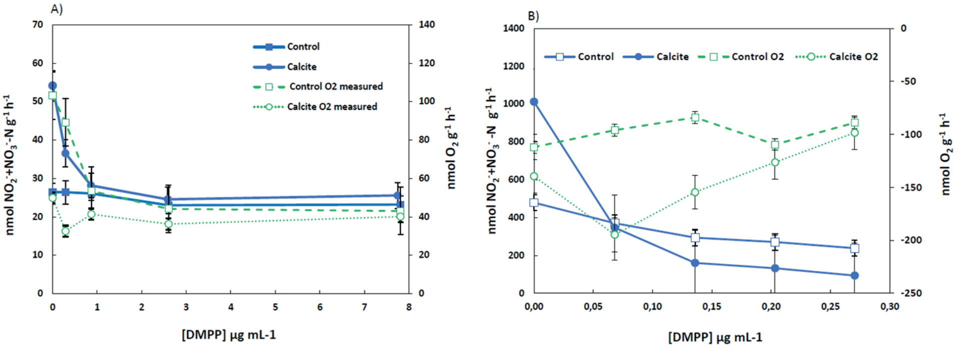

Figure 10. Nitrification rates and O_2 consumption rates measured over the course of 4 days expressed in nmol g^{-1} h^{-1} were measured from control (not limed) and calcite (bulk limed) in packed soil (A) and soil slurries (B). Note y-axis. Each treatment had four replicates (plots). Error bars are standard error (N=4). Differences between treatments in nitrification rates and oxygen consumption rates for both packed soil and soil slurries were significantly higher for limed soils ($p<0.01$).

DMPP decreased the nitrification rate in limed soil and this could mean that our soil is AOB dominated soil, as DMPP only works in AOB dominated soils (Gong et al., 2013). We believe that liming has increased AOBs abundance and activity and NH_3 availability, which is in agreement with observations by Nadeem et al. (2020) (Figure 10).

In respect to N_2O production, lowest dose of DMPP (0.28 µg mL-1) successfully inhibited N_2O production in calcite (bulk limed) to levels lower than of control (not limed) in packed soil (Figure 11 A). These results are similar to the findings of Das et al. (2022) who found that liming and DMPP combined reduced N_2O emissions by 90% from acid sugarcane soil. In soil slurries, higher dose of DMPP was needed to reduce the increased N_2O from calcite soil (Figure 11 B).

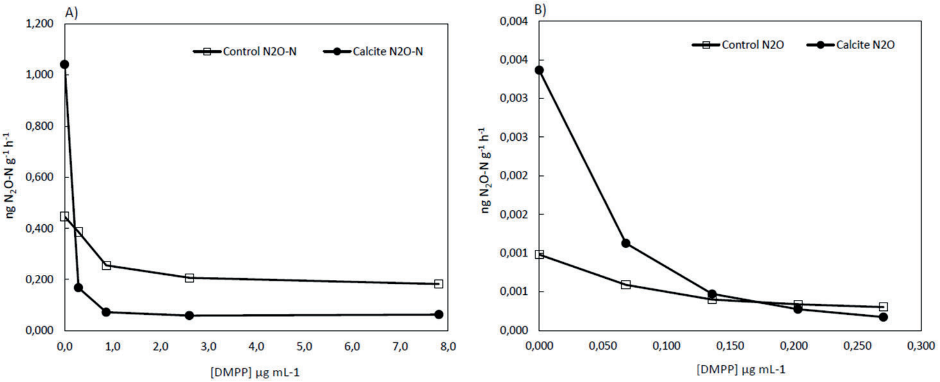

Figure 11. Response of N_2O production in packed soil (A) and soil slurries (B) for control (not limed) and calcite (bulk limed) at 60% WFPS measured over the course of 4 days. DMPP dose is expressed as µg mL-1. Note the different scaling of y-axis. Each treatment had four replicates (plots). Error bars are standard error (N=4). Differences between treatments in N_2O production for both packed soil and soil slurries were significantly higher for limed soils ($p<0.01$).

In soil slurries, N_2O production from limed and control (not limed) was successfully inhibited by 0.13 µg mL-1 of DMPP (Figure 11 A). This might mean that increase in N_2O due to increased nitrification after liming is and can be reduced by nitrification inhibitors in grassland soils that are dominated by AOBs.

A recent field study done by Tariq et al. (2022) investigated the mitigation potential of DMPP on N_2O emissions over a period of four months (less than one growing season) on arable soil. They concluded that DMPP did not increase the yields, but that the high N_2O emissions were successfully inhibited by the DMPP relative to the control. However, they point out that their study was limited only to one growing season and should be repeated over at least two years period including both growing and off-season.

Additionally, although DMPP is one of most used nitrification inhibitors in Europe, due to its efficiency and low toxicity (Zerulla et al., 2001) recovery of AOBs after decomposition of DMPP in the soil should be investigated and reported with threshold concentrations for various soils to avoid irrevocable inhibition of useful and important microorganisms (e.i. AOBs) (Benckiser et al., 2013).

5.3.5 Effect of maintenance liming on N_2O and CO_2 emissions (paper II)

Overall maintenance liming did not reduce N_2O emissions relative to bulk treatment over the course of 4.5 months although it did have lower N_2O emissions compared to the control (not limed), but none of these differences were significant (Table 7). However, lower emissions from bulk limed plots were significantly different from each other (difference within group calcite/dolomite/olivine, table 7). Most of variation in daily N_2O emissions over the course 4.5 months was explained by day-to-day flux variation (43%) and by plot number (13%). A lot of variation in N_2O emissions was left unexplained (43%).

Table 7. Parameter estimates of a linear mixed effects model for average daily N_2O-N emissions for fixed (treatment) and random effects (plots, day number). N_2O emissions were measured three to five times a week over a period of 4.5 months (25th June-5th November 2019, 2606 flux estimates). Each treatment had 4 replicates (plots). All treatments had lower N_2O emissions that the control, but this difference was only significant for the bulk treatment ($p<0.01$).

Fixed effects Treatment	**Coefficients estimates with standard error ($\mu g\ m^2\ d^{-1}$)**	**N_2O-N emissions P value ($p< 0.05$)**	**Random effects**	**Variance**
Control	5.28 ± 0.21		day number	0.77
Dolomite *maintenance*	4.61 ± 0.29	0.15	plot number	0.25
Calcite *maintenance*	4.55 ± 0.41	0.51	residuals	0.77
Olivine *maintenance*	4.75 ± 0.42	0.27		
Treatment *maintenance*	1.22 ± 0.59	0.91		
Dolomite *bulk*	4.45 ± 0.25	<0.01		
Calcite *bulk*	4.39 ± 0.25	<0.01		
Olivine *bulk*	4.59 ± 0.26	<0.01		

Pairwise comparison between bulk and maintenance liming revealed that there were no significant differences between treatments. Again, bulk limed treatments were significantly different from each other (Table 8).

Table 8. Pairwise parameter estimates of a linear mixed effects model for average daily N_2O-N emissions for fixed (treatments without the control, relative to each other- maintenance vs. bulk) and random effects (plots, day number). Same N_2O measuring period (25th June to 5th November 2019) and number of replicates (4) as mentioned in Table 7, just without the control. Bulk limed plots had lower N_2O emissions than maintenance limed, and this difference was not significant in each pairwise comparison.

Fixed effects **Treatment**	**Coefficients estimates with standard error** **(μg m^2 d^{-1})**	**N_2O-N emissions** **P value** **($p < 0.01$)**	**Random effects**	**Variance**
Dolomite *maintenance*	4.70 ± 0.20		day number	0.80
Calcite *maintenance*	4.57 ± 0.21		plot number	0.28
Olivine *maintenance*	4.77 ± 0.17		residuals	0.82
Dolomite *bulk*	4.54 ± 0.20	<0.01		
Calcite *bulk*	4.41 ± 0.21	<0.01		
Olivine *bulk*	4.61 ± 0.21	<0.01		
Treatment *maintenance*	0.16	0.31		

All emission peaks during the period of the first 14 days after liming were rainfall provoked. All treatments had lower N_2O-N emissions compared to control ($p<0.01$) during the period of the first 14 days and all treatments had an increase in CO_2

emissions compared to control, but this was only significant for calcite treatment ($p<0.01$). Similar results were obtained in a study by Aye et al. (2017) where they reported the highest CO_2 emissions from limed soils with the highest pH.

In terms of CO_2- equivalents the lower N_2O emissions were not large enough to offset the increased CO_2 emissions (Table 9). The magnitude of the increase in CO_2 emissions suggests that this increase may be due to stimulated decomposition of soil organic matter in limed soil (Grover et al., 2021) rather than solely the dissolution of carbonates (IPCC, 2006).

Grover et al. (2021) found in their study that limed arid and semi-arid soils have significantly higher turnover of newly added organic matter relative to not limed soil, and that the decomposition of extant soil organic carbon and decomposition of added crop residue were both positively correlated with soil pH. Aye et al. (2017) also reported a greater priming effect in soils that had a longer (35 years) liming history than recently limed (after 6 years) soils.

Based on this study findings and previous studies (Aye et al., 2017; Grover et al., 2021) this would mean that maintenance liming should be reapplied in periods longer than 5 years, to avoid negative effects on greenhouse gas (GHG) balance.

Table 9. Cumulative N_2O-N emissions expressed in CO_2 equivalents and CO_2-C emissions in kg m^2 over 14 days (24th June to 8th July 2019) for all treatments (no. flux estimates 600). Each treatment had four replicates. All treatments had an increase in CO_2 emissions relative to the control, but this was only significant for calcite maintenance ($p<0.01$). None of the treatments were able to off-set the increase in CO_2 emissions by lower N_2O emissions. Cumulative N_2O-N emissions were converted into CO_2 equivalents by multiplying N_2O-N emissions with 265 GWP (IPCC, 2006)

Lime type	Treatment	N_2O-N g m^2	N_2O in CO_2 eq. kg m^2	ΔN_2O (As CO_2 eq.) kg m^2	CO_2-C kg m^2	Δ CO_2 kg m^2	ΔCO_2 + ΔN_2O as CO_2 eq.* kg m^2
Control		1.19	0.49		816.16		
Calcite	Bulk	0.33	0.12	-0.37	1077.00	260.84	259.63
Calcite	Maintenance	0.35	0.14	-0.35	1133.11	316.95	315.65
Dolomite	Bulk	0.61	0.25	-0.24	1090.11	273.95	273.71
Dolomite	Maintenance	0.55	0.23	-0.27	1051.07	234.91	234.64
Olivine	Bulk	1.70	0.71	0.21	897.43	81.27	81.05
Olivine	Maintenance	1.44	0.59	0.10	820.86	4.7	4.8

Overall, liming effect on N_2O field emissions varied over the course of five years (2015-2019), with the highest emission peaks after fertilization after rainfall and freezing and thawing events. The pH effect-linear relationship between high pH and low N_2O emissions was only visible in the 2017 pot experiment. The same treatment effects were observed in all three years- with the lowest N_2O emissions in summer obtained by dolomite, calcite, and then olivine. The laboratory study shows that liming increased the nitrification rate, and thus possibly explains the increase in field N_2O field emissions after liming even after five years since bulk application. The magnitude of the increase in CO_2 emissions suggests an overall increase in priming and mineralization of limed soil relative to not limed.

5.4 Implications of the study

Currently, there is an increasing interest in liming globally, as in Norway, as a possible alternative and temporary measure to the shortage and recent high prices of mineral fertilizers. Increasing soils pH improves the sustainability of crop production and it may also act as a possible N_2O mitigating tool. Positive aspects of using lime are numerous and since there is an increasing trend in demand for lime due to frequent fertilizer use lowering arable soil pH, more studies on the long-term effect of liming are needed. One of the few questions that arise from this study; is liming a greenhouse gas source or a greenhouse gas sink? In a meta-study by Wang et al. (2021) it was concluded that liming is an overall GHG benefit due to positive effects on SOC stocks. However, studies summarized in Wang et al. had certain limitations as they reported GHG fluxes for only one or two growing seasons. Observed simultaneous increase in CO_2 and decrease in N_2O field emissions found in our two-week study would indicate that our soil is a greenhouse gas source. We also draw our conclusions based on a limited time-period (i.e. two weeks campaign), and therefore there is a need for a multi-year CO_2 and N_2O monitoring campaigns on fallow soil with investigations on SOC stock changes as well to test this. If the CO_2 increase is due to microbial activity, this might lead to an amendment of the 2006 IPCC's guidelines and calculation of emission factor (EF) from liming.

Another important question is the frequency of liming, or how long after applying bulk lime should one apply maintenance lime. In Norway it is common to lime the soil every 5-12 years, depending on the degree of soil leaching. As found in our study, liming has mitigative potential for N_2O emissions even after 5 years. Thus, it is important to find the optimum balance of reducing N_2O emissions and leveling off of CO_2 emissions by acclimatization of the microbial community to the existing pH, after liming.

Lastly, the question of using nitrification inhibitor(s) (3.4-DMPP) in the field as a possible solution to increased nitrification N_2O emissions in limed soils supporting high AOB/AOA. The Norwegian agricultural market seems still unfamiliar with nitrification inhibitors as a lone product. So far, they have been in use in some turf fields here in Norway, in form of slow-release NH_4 fertilizers. However, it might be the case that the use of nitrification inhibitors is not justified as a cost-efficiently soil management and N_2O mitigation measure, if soil is not being limed over $pH_{H2O}=6.5$, as N_2O emissions below this pH are believed to be denitrification dominated.

DMPP has been documented as an efficient and biodegradable nitrification inhibitor, however their application in the field, with their long-term effect on the active nitrifiers community, remains missing. This knowledge gap should be filled before recommending the wide use of such product commercially.

5.5 Conclusion

Siliceous minerals were not successful at achieving the desired soil pH increase, which might be due to their lower base cations content, and it might be that the application rates should have been higher to achieve the neutralizing effect like calcite. In addition, they did not show any clear trend in relation to N_2O emissions (except olivine), and as such they would not be recommended as an alternative to traditional calcareous liming treatments. Olivine should not be used due to its higher Ni content.

Calcite and dolomite on the other hand, were successful in increasing soils pH compared to control (unlimed soil). In relation to N_2O emissions, this soil pH increase at first did not translate into a negative linear relationship in the field experiment. However, by reducing the spatial variability of the field, and heavily inducing denitrification-conducive conditions in the pot experiment, this negative linear relationship between soil pH and N_2O emissions was achieved. With the highest pH obtaining the lowest N_2O emissions and vice versa.

With successfully increased soil pH, the nitrification potential of the soil increased as well, almost doubling 5 years after the bulk liming, and in turn, increased nitrification-derived N_2O emissions. This increase in nitrification rates 5 years after bulk liming, and lower N_2O emissions compared to maintenance liming, poses a question of repeated liming; how often maintenance liming should be applied. This study would suggest after more than 5 years, in order to not overstimulate N_2O emissions but again long-term measurements are needed.

Nevertheless, the increase in nitrification-derived N_2O emissions caused by liming, was successfully inhibited under laboratory conditions by using 3.4-DMPP nitrification inhibitor from calcite-limed soil. That suggests a high AOB/AOA ratio. However, this should be tested in the field.

Overall, liming of agricultural soils is an important and standard practice to counteract soil acidification, and is necessary to secure optimal soil health and crop productivity. However, liming would not be recommended as a management option to mitigate soil GHG emissions as the increase in the CO_2 emissions from calcareous material were not off-set by the decrease of N_2O emissions. This was concluded on a two-week measurement campaign and thus should be tested in a long-term field experiment on fallow soil along with investigations on SOC stock changes.

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Paper I

Effect of calcareous and siliceous amendments on N_2O emissions of a grassland soil

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Abstract

Liming of acidic agricultural soils has been proposed as a strategy to mitigate nitrous oxide (N_2O) emissions, as increased soil pH reduces the N_2O/N_2 product ratio of denitrification. The capacity of different calcareous (calcite and dolomite) and siliceous minerals to increase soil pH and reduce N_2O emissions was assessed in a two-year grassland field experiment. An associated pot experiment was conducted using homogenized field soils for controlling spatial soil variability. Nitrous oxide emissions were highly episodic with emission peaks in response to freezing- thawing and fertilization. Liming with dolomite caused a pH increase from 5.1 to 6.2 and reduced N_2O-emissions by 30% and 60% after fertilization and freezing-thawing events, respectively. Over the course of the two-year field trial, N_2O emissions were significantly lower in dolomite-limed than non-limed soil ($p<0.05$), although this effect was variable over time. Unexpectedly, no significant reduction of N_2O emission was found in the calcite treatment, despite the largest pH increase of all tested minerals. We tentatively attribute this to increased N_2O production by overall increase in nitrogen turnover rates (both nitrification and denitrification) following rapid pH increase in the first year after liming. Siliceous materials showed little pH effect and had no significant effect on N_2O emissions probably due to their lower buffering capacity and lower cation content. In the pot experiment using soils taken from the field plots three years after liming and exposing them to natural freezing-thawing, both calcite ($p<0.01$) and dolomite ($p<0.05$) significantly reduced cumulative N_2O emission by 50% and 30%, respectively relative to the non-limed control. These results demonstrate that the overall effect of liming is to reduce N_2O emission, although high lime doses may lead to a transiently enhanced emission.

Key words: soil pH, denitrification, liming, dolomite, siliceous minerals, grassland

1. Introduction

Acidity is well known to increase the N_2O/N_2 product ratio of denitrification (Nömmik, 1956; Simek & Cooper, 2002; Wijler & Delwiche, 1954) and therefore raising the pH of acidic soils by liming can decrease the N_2O emission and hence serve as a N_2O mitigation tool (Hénault et al., 2019; Kunhikrishnan et al., 2016; Page et al., 2009). Liming is a common agronomic practice used to counteract soil acidification by intensive cultivation, and to improve soil fertility of acidic soils (Fageria & Baligar, 2008). Most commonly used are calcareous materials (calcite and dolomite), but powdered siliceous mine tailings can be considered, as their dissolution under acidic conditions releases base cations which react with CO_2 or HCO_2^- and H^+ in the soil, thereby increasing the soil pH (Van Noort et al., 2018). Soil pH has direct and indirect effects on microbial nitrogen (N) transformations and associated production and consumption of N_2O. Directly, low pH inhibits the maturation of N_2O reductase in the periplasm (Bergaust et al., 2010). While the N_2O emission from denitrification decreases with increasing soil pH, the trend for nitrification appears to be opposite: the N_2O yield of nitrification (N_2O/NO_3^-) increases with increasing pH (Nadeem et al., 2020). Indirectly, liming may also control the potential of a soil to emit N_2O by affecting the microbial community composition over time (Braker et al., 2012; Jones et al., 2014) and, in the long run, by altering physical and chemical properties of the soil (Weil, R. & Brady, N., 2017).

In intensively managed grasslands, the N_2O budget is usually dominated by fertilizer-induced N_2O emissions, often triggered by rainfall (Dobbie & Smith, 2003). Soils experiencing cycles of drying and wetting emit N_2O mainly produced by denitrification (Congreves et al., 2018). After rewetting of dry soil, a flush of microbial activity depletes O_2, thus inducing denitrification and associated N_2O emissions (Saggar et al., 2013). Likewise, in colder climates, cycles of freezing and thawing can trigger N_2O emission peaks, which may contribute up to 50% of the total annual N_2O emission (Wagner-Riddle et al., 2017; Wallman et al., 2022). Freezing-thawing induced N_2O emission peaks are commonly attributed to the release of readily degradable organic matter from died-off microbial biomass and plant residues, which may fuel both nitrification and denitrification, and lower the O_2 availability (Byers et al., 2021; Christensen & Tiedje, 1990; Sturite et al., 2021).

While increasing soil pH can be expected to decrease N_2O emissions originating from denitrification, N_2O production by nitrification may increase (Nadeem et al., 2020), thus reducing or cancelling out the mitigation effect of liming. As with denitrification, liming has direct and indirect effects on nitrification and its N_2O yield: it instantaneously accelerates the nitrification rate due to shifting the NH_4^+/NH_3 equilibrium towards NH_3, and has an indirect effect by increasing the relative activity and abundance of ammonia oxidizing bacteria (AOB) over ammonia oxidizing archaea (AOA) (Hink, L. et al., 2018). AOB produce inherently more N_2O per unit oxidized NH_3 than AOA (Hink et al., 2017). Therefore, liming may increase N_2O emissions from nitrification.

Several other factors affect N_2O emissions such as soil texture, soil organic matter content, inorganic N availability, moisture, and functional microbial community composition (Abdalla et al., 2022; Wessén et al., 2011). Spatial variation of these factors may obscure the pH effect on N_2O emissions. For instance, a central factor for processes producing and consuming N_2O is soil moisture which greatly depends on local drainage conditions. Also, the trajectory of pH rise may affect N_2O processes; rapid pH rise by fast-reacting liming agents such as colloidal calcite may boost nitrification and increase available organic C, resulting in a transient increase in emission (Baggs et al., 2010). In addition, there still exists a gap in knowledge about impacts of liming on N_2O emissions from grasslands (Abdalla et al., 2022).

To expand on the two years of flux measurements after liming in the field experiment, we used homogenized field soils from the same liming treatments in the third year to perform a more controlled pot experiment, in which we simulated ploughing of grassland and mineral N addition, before exposing the pots to freeze-thaw conditions. Measurement campaigns were conducted both during the growing period, including fertilization and rain events, and during off-season in autumn and early spring to capture emission peaks triggered by freezing-thawing. We compared two calcareous and three siliceous liming treatments. Siliceous minerals were included as a possible alternative to traditional carbonates to explore the possibility for reducing N_2O emissions without liberating geochemically bound carbon as carbon dioxide (CO_2) (IPCC, 2006; Wang et al., 2021). To test the pH effect on N_2O emissions with less variability in drainage conditions as compared with the experimental field, we set up a freely draining, outdoor pot experiment, using excavated and homogenized soils from the field plots.

We hypothesized (1) that N_2O emissions decrease with increasing soil pH, (2) rapid pH increase after applying finely dispersed calcite to the soil would lead to transient increase of N_2O emissions, and (3) that the effect of more slowly dissolving dolomite or siliceous rock powder on N_2O emissions emerges gradually over time.

2. Materials and methods

2.1. Field experiment

A field liming experiment was established in 2014 at the experimental farm of the Norwegian University of Life Sciences (NMBU) in SE Norway, Ås (59° 49’ N, 10° 47’ E, 75 m a.s.l). The soil is classified as an Albeluvisol (WRB, 2006)(and has a clay loam texture, 27% clay, 48% silt, 25% sand – pH($CaCl_2$)= 5.0 before liming; total carbon 3%, total nitrogen content 0.28%, C:N= 11.03) and had been under a crop rotation since the 1950s (Manojlović et al., 2004). The field was last limed in the 1970s. The experimental layout consisted of five liming treatments and a control, with four replications per treatment randomly distributed in three rows. Calcareous materials were applied in autumn 2014 at rates chosen to achieve distinct pH values, whereas the mafic minerals were applied at a uniform rate of 30 t ha^{-1}. Larvikite, norite and calcite were by-product of mining industries and applied as slurries (Table 1). Dolomite was applied as granulate and olivine as a powder. To distribute the materials throughout the plough layer, liming was done in two consecutive rounds; first, half of the material was added and ploughed under to 20 cm. Thereafter, the other half was applied and harrowed to 10 cm depth.

Table 1. Liming materials: composition, producer, grain size and application rate

Treatment	**Dominant mineral***	**Producer**	**Grain size****	**Application**	**CCE (%)*****
Dolomite	$Ca(Mg)CO_3$	Franzenfoss	granulated	24 t ha^{-1****}	109
Calcite	$CaCO_3$	Hustad marmor, Elnesvågen, Norway	Finely dispersed slurry <0.4-1.5 μm	30 t ha^{-1}	100
Larvikite	32.2% feldspar, $(Na_{0.52}K_{0.29}Ca_{0.19}Al_{1.19}Si_{2.81}O_8)$, 24.5% pyroxene $(Mg_{0.24}Fe_{0.30}Ca_{0.46}SiO_3)$, 20.7% biotite, 17.5% nepheline$((Na_{0.78}K_{0.22})AlSiO_4)$, 2.9% calcite, and 2.2% sphene	Lundhs Real Stone, Larvik, Norway	Slurry < 63 μm	30 t ha^{-1}	47
Norite	65.3% plagioclase $(Na_{0.54}Ca_{0.46}A$	Titania,	Slurry < 300 μm	30 t ha^{-1}	61

	$l_{1.46}Si_{2.54}O_8$), 14.4% pyroxene, 11.4% ilmenite (containing some Mg),7.3% biotite, 1.0% magnetite, and 0.7% periclase	Hauge i Dalane, Norway			
Olivine	95.1% olivine ($Mg_{1.86}Fe_{0.14}SiO_4$), with 3.0% orthopyroxene and 1.9% chlorite	Silbeco, Åheim, Norway	Powder < 63 µm	30 t ha^{-1}	65.7

*Minerology and the dissolution rates have been described in a study by Van Noort et al. (2018)

**Particle size as mentioned by Nadeem et al. (2020)

***CCE Calcium carbonate equivalent or neutralizing value of the material compared to pure (100%) of $CaCO_3$. Calculated as a ratio of molecular weight of $CaCO_3$ and chemical composition of liming treatments (Havlin et al., 2014).

**** Dolomite was applied at a lower rate to adjust for its highest buffering capacity (which is 110% times of $CaCO_3$) to achieve the same targeted pH increase pH($CaCl_2$)= 6.5

Directly after liming in autumn 2014, winter wheat was sown. The wheat failed to establish, and the field was ploughed again in spring 2015. In early June 2015, a grass mixture (20% Timothy, 25% Perennial ryegrass, 25% Meadow fescue, 20% Tall fescue and 10% Meadow-grass) was undersown to spring barley, which served as a cover crop for grass establishment, receiving 100 kg N ha^{-1} as NPK fertilizer (YaraMila

NPK, 22:3:10). The cover crop was harvested on July 31st 2015. There was no grass harvests during the establishment year 2015. Instead, aboveground biomass was cut and mulched in July and October 2015. In spring 2016, the sward was fertilized with urea-N and NPK after the first and second harvest, respectively. A third cut occurred in September.

2.2. Pot experiment

In spring 2017, soil was excavated from 0-25 cm depth in each plot to set up a pot experiment. The soils were sieved (6 mm) and homogenized before placing each of them in two freely draining 9 L white plastic pots (total of 48 pots). To provide similar drainage and packing conditions (average 7.5 kg soil dry weight per pot), a layer of gravel, isolated by a cloth (to prevent root ingrowth) was placed beneath the soil, thus ensuring that the lower soil level would be above the drainage hole. Ryegrass was sown, to simulate forage production and the pots were kept outside, under natural conditions on white plastic sheets to prevent heating.

After establishing the sward throughout summer, the soil was removed from each pot and coarsely homogenized by hand (using a knife) before filling it back into the pot to mimic ploughing of a grass sward. Additional grass (500 g ryegrass m^{-2}) and KNO_3 (equivalent to 50 kg N ha^{-1}) was added to provoke conditions conducive to denitrification. Thereafter, the pots were placed outside and measured for N_2O emissions (1st Nov – 15th Dec; 44 days). Strong weather fluctuations led to repeated freezing and thawing of the pots. On 6th November time domain reflectometry (TDR) soil probes (Decagon, ECH2O GS3) were installed to measure soil moisture and temperature.

2.3. N_2O measurements and flux calculations

2.3.1. Field flux robot (FFR)

Fluxes were measured by an autonomous field flux robot (FFR), using a fast box chamber technique (Hensen et al., 2006) with on-board gas analytics. The robot is equipped with a weatherproof instrument chamber and powered by a lithium-ion battery pack. It navigates autonomously along predefined way points by real-time kinematic (RTK) GPS, achieving centimeter-level precise positioning.

The robot is an open platform designed to carry various equipment (Molstad et al., 2014). In our experiments, it was equipped with a tunable diode laser spectrometer (DLT-100, Los Gatos Research, Mountain View, CA, USA) that measures N_2O, CO and H_2O, and a CO_2/H_2O infrared gas analyzer (LI-840A, LI-COR Inc., Lincoln, NE, USA). The robot carries two static aluminum chambers (100 L) mounted to a 4 m wide vertical boom. To deploy the chambers, the boom is lowered automatically, pressing the chambers on the soil surface. Close contact with the surface is ensured by a ring of ribbed foam rubber attached to the soil surface with a predefined pressure. An additional ring of nylon brush hairs serves as wind break.

For each measurement, the chambers were deployed for 3 minutes, during which gas circulated through the chambers and the optical cells of the two instruments, alternating between the left and right chambers (20 seconds each), thus providing data for simultaneous estimation of N_2O and CO_2 fluxes from both chambers. A sonic anemometer mounted to the robot at 2.3 m height delivered continuous data on wind speed and direction.

Leakage testing was performed by deploying chambers on a steel plate with channels (to mimic uneven soil surface and rubber ring interference) and injecting a known concentration of N_2O into both of chambers (Molstad et al., 2014). Leakage was found to be negligible, affecting flux estimated by < 3% (unpublished results).

2.3.2.1. Flux calculations

N_2O concentrations measured (at ~1 Hz) during deployment were stored and postprocessed by a Python script to estimate flux rates (Molstad, 2015). For this, the first 6 s after each switch between the two chambers was omitted and a regression window of 126 s used to find the period of most linear CO_2 accumulation, which was then used to estimate the N_2O emission rate.

Raw data were plotted and inspected to identify outliers due to instrument failure, multiplexer failure, clogged tubing, or actual negative fluxes. All together 5% of the measured fluxes were omitted.

N_2O fluxes were calculated based on the linear slope estimates of N_2O concentration change over time, according to equation 1:

$$F_{N2O} = \frac{\mathrm{d}[\mathrm{N_2O}]}{\mathrm{d}t} \times \frac{V_c\, M_n}{A\, V_m}$$

Equation 1

where F_{N2O} is the flux (µg N_2O-N m^{-2} h^{-1}), $\mathrm{d}[\mathrm{N_2O}]/\mathrm{d}t$ the rate of N_2O concentration change in the chamber over time (ppmv h^{-1}), V_c the chamber volume (L), A the area covered by the chamber (m^2), M_n the molecular mass of N in N_2O (g mol^{-1}) and V_m the molar volume (L mol^{-1}) at chamber temperature (Tan et al., 2009). V_m was calculated according to the ideal gas law:

$$V_m = R\ \frac{T_c + 273.15}{P}$$

Equation 2

Where R is the ideal gas constant, T_c temperature in degrees Celsius, P pressure. Given the short deployment time, chamber temperature was assumed to be equal to air temperature, which was taken from downscaled meteorological data closest in time to the recorded flux estimate (MET, 2015-2016). For the pot experiment, equation 1 was modified to take account for the pot volume and the surface area of the pots.

Quality-checked data were used to calculate cumulative fluxes by linear interpolation (i.e., trapezoidal integration) in R studio, where values were sorted according to time order and aggregated to a cumulative flux (gasfluxes package version 0.4-1), R software 4.1.

2.4. Soil variables

Soil temperature and volumetric water content were measured by TDR probes (Decagon, ECH2O GS3, 0-10 cm depth, 70-MHz frequency), which were placed in the south and north part of the field. Water filled pore space (WFPS) was calculated as the ratio of volumetric soil water content to total soil porosity, as inferred from the field's average bulk density (Paul, 2015)(Equation 3).

$$\%WFPS = \frac{\text{soil water content x } 100}{1 - \dfrac{\text{bulk density}}{2.65\ \text{g/cm3}}}$$

Equation 3

The probes were removed during fertilization, harvests and sowing.

Total carbon content of soil was determined according to the dry combustion method as described in Nelson and Sommers (1983). Soil samples were crushed by a mortar before weighing and analyzing them on a Leco CHN628 element analyzer. The samples were dried at 55 ° C prior to analysis to remove residual moisture.

In 2013 prior to liming, soil samples were analyzed for texture by a Beckman Coulter LS 13 320, particle size analyzer, measuring the size distribution of the particles suspended in a liquid (Aqueous Liquid Module, ALM) after removing all organic material by H_2O_2 according to the method of Pye and Blott (2004). Nine samples were analyzed by the pipette method (Gee & Bauder, 1986) to calibrate the particle counter.

Throughout 2015 and 2016, composite samples for each plot were sampled twice or once a month for measuring pH both in water and 0.01 M $CaCl_2$ using a Thermo Scientific ROSS Ultra pH/ATC Triode electrode.

Soil was sampled prior to and after fertilization events (0-10 cm depth) for mineral nitrogen analysis. 25 g of fresh soil were extracted with 40 ml 1 M KCl. Subsamples of 1 mL were frozen for later analysis. The subsamples were thawed and centrifuged at 10 000 G for 15 minutes at 4 ^{0}C. NO_3^- and NO_2^- concentrations were determined by Griess reaction with Vanadium (III) chloride (Doane & Horwáth, 2003) using a microplate reader (Infinite F50, TECAN Austria GmbH) at 540 nm. NH_4^+ was analyzed calorimetrically by the sodium salicylate method at 660 nm (Keeney & Nelson, 1982), using the same microplate reader.

Soil texture, total carbon and total nitrogen were provided by Bleken (personal communication).

2.5. Ancillary variables

Precipitation and air temperature data were obtained from the nearby (~1 km to the east on a comparable field) NMBU weather station that is part of the Norwegian meteorological network (MET, 2015-2016).

2.6. Statistics

All flux rates were ln-transformed and tested for assumptions of linear relationship and multivariate normal distribution of residuals. An additive linear mixed effect model was used to test for treatments (fixed effects, six levels) on N_2O emissions. Variation in N_2O emissions between 24 different plots and between the 209 different sampling days was modelled by means of random effects. Pairwise comparison was used to compare the effects of each treatment to the unlimed control. The emission data were adjusted for multiplicity by Bonferroni correction (Bretz et al., 2016; Lenth et al., 2020). The same model was used to test differences between liming treatments in the pot experiment. The proportion of the random effect variance attributable to each random effect was found by dividing each random effect by the sum of all random effects (i.e., the total variance of the random effects).

Differences in soil pH were tested with a general linear model (GLM) with soil pH as response variable and liming treatments as predictor. The pH effect on cumulative N_2O emissions was tested with ANOVA factorial model using the anova function in R. All statistics were performed by R software, 4.1.2. R stats package (Hastie & Chambers, 1992), 4.0.2 package MASS (Venables & Ripley, 2002) for GLM and lme4 (Bates et al., 2014) for linear mixed-effects model.

3. Results

Soil pH

Liming with calcite ($p<0.01$) and dolomite ($p<0.01$) in 2014 resulted in significantly higher pH values in 2015 compared to the control (Fig. 1). Soil treated with silicious minerals in 2015 showed no increase in pH compared to control (pH 5.5). Except for the calcite treatment, soil pH increased throughout March to May, peaking shortly after fertilization (18th May) for all treatments. In late May, olivine started to separate from this grouping with slightly higher pH but decreased again during June and July. During the rest of the year 2015 there was a trend of decreasing pH in all treatments.

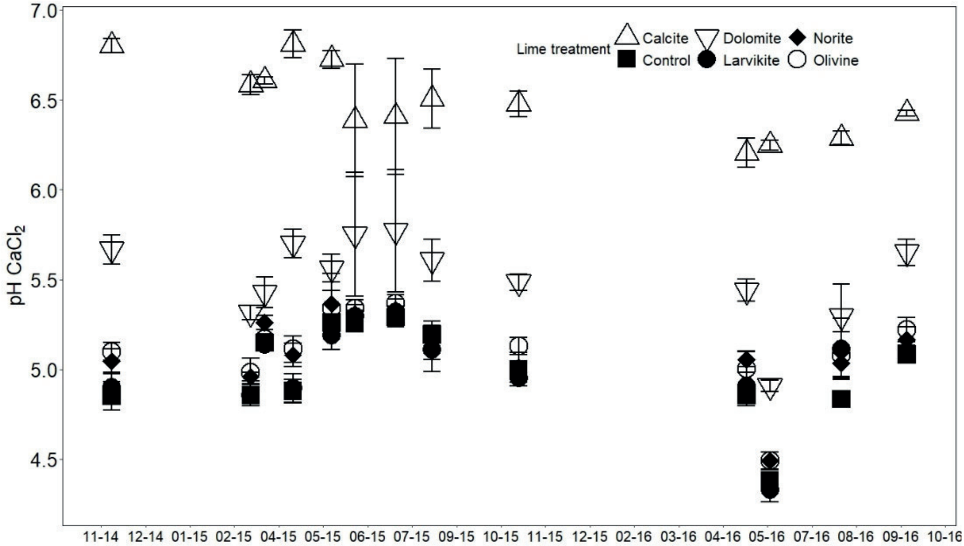

Figure 1. Average topsoil (0-15 cm) pH_{CaCl2} (+/- SE) from March 2015 to October 2016 for all treatments. N= 4 for each treatment and sampling date.

In May 2016 soil pH was similar to the last pH measurement in October 2015 and there was a positive trend over the year, but not significant (Fig. 1). In May 2016, there was on average half a pH unit decrease in norite, control, larvikite and olivine between 17th April (before fertilization with urea) and 20th May (after fertilization with urea). During this time dolomite decreased soil pH by almost one pH unit (approx. 0.8), while calcite remained stable. Later in July mafic minerals and dolomite returned to their pre-urea fertilization pH values. In September dolomite, calcite and olivine increased pH, while norite, larvikite and control were stable, showing similar values as in July.

N_2O emissions

Field experiment

Over the course of the two-year field experiment, N_2O emissions showed distinct emission peaks (Fig. 2A), which were related to harvesting (31st July 2015 and 14th June 2016), fertilization (22nd October 2015, 10th May and 14th June 2016) and freezing and thawing events (23rd to 22nd November 2015 and 16th to 30th March 2016). The first peak in 2015 was observed after harvesting the cover crop (31st July

2015) coinciding with increasing WFPS due to abundant rain (Fig. 2 B). Another pronounced emission peak in 2015 was observed after mulching of the grass sward on 10th October 2015 and fertilization on 20th October which coincided with a period of diurnal freeze-thaw cycles, eliciting a large N_2O emission peak.

Fertilization with urea increased N_2O emissions in early May 2016 and even more in June 2016 (after 1st harvest). During the latter event the highest emission peak was recorded for both years (~2300 µg N_2O-N m^2 d^{-1}). This peak was triggered by rainfall after fertilization. Fertilization after the second harvest did not result in any distinct emission peaks.

Emission peaks at the end of November 2015 coincided with some rain and decreasing soil temperatures, followed by two smaller emission peaks triggered by freezing and thawing cycles (highest peak ~900 µg N_2O-N m^2 d^{-1}). During snowmelt and spring thaw in March 2016, N_2O emissions strongly fluctuated with peak emissions of up to ~1300 µg N_2O-N m^2 d^{-1}, almost double the magnitude of the highest emission peak observed in autumn 2015 (Fig. 2 A). By contrast, highest recorded emission for the first freezing and thawing event in autumn 2016 did not exceed 8.75 µg m^2 d^{-1} N_2O-N.

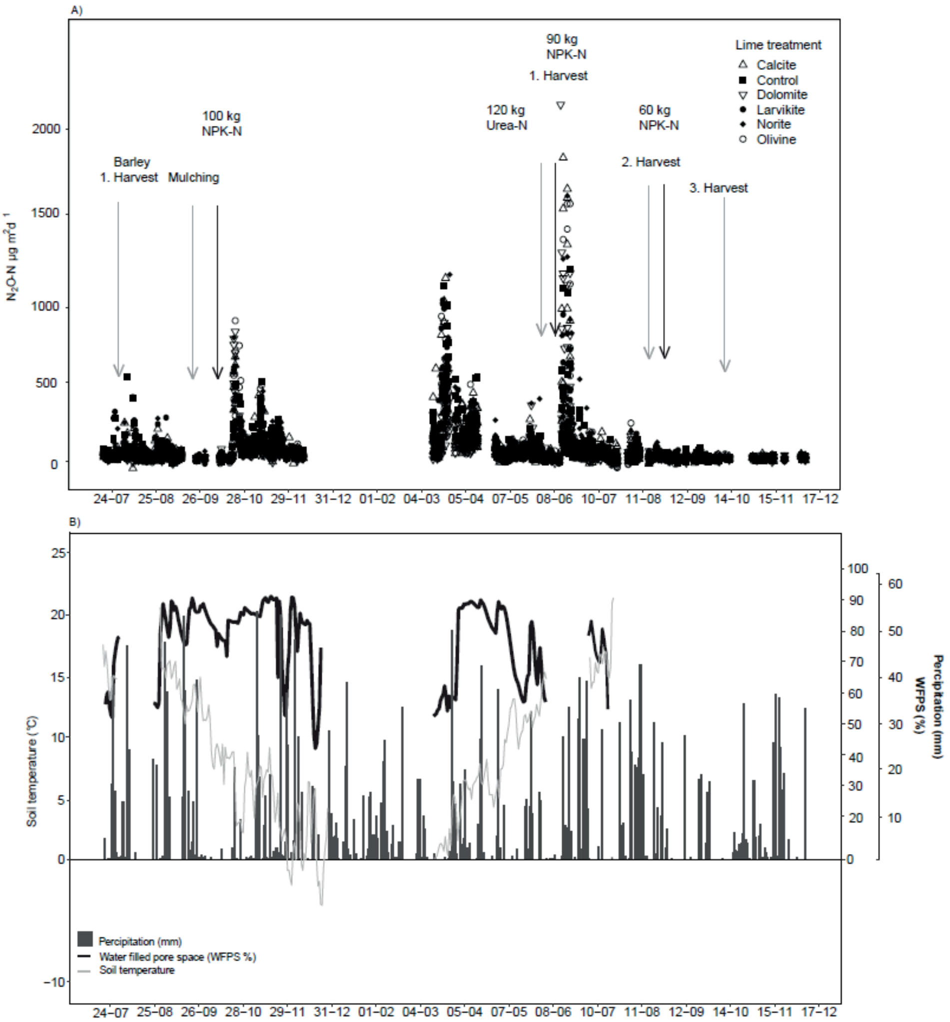

Figure 2. Mean N_2O emission in the field experiment (in µg N m^2 h^{-1}) in 2015 (July to December) and 2016 (March to December) (A); for clarity, no standard deviations are shown; with no significant differences in emission rates. B) Average daily soil temperature 0-10 cm depth, water filled pore space (%) and daily precipitation in mm.

Treatment effects

There was no overall significant ($p=0.54$) relationship between N_2O emissions and soil pH for the field trial based on cumulative N_2O emissions. However, N_2O emissions in the dolomite treatment were significantly lower than in the control ($p<0.05$; Table

2), while no other liming treatment affected N_2O emissions significantly. Most of the variation seen in N_2O emissions was explained by day-to-day flux dynamics (day number, 56 % of the variation). A large part of the variation was left unexplained (26%) and plot number explained only 1.3% of the variation in N_2O emissions.

Table 2. Parameter estimates of a linear mixed effects model for ln-transformed mean N_2O emissions (µg N_2O-N m^2 d^{-1}) in the field experiment for fixed (treatment) and random effects (plots, day number). P values indicate differences between treatments and control (not limed).

2015 - 2016				
Fixed effects **Treatment**	**Estimate**	**N_2O-N emissions P value** **($p < 0.05$)**	**Random effects**	**Variance**
Dolomite	-0.28 ± 0.07	0.03	day number	1.28
Larvikite	0.05 ± 0.35	0.85	plot number	0.03
Calcite	-0.04 ± 0.54	0.67	residuals	0.62
Norite	0.10 ± 0.27	0.38		
Olivine	-0.06 ± 0.51	0.64		

N_2O cumulative emissions across seasons revealed that dolomite had generally smaller N_2O emissions compared to the control (Figs. 3 A-C), although this was not statistically significant. Calcite, which was the treatment with the highest pH raise (Fig. 1) appeared to emit less N_2O than the control in the first growing season (Fig. 3A), but this difference was not significant due to the high variability of emission fluxes in the control. In the following seasons, the average cumulative N_2O emission of the calcite treatment was numerically almost equal that of the control.

Overall, silicious minerals had no significant effect on N_2O emissions (Table 2 and 3). Olivine had smaller, though not statistically significant, emissions than the control in the first summer, but this effect disappeared throughout the following seasons. (Fig. 3). Larvikite and norite had variable effects on N_2O emissions. Growing season emissions in 2016 did not show any treatment effect (Fig. 3 D).

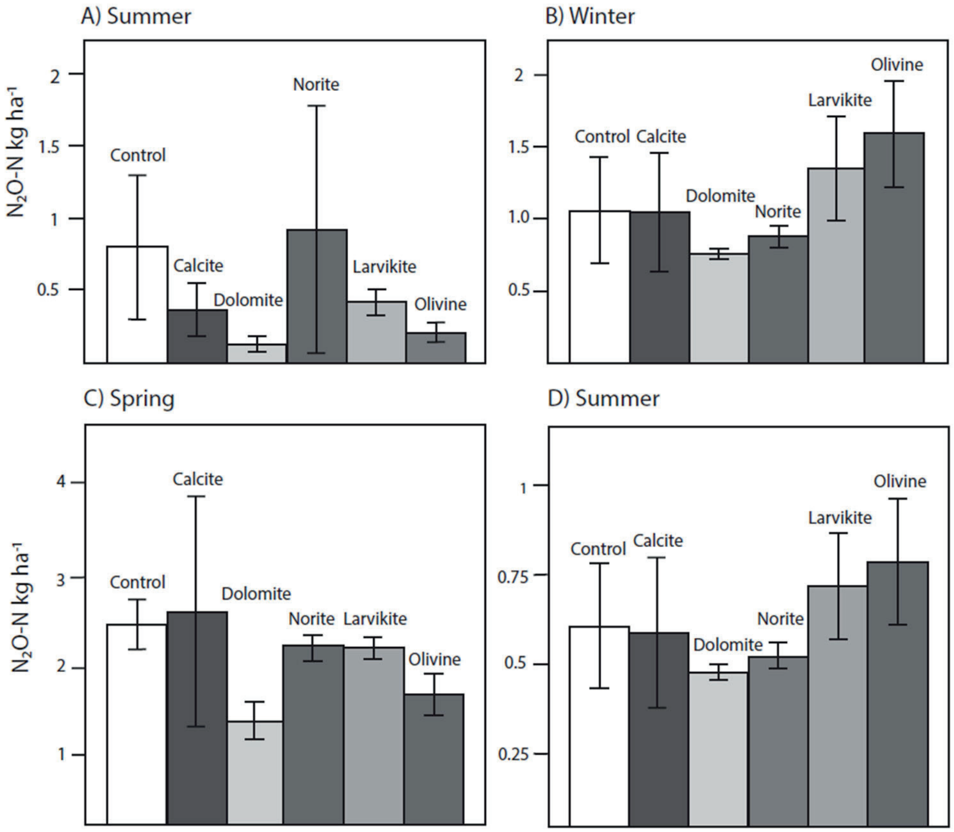

Figure 3. Cumulative N_2O emissions (N kg ha^{-1} period^{-1}) in the field experiment: A) growing period 2015 (20th July to 14th September 2015); B) Fall 2015 (20th October to 20th November 2015); C) Spring thaw 2016 (14th March to 15th April 2016); D) Growing-season 2016 (20th July to 15th September 2016). Error bars are standard error (N=4).

Pot experiment

In the pot experiment, frequent freezing and thawing triggered exceptionally high emission rates (Fig S2. A). Cumulated over the entire experimental period (November-December 2017) N_2O emissions were strongly affected by pH (Fig. 4) with calcite ($p<0.01$) and dolomite ($p<0.05$) having 50% and 30%, respectively, smaller emissions than the control (Figure 4).

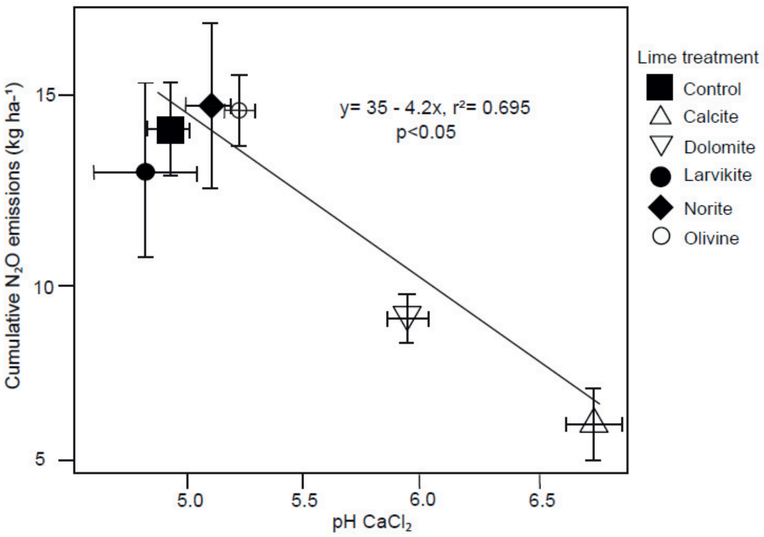

Figure 4. Relationship between cumulative N_2O emissions and measured soil pH in the pot trial. Shown are average cumulative N_2O-N fluxes in kg ha^{-1} over a period of 44 days (1st November to 15th December 2017) plotted over average soil pH in 0.01 M $CaCl_2$ with standard errors for both variables.

The variation in N_2O emissions explained by treatment was by approximately 15 % higher in the pot experiment than in the field experiment (Table 2 and 3). Day to day flux variation (day number) explained 70%, plot number 1% and unexplained variation was 29%. It is important to note that the field experiment consisted of 209 sampling dates while the pot experiment was sampled in only 44 dates, which inherently reduces day-to-day variation and overall unexplained variability (error).

Table 3. Parameter estimates of a linear mixed effects model for ln-transferred N_2O emissions (µg N_2O-N m^2 h^{-1}) in the pot experiment for fixed (all treatments) and random effects (plots, day number). P values indicate differences between treatments and control (not limed).

Pot experiment				
1st November - 15th December 2017				
Fixed effects **Treatment**	**Estimate**	**N_2O-N emissions** **P value** **($p < 0.05$)**	**Random effects**	**Variance**
Dolomite	-0.40 ± 0.32	0.02	Day number	2.88
Larvikite	0.15 ± 0.80	0.35	Plot number	0.04
Calcite	-0.66 ± 0.21	<0.01	Residual	1.18
Norite	0.26 ± 0.77	0.13		
Olivine	0.18 ± 0.39	0.27		

4. Discussion

Based on previous laboratory studies of denitrification in soil (Simek & Cooper, 2002; Wijler & Delwiche, 1954), and subsequent documentation that the maturation of N_2O reductase is hindered by low pH (Bergaust et al., 2010; Liu et al., 2014) we hypothesized that N_2O emissions would be negatively related with soil pH. Such a negative relationship has also been documented for field fluxes in a meta study by Wang et al. (2018). In a review paper by Abdalla et al. (2022), in four out of ten field studies N_2O emissions decreased after increased pH by liming. However, under field conditions, the pH effect may be overridden by other factors. Particularly under conditions of shifting O_2 availability, the well-documented suppression of N_2O emission from denitrification by pH rise may be cancelled out by increased N_2O emissions from nitrification. Nadeem et al. (2020), using soils from the same field plots in a laboratory study, demonstrated that, after NH_4^+ addition, soil moisture played a key role for the overall pH effect on N_2O emissions; at lower soil moistures, nitrification prevailed and N_2O emission was positively correlated with soil pH, while at higher soil moistures, O_2 consumption by nitrification induced coupled nitrification-denitrification, the N_2O production of which was negatively correlated with soil pH. Under field conditions, a multitude of factors determines O_2 availability in the soil (root respiration, C availability, NH_4^+ availability, soil moisture), which makes it difficult to predict dynamics of nitrification and denitrification *in situ*. Next to the temporal dynamics of nitrification and denitrification, their relative importance may show considerable spatial variation, which compromises the statistical power of plot trials. In the present study, large differences in soil texture and hence aeration conditions, were detected across the field plots (Supplementary data, Fig. 1). This may explain the large standard errors of cumulative N_2O emissions we found, for instance, in the unlimed control (Fig. 3) and the overall low explanatory power of treatment in the mixed effect models (Tables 2 and 3). The high spatial variability of N_2O fluxes in the field trial prompted us to conduct a more controlled pot experiment, in which we attempted to equalize drainage conditions by letting the pots freely drain and, in addition, stimulated denitrification by mixing in grass litter and NO_3^-. Exposing the pots to natural freezing-thawing cycles elicited vigorous N_2O emissions which, when cumulated, showed a pH-proportional response with significantly less N_2O emissions the higher the pH (Fig. 4). This corroborated that liming has a direct effect on N_2O emissions when originating from denitrification.

Another critical factor for the effect of liming on N_2O emissions may be the trajectory of pH rise. While granulated dolomite dissolved slowly, the finely dispersed calcite slurry used in our study instantly raised the pH from pH 5.00 to pH 6.62 (Fig. 1). Rapid pH raise has multiple effects in soil, among them release of adsorbed DOC to the soil solution, increased microbial activity and, most notably, a strong stimulation of nitrification (Li et al., 2020) . Overshooting NH_3 oxidation may result in transient nitrite (NO_2^-) accumulation as shown for our soils in the laboratory experiment by Nadeem et al. (2020). Nitrite accumulation, in turn, may induce additional N_2O production by chemical hydroxylamine oxidation (Liu et al., 2019), hybrid N_2O formation (Terada et al., 2017) or nitrifier-denitrification (Kremen et al., 2005). In our field experiment, calcite addition caused variable effects resulting in large standard errors and non-significant differences to the control. We therefore conclude that rapid pH raise by calcite slurries is not suitable for mitigating N_2O emissions, most likely because pH raise perturbs N cycling in general, and nitrification in particular.

It is noteworthy that liming did not affect N_2O emissions during freezing-thawing cycles in autumn 2015 (shortly after mineral N addition) nor in spring 2016 (spring thaw). A possible explanation for the lack of a pH effect on N_2O emissions during freezing-thawing may be strongly reductive conditions in partly frozen soils (Öquist et al., 2004). As shown by Byers et al. (2021), N_2O lingers in the subsoil for extended periods while pO_2 declines and also *de novo* production of N_2O during thawing often occurs when soils are fully saturated. Under these conditions, N_2O reductase may be induced unconditionally due to a lack of electron acceptors, thus overriding the effect of pH on denitrification product stoichiometry. Interestingly, Russenes et al. (2016) working in a wheat stubble field in SE Norway found a negative relationship between N_2O emissions and naturally occurring pH variation during spring thaw, probably reflecting less reductive conditions in a wheat stubble as compared to a grass ley. More research is needed to elucidate whether pH interactions with non-growing season N_2O processes differ in annual and perennial cropping systems.

Contrary to our field experiment, we found a significant negative relationship between soil pH and N_2O in the pot experiment (Fig. 4). This was partly because allowing for free drainage removed some of the intra-treatment variability observed in the field experiment as can be seen from a slight decrease in the proportion of variance explained by plot number between the pot and field experiment, 1% and 1.3%, respectively. More importantly, there was a clear pH effect because addition of plant litter and nitrate and exposure to freezing-thawing triggered denitrification as the dominant N_2O source. This confirms that the recurrently reported observation of a liming effect on denitrification product stoichiometry can be reproduced under near-field conditions.

Despite the fact that N_2O field emissions did not scale proportionally with soil pH, we found that dolomite reduced N_2O emissions in all fertilization events by on average 30% (Fig. 1 A, four fertilization events total) which is in accordance with results of Hénault et al. (2019) who found that liming of acidic soils to neutrality reduces N_2O emissions after fertilizing events. However, this fertilization event coincided with an autumn freezing and thawing event (Fig. 1) and therefore it is hard to conclude if the dolomite led to reduced N_2O emissions derived from fertilization and/or freezing-thawing event. Nevertheless, dolomite successfully reduced fertilization induced emissions and we believe these emissions were predominantly denitrification induced, due to a combination of rain and NH_4^+ induced O_2 consumption.

Overall, we conclude that liming effects on field N_2O emissions are variable, depending on season, fertilizer application rates and winter conditions. Siliceous minerals did not show promising results as an alternative to traditional limes (calcite and dolomite). Rapid pH increase, as seen for finely dispersed calcite, has shown inconclusive results, despite its strong effect on soil pH. High lime doses should be avoided as they may lead to enhanced emissions.

Acknowledgments

We would like to thank Trygve Fredriksen and Øyvind Peder Vartdal for their help with field and laboratory work. This research was financed by Norwegian Research Council project number 234382 (MIGMIN) and the FACCE ERA-GAS project, MAGGE pH under Grant Agreement No. 696356.

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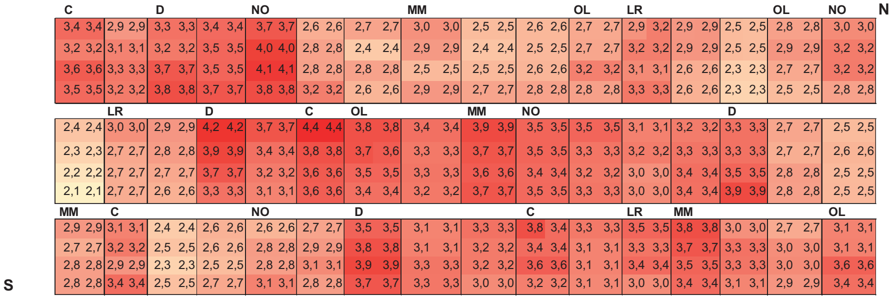
Supplementary data

A)



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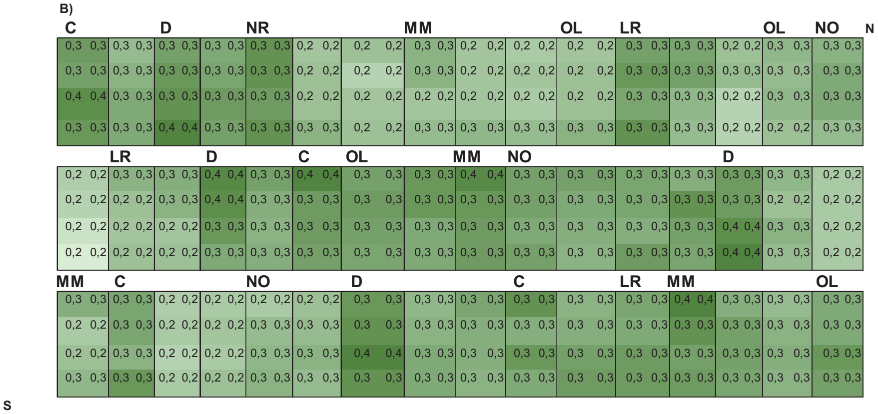
3,2 3,2	3,1 3,1	3,2 3,2	3,5 3,5	4,0 4,0	2,8 2,8	2,4 2,4	2,9 2,9	2,4 2,4	2,5 2,5	2,7 2,7	3,2 3,2	2,9 2,9	2,5 2,5	2,9 2,9	3,2 3,2
3,6 3,6	3,3 3,3	3,7 3,7	3,5 3,5	4,1 4,1	2,8 2,8	2,8 2,8	2,5 2,5	2,5 2,5	2,6 2,6	3,2 3,2	3,1 3,1	2,6 2,6	2,3 2,3	2,7 2,7	3,2 3,2
3,5 3,5	3,2 3,2	3,8 3,8	3,7 3,7	3,8 3,8	3,2 3,2	2,6 2,6	2,9 2,9	2,7 2,7	2,8 2,8	2,8 2,8	3,3 3,3	2,6 2,6	2,3 2,3	2,5 2,5	2,8 2,8

	LR		D		C	OL		MM	NO				D		
2,4 2,4	3,0 3,0	2,9 2,9	4,2 4,2	3,7 3,7	4,4 4,4	3,8 3,8	3,4 3,4	3,9 3,9	3,5 3,5	3,5 3,5	3,1 3,1	3,2 3,2	3,3 3,3	2,7 2,7	2,5 2,5
2,3 2,3	2,7 2,7	2,8 2,8	3,9 3,9	3,4 3,4	3,8 3,8	3,7 3,6	3,3 3,3	3,7 3,7	3,5 3,5	3,3 3,3	3,2 3,2	3,3 3,3	3,3 3,3	2,7 2,7	2,6 2,6
2,2 2,2	2,7 2,7	2,7 2,7	3,7 3,7	3,2 3,2	3,6 3,6	3,5 3,5	3,3 3,3	3,6 3,6	3,4 3,4	3,2 3,2	3,0 3,0	3,4 3,4	3,5 3,5	2,8 2,8	2,5 2,5
2,1 2,1	2,7 2,7	2,6 2,6	3,3 3,3	3,1 3,1	3,6 3,6	3,4 3,4	3,2 3,2	3,7 3,7	3,5 3,5	3,3 3,3	3,0 3,0	3,4 3,4	3,9 3,9	2,8 2,8	2,5 2,5

MM	C			NO		D			C		LR	MM			OL
2,9 2,9	3,1 3,1	2,4 2,4	2,6 2,6	2,6 2,6	2,7 2,7	3,5 3,5	3,1 3,1	3,3 3,3	3,8 3,4	3,3 3,3	3,5 3,5	3,8 3,8	3,0 3,0	2,7 2,7	3,1 3,1
2,7 2,7	3,2 3,2	2,5 2,5	2,6 2,6	2,8 2,8	2,9 2,9	3,8 3,8	3,1 3,1	3,2 3,2	3,4 3,4	3,1 3,1	3,3 3,3	3,7 3,7	3,3 3,3	3,0 3,0	3,1 3,1
2,8 2,8	2,9 2,9	2,3 2,3	2,5 2,5	2,8 2,8	3,1 3,1	3,9 3,9	3,3 3,3	3,2 3,2	3,6 3,6	3,1 3,1	3,4 3,4	3,5 3,5	3,3 3,3	3,0 3,0	3,6 3,6
2,8 2,8	3,4 3,4	2,5 2,5	2,7 2,7	3,1 3,1	2,8 2,8	3,7 3,7	3,3 3,3	3,0 3,0	3,2 3,2	3,1 3,1	3,0 3,0	3,4 3,4	3,1 3,1	2,9 2,9	3,4 3,4

B)



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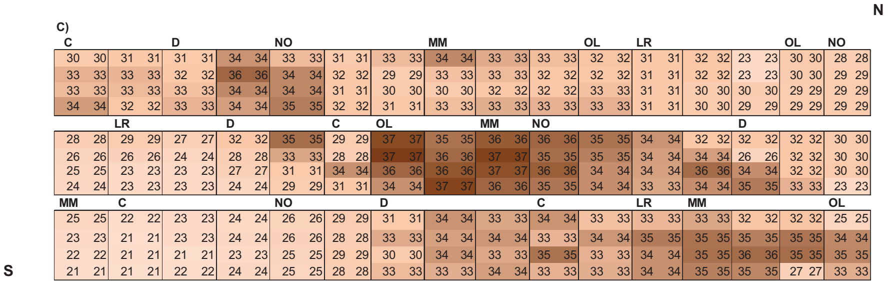
0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,2 0,2	0,2 0,2	0,3 0,3	0,2 0,2	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3
0,4 0,4	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,2 0,2	0,2 0,2	0,2 0,2	0,2 0,2	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,2 0,2	0,3 0,3	0,3 0,3
0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,3 0,3	0,3 0,3	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,2 0,2	0,2 0,2	0,3 0,3

	LR		D		C	OL		MM	NO				D		
0,2 0,2	0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,4 0,4	0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,2 0,2
0,2 0,2	0,2 0,2	0,3 0,3	0,4 0,4	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,2 0,2	0,2 0,2
0,2 0,2	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,2 0,2
0,2 0,2	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,2 0,2

MM	C			NO		D			C		LR	MM			OL
0,3 0,3	0,3 0,3	0,2 0,2	0,2 0,2	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,3 0,3	0,3 0,3
0,2 0,2	0,3 0,3	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3
0,2 0,2	0,3 0,3	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,4 0,4	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3
0,3 0,3	0,3 0,3	0,2 0,2	0,2 0,2	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3	0,3 0,3

C)



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33 33	33 33	32 32	36 36	34 34	32 32	29 29	33 33	33 33	32 32	32 32	31 31	32 32	23 23	30 30	29 29
33 33	33 33	33 33	34 34	34 34	31 31	30 30	30 30	32 32	32 32	33 33	31 31	30 30	30 30	29 29	29 29
34 34	32 32	33 33	34 34	35 35	32 32	31 31	33 33	33 33	33 33	33 33	31 31	30 30	29 29	29 29	29 29

	LR		D		C	OL		MM	NO				D		
28 28	29 29	27 27	32 32	35 35	29 29	37 37	35 35	36 36	36 36	35 35	34 34	32 32	32 32	32 32	30 30
26 26	26 26	24 24	28 28	33 33	28 28	37 37	36 36	37 37	35 35	35 35	34 34	34 34	26 26	32 32	30 30
25 25	23 23	23 23	27 27	31 31	34 34	36 36	36 36	37 37	36 36	34 34	34 34	36 36	34 34	32 32	30 30
24 24	23 23	23 23	24 24	29 29	31 31	34 34	37 37	36 36	35 35	34 34	33 33	34 34	35 35	33 33	23 23

MM	C			NO		D			C		LR	MM			OL
25 25	22 22	23 23	24 24	26 26	29 29	31 31	34 34	33 33	34 34	33 33	33 33	33 33	32 32	32 32	25 25
23 23	21 21	23 23	24 24	26 26	28 28	33 33	34 34	34 34	33 33	34 34	35 35	35 35	35 35	35 35	34 34
22 22	21 21	21 21	23 23	25 25	29 29	30 30	34 34	33 33	35 35	33 33	34 34	35 35	36 36	35 35	35 35
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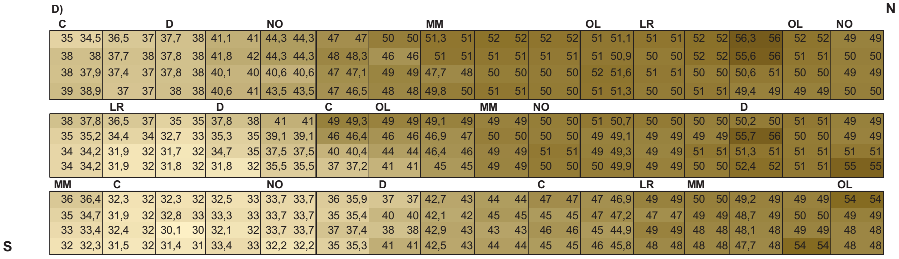
D)



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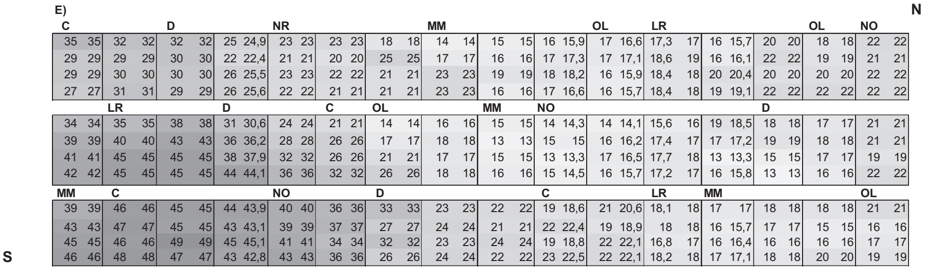
38 38	37,7 38	37,8 38	41,8 42	44,3 44,3	48 48,3	46 46	51 51	51 51	51 51	51 50,9	50 50	52 52	55,6 56	51 51	50 50
38 37,9	37,4 37	37,8 38	40,1 40	40,6 40,6	47 47,1	49 49	47,7 48	50 50	50 50	52 51,6	51 51	50 50	50,6 51	50 50	49 49
39 38,9	37 37	38 38	40,6 41	43,5 43,5	47 46,5	48 48	49,8 50	51 51	50 50	51 51,3	50 50	51 51	49,4 49	49 49	50 50

	LR		D		C	OL		MM	NO				D		
38 37,8	36,5 37	35 35	37,8 38	41 41	49 49,3	49 49	49,1 49	49 49	50 50	51 50,7	50 50	50 50	50,2 50	51 51	49 49
35 35,2	34,4 34	32,7 33	35,3 35	39,1 39,1	46 46,4	46 46	46,9 47	50 50	50 50	49 49,1	49 49	49 49	55,7 56	50 50	49 49
34 34,2	31,9 32	31,7 32	34,7 35	37,5 37,5	40 40,4	44 44	46,4 46	49 49	51 51	49 49,3	49 49	51 51	51,3 51	51 51	51 51
34 34,2	31,9 32	31,8 32	31,8 32	35,5 35,5	37 37,2	41 41	45 45	49 49	50 50	50 49,9	49 49	50 50	52,4 52	51 51	55 55

MM	C			NO		D			C		LR	MM			OL
36 36,4	32,3 32	32,3 32	32,5 33	33,7 33,7	36 35,9	37 37	42,7 43	44 44	47 47	47 46,9	49 49	50 50	49,2 49	49 49	54 54
35 34,7	31,9 32	32,8 33	33,3 33	33,7 33,7	35 35,4	40 40	42,1 42	45 45	45 45	47 47,2	47 47	49 49	48,7 49	50 50	49 49
33 33,4	32,4 32	30,1 30	32,1 32	33,7 33,7	37 37,4	38 38	42,9 43	43 43	46 46	45 44,9	49 49	48 48	48,1 48	49 49	48 48
32 32,3	31,5 32	31,4 31	33,4 33	32,2 32,2	35 35,3	41 41	42,5 43	44 44	45 45	46 45,8	48 48	48 48	47,7 48	54 54	48 48



C				D				NR						MM						OL		LR						OL		NO	
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29	29	29	29	30	30	22	22,4	21	21	20	20	25	25	17	17	16	16	17	17,3	17	17,1	18,6	19	16	16,1	22	22	19	19	21	21
29	29	30	30	30	30	26	25,5	23	23	22	22	21	21	23	23	19	19	18	18,2	16	15,9	18,4	18	20	20,4	20	20	20	20	22	22
27	27	31	31	29	29	26	25,6	22	22	21	21	21	21	23	23	16	16	17	16,6	16	15,7	18,4	18	19	19,1	22	22	22	22	22	22
		LR				D				C		OL				MM		NO								D					
34	34	35	35	38	38	31	30,6	24	24	21	21	14	14	16	16	15	15	14	14,3	14	14,1	15,6	16	19	18,5	18	18	17	17	21	21
39	39	40	40	43	43	36	36,2	28	28	26	26	17	17	18	18	13	13	15	15	16	16,2	17,4	17	17	17,2	19	19	18	18	21	21
41	41	45	45	45	45	38	37,9	32	32	26	26	21	21	17	17	15	15	13	13,3	17	16,5	17,7	18	13	13,3	15	15	17	17	19	19
42	42	45	45	45	45	44	44,1	36	36	32	32	26	26	18	18	16	16	15	14,5	16	15,7	17,2	17	16	15,8	13	13	16	16	22	22
MM		C						NO				D						C				LR		MM						OL	
39	39	46	46	45	45	44	43,9	40	40	36	36	33	33	23	23	22	22	19	18,6	21	20,6	18,1	18	17	17	18	18	18	18	21	21
43	43	47	47	45	45	43	43,1	39	39	37	37	27	27	24	24	21	21	22	22,4	19	18,9	18	18	16	15,7	17	17	15	15	16	16
45	45	46	46	49	49	45	45,1	41	41	34	34	32	32	23	23	24	24	19	18,8	22	22,1	16,8	17	16	16,4	16	16	16	16	17	17
46	46	48	48	47	47	43	42,8	43	43	36	36	26	26	24	24	22	22	23	22,5	22	22,1	18,2	18	17	17,1	18	18	20	20	19	19

Figure 12. Spatial variability of total carbon, pH and texture in the field trial: A) Total carbon in 2013 (C= control, D= dolomite, NO= norite, MM= calcite, OL= olivine, LR= Larvikite); B) Soil pH (H_2O) in 2013 (before liming); C) Clay content; D) Silt content; E) Sand content

Northwest plots have higher sand content (Fig. 2E) and lower clay (Fig.2 C). Northmost plots have lower sand content but high loam content (Fig.2 D). In a diagonal transect of the field there is an accumulation of clay (Fig.2 C) in the middle of the field from first row of North plots to Northeast plots.

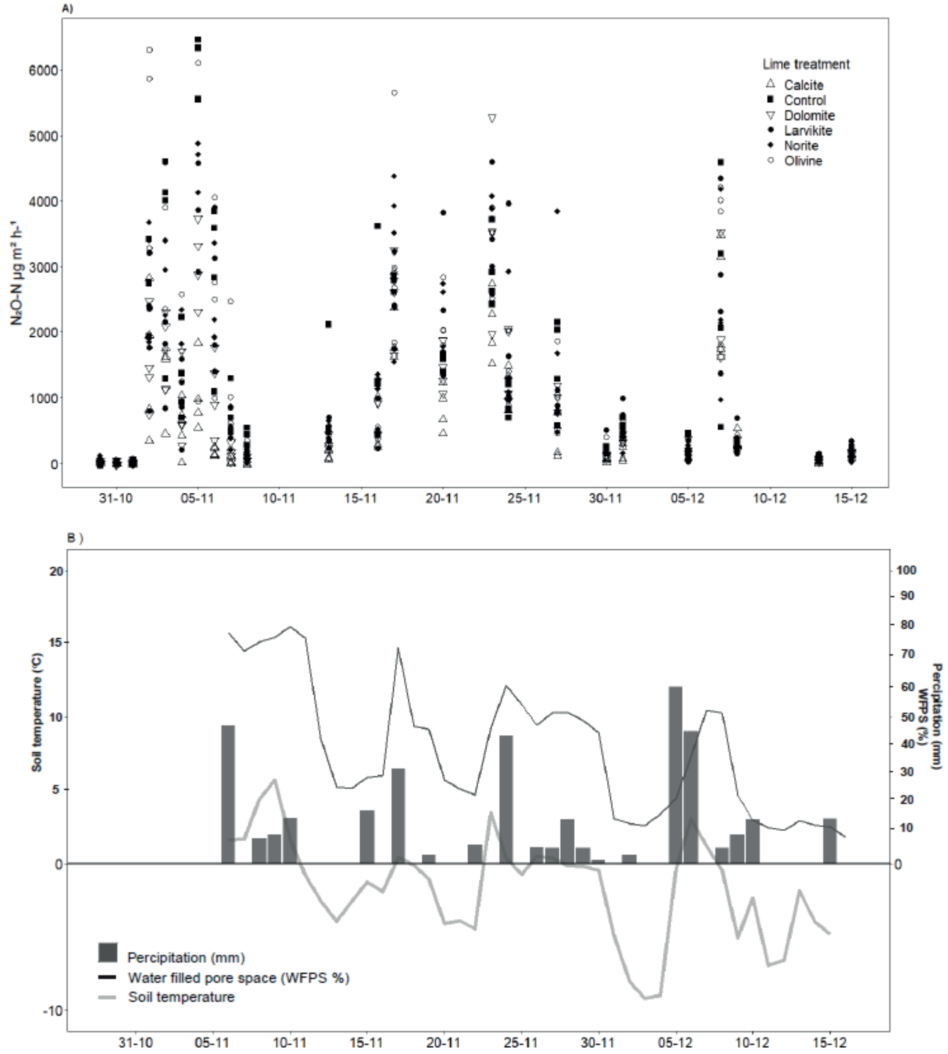

Figure S2. Hourly N_2O-N emission in the pot experiment in 2017 (1st November to 15th December 2017). Each treatment had four replicates (pots). (A); shown are single pot values. B) Average daily soil temperature 0-10 cm depth, water filled pore space (%) and daily precipitation in mm.

Paper II

Effects of maintenance liming on growing season N_2O emissions in an arable soil of SE Norway

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Abstract

Soil pH management has recently received attention as a possible mitigation tool for N_2O emissions. There has been growing evidence that liming acidic soils may reduce N_2O emissions in the short term, but few studies have investigated long-term effects of soil pH management on N_2O emissions in arable soils. Here we report effects of bulk and maintenance liming on growing season N_2O emissions five years after bulk liming in an acidic clay loam in SE Norway. Bulk lime was applied in fall 2014 with conventional aglime (dolomite), finely dispersed calcite and olivine, the field was sown to a grass ley. In summer 2019, we re-applied 1.7 t ha^{-1} of dolomite, 1.2 t ha^{-1} calcite and 5 t ha^{-1} olivine to subplots of the previously bulk-limed plots. Frequent N_2O and CO_2 flux measurements by a field flux robot were started immediately to capture the CO_2 response of the not yet vegetated field. Adjacent plots with bulk, maintenance lime and control (not limed) were measured sequentially to allow direct plot-wise comparison of liming effects.

Only calcite maintenance treated plots had an increase in soil pH ($p<0.01$) but overall, there was no difference between bulk and maintenance limed effect on N_2O emissions ($p=0.91$). Comparing adjacent bulk- and maintenance limed plots showed that the N_2O mitigation effect of re-liming was confined to rain-driven emission peaks, suggesting that maintenance liming has the potential to facilitate more complete denitrification to N_2, irrespective of soil pH. Global warming potential calculated for the first two weeks after lime application revealed that none of the treatments decrease in N_2O emissions could offset increased CO_2 emissions. The magnitude of increase in CO_2 emissions two weeks after liming indicates that the increase is due to increased priming and not purely dissolution of carbonates.

Our results suggest that maintenance liming, as a component of good agricultural practice, may overstimulate N turnover rates and thus increase N_2O emissions. However, the amount of CO_2 released by dissolution of lime should be investigated over longer periods to fully explore the net mitigation potential of soil pH management for GHG emissions in crop production.

Key words: liming, N_2O emissions, soil pH, CO_2 emissions

1. Introduction

Liming is a common practice used in agriculture to counteract negative impacts of soil acidification (Weil, R. & Brady, N., 2017). Apart from improving yields and increasing P availability (Haynes, 1982), liming can also increase microbial activity (Shah et al., 1990) and in the long-term can increase carbon pool size and therefore decrease global warming (Fornara et al., 2011). Recently, liming has received raising attention as a possible mitigation tool for reducing denitrification derived N_2O emissions (Qu et al., 2014; Russenes et al., 2016). While some authors report decreases in N_2O emissions after application of lime (Hénault et al., 2019) others experienced no change (Barton et al., 2013; Feng et al., 2003; Hüppi et al., 2015) or an increase (Han et al., 2011; Zaman et al., 2008), which they attributed to either microbial activity shift from more complete denitrification to NH_3 oxidation (Baggs et al., 2010) or an increase in net nitrogen turnover rates (Byers et al., 2021). Most field studies on the N_2O reducing effect of liming so far focus on flux measurments of season right after bulk lime application (Hénault et al., 2019; Vázquez et al., 2020) but do not address the short-term effect of soil pH management, which can be controlled by maintenance liming. As field N_2O emissions are highly variable depending on season, weather and winter conditions more long-term field measurment campaigns are needed. In addition, production of N_2O is tightly coupled to CO_2 emissions (Sahrawat & Keeney, 1986) and carbon-sequestration may increase N_2O emissions (Li et al., 2005).

According to Tier 1 methodology by IPCC (2006) all applied lime is supposed to be lost as CO_2 emissions. However, there are studies suggesting that liming reduces soil organic carbon short-term by increasing soil microbial activity (Grover et al., 2017).

In the present study we evaluated short-term (maintenance) and long-term (bulk liming) effects of liming with dolomite, calcite and olivine on N_2O emissions throughout a growing season and CO_2 emissions the first 14 days after application of lime.

2. Materials and methods

Site and soil

The field site is located in SE Norway on a research farm of the Norwegian University Life Sciences (59° 49' N, 10° 47' E, 75 m a.s.l). The soil is classified as Retisols (WRB) and is artificially drained at appr. 1 m depth. The soil texture is 27% clay, 48% silt and 25% sand with a bulk densities of 1.18 g cm^{-3} at 10-15 cm depth (Bleken unpublished data). Mean annual temperature is 7°C, with mean annual precipitation of 1083 mm (Wolff et al., 2020).

The site was under crop rotation from 1954 to 2013 with no liming since the 1980s (Cuvardic et al., 2004). In 2013 the layout of the experiment consisted of 4x4 plots with three treatments (calcite, dolomite and olivine) and a control. Bulk lime was applied in 2013 to the field at a rate of 30 t ha^{-1} (except dolomite 24 t ha^{-1}). In 2014, it was sown to a grass mixture, which was ploughed under in autumn 2018 and kept as a fallow throughout winter and spring. In the beginning of June 2019, glyphosate was applied to kill regrowth and weeds before harrowing the field on 24th of June 2019 (0-15 cm), sowing it to winter wheat (*Triticum Aevestium*) and fertilized with 90 kg NPK-N ha-1 (YaraMila, 22:3:10). Maintenance lime was applied by hand to 2x2 m subplots in the already existing liming treatments (n=4) on the 24th June 2019 same day as the field was fertilized and sown. Lime was applied at rates of 1.7 (dolomite), 1.2 (calcite) and 5.0 (olivine) tons ha^{-1} in same form as in 2014. Dolomite and olivine were finely ground powder while calcite was applied pelleted, targeting a soil pH(H_2O) of 7.5 (Table 1). There was no attempt to incorporate the lime into the soil.

Aboveground biomass was mulched two times (19th August and 20th September), as we were not interested in agronomic yields in this experiment.

Table 1. Liming materials- composition, producer, grain size and application rate

Treatment	Dominant mineral	Producer	Grain size	Application (t ha^{-1})	CCE (%)
Dolomite	$Ca(Mg)CO_3$		powder	1.7	109
Calcite	$CaCO_3$		granulated	1.2	100
Olivine	95.1% olivine ($Mg_{1.86}Fe_{0.14}SiO_4$), with 3.0% orthopyroxene and 1.9% chlorite	Silbeco, Åheim, Norway	powder	5	65.7

Experimental design

Since the liming plots were embedded in a larger field trial without clear pH effect on N_2O (Todorcic Vekic et al., manuscript), plots for maintenance liming were established as subplots in originally bulk-limed plots. Using an autonomous field flux robot (see below) which moves from plot to plot, this allowed us to compare N_2O and CO_2 emissions plot-wise for bulk and bulk + maintenance liming (Fig. 1).

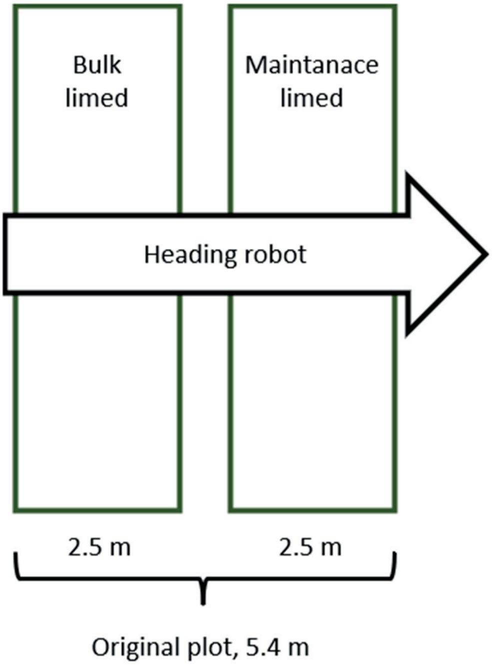


Figure 1. Layout of old limed (bulk) and re-limed limed plots (maintenance) and orientation of field flux robot's trajectory in the field.

N_2O and CO_2 measuring campaign

Measuring of N_2O emissions started on 26th June 2019 and lasted until 5th November 2019, consisting of 2618 flux estimates. CO_2 emissions were measured the first three weeks after lime application, 26th June to 15th July, before wheat started to germinate. Measurements were done with a field flux robot (FFR1) three times a week except for a short break in September.

Field flux robot

Field flux robot is an autonomous robot (Bakken Lars et al., 2015) powered by a lithium-ion battery pack (2 X 20 V) and consists of: (1) weatherproof instrument chamber (52x140x200 cm), (2) high precision real-time kinematic (RTK) GPS, (3) an inertial measurement unit (IMU) system, proprietary to Adigo AS (Figure 2). In our experiment the robot was equipped with a tunable diode laser spectrometer (DLT-100, Los Gatos Research, Mountain View, CA, USA) that measured N_2O, CO and H_2O, and a CO_2/H_2O infrared gas analyzer (LI-840A, LI-COR Inc., Lincoln, NE, USA). More details on functioning of the robot is described by Molstad et al. (2014).

Figure 2. Field flux robot (FFR) (Bakken Lars et al., 2015)

Flux calculations

N_2O fluxes were calculated based on the slope estimates of N_2O and CO_2 change during enclosure time, according to equation 1:

$$F_{N2O} = \frac{\mathrm{d}[\mathrm{N_2O}]}{\mathrm{d}t} \times \frac{V_c\, M_n}{A\, V_m}$$

Equation 1

where F_{N2O} is the flux (µg N_2O-N m^{-2} h^{-1}), $\mathrm{d}[\mathrm{N_2O}]/\mathrm{d}t$ the rate of N_2O concentration change in the chamber over time (ppmv h^{-1}), V_c the chamber volume (L), A the area covered by the chamber (m^2), M_n the molecular mass of N in N_2O (g mol^{-1}) and V_m the molar volume (L mol^{-1}) at chamber temperature (Tan et al., 2009). V_m was calculated according to the ideal gas law:

$$V_m = R\ \frac{T_c + 273.15}{P}$$

Equation 2

Where R is the ideal gas constant, T_c temperature in degrees Celsius, P pressure.

Given the short deployment time, the chamber temperature was assumed to be equal to the air temperature, which was taken from Yr database (MET, 2019).

Cumulative fluxes were calculated by linear interpolation in R studio (gasfluxes package version 0.4-1), R software 3.5.2.

Ancillary variables

Air temperature and precipitation data were obtained by a nearby weather station located in Ås (MET, 2019). Soil temperature and volumetric water content was measured with TDR soil probes (Decagon, ECH2O GS3, 0-10 cm, 70-MHz frequency), installed in 5 cm depth. Water filled pore space was calculated as a ratio of volumetric water content to known bulk density and particle density (2.65 g cm^3).

Mineral nitrogen and pH

Soil was sampled from 0-10 cm depth with a 15 mm soil corer once a week for a whole of July (3rd, 10th,18th and 27th), then once in August (2nd Aug) and once in September (19th Sep). Four cores were taken per plot and composited for determination of mineral N. Mineral nitrogen was determined colorimetrical by microplate reader (Infinite F50, TECAN Austria GmbH) at 540 nm in 1m KCl extracts. pH was determined in 0.1 mM $CaCl_2$ and water once a week from June to July and then once a month until September.

Statistics

All emission data was ln-transformed and tested for assumptions of linear relationship and multivariate normal distribution of residuals. A linear mixed effect model was used to test for treatments (fixed effects, four levels) effect on N_2O and CO_2 emissions.

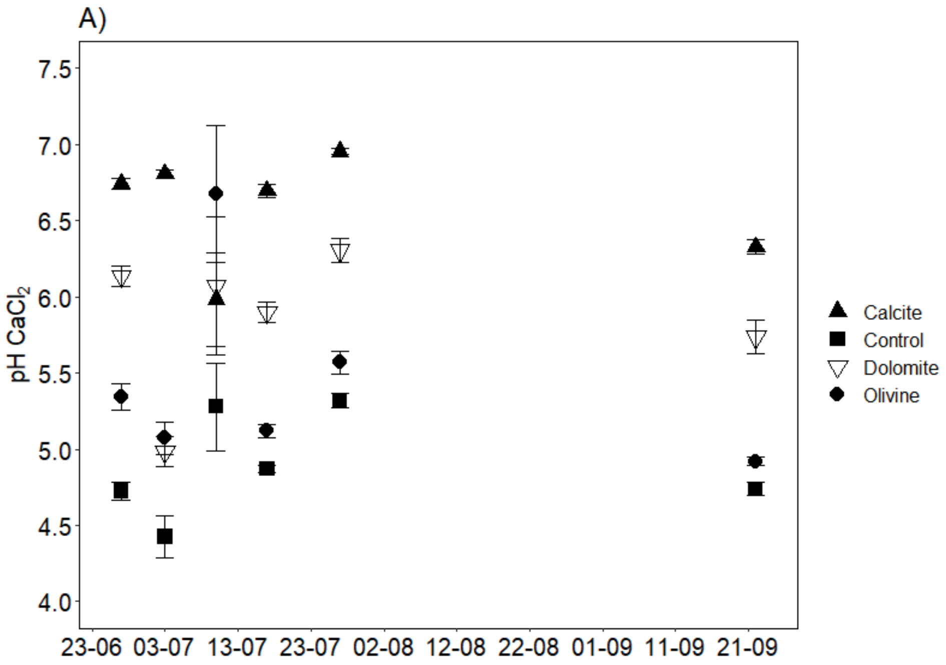
Variation in N_2O and CO_2 emissions between the 36 different plots and between 41 sampling days was modelled by means of random effects. Pairwise comparison was done to compare the effects of each treatment (bulk/maintenance), and to compare the effects of each treatment (bulk/maintenance) to the control. The emission data were adjusted for multiplicity by Bonferroni test (Bretz et al., 2016; Lenth et al., 2020). The proportion of the random effect variance attributable to each random effect was found by dividing each random effect by the sum the random effects (i.e., the total variance of the random effects). Differences in soil pH were tested with an additive general linear model (GLM) with soil pH as response variable and liming treatments and time as predictor.

All statistics were performed by R software, 4.1.2. R stats package (Hastie & Chambers, 1992), 4.0.2 package MASS (Venables & Ripley, 2002) for GLM and lme4 (Bates et al., 2014) for linear mixed-effects model.

3. Results

Soil pH

Over the course of three months the pH remained constant for all three treatments. There were significant differences among treatments over time for both maintenance (p=0.01) and bulk limed (p=0.01). Maintenance liming increased soil pH relative to bulk liming only in calcite treatment (p <0.01).



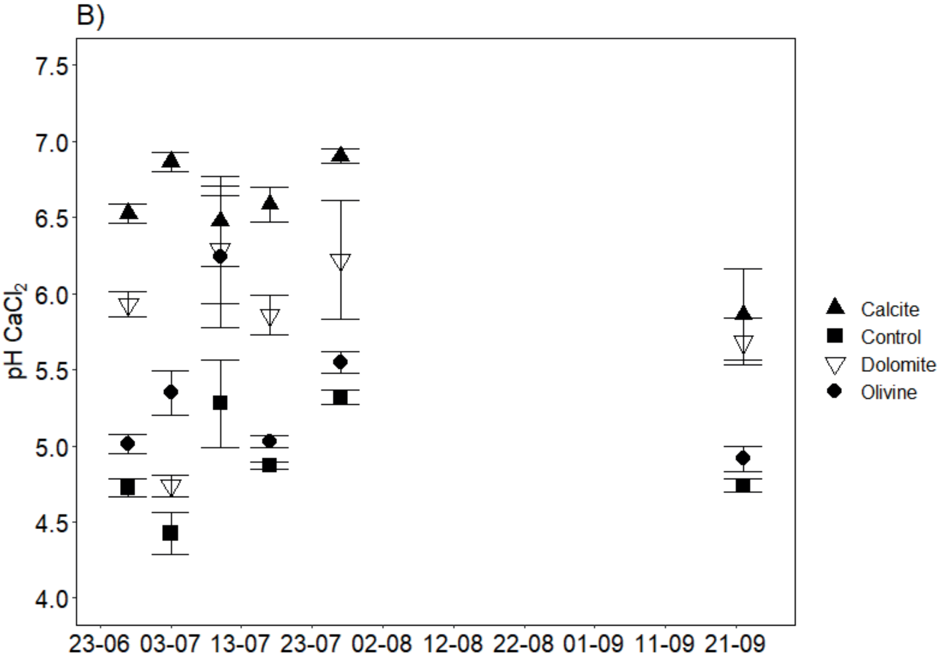


Figure 1. Soil pH in 0.01 M $CaCl_2$ for maintenance (A) and bulk limed (B) treatments from 25th June to 22nd September 2019.

N_2O emissions

Over the period of 4.5 months (24th June-5th November 2019) there were four consecutive emission peaks. The first emission peak was recorded on 24th June after fertilization, liming and sowing which was followed by precipitation (approx. 12 mm) and lasted for seven days. During the first emission peak control (not limed) had the highest daily flux with 966.5 µg N_2O-N m^2 d^{-1}, which was the highest recorded daily emission flux over the whole period of measurment campaign. The second emission peak occurred on 22nd July, after four days of rain (total 24 mm), during which dolomite- maintenance had the highest emission flux 599.8 µg N_2O-N m^2 d^{-1}. No measurements were conducted between 22nd July and 5th August; therefore it is uncertain for how long the second peak lasted. The third emission peak began on 30th August after 28 mm of rain and continued until 29th September. At the beginning of the third peak all treatments showed high emissions. The fourth emission peak began on 16th October after approx.40 mm rainfall between 17th and 20th October. Highest emission flux during forth peak was recorded for olivine-bulk 332.7 μg N_2O-N m^2 d^{-1}. We assume the fourth peak continued even after our last recorded measurements on 5th November, with first three days of freezing and thawing of the soil being recorded between 27th and 5th November. There were no fluxes recorded between 23rd and 26th October.

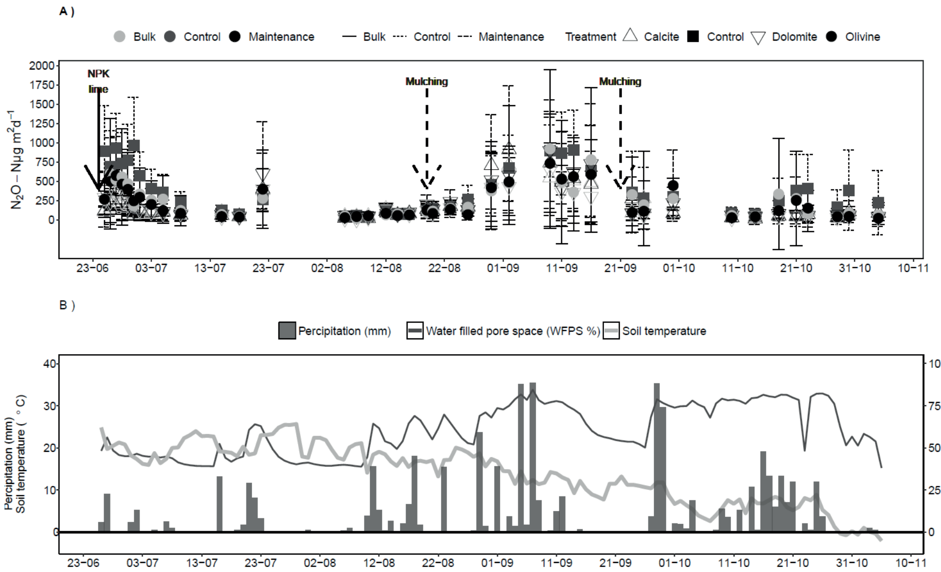


Figure 2. A) Mean N_2O-N μg m^2 d^{-1} emissions (n=4) in bulk lime, maintenance lime and control soil for calcite, dolomite and olivine; B) TDR soil probe hourly soil temperature, daily rainfall (mm) and water filled pore space (WFPS %) in top 10 cm.

There were no significant differences within group for maintenance lime (calcite/dolomite/olivine). However, lower emissions from bulk limed plots were significantly different from each other (difference within group calcite/dolomite/olivine, table 2)).

Table 2. Parameter estimates of a linear mixed effects model for average daily N_2O emissions for fixed (treatment) and random effects (plots, day number). P values indicate differences between treatments and control (not limed).

Fixed effects **Treatment**	**Estimate**	**N_2O-N emissions P value** **($p< 0.05$)**	**Random effects**	**Variance**
Control	5.28 ± 0.21		day number	0.77
Dolomite *maintenance*	4.61 ± 0.29	0.15	plot number	0.25
Calcite *maintenance*	4.55 ± 0.41	0.51	residuals	0.77
Olivine *maintenance*	4.75 ± 0.42	0.27		
Treatment *maintenance*	1.22 ± 0.59	0.91		
Dolomite *bulk*	4.45 ± 0.25	<0.01		
Calcite *bulk*	4.39 ± 0.25	<0.01		
Olivine *bulk*	4.59 ± 0.26	<0.01		

Most of variation is explained by day-to-day flux variation (43%) and by plot number (13%). A lot of variation in N_2O emissions was left unexplained (43%).

Overall, there were no statistical differences between maintenance and bulk liming, but each pair-wise comparison is significant (Table 3).

Table 3. Pairwise parameter estimates of a linear mixed effects model for average daily N_2O emissions for fixed (treatment) and random effects (plots, day number). P values indicate differences between treatments (without control).

Fixed effects **Treatment**	**Coefficients estimates** **with standard error**	**N_2O-N emissions** **P value** **($p< 0.01$)**	**Random effects**	**Variance***
Dolomite *maintenance*	4.70 ± 0.20		day number	0.80
Calcite *maintenance*	4.57 ± 0.21		plot number	0.28
Olivine *maintenance*	4.77 ± 0.17		residuals	0.82
Dolomite *bulk*	4.54 ± 0.20	<0.01		
Calcite *bulk*	4.41 ± 0.21	<0.01		
Olivine *bulk*	4.61 ± 0.21	<0.01		
Treatment *maintenance*	0.16	0.31		

*Ratio of measured variance and total variance expressed as percentage (%)

In pairwise comparison a large part of the variation in N_2O emissions was explained by day-to-day flux (42%), while plot number explained only 15% of the variation in N_2O emissions and 43% of the variation was left unexplained (residuals).

N_2O and CO_2 emissions

Maintenance limed

After liming N_2O and CO_2 emissions were measured simultaneously for the first three weeks of measurements, from 24th June to 15th July, while the soil was still fallow. Differences between treatments in emission patterns for N_2O and CO_2 emissions for clarity reasons are expressed in percentage of control (100%). N_2O and CO_2 emission peaks for all treatments were rainfall triggered. Overall, all treatments had lower N_2O-N emissions compared to 100% of control ($p<0.01$) and all treatments had an increase in CO_2-C emissions compared to control ($p>0.05$). Only calcite had significantly higher CO_2-C emissions compared to control ($p<0.01$).

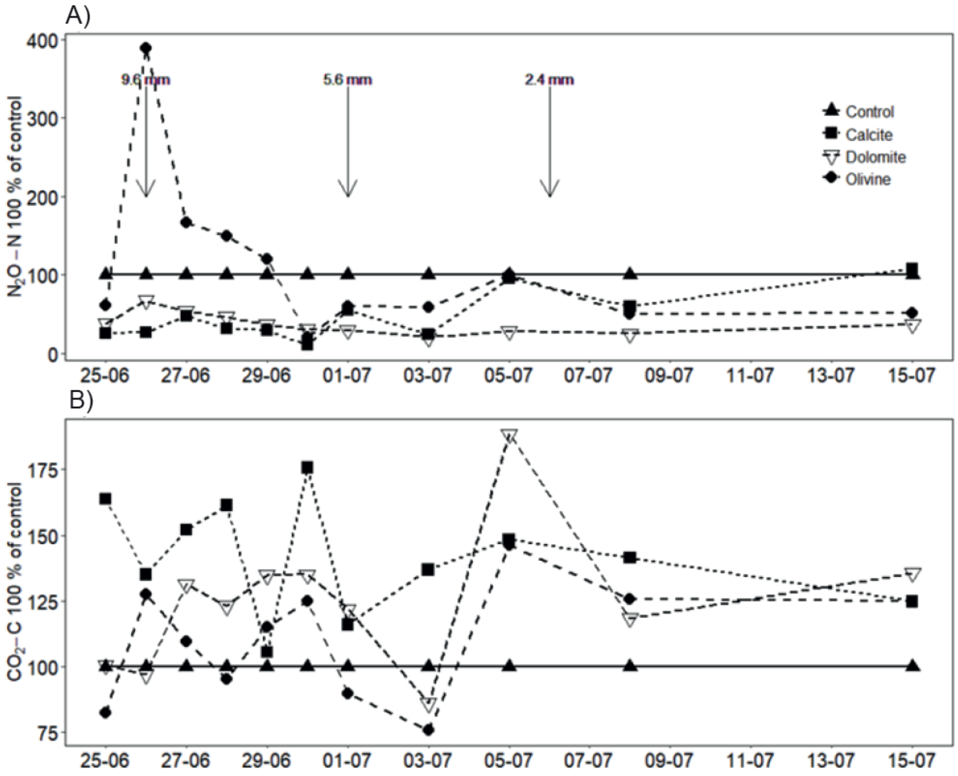


Figure 3. Maintenance limed N_2O-N (A) and CO_2-C emissions (B) expressed as 100 % of control.

Bulk limed

Plots limed in 2014, experienced similar emission peak trends as maintenance limed plots, but lower in N_2O rates. All treatments had lower N_2O-N emissions compared to control ($p<0.01$) and an increase in CO_2-C emissions compared to control ($p>0.05$). Again, only calcite had significantly higher CO_2-C emissions compared to control ($p<0.01$).

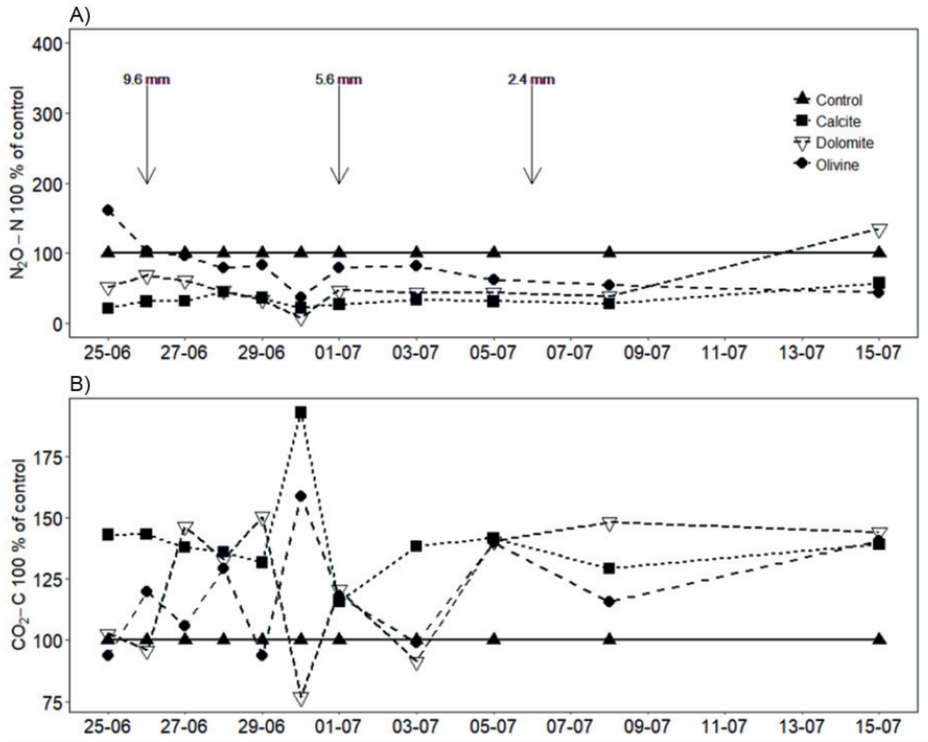


Figure 4. Bulk limed N_2O-N (A) and CO_2-C emissions (B) expressed as 100 % of control.

When converting N_2O emissions into CO_2 equivalents, for the first two weeks after liming (24th June to 8th July) we saw that all treatments had an increase in CO_2 so high that could not be offset by the decrease in N_2O emissions after liming application (Table 4.). Only olivine (both treatments) had the lowest increase in CO_2 emissions compared to control and other treatments ($p<0.01$).

Table 4. Change in N_2O emissions expressed as CO_2 equivalent relative to control, CO_2-C emissions in kg m^2 and sum of change in CO_2 and N_2O emissions as CO_2 equivalent over 14 days (24th June to 8th July 2019) for all treatments. Global warming potential of 265 was used for conversion of N_2O to CO_2 equivalent (IPCC, 2014).

Lime type	Treatment	N_2O-N g m^2	N_2O in CO_2 equivalent kg m^2 (GWP)	Δ N_2O (as CO_2 equivalent) kg m^2	CO_2-C kg m^2	Δ CO_2 kg m^2	Δ CO_2 + Δ N_2O as CO_2 equivalent* kg m^2
Control		1.19	0.495 kg		816.16		
Calcite	Bulk	0.33	0.128 kg	-0.367	1077.00	260.84	259.63
Calcite	Maintenance	0.35	0.145	-0.349	1133.11	316.95	315.65
Dolomite	Bulk	0.61	0.251	-0.244	1090.11	273.95	273.71
Dolomite	Maintenance	0.55	0.227	-0.268	1051.07	234.91	234.64
Olivine	Bulk	1.70	0.707	0.212	897.43	81.27	81.05
Olivine	Maintenance	1.44	0.599	0.103	820.86	4.7	4.8

4. Discussion and conclusions

Dolomite and calcite maintenance limed plots showed initially a similar increase in soil pH, but only calcite maintenance significantly ($p<0.01$) increased soils pH after 3 months. We believe the fluctuation of soils pH for both maintenance and bulk liming during the three months campaign was chiefly influenced by activities in the field and weather conditions, i.e. mulching, rainfall and fluctuating WFPS (Fig. 2) regardless of liming materials and their finesses (Tab. 1). As the instant rise in soil pH has effect on increasing soil microbial activity, priming effect and simulation of nitrification (Li et al., 2020) we assume this all had an impact on almost no change in soils pH.

We suspected we would see a negative relationship between N_2O emissions and soil pH (Hénault et al., 2019; Wang et al., 2018) as a result of full maturation of N_2O reductase (Bergaust et al., 2010; Liu et al., 2014), with maintenance limed treatments increasing soil pH and resulting in lower N_2O emissions, compared to bulk lime and control (not lime) but we did not find this effect. We did see overall lower N_2O emissions by maintenance limed compared to control, but this difference was not significant (Tab. 2). We believe that pH effect under field conditions in our study may have been obscured by the indirect influence of increased microbial activity and nutrient turnover rates that resulted in an increase in N_2O emissions rather than decrease (Baggs et al., 2010; Byers et al., 2021; Vekic et al., 2022).

Overall N_2O emissions peaks varied over the period of 4.5 months with highest emission peaks occurring after liming, sowing and fertilization, after rainfall. There were no significant differences between treatments during these events ($p>0.05$). Maintenance liming had higher N_2O emissions compared to bulk liming and lower than control but none of these differences were significant (Tab.2 and 3). Similar N_2O emission trends were observed on the same field in a study by Vekic et al. (2022) one, two and three years after bulk liming where highest emission peaks were fertilization and rainfall and freezing and thawing provoked. Towards the end of our study, in October 2019 it seems as if first freezing and thawing event began as soil temperature began to fall below 0℃ (Fig. 2), but the campaign ended too shortly after to be able to say if there was an effect of maintenance liming on magnitude of freezing and thawing induced N_2O emissions.

Additionally, CO_2 emissions may increase after application of lime due to dissolution of carbonates (IPCC, 2006) and stimulated priming (Grover et al., 2021). Thus, we measured simultaneously N_2O and CO_2 emissions for the first two weeks after liming. Both maintenance and bulk limed soils had an increase in CO_2 relative to the control, and decrease in N_2O emissions, with only calcite (both treatments) having this increase in CO_2 emissions statistically significant compared to control (Fig 2. and 3.). From the beginning of measured campaign, calcite had the highest pH and we believe high CO_2 emissions are a result of higher turnover rates as was concluded in a study by Vekic et al. (2022) done on the same field three and four years ago after bulk liming.

When converting N_2O emissions into CO_2 equivalents we saw that all treatments could not off-set the increase in CO_2 emissions with their lower N_2O emissions (Fig. 5). This poses a question of frequency of maintenance lime application. In Norway, it is common to lime the soil every 5-12 years depending on soil leaching (Nesheim, 2014). In our study, maintenance lime was applied 5 years after bulk lime, and even after 5 years, there was still an increase in CO_2 emissions relative to control from bulk treated plots. We believe than for all treatments, especially bulk treated plots, the magnitude of increase in CO_2 emissions could not be purely due to dissolution of carbonates and therefore we assume this increase is a result of enhanced priming caused by higher pH (liming) relative to control (not limed)(Grover et al., 2021). In addition, olivine is not a calcareous mineral and yet olivine bulk treated plots have had higher CO_2 emissions relative to control (Tab. 4) additionally corroborating the priming hypothesis. However, we conclude this based on a period of two weeks measurment campaign and thus this should be tested over longer period of time on fallow soil to fully explore the net mitigation potential of soil pH management for GHG emissions in crop production.

Based on our findings, we conclude that maintenance lime with carbonates should be applied after more than 5 years and in moderate rates to avoid overstimulation of nutrient turnover and high N_2O and CO_2 emissions.

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Paper III

3,4-dimethylpyrazole phosphate (DMPP) inhibition of nitrification and its N_2O yield in remolded soil and soil slurries

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Abstract

Application of 3,4-dimethylpyrazole phosphate (DMPP) has been proposed as mitigation option to reduce N_2O from soils with high pH by postponing NO_3^- oxidation in soils. There has been mounted evidence how DMPP affect alkaline soils in reducing their nitrification rates but how does this inhibitor affect recently limed soils it is yet unclear. Here we report a laboratory investigation of efficiency of DMPP inhibitor on three contrasting soils- Chinese alkaline oxisol, acid soil and recently limed (~4 years) clay loam soil from SE Norway in remoulded soils and slurries. Over period of 72 hours, nitrification rate, and N_2O emissions were measured in slurries and remoulded soils. Smallest dosage of DMPP (0.5% of added N) successfully inhibited ~60% N_2O from recently limed soil and ~80% of N_2O in Chinese alkaline soil in soil slurries, and ~80% in remoulded recently limed clay loam and ~90% in Chinese alkaline soil. Moderately acidic soil in remoulded incubation had ~10% inhibition and soil slurries ~30%, and very acid soil had almost no response in remoulded incubation and ~50%

inhibition by 0.5% DMPP of added N. We conclude that low dosage of DMPP successfully inhibits nitrification and N_2O production from recently limed soils, and alkaline soils but field investigations are needed as a proof of concept.

Key words: nitrification, N_2O, DMPP, soil pH, liming

1. Introduction

Nitrification inhibitors (NIs) have been suggested to increase nitrogen use efficiency (NUE) and their use has been recommended to reduce negative environmental impacts in agriculture due to mineral fertilizer application i.e. nitrate leaching and N_2O emissions (Chen et al., 2010; Zaman et al., 2009). In the past decade nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), has been used to do just that- reduce nitrogen loss and improve NUE (Gilsanz et al., 2016; Lam et al., 2017). In fertilized soils autotrophic nitrification is usually dominate source of nitrification activity, performed by ammonia oxidizing bacteria (AOB), nitrifying oxidizing bacteria (NOB) and ammonia oxidizing archaea (AOA), unless autotrophic nitrifiers are chemically inhibited (Robertson & Groffman, 2015), then heterotrophic nitrification may proceed by oxidation of NH_4^+ or organic-N (Laughlin et al., 2008; Mori et al., 2019; van Niel et al., 1993). Comammox (complete ammonium oxidation) microorganisms have been detected in soils but their quantitative importance is uncertain (Koch et al., 2019).

On the other hand, ammonia-oxidizing bacteria (AOB) have been identified as being a big source of N_2O in mineral soils as they have a much higher N_2O/NO_2^- than AOA's and as such pose a big environmental threat especially during growing season. In addition, some soils receive liming as a good agronomic practice to counteract excess acidity from growing season, which may stimulate both AOB's and AOA's growth. To selectively counteract this increase in microbial growth of AOB's, DMPP may be used to inhibits ammonia oxidizing bacteria (AOB) while ammonia oxidizing archaea (AOA) would be unaffected by this NI (Gong, P. et al., 2013). Mechanism of inhibition by DMPP is trough suppression of oxidation of NH_3 to hydroxylamine (ammonia monooxygenase AMO), possibly by removing copper (co-factor of ammonium monooxygenase) as a metal chelator (Ruser & Schulz, 2015). Variable inhibition by

DMPP has been observed, apparently depending on factors such as: by edaphic factors (Gilsanz et al., 2016), soil temperature (Gong, P. et al., 2013), nitrification potential of the soil (Mukhtar & Lin, 2019) and soil pH (Shi et al., 2016). Additionally, soil pH plays an important role as it is a niche determinant of AOA and AOB abundance in soils, with former dominating lower pH and latter higher pH environments (Hink, L. et al., 2018). Hence, lower DMPP efficiency in low-pH conditions for it affects AOBs, which are less abundant microbes in low pH soils. Since low pH soils are associated with low NUE, poorer yields and higher N_2O emissions losses due to denitrification liming has been proposed as mitigation tool (Hénault et al., 2019) for denitrification derived emissions as well increasing nutrient status of the soil. However, increasing soil pH results in increase of nitrification activity and thus N_2O emissions. Liming may induce nitrification driven N_2O emissions for two reasons: (i) enhance AOB over AOA, and this in itself will increase the N_2O production by nitrification because the two groups have widely different N_2O/NO_2^- product ratios: 0.01-0.1% for AOA and 0.1-1% for AOB and (ii) increase the nitrification rates, hence the potential for nitrification-induced heterotrophic denitrification. To our knowledge, thus far investigations on DMPP efficiency has been investigated in various cropped and not-cropped soils but no investigations have been done on recently limed soils.

Therefore, we aimed to study the influence of minimum added dose of DMPP of three contrasting soils: recently limed and unlimed clay loam soil from boreal climate, subtropical clay loam and temperate alkaline loess soil and its effect on nitrification rate, N_2O production and N_2O yields.

2. Materials and methods

Nitrification rates and N_2O production were determined in microcosms containing SE Norwegian grassland soil from a liming experiment (clay loam; pH 5.5 and 7.0), an alkaline loess soil from the North-China Plain (pH 8.3) and an acidic soil from subtropical China (pH 5.1). Ammonium amended microcosms were incubated for 4 days with increasing doses of 3.4-Dimethylpyrazole phosphate (DMPP), both as soil slurries and packed soils to assess the effect of DMPP on nitrification and its N_2O

production. An acetylene control was included (0.1 vol% C_2H_2) to inhibit NH_4^+ oxidation by both AOB and AOA where possible (Walter et al., 1979).

The state of the soils differed in that Chinese soils were air dried and sieved some time ago (2 weeks prior), whereas the Norwegian soils were sampled freshly, air dried, sieved (2 mm) right before the slurry experiment. Therefore, the Chinese soil was given 3 days after remoistening, to overcome any respiratory flush.

In both experiments, DMPP doses were expressed as µg DMPP mL^{-1}. However, the effective DMPP concentration in the soil slurries was highly diluted (~4 g dw soil in 40 ml 1mM NH_4Cl) resulting in concentrations of 0 – 0.27 µg DMPP ml^{-1}. When planning the experiment with remoulded soils, it was decided to allow for much higher effective DMPP concentrations in the soil solution as DMPP was expected to not readily and evenly diffuse through the entire soil volume. Nominal DMPP concentrations in the experiment with remoulded soil ranged from 0 – 6.75 µg mL^{-1}. The target soil moisture for the remoulded soil was 60% WFPS with a target bulk density of 1.1 g cm^3. However, we did not manage to compact the soil to this density (except for Norwegian limed, NL) without clumping the soil. Moreover, we corrected for soil moisture values (when calculating DMPP doses), resulting in the below set up for remoulded soil:

Table 1. Packed mesocosms properties

Short name	Soil	grav. WC before addition	dw soil in assays	Bulk density	WFPS	Added $NH4^+$-N per vial (µM)
NC	NOR control	0.20	10	0.99	0.6	1000
NL	NOR calcite	0.23	10	1.09§	0.6	1000
CO	CN oxisol	0.25	9.6*	0,87	0.6	960¤
CA	CN alkalic	0.26	9.8*	0,89	0.6	976

*because of erroneous gravimetric water content when packing the tubes

§ soil was clumped

¤ to avoid preparing various NH_4^+ stocks

The nitrification assay was run in 60 ml screw cap tubes, the accompanying N_2O assay in 120 ml serum bottles. The gas assay had a sixth treatment, namely zero DMPP + ~1 vol% acetylene. All tubes/flasks were incubated closed at room temperature (24°C). Nitrification tubes were sampled 4 times in triplicate in ca. 24 h intervals. Gases were measured twice a day. Together, there were 240 tubes and 72 gas bottles. Gas bottles were extracted with 1m KCl at the end of the experiment (ca. 72 hrs).

Nitrification rates were determined from NO_2^- and NO_3^- accumulation over time. Both NO_3^- and NO_2^- were measured colorimetrically using a microplate reader (Infinite F50, TECAN Austria GmbH) at 540 nm by Griess reaction (Keeney and Nelson, 1982), with NO_3 being converted to NO_2 by vanadium chloride (Doane and Horwath, 203). The N_2O kinetics were measured by the robotized incubation system, where samples are placed in a water bath securing constant room temperature (23°C) while being stirred at 550 rpm for 2 hours per day. After each run, the bottles were placed on a reciprocal shaker (125 rpm).

Soil slurries

A total of 36 bottles (Norwegian soil *3 replicates/treatment) and 39 (Chinese soil*3 replicates/treatment + additional treatment of high NH_4) was prepared. 5 g of each soil (fresh weight) were suspended in 40 mL $^{-1}$ mM NH_4Cl and an extra three bottles of Chinese alkalic soils in 2 mM 40 mL NH_4Cl. Over the course of 4 days they were measured for N_2O production once a day (1 ml) for NO_2^-+ NO_3^- analysis.

Both nitrification and N_2O production rates were corrected for “heterotrophic nitrification” or “$NO_2^-NO_3^-N$ immobilization” based on acetylene controls; this was not possible with packed soils, as no C_2H_2 could be applied to the plastic tubes.

Nitrification rate

Soils differed in inducible nitrification (without DMPP) following the order Chinese alkaline (CA)> Norwegian limed (NL)> Norwegian acid (NC)> Chinese acid (CO). This in fact reproduces the pattern observed with remoulded soil. However, comparing absolute rate, nitrification in agitated slurries was 1000 to 6000 times higher than in remoulded soil. We compared the rates obtained in slurries with those reported by Nadeem et al (2020), and they corresponded very well.

Inhibition was calculated as sum of DMPP sensitive (Vmsens) and insensitive ammonia oxidizers nitrification rate (Vi, presumably AOAs) and described by non-competitive inhibition model, which was used to estimate the half-inhibition coefficient, K_i by fitting the model to measured rates (minimizing the squared difference between model and measurements).

Modelled values calculated for non-competitive inhibition of the sensitive nitrifiers by the following equation 1:

$$V=Vi+Vmsens/(1+[DMPP]/ki)$$

Equation 1.

where Vi=nitrification rate of DMPP-insensitive ammonia oxidizers, Vmsens= maximum rate (in control) of the DMPP-sensitive ammonia oxidizers, and ki= is the “half saturation” constant for inhibition.

3. Results

Soil properties

Soils used in our study varied in their type, soil pH, land use and climate (Tab. 2). Both Chinese soils had moderately similar soil organic carbon, total nitrogen, and C/N, while Norwegian soils had slightly higher SOC compared to Chinese soils.

Table 2. Soil properties

Site	Handan, China	Changsha, China	Ås, Norway	Ås, Norway
Short name	CA	CO	NC	NL
Latitude	36^{o}52’N	28^{o}32’N	59^{o} 49’N	59^{o} 49’N
Longitude	115^{o}10’E	113^{o}19’E	10^{o} 47’E	10^{o} 47’E
T (°C)	14.3	17.4	7	7
P (mm)	502	1428	1038	1038
Climate	Temperate monsoon	Subtropical monsoon	Boreal	Boreal
Land use (crop)	Wheat-maize rotation	Rape-maize rotation	Not grazed grassland	Not grazed grassland
Soil type	Calcaric Cambisol	Haplic Acrisol	Albeluvisol	Albeluvisol
Soil texture	Sandy loam	Loam clay	Clay loam	Clay loam
pH	8.3	5.1	5.5	6.5
SOC (g kg^{-1})	10.2±0.1	10.7±0.2	13.5±1.4	14.8±1.4
TN (g kg^{-1})	1.1±0.1	1.1±0.1	0.28±0.04	
C/N	9.27±0.65	9.73±0.41	11.1±0.6	
NH_4^+-N (μg g^{-1})	8.40±0.67	32.20±2.72	46±5.16	51.08±7.55

NO_3^--N (µg g^{-1})	24.50±0.31	23.99±0.43	10.08±0.76	21.24±1.68
N rate (kg N ha^{-1})	Wheat: 200, Maize: 180 (Urea)	Maize:150 (Manure+Urea)	NPK-N (NH_4NO_3): 270	

Apart from difference in climate, Chinese alkaline soil has been fertilized with urea, while Chinese acidic was both with manure and urea. Rates of application were highest in Norwegian soils with 270 kg NPK-N ha^{-1} as ammonium nitrate. Both Chinese soils nitrification rates are similar, while in Norwegian soils, the limed soil has almost the double the nitrification rate after 4 years of liming that of control.

Remoulded soils

Nitrification rates

Chinese alkaline soils seemed to have a very high nitrification (Figure 1. B), whereas the other 3 soils (Chinese acid, Norwegian acid and neutral) have had similar (unrestricted) nitrification rates (Figure 1., A and B).

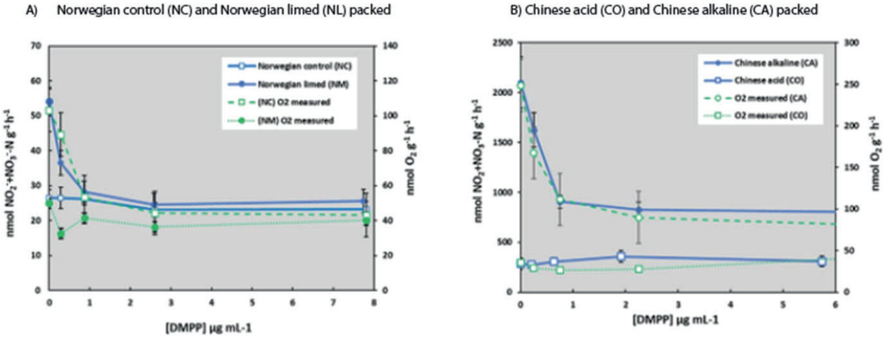

Figure 1. Nitrification rates and O_2 production rates expressed in nmol g^{-1} h^{-1} measured from (A) Norwegian control (NC) and Norwegian limed (NL); (B) in Chinese alkaline (CA) and Chinese acid (CO) in packed soils

DMPP response of nitrification

Chinese alkaline soils responded strongly; $DMPP_{max}$ 0,96 µg mL^{-1}; ca 1/3 of the nitrification activity could be inhibited by DMPP (Figure 1. B) while Norwegian neutral soils responded strongly; $DMPP_{max}$ at DMPP of 2,9 µg mL ^{1}; ca 50% of the nitrification activity could be inhibited by DMPP (Figure 1. A). Both acidic soils (CO and NC) showed no effect of DMPP on nitrification rates (Figure 1.).

N_2O production

N_2O production was measured independently from nitrification rates.

Chinese alkaline soils had abnormally high N_2O production. All other soils had similar (unrestricted by DMPP) N_2O production rates at around 1 ng N g^{-1} h^{-1}

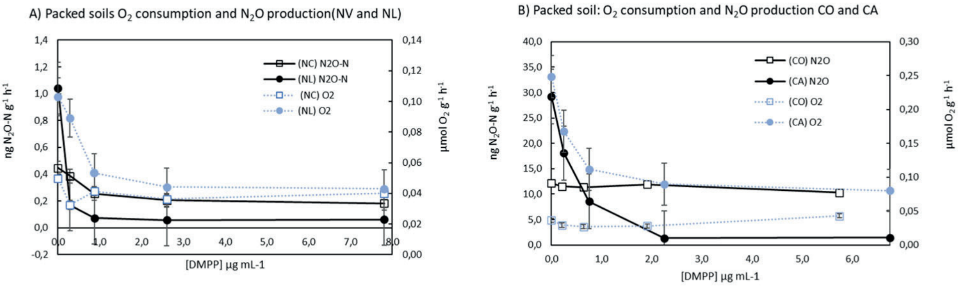

Figure 2. Response of N_2O and O_2 consumption in remoulded soil from (A) Norwegian and (B) Chinese soils at 60% WFPS. DMPP dose is still expressed as µg mL-1. Note different scaling of y-axis. NC: Norwegian control (acid soil); NL: Norwegian limed (neutral soil); CO: Chinese acid soil; CA: Chinese alkaline soil

DMPP response of N_2O production

Chinese alkaline soils showed strong DMPP response. The lowest DMPP dose inhibited a fair share of nitrification activity (ca. 50%).

Norwegian limed soil had a strong inhibition response $DMPP_{max}$ 80% of the N_2O production activity could be inhibited. Chinese acidic (CO) soil had no effect of DMPP on N_2O production and Norwegian control (NC) soil had an ordinary inhibition response; $DMPP_{max}$ ca 50% of the N_2O production activity could be inhibited.

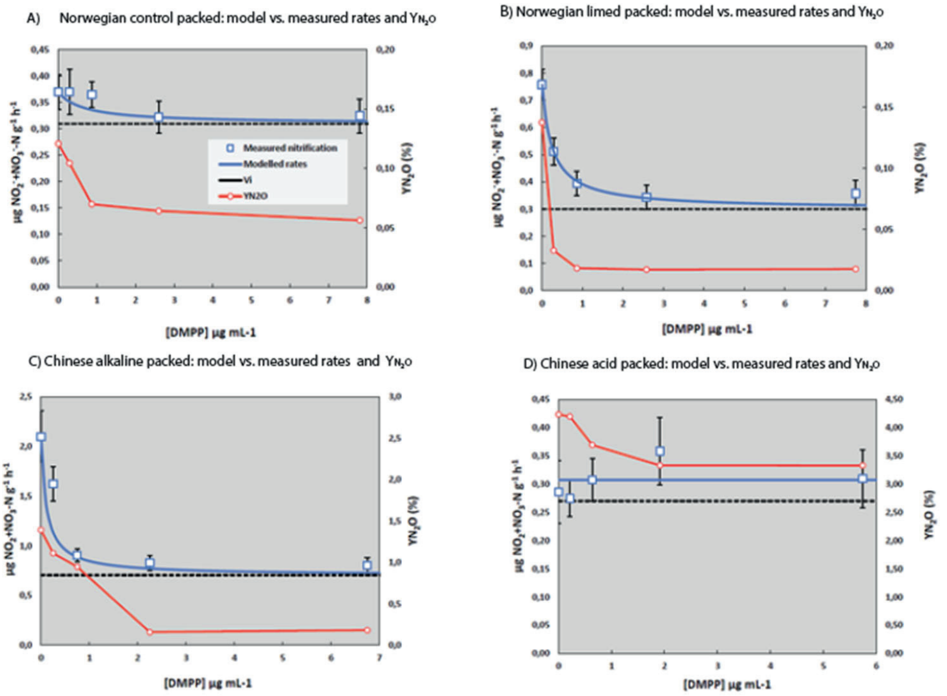

Figure 3. A) Modelled versus measured values and *Y_{N2O}* for Norwegian control packed (NC); B) Modelled versus measured values and *Y_{N2O}* for Norwegian limed packed (NL); C) Modelled versus measured values and *Y_{N2O}* for Chinese alkaline packed (CA); D) Modelled versus measured values and *Y_{N2O}* for Chinese acid packed (CO)

In both alkaline soils it appeared that, the higher the nitrification, the more efficient DMPP inhibition was, both with respect to $DMPP_{max}$ and completeness of inhibition. Rates were not statistically significant from each other. Norwegian control (NC) soil without DMPP had somewhat lower yield (*Y_{N2O}*) than Norwegian limed soil. *Y_{N2O}* was sensitive to DMPP. Modelled values for Norwegian limed and Chinese alkaline followed nicely the estimated rates (Fig. 3 B and C), while Chinese acid did not at all (Fig. 3, D).

Soil slurries

Nitrification rate

Soils differed in inducible nitrification (without DMPP) following the order Chinese alkaline > Norwegian limed > Norwegian control > Chinese acid soil. This in fact reproduces the pattern observed with remoulded soil. We compared the rates obtained in slurries with those reported by Nadeem et al (2020), and they corresponded very well. Chinese alkaline soils demonstrated gradual inhibition of nitrification with increasing DMPP, achieving maximum inhibition at 0.72 µg mL^{-1} DMPP (of added N) (Figure 4. B); for comparison: max inhibition in packed alkaline soils was reached with 0.75 µg ml-1 DMPP (Figure 1.B); this can be explained by higher dilution, hence lower effective DMPP concentrations in the slurries (obviously, the increased contact to the inhibitor in the slurry did not outweigh the reduced concentration). In alkaline slurries, a maximum of ~70% of nitrification was inhibited, which was about the same as in the packed alkaline soil.

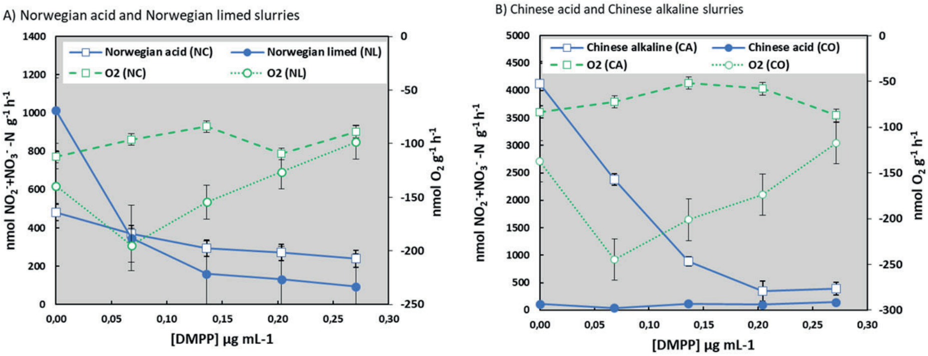

Figure 4. Nitrification rates and O_2 consumption rates in soil slurries: (A) Norwegian acid (NC) and Norwegian limed (NL); (B) Chinese acid (NC) and Chinese alkaline (CA). Note y-axis.

In Norwegian limed like in Chinese alkaline, the inhibition of nitrification by DMPP seems to be somewhat more gradual as compared with its packed counterpart (Figure 1 and 4, both A). In contrast to Chinese alkaline, the maximum achievable inhibition in soil slurries (~84%) was higher than in packed Norwegian neutral soil (~60%). As with packed soils, there was little inhibition by DMPP in Norwegian control and Chinese acid slurries (Figure 1 and 3, both A).

N_2O production

Chinese alkaline soil slurries without DMPP had much lower N_2O production (Figure 5. B). Norwegian limed soil had higher N_2O production but were more inhibited by DMPP than acidic soil (Figure 5. A). Adding DMPP to Chinese acid soils strongly increased N_2O production before it fell back to low rates at higher DMPP doses (Figure 5 B).

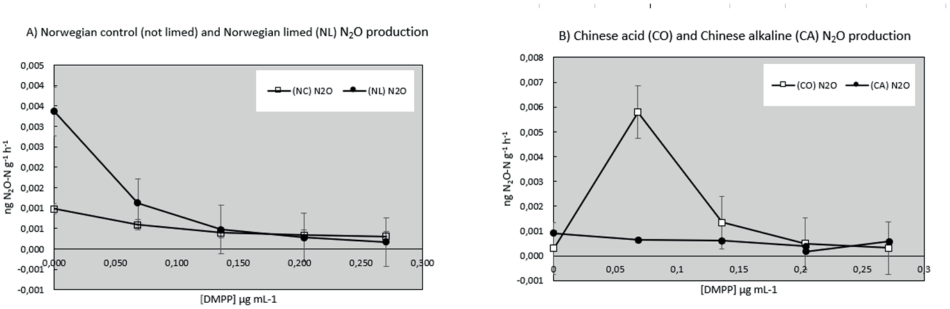

Figure 5. Response of N_2O production in soil slurries from (A) Norwegian and (B) Chinese soils at 60% WFPS. DMPP dose is still expressed as µg mL^{-1}. Note different scaling of y-axis. NC: Norwegian control (acid soil); NL: Norwegian limed (neutral soil); CO: Chinese acid soil; CA: Chinese alkaline soil.

In Chinese soil slurries, O_2 consumption was lower in the Chinese acid than the alkaline loess soil. Which was also the case in packed Chinese soil; higher O_2 consumption rates in alkaline soil than acidic soil.

Measured vs modeled measurements and Y_{N2O}

In Chinese alkaline soil slurries Y_{N2O} decreased from very low values (~0.0002%) to values (0.00010%) (Figure 6. C). The Chinese acid soils, Y_{N2O} without DMPP was 0.00022 but increased to 0.011% with the addition of 0.068 µg DMPP mL^{-1} before decreasing with increasing DMPP to almost control levels. Also, Chinese acid slurries demonstrated NO_2^- accumulation (Supplementary data Fig. 4). Two out of three controls had substantial NO_2^- accumulation (black squares), whereas all other treatments accumulated some NO_2^- with 0.068 µg mL^{-1} DMPP and less with higher DMPP doses. 50 nmol NO_2^- corresponds appr. to 0.7 µg N g^{-1} which is in the same order of magnitude as observed with packed Chinese acid soil (Supplementary data Fig. 2).

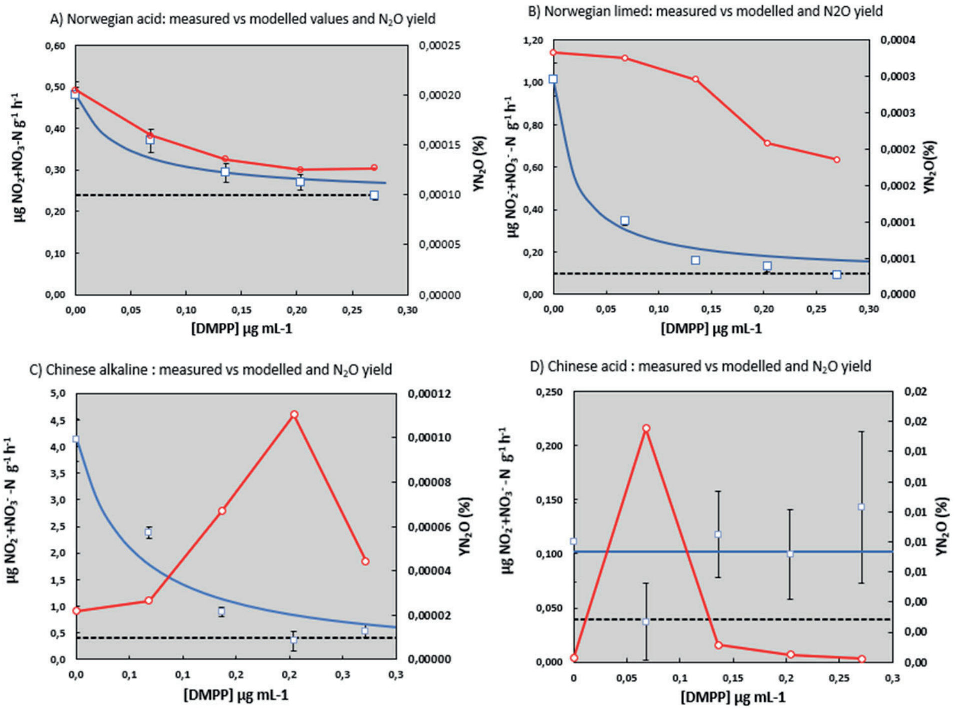

Figure 6. A) Modeled versus measured values and Y_{N2O} for Norwegian control slurries; B) Modeled versus measured values and Y_{N2O} for Norwegian limed slurries; C)) Modeled versus measured values and Y_{N2O} for Chinese alkaline slurries; D) Modeled versus measured values and Y_{N2O} for Chinese acid slurries

Y_{N2O} in slurries with Norwegian limed and control soil behaved like expected, both with respect to Y_{N2O} level and DMPP response. Like with packed Norwegian soil, DMPP reduced Y_{N2O} of limed Norwegian soil (Figure 6 A). The increase in Y_{N2O} of Chinese acid slurries upon adding DMPP followed with decreased O_2 consumption (Figure 5 B).

Table 3. Parameters of measured (rate) values and modeled values for all soils in remolded and soil slurries (Figures 1 and 3). Ki is the DMPP concentration that is needed to reach 50% inhibition.

Rates, μg N g^{-1} h^{-1}

Soil	Treatment	DMPP (μg ml-1)	Vi	Vmsens	Ki	Vtot
			insensitive	sensitive		
NC	packed	0-7,81	0,31	0,06	0,67	0,37
pH=5.5	slurry	0-0,27	0,24	0,24	0,04	0,48
NL	packed	0-7,74	0,30	0,46	0,25	0,76
pH=6.5	slurry	0-0,27	0,10	0,91	0,02	1,01
CA	packed	0-6,75	0,71	1,40	0,11	2,10
pH=8.3	slurry	0-0,27	0,00	4,19	0,05	4,19
CO	packed	0-5,74	0,31	0	Nd*	0,31
pH=5.1	slurry	0-0,27	0,10	0	Nd*	0,11

* not detectable

Ki was not determined for the Chinese acid (both soil and slurry, see table 3). We could not determine Ki for this soil because the activity of DMPP-sensitive bacteria was not detectable (the rate did not decline with increasing concentrations of DMPP)

4. Discussion

It is difficult to work with nitrification N_2O production across soils with widely differing nitrification potentials, because soils with high nitrification potentials may induce denitrification when nitrifying unconstrained by DMPP demonstrating the potential of NIs for N_2O mitigation (Torralbo et al., 2017).

It is unclear which processes mediate nitrification in the subtropical Chinese acidic soil: even though there was a decent measurable nitrification activity (0.38 μg N g^{-1} h^{-1}packed and 0.13 μg N g^{-1} h^{-1} slurry), DMPP had no measurable effect (Figure 1 and 3

D). Acetylene treatments in the soil slurry experiment (Fig.3 D) suggested that ~68% of the total nitrification related N_2O production was by 'heterotrophic' nitrification, which would explain the apparent insensitivity of nitrification to DMPP by dominance of heterotrophic nitrification. The fraction of DMPP-sensitive nitrification (presumably AOB) increases with soil pH (Table 3.) and is higher in the slurry than in intact soil. Inhibition coefficient (Ki) in slurries is much lower than in intact soils and appears to be unaffected by pH. Ki estimate for NL is very uncertain.

With respect to pH, the choice of soils is a bit unlucky, as we compare soils with contrasting pH from a liming trial (Norway; same soil, different pH) and from geographically distinct locations (China; grossly different soils, extremes). Potential nitrification rate (without DMPP) increased with soil pH (Figure 1 and 4, note different y-axis), with nitrification in slurry (Figure 4.) being higher than in remoulded soils (Figure 1.) except for the most acid soil (CO).

In packed soils Chinese alkaline soils, the unconstrained nitrification rate was 0.305 $\mu g\ N\ g^{-1}\ h^{-1}$; and high Y_{N2O} of ~ 2% could be due to this. We interpret this as some sort of induced coupled nitrifier denitrification (Shi et al., 2016; Shi et al., 2017). This would mean that DMPP may be very efficient in preventing coupled nitrification-denitrification after NH_4^+ fertilization under field conditions, as it suppresses excessive O_2 consumption by nitrification. The Norwegian soils showed a decrease in Y_{N2O} both for limed and acidic (Figures 3 and 6, A-B). DMPP suppressed N_2O in acidic soil, even though no effect was seen on nitrification, which may have to do with contrasting sensitivities for the two endpoints (nitrification versus N_2O). In general, Y_{N2O} of soils with high pH, likely AOB dominated, nitrification is more sensitive to DMPP than Y_{N2O} of soils with smaller nitrification potential (Mukhtar & Lin, 2019; Shi et al., 2016). This is in line with the idea that DMPP acts predominately on AOB (high inherent Y_{N2O}) and not on AOA (low inherent Y_{N2O}) (Dong et al., 2013; Shen et al., 2008). Hence, DMPP should be an efficient inhibitor in cultivated, limed soils supporting high AOB/AOA ratios (Mukhtar & Lin, 2019).

In Chinese alkaline-packed soil, Y_{N2O} increased from very low values (~2%) to values (0.015%) expected for AOB-dominated soil. We have no idea what caused the atypical low N_2O production potential in Chinese alkaline control soil. The shaking has been implicated to selectively inhibit AOA (Laanbroek et al., 2013), in the Chinese alkalic, there should hardly be any AOA. The Chinese acid, by contrast, showed another anomaly with DMPP: Y_{N2O} without DMPP was 0.17 ± 0.03, which is a bit high for a putatively AOA-dominated soil. The addition of 0.68 µg mL^{-1} DMPP resulted in a sharp increase of Y_{N2O} to 0.01% before decreasing with increasing DMPP to control levels. We interpret this as chemodenitrification due to the observed NO_2^- accumulation in the Chinese acid slurries. Two of the three controls (no DMPP) had substantial NO_2^- an accumulation (Supplementary data Figure 4), whereas all other treatments accumulated some NO_2^- with 0.68 µg mL-1 DMPP and less with higher DMPP doses. With 50 nmol NO_2^- corresponds appr. to 0.7 µg N g^{-1} which is in the same order of magnitude as observed with packed Chinese acid soil. Nitrite under acidic conditions may chemically decompose to N_2O and may thus explain the erratically high N_2O production rates, probably because DMPP affects NOBs, which may result in transiently increased NO_2^- pool sizes. Most intriguingly, the increase in Y_{N2O} of Chinese acid soil slurries upon adding DMPP "correlated" with increased O_2 consumption. In Chinese soil slurries, O_2 consumption was higher in the acid soils than in the alkaline loess soil. This was not the case in packed Chinese soil; here alkalic soil had much higher O_2 consumption rates than acidic soil. This may point towards some wired O_2 sink in the acid slurries, probably related to the oxidation of trace metals which are very abundant in this type of soil. In Norwegian limed slurries Y_{N2O} and control-acid soil behaved as expected, both with respect to Y_{N2O} level and DMPP response. Like with packed Norwegian soil, DMPP reduced the Y_{N2O} of neutral Norwegian soil.

We conclude that the application of low concentration of DMPP (0.68 µg mL^{-1}) in recently limed soils (~4 years) can inhibit up to 50% of nitrification and N_2O yield, but this effect should be also tested in the field*.* The addition of DMPP in Chinese acid soil seem to have induced chemo denitrification, while exceptionally high nitrification rate in Chinese loess alkaline soil, which we attribute to nitrifier denitrification. In both higher pH soils, Chinese alkaline and clay loam Norwegian limed soil, DMPP was successful in significantly reducing N_2O emissions ($p<0.05$).

5. References

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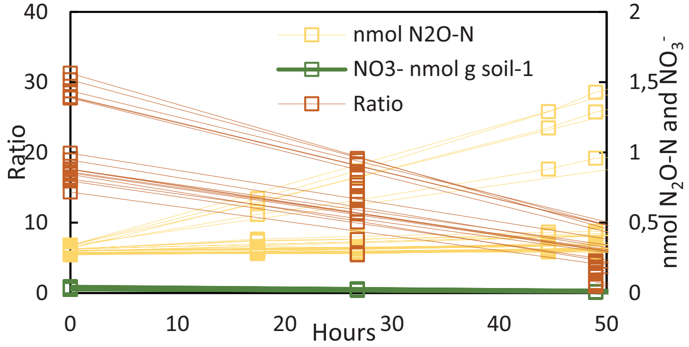
Supplementary data

A)



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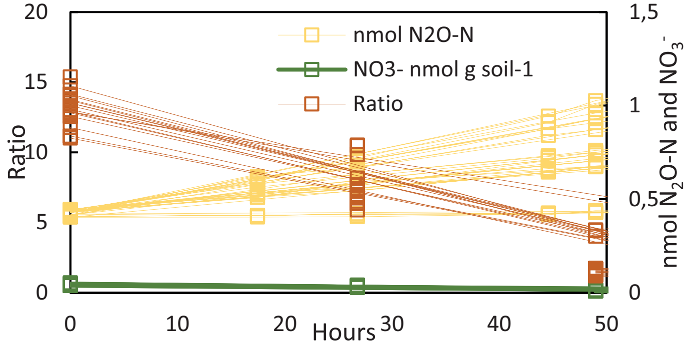

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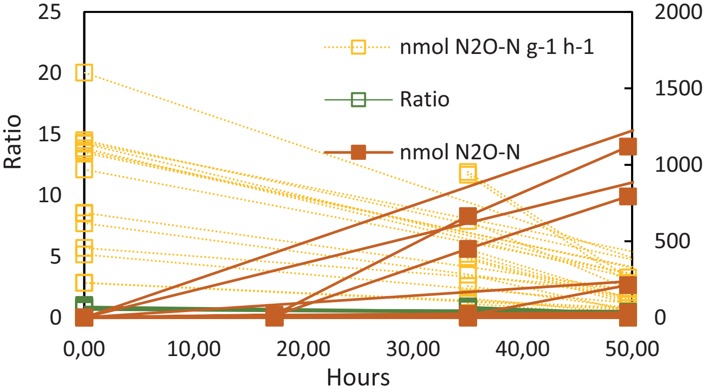


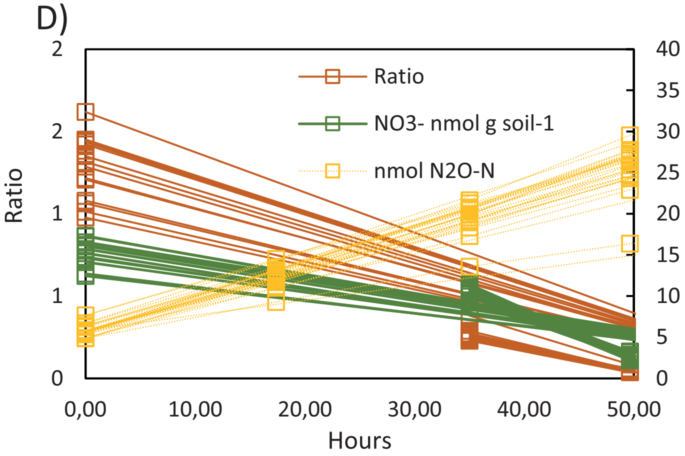
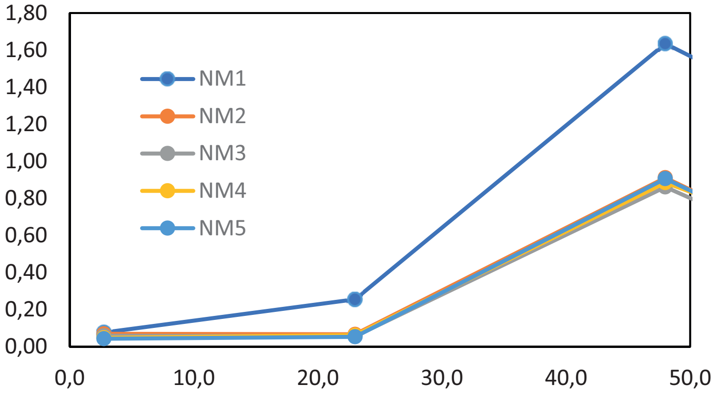
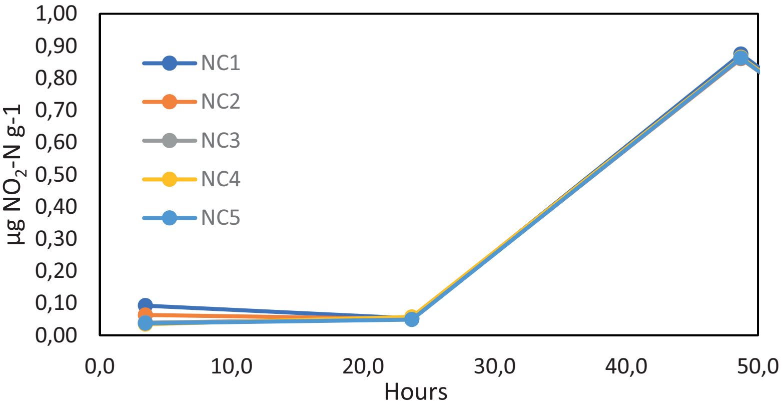


Figure 1. Raw [NO_3^-] and N_2O in remolded soils for (A) Norwegian control (NV); (B) Norwegian limed soil (NL); (C) Chinese acid (CO) and (D) Chinese alkaline (CA) soil

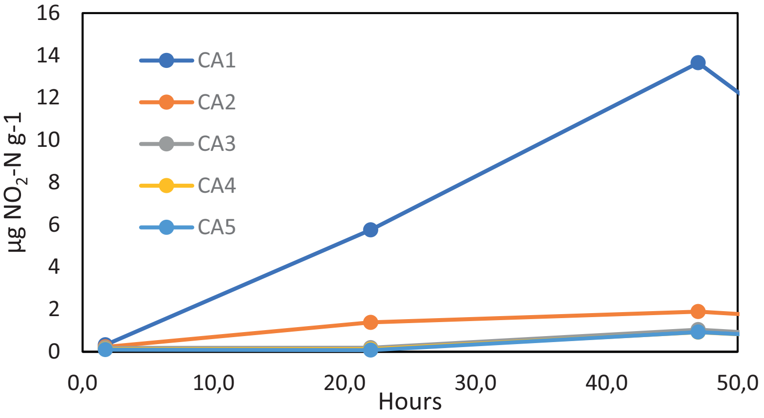
A)


1,80
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
0,0
10,0
20,0
30,0
40,0
50,0
NM1
NM2
NM3
NM4
NM5

B)


μg NO_2-N g-1
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
0,0
10,0
20,0
30,0
40,0
50,0
Hours
NC1
NC2
NC3
NC4
NC5

C)


μg NO_2-N g-1
16
14
12
10
8
6
4
2
0
0,0
10,0
20,0
30,0
40,0
50,0
Hours
CA1
CA2
CA3
CA4
CA5

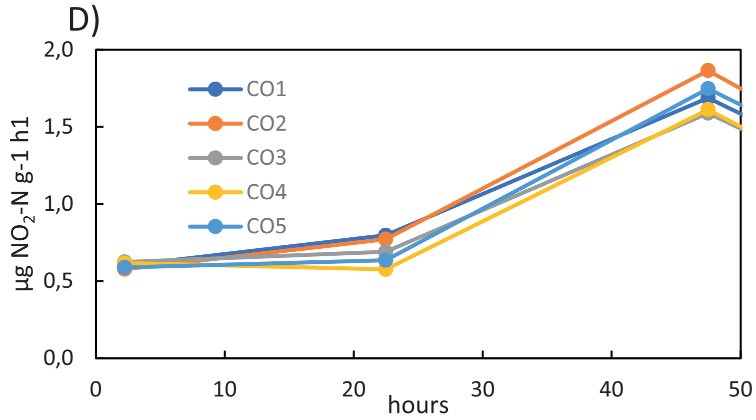
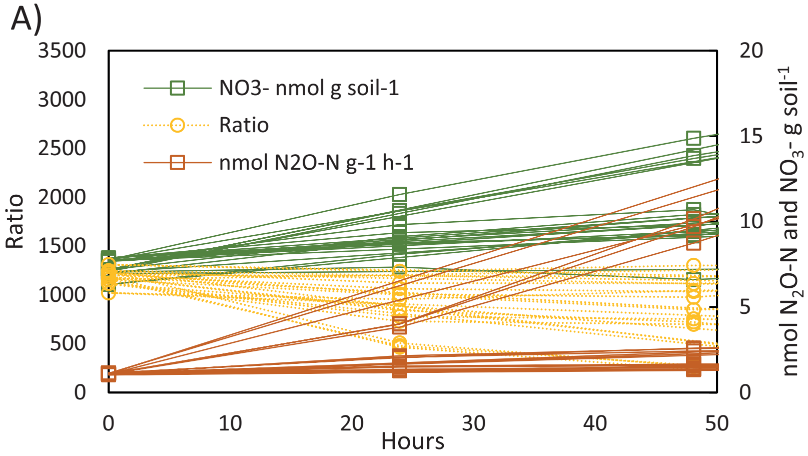


Figure 2. [NO_2^-] in remolded soils for (A) Norwegian control (NV); (B) Norwegian limed soil (NL); (C) Chinese acid (CO) and (D) Chinese alkaline (CA) soil



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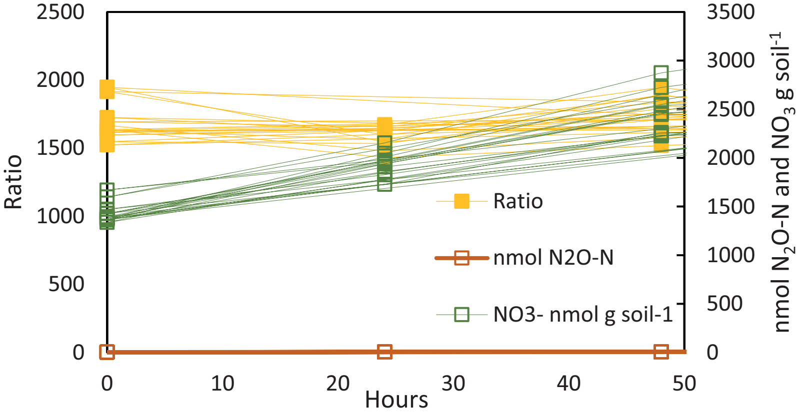
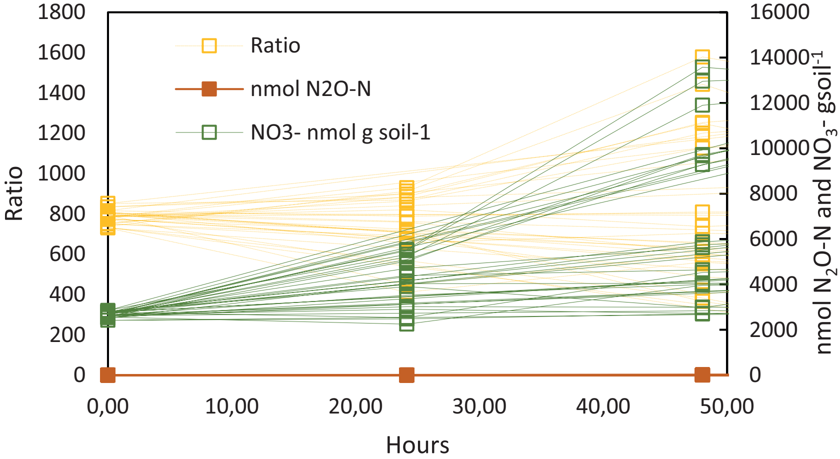

2500
2000
1500
1000
500
0
Ratio
3500
3000
2500
2000
1500
1000
500
0
nmol N_2O-N and NO_3 g soil^{-1}
0
10
20
30
40
50
Hours
Ratio
nmol N2O-N
NO3- nmol g soil-1

C)


1800
1600
1400
1200
1000
800
600
400
200
0
Ratio
16000
14000
12000
10000
8000
6000
4000
2000
0
nmol N_2O-N and NO_3^- gsoil^{-1}
Ratio
nmol N2O-N
NO3- nmol g soil-1
0,00
10,00
20,00
30,00
40,00
50,00
Hours

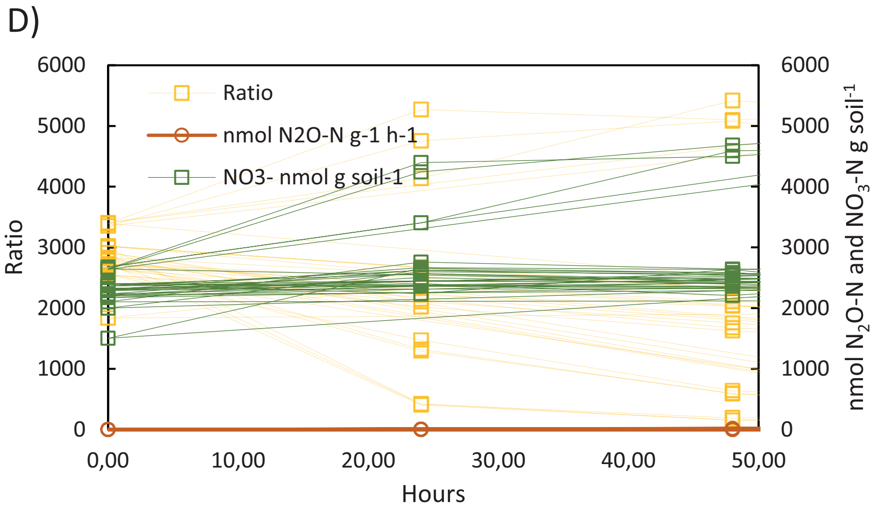
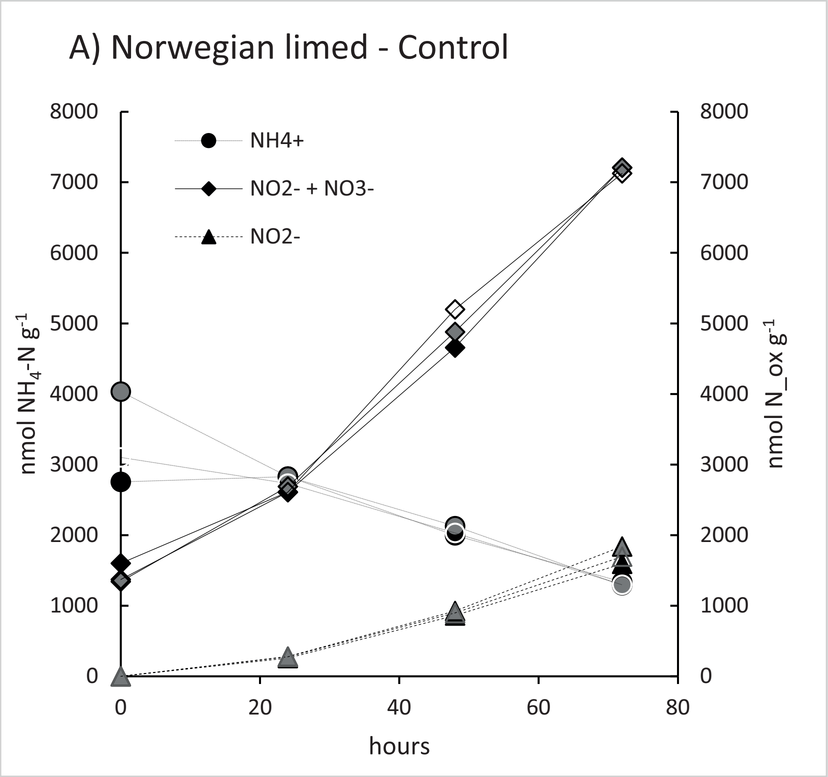
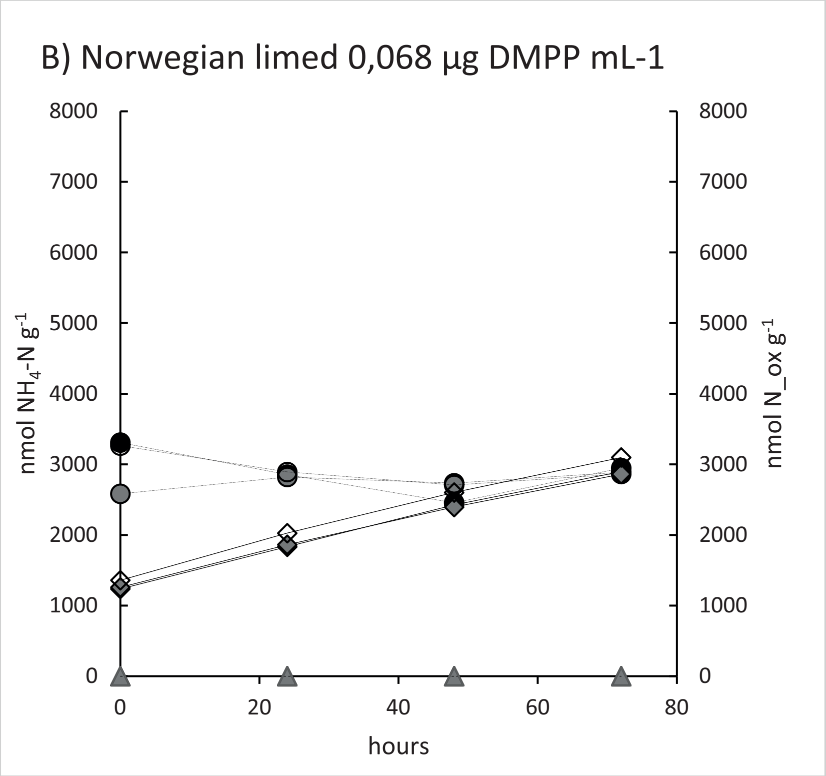
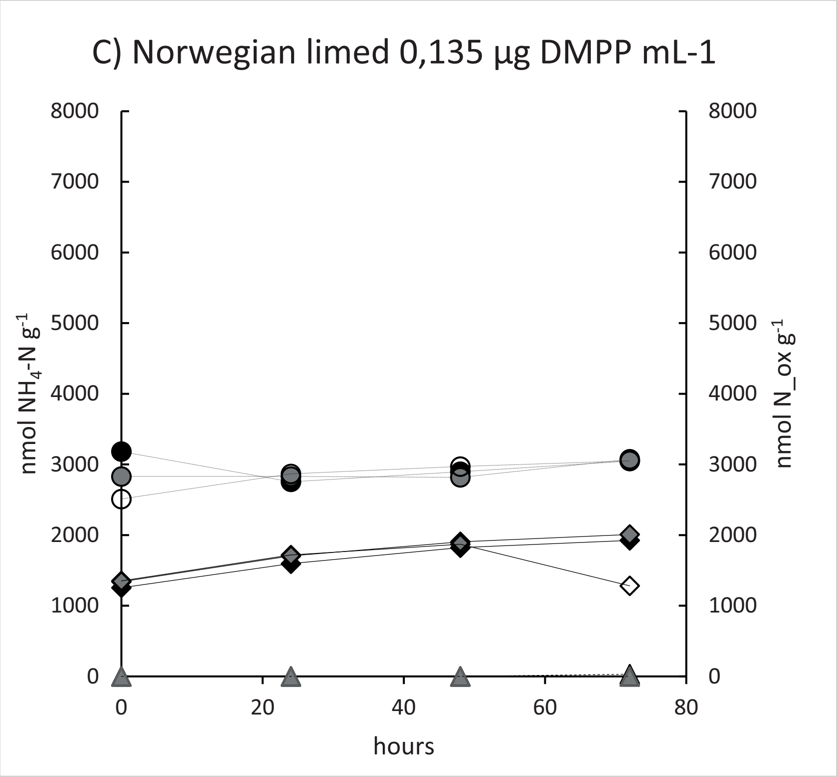
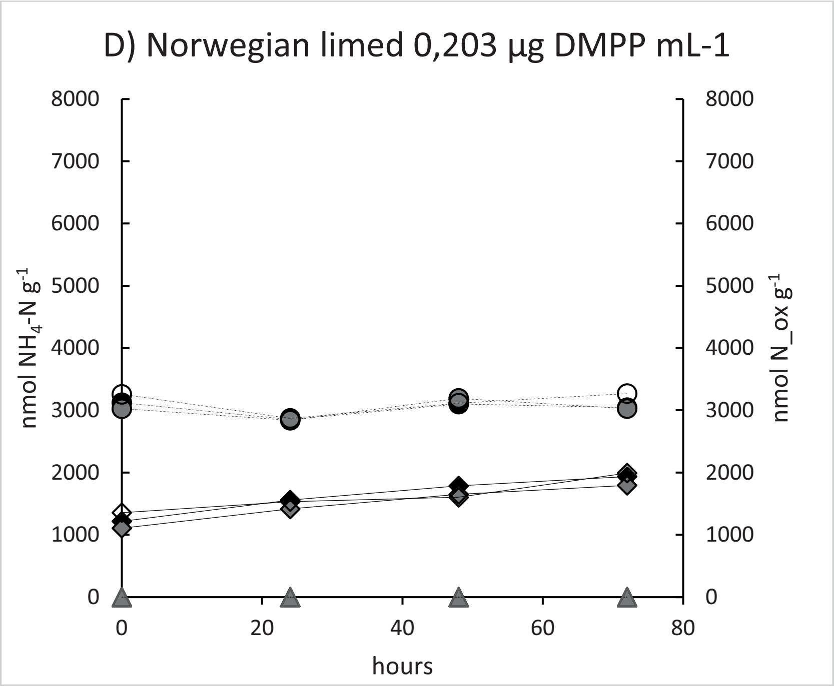


Figure 3. Raw [NO_3^-] and N_2O in soil slurries for (A) Norwegian control (NV); (B) Norwegian limed soil (NL); (C) Chinese acid (CO) and (D) Chinese alkaline (CA) soil




B) Norwegian limed 0,068 µg DMPP mL-1
nmol NH4-N g-1
nmol N_ox g-1
hours
0
1000
2000
3000
4000
5000
6000
7000
8000
0
20
40
60
80


C) Norwegian limed 0,135 µg DMPP mL-1
8000
7000
6000
5000
4000
3000
2000
1000
0
nmol NH4-N g-1
nmol N_ox g-1
0
20
40
60
80
hours



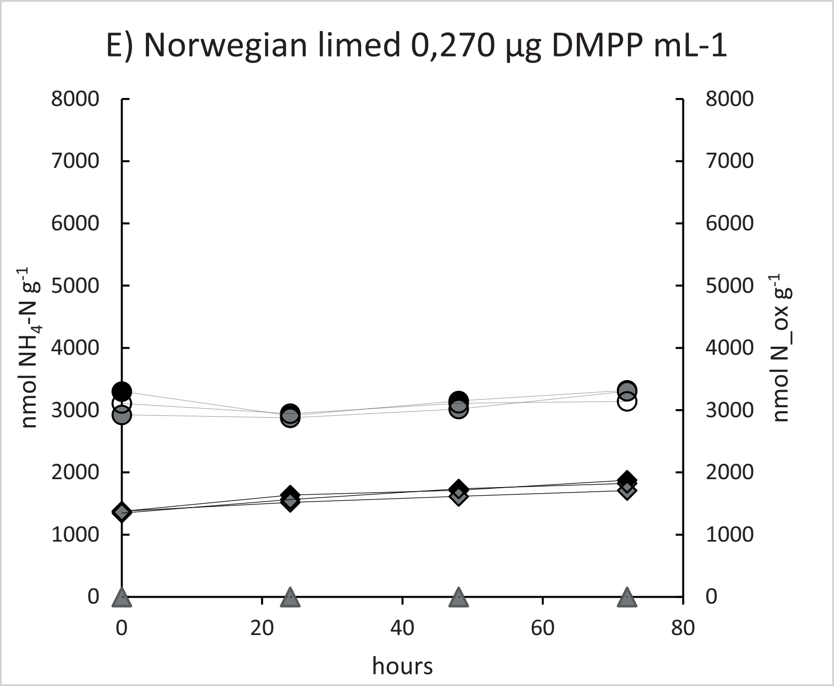
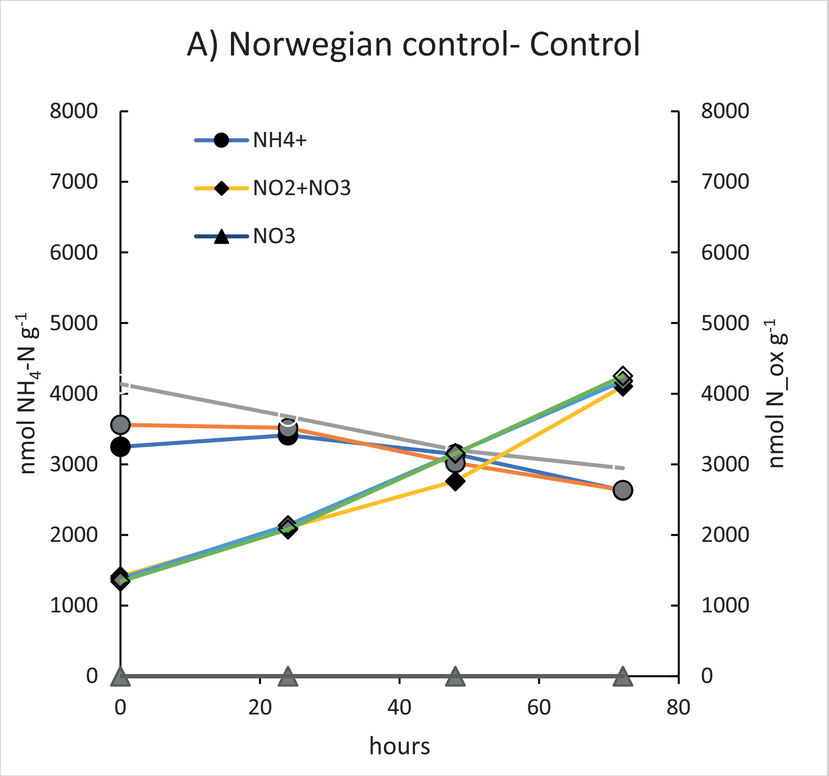
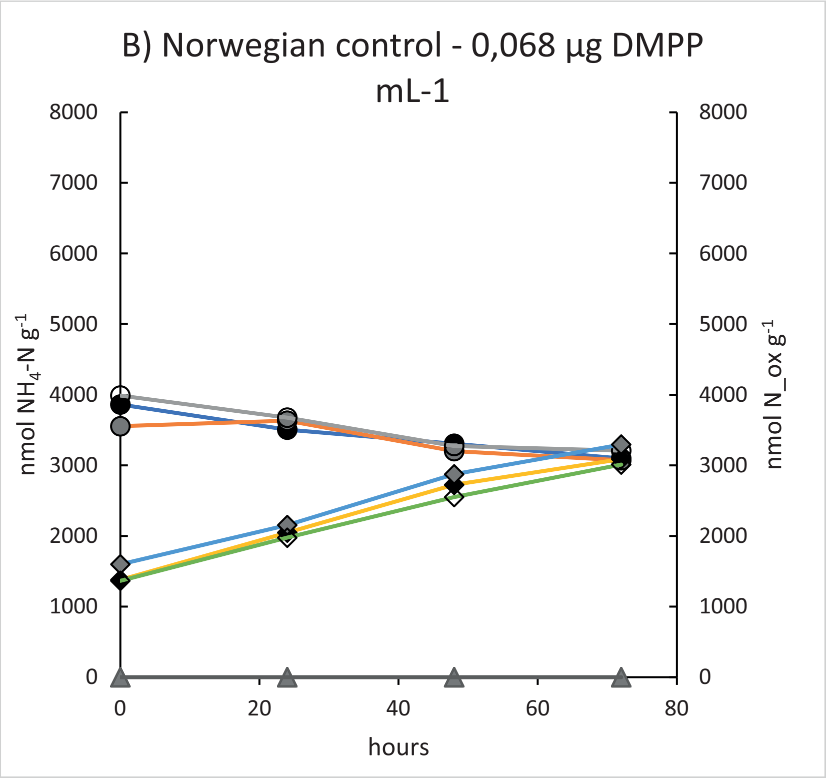
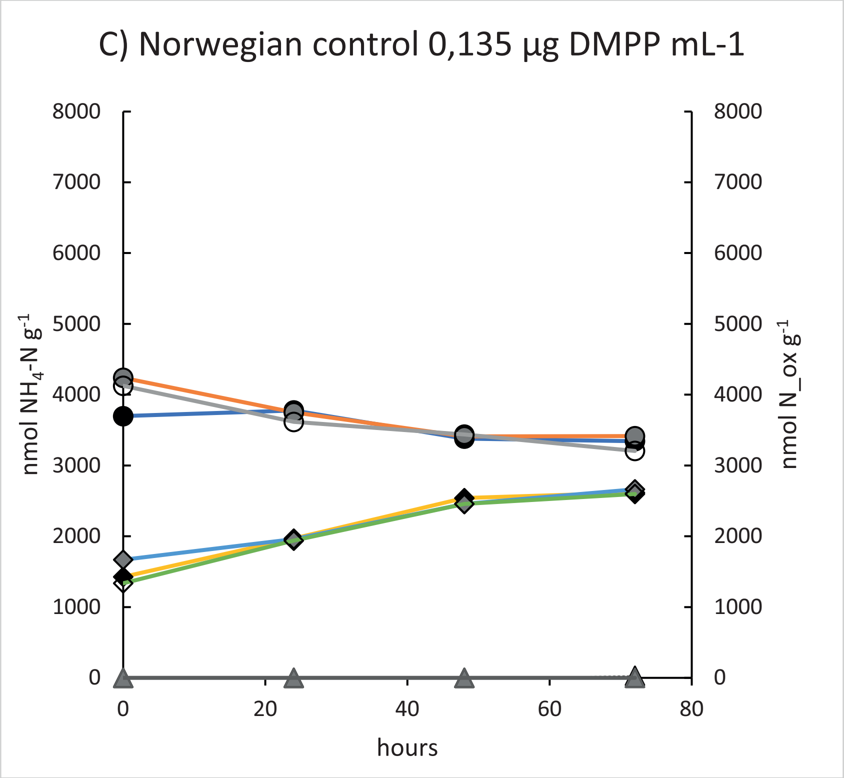


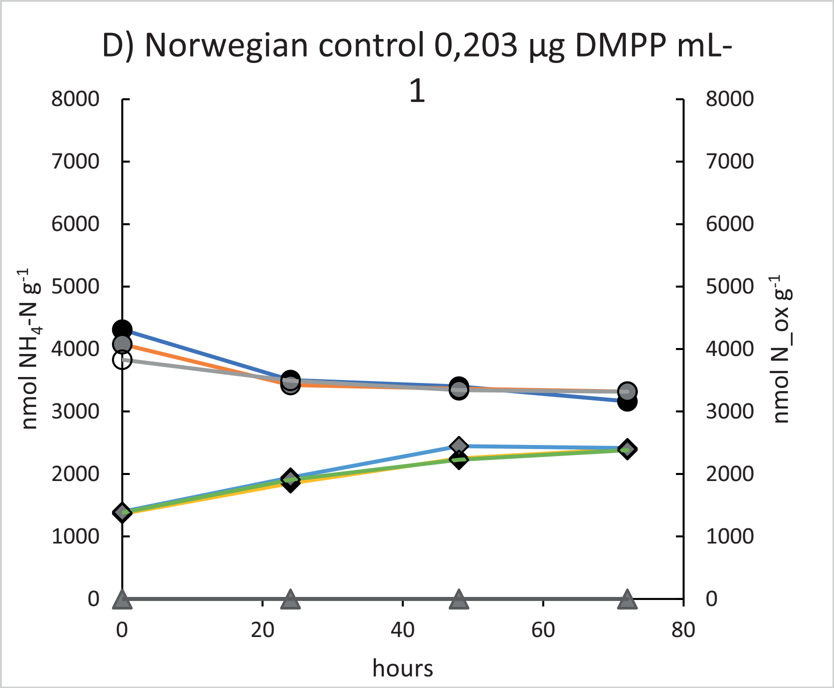
Figure 4. Raw [NH_4^+], [NO_3^-+NO_2^-] and [NO_2^-] g soil $^{-1}$ in soil slurries for Norwegian limed


A) Norwegian control- Control
NH4+
NO2+NO3
NO3
nmol NH4-N g-1
nmol N_ox g-1
hours
0
1000
2000
3000
4000
5000
6000
7000
8000
0
20
40
60
80


B) Norwegian control - 0,068 µg DMPP mL-1
nmol NH4-N g-1
nmol N_ox g-1
hours
0
1000
2000
3000
4000
5000
6000
7000
8000
0
20
40
60
80



C) Norwegian control 0,135 µg DMPP mL-1



D) Norwegian control 0,203 µg DMPP mL-1

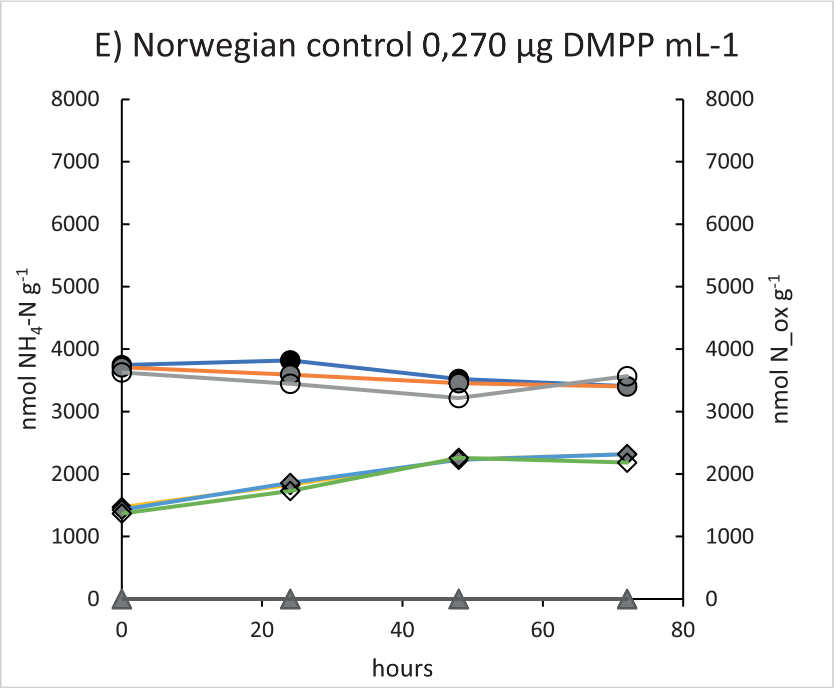
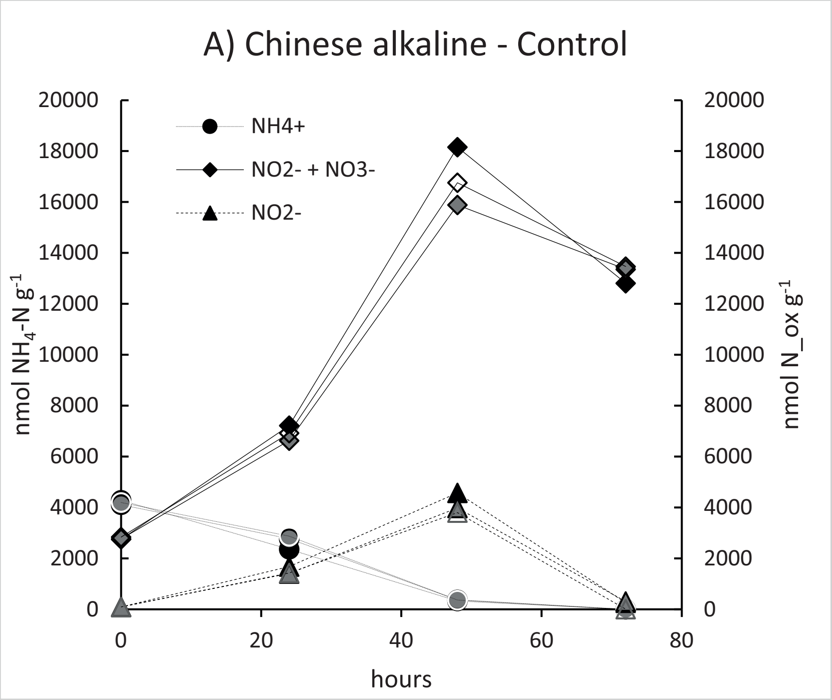
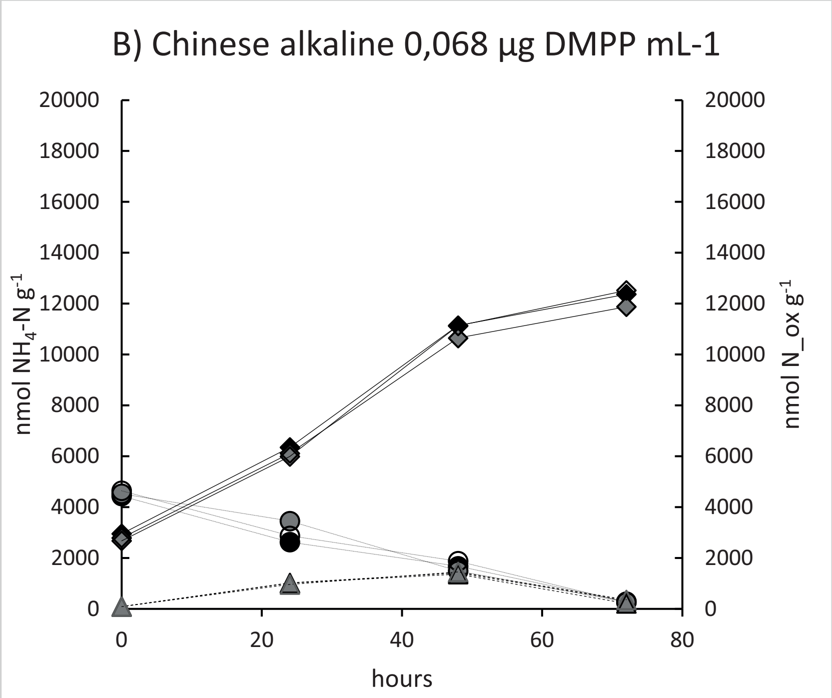
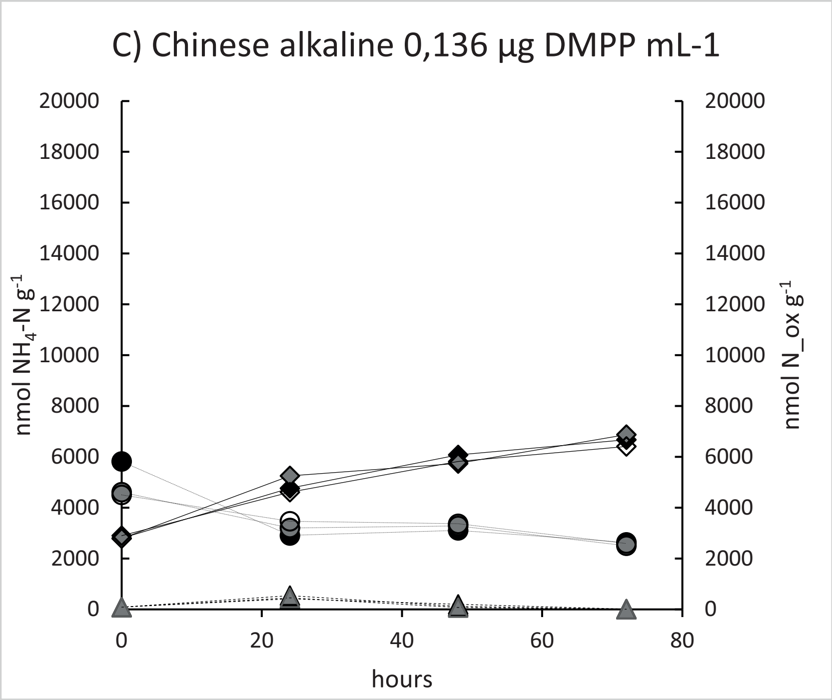


Figure 5. [NH_4^+-N], [NO_3^-+NO_2^--N], [NO_2^--N] in soil slurries for Norwegian control


A) Chinese alkaline - Control
NH4+
NO2- + NO3-
NO2-
nmol NH$_4$-N g^{-1}
nmol N_ox g^{-1}
hours
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
0
20
40
60
80


B) Chinese alkaline 0,068 µg DMPP mL-1
nmol NH4-N g-1
nmol N_ox g-1
hours
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
0
20
40
60
80


C) Chinese alkaline 0,136 µg DMPP mL-1
nmol NH4-N g-1
nmol N_ox g-1
hours
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
0
20
40
60
80

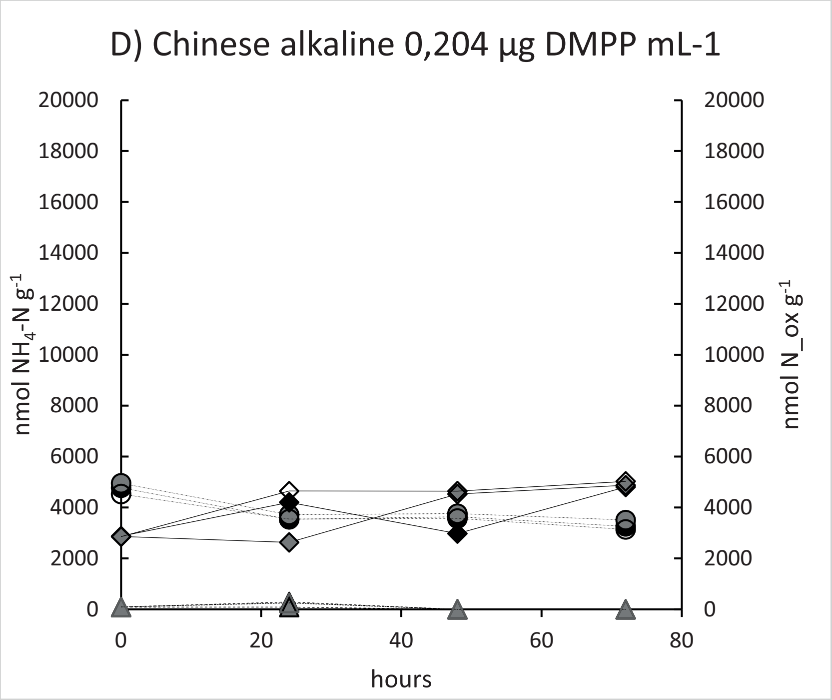
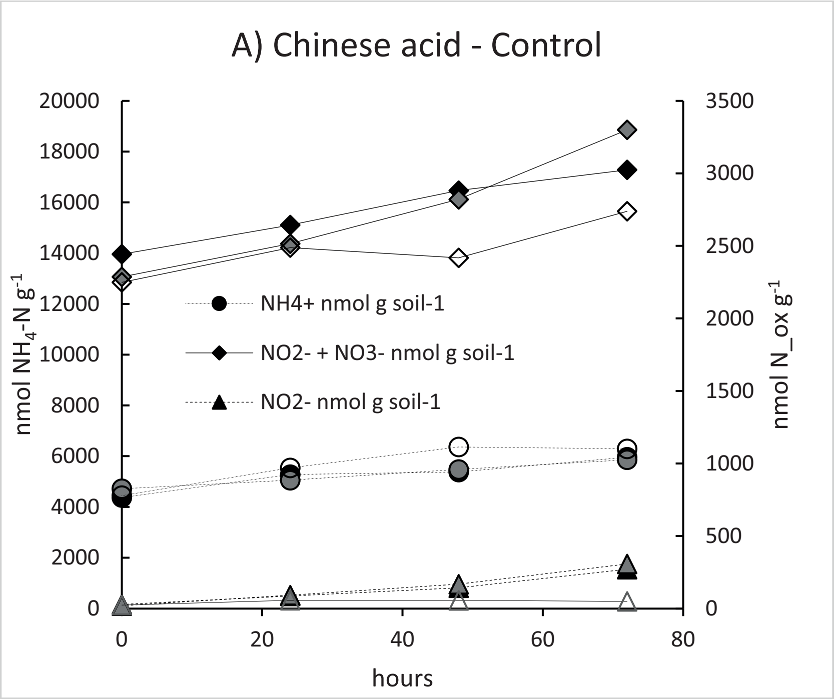
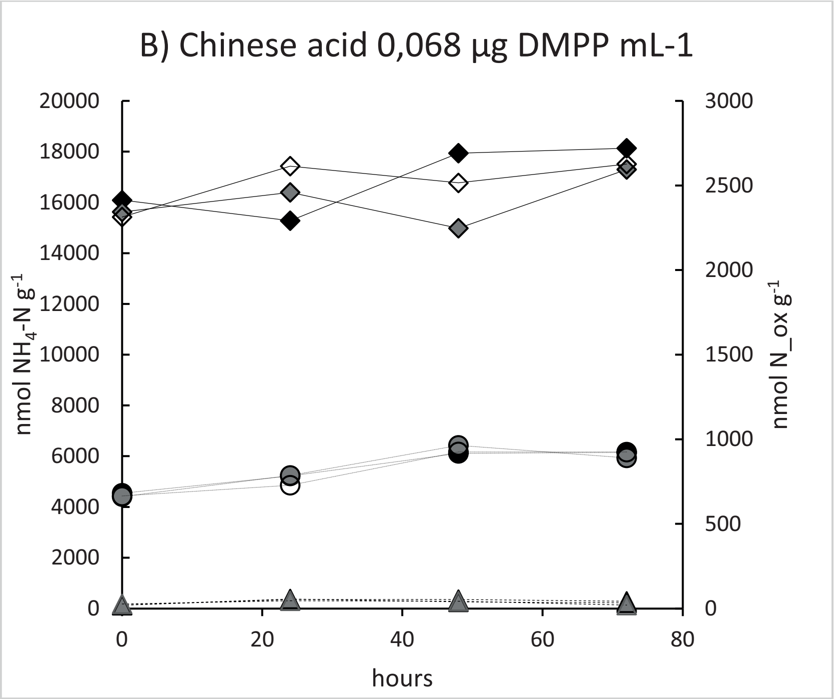
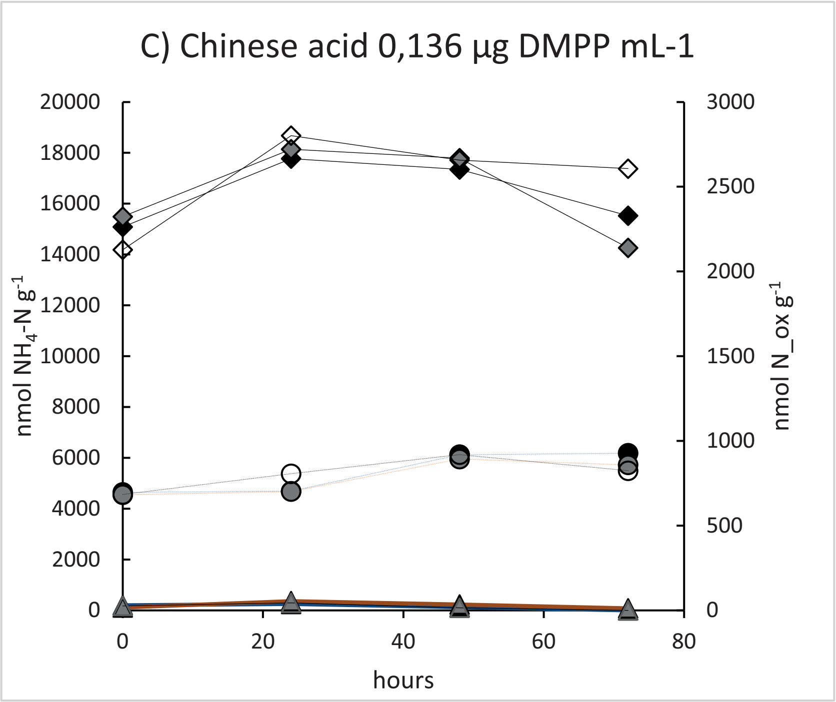
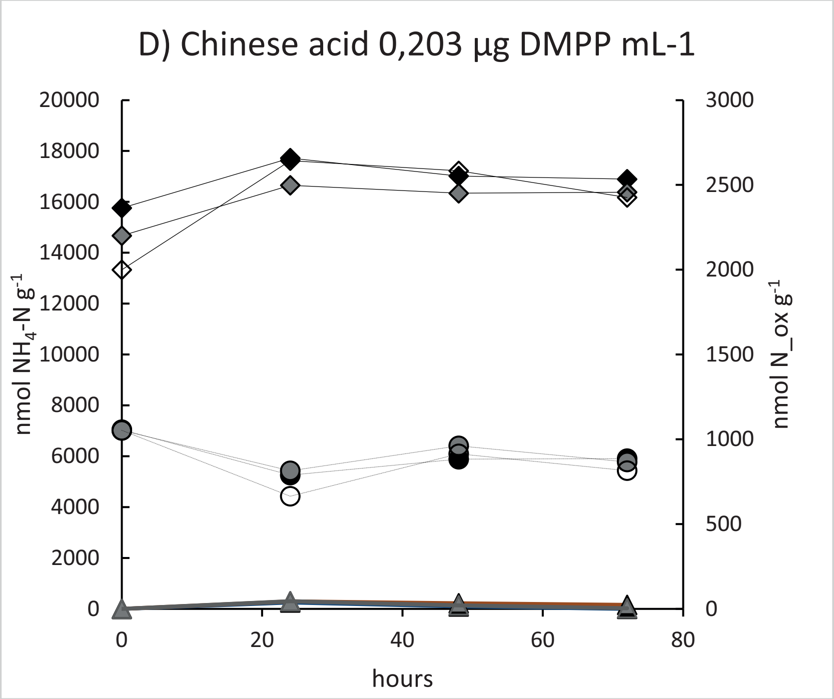


Figure 6. [NH_4^+-N], [NO_3^-+NO_2^--N], [NO_2^--N] in soil slurries for Chinese alkaline soil

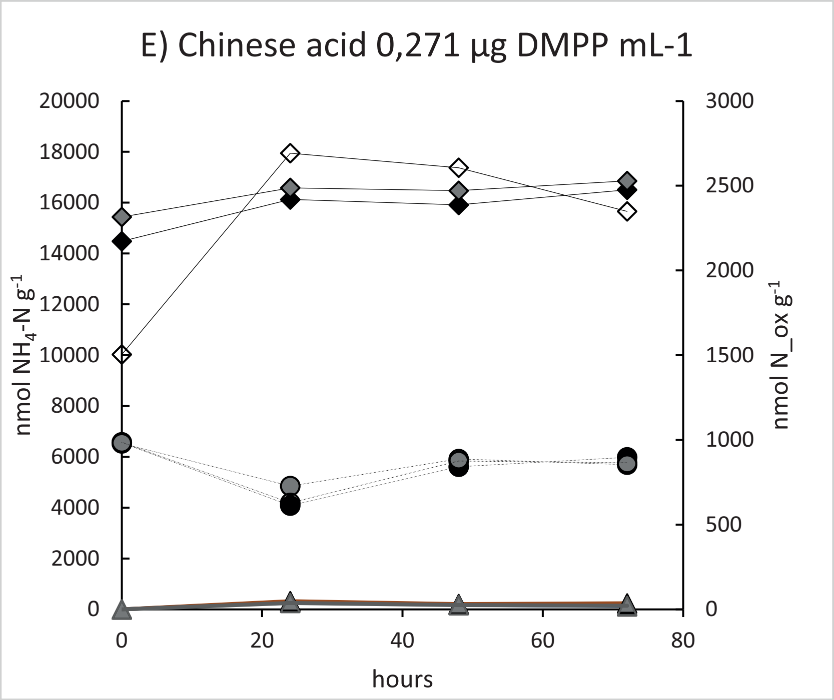

A) Chinese acid - Control
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
nmol NH_4-N g^{-1}
3500
3000
2500
2000
1500
1000
500
0
nmol N_ox g^{-1}
0
20
40
60
80
hours
NH4+ nmol g soil-1
NO2- + NO3- nmol g soil-1
NO2- nmol g soil-1


B) Chinese acid 0,068 µg DMPP mL-1
nmol NH4-N g-1
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
nmol N_ox g-1
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
hours


C) Chinese acid 0,136 µg DMPP mL-1
nmol NH4-N g-1
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
nmol N_ox g-1
3000
2500
2000
1500
1000
500
0
0
20
40
60
80
hours



D) Chinese acid 0,203 µg DMPP mL-1



E) Chinese acid 0,271 µg DMPP mL-1

Figure 7. [NH_4^+-N], [NO_3^-+NO_2^--N], [NO_2^--N] in soil slurries for Chinese acid soil

ISBN: 978-82-575-2031-1
ISSN: 1894-6402

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