frontiers in Environmental Science

ORIGINAL RESEARCH
published: 04 December 2020
doi: 10.3389/fenvs.2020.598513

Contingent Effects of Liming on N_2O-Emissions Driven by Autotrophic Nitrification

Shahid Nadeem[1], Lars R. Bakken[2], Åsa Frostegård[2], John C. Gaby[2] and Peter Dörsch[1]*

[1] Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences (NMBU), Ås, Norway, [2] Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences (NMBU), Ås, Norway

OPEN ACCESS

Edited by:
Muhammad Shaaban, Bahauddin Zakariya University, Pakistan

Reviewed by:
Alessandro Florio, Institut National de Recherche pour l'Agriculture, l'alimentation et l'Environnement (INRAE), France
Meihua Deng, ZheJiang Academy of Agricultural Sciences, China

***Correspondence:**
Lars R. Bakken
lars.bakken@nmbu.no

Specialty section:
This article was submitted to Soil Processes, a section of the journal Frontiers in Environmental Science

Received: *24 August 2020*
Accepted: *11 November 2020*
Published: *04 December 2020*

Citation:
Nadeem S, Bakken LR, Frostegård Å, Gaby JC and Dörsch P (2020) Contingent Effects of Liming on N_2O-Emissions Driven by Autotrophic Nitrification. Front. Environ. Sci. 8:598513. doi: 10.3389/fenvs.2020.598513

Liming acidic soils is often found to reduce their N_2O emission due to lowered $N_2O/(N_2O + N_2)$ product ratio of denitrification. Some field experiments have shown the opposite effect, however, and the reason for this could be that liming stimulates nitrification-driven N_2O production by enhancing nitrification rates, and by favoring ammonia oxidizing bacteria (AOB) over ammonia oxidizing archaea (AOA). AOB produce more N_2O than AOA, and high nitrification rates induce transient/local hypoxia, thereby stimulating heterotrophic denitrification. To study these phenomena, we investigated nitrification and denitrification kinetics and the abundance of AOB and AOA in soils sampled from a field experiment 2–3 years after liming. The field trial compared traditional liming (carbonates) with powdered siliceous rocks. As expected, the $N_2O/(N_2O + N_2)$ product ratio of heterotrophic denitrification declined with increasing pH, and the potential nitrification rate and its N_2O yield (Y_{N2O}: N_2O-N/NO_3^--N), as measured in fully oxic soil slurries, increased with pH, and both correlated strongly with the AOB/AOA gene abundance ratio. Soil microcosm experiments were monitored for nitrification, its O_2-consumption and N_2O emissions, as induced by ammonium fertilization. Here we observed a conspicuous dependency on water filled pore space (WFPS): at 60 and 70% WFPS, Y_{N2O} was 0.03-0.06% and 0.06–0.15%, respectively, increasing with increasing pH, as in the aerobic soil slurries. At 85% WFPS, however, Y_{N2O} was more than two orders of magnitude higher, and decreased with increasing pH. A plausible interpretation is that O_2 consumption by fertilizer-induced nitrification cause hypoxia in wet soils, hence induce heterotrophic nitrification, whose Y_{N2O} decline with increasing pH. We conclude that while low emissions from nitrification in well-drained soils may be enhanced by liming, the spikes of high N_2O emission induced by ammonium fertilization at high soil moisture may be reduced by liming, because the heterotrophic N_2O reduction is enhanced by high pH.

Keywords: soil pH, nitrification, N_2O-reductase, N_2O, moisture

INTRODUCTION

Although soils are buffered by ion exchange reactions and weathering of minerals (Chadwick and Chorover, 2001), they become gradually acidified by cultivation, primarily due to ammoniacal nitrogen fertilization and loss of base cations (von Uexküll and Mutert, 1995; Guo et al., 2010). Soil acidification is commonly counteracted by carbonate addition, either as frequent low doses or as infrequent heavy dressings (Shaaban et al., 2014; Goulding, 2016). Crop plants display substantial variation in acid tolerance (Goulding, 2016), and even cultivars within one species may vary in their tolerance to soil acidity (Kochian et al., 2004). Thus, the target pH for sustaining high yields will depend on the crop, with the minimum given by its tolerance to acidity and the maximum by the micronutrient availability of the soil at elevated pH (White and Robson, 1989). Liming may also serve other purposes than securing an agronomic minimum pH for crop production. Heavy dressings of CaO (or $Ca(OH)_2$) reduce soil erosion by improving the structure of heavy clay soils (Ulén and Etana, 2014), while the effect on crop growth appears variable, possibly due to reduced manganese availability at high pH in some soils (Blomquist et al., 2018).

Liming beyond the minimum for crops has also been proposed as a means of reducing N_2O emissions, since soil acidification leads to high N_2O/N_2 product ratios of denitrification under standardized anoxic incubations. This was first observed by Wijler and Delwiche (1954), and corroborated by subsequent investigations, although the reason remained obscure (Čuhel and Šimek, 2011). Recent studies of transcription and enzyme kinetics have provided compelling evidence that this is a post-transcriptional phenomenon: the making of functional N_2O-reductase is increasingly impaired with increasing proton concentrations (Liu et al., 2014).

Numerous field and microcosm experiments have been conducted to determine if N_2O emission can be reduced by increasing soil pH, be it by carbonates (Shaaban et al., 2014, 2015, 2018; Oo et al., 2018), or biochar (reviewed by Cayuela et al., 2014). Although the investigations generally corroborate the hypothesis, there are also cases where N_2O emissions were either unaffected or even increased in response to elevated soil pH (see review by Qu et al., 2014). One reason for the variable effect of liming on N_2O emissions from soils could be the production of N_2O by ammonia oxidizing organisms. The three known groups of ammonia oxidizing organisms are the ammonia oxidizing bacteria (AOB), the ammonia oxidizing archaea (AOA) and the recently discovered comammox group (Daims et al., 2015), which oxidizes NH_3 all the way to NO_3^- While little is known about the ecology and N_2O production of comammox, the biochemistry and ecophysiology of AOB and AOA and their N_2O production have been subject to intense studies. AOA are frequently found to outnumber AOB in soils with low availability of NH_4^+ and low pH, while AOB appear to thrive in response to high pH and fertilization with NH_4^+ (Nicol et al., 2008). Thus, liming acid soils should favor AOB over AOA, which would enhance N_2O production by nitrification because AOB produce much more N_2O per mole N oxidized than AOA, as pointed out by Hink et al. (2017a).

Next to the prevalence of AOB and AOA, the amount of N_2O produced per mole N oxidized could also be affected by the rate of nitrification: nitrification consumes oxygen and produces NO_2^- and NO_3^-, hence potentially inducing denitrification, either by heterotrophic or nitrifying organisms (Kool et al., 2011a,b; Huang et al., 2014). Isotope tracing data have been taken to suggest that *nitrifier denitrification* is more significant than heterotrophic denitrification in soils (Zhu et al., 2013; Wrage-Mönning et al., 2018), but the validity of this has been challenged recently by Bakken and Frostegård (2017), who claim that the isotope tracing method cannot be used to distinguish between the two pathways. This distinction is important in the context of pH management because the net production of N_2O by heterotrophic denitrification is strongly dependent on pH, while that by *nitrifier denitrification* is not: denitrification by nitrifying organisms produces pure N_2O regardless of pH, because they lack the gene for N_2O reductase (*nosZ*) (Klotz and Stein, 2011).

While increasing soil pH was expected to reduce the N_2O emission from denitrification by enhancing the synthesis of functional N_2O reductase, we hypothesized that it would have the opposite effect on N_2O emission from nitrification, both by increasing its N_2O yield (mol N_2O per mol NO_3^- produced) and by enhancing the potential nitrification rate. The latter would lead to high oxygen consumption after fertilization with ammonium, hence increasing the risk for hypoxia and denitrification if the soil moisture content is high. Such nitrification induced denitrification would be a strong N_2O source, hypothetically enhanced by increasing the soil pH, unless effectively counteracted by enhanced synthesis of N_2O reductase at high pH. To shed light on the potential stimulation of N_2O emission from nitrification after liming, we conducted a series of laboratory incubations with soils sampled over a period of 2 years from a field trial, in which carbonates were compared with siliceous rock powders as agents to increase the soil pH (Nadeem et al., 2015). The motive for testing powdered siliceous rocks was to explore the possibility of using carbonate-free minerals, thus avoiding the emission of carbonate-CO_2. In addition, we describe the effect of the rock powders on the AOB/AOA ratio as a function of increasing soil pH.

While corroborating the well-known effect of pH-increase on the synthesis of functional N_2O reductase (hence reducing N_2O emission from denitrification), the strictly oxic incubations demonstrated that the rate of nitrification and its N_2O yield increased with soil pH. We further demonstrate that nitrification-induced denitrification increases with soil moisture, and provide evidence that oxygen consumption by nitrification induces heterotrophic denitrification, rather than *nitrifier-denitrification*. The results are important because they explain why the effect of liming on the N_2O emission induced by ammonium fertilization depends on the soil moisture content: at very high soil moisture content, ammonium fertilization may cause high N_2O emission by inducing heterotrophic denitrification, and this emission can be reduced by liming. At modest/low moisture content, ammonium fertilization induces modest

N_2O emission from nitrification alone, and this is enhanced by liming.

MATERIALS AND METHODS

Experimental Site and Soil Sampling

Soils were sampled from a field trial established in autumn 2014, to test the effect of finely ground siliceous minerals as alternatives to liming. The trial is situated on the research farm of the Norwegian University of Life Sciences (NMBU) at Ås (59° 49′ N, 10° 47′ E, 75 m a.s.l) in southeast Norway. Mean annual temperature is 7.7°C and mean annual precipitation is 1,083 mm (Hansen and Grimenes, 2015). The field had been under crop rotation including leys since 1953, and the soil (clay loam) had not been limed since 1970. The field trial consists of six treatments: two types of calcareous lime (calcite and dolomite), and three types of siliceous rock powders (olivine, norite, and larvikite) and an untreated control. Each treatment was replicated four times and randomly distributed in two blocks (same blocks as the previous crop rotation experiment). Soil pH and content of soil organic matter measured prior to liming is given in **Supplementary Table 1**. Minerals were applied during autumn 2014 at a rate of 30 t ha^{-1} (siliceous rock powders and calcite) and 23 t ha^{-1} (dolomite). The siliceous minerals were Olivine: Blueguard 63, particle size <63 μm, from the company Sibelco Nordic AS Norway; Larvikite: rock cutting dust from Lundhs AS Norway, which was further ball milled and wet sieved to achieve a particle size <63 μm; Norite: waste material from titanium enrichment process (cyclone removal of particles), from Titania AS Norway, particle size <300 μm (∼50% W/W <63 μm); Calcite obtained as a suspension of colloidal particles (0.4–1.5 μm) from OMYA-Hustadmarmor AS; and Dolomite, obtained as an agricultural lime from Franzefoss A/S Norway. The mineral materials were mixed evenly into the upper 20 cm of the soil by plowing after adding half of the material, then harrowing after adding the second half. Winter wheat was sown in late autumn 2014, but failed to establish, and the field was plowed again in the late spring of 2015. Barley (*Hordeum vulgar*, cultivar Sunita) was sown on June 5 as a cover crop together with a grass mixture (20% *Phleum pratense*, 25% *Lolium perenne*, 25% *Festuca pratensis*, 20% *Festuca arundinacea schreb*, 10% *Poa pratense L*), fertilized with 100 kg N ha^{-1} (mineral fertilizer, NPK 22:3:10). The cover crop was harvested after 8 weeks. Ley growth was poor in the year of establishment, but improved in subsequent years. The ley was fertilized with 270 kg N ha^{-1} y^{-1} as NPK or CAN, split into 120 kg N in spring, 90 kg N after the 1st harvest and 60 kg N after the 2nd harvest. Soil samples were collected in 2015 and 2016 (during summer and late autumn), at 15, 21, and 27 months after liming. A composite sample (2–10 cm) was taken from each replicate plot by pooling at least 6 auger cores per plot. The soil samples were immediately transferred to the laboratory, sieved (2 mm), and stored in plastic bags at 4°C, until used for analyses and laboratory incubations (1–5 days after collection).To determine soil pH, 10 g soil were thoroughly dispersed in 25 mL 0.01 M $CaCl_2$ (hand shaken). The slurry was left to settle overnight, then shaken again, and finally left to settle for 15 min before measuring the pH.

Nitrification and Denitrification Kinetics

Soil Slurry Experiments

Soil slurries were used for potential nitrification and denitrification experiments. Equivalents of 4 g dry weight soil were transferred to 120 ml serum flasks and dispersed in 40 ml 1 mM NH_4Cl (for nitrification) or KNO_3 solution (for denitrification). All flasks were equipped with Teflon coated magnetic stirrers, and crimp-sealed with Teflon-coated silicone septa for nitrification experiments (to avoid inhibition of nitrification), and butyl rubber septa for denitrification experiments.

To ensure low initial NO_3^- concentrations in the nitrification assay, the soils were first flooded with deionized water and drained by vacuum in 500 ml filter funnels (Millipore) with 0.45 μm filters, then transferred to the vials (4 g dry weight equivalents). The nitrification assay was performed at 23°C, while shaking the flasks horizontally on a reciprocal shaker (125 rpm) to ensure that fully oxic condition were maintained throughout the incubation. To measure N_2O, the bottles were removed from the shaker once or twice per day and placed for a short period in a temperature-controlled water bath (23°C) of the incubation robot (Molstad et al., 2007). The robot sampled the headspace (∼1 mL) with a hypodermic needle, to determine O_2 and N_2O concentrations. After each sampling, an equal volume of He was pumped back to the headspace to keep the flask pressure at ∼1 atm. We used the new version of the robot, described by Molstad et al. (2016), equipped with an Agilent GC-7890A gas chromatograph with three detectors (FID, TCD, ECD) for determining the concentrations of O_2, N_2, N_2O, and CO_2, and with a chemiluminescence detector (Teledyne NO/NO_x analyzer mod 200E) for detection of NO. After each gas analysis, the flasks were returned to the reciprocal shaker. Oxygen concentrations in the headspace were monitored throughout, and pure oxygen was added when O_2 concentration fell below 16 vol% in the headspace, thus maintaining O_2 concentration between 15 and 20 vol%.

Denitrification kinetics were measured by frequent sampling from the headspace of 120 ml serum flasks containing 4 g dry weight soil and 40 ml of 1 mM KNO_3. The flasks were placed in the water bath of the incubation robot, and the slurries were stirred continuously (400 rpm). Before the incubation, all flasks were washed with He by repeated evacuation and filling. The final He overpressure was released by using a syringe without plunger containing water. The observed gas kinetics were corrected for dilution and leakage as outlined by Molstad et al. (2007). Soil pH for each replicate sample was measured at the beginning and end of the incubation, and the average of these two values was used for further calculations.

NO_2^- and NO_3^- Measurements

Nitrification rates were determined from the accumulation of NO_3^- and NO_2^- in the soil slurries over time. Once per day, 0.5 ml of the soil slurry was removed with a syringe and centrifuged at 10,000 *g* for 15 min at 4°C. NO_3^- and NO_2^- concentrations in the supernatant were determined immediately by colorimetry using a microplate reader (Infinite F50, TECAN Austria GmbH) at 540 nm. Both NO_3^- and NO_2^- were measured by Griess reaction (Keeney and Nelson, 1982),

with NO_3^- being converted to NO_2^- by vanadium chloride (Doane and Horwath, 2003).

Calculations

Nitrification rates were calculated from $NO_2^- + NO_3^-$ accumulation, while denitrification rates were calculated as the sum of NO, N_2O and N_2 accumulation. N_2O emission potentials from each process were estimated by calculating the N_2O yield as $N_2O/(NO_2^- + NO_3^-)$ for nitrification and the N_2O production index (I_{N2O}) for denitrification. The N_2O index of denitrification was calculated as described in Liu et al. (2010).

$$I_{N2O} = \frac{\int_0^T N_2O\, dt}{\int_0^T (N_2O + N_2) dt} \quad (1)$$

where N_2O (t) is the accumulated flux of N_2O at any time t, N_2 (t) is the accumulated flux of N_2 at any time, and T is the time when a certain amount of NO_3^--N kg^{-1} soil was recovered as gaseous N (NO_2^-, NO, N_2O, and N_2). I_{N2O} was calculated for two time periods, T_{10} and T_{25}; i.e., the time point when 10 and 25 μmol NO_3^-, respectively, had been denitrified to gaseous *N*.

Incubation Experiments With Remolded Soil

To study nitrification and denitrification under more realistic conditions than in slurries, we conducted a series of incubations with remolded soils.

Aerobic incubation was used to study nitrification (and its N_2O emission): 10 g of sieved soil from each plot (15 months after liming) was added to 120 ml serum flasks, packed to a bulk density of 1 g cm^{-3} and adjusted to 60% water filled pore space (WFPS). To induce nitrification, the soil was amended with a concentrated $(NH_4)_2SO_4$ solution equivalent to 110 mg N kg^{-1} soil by carefully distributing the solution throughout the soil volume, by using a syringe with a long needle. The flasks were incubated with air in the headspace at 20°C for 150 h, and O_2 consumption and N_2O production were monitored by the robotized incubation system described above. Nitrification was estimated from NO_3^- and NO_2^- accumulation in parallel offline incubation flasks covered with aluminum foil, which were subsampled periodically for determination of NO_3^- and NO_2^- concentrations (0.5 g soil was transferred to Eppendorf tubes with 1 ml deionized water, shaken, and centrifuged). The experiment was repeated at higher WFPS values (70 and 85%) with soils collected 27 months after liming to quantify the net N_2O emissions under conditions supporting coupled nitrification-denitrification, i.e., the reduction of NO_3^- or NO_2^- from nitrification by denitrification.

Anaerobic incubation of remolded soil was used to study denitrification: Prior to incubation, the soils were flooded with 2 mM KNO_3 solution and then drained, as described by Liu et al. (2010). After drainage an equivalent of 20 g dry weight soil was loosely packed into 120 ml serum flasks. Headspace gas was replaced with He. Headspace gasses were monitored as described above.

SSU rRNA Gene Sequence Analysis

DNA was extracted according to the protocol of Lim et al. (2016). To determine the relative proportion of AOB vs. AOA, we conducted amplicon sequencing of the SSU rRNA gene from the microbial communities in the different field plots as sampled December, 2016. Briefly, the V4 region of the 16S rRNA gene was amplified with the 515f (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806rB (5′-GGACTACNVGGGTWTCTAAT-3′) primers (Apprill et al., 2015; Parada et al., 2016) by following the Earth Microbiome Project protocol[1], and amplicons were sequenced on an Illumina MiSeq instrument using a 600 cycle kit, v3 (2 × 300 bp paired-end reads). A total of 36 samples were sequenced, which includes field plots that were sequenced in triplicate (**Supplementary File 1**) as technical replicates. The number of sequence reads per sample ranged from 81,276 to 457,377 with a mean of 299,296 and standard deviation of 99,835. The demultiplexed FASTQ files were obtained from the sequencer and analyzed in the statistical software R^2 using the packages DADA2 (Callahan et al., 2016) and Phyloseq (McMurdie and Holmes, 2013). Taxonomy of the resultant Amplicon Sequence Variants (ASVs) was established by reference to the Silva SSU rRNA database release 132 (Yilmaz et al., 2014). We then identified ammonia-oxidizers by searching for the term "nitroso" among all levels of the taxonomic hierarchy, whereby we determined that the ammonia-oxidizing *Archaea* consisted solely 42 ASVs in 2 classes of the phylum *Thaumarchaeota*, *Nitrososphaeria* and "Group_1.1c". Similarly, we identified ammonia-oxidizing *Bacteria* as consisting of 77 ASVs within the family *Nitrosomonadaceae* within the phylum *Gammaproteobacteria*. The abundances of these ASVs in the different samples were tallied to obtain the relative abundance of AOB and AOA and thus to determine the AOB/AOA ratio. To identify nitrite oxidizing bacteria, we similarly searched for their taxonomic descriptors and identified *Nitrospira*, *Nitrobacter*, and *Candidatus* Nitrotoga as present in the dataset. A total of 20 *Nitrospira* ASVs were detected while only one sample contained one *Nitrobacter* ASV and another sample contained a single *Candidatus* Nitrotoga ASV.

[1]http://www.earthmicrobiome.org/emp-standard-protocols/16s/

TABLE 1 | Soil pH as affected by the mineral treatments.

Treatment	Soil pH_{CaCl2}	ΔpH
Control	5.01 ± 0.05	–
Larvikite	5.02 ± 0.04	0.01 ± 0.01
Norite	5.12 ± 0.04	0.11 ± 0.02
Olivine	5.17 ± 0.03	0.16 ± 0.03
Dolomite	5.55 ± 0.04	0.58 ± 0.04
Calcite	6.62 ± 0.06	1.57 ± 0.08

The table shows the average soil pH_{CaCl2} (n = 14 ± SE) throughout the 2 years after incorporation of lime and minerals, and the average increase in pH by each mineral treatment relative to the control (ΔpH). Pairwise t-tests for difference between mineral treatments and control (ΔpH) showed significant effects ($p < 0.05$) for all minerals except Larvikite. Fluctuations in pH and ΔpH throughout the 2 years are shown in ***Supplementary Figure 1****.*

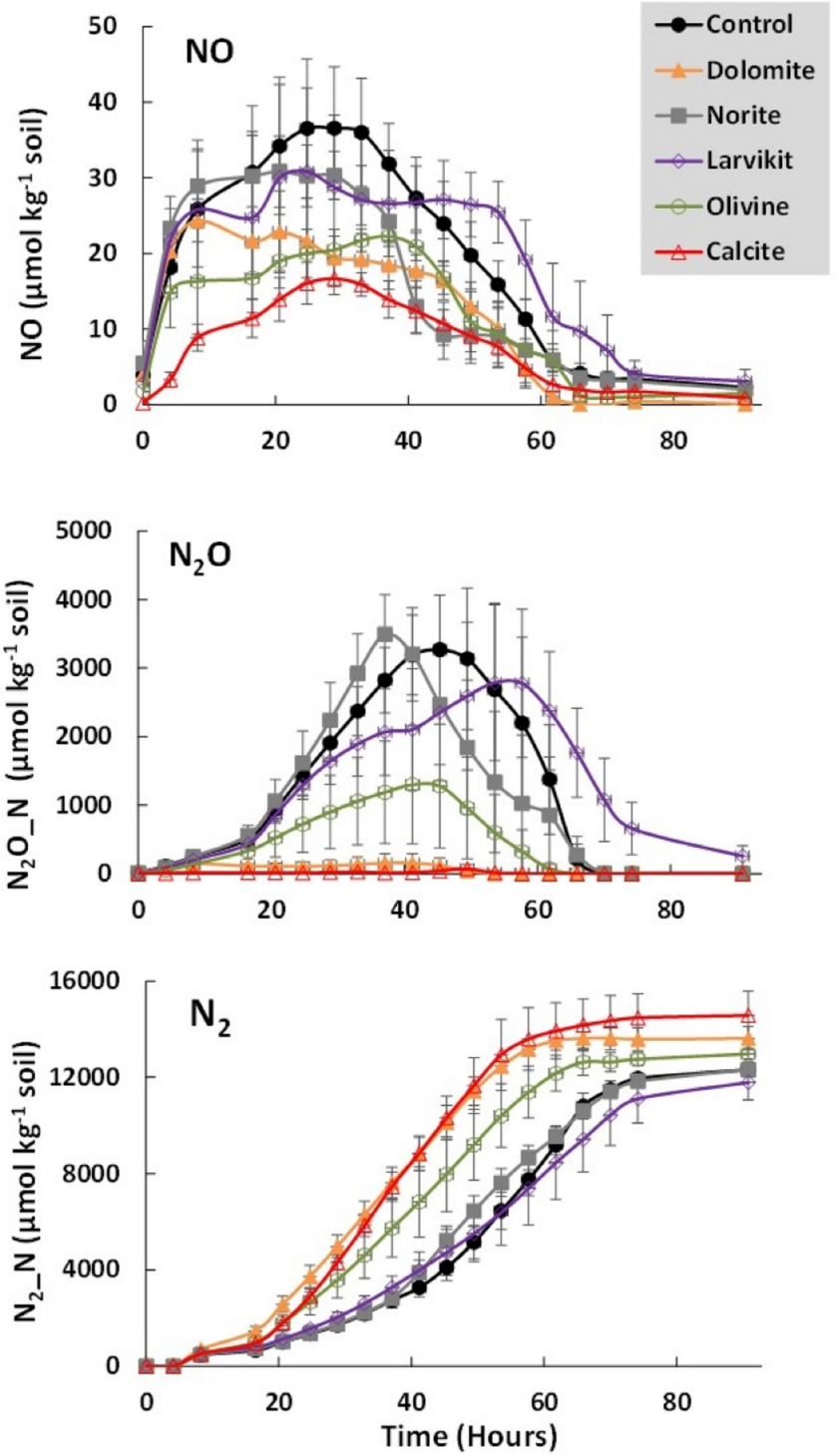

FIGURE 1 | Denitrification in soil slurries. NO and N_2O are shown as measured, while N_2 is the cumulative production (measured values corrected for sampling loss, see Molstad et al., 2007). Plotted values are means and standard errors of four field-replicates for each treatment, sampled 27 months after mineral applications in the field experiment. Samples taken 15 months after mineral treatment showed very similar response to soil pH whereby the N_2O production index (I_{N2O}) declined with soil pH. I_{N2O} for individual soil samples in both experiments are shown in **Supplementary Figure 4**.

Accession Numbers

The sequence data analyzed in this study is available for download at the Sequence Read Archive (SRA) under BioProject accession PRJNA541961, which corresponds to BioSample accessions SAMN12414160 to SAMN12414195 and SRA accessions SRS5197145 to SRS5197180.

RESULTS

Soil pH

Average soil pH measured in the field plots throughout the 26 months after application of lime and siliceous minerals are shown in **Table 1**. The pH increased 1.57 units in the calcite treatment, 0.58 in the dolomite, 0.16 in the olivine and 0.11 in the norite treatment. Larvikite had no significant effect on the soil pH ($p = 0.12$ for pairwise t-test of larvikite versus control). The calcite effect decreased over time by 0.5 units (**Supplementary Figure 1**).

Denitrification Kinetics in Anoxic Slurry Incubations

The gas kinetics in anoxic slurries of soils sampled 27 months after incorporation of lime and rock powder (**Figure 1**) showed transient accumulation of both NO and N_2O which clearly declined in magnitude with increasing soil pH, more dramatically so for N_2O than for NO. Stable plateaus of N_2, reached after ∼60 h indicated depletion of N oxides since the levels largely accounted for the initial amount of NO_3^--N present in the flasks (∼14 mmol N kg^{-1}; the soil contained ∼4 mmol NO_3^- kg^{-1}). **Table 2** summarizes the maximum denitrification rates, maximum NO and N_2O accumulation, and the N_2O index (I_{N2O}) for incubation experiments carried out with soils sampled 15, 21, and 27 months after liming. Denitrification rates were lowest in samples taken during winter 2015 (the year of ley establishment), and largest in summer of the first production year, after which they decreased again in the following winter. Denitrification rates exhibited a slight but statistically significant increase with soil pH (**Supplementary Figure 3**), while the transient accumulation of NO declined with pH. The influence of soil pH on the $N_2O/(N_2O + N_2)$ ratio of denitrification was examined by plotting the N_2O index (I_{N2O}) across pH, revealing a marked decrease with increasing pH (**Supplementary Figure 4**).

Nitrification and N_2O Yield in Oxic Slurry Incubations

Figure 2 shows the kinetics of oxygen consumption, transient accumulation of NO_2^- and the accumulation of $N_2O + NO_3^-$ in oxic slurries of soils sampled in December 2015 and 2016 (15 and 27 months after incorporation of lime and siliceous minerals). Similar kinetics were observed for soils sampled 21 months after liming and **Table 3** summarizes essential variables for all three sampling dates. The oxygen consumption rate was largest in calcite and dolomite treated soils, followed by olivine

TABLE 2 | Denitrification rates and transient NO and N_2O accumulation in anoxic slurry incubations.

Treatment	Denitrification rate μmol N kg^{-1} h^{-1}	Max NO μmol N kg^{-1}	Max N_2O μmol N kg^{-1}	I_{N2O25}
December 2015 (15 months)				
Control	69 ± 3	46 ± 6	1749 ± 351	0.22 ± 0.04
Larvikite	63 ± 3	41 ± 3	906 ± 185	0.11 ± 0.03
Norite	60 ± 5	47 ± 4	1390 ± 428	0.20 ± 0.07
Olivine	69 ± 6	45 ± 7	1460 ± 451	0.19 ± 0.05
Dolomite	90 ± 6	26 ± 2	247 ± 77	0.03 ± 0.01
Calcite	91 ± 7	16 ± 1	5 ± 1	<0.01
July 2016 (21 months)				
Control	376 ± 18	40 ± 4	5724 ± 665	0.33 ± 0.04
Larvikite	354 ± 29	40 ± 1	5384 ± 853	0.26 ± 0.05
Norite	409 ± 20	43 ± 3	5886 ± 1454	0.22 ± 0.02
Olivine	398 ± 9	38 ± 3	2844 ± 832	0.18 ± 0.04
Dolomite	424 ± 15	35 ± 3	224 ± 54	0.10 ± 0.03
Calcite	413 ± 7	27 ± 2	111 ± 74	0.01 ± 0.00
December 2016 (27 months)				
Control	204 ± 15	39 ± 8	3620 ± 748	0.42 ± 0.04
Larvikite	191 ± 17	35 ± 11	3201 ± 893	0.34 ± 0.06
Norite	207 ± 12	35 ± 4	3627 ± 539	0.44 ± 0.08
Olivine	255 ± 23	26 ± 4	1421 ± 870	0.18 ± 0.10
Dolomite	263 ± 26	27 ± 4	265 ± 102	0.04 ± 0.01
Calcite	293 ± 33	18 ± 2	97 ± 60	<0.01 ± 0.00

Denitrification rates (μmol N kg^{-1} soil h^{-1}) are given as average values (n = 4, ±SE) for the first 50 h of incubation. Maximum amounts of NO and N_2O are given as μmol N kg^{-1} soil. The N_2O index (I_{N2O}) is a proxy for the propensity of N_2O emission from denitrification calculated by equation (1) for the period until the total amount of gaseous N ($NO + N_2O + N_2$) accumulation reaches the specific limit indicated by the subscript (I_{N2O25}: 25 μmol N-gas $vial^{-1}$ = 6.3 mmol N-gas kg^{-1} soil). Rates and I_{N2O25} for individual soil samples in both experiments are shown in ***Supplementary Figures 3, 4****, respectively.*

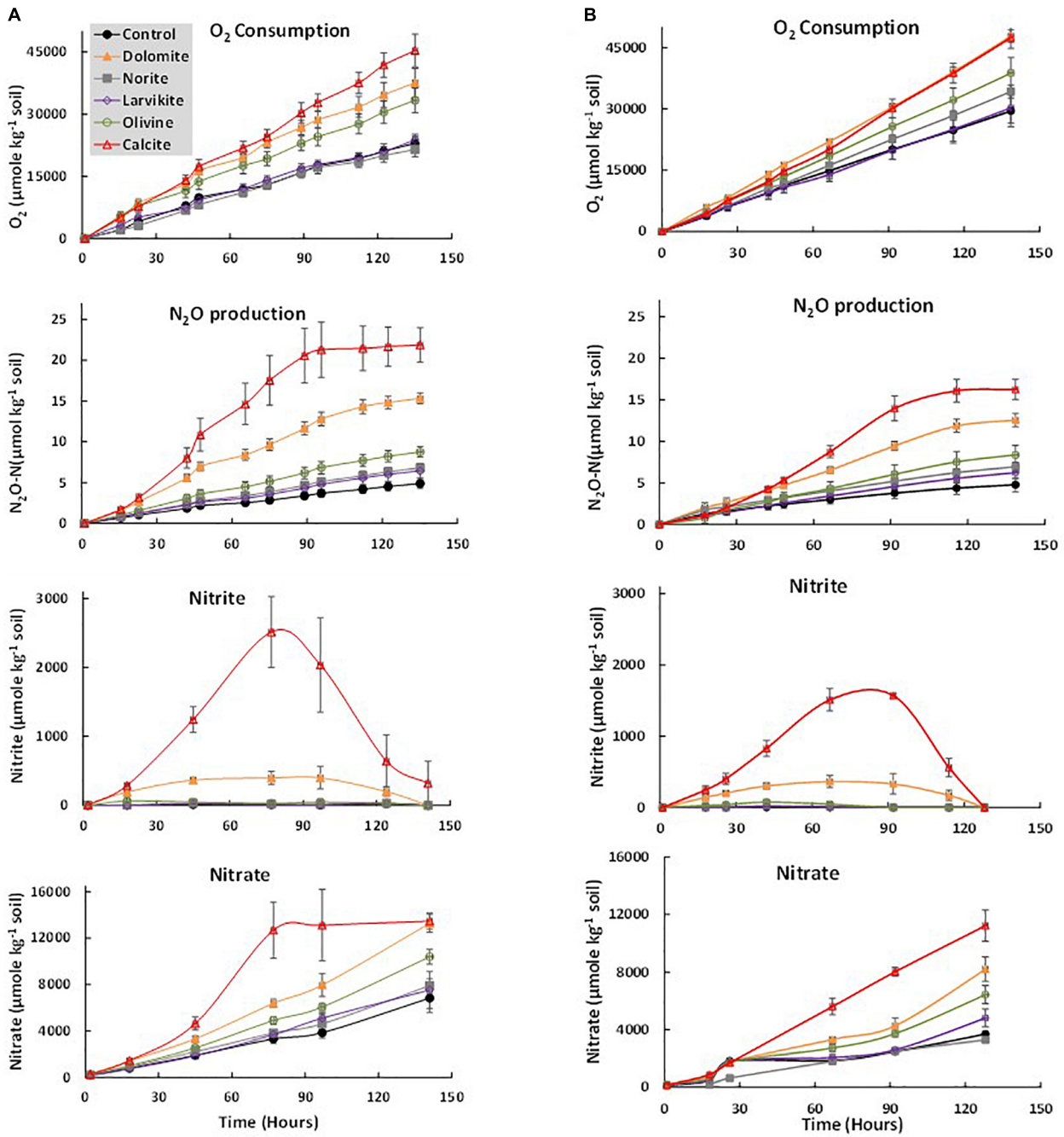

FIGURE 2 | Nitrification in oxic soil slurries. Oxygen consumption (cumulative), and production of N_2O nitrite and nitrate oxic soil slurries. Plotted values are the means and standard errors of four field replicates of each mineral treatment. Plotted gas kinetics are from two incubations experiments with soil collected from the field December 2015 (Panel **A**) and 2016 (Panel **B**) (15 and 27 months after application of minerals).

($p < 0.0005$), whereas larvikite and norite treatments were indistinguishable from the control.

The NH_4^+ oxidation rates (measured as $NO_2^- + NO_3^-$ accumulation) increased linearly with pH (**Figure 3**). NH_4^+ was evidently not depleted during the incubation, except for the calcite-amended soil sampled 15 months after mineral application (**Figure 2**: the NO_3^- plateaus reached after 60–90 h match the initial amounts of NH_4^+, which was 1.4 mmol NH_4^+ kg^{-1}). Transient accumulation of NO_2^- was substantial in the high pH soils (calcite > dolomite), whereas it was marginal in the other treatments and was found to increase exponentially with pH (**Supplementary Figure 4**). The calculated N_2O yield (Y_{N2O}) ranged from 0.09 to 0.34% and was significantly higher in the calcite and dolomite treatments than in the other treatments.

TABLE 3 | Rates of O_2 consumption, ammonium oxidation, N_2O production, NO_2^- accumulation and N_2O yield (Y_{N2O}) in oxic slurry incubations.

Treatment	O_2 μmol kg^{-1} h^{-1}	NH_4^+ oxid. † μmol N kg^{-1} h^{-1}	N_2O prod. † μmol N kg^{-1} h^{-1}	max NO_2^- μmol N kg^{-1}	Y_{N2O} (%)
December 2015 (15 months)					
Control	176 ± 19	41 ± 6	0.04 ± 0.01	25 ± 7	0.09 ± 0.01
Larvikite	181 ± 8	61 ± 2	0.05 ± 0.01	31 ± 3	0.08 ± 0.01
Norite	168 ± 16	51 ± 5	0.05 ± 0.01	36 ± 5	0.10 ± 0.00
Olivine	238 ± 20	69 ± 6	0.07 ± 0.01	110 ± 66	0.10 ± 0.01
Dolomite	276 ± 23	86 ± 9	0.14 ± 0.01	471 ± 133	0.14 ± 0.01
Calcite	340 ± 37	165 ± 28	0.25 ± 0.04	2687 ± 550	0.15 ± 0.01
July 2016 (21 months)					
Control	189 ± 22	32 ± 3	0.04 ± 0.00	nd††	0.12 ± 0.00
Larvikite	198 ± 47	31 ± 3	0.05 ± 0.01	nd	0.15 ± 0.01
Norite	207 ± 24	38 ± 4	0.05 ± 0.01	nd	0.14 ± 0.01
Olivine	198 ± 20	49 ± 4	0.10 ± 0.01	nd	0.19 ± 0.01
Dolomite	249 ± 9	77 ± 3	0.22 ± 0.01	423 ± 78	0.28 ± 0.03
Calcite	269 ± 53	99 ± 2	0.35 ± 0.04	1461 ± 135	0.34 ± 0.06
December 2016 (27 months)					
Control	213 ± 26	24 ± 5	0.04 ± 0.01	1 ± 1	0.14 ± 0.01
Larvikite	216 ± 27	31 ± 5	0.05 ± 0.00	17 ± 12	0.16 ± 0.02
Norite	246 ± 13	39 ± 2	0.06 ± 0.00	23 ± 7	0.13 ± 0.01
Olivine	281 ± 25	44 ± 4	0.07 ± 0.01	71 ± 22	0.15 ± 0.01
Dolomite	343 ± 11	64 ± 5	0.10 ± 0.00	411 ± 107	0.16 ± 0.01
Calcite	347 ± 22	84 ± 7	0.13 ± 0.01	1563 ± 66	0.18 ± 0.03

Data were averaged over the entire incubation period. Results for three experiments with soil sampled 15, 21, and 27 months after incorporation of lime and rock powders. Presented values are means of four field replicates and standard error. Maximum NO_2^- concentrations for individual field plot samples plotted against soil pH are shown in ***Supplementary Figure 4****. †NH_4^+ oxid., ammonium oxidation rate; N_2O prod., N_2O production rate.††nd, not detected.*

Assuming that the oxidation of 1 mole of NH_3 to NO_3^- consumes 2 moles of O_2, the measured nitrification accounted for 20–90% of the measured oxygen consumption rate (consistently highest for calcite).

Anoxic Incubations of Remolded Soil

For loosely remolded soil sampled 27 months after liming and incubated under fully anoxic conditions, denitrification gas kinetics exhibited similar N gas accumulation patterns as observed in the anoxic soil slurries. Calcite and dolomite treated soils accumulated less N_2O than any other treatment (**Figure 4**). Denitrification rate, maximum N_2O and N_2O index (I_{N2O}) in remolded soil experiments are shown in **Table 4**. Denitrification rates were calculated for the period 0–50 h of incubation, and the N_2O index for the time point when 500 μmol N kg^{-1} soil was recovered as NO + N_2O + N_2-N.

Oxic Incubations of Remolded Soils

The O_2 consumption rates, NH_4^+ oxidation rates, maximum NO_2^- concentrations, and the N_2O yields for these incubations are all summarized in **Table 5**, and the N_2O kinetics is shown in **Figure 5A**. The O_2 consumption rate increased gradually with increasing soil moisture content: the average O_2 consumption rates (all treatments) were 75, 106, and 153 μmol O_2 kg^{-1} soil h^{-1} at 60, 70, and 85% WFPS, respectively. The higher O_2 consumption rate at 70% than at 60% WFPS likely reflects a higher concentration of available organic carbon: soils used for the 60% WFPS experiments were sampled in December 2015, while the 70 and 85% WFPS experiments used soil samples taken in December 2016, when the ley was fully established. This is confirmed by oxygen consumption rates measured in oxic soil slurries (**Table 3**): the average rates for the December samples were 230 and 274 μmol O_2 kg^{-1} soil h^{-1} in 2015 and 2016, respectively, i.e., a 20% increase from 2015 to 2016.

The percentage of O_2 consumption theoretically accounted for by NH_3 oxidation (assuming 2 mol O_2 consumed per mol NH_4^+ oxidized) varied between 20 and 40% at 60% WFPS, which is somewhat lower than in the soil slurries. For the 85% WFPS treatment, much lower percentages were calculated, reflecting that NO_3^- consumption by denitrification resulted in grossly underestimated nitrification rates.

On average, the estimated N_2O yield (Y_{N2O}) at 70% WFPS was twice as high as at 60% WFPS (0.088 versus 0.043%, **Table 5**). The Y_{N2O} values for 70% WFPS are uncertain, however, since they are based on the assumption that nitrification rates were the same as at 60% WFPS. The 85% WFPS treatment differed markedly from the two other moisture levels in that O_2 consumption rates were larger, rates of NO_2^- + NO_3^- accumulation were lower, and N_2O production rates were two orders of magnitude higher. The apparent N_2O yields calculated for the initial 75 h (**Table 5**) were high, ranging from 2 to 15%, and approximately twice as high if calculated for the first 45 h of incubation, except for calcite, for which the N_2O production was low compared to the others, and more constant throughout. The pH response of the N_2O production rate in the 85% WFPS treatment differed fundamentally from those of the lower WFPS treatments (**Figure 5B**): while the N_2O production rates increased with pH at 60 and 70% WFPS, they declined sharply at 85% WFPS.

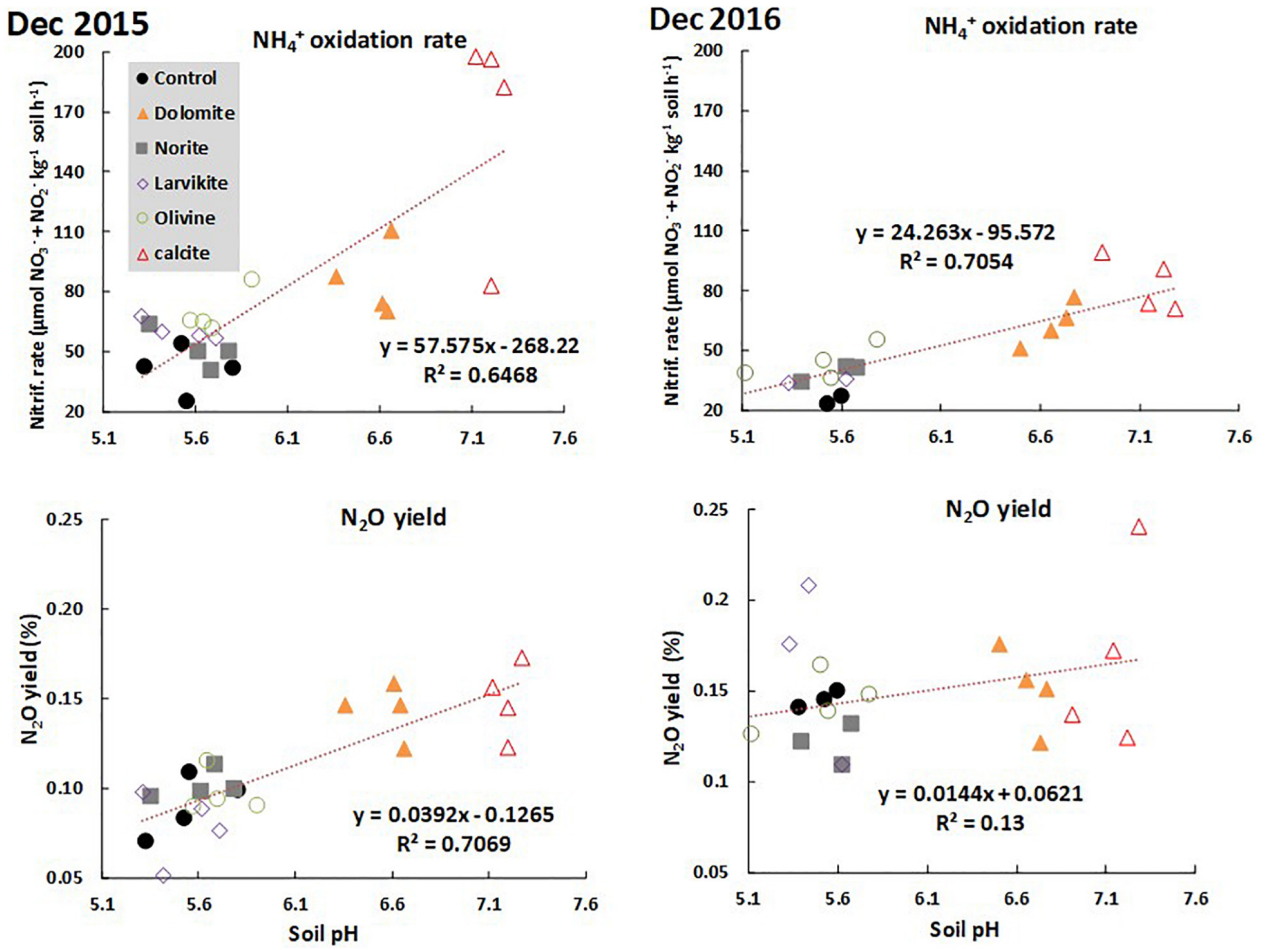

FIGURE 3 | Relationship between soil slurry pH, NH_4^+ oxidation rate and N_2O yield (% of oxidized N emitted as N_2O-N) in oxic nitrification assays. The plotted values are single flask values for four field replicates of each treatment. Plotted values are from two incubation experiments with soils collected from field 15 and 27 months after application of minerals.

AOA, AOB, and Nitrite Oxidizer Abundances

The relative abundance of AOA-, AOB-, and NOB-SSU in the overall bacterial community, as indicated by SSU rRNA amplicon sequencing, are shown in **Figure 6**. AOA identified in the samples consisted of 42 ASVs in the phylum *Thaumarchaeota* including the genera *Candidatus Nitrosotalea*, *Nitrososphaera* and *Nitrocosmicus*, as well as undescribed taxa within the phylum (**Supplementary File 1**). The identified AOB consisted of 77 ASVs from the family *Nitrosomonadaceae* within the phylum *Proteobacteria* and consisted of the genera *Nitrosomonas* and *Nitrosospira*, as well as several uncharacterized genera (**Supplementary File 1**). We also identified SSU rRNA genes affiliated to the phylum *Nitrospirae* and the genus *Nitrospira*, which are nitrite oxidizers (**Supplementary File 1** and **Supplementary Figure 8**). Regression analyses showed that the relative abundances of AOB and NOB increased significantly with soil pH ($p < 0.001$ for both), while AOA declined with pH ($p = 0.02$), and the AOB/AOA abundance ratio increased with pH ($p < 0.001$). SSU rRNA identified as *Nitrospira* may include *comammox*, i.e., organisms that oxidize ammonium to nitrate (Daims et al., 2015), but the ability of a given *Nitrospira* to carry out comammox as opposed to only nitrite oxidation may only be discerned by analysis of their functional genes (Daims et al., 2015). Hence, our SSU rRNA analysis cannot discern whether the *Nitrospira* ASVs detected are simple NOB or comammox.

DISCUSSION

It has become increasingly clear that the reason why emissions of N_2O from soils increase with acidity (Wang et al., 2018) is that the synthesis of functional N_2O reductase is impeded by low pH (Liu et al., 2014). Therefore, liming of acidic soil should reduce N_2O emissions and would justify the use of high doses of lime/biochar to mitigate these emissions, hence reducing the climate forcing that occurs as a result of fertilizer use in crop production. In theory, this beneficial effect of liming on climate forcing could be outweighed by emission of carbonate-CO_2,

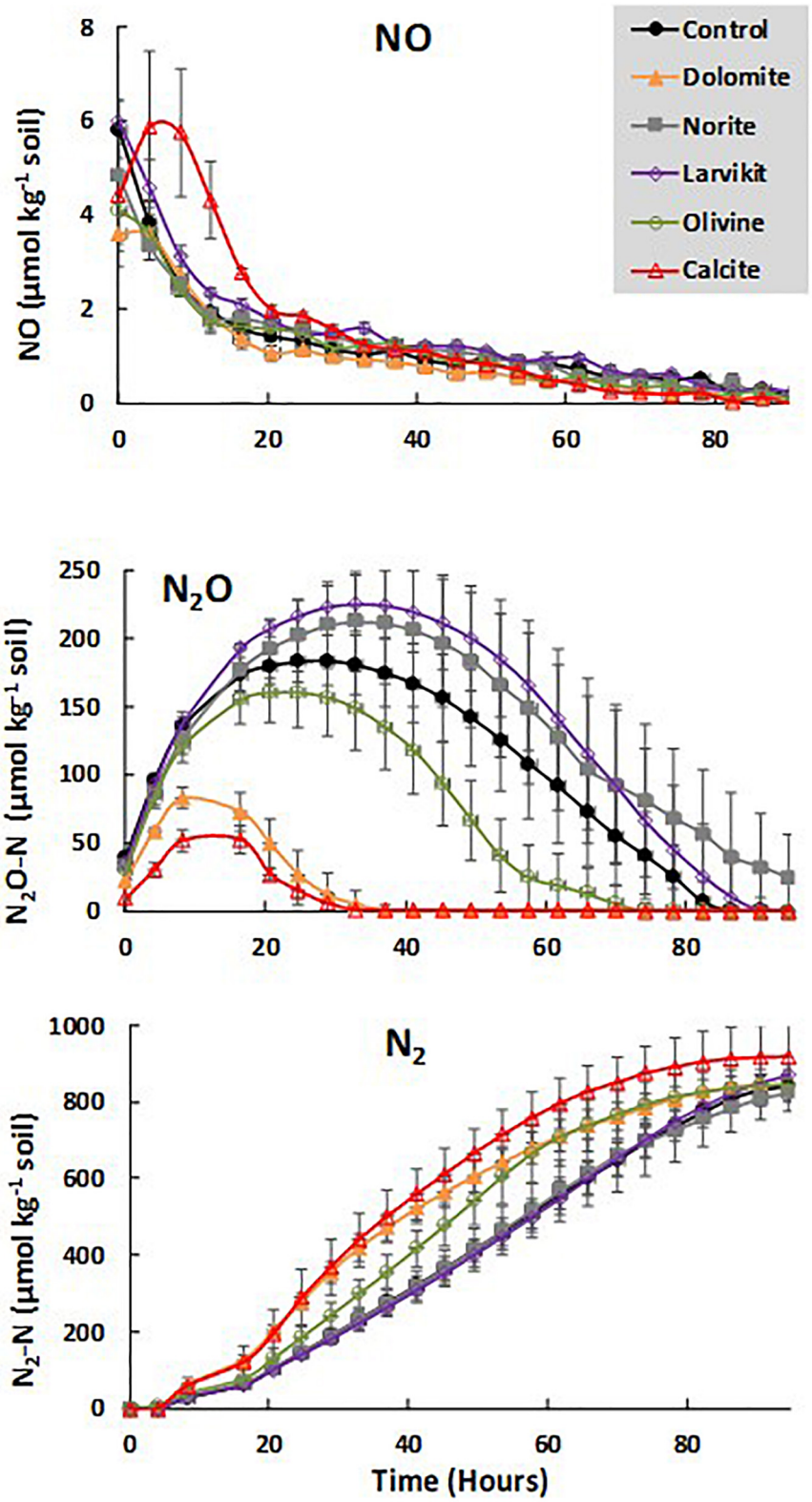

FIGURE 4 | Gas kinetics of denitrification in remolded soil under anoxic conditions. Plotted values are the means and standard error of four field replicates. Soil samples were collected in December 2016 (27 months after application of minerals).

TABLE 4 | Denitrification rate and production of NO and N_2O during anoxic incubation of remolded soils amended with nitrate.

Treatment	Denitrification μmol N kg^{-1} soil h^{-1}	Max. NO μmol N kg^{-1} soil	Max N_2O μmol N kg^{-1} soil	$I_{N2O}10$ (10)
Control	13 ± 0.4	6 ± 0.6	193 ± 20	0.43 ± 0.04
Larvikite	13 ± 0.6	6 ± 0.5	234 ± 29	0.50 ± 0.04
Norite	13 ± 1.4	5 ± 0.8	228 ± 33	0.46 ± 0.07
Olivine	15 ± 1.0	4 ± 0.6	166 ± 27	0.34 ± 0.06
Dolomite	14 ± 0.8	5 ± 0.4	90 ± 11	0.10 ± 0.05
Calcite	14 ± 1.8	6 ± 1.5	62 ± 10	0.10 ± 0.02

The N_2O index (I_{N2O}) is a proxy for the propensity of N_2O emission calculated by equation (1) for the period until the total amount of gaseous N ($NO + N_2O + N_2$) reached a specific limit indicated by the subscript (I_{N2O10}: 10 μmol N vial = 500 μmol kg^{-1} soil). Mean values of four field replicates (±SE) are given.

TABLE 5 | Rates of O_2 consumption, net $NO_2^- + NO_3^-$ accumulation, maximum NO_2^- accumulation and N_2O production rate during oxic incubation of remolded soils amended with ammonium at 60, 70, and 85% WFPS.

Treatment	O_2 rate μmol kg^{-1} soil h^{-1}	$NO_2^- + NO_3^-$ accum.-rate μmol N kg^{-1} soil h^{-1}	N_2O rate μmol N kg^{-1} soil h^{-1}	Max NO_2^- μmol N kg^{-1} soil	Y_{N2O} %
December 2015 (60% WFPS)					
Control	77 ± 9	22 ± 3	0.007 ± 0.0001	nd†	0.03 ± 0.004
Larvikite	63 ± 8	19 ± 2	0.007 ± 0.0004	nd	0.04 ± 0.004
Norite	62 ± 8	25 ± 5	0.007 ± 0.0008	nd	0.04 ± 0.007
Olivine	88 ± 6	27 ± 4	0.015 ± 0.0037	2 ± 2	0.06 ± 0.013
Dolomite	69 ± 6	26 ± 2	0.008 ± 0.0003	5 ± 5	0.03 ± 0.003
Calcite	90 ± 15	40 ± 5	0.021 ± 0.0025	117 ± 40	0.06 ± 0.014
December 2016 (70% WFPS)					
Control	102 ± 8	nd†	0.015 ± 0,004	nd	0.07††
Larvikite	86 ± 3	nd	0.018 ± 0.002	nd	0.10
Norite	96 ± 4	nd	0.014 ± 0.002	nd	0.06
Olivine	105 ± 7	nd	0.016 ± 0.004	nd	0.07
Dolomite	126 ± 6	nd	0.021 ± 0.002	nd	0.08
Calcite	131 ± 9	nd	0.050 ± 0.012	nd	0.15
December 2016 (85% WFPS)					
Control	142 ± 17	4 ± 1	0.74 ± 0.47	nd	8 ± 4
Larvikite	123 ± 14	3 ± 0	1.48 ± 0.86	nd	15 ± 9
Norite	137 ± 13	4 ± 1	1.24 ± 1.20	nd	8 ± 7
Olivine	166 ± 12	3 ± 1	0.45 ± 0.25	nd	6 ± 3
Dolomite	193 ± 6	4 ± 0	0.43 ± 0.22	nd	5 ± 2
Calcite	157 ± 29	3 ± 0	0.06 ± 0.03	213 ± 40	2 ± 1

*The N_2O yield (Y_{N2O}) is the percentage of N_2O-N relative to net accumulation of oxidized N ($NO_2^- + NO_3^- + N_2O$) at the end of the incubation. The N_2O emission from soils without NH_4^+ amendment was ≤10% of that with NH_4^+ (**Supplementary Table 3**). Presented values are means and standard error of four field replicates. †nd, not detected. ††NO_2^- and NO_3^- were not measured in this incubation. Y_{N2O} was estimated assuming identical nitrification rates as measured at 60% WFPS, hence standard deviations were not estimated.*

although the assumption that all carbonate-C is released as CO_2 has been contested (Hamilton et al., 2007; Page et al., 2009). Nevertheless, the emission of carbonate-CO_2 was our motive for testing finely ground siliceous minerals as alternatives to carbonates. The weathering of siliceous minerals will increase the pH of acidic soils, but our results so far are discouraging: while the carbonate materials caused a substantial increase in soil pH, larvikite had no significant effect, and norite and olivine increased the pH by only 0.11 and 0.16 pH units, respectively (**Table 1** and **Supplementary Figure 1**). Thus, although demonstrating the potential for two of the siliceous minerals, the weathering rates were obviously too low to achieve substantial increase in soil pH within 3 years after application. Improvement could have been achieved by grinding the minerals to finer particle size, since the weathering rate is proportional to the surface area, but the cost would be prohibitively high (van Noort et al., 2018).

N_2O emission from denitrification typically occurs during hypoxic/anoxic spells and is caused by transiently high soil moisture, high oxygen consumption rates, or both. The N_2O emissions during such "hot events," or in "hot spots" if localized in microsites with high oxygen consumption rates (Schlüter et al., 2019) depends on the enzyme kinetics: early/efficient synthesis of active N_2O reductase secures a low $N_2O/(N_2 + N_2O)$ product ratio, hence minimizing emission of N_2O, whereas delayed synthesis will lead to a high N_2O product ratio and emission. Since the synthesis of functional N_2O reductase is severely impeded/delayed by low pH (Liu et al., 2014),

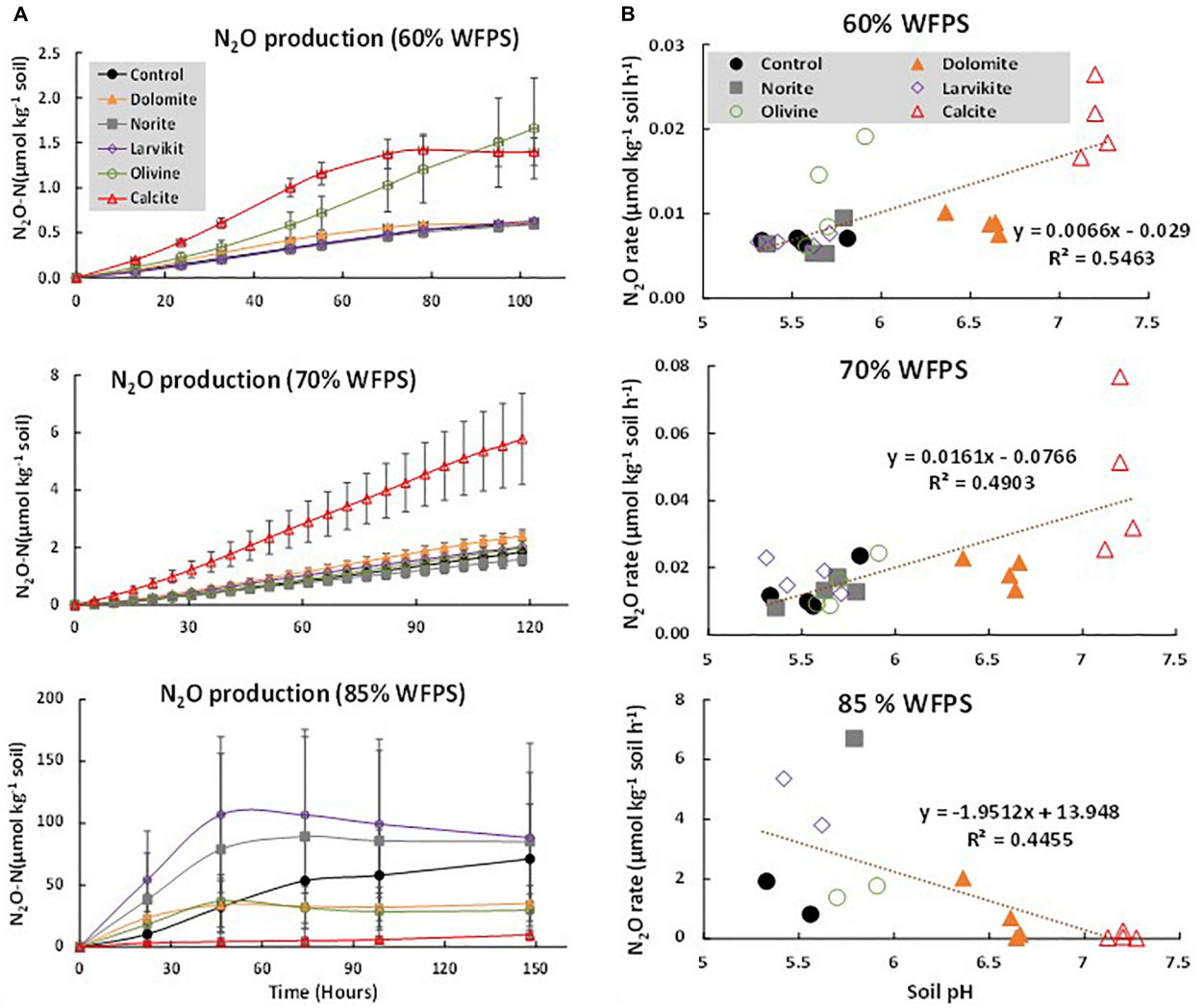

FIGURE 5 | N_2O accumulation during oxic incubations of remolded soils amended with NH_4^+. Panel **(A)** shows the N_2O kinetics throughout the incubations at the three moisture levels (means and standard error, $n = 4$). Panel **(B)** shows the N_2O production rates during the first 50 h of incubation plotted against the pH of the soils (individual flasks). Soils were collected from the field 15 months after liming (December 2015) for the 60% WFPS experiment and 27 months after liming (December 2016) for the 70 and 85% WFPS experiment. The NO_2^- + NO_3^- accumulation throughout the incubations at 60 and 85% WFPS are shown in **Supplementary Figure 6**.

the emission of N_2O will decrease with increasing soil pH. Anoxic soil incubations are commonly used to mimic anoxic spells, and such experiments have invariably shown that the transient accumulation of N_2O is controlled by pH (Čuhel and Šimek, 2011; Raut et al., 2012; Qu et al., 2014). The present result is no exception: increasing the pH by liming clearly lowered the $N_2O/(N_2O + N_2)$ product ratio during anaerobic incubations of both soil slurries (**Figure 1** and **Table 2**) and remolded soil (**Figure 4**). Thus, our findings corroborate previous investigations of denitrification as affected by pH. Our main aim, however, was to investigate the effect of liming on both denitrification and nitrification, and their contribution to N_2O emission.

Based on Hink et al. (2018) we hypothesized that increasing the pH by using lime or siliceous minerals would increase the potential nitrification rates in the soil, and selectively stimulate bacterial (AOB) over archaeal ammonia oxidizers (AOA). We further hypothesized that the N_2O yield of nitrification (Y_{N2O}) would increase with soil pH (as observed previously by Mørkved et al., 2007), because AOB have an inherently higher Y_{N2O} than AOA (Hink et.al. 2017a). The SSU rRNA gene quantification corroborated the hypothesis regarding selective stimulation of AOB (**Figure 6**). The effect of soil pH on AOA and AOB abundances has also been studied by Hu et al. (2013), who observed that the qPCR-determined ratio of AOA/AOB decreased with increasing soil pH, and hence the inverse of that ratio, or

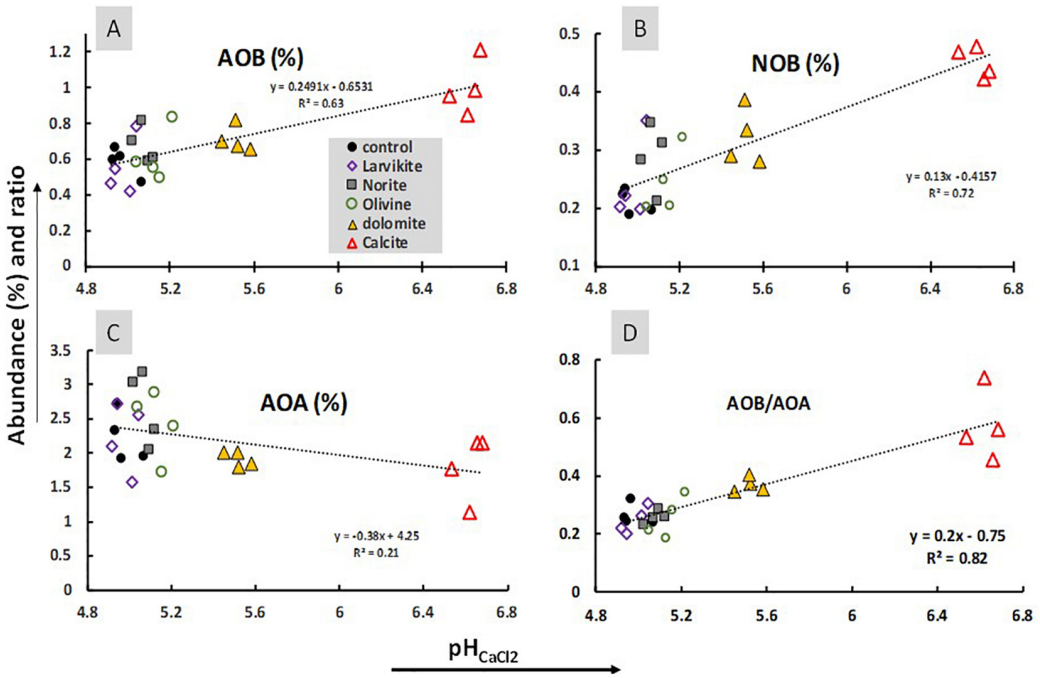

FIGURE 6 | The relative abundance (%) of nitrifying organisms as affected by soil pH. The figures show the relative abundance SSU rRNA copies (%) for AOB **(A)**, NOB **(B)** and AOA **(C)**, and the ratio between AOB and AOA abundance **(D)**; all plotted against pH measured in 0.01 M $CaCl_2$. Each point represents one individual field plot. The equation for the linear regression is included (all were significant, $p < 0.05$). The abundance of dominating groups within AOA and AOB are shown in **Supplementary Table 4**. The N_2O yield of nitrification in soil slurries (Y_{N2O}. mol N_2O-N per mol oxidized NH_4^+) correlated strongly with the AOB/AOA ratio (**Supplementary Figure 7**).

the AOB/AOA ratio increases, which supports our findings (**Figure 6**). The AOA taxa included several candidatus genera as well as a number of uncharacterized taxa, suggesting that there remains considerable diversity to be explored among AOA, whereas for the AOB, all the ASVs were from the *Nitrosomonadaceae* and mostly affiliated with named genera, although most were apparently uncultivated phylotypes such as "mle1-7," "MND1," etc. (**Supplementary File 1**). In addition, the slurry experiments confirmed the effect on nitrification rates and N_2O yields (**Figure 3**). The soil slurry experiments were designed to secure oxic conditions, thus eliminating denitrification as a source of N_2O. This is important, because it means that the N_2O produced in these experiments largely reflects the inherent N_2O production by the ammonia oxidation pathways. A similar increase in abundance of the nitrite-oxidizing *Nitrospira* (**Supplementary Figure 8**) with increasing pH was observed by Rousk et al. (2010), although in that study the r^2 between pH and *Nitrospira* relative abundance was 0.16 with $P = 0.20$.

We further hypothesized that the enhancement of nitrification by liming would increase the risk that the oxygen consumption by nitrification creates hypoxic/anoxic microsites in soil, thus inducing heterotrophic denitrification. This would be aggravated by high soil moisture content (Schurgers et al., 2006). The results with remolded soils shed some light on this (**Table 5**): at low soil moisture content (60 and 70% WFPS), Y_{N2O} was lower than in the soil slurries, but increased with soil pH (as in the soil slurries) thus suggesting that nitrification was the main dominating process. At high soil moisture content (85% WFPS), the apparent Y_{N2O} was 2–3 orders of magnitude higher than in the low moisture (60 and 70% WFPS) experiments, plausibly because oxygen consumption by nitrification along with high WFPS resulted in hypoxic/anoxic microsites and hence denitrification. This N_2O-production by denitrification could be expected to be promoted by high soil pH because of the higher nitrification rate (providing NO_2^- and NO_3^- for denitrification), but the opposite was the case; the N_2O production rates clearly declined with soil pH. Our interpretation of this finding is that, while denitrification was induced at all pH levels, the $N_2O/(N_2 + N_2O)$ product ratio declined with increasing soil pH due to more efficient synthesis of functional N_2O reductase at high pH (Liu et al., 2014). This necessarily implies that heterotrophic denitrification, rather than *nitrifier denitrification* must have been dominating, because ammonia oxidizing bacteria lack the gene for N_2O-reductase. A recent study of the electron flow to denitrification in ammonia oxidizing bacteria support this interpretation, showing that although the electron flow to nitrite- and nitric oxide reductase in these organisms increased in response to hypoxia, it never amounted to more than 1.2% of the total electron flow (Hink et al., 2017b). This finding lends little support to the common notion that *nitrifier denitrification* is a

[2]https://www.r-project.org/

strong source of N_2O in soils under partially hypoxic conditions (Zhu et al., 2013; Mushinski et al., 2019). As argued elsewhere (Bakken and Frostegård, 2017), the evidence for significant N_2O production by *nitrifier denitrification* is in fact rather weak. The notion emerged as a result of circumstantial evidence (co-occurrence, and selective inhibition of ammonia oxidation), and later by employing $^{18}O/^{15}N$ tracing to differentiate between N_2O from *nitrifier denitrification* and heterotrophic denitrification (Wrage-Mönning et al., 2018). The differentiation is based on the assumption that nitrite produced by ammonia oxidizers is exclusively reduced by the ammonia oxidizers themselves, because heterotrophs prefer nitrate. This is highly improbable, however: a fraction of the denitrifying heterotrophs carrying nitrite reductase lack nitrate reductase (Lycus et al., 2017), and those with nitrate reductase will reduce external nitrite along with nitrate because nitrite is a free intermediate that is reduced outside the cytoplasmic membrane. This, together with the fact that heterotrophic denitrifiers grossly outnumber ammonia oxidizing organisms, and that nitrifiers allocate miniscule fractions of the electron flow to denitrification (Hink et al., 2017a), suggests the opposite: under hypoxic conditions the nitrite produced by ammonia oxidizers is more likely to be reduced by heterotrophs than by ammonia oxidizers themselves.

Our results are important because they explain why increasing the pH of acidic soils (by lime or biochar) has a contingent effect on their N_2O emission. Highly variable effects may be expected for systems/conditions where N_2O emission is driven by high ammonium oxidation rates such as urine patches (Clough et al., 2004; Carter, 2007; Khan et al., 2011), while consistent reduction of the N_2O emission can be expected for systems/conditions where heterotrophic denitrification is dominant. Laboratory experiments may be designed to isolate nitrification- and denitrification driven N_2O emissions, but in agronomically realistic field experiments, the contributions of nitrification and denitrification merge and fluctuate (Russenes et al., 2016), being further modulated by a plethora of other factors (Butterbach-Bahl et al., 2013; Saggar et al., 2013; Rochette et al., 2018). As a result, the net effect of pH management on N_2O emissions in single field experiments is to some extent anecdotic, and the evaluation of pH management as a mitigation option should be based on ensembles of well-designed field experiments. Unfortunately, most of the existing emission data are from field experiments designed to test other factors than pH. Nevertheless, a recent meta-study by Wang et al. (2018), which used emission data from 117 studies worldwide, demonstrated an overall reduction of N_2O emission by increasing soil pH, creating a basis for recommending liming for mitigating N_2O emissions from acidified soils.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SN: experimental design and writing, did the laboratory work, and analyses of kinetics. LB: experimental design and writing. ÅF: molecular analyses and writing. JG: bioinformatic analyses and writing. PD: experimental design and writing. All authors contributed to the article and approved the submitted version.

FUNDING

This research was financed by the Norwegian Research Council; project number 234382 (MIGMIN). PD received funding from the FACCE-ERA-GAS project MAGGE-pH under the Grant Agreement No. 696356.

ACKNOWLEDGMENTS

We thank Trygve Fredriksen for support during the field sampling, and Natalie Y. N. Lim for conducting the DNA extractions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2020.598513/full#supplementary-material

REFERENCES

Apprill, A., Mcnally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. doi: 10.3354/ame01753

Bakken, L. R., and Frostegård, Å (2017). Sources and sinks for N2O, can microbiologist help to mitigate N2O emissions? *Environ. Microbiol.* 19, 4801–4805. doi: 10.1111/1462-2920.13978

Blomquist, J., Simonsson, M., Etana, A., and Berglund, K. (2018). Structure liming enhances aggregate stability and gives varying crop responses on clayey soils. *Acta Agric. Scand. B Soil Plant Sci.* 68, 311–322. doi: 10.1080/09064710.2017.1400096

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

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869

Carter, M. S. (2007). Contribution of nitrification and denitrification to N_2O emissions from urine patches. *Soil Biol. Biochem.* 39, 2091–2102. doi: 10.1016/j.soilbio.2007.03.013

Cayuela, M. L., Zwieten, L., Singh, B. P., Jeffery, S., Roig, A., and Sanchez-Mondero, M. A. (2014). Biochar's role in mitigating soil nitrous oxide emissions: a review and metaanalysis. *Agric. Ecosyst. Environ.* 191, 5–16. doi: 10.1016/j.agee.2013.10.009

Chadwick, O. A., and Chorover, J. (2001). The chemistry of pedogenic thresholds. *Geoderma* 100, 321–353. doi: 10.1016/s0016-7061(01)00027-1

Clough, T. C., Kelliher, F. M., Sherlock, R. R., and Ford, C. D. (2004). Lime and soil moisture effects on nitrous oxide emissions from a urine patch. *Soil Soc. Am. J.* 68, 1600–1609. doi: 10.2136/sssaj2004.1600

Čuhel, J., and Šimek, M. (2011). Proximal and distal control by pH of denitrification rate in a pasture soil. *Agric. Ecosyst. Environ.* 141, 230–233. doi: 10.1016/j.agee.2011.02.016

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., et al. (2015). Complete nitrification by *Nitrospira bacteria*. *Nature* 528, 504–509. doi: 10.1038/nature16461

Doane, T. A., and Horwath, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* 36, 2713–2722. doi: 10.1081/al-120024647

Goulding, K. W. T. (2016). Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. *Soil Use Manag.* 32, 390–399. doi: 10.1111/sum.12270

Guo, J., Liu, X., Zhang, Y., Shen, J., Han, W., Zhang, W., et al. (2010). Significant acidification in major Chinese croplands. *Science* 327, 1008–1010. doi: 10.1126/science.1182570

Hamilton, S. K., Kurzman, A. L., Arango, C., Jin, L., and Robertson, G. P. (2007). Evidence for carbon sequestration by agricultural liming. *Glob. Biogeochem. Cycles* 21:GB2021. doi: 10.1029/2006GB002738

Hansen, V. T., and Grimenes, A. A. (2015). *Meteorologisk Data for Ås 2014*. Oslo: Norwegian Meteorological Institute.

Hink, L., Gubry-Rangin, C., Nicol, G. W., and Prosser, J. I. (2018). The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidizers for nitrous oxide emissions. *ISME J.* 12, 1048–1093.

Hink, L., Lycus, P., Gubry-Rangin, C., Frostegård, Å, Nicol, G. W., Prosser, J. I., et al. (2017b). Kinetics of NH3-oxidation, NO-turnover, N2O production and electron flow during oxygen depletion in model bacterial and archaeal ammonia oxidisers. *Environ. Microbiol.* 19, 4882–4896. doi: 10.1111/1462-2920.13914

Hink, L., Nicol, G. W., and Prosser, J. I. (2017a). Archaea produce lower yields of N2O than bacteria during aerobic ammonia oxidation in soil. *Environ. Microbiol.* 19, 4829–4837. doi: 10.1111/1462-2920.13282

Hu, H. W., Zhang, L. M., Dai, Y., Di, H. J., and He, J. Z. (2013). pH-dependent distribution of soil ammonia oxidizers across a large geographical scale as revealed by high-throughput pyrosequencing. *J. Soils Sediments* 13, 1439–1449. doi: 10.1007/s11368-013-0726-y

Huang, T., Gao, B., Hu, X. K., Lu, X., Well, R., Christie, P., et al. (2014). Ammonia-oxidation as an engine to generate nitrous oxide in an intensively managed calcareous Fluvo-aquic soil. *Sci. Rep.* 4:3950. doi: 10.1038/srep03950

Keeney, D. R., and Nelson, D. W. (1982). "Nitrogen inorganic forms," in *Methods of Soil Analysis, Part 2- Chemical and Microbiological Properties*, eds A. L. Page, R. H. Miller, and D. R. Keeney (Madison, WI: American Society of Agronomy Inc), 643–698. doi: 10.2134/agronmonogr9.2.2ed.c33

Khan, S., Clough, T. J., Goh, K. M., and Sherlock, R. R. (2011). Influence of soil pH on NOx and N2O emissions from bovine urine applied to soil columns. *New Zeal. J. Agric. Res.* 54, 285–301. doi: 10.1080/00288233.2011.607831

Klotz, M. G., and Stein, L. Y. (2011). "Genomics of ammonia−oxidizing bacteria and insights into their evolution," in *Nitrification*, eds B. B. Ward, D. J. Arp, and M. G. Klotz (Washington, DC: ASM Press), doi: 10.1128/9781555817145.ch4

Kochian, L. V., Hoekenga, A., and Pineros, M. A. (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.* 55, 459–493. doi: 10.1146/annurev.arplant.55.031903.141655

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

Kool, D. M., van Groenigen, J. W., and Wrage, N. (2011a). Source determination of nitrous oxide based on nitrogen and oxygen isotope tracing: dealing with oxygen exchange. *Methods Enzymol.* 496, 139–160. doi: 10.1016/b978-0-12-386489-5.00006-3

Lim, N. Y. N., Roco, A. C., and Frostegård, Å (2016). Transparent DNA/RNA co-extraction workflow protocol suitable for inhibitor-rich environmental samples that focuses on complete DNA removal for transcriptomic analyses. *Front. Microbiol.* 7:1588. doi: 10.3389/fmicb.2016.01588

Liu, B., Frostegård, Å, and Bakken, L. R. (2014). Impaired reduction of N2O to N2 in acid soil is due to a post-transcriptional interference with the expression of nosZ. *mBio* 5:e01383-14. doi: 10.1128/mBio.01383-14

Liu, B., Mørkved, P. T., Frostegård, Å, and Bakken, L. R. (2010). Denitrification gene pools, transcription and kinetics of NO, N2O and N2 production as affected by soil pH. *FEMS Microbiol. Ecol.* 72, 407–417. doi: 10.1111/j.1574-6941.2010.00856.x

Lycus, P., Bøthun, K. L., Bergaust, L., Shapleigh, J. P., Bakken, L. R., Frostegård, Å., et al. (2017). Phenotypic and genotypic richness of denitrifiers revealed by a novel isolation strategy. *ISME J.* 11, 2219–2232. doi: 10.1038/ismej.2017.82

McMurdie, P. J., and Holmes, S. (2013). Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217

Molstad, L., Dörsch, P., and Bakken, L. R. (2007). Robotized incubation system for monitoring gases (O2, NO, N2O and N2) in denitrifying cultures. *J. Microbiol. Methods* 71, 202–211. doi: 10.1016/j.mimet.2007.08.011

Molstad, L., Dörsch, P., and Bakken, L. R. (2016). *Report: New Improved Robot for Gas Kinetics in Batch Cultures*. Berlin: Research Gate, doi: 10.13140/RG.2.2.30688.07680

Mørkved, P. T., Dörsch, P., and Bakken, L. R. (2007). The N2O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biol. Biochem.* 39, 2048–2057. doi: 10.1016/j.soilbio.2007.03.006

Mushinski, R. M., Phillips, R. P., Payne, Z. C., Avney, R. B., Jo, I., Fei, S., et al. (2019). Microbial mechanisms and ecosystem flux estimation for aerobic NOy emissions from deciduous forest soils. *Proc. Natl. Acad. Sci. U.S.A.* 116, 2138–2145. doi: 10.1073/pnas.1814632116

Nadeem, S., Bakken, L. R., Köster, J. R., Mørkved, P. T., Simon, N., and Dörsch, P. (2015). Soil pH management without lime, a strategy to reduce greenhouse gas emissions from cultivated soils. *Geophys. Res. Abstracts* 17, EGU2015–EGU9790.

Nicol, G. W., Leininger, S., Schleper, C., and Prosser, J. I. (2008). The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environ. Microbiol.* 10, 2966–2978. doi: 10.1111/j.1462-2920.2008.01701.x

Oo, A. Z., Sudo, S., Akiyama, H., Win, K. T., Shibata, A., Yamamoto, A., et al. (2018). Effect of dolomite and biochar addition on N2O and CO2 emissions from acidic tea field soil. *PLoS One* 13:e0192235. doi: 10.1371/journal.pone.0192235

Page, K. L., Allen, D. E., Dalal, R. C., and Slattery, W. (2009). Processes and magnitude of CO2, CH4, and N2O fluxes from liming of Australian acidic soils: a review. *Soil Res.* 47, 747–762. doi: 10.1071/sr09057

Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. doi: 10.1111/1462-2920.13023

Qu, Z., Wang, J., Almøy, T., and Bakken, L. R. (2014). Excessive use of nitrogen in Chinese agriculture results in high N2O/(N2O+ N2) product ratio of denitrification, primarily due to acidification of the soils. *Glob. Change Biol.* 20, 1685–1698. doi: 10.1111/gcb.12461

Raut, N., Dörsch, P., Sitaula, B. K., and Bakken, L. R. (2012). Soil acidification by intensified crop production in South Asia results in higher $N_2O/(N_2 + N_2O)$ product ratios of denitrification. *Soil Biol. Biochem.* 55, 104–112. doi: 10.1016/j.soilbio.2012.06.011

Rochette, P., Liang, C., Pelster, D., Bergeron, O., Lemke, R., Kroebel, R., et al. (2018). Soil nitrous oxide emissions from agricultural soils in Canada: exploring relationships with soil, crop and climate variables. *Agric. Ecosyst. Environ.* 254, 69–81. doi: 10.1016/j.agee.2017.10.021

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., et al. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340–1351. doi: 10.1038/ismej.2010.58

Russenes, A. L., Korsaeth, A., Bakken, L. R., and Dörsch, P. (2016). Spatial variation in soil pH controls off-season N2O emission in an agricultural soil. *Soil Biol. Biochem.* 99, 36–46. doi: 10.1016/j.soilbio.2016.04.019

Saggar, S., Jha, N., Deslippe, J., Bolan, N. S., Luo, J., Giltrap, D. L., et al. (2013). Denitrification and N2O:N2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. *Sci. Total Environ.* 465, 173–195. doi: 10.1016/j.scitotenv.2012.11.050

Schlüter, S., Zawallich, J., Vogel, H. J., and Dörsch, P. (2019). Physical constraints for respiration in microbial hotspots in soil and their importance for denitrification. *Biogeosciences* 16, 3665–3678. doi: 10.5194/bg-2019-2

Schurgers, G., Dörsch, P., Bakken, L. R., Leffelaar, P., and Haugen, L. E. (2006). Modelling soil anaerobiosis from water retention characteristics and soil respiration. *Soil Biol. Biochem.* 39, 2637–2644. doi: 10.1016/j.soilbio.2006.04.016

Shaaban, M., Peng, Q., Hu, R., Wu, Y., Lin, S., and Zhao, J. (2015). Dolomite application to acidic soils: a promising option for mitigating N2O emissions. *Environ. Sci. Pollut. Res.* 22, 19961–19970. doi: 10.1007/s11356-015-5238-4

Shaaban, M., Peng, Q., Lin, S., Wu, Y., Zhao, J., and Hu, R. (2014). Nitrous oxide emission from two acidic soils as affected by dolomite application. *Soil Res.* 52, 841–848. doi: 10.1071/sr14129

Shaaban, M., Wu, Y., Khalid, M. S., Peng, Q., Xu, X., Wu, L., et al. (2018). Reduction in soil N2O emissions by pH manipulation and enhanced nosZ gene transcription under different water regimes. *Environ. Pollut.* 235, 625–631. doi: 10.1016/j.envpol.2017.12.066

Ulén, B., and Etana, A. (2014). Phosphorus leaching from clay soils can be counteracted by structure liming. *Acta Agric. Scand. B Soil Plant Sci.* 64, 425–433. doi: 10.1080/09064710.2014.920043

van Noort, R., Mørkved, P. T., and Dundas, S. H. (2018). Acid neutralization by mining waste dissolution under conditions relevant for agricultural applications. *Geosciences* 8:380. doi: 10.3390/geosciences8100380

von Uexküll, H. R., and Mutert, E. (1995). Global extent, development and economic impact of acid soils. *Plant Soil* 171, 1–15. doi: 10.1007/bf00009558

Wang, Y., Guo, J., Vogt, R. D., Mulder, J., Wang, J., and Zhang, X. (2018). Soil pH as the chief modifier for regional nitrous oxide emissions: new evidence and implications for global estimates and mitigation. *Glob. Change Biol.* 24, e617–e626.

White, P. F., and Robson, A. D. (1989). Rhizosphere acidification and Fe^{3+} reduction in lupins and peas: Iron deficiency in lupins is not due to a poor ability to reduce Fe^{3+}. *Plant and Soil* 119, 163–175. doi: 10.1007/BF02370281

Wijler, J., and Delwiche, C. C. (1954). Investigations on the denitrifying process in soil. *Plant Soil* 5, 155–169. doi: 10.1007/bf01343848

Wrage-Mönning, N., Horn, M., Well, R., Müller, C., Velthof, G., and Oenema, O. (2018). The role of nitrifier denitrification in the production of nitrous oxide revisited. *Soil Biol. Biochem.* 123, A3–A16.

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., et al. (2014). The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. *Nucleic Acids Res.* 42, D643–D648. doi: 10.1093/nar/gkt1209

Zhu, X., Burger, M., Doane, T. A., and Horwath, W. R. (2013). Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2O and NO under low oxygen availability. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6328–6333. doi: 10.1073/pnas.1219993110

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.