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# Impact of liming and maize residues on $N_2O$ and $N_2$ fluxes in agricultural soils: an incubation study

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## Abstract

Since it is known that nitrous oxide ($N_2O$) production and consumption pathways are affected by soil pH, optimising the pH of agricultural soils can be an important approach to reduce $N_2O$ emissions. Because liming effects on $N_2O$ reduction had not been studied under ambient atmosphere and typical bulk density of arable soils, we conducted mesoscale incubation experiments with soils from two liming trials to investigate the impact of long-term pH management and fresh liming on N transformations and $N_2O$ production. Soils differed in texture and covered a range of pH levels (3.8–6.7), consisting of non-limed controls, long-term field-limed calcite and dolomite treatments, and freshly limed soils. Both soils were amended with $^{15}N$-labelled potassium nitrate ($KNO_3$) and incubated with and without incorporated maize litter. Packed soil mesocosms were cycled through four phases of alternating temperatures and soil moistures for at least 40 days. Emissions of $N_2O$ and dinitrogen ($N_2$) as well as the product ratio of denitrification $N_2O/(N_2O+N_2)$, referred to as $N_2Oi$ were measured with the $^{15}N$ gas flux method in $N_2$-reduced atmosphere. Emissions of $N_2O$ increased in response to typical denitrifying conditions (high moisture and presence of litter). Increased temperature and soil moisture stimulated microbial activity and triggered denitrification as judged from $^{15}NO_3^-$ pool derived $N_2O+N_2$ emissions. Fresh liming increased denitrification in the sandy soil up to 3-fold but reduced denitrification in the loamy soil by 80%. $N_2Oi$ decreased throughout the incubation in response to fresh liming from 0.5–0.8 to 0.3–0.4, while field-limed soils had smaller $N_2Oi$ (0.1–0.3) than unlimed controls (0.9) irrespective of incubation conditions. Our study shows that the denitrification response (i.e., $N_2O+N_2$ production) to liming is soil dependent, whereas liming effects on $N_2Oi$ are consistent for both long- and short-term pH management. This extends previous results from anoxic slurry incubation studies by showing that soil pH management by liming has a good mitigation potential for agricultural $N_2O$ emissions from denitrification under wet conditions outside of cropping season.

**Keywords** Soil pH management · Field trials · Denitrification · Nitrous oxide · Dinitrogen · $^{15}N_2$ flux method

## Introduction

Soil pH affects all chemical, biological and physical processes in soil. Many high-latitude agricultural soils are prone to acidification due to their siliceous mineralogy and precipitation exceeding evapotranspiration (Fabian et al. 2014). Acidification results from removal of base cations ($Ca^{2+}$, $Mg^{2+}$, $K^+$ and $Na^+$) by leaching and crop export and is exacerbated by application of ammoniacal fertilisers, acid rain and atmospheric N deposition, nutrient uptake by plants, root exudates and mineralisation of organic matter (Aquilina et al. 2012; Goulding 2016). Soil acidification decreases soil fertility, structural stability (Goulding 2016) and microbial diversity (Fierer and Jackson 2006). Together, this may lead to reduced yields. Acidic soils have also been reported to have large emissions of nitrous oxide ($N_2O$), which account for a major share of the greenhouse gas (GHG) footprint of crop production (Kunhikrishnan et al. 2016; Hénault et al. 2019; WMO 2019). Nitrous oxide has a global warming potential 273 times stronger than that of carbon dioxide ($CO_2$) on a 100-year timeline and is presently the strongest known contributor to stratospheric ozone

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depletion (Ravishankara et al. 2009; Revell et al. 2015; IPCC 2021).

Nitrous oxide is produced in soils mainly by microbial nitrification and denitrification (Butterbach-Bahl et al. 2013; Van Groenigen et al. 2015), with denitrification being the quantitatively dominant source. Denitrification is the dissimilatory reduction of nitrate ($NO_3^-$) or nitrite ($NO_2^-$) in the absence of oxygen mediated by facultatively anaerobic bacteria and fungi. Denitrification is a modular process, producing nitric oxide (NO), $N_2O$ and eventually $N_2$ (Shapleigh 2006). The process is controlled by proximal and distal regulators (Groffman et al. 1988; Saggar et al. 2013). Proximal regulators such as temperature and availability of $NO_3^-$, organic carbon (C) and oxygen ($O_2$) affect denitrifying organisms and their denitrification rates directly, whereas distal regulators, e.g., soil texture, water availability, plant growth and management practices, have an indirect effect. In this respect, soil pH can act both as a proximal and a distal regulator for denitrification (Čuhel and Šimek 2011).

Soil microbial activity is positively correlated with pH up to neutrality. Increasing pH results in increased respiration which, in turn, leads to more $O_2$ consumption and, depending on the soil water content, to anaerobic conditions that favour denitrification (Saggar et al. 2013). Also, mineralisation and nitrification are enhanced at higher pH (Zhang et al. 2023) and thus increase substrate availability ($NO_3^-$) for denitrifiers while also consuming $O_2$, which may lead to nitrification-induced denitrification (Kunhikrishnan et al. 2016; Senbayram et al. 2019; Nadeem et al. 2020; Cheng et al. 2021; Wang et al. 2023). Acidic soils emit more $N_2O$ than alkaline soils because the $N_2O/(N_2O+N_2)$ product ratio increases with soil acidity (Šimek and Cooper 2002; Bakken et al. 2012; Saggar et al. 2013; Liu et al. 2014). Therefore, soil pH not only positively affects the denitrification rate, but also the product ratio of $N_2O$ (Butterbach-Bahl et al. 2013; Šimek and Cooper 2002). The mechanisms, by which $N_2O$ reduction to $N_2$ are inhibited at low pH, are still unclear, but several studies (Bakken et al. 2012; Liu et al. 2014) point at impairment of post-transcriptional assemblage of functional $N_2O$ reductase ($N_2OR$) at low pH, rather than inhibition of the enzyme's activity (Šimek and Cooper 2002). In general, complete denitrification to $N_2$ is promoted by neutral to slightly alkaline soil pH, high soil water content, high soil temperature, low rates of $O_2$ diffusion and the presence of labile C as well as available copper (Cu) as an essential component of $N_2OR$ (Saggar et al. 2013; Shen et al. 2020).

Liming of cultivated soils with limestone, dolomite or quicklime is a common practice to sustain pH-dependent soil fertility within an optimal range for plant nutrition while preventing the mobilisation of heavy metals (Goulding 2016; Holland et al. 2018). Liming also affects soil processes such as soil organic matter mineralisation, $CO_2$ emissions from respiration and lime dissolution (Hamilton et al. 2007), N mineralisation, nitrification and gaseous N emissions (Curtin et al. 1998; Wang et al. 2021). These factors and their interactions affect $N_2O$ dynamics in complex ways (Wang et al. 2021), which need to be considered when interpreting direct and indirect pH-effects on denitrification and $N_2O$ reduction.

Russenes et al. (2016) showed that the $N_2O$ product ratio of denitrification determined in the laboratory was positively correlated with $N_2O$ emissions in a cereal field after harvest, and that both variables were driven by small-scale variation in soil pH. This suggests that the pH effect on the product stoichiometry of denitrification $N_2Oi$ may affect field emissions of $N_2O$ under certain conditions and that raising the soil pH can be used to mitigate these emissions. Similarly, Hénault et al. (2019) found in laboratory and field experiments that liming improved $N_2O$ reduction and decreased soil $N_2O$ emissions. However, liming has also been reported to stimulate soil $N_2O$ emissions due to enhanced nitrification and nitrifier $N_2O$ production (Baggs et al. 2010; Nadeem et al. 2020), increased $NO_3^-$ availability (Clough et al. 2004; Wang et al. 2021) or by inducing coupled nitrification-denitrification (Nadeem et al. 2020). Liming may also affect C dynamics, by stimulating mineralisation activity which could decrease soil organic C (SOC) stocks (Paradelo et al. 2015; Wang et al. 2021). At the same time, liming promotes plant growth and C inputs via litter and root exudation, thus counteracting SOC losses (Ahmad et al. 2013; Liang et al. 2017; Abalos et al. 2020). Finally, liming ameliorates soil structure and improves soil aggregate stability by strengthening the clay-organic matter bonds. This contributes to physicochemical protection of SOC and decreases mineralization rates and microbial respiration (Holland et al. 2018; Wang et al. 2021).

Overall, liming supports agricultural production by optimising soil properties, nutrient cycles and yields (Goulding 2016; Holland et al. 2018; Wang et al. 2021). Still, for devising soil pH management as a GHG mitigation tool, better understanding of the mechanisms and net effects of liming on soil N transformations and gaseous losses is needed.

Most studies investigating the impact of pH on the $N_2O$ product ratio of denitrification have been conducted under standardised laboratory conditions with anoxic cultures of model organisms or soil slurries (Liu et al. 2010; Bergaust et al. 2010; Qu et al. 2014; Nadeem et al. 2020). On the other hand, previous field studies involving pH treatments did not quantify denitrification and $N_2$ emissions (Hénault et al. 2019; Abalos et al. 2020; Žurovec et al. 2021). This might be because the final product of denitrification, $N_2$, is difficult to determine accurately at the field scale due to its high

atmospheric background (Groffman et al. 2006; Scheer et al. 2020). Therefore, to the best of our knowledge, the effect of pH on $N_2$ fluxes has not been investigated at the field scale. Quantifying $N_2$ production to determine the product ratio of denitrification requires sophisticated experimental and technical approaches, such as $^{15}N$ tracing or headspace flushing with $N_2$-depleted atmosphere which is challenging under field conditions (Well et al. 2019), which can be more easily accomplished in an incubation setup (Kemmann et al. 2021; Senbayram et al. 2019).

The objective of the present study was to investigate the effect of long-term soil pH management and fresh liming on denitrification and $N_2Oi$ under controlled but variable environmental conditions. Specifically, we aimed to evaluate the interaction of soil moisture, temperature, litter amendment and liming, while excluding plant effects (i.e., representing periods of fallow common in cereal cropping). We used temperature and moisture controlled mesocosms with repacked soil which we subjected to the $^{15}N$ gas flux method ($^{15}NGF$) in a $N_2$-depleted atmosphere (Lewicka-Szczebak et al. 2013; Kemmann et al. 2021) to quantify both $N_2O$ and $N_2$ from denitrification as well as $N_2O$ produced by other processes, e.g., nitrification. This setup is referred to as mesoscale because the size of the mesocosms exceeds the amount of soil typically used in batch incubations and natural conditions could be mimicked to some extent in terms of headspace gas concentrations, litter and water input. The soil columns were incubated under ambient $O_2$ conditions with and without addition of straw under variable soil moisture and temperature. In this way, we mimicked soil conditions after tillage with and without retention of crop residue.

We hypothesised that (i) adding lime to acidic soils would increase total denitrification due to enhanced mineralisation and associated $O_2$ consumption, transiently increasing $N_2O$ emissions; (ii) denitrification in field-limed soils would have a lower $N_2Oi$ than its unlimed counterpart, leading to reduced $N_2O$ emissions under warm-wet conditions and (iii) that the overall reduction of $N_2O$ emissions by liming depends on the net effect of increasing $N_2+N_2O$ fluxes and increased reduction of $N_2O$ to $N_2$ as well as $N_2O$ production from other processes, such as nitrification.

**Table 1** Properties of the sand and loam soil sampled from long-term field trials (more information in Table S2)

| | Sand (Denmark) | Loam (Norway) |
|---|---|---|
| Soil type | Humic Podzol | Stagnic Albeluvisol |
| Sand (%) | 92 | 39 |
| Silt (%) | 4 | 40 |
| Clay (%) | 4 | 21 |
| Bulk density [g $cm^{-3}$] | 1.41 | 1.14 |
| pH ($CaCl_2$) - unlimed | 3.8 | 4.8 |
| pH ($CaCl_2$) - limed | 6.9 | 6.0 / 6.7 |
| C/N ratio | 12.6 | 11.6 |

## Materials and methods

### Soil sampling and properties

Soil was sampled from two long-term liming experiments in Denmark and Norway differing in soil type, crop and liming history (Table 1). Both field experiments include an unlimed control. The sandy site is part of a long-term liming experiment at Jyndevad, Denmark (54°53'20"N, 9°07'40"E) established in 1942 with continuous cereal cropping (Abalos et al. 2020). The sandy soil was sampled in February 2019 from three replicates of unlimed control plots (pH 3.8) and from plots limed with 12 Mg $ha^{-1}$ dolomite every 6–9 years (pH 6.9) since 1942. The loamy site at Ås, Norway (59°39'47"N, 10°45'42"E) was established in 2014 and compares (among others) unlimed control plots (pH 4.8) with limed plots, which had received 23 Mg $ha^{-1}$ dolomite (pH 6.0) or 30 Mg $ha^{-1}$ calcite (pH 6.7) in autumn 2014. Dolomite was applied as granulate, while calcite was applied as a finely dispersed slurry resulting in a fast pH raise (Nadeem et al. 2020). The loamy soil was sampled in May 2019. At both locations, soil was sampled from 0 to 20 cm, sieved at 10 mm before shipping, and stored at 15 °C in darkness before packing the columns.

### Experimental design: treatments and incubation setup

The sand and the loam soils were incubated in two separate experiments. The setups comprised six pH-management/litter combinations for the sand (unlimed, freshly limed and long-term limed, each with and without addition of 2 g maize straw $kg^{-1}$ dried soil) and eight pH-management/litter combinations for the loam (unlimed, freshly limed and long-term limed by calcite or dolomite, each with and without addition of maize straw), giving a total of 14 treatments. To study the direct impact of liming on denitrification and $N_2Oi$, a subset of the unlimed control soil from both locations was freshly limed with 8.5 and 10.5 mg $kg^{-1}$ soil finely ground limestone ($CaCO_3$; particle size $\leq$ 1 mm), for sand and loam, respectively. The maize straw (C/N ratio 34.5) was obtained by cutting stem and leaves of adult plants into $\leq$2 cm pieces and drying them. The lime and litter were mixed into the soil and preincubated without compaction in covered boxes at 15 °C for 8 to 10 days. Gravimetric water content (GWC) during preincubation (mean ± standard deviation, SD) was 11.7 ± 0.8% for sand ($n$ = 3) and 26.4 ± 1.5% (w/w) for loam ($n$ = 9).

After preincubation, the soils were amended with 71 mg $KNO_3$-N $kg^{-1}$ DM (sand) and 87 mg $KNO_3N$ $kg^{-1}$ DM (loam) equivalent to a N fertilisation rate of 100 kg N $ha^{-1}$ based on the field bulk densities given in Table 1 for the

depth of 0–10 cm. The target $NO_3^-$ enrichment of 60 atom% (at%) $^{15}N$ was achieved by mixing 98 at% labelled $KNO_3$ with unlabelled $KNO_3$ taking into account the initial soil nitrate content. Fertiliser was applied and soil moisture adjusted one day before packing the mesocosms by spraying the fertiliser solution onto the soil while mixing it. Target bulk densities were based on bulk densities measured in the field, i.e., 1.41 and 1.14 g $cm^{-3}$ for sand and loam, respectively. Packed soil columns had a final measured bulk density of $1.37 \pm 0.03$ g $cm^{-3}$ for the sand (mean ± SD, $n = 30$) and $1.01 \pm 0.04$ g $cm^{-3}$ for the loam (mean ± SD, $n = 40$). The soil was packed into Plexiglass cylinders (18 cm high, 14.4 cm i.d.) to a height of 10 cm, leaving a headspace of 1303 $cm^3$. Compaction was achieved by pressing the soil with a piston fitting the column diameter to the target volume. The soil columns were adjusted to respective target initial soil moisture (Table 2) by distributing 0.01 M calcium chloride solution ($CaCl_2$) evenly on the soil surface with a syringe. $CaCl_2$ solution instead of plain water was applied because of the stabilizing effect of $Ca^{2+}$ to prevent excessive particle dispersion (Klute and Dirksen 1986). The cylinders were closed with gas-tight Plexiglass-tops equipped with irrigation nozzles as described in Kemmann et al. (2021). Each treatment had four replicates, with one additional replicate used for destructive sampling to measure water content and mineral N content ($N_{min} = NO_3^-$-N + $NH_4^+$-N) after half of the incubation period (day 20 for sand and day 19 for loam). This resulted in a total of 30 mesocosms for the sand and 40 mesocosms for the loam. Mesocosms were packed and installed in the incubation system within one day, connecting them to a gas chromatograph (GC-2014, Shimadzu) with an automated sampling and online measurement system (Kemmann et al. 2022).

To simulate seasonal conditions typical for the two sites, we changed temperature and soil moisture dynamically throughout the incubation. Two temperature and two soil moisture regimes were applied in four consecutive phases (Table 2). To mimic typical winter and summer soil temperatures, incubation at 5 °C and 15 °C, respectively, was chosen. Initial soil moistures were set close to field capacity (-100 hPa) of the soils which corresponded to 45% water-filled pore space (WFPS, equivalent to 15% GWC) and 52% WFPS (equivalent to 28% GWC) for sand and loam, respectively. Irrigation with 0.01 M $CaCl_2$ after half of the incubation increased WFPS to 57% and 62% in sand and loam, respectively (Table 2).

The duration of each incubation regime was adapted to measured N emissions, i.e., to approach steady-state $N_2O$ fluxes. For instance, the warm-wet phase of the sand incubation was prolonged due to increasing $N_2O$ emissions during that phase, while the cool dry phase of the loam experiment was shortened because there were no changes in emissions. As a result, the timelines differed between soils. Technical problems led to minor disruptions of the temperature regimes, but these were quickly resolved within one day. In total, the sand was incubated for 47 days and the loam for 41 days.

## Soil analyses

After packing of mesocosms, the remaining soil was extracted to measure initial soil moisture, $N_{min}$ contents and pH ($n = 1$ per treatment). This was assumed to be representative for the soil filled in the mesocosms because the entire batch was thoroughly mixed before packing. Destructive sampling of soil mesocosms occurred after phase 2 (half-point throughout the incubation, $n = 1$) and at the end of the incubation ($n = 4$) to determine water content, $N_{min}$ and $^{15}N$ enrichment of $NO_3^-$-N. Soil properties were determined for a subsample of each individual replicate at the end of the incubation. For this, the soil was removed from the column, thoroughly mixed and stored frozen at -18 °C. Subsamples were air-dried for pH analysis and dried at 40 °C for total C and N analysis.

Water content was determined gravimetrically by drying 30 g of soil overnight at 105 °C. WFPS was calculated from bulk density, gravimetric water content and an assumed particle density of 2.65 g $cm^{-3}$. Soil pH was measured with a glass electrode (FE20, Mettler Toledo, Urdorf, Switzerland) in a 1:5 slurry with 0.01 M $CaCl_2$. To analyse $N_{min}$, soil was extracted with 2 M potassium chloride (KCl) in a soil-to-solution ratio of 1:5 (w/w). After filtration (MN 614¼ filters, Macherey & Nagel, Düren, Germany), the extracts were analysed colorimetrically with a continuous flow analyser (SA 5000, Skalar Analytical, Netherlands). Total organic and inorganic C and N analyses were conducted by dry combustion using an elemental analyser (LECO Tru-Mac CN, St. Joseph, MI, USA).

**Table 2** Soil moisture and temperature regimes applied in four consecutive phases. Moisture levels were changed between phases 2 and 3. WFPS is given as final value of the dry (1 + 2) and wet (3 + 4) moisture phases after sampling

| Phase | | 1 – warm dry | 2 – cool dry | 3 – warm wet | 4 – cool wet | |
|---|---|---|---|---|---|---|
| **WFPS** | Sand | 45.4 ± 5.2% | | 57.4 ± 1.0% | | |
| | Loam | 52.0 ± 0.3% | | 61.7 ± 0.3% | | |
| **Temperature** | | 15 °C | 5 °C | 15 °C | 5 °C | |
| **Duration [d]** | Sand | 9 | 11 | 17 | 10 | Σ 47 |
| | Loam | 13 | 6 | 11 | 11 | Σ 41 |

## Gas emission measurements

Concentrations of $CO_2$, $N_2O$, $CH_4$, $N_2$ and $O_2$ in the headspace gas of each mesocosm were measured every four (sand) to six hours (loam) with a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan), which sampled the outflowing headspace air using automated valves. Gas flow rates for each mesocosm were measured every two hours by a digital flowmeter (Kemmann et al. 2021). The flow was set to 20 ml $min^{-1}$. Empty incubation vessels served as blank controls to monitor the background concentrations and isotopic values of the gas supply (see below). Blanks and gas standards for calibrations were regularly integrated into the measuring sequence. The analytical precision was determined by repeated measurements of standards (0.33, 0.55, 2.01, 6.94, 40.4, 130 ppm $N_2O$, 388, 704 2479, 10,000, 20,100, 50,800 ppm $CO_2$) and the coefficient of variation (CV) was consistently < 2% for all gases. To enhance the detection limit of the $^{15}N$ gas flux method, the headspace of the mesocosms was flushed continuously with 20 ml $min^{-1}$ $N_2$-depleted synthetic air (2% $N_2$, 20% $O_2$, 78% He) (Lewicka-Szczebak et al. 2017) using a gas mixer (HovaGAS digital G8, IAS GmbH, Frankfurt, Germany). For the incubation of the loam soil, 400 ppm $CO_2$ and 330 ppb $N_2O$ were added to the gas mixture in order to sustain approximately natural atmospheric levels in the mesocosms and to provide sufficient $N_2O$ for isotope analysis. Additionally, nitric oxide (NO) was measured in the exhaust flow of the mesocosms using a modular gas analyser (CLD 88 Yp, Eco Physics GmbH, Hürth, Germany). All gas fluxes were checked manually and faulty values due to technical problems with the gas supply removed (< 10% of all fluxes) before calculating cumulative fluxes by linear interpolation. The first five days of each phase were excluded from cumulative flux calculations because conditions after packing and phase transition were considered unstable.

## Stable isotope analysis

To estimate total denitrification, the $^{15}NGF$ method was applied (Lewicka-Szczebak et al. 2013). For this, the $NO_3^-$ pool was labelled to ~ 60 at% at the beginning of the experiment. Gas for $^{15}N$ isotope analysis of $N_2$ and $N_2O$ was sampled by serially inserting two 12 ml exetainer vials (Labco Ltd., Lampeter, UK) with rubber septa in the outlet gas flow from the soil mesocosms. Samples from each mesoscosm plus one blank were collected daily after an event (fertilisation, change of temperature, irrigation) for one week and then every second day. Exetainers were thus flushed approximately 2400 times (24 h) before they were disconnected from the gas flow.

Isotope ratio mass spectrometry (IRMS) analysis was conducted as described previously in Lewicka-Szczebak et al. (2017). Samples were chosen based on temporal flux dynamics of $N_2O$ and $CO_2$ as informed by continuous automated GC measurements. Gas samples were analysed using a modified GasBench II (Thermo Scientific, Bremen, Germany) preparation system and automated sampling (PAL Systems, Zwingen, Switzerland) coupled to an isotope ratio mass spectrometer (MAT 253, Thermo Fisher Scientific, Bremen, Germany) according to Lewicka-Szczebak et al. (2013). Prior to analysis, $N_2O$ was reduced to $N_2$ gas in a Cu oven, allowing determination of N isotope mass ratios $^{29}R$ ($^{29}N_2/^{28}N_2$) and $^{30}R$ ($^{30}N_2/^{28}N_2$) from $N_2$, $N_2+N_2O$ and $N_2O$. Fractions of gas species originating from the $^{15}N$-labelled $NO_3^-$ pool undergoing denitrification were quantified for $N_2$ ($fp\_N_2$), $N_2+N_2O$ ($fp\_N_2+N_2O$) and $N_2O$ ($fp\_N_2O$) according to Spott et al. (2006), where the fp notation refer to fraction (f) from the pool (p) of $^{15}NO_3^-$. Replicates of four standard gas samples (compressed air) were analysed together with the samples. The IRMS had an analytical precision (CV) of < 7% (SD < $1*10^{-6}$) for $^{30}R$ and of < 0.01% (SD < $5*10^{-7}$) for $^{29}R$.

## Calculations and statistical analyses

Calculations and statistical analyses were performed with the software R (R Core Team, 2022; version 4.2.2). $CO_2$ and total $N_2O$ ($N_2Ot$) fluxes were calculated per mass of dry soil and time (mg C $kg^{-1}$ $d^{-1}$ and µg N $kg^{-1}$ $d^{-1}$) by multiplying the GC-measured concentration with the flow rate. $N_2O$ emissions measured by GC are named "total" as they comprise $N_2O$ from all pools as opposed to $fp\_N_2O$ from the $^{15}N$-labelled $NO_3^-$ pool (hereafter referred to as $^{15}NO_3^-$ pool).

$N_2O$ and $N_2$ fluxes derived by denitrification from the $^{15}NO_3^-$ pool ($fp\_N_2+N_2O$, $fp\_N_2$, $fp\_N_2O$) and from other sources ($fn\_N_2O$) can be calculated in different ways using either the fractions obtained from isotope ratios by IRMS analysis (fp) or by combining these fractions with total $N_2O$ fluxes obtained by GC analysis. Denitrification calculated from isotopic results may be biased due to inhomogeneity of $^{15}N$ in the labelled pool and because of the possible formation of hybrid $N_2$ and $N_2O$ from anammox or co-denitrification (Zaman et al. 2021) which cannot strictly be distinguished from denitrification derived gaseous N. Moreover, the detection limit for the isotope-based fractions is lower than that for total $N_2Ot$ measured by GC. Finally, when $^{15}NO_3^-$ pool derived $N_2$ and $N_2O$ fluxes are low, some or all of the various fractions ($fp\_N_2$, $fp\_N_2+N_2O$, $fp\_N_2O$) can be below the detection limit. In such cases, missing fractions can be inferred by combining total $N_2O$ fluxes

with detectable isotopic fractions. If all three fractions ($fp\_N_2 + N_2O$, $fp\_N_2$ and $fp\_N_2O$) were below detection, the gap was filled for $fp\_N_2 + N_2O$ with half of the limit of detection (LOD/2) by IRMS analysis (Buchen-Tschiskale et al. 2023; Kemmann et al. 2022). LOD was calculated by 2*SD for $^{29}R$ und $^{30}R$ for each daily sample run of the mass spectrometer. Values of $fp\_N_2O$ can also be calculated from the share of pool derived $N_2O$ relative to total $N_2O$ in the gas sample ($Fp\_N_2O$) and the GC-measured $N_2Ot$ ($fp\_N_2O = N_2Ot$ * $Fp\_N_2O$). We tested the validity of this alternative calculation based on the samples with $N_2Ot$ and $fp\_N_2O > 0.5$ ppm, assuming that above these threshold values robust results from both calculations can be expected. Close agreement of both $fp\_N_2O$ values (Fig. S4) shows that estimating $fp\_N_2O$ from $N_2Ot$ and $Fp\_N_2O$ is a valid approach. When $fp\_N_2 + N_2O$ was smaller than $fp\_N_2O$ after recalculation, we set it to the same level as $fp\_N_2O$. Further details on applied gap filling procedures are given in the supplementary information (SI) (Table S1).

Cumulative gas fluxes were calculated using linear interpolation divided by the number of hours of incubation to account for different incubation periods. Further statistical analyses were conducted with cumulative $CO_2$ and total $N_2O$ fluxes as well as with the $N_2O$, $N_2O + N_2$ and $N_2$ fluxes from the $^{15}N$-labelled $NO_3^-$ pool. The product ratio of denitrification ($N_2Oi = fp\_N_2O / fp\_N_2 + N_2O$) and the ratio of pool derived $N_2O$ relative to total $N_2O$ in the gas sample ($Fp\_N_2O = fp\_N_2O / N_2Ot$) were calculated for each sample and as well as for cumulative fluxes for all treatments and all phases. Occasional $N_2Oi$ and $Fp\_N_2O$ values of > 1 were set to 1. $N_2O$ fluxes from other sources than the labelled pool ($fn\_N_2O$) were calculated as (1–$Fp\_N_2O$) * $N_2Ot$. This is more accurate than calculating $fn\_N_2O$ as $N_2Ot$ minus $fp\_N_2O$, because $Fp\_N_2O$ values are several orders of magnitude higher (typically between 0.5 and 1) than $fp\_N_2$ and $fp\_N_2 + N_2O$ values (typically below $10^{-5}$) because the $N_2O$ background is typically below 10 ppm, while the $N_2$ background was set to 20.000 ppm (2 vol%). Where $Fp\_N_2O$ could not be obtained directly from IRMS analysis, but total $N_2O$ and $fp\_N_2O$ was available, $fn\_N_2O$ was calculated as $N_2Ot$ minus $fp\_N_2O$. Due to the different gap filling methods, there is no exact match between cumulative fluxes of $fp\_N_2 + N_2O$, $fp\_N_2O$, $fp\_N_2$ and $fn\_N_2O$, i.e., cumulative $fp\_N_2O$ is not identical to cumulative $fp\_N_2 + N_2O$ minus cumulative $fp\_N_2$ and $fn\_N_2O$ is not identical to cumulative $N_2Ot$ minus cumulative $fp\_N_2O$.

Changes of $NO_3^-$ content (final $NO_3^-$ minus initial $NO_3^-$) during incubation (Davidson et al. 1991; Deppe et al. 2017) were calculated as an estimate for net nitrification.

To test for differences in cumulative emissions between treatments (liming + litter), phases and soils, analysis of variance (ANOVA) was performed. Cumulative N emissions were log10-transformed to handle variance heterogeneity. Cumulative fluxes were analysed using linear models. When the assumption of homoscedasticity was violated a variance structure was implemented in a generalised least square model (nlme) (Pinheiro et al. 2023). For testing of differences in $N_2Oi$ and $Fp\_N_2O$ between treatments, a beta regression model was applied. Posthoc multiple pairwise comparisons of estimated marginal means were performed with Tukey p value adjustment (emmeans) between treatments and phases.

To test for the impact of pH on $N_2Oi$ of cumulative fluxes, a generalised linear model of the beta-distribution family was used together with a logit link function (R package betareg; Cribari-Neto and Zeileis 2010). The precision parameter of the beta-distribution was modelled in dependence of the soil. Only the treatments with litter were modelled as their isotope signals were more consistent due to higher emissions. The freshly limed treatments were not included in this analysis.

The net effect of pH on $N_2O$ emissions was investigated by regression analysis of fluxes from litter treatments. First, the effect of pH on total denitrification was modelled with a $\log_{10}$-linear model:

$$\log_{10}\left(f_{P,N_2+N_2O}\right) = \alpha_{0,soil,phase} + \alpha_{1,soil,phase} \cdot pH + \varepsilon, \varepsilon \sim N\left(0, \sigma^2\right) \quad (1)$$

where $f_{P,\ N2+N2O}$ (µg N $m^{-2}$ $h^{-1}$) is the flux of $N_2O + N_2$ from the $^{15}NO_3^-$ pool, $\alpha_{i,\ soil,\ phase}$ are regression coefficients stratified by soil and temperature/moisture phase and ε are normal-distributed residuals.

Using the results of this fit, the total $N_2O$ flux was then modelled as:

$$\log_{10}\left(N_2O_{total}\right) = \beta_0 + \beta_1 \cdot pH + \beta_2 \cdot \log\left(f_{P,N_2+N_2O}\right) + \beta_3 \cdot pH \cdot \log\left(f_{P,N_2+N_2O}\right) + \varepsilon, \varepsilon \sim N\left(0, \sigma^2\right) \cdot \quad (2)$$

where $N_2O_{total}$ (µg N $m^{-2}$ $h^{-1}$) is the total (GC-measured) flux of $N_2O$, $\beta_i$ are regression coefficients and ε are normal-distributed residuals.

The parameters of both models were estimated from the data and the parameterized Eq. 1 substituted into the parameterized Eq. 2. This results in a quadratic function describing $\log(N_2O_{total})$ in dependence of pH:

$$\log_{10}\left(N_2O_{total}\right) = \left(\beta_0 + \beta_2 \cdot \alpha_{0,soil,phase}\right) + \left(\beta_1 + \beta_2 \cdot \alpha_{1,soil,phase} + \beta_3 \cdot \alpha_{0,soil,phase}\right) \cdot pH + \left(\beta_3 \cdot \alpha_{1,soil,phase}\right) \cdot pH^2. \quad (3)$$

Using some elementary function analysis, the gradient of these functions within typical soil pH ranges was

**Table 3** Soil pH measured in 0.01 M $CaCl_2$ at start ($n=1$), mid ($n=1$) and end of experiment (mean ± standard deviation, $n=4$)

| | Treatment | Start | Mid | End |
|---|---|---|---|---|
| **Sand** | Control | 3.82 | 3.78 | 3.73 ± 0.11 |
| | Control + Litter | 3.80 | 3.82 | 3.79 ± 0.02 |
| | Freshly limed | 6.29 | 6.57 | 6.54 ± 0.09 |
| | Freshly limed + Litter | 6.42 | 6.62 | 6.59 ± 0.04 |
| | Dolomite | 6.27 | 7.04 | 6.77 ± 0.24 |
| | Dolomite + Litter | 6.63 | 6.77 | 6.54 ± 0.01 |
| **Loam** | Control | 4.77 | 4.70 | 4.54 ± 0.13 |
| | Control + Litter | 4.76 | 4.80 | 4.55 ± 0.20 |
| | Freshly limed | 5.91 | 6.09 | 6.35 ± 0.10 |
| | Freshly limed + Litter | 6.01 | 6.18 | 6.51 ± 0.05 |
| | Dolomite | 5.68 | 5.75 | 5.52 ± 0.22 |
| | Dolomite + Litter | 5.76 | 5.73 | 5.65 ± 0.29 |
| | Calcite | 6.63 | 6.73 | 6.63 ± 0.08 |
| | Calcite + Litter | 6.70 | 6.75 | 6.66 ± 0.05 |

investigated regarding its direction (positive or negative correlation between $N_2O$t and pH).

All significance levels were set to $\alpha = 0.05$ and measures of central tendency and dispersion are given as mean and standard deviation (SD, $n=4$) unless otherwise indicated.

## Results

### Soil parameters

#### Soil pH

At the end of the experiment soil pH in the long-term unlimed controls was 3.8 ± 0.1 and 4.5 ± 0.2 for the sandy and loamy soil, respectively, including both litter treatments. Fresh liming increased the pH to 6.6 ± 0.1 (sand) and 6.4 ± 0.1 (loam). The pH of the dolomite field-limed treatment was 6.7 ± 0.2 in sand and 5.6 ± 0.2 in loam, while the calcite field-limed loamy soil had a pH of 6.6 ± 0.1 (Table 3).

During the incubation, pH values were relatively stable for all treatments. An exception was the freshly limed treatment, where pH increased by 0.2 (sand) and 0.5 units (loam), from first to last sampling, and the dolomite treatment of the sandy soil with a pH increase of 0.5. The litter addition did not affect soil pH compared to the equivalent treatments without litter.

#### Mineral N

In the sandy soil, $NO_3^-$ content increased in all treatments, apart from the control (Table 4). Freshly limed sand showed the highest increase with and without litter addition. Litter addition to long-term dolomite limed and unlimed sandy soil resulted in lower $NO_3^-$ contents than without litter and no increase during incubation. In the loam, $NO_3^-$ contents increased in all treatments, also those with litter addition. Highest increases of $NO_3^-$ occurred in unlimed and calcite limed loamy soil without litter while $NO_3^-$ did not increase as much in freshly limed and calcite limed soil with litter.

KCl-extractable $NH_4^+$ was generally low, particularly in the loam (Table 4). In unlimed and dolomite limed sandy soil, $NH_4^+$ contents tended to increase during incubation. Freshly limed soils had the highest initial $NH_4^+$ contents which decreased significantly without litter until the end of the experiment.

**Table 4** Mineral N contents ($NO_3^-$-N and $NH_4^+$-N) at start ($n=1$), mid ($n=1$) and end ($n=4$) of the incubation experiment. Mean daily changes in $NO_3^-$ concentrations given in mg N $kg^{-1}$ soil. DM, dry matter; b.d., below detection limit

| | Treatment | $NO_3$ [mg N $kg^{-1}$ DM] | | | $NH_4$ [mg N $kg^{-1}$ DM] | | | $NO_3^-$ changes [mg N $kg^{-1}$ $d^{-1}$] |
|---|---|---|---|---|---|---|---|---|
| | | Start | Mid | End | Start | Mid | End | |
| **Sand** | Control | 69.2 | 73.2 | 67.1 ± 7.7 | 0.34 | 5.95 | 5.46 ± 0.92 | -0.04 ± 0.16 |
| | Control + Litter | 65.2 | 78.5 | 65.2 ± 4.9 | 0.52 | 0.61 | 3.10 ± 0.34 | 0.00 ± 0.10 |
| | Freshly limed | 66.1 | 66.1 | 83.4 ± 2.4 | 2.21 | 4.72 | 0.84 ± 0.05 | 0.37 ± 0.05 |
| | Freshly limed + Litter | 67.4 | 79.8 | 80.9 ± 9.4 | 1.88 | 1.25 | 1.80 ± 0.48 | 0.29 ± 0.20 |
| | Dolomite | 72.0 | 79.5 | 83.1 ± 5.2 | b.d. | 0.89 | 1.52 ± 0.34 | 0.24 ± 0.11 |
| | Dolomite + Litter | 65.9 | 69.0 | 65.1 ± 1.1 | b.d. | 1.50 | 2.15 ± 0.56 | -0.02 ± 0.02 |
| **Loam** | Control | 79.5 | 88.1 | 99.1 ± 4.8 | b.d. | b.d. | b.d. | 0.48 ± 0.12 |
| | Control + Litter | 71.3 | 76.8 | 85.9 ± 5.7 | b.d. | b.d. | b.d. | 0.36 ± 0.14 |
| | Freshly limed | 77.7 | 86.3 | 92.1 ± 5.9 | b.d. | b.d. | b.d. | 0.35 ± 0.14 |
| | Freshly limed + Litter | 73.5 | 73.4 | 81.6 ± 8.4 | b.d. | b.d. | 0.29 ($n=1$) | 0.20 ± 0.20 |
| | Dolomite | 75.4 | 85.8 | 91.6 ± 2.9 | b.d. | b.d. | b.d. | 0.39 ± 0.07 |
| | Dolomite + Litter | 77.4 | 79.3 | 94.2 ± 6.7 | b.d. | b.d. | b.d. | 0.41 ± 0.16 |
| | Calcite | 71.8 | 76.9 | 98.2 ± 6.2 | b.d. | b.d. | b.d. | 0.64 ± 0.15 |
| | Calcite + Litter | 66.7 | 64.5 | 74.3 ± 4.1 | b.d. | b.d. | b.d. | 0.10 ± 0.10 |

## Soil gas emissions

### $CO_2$

$CO_2$ emissions from both soils were clearly enhanced by the freshly added lime, particularly so in the first warm-dry phase after which they followed temperature and soil moisture changes (Fig. 1). Largest $CO_2$ emissions of up to 50 mg C $kg^{-1}$ $d^{-1}$ were recorded in the freshly limed treatments at the start of the incubation, which levelled off during the first 2–5 days before becoming more stable during the initial warm-dry phase. Lower temperatures (phase 2 and 4) led to smaller emissions while increase of temperature and soil moisture by irrigation (phase 3) resulted in increased $CO_2$ fluxes.

ANOVA showed that temperature and moisture as well as their interaction were highly significant drivers of mean cumulative $CO_2$ emissions ($p < 0.05$). Litter addition more than doubled the $CO_2$ emission rates in long-term limed and unlimed soils when integrating over all phases, with a smaller increase in freshly limed soils (~ 1.5-fold). Generally, the $CO_2$ fluxes were largest in freshly limed treatments, followed by calcite and dolomite field-limed soils, while the least $CO_2$ was emitted from non-limed soil (Table 5, Table S4) regardless of litter addition.

### NO

Highest nitric oxide (NO) emissions (only measured in loam) were observed in the unlimed control treatment (Fig. S1). Liming treatments substantially decreased NO emissions ($p < 0.001$). NO fluxes responded to the temperature changes with highest emissions in the warm phases. Litter addition significantly increased emissions from the control soil ($p < 0.001$; Table 5). The mean total NO emissions of the limed treatments were around 0.1 µg N $kg^{-1}$ $d^{-1}$ with very little changes over the phases for both litter treatments. By contrast, the unlimed soil emitted on average 0.4 µg NO-N $kg^{-1}$ $d^{-1}$ without litter and 0.7 µg NO-N $kg^{-1}$ $d^{-1}$ with litter (Table 5).

The mean NO/$N_2O$ ratio (NOi, Fig. S2, Table S5) calculated from cumulative fluxes was generally higher in treatments without litter compared to added litter. While the

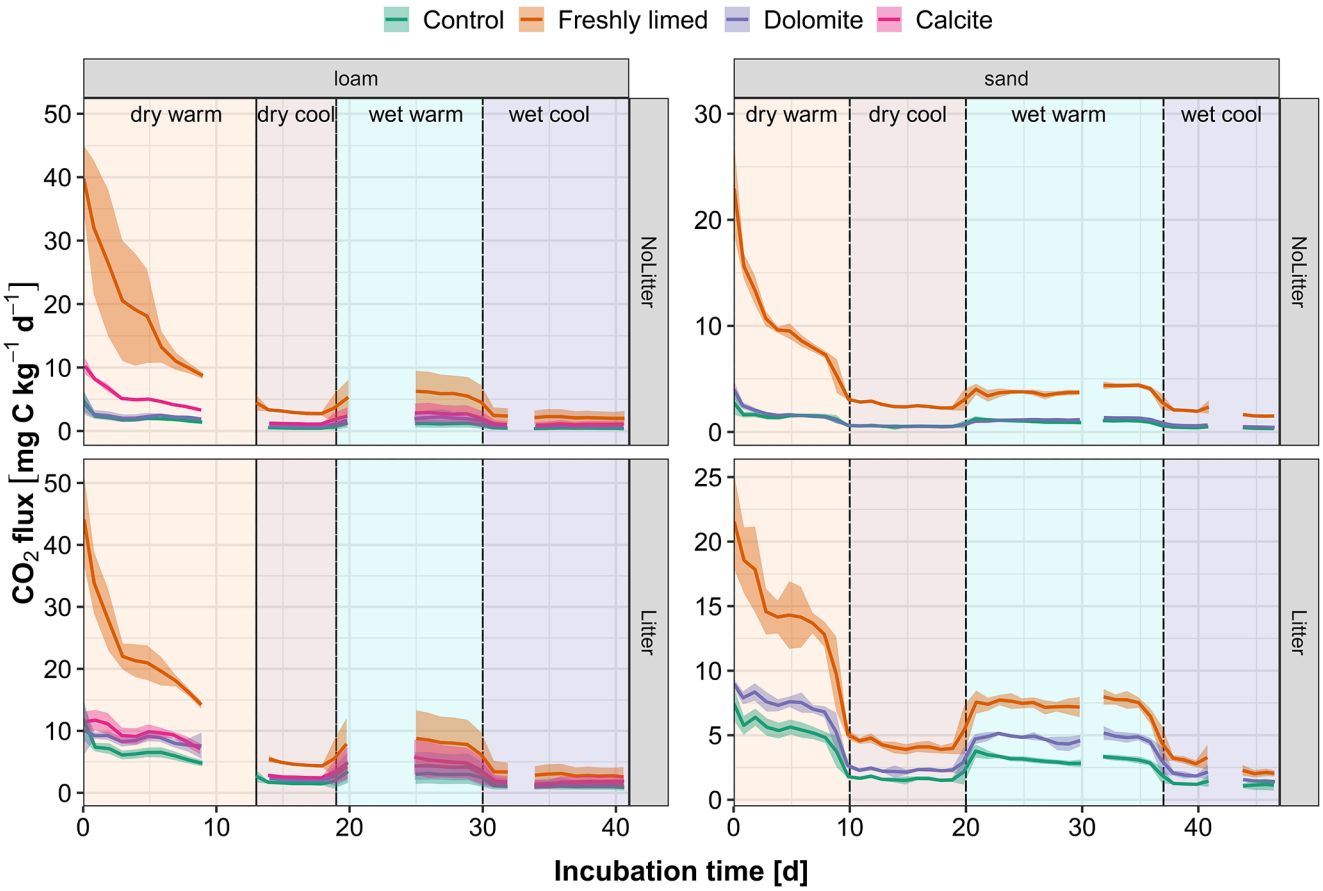


**Fig. 1** Temporal dynamics of $CO_2$ emissions in loam (left) and sand (right) throughout different phases of the incubation (given as mean daily flux rate). Each panel compares fluxes without (upper subpanel) and with maize straw addition (lower subpanel). Note the difference in y-axis scales. Error bands depict ± 1 standard deviation. Data gaps were caused by technical issues

unlimed soil without litter had the highest mean NOi of 0.8, the same unlimed soil with litter had the lowest NOi of 0.02.

### Total $N_2O$

Total $N_2O$ emissions ($N_2Ot$) measured by GC (Fig. 2, Table S6) were low in treatments without litter, except for the second half of phase 3 (warm wet) for the freshly-limed sandy soil, when emissions reached a maximum of 4 µg $N_2O$-N $kg^{-1}$ $d^{-1}$. Emissions were generally larger in treatments with litter addition, exhibiting clearer differences between liming treatments as well as temperature and soil moisture regimes. All fluxes decreased with decreasing temperatures while still showing differences between liming treatments. Emissions increased with increasing soil moisture in all treatments. Litter addition to unlimed soil resulted in emissions of up to 18 µg $N_2O$-N $kg^{-1}$ $d^{-1}$ and 40 µg $N_2O$-N $kg^{-1}$ $d^{-1}$ during the warm-wet phase in the unlimed sand and the loam, respectively. The strongest pH effect was observed in the litter amended loam with higher emissions in the unlimed control throughout all phases.

No significant differences in $N_2O$ emissions were observed between no-litter limed treatments in loamy soil (Table 5), but in sandy soil, the freshly limed soil without litter emitted significantly more $N_2Ot$ ($p < 0.007$) than the other two treatments. Litter addition significantly increased $N_2Ot$ in all liming treatments in both soils, except for calcite-limed loamy soil.

Overall, liming strongly suppressed $N_2Ot$ emissions. The calcite field-limed loam and the dolomite field-limed sand had the lowest emissions, followed by freshly limed soils. Unlimed controls had the highest emissions in both soils irrespective of incubation phase. Liming of loamy soil caused significant (15- to 45-fold) reduction of $N_2Ot$ emissions for all limed treatments with litter. Lime addition to sandy soil with litter still decreased $N_2Ot$ emissions significantly by more than half up to two thirds.

### $N_2$ and $N_2O$ emissions from the $^{15}N$-labelled $NO_3^-$ pool

Denitrification derived $N_2 + N_2O$ emissions followed the pattern of GC-measured total $N_2O$ emissions in relation to temperature and moisture conditions (Fig. 3). Treatments without litter addition had low fluxes of $^{15}NO_3^-$ pool derived $fp\_N_2$ and $fp\_N_2O$, except for freshly limed soil in

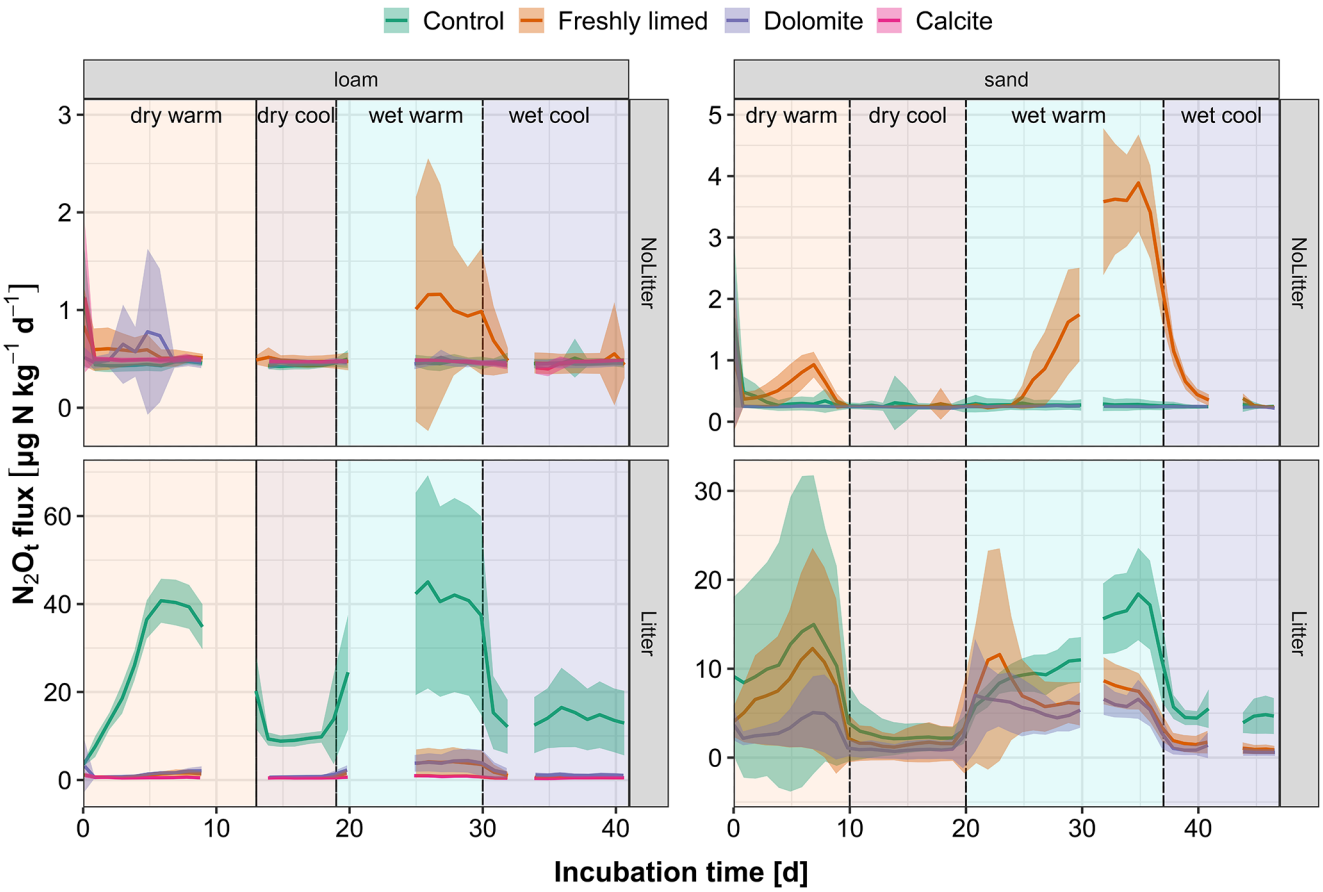


**Fig. 2** Temporal dynamics of $N_2Ot$ emissions of loam (left) and sand (right) throughout different phases of the incubation (given as mean daily flux rate). Note the difference in y-axis scales. Error bands depict ± 1 standard deviation. Data gaps were caused by technical issues

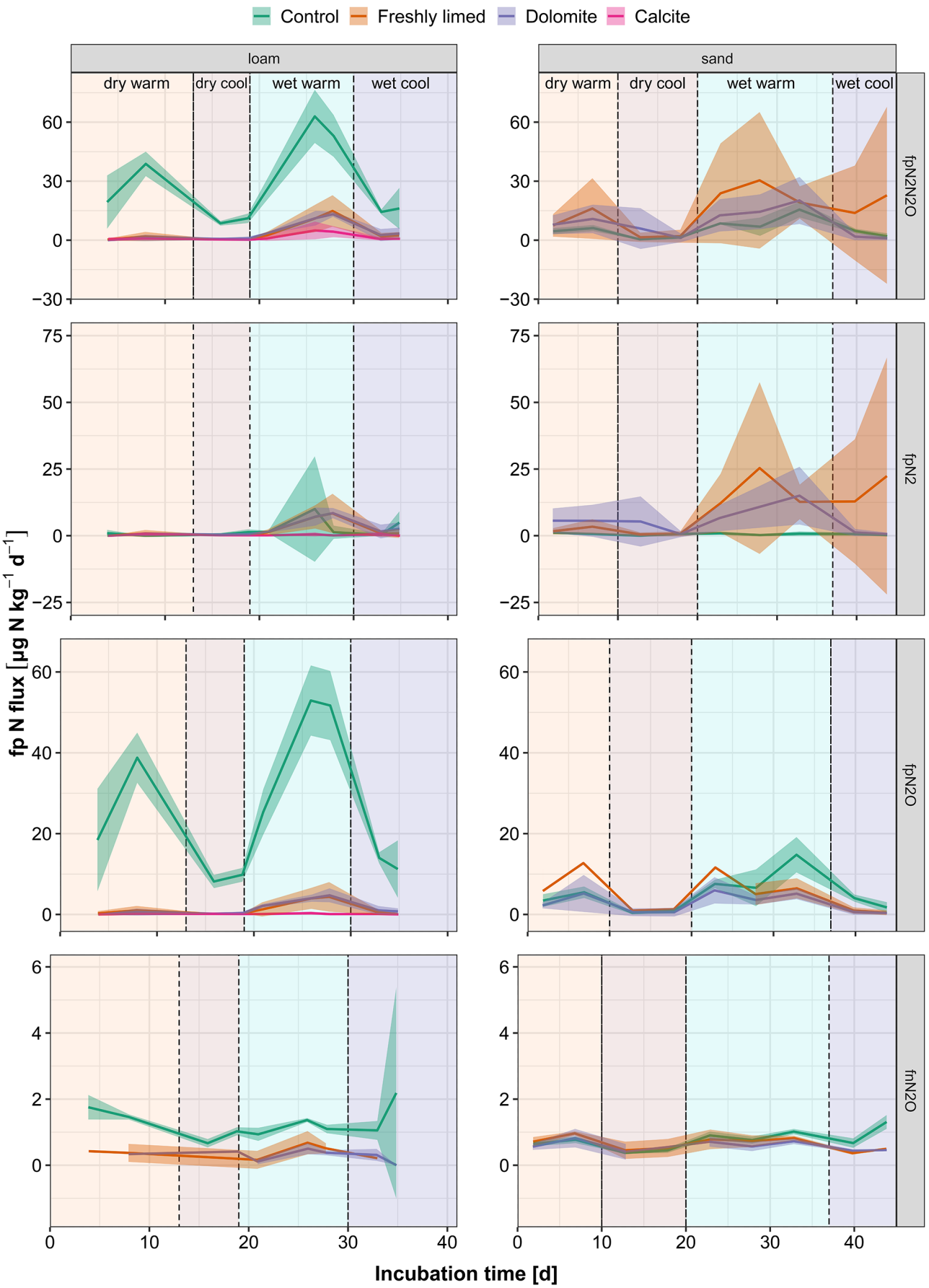
Control
Freshly limed
Dolomite
Calcite
loam
sand
dry warm
dry cool
wet warm
wet cool
fpN2N2O
fpN2
fpN2O
fnN2O
fp N flux [µg N kg$^{-1}$ d$^{-1}$]
Incubation time [d]

**Fig. 3** Mean fluxes derived from the $^{15}N$-labelled $NO_3^-$ pool of $fp\_N_2+N_2O$, $fp\_N_2$ and $fp\_N_2O$, and $N_2O$ from non-labelled N pools ($fn\_N_2O$) from loam (left) and sand (right) for liming treatments with litter. Error bands depict ± 1 standard deviation. All data is based on mass spectrometry measurements

**Table 5** $CO_2$, total $N_2O$ ($N_2Ot$) and NO fluxes (by GC analysis), fluxes derived from the $^{15}N$-labelled $NO_3^-$ pool (by IRMS analysis) of $fp\_N_2+N_2O$, $fp\_N_2$ and $fp\_N_2O$, and $fn\_N_2O$ fluxes from non-labelled N pools (by combining GC and IRMS data). Mean values of the incubation period were derived by dividing cumulative fluxes by incubation time. Given are means of replicate values ± SD ($n=4$) per treatment over all temperature and humidity phases. Letters a-f identify statistic significances for each gas flux. (n. m. – not measured, n. d. – not determinable by (1–$Fp\_N_2O$) * $N_2Ot$). Imbalance between fp fluxes (i.e., $fp\_N_2+fp\_N_2O \neq fp\_N_2+N_2O$) is due to gap-filling and calculation of fluxes by IRMS results only or a combination of IRMS and GC results (see supplement, Table S1)

| Soil | Treatment | pH | $CO_2$ flux [mg C $kg^{-1}$ $d^{-1}$] | $N_2Ot$ flux [µg N $kg^{-1}$ $d^{-1}$] | NO flux [µg N $kg^{-1}$ $d^{-1}$] | $fp\_N_2+N_2O$ [µg N $kg^{-1}$ $d^{-1}$] | $fp\_N_2$ [µg N $kg^{-1}$ $d^{-1}$] | $fp\_N_2O$ [µg N $kg^{-1}$ $d^{-1}$] | $fn\_N_2O$ [µg N $kg^{-1}$ $d^{-1}$] | $N_2Oi$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sand** | Control | 3.7 | 0.80 ± 0.07 $^{d}$ | 0.28 ± 0.05 $^{c}$ | n. m. | 0.16 ± 0.12 $^{c}$ | 0.16 ± 0.12 $^{cd}$ | 0.004 ± 0.003 $^{d}$ | n. d. | 0.02 ± 0.0002 $^{d}$ |
| | Control + Litter | 3.8 | 2.53 ± 0.27 $^{c}$ | 9.69 ± 5.17 $^{a}$ | n. m. | 6.02 ± 1.42 $^{ab}$ | 0.57 ± 0.19 $^{bc}$ | 5.44 ± 1.48 $^{a}$ | 0.74 ± 0.08 $^{a}$ | 0.90 ± 0.05 $^{a}$ |
| | Freshly limed | 6.5 | 3.61 ± 0.23 $^{b}$ | 1.00 ± 0.37 $^{b}$ | n. m. | 2.03 ± 1.07 $^{b}$ | 1.31 ± 0.79 $^{b}$ | 0.54 ± 0.24 $^{b}$ | 0.64 ± 0.02 $^{a}$ | 0.28 ± 0.04 $^{b}$ |
| | Freshly limed + Litter | 6.6 | 6.31 ± 0.67 $^{a}$ | 4.33 ± 3.11 $^{ab}$ | n. m. | 16.75 ± 18.93 $^{a}$ | 11.47 ± 14.22 $^{a}$ | 5.28 ± 4.75 $^{a}$ | 0.70 ± 0.07 $^{a}$ | 0.38 ± 0.11 $^{b}$ |
| | Dolomite | 6.8 | 0.88 ± 0.07 $^{d}$ | 0.24 ± 0.01 $^{c}$ | n. m. | 0.28 ± 0.11 $^{c}$ | 0.10 ± 0.04 $^{d}$ | 0.04 ± 0.01 $^{c}$ | n. d. | 0.16 ± 0.09 $^{c}$ |
| | Dolomite + Litter | 6.5 | 3.64 ± 0.12 $^{b}$ | 2.79 ± 1.77 $^{ab}$ | n. m. | 9.12 ± 5.53 $^{a}$ | 6.23 ± 3.99 $^{a}$ | 2.89 ± 1.55 $^{a}$ | 0.62 ± 0.12 $^{a}$ | 0.33 ± 0.04 $^{b}$ |
| **Loam** | Control | 4.5 | 1.06 ± 0.18 $^{f}$ | 0.45 ± 0.02 $^{c}$ | 0.37 ± 0.03 $^{b}$ | 0.50 ± 0.13 $^{d}$ | 0.25 ± 0.09 $^{b}$ | 0.12 ± 0.02 $^{d}$ | n. d. | 0.26 ± 0.11 $^{bc}$ |
| | Control + Litter | 4.6 | 3.26 ± 0.67 $^{d}$ | 30.45 ± 4.40 $^{a}$ | 0.68 ± 0.09 $^{a}$ | 29.57 ± 3.81 $^{a}$ | 1.83 ± 2.21 $^{ab}$ | 27.73 ± 4.80 $^{a}$ | 1.15 ± 0.13 $^{a}$ | 0.94 ± 0.08 $^{a}$ |
| | Freshly limed | 6.4 | 6.06 ± 0.41 $^{b}$ | 0.65 ± 0.16 $^{c}$ | 0.11 ± 0.01 $^{de}$ | 0.96 ± 1.31 $^{bcd}$ | 0.75 ± 1.01 $^{ab}$ | 0.20 ± 0.30 $^{cde}$ | 0.53 $^{ab}$ | 0.19 ± 0.02 $^{bc}$ |
| | Freshly limed + Litter | 6.5 | 9.21 ± 1.22 $^{a}$ | 1.82 ± 1.10 $^{bc}$ | 0.12 ± 0.01 $^{d}$ | 4.06 ± 2.57 $^{bc}$ | 2.27 ± 1.75 $^{a}$ | 1.42 ± 1.20 $^{bc}$ | 0.37 ± 0.20 $^{b}$ | 0.32 ± 0.14 $^{b}$ |
| | Dolomite | 5.5 | 1.56 ± 0.15 $^{e}$ | 0.48 ± 0.06 $^{c}$ | 0.11 ± 0.02 $^{ef}$ | 0.65 ± 0.55 $^{bcd}$ | 0.20 ± 0.09 $^{b}$ | 0.20 ± 0.11 $^{cde}$ | n. d. | 0.39 ± 0.20 $^{b}$ |
| | Dolomite + Litter | 5.7 | 4.59 ± 0.81 $^{c}$ | 2.31 ± 0.51 $^{b}$ | 0.15 ± 0.02 $^{c}$ | 4.03 ± 0.70 $^{b}$ | 2.47 ± 0.35 $^{a}$ | 1.56 ± 0.40 $^{b}$ | 0.32 ± 0.05 $^{b}$ | 0.38 ± 0.04 $^{b}$ |
| | Calcite | 6.6 | 2.49 ± 0.15 $^{d}$ | 0.47 ± 0.01 $^{c}$ | 0.09 ± 0.01 $^{f}$ | 0.63 ± 0.17 $^{d}$ | 0.32 ± 0.11 $^{b}$ | 0.07 ± 0.01 $^{e}$ | n. d. | 0.12 ± 0.02 $^{c}$ |
| | Calcite + Litter | 6.7 | 5.10 ± 0.66 $^{c}$ | 0.63 ± 0.06 $^{c}$ | 0.10 ± 0.004 $^{ef}$ | 1.52 ± 0.35 $^{c}$ | 0.32 ± 0.02 $^{b}$ | 0.14 ± 0.04 $^{d}$ | n. d. | 0.10 ± 0.06 $^{c}$ |

the warm wet phase. Litter addition significantly increased the $^{15}NO_3^-$ pool derived N emissions by more than one order of magnitude, particularly in unlimed soils (Table 5, Tables S7-S9). Differences between liming treatments as well as fluctuations due to temperature and soil moisture were more pronounced in litter treatments. During the incubation, $fp\_N_2+N_2O$ decreased with temperature and was highest in the warm wet phase (Fig. 3). In the litter treatments, $^{15}NO_3^-$ pool derived nitrogen from unlimed soils was predominantly released as $N_2O$, whereas $N_2$ emissions were enhanced in all liming treatments of the sandy soil. Total denitrification ($fp\_N_2+N_2O$) was lowest in the long-term calcite treatment of the loamy soil.

In the sandy soil, the largest $fp\_N_2+N_2O$ emissions were observed for the freshly limed soil whereas unlimed control had the smallest emissions. Conversely, for the loamy soil, the unlimed control had the largest $fp\_N_2+N_2O$ emissions (up to 60 µg N $kg^{-1}$ $d^{-1}$) while fluxes from the other liming treatments were significantly smaller. Total pool-derived $fp\_N_2+N_2O$ emissions from the unlimed control with litter were 6.0 and 29.6 µg N $kg^{-1}$ $d^{-1}$ in sandy and loamy soil, respectively (Table 5, Table S7). The increase of total emissions due to fresh liming was significant in sandy soil without litter, but not with litter. In contrast, liming treatments of the loamy soil with litter decreased emissions by 82 to 98% compared to the unlimed controls, with calcite-limed loam showing the largest reduction in total denitrification

due to liming. No significant differences in total denitrification were found between fresh and long-term liming of the loam. All experimental factors (liming, litter, phase) and their first-order interactions had a significant effect on total emissions from denitrification ($p < 0.001$, Table S12): $N_2 + N_2O$ increased when liming occurred, litter was added and the phase conditions were warm and/or wet.

$N_2O$ fluxes from other sources than the $^{15}N$-labelled $NO_3^-$ pool ($fn\_N_2O$) were low and in treatments without litter could only be derived for freshly limed soils in the warm wet phase (Table S10). Litter addition did not lead to significantly higher $fn\_N_2O$ fluxes in freshly limed treatments, but was essential to produce emissions from all other pH treatments. In both soils, most $fn\_N_2O$ was produced from unlimed controls amended with litter. There was no significant difference in sand between unlimed and limed treatments, but loam had significantly lower $fn\_N_2O$ from freshly and dolomite limed soil and no detectable flux from calcite treatments (Table S10).

The mean contribution of denitrification to total $N_2O$ emissions ($Fp\_N_2O$) in the litter treatments was 0.73 in the sand and increased in freshly and dolomite limed sand. In contrast, in the loamy control soil, $Fp\_N_2O$ was 0.76 and decreased with liming treatments (Table 6). Litter addition increased $Fp\_N_2O$ significantly in all treatments of sand and in unlimed loam, but not in freshly and field-limed loam.

**Table 6** Fraction of pool derived $N_2O$ of total $N_2O$ ($Fp\_N_2O = fp\_N_2O/N_2Ot$) per treatment calculated from cumulative fluxes

| | | $Fp\_N_2O$ | |
|---|---|---|---|
| | | No Litter | With Litter |
| **Sand** | Control | $0.01 \pm 0.01^{e}$ | $0.73 \pm 0.06^{b}$ |
| | Freshly limed | $0.46 \pm 0.10^{c}$ | $0.92 \pm 0.10^{a}$ |
| | Dolomite | $0.15 \pm 0.04^{d}$ | $0.86 \pm 0.08^{b}$ |
| **Loam** | Control | $0.23 \pm 0.05^{c}$ | $0.76 \pm 0.04^{a}$ |
| | Freshly limed | $0.22 \pm 0.29^{c}$ | $0.54 \pm 0.20^{ab}$ |
| | Dolomite | $0.36 \pm 0.18^{bc}$ | $0.54 \pm 0.04^{ab}$ |
| | Calcite | $0.13 \pm 0.02^{c}$ | $0.19 \pm 0.05^{c}$ |

## The $N_2O/(N_2 + N_2O)$ product ratio of denitrification ($N_2Oi$)

The $N_2Oi$ values (Fig. 4) of the unlimed treatment were close to 1 for both soils (Table 5, Table S11). Thus, only a small fraction of the $N_2O$ produced by denitrification was reduced to $N_2$. The long-term dolomite (sand) and calcite (loam) treatments exhibited the lowest $N_2Oi$ (between 0.1 in loam and 0.4 in sand), meaning that more than 60% of

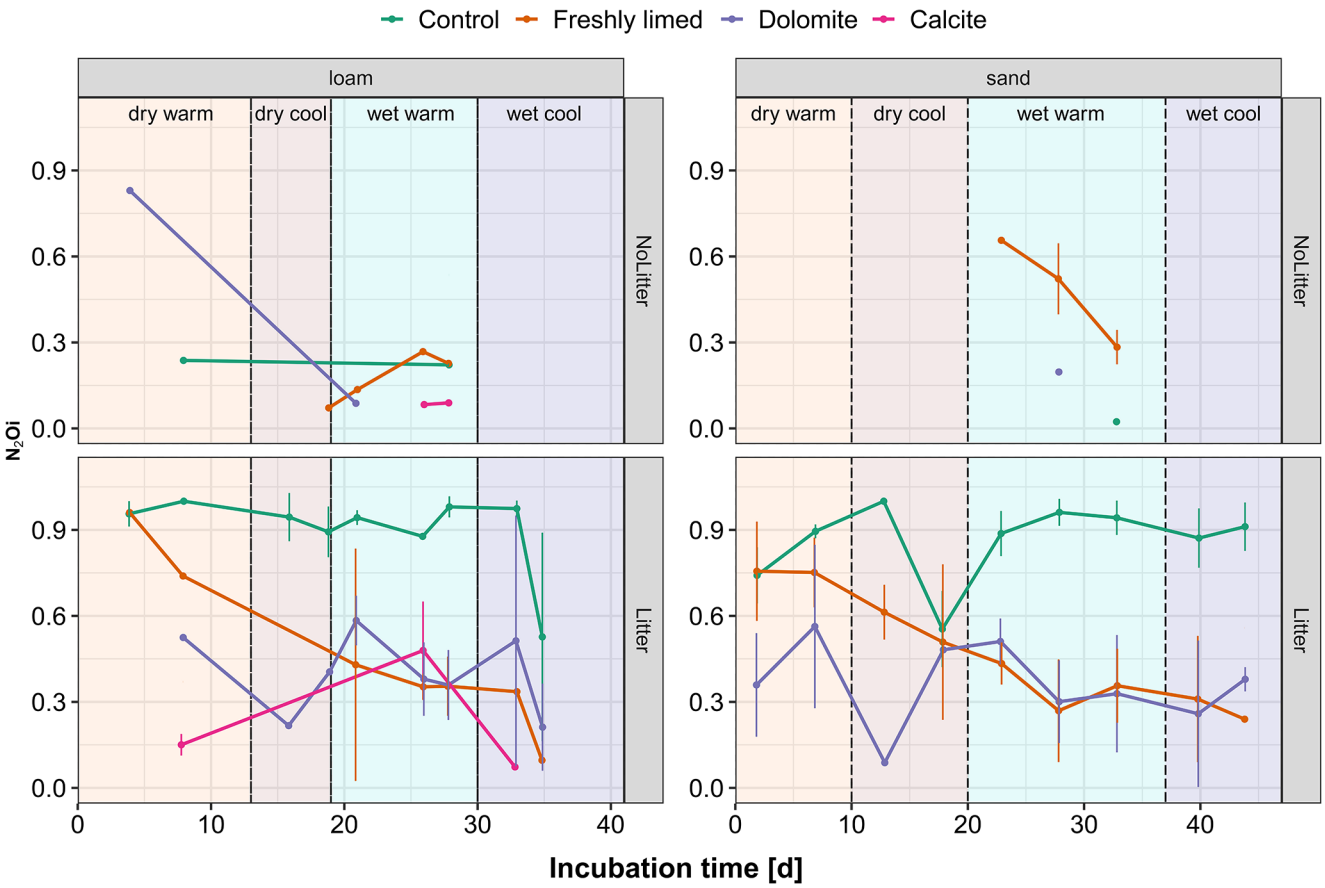


**Fig. 4** Changes of the mean $N_2O/(N_2 + N_2O)$ product ratio of denitrification ($N_2Oi$) during incubation

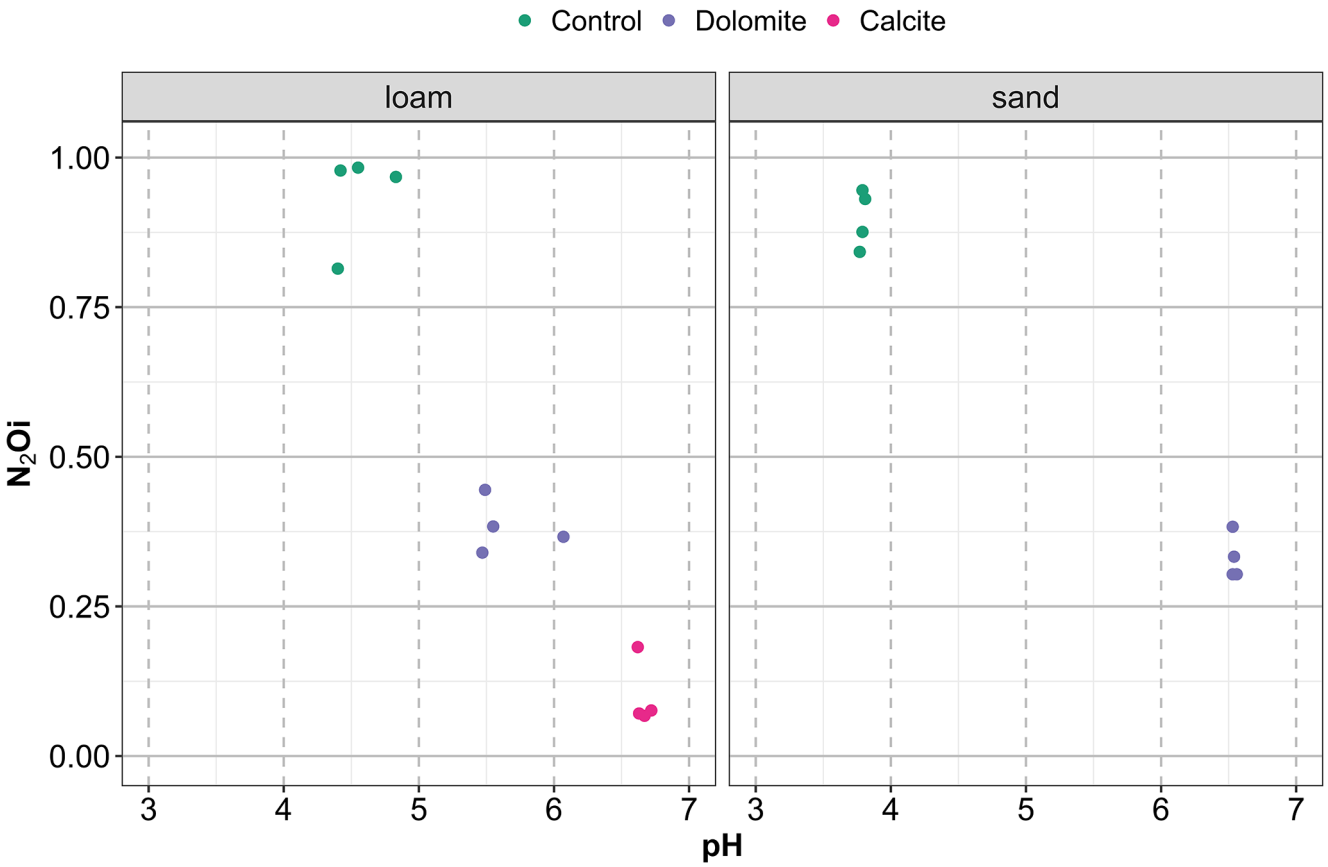


**Fig. 5** $N_2Oi$ as a function of soil pH for long-term unlimed and limed treatments with added litter. Results are shown for the loam (left panel) and sandy soil (right panel)

the denitrification products were emitted as $N_2$. The $N_2Oi$ values of freshly limed treatments in both soils declined steadily throughout the incubation irrespective of temperature or moisture regime.

For both soils, a clear negative correlation between $N_2Oi$ of field-limed soil and soil pH was found (Fig. 5). Unlimed soil was characterised by low pH and high $N_2Oi$ whereas all field-limed treatments exhibited lower $N_2Oi$ values. According to beta regression analysis (Table S13), soil pH had a major effect on $N_2Oi$ ($p < 0.001$) irrespective of soil type. However, soil type had a significant impact on the variance i.e., the precision parameter of the beta-distribution, which was higher in the loamy soil ($p < 0.001$). The model confirms the hypothesis that $N_2Oi$ decreases with increasing pH.

## Net effect of pH on total denitrification and $N_2O$ emissions

The mean total $N_2O$ fluxes correlated negatively with soil pH. This effect was more pronounced in the loamy than the sandy soil. Disaggregating total $N_2O$ fluxes into the pool derived N fluxes (Table 5) revealed contrasting dynamics for the two soils. In the loamy soil, high pH was associated with lower total denitrification ($fp\_N_2+N_2O$) and lower $fp\_N_2O$ but relatively stable $fp\_N_2$. In contrast, in the sandy soil $fp\_N_2+N_2O$ and $fp\_N_2$ increased with pH while $fp\_N_2O$ declined slightly with increasing pH. Thus, the stimulation of $N_2O$ production by denitrification at higher pH in the sandy soil was offset by a decreasing $N_2Oi$. This was also true if pH increase resulted in enhanced $N_2+N_2O$ fluxes, showing that in these cases the pH-induced decrease in $N_2Oi$ overcompensated for an increase in $N_2O$ emission due to increased total denitrification ($N_2+N_2O$). This was confirmed by the regression analysis (Eq. 1 to 3). Fitting the models for $N_2+N_2O$ (Eq. 1) and $N_2O$ (Eq. 2) fluxes resulted in $R^2$ values of 0.74 and 0.75, respectively. Except for the "wet cool" treatment, the coefficients $\alpha_{1,soil,\ phase}$ in Eq. 1 were positive for the sandy soil which indicates an increase in total denitrification with increasing pH. Conversely, for the loamy soil, results indicate a decrease of total denitrification with increasing pH (negative $\alpha_{1,soil,\ phase}$). NO fluxes, which were not included in the models, were also highest from the low-pH control treatment in the loamy soil. For the final $N_2O$ model (Eq. 3), the gradient was negative for all phases in both soils and the vertex of the parabola was always very far outside the possible pH range, i.e., the

combined effect of increasing pH on total denitrification and the product ratio of denitrification can be expected to always decrease $N_2O$ emissions in the two soils under the studied environmental conditions if crop residues are available.

## Discussion

### Liming impact on total denitrification and non-denitrification processes

Although pH increase through liming is a factor that has been claimed to increase denitrification in line with a general increase of microbial activity (Čuhel et al. 2010; Saggar et al. 2013), we did not detect significant increases in total denitrification ($fp\_N_2+N_2O$) from our fresh liming treatments for loamy or sandy soil, except in sand without litter. Litter addition to the loamy soil strongly stimulated total denitrification ($fp\_N_2+N_2O$), and unexpectedly, the unlimed control had the highest total denitrification rates, with notably 90% of the emissions occurring as $N_2O$-N. The observation that liming decreased total denitrification in the loamy soil contradicts our hypothesis that liming and high pH would increase total denitrification. Liming of the sandy soil in the presence of residues apparently increased denitrification (Table S7, Fig. 3) but due to heterogeneity of $N_2O$ fluxes this increase was not statistically significant. High variability of denitrification in unsaturated soils is known to result from the fact that the process is typically limited to anoxic hot-spots (Groffman et al. 2009; Rohe et al. 2021) often linked to the heterogenous distribution of labile organic C, e.g. plant residues (Parkin 1987). The extremely high variability in the sandy soil could be because the spatial distribution of litter might have higher impact in the sand than in the loamy soils due to the higher gas diffusivity of the sand, where patchiness or clumping of litter might be prerequisite for development of anoxic microsites (Loecke and Robertson 2009; Well et al. 2023).

Liming increases microbial activity which leads to increased mineralisation, which in turn leads to higher substrate availability for nitrification and denitrification and local oxygen depletion (Saggar et al. 2013; Shaaban et al. 2020). A tendency toward increased N fluxes in freshly limed treatments versus long-term dolomite-limed treatments was seen for both soils, but this effect was only significant for the sandy soil without litter in moist phases. Surprisingly, lime addition to the acidic loam had a negative effect on denitrification. In the presence of litter, $fp\_N_2+N_2O$ decreased instantly to the level of field-limed soils. One reason might be the high clay content of this soil. Liming stabilises soil structure by providing $Ca^{2+}$ and $Mg^{2+}$ ions which improve aggregate stability and soil aeration (Mordhorst et al. 2018; Frank et al. 2019). Packing and wetting of the soil might have induced disturbance of microstructure in unlimed soil but to lesser extent in limed soil. Hence, the observed lower denitrification rates of limed loamy soil could result from better aeration due to more stable pore structure and thus lower anaerobic soil volume fraction by improved $O_2$ exchange. The structure effect on $O_2$ diffusivity is known to be relevant in soils with higher clay content only, which might explain the contrast to the sandy soils (Frank et al. 2021), but so far, the significance of this effect for denitrification has not been studied in detail. We can rule out that abiotic $N_2O$ production might have contributed to this effect since this process can be relevant only at low pH (Venterea 2007; Wei et al. 2019).

In contrast to our results, Senbayram et al. (2019) found a clear increase in $N_2+N_2O$ with pH. However, in that study, soils were not packed to field bulk density and clay content was low. We suspect that in contrast to our study, a liming effect on soil structure was thus missing or was less pronounced. They also observed no mitigation effect of liming in $NO_3^-$ treated soils during oxic incubation but only under prolonged anoxic conditions (Senbayram et al. 2019). This might be in line with our results as we observed a liming effect on $N_2O$ emissions, i.e., increasing the share of $N_2O$ completely reduced to $N_2$, only in the litter treatments with presumably larger anaerobic soil volume fraction. Also, $N_2O$ from other sources than denitrification was not assessed by Senbayram et al. (2019), thus liming could have favoured $N_2O$ fluxes from nitrification (Prosser et al. 2020) and it is not clear to which extent the lack of $N_2O$ mitigation was due to other factors overriding pH effects on $N_2Oi$.

### Impact of liming on the $N_2Oi$ product ratio of denitrification

Liming stimulated complete denitrification by promoting the overall reduction of $NO_3^-$ via $N_2O$ to $N_2$. This was reflected by higher $N_2$ emissions and thus lower $N_2Oi$ values in limed than unlimed soils. As hypothesised, $N_2Oi$ was close to 1 in non-limed, acidic controls and decreased with increasing soil pH, regardless of long-term or fresh liming. This was true for all liming treatments of both soils. We expected fresh liming to have variable effects on $N_2Oi$, but found a clear trend of decreasing $N_2Oi$ values throughout the different phases also in freshly limed soil. This finding is in accordance with denitrification experiments conducted with anoxic incubations (Liu et al. 2010; Bergaust et al. 2010; Raut et al. 2012; Qu et al. 2014) and is supported by a similar slope of $N_2Oi$ as a function of pH reported by Qu et al. (2014) (Fig. 6), notwithstanding the fact that incubation conditions differed strongly.

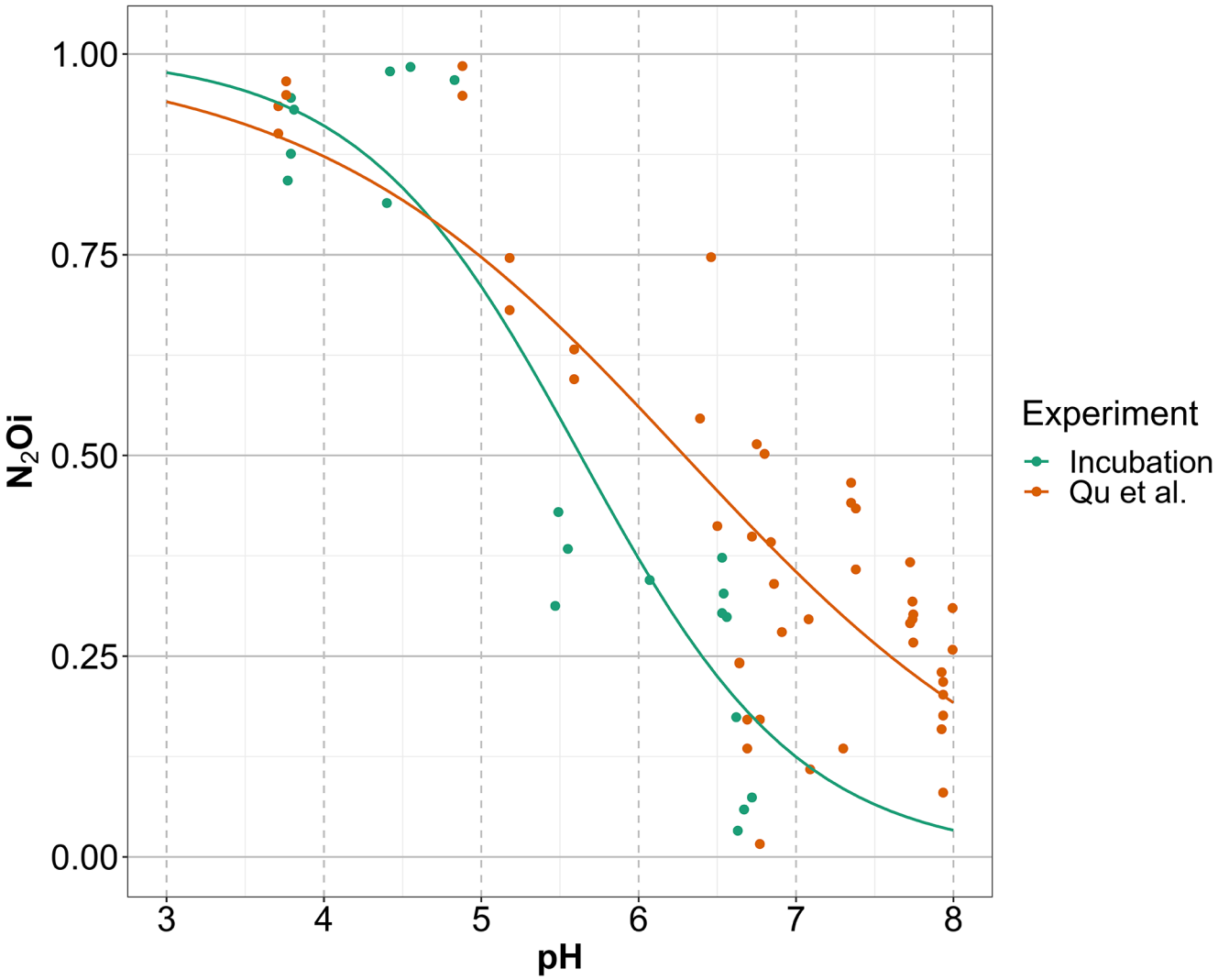


**Fig. 6** Dependency of $N_2Oi$ on soil pH for data from Qu et al. (2014; orange) and this study (green). Green dots are average mesocosm $N_2Oi$ values ($n = 20$) of unlimed and field-limed treatments with litter of both sand and loam soils and over all phases with modelled beta regression (green line). Orange dots are data of five Chinese soils with different fertilisation treatments from study of Qu et al. (2014)

Our incubation was designed to include aerobic conditions in packed soils during dry phases, which would inhibit denitrification. During the wet phases, increased mineralisation and respiration due to liming and litter in combination with reduced diffusive $O_2$ exchange induced by high WFPS likely promoted denitrifying activity in anoxic microsites (Schlüter et al. 2018). Soil structure is an important factor in this context as it controls the diffusivity of $O_2$. We worked with repacked soil cores adjusted to typical bulk densities and incubated under ambient $O_2$ concentration, thus mimicking soil porosity and aeration under field conditions. By contrast, Qu et al. (2014) worked with 4-mm sieved soils without repacking and determined $N_2Oi$ in fully anoxic batch incubations, thereby inducing potential denitrification rates which rarely occur in arable soils. Low $O_2$ is known to increase $N_2O$ reductase activity (Morley et al. 2008) and $N_2Oi$ might also depend on the actual denitrification rate and its impact on substrates ($NO_3^-$ and labile organic C) (Senbayram et al. 2019, 2022). Using field bulk densities allows to keep $N_2O$ residence times in the soil similar to those in the field, which is a crucial factor determining the chance for $N_2O$ to be reduced to $N_2$ (Ball 2013). The question arises whether the dependence of $N_2Oi$ to pH under field conditions is similar to the observed effects under anoxic conditions with disturbed soil structure which has not been tested experimentally so far. Our study extends previous work by evaluating to which extent the regulation of $N_2Oi$ by soil pH described for fully anoxic conditions also holds for field-like conditions. In dry phases and/or treatments without litter, the pH effect was less evident (Fig. 4), presumably due to low denitrification rates. We could confirm the pH effect on $N_2Oi$ only in litter treatments, i.e., under conditions where

the anaerobic soil volume was relatively large, likely making denitrification the dominant source of $N_2O$. Our results confirm that liming has a strong effect on denitrification and $N_2Oi$, implying that the overall mitigation effect of liming on $N_2O$ emission will be relevant under conditions favouring denitrification. When denitrification plays a minor role, $N_2O$ fluxes from other sources may dominate.

In a study with long-term limed acidic sandy soils, Senbayram et al. (2019) argued that soil nitrate concentration is the dominating factor for $N_2Oi$ rather than soil pH and determined a threshold nitrate concentration of 45 mg N $kg^{-1}$ soil above which the $N_2O$ reduction to $N_2$ is almost completely inhibited (> 90%) regardless of soil pH. Our study showed a pronounced pH effect despite high nitrate availability from the start (> 65 mg N $kg^{-1}$) and nitrification enhanced by liming, as evidenced by increased nitrate concentrations towards the end of the incubation.

While the microbial communities of the long-term limed and unlimed soils are adapted to the prevailing soil pH, fresh liming requires the microbiome to adapt to the new pH conditions. Šimek et al. (2002) concluded from their study with long-term and short-term limed soils that it takes about two days until denitrifiers have adapted to changed pH. They proposed this could either be due to the development of a community of denitrifiers which can grow better at higher pH or due to accommodation of the existing populations to new conditions, i.e., better functioning of the $N_2O$ reductase (Šimek et al. 2002). By contrast, Dörsch et al. (2012) and Brenzinger et al. (2015) found that parental pH has a longer-lasting effect on the denitrification product stoichiometry ($N_2Oi$) in extracted microbial communities after pH upshift. In our study, $N_2Oi$ in freshly limed treatments declined steadily throughout 41–47 days of incubation in both soils, suggesting that the denitrifier community changed gradually with time towards more complete denitrification. This indicates either a prolonged adaptation time of the microbial community to pH upshift after liming, a gradual change in pH due to ongoing lime dissolution, or it might be the result of enhanced denitrifying conditions in the second half of the incubation, allowing for de novo synthesis of functional $N_2O$ reductase. The apparent increase in pH during the experiment in the freshly limed treatments (Table 3) indicates that the temporal pH effect was relevant.

## Net effect of liming on $N_2O$ fluxes

We observed that the impact of pH was consistently strong with respect to $N_2Oi$ and variable with respect to $fp\_N_2O + N_2$ (total denitrification). $N_2O$ emissions were negatively related to pH due to the pH effect on $N_2Oi$ and because fresh liming did not significantly increase $fp\_N_2 + N_2O$ to counteract the shift on $N_2Oi$ as hypothesised, although there was more denitrification in freshly limed sand. On the contrary, in the loamy soil liming lowered the $fp\_N_2 + N_2O$ flux, further enhancing the $N_2O$ reducing effect of pH rise. A stimulation of total denitrification by pH raise could only be seen as a non-significant trend in the sandy soil, whereas in the loamy soil, total denitrification was highest in unlimed soil with $N_2O$ as the main product.

Our approach allowed to quantify not only $N_2Oi$, but also the share of $N_2O$ originating from labelled and non-labelled sources ($Fp\_N_2O$, $1-Fp\_N_2O$). This raises the question how $N_2O$ fluxes from processes other than denitrification of the initial nitrate pool were affected by the liming treatments and if these effects have relevance in addition to the expected main effects on $N_2O$ reduction to $N_2$ by denitrification. Because hypoxia is crucial for denitrification to occur, presence of $O_2$ (i.e., during dry phases) should increase the fraction of $N_2O$ produced by nitrification which is an aerobic and strongly $O_2$-consuming process. Although we did not add $NH_4^+$-N, we observed a significant increase in $NO_3^-$ content throughout incubation in all treatments except for control treatments of sandy soil. This shows that nitrification was relevant, oxidizing $NH_4^+$ released by mineralisation. Treatments without litter exhibited a large share of $N_2O$ which did not originate from the $^{15}N$-labelled $NO_3^-$ pool (low $Fp\_N_2O$ values, Table 6) which might thus have been produced via autotrophic or heterotrophic nitrification, coupled nitrification-denitrification or nitrifier denitrification (Wrage-Mönnig et al. 2018). In both soils, $fn\_N_2O$ was below detection in treatments without litter except for freshly limed treatments in the wet-warm phase. However, the contribution of these sources to the total flux ($Fp\_N_2O$, Table 6) was lower in the litter treatments, because litter increases the fluxes from the labelled source, i.e., from denitrification of the initial nitrate pool due to the litter impact on denitrification. In absence of litter, fresh liming increased $N_2O$ fluxes from other sources than $NO_3^-$ ($fn\_N_2O$, Table 5 & S10), which was probably due to enhancement of N mineralisation and subsequent nitrification by liming (Nadeem et al. 2020; Prosser et al. 2020; Vekic et al. 2023). This is supported by the positive slope of $NO_3^-$ accumulation as a function of pH for loam and sand in the absence of litter (Fig. S3) since we assume that changes in nitrate content are related to nitrification.

NO fluxes indicated that nitrification was not the dominating process of $N_2O$ production in most of the treatments, as the $NO/N_2O$ flux ratios (NOi) were below the threshold of < 1 (Pérez et al. 2006). Only in unlimed loamy soil with litter, NOi was > 1 and $fn\_N_2O$ was significantly higher than in the limed treatments. Zhu et al. (2013) reported that nitrification is not exclusively bound to fully aerobic conditions, but can significantly contribute to $N_2O$ emissions even under limited $O_2$ availability, e.g., in relation to litter

amendment followed by $O_2$ consumption. This could imply that processes other than denitrification are substantial contributors to total $N_2O$ emissions in unlimed, low pH soil.

### Comparison with field emissions

Our study bridges the gap between fully anoxic denitrification process studies (as in Qu et al. 2014) and net $N_2O$ emissions measured in the field by distinguishing pH effects on $N_2Oi$ and $N_2O$ from other processes. Wang et al. (2018) showed in their global meta-analysis that acidic soils release significantly more $N_2O$ than alkaline soils with the same N input. In the years 2018 and 2019, field measurements of the sandy soil used in our study showed a reduction in $N_2O$ emissions of approximately 40–80% due to liming, similar to the results in our incubation (Abalos et al. 2020). In a combined laboratory and field study, Hénault et al. (2019) found that $N_2O$ reduction was promoted by increased pH resulting in decreased $N_2O$ emissions. Results from a long-term limed grassland trial by Žurovec et al. (2021) also showed a decrease in $N_2O$ emissions of up to 39% in limed compared to unlimed plots while also observing peak $N_2O$ emissions after fertilisation when soil moisture was high with WFPS values between 60 and 80%. Likewise, our study proved denitrification to be the dominant source of $N_2O$ in wet soil with litter amendment, but $N_2O$ from other sources, such as nitrification, to be enhanced when litter was not present. Flux measurements outside the cropping season in a field study of natural pH variation (5.4–5.9) by Russenes et al. (2016) correspondingly showed that emissions during autumn were dominated by denitrification enhanced by decomposition of crop residues and seasonally high moisture. Besides, the same inverse relationship between soil pH on $N_2O$ emissions was confirmed where already small spatial variation of natural pH influenced $N_2Oi$ and $N_2O$ emissions significantly.

Together, these findings are in line with the results of our study which show that liming is effective to lower $N_2O$ emissions from denitrification under conditions favouring this process i.e., during wet phases and after crop residue incorporation. Yet, our experiment was a short-term study which excluded effects from growing crops to control other factors. Therefore, our data may not fully explain the often observed lowering of $N_2O$ fluxes in agricultural soils by liming. The dynamic incubation resulted in flux dynamics similar to field emissions when N substrates are not limiting, such as in soil between crop rows with weak rhizosphere (Kemmann et al. 2021). However, growing plants induce multiple effects on $N_2O$ fluxes (Rummel et al. 2020; Maurer et al. 2021) which interact with pH and liming, which have not yet been addressed to the best of our knowledge.

## Conclusions

We investigated two remoulded soils with different textures and a range of pH values under varying conditions in mesoscale incubation experiments to determine the effect of liming on $N_2O$ producing processes and $N_2$ emissions. Our experiments extend previous findings obtained with fully anoxic slurry incubations, since we studied liming effects under natural $O_2$ conditions and with typical bulk density of arable soil while simultaneously assessing source-specific $N_2O$ fluxes. Our results show that under ambient atmosphere, the previously reported inverse relationship between soil pH and $N_2O$ emissions can only be expected if denitrifying conditions are induced, i.e., facilitated by high temperature, high moisture and added plant litter. This effect was linked to a direct pH effect on $N_2Oi$ in both soils, decreasing the proportion of $N_2O$ in denitrification with increasing pH, irrespective of from long-term or fresh liming. We therefore conclude that the pH effect on the product stoichiometry of denitrification is soil independent. By contrast, the effect of liming on overall denitrification appears to be soil dependent. The expected increase in total denitrification due to rising pH did not occur; instead, we found highest total emissions from the low pH loamy soil. We suspect an underlying effect of liming on soil structure leading to better aeration after liming this soil. Fresh lime addition led to higher denitrification and $N_2O$ emissions compared to long-term limed soils, but the continuous decline of $N_2Oi$ during incubation points to an adaptation of denitrifiers to altered pH conditions within a few days, mediating a more complete reduction of $N_2O$ to $N_2$. In the absence of conditions promoting denitrification, we did not observe significant liming or pH effects on $N_2O$ fluxes and processes. Further studies are needed to study pH and liming effects on $N_2O$ transformations in liming trials at the field scale, including growing crops.

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**Author contributions** All authors contributed to the study conception and design. Material preparation, data collection and laboratory work were performed by LP. Data analyses were performed by LP, RW and

RF. The first draft of the manuscript was written by LP and all authors commented on previous versions of the manuscript. All authors provided critical input on drafts and approved the final manuscript.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.



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