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Application of a triple ^{15}N tracing technique to elucidate N transformations in a UK grassland soil

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ABSTRACT

To identify the production and consumption pathways and temporal dynamics of N_2O emitted from soil, this study uses ^{15}N-labelled substrate-N to quantify the underlying gross N transformation rates using the *Ntrace* analysis tool and link them to N-emissions. In three experiments twelve soil cores each were incubated in a lab incubation system to measure gaseous emissions, while parallel incubations under the same conditions were set up for destructive soil sampling at 7 time points. Using the triple labelling technique (applying NH_4NO_3 with either the NH_4^+-N or the NO_3^--N, or both being ^{15}N labelled), this study investigated the effects of 55, 70 and 85% water filled pore space (deemed to promote nitrification, both nitrification and denitrification, and denitrification, respectively) in a clay soil on gaseous N emissions and investigates the source and processes leading to N_2O emissions.

To assess the utilisation of applied NO_3^- vs. nitrified NO_3^- from applied NH_4^+, the ^{15}N tracing tool *Ntrace* was used to quantify the rates of immobilisation of NO_3^- and NH_4^+, oxidation of NH_4^+, mineralisation of organic N and subsequent nitrification by the analysis of the ^{15}N in the soil. Gross transformation rates were calculated, indicating the relative importance of added NO_3^- and NO_3^- derived from nitrified added NH_4^+.

Results show an important contribution of heterotrophic nitrification (organic N oxidation to NO_3^-) which was highest at the 55% water filled pore space (WFPS), decreasing in its contribution to N-transformation processes with increasing WFPS, while nitrification (NH_4^+ oxidation to NO_3^-) was contributing the most at 70% WFPS. The contribution of denitrification increased with increasing WFPS, but only became dominant at 85% WFPS. While denitrification still showed to be most important at high and nitrification at lower WFPS, the actual % WFPS values were not as expected and highlight the fact that WFPS is a contributor, but not the sole/most important parameter determining the type of N-transformation processes taking place.

1. Introduction

Nitrous oxide (N_2O) is an important greenhouse gas (GHG) accounting for approximately 6% of the current global warming (WMO, 2018). The atmospheric N_2O concentration has been increasing since the Industrial Revolution, with soils representing its major source, making the understanding of its sources and removal processes important for the development of mitigation strategies.

Several processes have been studied to determine their contribution to N_2O production in soils: (i) nitrification, which has been reported as autotrophic (NH_4^+ oxidation) and heterotrophic (organic N oxidation) (Zhang et al., 2015); (ii) denitrification, due to the incomplete denitrification of nitrate (NO_3^-) under anaerobic conditions (Attard et al., 2011); (iii) nitrifier denitrification (Zhu et al., 2013); and (iv) chemodenitrification as a non-biological process (Van Hecke et al., 1990).

It has been found that N_2O is mainly produced via biological

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processes and that emissions through nitrification and denitrification produce up to 70% of the annual emitted N_2O worldwide (Butterbach-Bahl et al., 2013). Several studies aimed to distinguish the main pathway responsible for N_2O emissions (Khalil et al., 2004; Bateman and Baggs, 2005), and identify a predominant process under certain conditions. While nitrification requires O_2, denitrification relies on its absence or limitation and has been attributed to anoxic conditions (Khalil et al., 2004). It is therefore generally agreed that water filled pore space (WFPS) is one of the key factors affecting which process dominates N_2O production. The higher the WFPS the more air in pores is replaced by water, thereby removing O_2 from the soil. However, it is also thought that several processes can occur simultaneously in different microsites of the same soil (Arah, 1997) due to the generation of local differences in soil aggregates.

It is well known that N_2O is produced by microorganisms who are dependent on several factors, such as environmental conditions, nutrient availability etc. (Saggar et al., 2013), which suggests that it is also likely that the N_2O-source processes themselves change over time due to changes in limiting factors such as soil moisture and carbon availability, allowing newly formed N-species to become new sources. As an example, in addition to added NO_3^-, the native soil NO_3^- and that produced from nitrification of applied or soil NH_4^+, can also be a source of N_2O via denitrification following nitrification.

Different methods have been applied to identify the occurrence and importance of different processes under different conditions. Amongst those are ^{15}N-labelling techniques (Stark, 2000), as well as isotopologue analyses of N_2O and O_2 ($^{15}N/^{18}O$) (Meijide et al., 2010; Bergstermann et al., 2011; Wu et al., 2016).

When aiming to determine how important different processes are under certain environmental conditions and management (e.g. soil moisture, C and N applications, etc), incubation experiments, where single factors and combinations of these can be manipulated, are the methodology of choice. Automated systems such as the denitrification incubation system, DENIS (Cárdenas et al., 2003) at Rothamsted have proven useful for process determination. In the DENIS, soil cores are incubated under an N_2-free atmosphere, allowing direct measurements of all emitted N gases (NO, N_2O and N_2) as well as CO_2. The transformation of N in soils and particularly the production of N_2O from different sources, such as fertilisers or animal excreta, has been studied through a series of laboratory incubation experiments (i.a. Meijide et al. (2010), Bergstermann et al. (2011), Loick et al. (2017)) using this system. The advantage of this system, when looking at N_2O source processes is, that under an N_2 free atmosphere it is possible to measure N_2 which, depending on the initial conditions, can only be produced via complete denitrification.

In order to fully investigate transformations leading to N_2O production and removal, quantifying their contributions and assessing the potential for change of processes, a combination of laboratory experiments with models/analysis tools at the same scale offer great potential.

One process model/analysis tool using ^{15}N distribution in the data obtained from ^{15}N labelling experiments has been developed by Müller et al. (2004; 2007). This analysis tool, represents an improvement of the dilution model by Kirkham and Bartholomew (1954), and includes soil nitrite and gaseous compounds emitted. It traces ^{15}N applied to soil and quantifies the gross N rates based on measurements of the partition of ^{15}N in soil pools from dual or triple isotope labelling of the source. The model determines the most suitable dynamics through the best Akaike's Information Criterion (AIC). The objective of this study is to show how N-transformation processes leading to N_2O emissions change over time and how WFPS can influence the initial dominance of certain processes but does not necessarily determine a sole process. The advantage of the triple labelling technique is that production of N_2O from an organic (unlabelled) source outside the mineral N pools can be unambiguously determined because if all relevant mineral N pools are labelled then a dilution of the N_2O has to arrive from outside that system. Also, for the parameter optimisation techniques it provides additional observations which reduce the danger of over parameterisation during parameter optimisation

To achieve this the triple labelling technique using Ammonium Nitrate (NH_4NO_3) was applied as a substrate with the N being labelled with ^{15}N in its different positions. Changes in soil N (NO_2^-, NO_3^-, and NH_4^+) were measured to quantify the underlying gross N transformation rates using the *Ntrace* analysis tool (Müller et al., 2007) with the measured emissions to then identify sub-rates based on the ^{15}N distribution in the data. This was linked to gaseous N-emissions to identify the production and consumption pathways and temporal dynamics of N_2O. In order to determine the source of N_2O from the triple labelling experiment, the DENIS was extended by connecting it to a GC–MS to include continuous measurements of emitted ^{15}N-N_2O.

We will test the following hypothesis: 1) that NO and N_2O losses at different soil moisture levels will decrease at higher moisture values due to easier diffusion and conversion to N_2; 2) that at the highest soil moisture N_2O is mostly derived from NO_3^- whilst at the low moisture from NH_4^+; 3) that nitrification and denitrification are the main sources of N_2O at all moistures.

2. Materials and methods

2.1. Soil preparation

A clayey pelostagnogley soil of the Hallsworth series (Clayden and Hollis, 1984) (44% clay, 40% silt, 15% sand (w/w), Table 1) was collected on the 26th of May 2015 from a typical grassland in SW England, located at Rothamsted Research, North Wyke, Devon, UK (50°46′10″N, 3°54′05″W). Spade-squares (20 × 20 cm to a depth of 15 cm) of soil were taken from 12 locations along a 'W' line across a field of 600 m^2 size, which had not had any grazing animals on it, nor received any fertiliser input for over 20 years. After sampling, the soil was air dried to ~30% H_2O (gravimetric moisture content), roots and plant residues were removed, and the soil sieved to <2 mm and stored at 4 °C before packing into cores and starting the incubation.

Initial soil characteristics are given in Table 1.

2.2. Experimental design

The incubation experiment was carried out using the DENIS, a specialized gas-flow-soil-core incubation system (Cárdenas et al., 2003) in which environmental conditions can be tightly controlled. The DENIS simultaneously incubates a maximum of 12 vessels containing one soil core each. Cores were packed to a bulk density of 0.8 g cm^{-3} to reflect field conditions, to a height of 75 mm into stainless steel sleeves of 141 mm diameter. Due to the limited space within the DENIS and the requirement for replication, three experiments (see below) were performed directly one after another under the same tightly controlled conditions (i.e. temperature, gas flow, amendment application). All soil was kept in the fridge (4℃) until needed and treated to the same time scales to prevent any changes in soil characteristics.

Table 1
Soil characteristics (before amendment application).

Mean ± standard error ($n = 3$).	
Parameter	Amount
pH water [1:2.5]	5.6 ± 0.27
BD (g cm^{-2})	0.8 ± 0.0005
Available Magnesium (mg kg^{-1} dry soil)	100.4 ± 4.81
Available Phosphorus (mg kg^{-1} dry soil)	10.4 ± 1.10
Available Potassium (mg kg^{-1} dry soil)	97.5 ± 12.83
Available Sulphate (mg kg^{-1} dry soil)	51.7 ± 0.62
Total N (g kg^{-1} dry soil)	5.0 ± 0.10
Total Extractable Oxidised N (mg kg^{-1} dry soil)	15.1 ± 0.07
Ammonium N (mg kg^{-1} dry soil)	9.2 ± 0.09
Total Organic Carbon (% w/w)	6.79 ± 0.17

To promote nitrification-, denitrification- or a combination of both, each experiment was performed at a different WFPS (Bollmann and Conrad, 1998; Butterbach-Bahl et al., 2013). The soil moisture was adjusted to 55%, 70% or 85% WFPS, respectively, taking the amendment with nutrient solution into account. To measure N_2 fluxes, the native N_2 was removed from the soil and headspace without limiting O_2 levels that would be present in air. This was achieved by using a helium–oxygen mixture He:O_2 of 80:20. First the soil cores were flushed from the bottom at a flow rate of 30 ml min^{-1} for 14 h. To measure baseline emissions, flow rates were then decreased to 12 ml min^{-1} and the flow re-directed over the surface of the soil core for three days before amendment application and for the remaining experimental period. The vessels were kept at 20 °C during flushing as well as for the 13-day incubation period after amendment application.

Three incubations were needed to accommodate the different ^{15}N treatments and soil moisture levels. Each incubation involved the following three treatments of NH_4NO_3 (Sigma-Aldrich, St. Louis, MO, USA), with three replicate vessels per treatment: i) $^{15}NO_3$ = cores amended with single labelled $NH_4^{15}NO_3$ at 50 atom%; ii) $^{15}NH_4$ = cores amended with single labelled $^{15}NH_4NO_3$ at 50 atom%; iii) $^{15}NO_3^{15}NH_4$ = cores amended with double labelled $^{15}NH_4^{15}NO_3$ at 50 atom%. Considering the total surface area of the vessel, N was applied at a rate of 75 kg N ha^{-1}. The applied rate of N equates to 125 mg N kg^{-1} dry soil, which was dissolved in 50 ml of H_2O before being applied to the soil. To maintain the incubation conditions, the amendment was applied to each of the three cores via a sealed amendment container on top of the incubation vessel. Before amendment application the headspace of the amendment vessel was flushed with He to prevent any atmospheric N_2 entering the system.

Additionally, a parallel incubation only for destructive soil sampling at 7 time-points after treatment application (5 h, days 1, 2, 3, 4, 7, 10) with 3 replicates of each was performed each time. For logistical reasons smaller cores (4.5 cm diameter) had to be used, which were packed with the same soil and to the same specifications used for the DENIS incubation and kept under the same controlled conditions. At the sampling time, soil was analysed for extractable Ammonium (NH_4^+), Nitrate (NO_3^-), Nitrite (NO_2^-) concentrations and ^{15}N-enrichment of those molecules ($^{15}NH_4^+$, $^{15}NO_3^-$, $^{15}NO_2^-$).

2.3. Gas analyses

Gas samples were taken every four hours for each vessel from the Denis system. Fluxes of N_2O and CO_2 were quantified using a Perkin Elmer Clarus 500 gas chromatograph (Perkin Elmer Instruments, Beaconsfield, UK) equipped with an electron capture detector (ECD) for N_2O and CO_2. N_2 emissions were measured by gas chromatography with a helium ionisation detector (VICI AG International, Schenkon, Switzerland), while NO concentrations were determined by chemiluminescence (Sievers NOA280i, GE Instruments, Colorado, USA). All gas concentrations were corrected for the surface area and flow rate going through the vessel (measured daily). Fluxes were calculated on a kg N or C ha^{-1} day^{-1} basis. Isotopic signatures were determined via isotope ratio mass spectrometer (PDZ Europa 20-20 Stable Isotope Analyser, Sercon, Crewe, UK) linked to an ANCA-TGII gas preparation system (Sercon, Crewe, UK).

2.4. Soil analyses

The initial soil N was measured at the start of each incubation by randomly taking three 100 g samples from the bulk soil before core packing and WFPS adjustment. This soil was analysed for total extractable oxidised N (TO_xN, combined amount of NO_2^- and NO_3^-) and NH_4^+. Soil samples (100 g) from the parallel incubation were analysed for extractable NO_2^-, NO_3^- and NH_4^+ concentrations at each time point. WFPS was calculated from soil moisture contents by drying a subsample (50 g) at 105C overnight. Soil extractable NO_2^-, NO_3^- and NH_4^+, concentrations were analysed after blending the samples with 2 M KCl at pH 8 following the method of Stevens and Laughlin (1995). The extracts were analysed by colourimetry using a Spectrophotometer (Cecil Instruments, Cambridge, UK) for the analysis of NO_2^-, or an Aquakem 250 discrete photometric analyser (Thermo Fisher Scientific, Hemel Hempsted, UK) for the analysis of NO_3^- and NH_4^+. The ^{15}N abundances of the NO_2^-, NO_3^- and NH_4^+ were determined by methods based on the generation of N_2O for isotope ratio mass spectrometry (IRMS). The production of N_2O from NO_2^- and NO_3^- is based on the reaction between NO_2^- and NH_2OH under acid conditions and the NO_3^- having been reduced to NO_2^- with Cd (Stevens and Laughlin, 1994). The production of N_2O from NH_4^+ consists of a diffusion stage where ammonia (NH_3) is absorbed into H_2SO_4 followed by an oxidation step where recovered $(NH_4)_2SO_4$ is oxidised to N_2 by alkaline NaOBr, during which N_2O is produced as a by-product (Laughlin et al., 1997). In each case, the resulting N_2O was transferred to an Exetainer (Labco Ltd, Lampeter, Wales). The N_2O enrichment was determined using a Gilson Autosampler (Gilson UK, Dunstable, UK) by IRMS as described in the gas analyses section.

2.5. Statistical analysis

Statistical analysis was performed using GenStat 16th edition (VSN International Ltd). Prior to the statistical tests all data were analysed to proof their normal distribution (Kolmogorove-Smirnov test) and equality of variance (Levene test). Cumulative emissions of NO, N_2O, N_2 and CO_2 were calculated from the area under the curve (time vs flux as shown in Fig. 2) after linear interpolation between sampling points.

3. Analysis of N_2O source contribution

To determine the contribution of different sources to N_2O emissions the *Ntrace*$_{basic}$ analysis tool by Müller et al. (2007) was used. This analysis tool represents an extension of the dilution approach of Kirkham and Bartholomew (1954) and quantifies gross N rates based on measured data. To achieve this, a model is used to quantify the individual gross rates, connecting the various soil N pools by parameter optimization routines.

The gross N transformation rates quantified where:

$M_{Nrec,}$ mineralization of recalcitrant organic N to NH_4^+;

$M_{Nlab,}$ mineralization of labile organic N (e.g., monomolecular organic N, amino acids, proteins) to NH_4^+;

$I_{NH4Nrec}$, immobilization of NH_4^+ to recalcitrant organic N;

$I_{NH4Nlab}$, immobilization of NH_4^+ to labile organic N;

A_{NH4}, adsorption of NH_4^+ on exchange sites;

R_{NH4a}, release of adsorbed NH_4^+;

O_{NH4}, oxidation of NH_4^+ to NO_3^-;

O_{Nrec}, oxidation of organic N to NO_3^-; (heterotrophic nitrification)

as well as the following 4 rates, which were, however, negligible:

I_{NO3}, immobilization of NO_3^- to recalcitrant organic N;

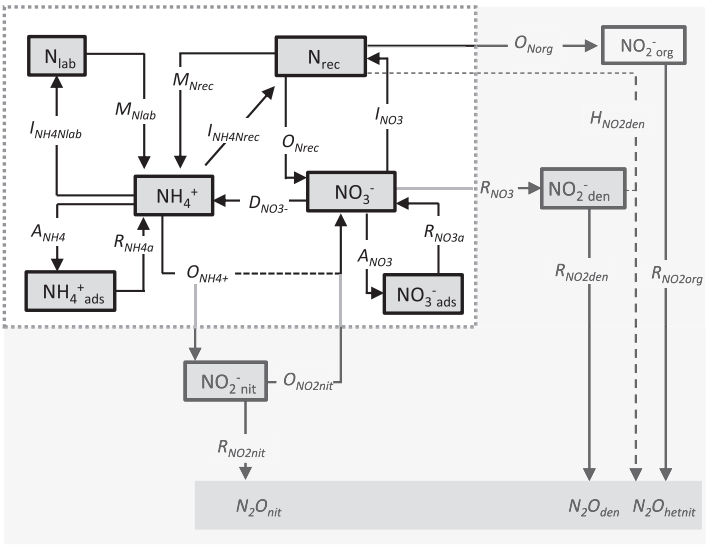
D_{NO3}, dissimilatory reduction of NO_3^- to NH_4^+;

A_{NO3}, adsorption of NO_3^- to labile organic N;

$R_{NO3,}$ release of adsorbed NO_3^-

One feature of *Ntrace* is to identify the simplest model structure that is sufficient and adequate to explain the measured data. Therefore, a range of different model versions (including/ excluding certain transformation rates) and/or kinetic setting are tested. The most suitable model is then identified by comparing the AIC of each model run which takes the goodness of fit and the number of parameters used into account. Thus, this tool also identifies rates which are not needed to explain the overall dynamics (e.g. the mineralization of labile organic N in our case). Fig. 1 shows the full conceptual model according to Müller et al. (2014) indicating the rates used based on the 2007 model (Müller et al., 2007) in the top left area.

Pathway specific N_2O emissions were determined by assuming that N_2O originated from the NH_4^+, organic N and NO_3^- pool (Fig. 1) (Stange et al., 2009; Müller et al., 2014). The contributions of these three pools



Parameter Discription	
Parameter	Description
M_x	Mineralisation of X
I_x	Immobilisation of X
O_x	Oxidation X
R_x	Release of X
D_x	Dissimilatory reduction of X
H_x	X transformation by Heterotrophs
A_x	Adsorption of X
X_{org}	Organic X
X_{lab}	Labile X
X_{rec}	Recalcitrant X
X_a	Adsorbed X
X_{nit}	X from Nitrification
X_{den}	X from Denitrification
X_{hetnit}	X from heterotrophic nitrification

Fig. 1. Conceptual ^{15}N tracing model of N transformations according to Müller et al. (2014). See table for description of the parameters/processes.

were calculated by the parameter identification routine described by Rütting et al. (2010):

$$a_{N2O} = C_{NH4} \times a_{NH4} + C_{ON} \times a_{ON} + C_{NO3} \times a_{NO3} \quad (1)$$

$$C_{NH4} + C_{ON} + C_{NO3} = 1 \quad (2)$$

where a_{N2O} is the ^{15}N abundance of N_2O produced during incubation, a_{NH4}, $a_{ON}$and a_{NO3} are the ^{15}N abundance of NH_4^+, organic N and NO_3^-, respectively, and C_{NH4}, C_{ON} and C_{NO3} are the contributions from oxidation of NH_4^+ to NO_3^-, oxidation of organic N to NO_3^- and reduction of NO_3^- to total N_2O production, respectively.

4. Results

4.1. Fluxes of N gases and CO_2

Nitric oxide emissions increased in all treatments (Fig. 2a) during the incubation period. At the highest moisture of 85% WFPS, NO emissions reach a plateau after 6 days and start to decrease after 10 days. For the 2 lower moisture levels emissions were increasing over the whole course of the experiment. Emissions increased significantly with WFPS, as shown.

Nitrous oxide emissions (Fig. 2b) were very low and near the detection limit (N_2O: 0.5 ppm, equivalent to a flux of 0.00027 kg N $ha^{-1}h^{-1}$) in the two lower WFPS treatments. In the 85% WFPS treatment N_2O emissions were significantly higher ($p < 0.05$) than the other 2 treatments and showed a peak at day 1 of around 14 g N $ha^{-1}h^{-1}$ after which emissions decreased to around 3 g N $ha^{-1}h^{-1}$ by the end of the experiment. At the lower WFPS of 55 and 70%, N_2O emissions were not significantly different between the WFPS treatments.

Nitrogen gas emissions (Fig. 2c) were low in the 55% and 70% WFPS treatments and did not show a peak. Higher N_2 emissions were detected in the 85% WFPS treatment with a peak at around day 2. After day 5, N_2 emissions were low as in the other two treatments. Some N_2 was introduced into the system when the amendment was applied. This took about 1 day to disappear (see high soil moisture treatment) (see Fig. 2).

The total amounts of N emitted as NO, N_2O and N_2 show an increase with increasing WFPS (Table 2). However, total amounts of NO-N were almost insignificant making up less than 0.04% of total N emissions. Total emissions of N_2O were low in the 55% and 70% WFPS treatment (<3% of total N emissions), but significantly higher at the highest WFPS of 85% (21.3% of total N emissions). N_2 emissions was only any significantly different at the high soil moisture. The N_2-N represented the largest component of the emitted N at least 80%. The N_2O-N to N_2-N ratios were smaller at the middle soil moisture (0.03) compared to 0.27 at 85% WFPS.

Carbon dioxide emissions (Fig. 2d) increased immediately after the application of NH_4NO_3 and showed a maximum on day 2 in the 55% and 85% WFPS treatments decreasing afterwards. In the 70% WFPS treatment emissions seem to have decreased in the first day to recover in day 2 which was followed by a steady decrease similarly to the other 2 treatments. Values for the 70% WFPS treatment were the lowest during all the incubation compared to the other 2 treatments.

4.2. Proportion of N_2O from added N

Results of the estimation of the proportion of N_2O derived from the applied treatments showed that initially, at 55% WFPS, very little N_2O emissions derived from added single-labelled NH_4^+ (Fig. 3a, ○). Larger amounts derived from added labelled ^{15}N, were found in the other ^{15}N-treatments within the first day (up to 50% from $^{15}NH_4^{15}NO_3$). Those rapidly decreased and became similar to the $^{15}NH_4^+$ treatment after 24 h. For the rest of the incubation similar proportions of N_2O derived from all labelled amendments. Those proportions increased until day 12 when they reached about 10%.

The trends changed in the 70% moisture treatment (Fig. 3b), where the proportion of N_2O from the added ^{15}N initially increased for all ^{15}N amendments. After day 1 the proportion remained the same for the $^{15}NO_3^-$ amendment (▲) but kept increasing steadily for the other ^{15}N-amendments reaching 25 and 30% for $^{15}NH_4^+$ and $^{15}NH_4^{15}NO_3$, respectively.

For the highest moisture treatment (Fig. 3c), the proportion of N_2O from labelled N also increased on the first day for all treatments, however, with $^{15}NO_3$ and $^{15}NH_4^{15}NO_3$ the increase was significantly higher than with $^{15}NH_4$ (○; up to 50%). After this day, the contribution of the labelled amendment to N_2O emissions decreased for those amendments, reducing to 20 and 40% for $^{15}NO_3$ and $^{15}NH_4^{15}NO_3$, respectively on day 13. In the $^{15}NH_4$ treatment on the other hand, N_2O emissions decreased slightly after the maximum in day 1 and then continued to increase,

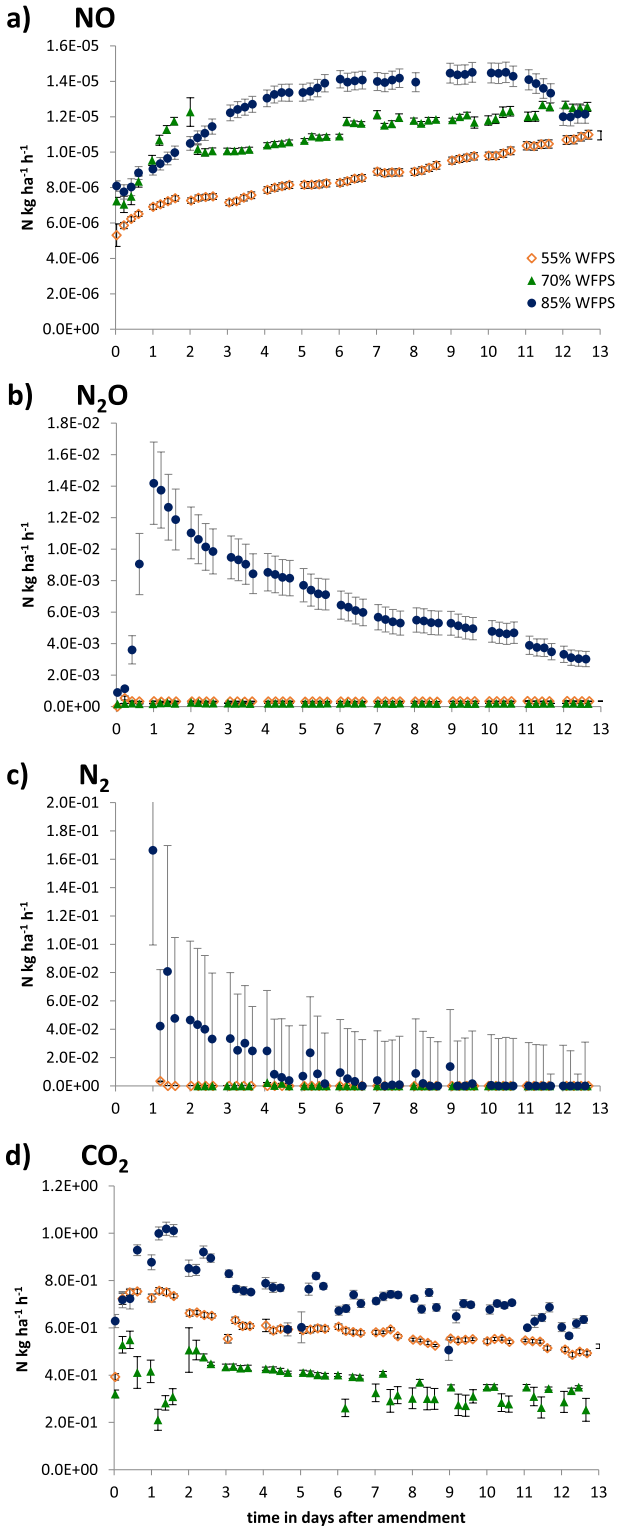


Fig. 2. Average fluxes of NO, N_2O, N_2 and CO_2 at the three WFPS of 55%, 70% and 85% (n = 9). Fluxes are averaged over the three differently labelled NH_4NO_3 treatments.

reaching 20% on day 13.

4.3. Soil N concentrations and ^{15}N enrichment

Analysis of the soil N before each incubation and before core packing

Table 2
Average cumulative emissions of NO, N_2O over the experimental period and N_2 from day 2.6 (after flushing out of N_2 introduced with amendment) in kg N ha^{-1}.

Mean ± standard error (n = 9). Different letters indicate significant differences in emissions between the WFPS treatments ($p < 0.05$)

WFPS	*NO-N*	*N_2O-N*	*N_2-N*	*total N*	*%N as NO-N*	*%N as N_2O-N*	*% N as N_2-N*
55%	1.09E-04 ± 6.28E-06^{c}	4.16E-03 ± 2.35E-04^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00	na	na	na
70%	1.41E-04 ± 7.32E-07^{b}	2.69E-03 ± 4.28E-05^{a}	0.08 ± 0.08^{a}	0.09 ± 0.08	0.16	3.0	89
85%	1.61E-04 ± 5.71E-06^{a}	8.51E-02 ± 3.52E-03^{c}	0.32 ± 0.30^{a}	0.40 ± 0.31	0.04	21.2	80

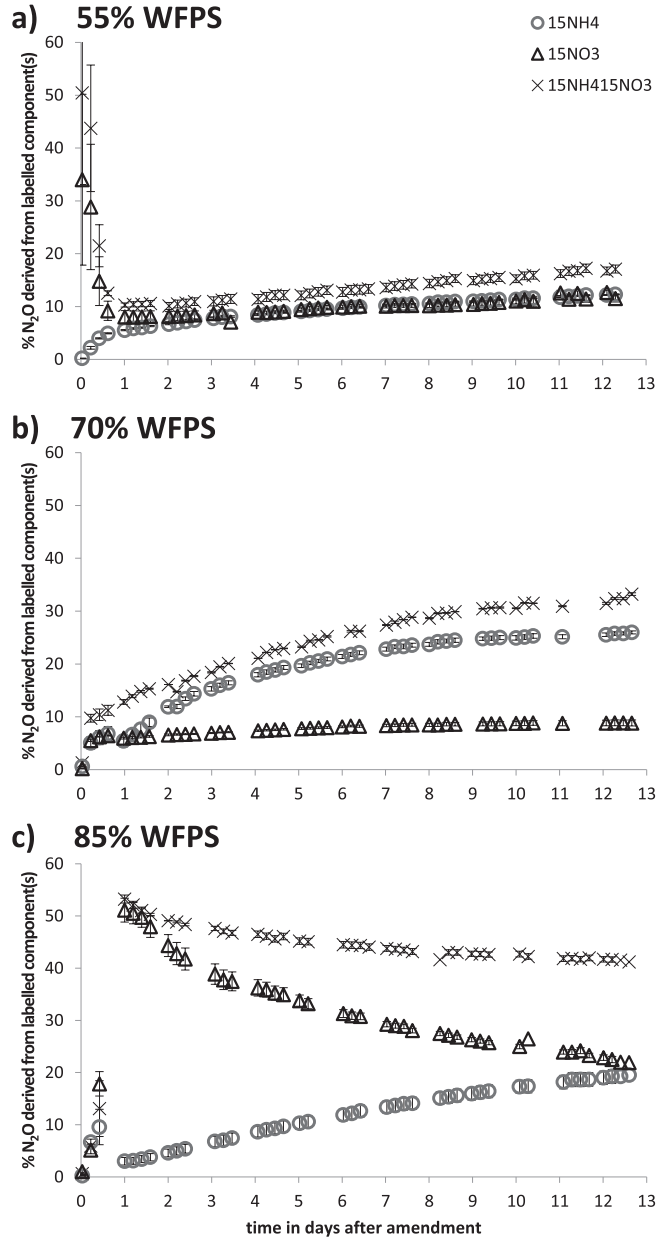


Fig. 3. Proportion of emitted N_2O that derived from the labelled components of the different treatments (n = 3) separated for the different WFPS'.

showed the following values of TO_xN: 0.0681 (±0.001), 0.1335 (±0.0112) and 0.0844 (±0.0096) mg g^{-1} dry soil for 55, 70 and 85% WFPS-incubations, respectively. For NH_4^+, values were 0.0869 (±0.0044), 0.0485 (±0.0010) and 0.0957 (±0.0017) mg g^{-1} dry soil for 55, 70 and 85% WFPS-incubations, respectively.

Fig. 4 shows the dynamics of the analysed N forms in the soil throughout the experiment. Soil NO_2^- was of the order of 0.1 µg N g^{-1} dry soil during the incubation period and slightly higher in the 85% WFPS treatment. Soil NH_4^+ and NO_3^- concentrations were around 1000 times higher than NO_2^-, with more NO_3^- than NH_4^+ in the 70% and 85% WFPS treatments, while no differences in soil NH_4^+ and NO_3^- could be detected in the 55%WFPS treatment.

The 70 and 85% WFPS treatments showed larger changes in the time series with soil NO_3^- increasing and NH_4^+ decreasing, while those concentrations remained relatively constant and of similar magnitude (around 0.15 mg N g^{-1} dry $soil^{-1}$) in the 55% moisture treatment.

The ^{15}N-enrichment of soil NO_2^-, NO_3^- and NH_4 is shown in Fig. 5. The lowest ^{15}N-enrichment of soil NO_2^- and NO_3^- was from the $^{15}NH_4$ amendment (●) (Fig. 5a and b) for all moisture treatments while a higher enrichment of those two soil components was found when $^{15}NO_3$ (▲) or $^{15}NH_4^{15}NO_3$ (■) were applied (Fig. 5d,e,g and h). Values of enriched NO_2^- were generally lower than those of enriched NO_3^- (5 vs. 20 atom%) (Fig. 5a and b). Soil ^{15}N-enrichment of NO_3^- was generally in the order 85%>55%>70% WFPS (*solid blue, dotted orange, dashed green*) when the soil was amended with $^{15}NO_3$ or $^{15}NH_4^{15}NO_3$ (Fig. 5e and h).

The amendment with $^{15}NO_3$ (▲) resulted in lowest soil NH_4^+ enrichment (Fig. 5f) at 70 and 85% WFPS, while the opposite was found for the initial 4 days when soil was at 55% WFPS. Here treating the soil with $^{15}NO_3$ resulted in higher soil NH_4^+ enrichment than soil treated with $^{15}NH_4$ or $^{15}NH_4^{15}NO_3$. There was no significant difference in the enrichment of the soil NH_4^+ depending on whether the soil was amended with $^{15}NH_4$ or $^{15}NH_4^{15}NO_3$; enrichment was higher for the 70 and 85% WFPS treatments than the 55% one (Fig. 5c and i).

As previously mentioned, compared to the other amendments the addition of $^{15}NH_4$ resulted in significantly lower enrichment of ^{15}N-labelled NO_2^- as well as NO_3^- for all WFPS treatments and a significant decrease in $^{15}NH_4^+$ at the lower WFPS values of 55 and 70%.

When applying $^{15}NO_3$ the only significant changes in the enrichment of ^{15}N-labelled compounds was found at 85% WFPS where ^{15}N-labelled NO_3^- enrichment was significantly lower at the end of the 10-day experiment and at 55% WFPS where ^{15}N-labelled NH_4^+ enrichment was also significantly lower at the end of the experimental period (Fig. 5d-f).

Applying $^{15}NH_4^{15}NO_3$ did not result in any significant changes in the enrichment of ^{15}N-labelled NO_2^- or NO_3^- at any of the WFPSs. However, a significantly lower enrichment of ^{15}N-labelled NH_4^+ between the beginning and end of the experimental period was found for all WFPS values (Fig. 5g-i).

4.4. Analysis of transformation rates

The results of the *Ntrace* analysis tool (Fig. 1) showed that gross transformation rates of NO_3^- and NH_4^+ and Mineralisation of labile N to NH_4^+ were generally highest at 55% WFPS and mostly decreased with increasing WFPS (Fig. 6a–c). Oxidation of recalcitrant N to NO_3^-, however increased with increasing WFPS (Fig. 6d). Desorption of adsorbed NH_4^+ as well as NO_3^- was highest at 70% WFPS (Fig. 6e), although not statistically significant, while the transformation of NH_4^+ to NO_3^- was significantly lower at this WFPS than at the higher or lower WFPS (Fig. 6a).

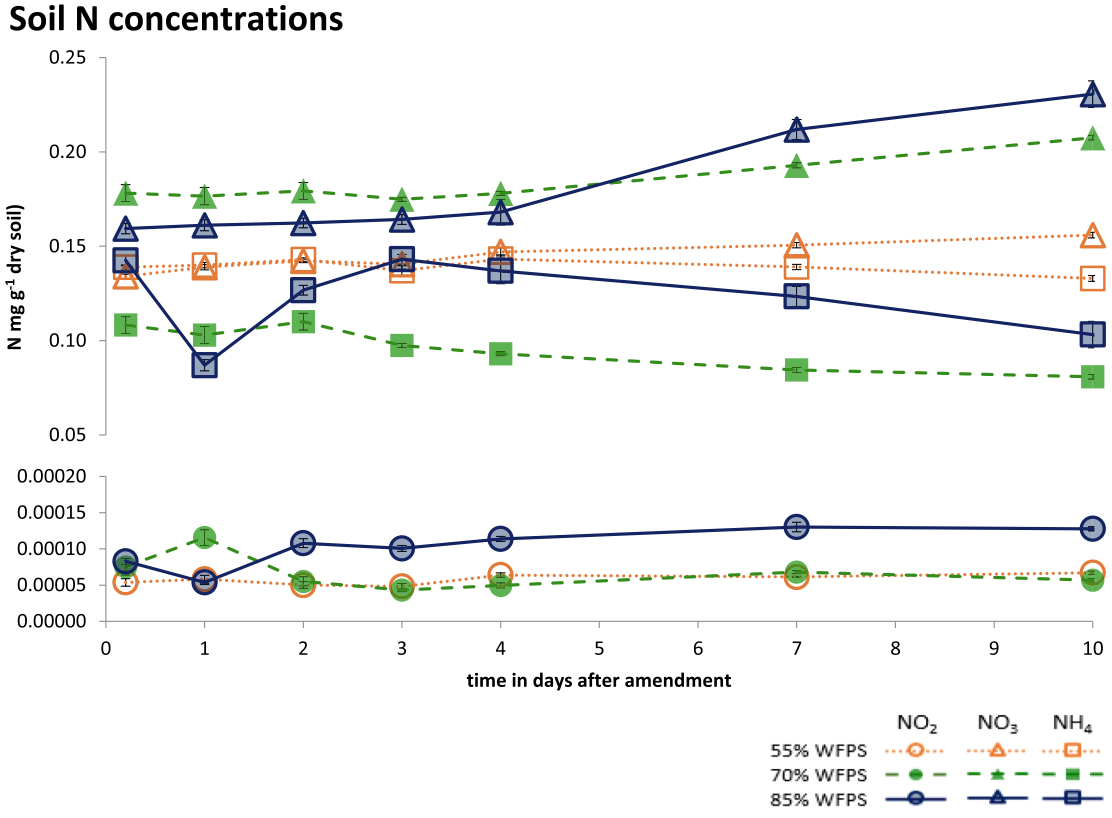


Fig. 4. Soil concentrations of NO_2^-, NO_3^-, and NH_4^+ in mg N per g dry soil (n = 9). Values are averaged over all three labelled NH_4NO_3 treatments, but separated by WFPS.

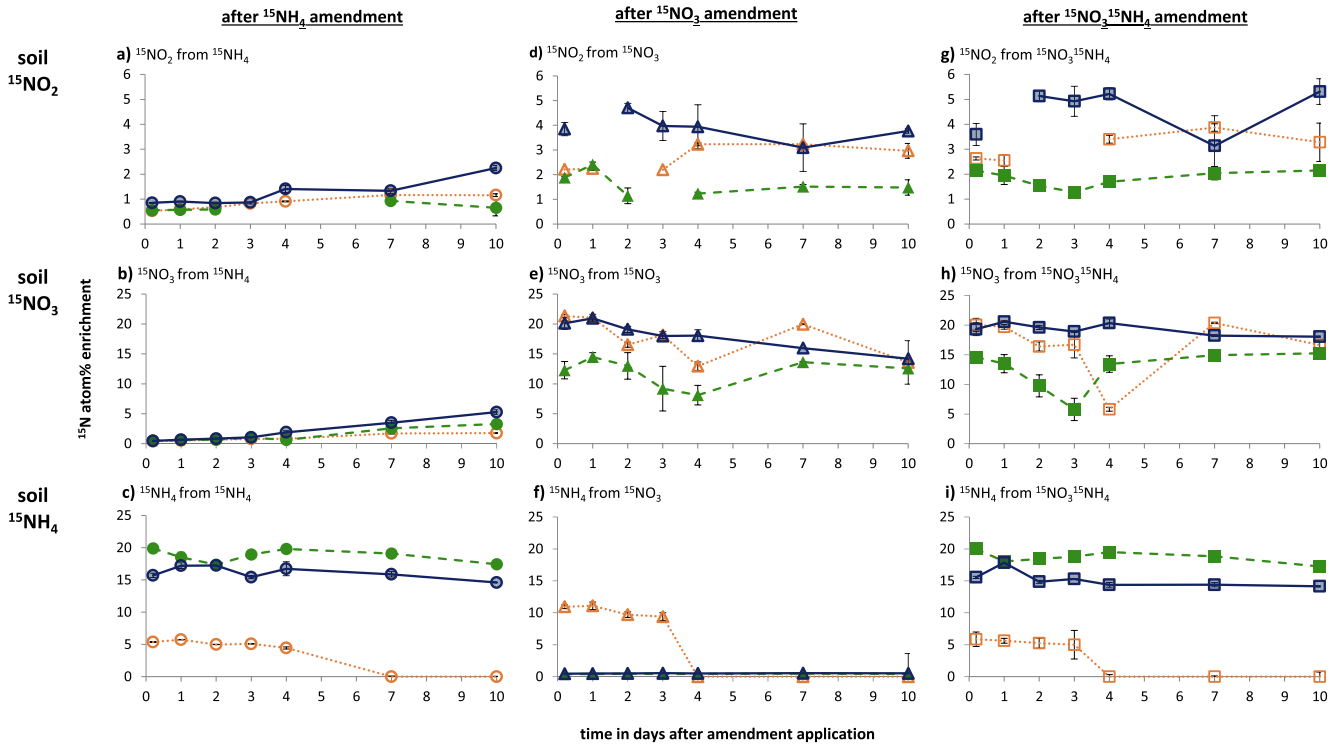


Fig. 5. Enrichment of soil NO_2^-, NO_3^- and NH_4^+ of the different treatments (n = 3) separated for the different WFPS'.

4.5. *Apportioning of N_2O emissions*

Fig. 7 shows the resulting apportioning of the N_2O emissions to the three different processes: heterotrophic nitrification, denitrification and nitrification. At 55% WFPS, an initial large contribution of denitrification is shown, which quickly decreased in favour of heterotrophic nitrification (30%) by the end of day 1. Heterotrophic nitrification remained the dominant process throughout the incubation except on days 4 and 10, when the sum of denitrification and autotrophic nitrification where approximately 50%.

At 70% WFPS, heterotrophic nitrification dominated at the start of the incubation vs denitrification (70 vs 30%) but decreased in importance with time to almost zero at the end of the incubation, when autotrophic nitrification became more dominant (65%).

At 85% WFPS, heterotrophic nitrification is only relevant on the first day (80%); from then on, denitrification dominated (~100% on days 1–2) and remained at about 60–80% with the rest of the contribution coming from autotrophic nitrification.

The summary graph (Fig. 8) shows the average contribution of each process to N_2O emissions as total amounts of N_2O-N emitted, as well as percentage of N_2O emitted by each of the three processes. With increasing soil moisture, an increase in the contribution from denitrification to N_2O emissions was found, whilst the contribution from heterotrophic nitrification decreased. For autotrophic nitrification, however, the largest contribution was at the intermediate soil moisture of 70% WFPS.

5. Discussion

In a recent literature review and *meta*-analysis, Barrat et al. (2020) found that WFPS was a significant explanatory variable for N_2O emissions and this was affected by the prior moisture status of the soil. In our experiments, the soils were prepared in a standard manner, so only the final moisture status at the start of the incubation differed. Therefore in our study, we investigated the relative differences between the 3 soil moisture status (or WFPS) on N partitioning in the soil N compounds and the N emitted compounds, and the apportioning of N_2O emissions to different processes.

5.1. *Process dependent N-emissions at different WFPS*

Denitrification, if complete, transforms the produced N_2O into N_2. Denitrification is commonly incomplete with N_2O not being transformed to N_2 due to a lack of N_2O reductase (Nos) in the microbial community, or due to a sufficient supply of NO_3^- whose reduction is energetically more favourable than the reduction of N_2O to N_2 (Saggar et al., 2013). Due to incomplete denitrification, highest N_2O production is expected from denitrification and consequently from soils with a relatively higher WFPS. However, the importance and dominance of certain processes ultimately depends on the microbial community present in the soil and its activity which is influenced by the soil conditions. In our study we used a grassland soil that, has not had any fertiliser input, nor been grazed and therefore has not received animal excrements as a nutrient source for over 20 years. We assume that due to the management of the field lacking regular supply of nutrients, the microbial community within the soil would have differed from those communities found in other grasslands (Denef et al., 2009). This would have had an influence on the N-transformation processes in this soil.

Additionally, it has been shown that soil moisture content influences nutrient availability and movement through the soil (Misra and Tyler, 1999) therefore influencing access of those nutrients transported within a solution to the present microbial community and subsequently influencing N transformation processes.

In addition, the contributions observed from the treatments applied to the emitted N_2O were generally<50%, implying that the soil N pool was a larger contributor. We had no zero N treatment in our experimental design to confirm this, however, even if we had this, it is possible that the soil microbial community was primed by added N (Müller and Clough, 2014), so more of the soil N would have been utilised in the N treated soils, than in a zero N control.

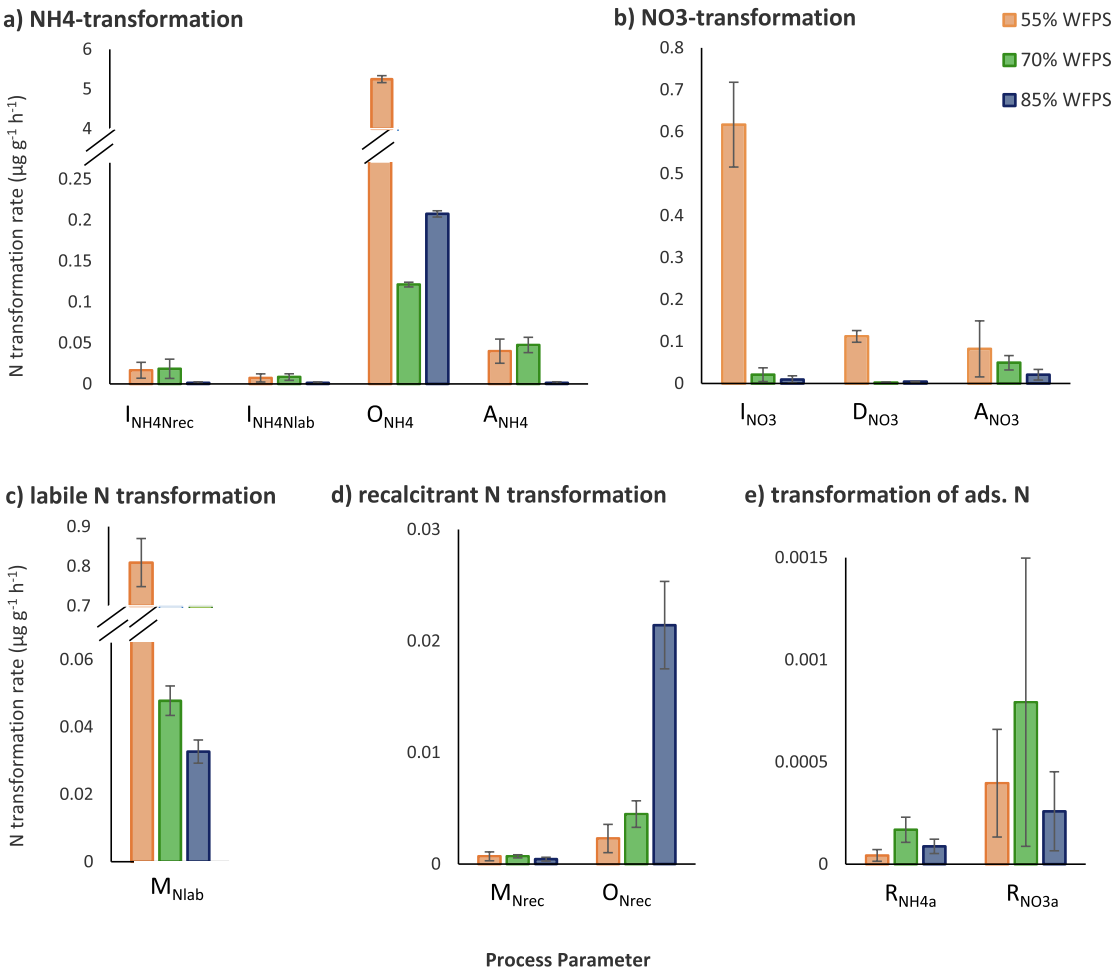


Fig. 6. Gross soil-N transformation rates in µg per g and h as determined by the model by Müller et al. (2014). The header of each graphs gives the reactant with the product and the process (square brackets) on the x-axis. Please refer to the tabulated parameter descriptions in Fig. 1 for a description of the process parameters.

5.1.1. N-emission processes at 85% WFPS

In our study, the highest N_2O emissions were found at WFPS of 85% and these emissions decreased over time. At this high WFPS the dual labelling analysis showed that more N_2O was derived from the applied NO_3^- (Fig. 3c, initially $^{15}NO_3^-$ contributed over 50% while $^{15}NH_4^+$ contributed less than 5%), indicating that denitrification was the dominant process in our experiment. Over the course of the experiment at 85% WFPS, the proportion of N_2O from the ^{15}N labelled NO_3^- decreased, while that of NH_4^+ increased.

A possible explanation for the increased contribution of applied ^{15}N-NH_4^+ in N_2O emissions could be that the measured ^{15}N-N_2O derived from $^{15}NO_3^-$ which had previously been produced via nitrification from the added $^{15}NH_4^+$. The results of soil NO_3^- agree with this as there was an increase during the incubation coinciding with a decrease in soil NH_4^+. The initial increase in CO_2 reflects aerobic respiration after the treatments were applied that settles at the end of the peak at about days 3–4. The N_2 fluxes up till day 4 in the highest soil moisture treatment can be explained by an increase in anaerobicity during this period promoting denitrification. It is possible, that O_2 concentrations recover with time, changing conditions from promoting denitrification to promoting nitrification where N_2O is produced from hydroxylamine NH_2OH. Nitrifying conditions might have also developed at the surface by drying of the upper layers of the soil. Though moisture contents of the soil cores used in this experiment did not change significantly over time, it has been shown in previous experiments that water can redistribute from top to bottom creating more aerobic, nitrification promoting conditions at the surface where gas exchange with the atmosphere takes place (Loick et al., 2016). However, our results suggest that most of the detected N_2O came from denitrification of the NO_3^- produced via nitrification of the applied $^{15}NH_4^+$ due to the increase in NO_3^- and a general decrease in NH_4^+ at 85% WFPS (Fig. 4). Therefore, while nitrification is taking place even under this high WFPS, denitrification is still the dominant process producing N_2O. This is further supported by soil ^{15}N analysis (Fig. 5), where results show a significant increase in soil $^{15}NO_3^-$ in the $^{15}NH_4^+$ treatments, while the enrichment of $^{15}NH_4^+$ in the same treatment significantly decreased.

Emissions of other N-gases produced during N transformation processes provide additional support that denitrification was most important at the highest WFPS of 85%. Higher emissions of N_2 (Fig. 2c), the final product of denitrification indicate that complete denitrification had been achieved for some of the available NO_3^-.

5.1.2. N-emission processes at 70% WFPS

At the intermediate WFPS of 70% it was expected that nitrification and denitrification would be equally important. In fact, the results of the *Ntrace* analysis tool show an equal contribution of denitrification, nitrification and heterotrophic nitrification at 70% WFPS. ^{15}N soil analysis also supports a near equal distribution of nitrification and denitrification with $^{15}NH_4^+$ showing a decrease and $^{15}NO_3^-$ a corresponding increase when $^{15}NH_4^+$ was added (Fig. 5b/c). The analysis of $^{15}N_2O$ (Fig. 3b) revealed an approximately 3 times higher contribution of the added $^{15}NO_3^-$ to N_2O emissions than that of added $^{15}NH_4^+$,

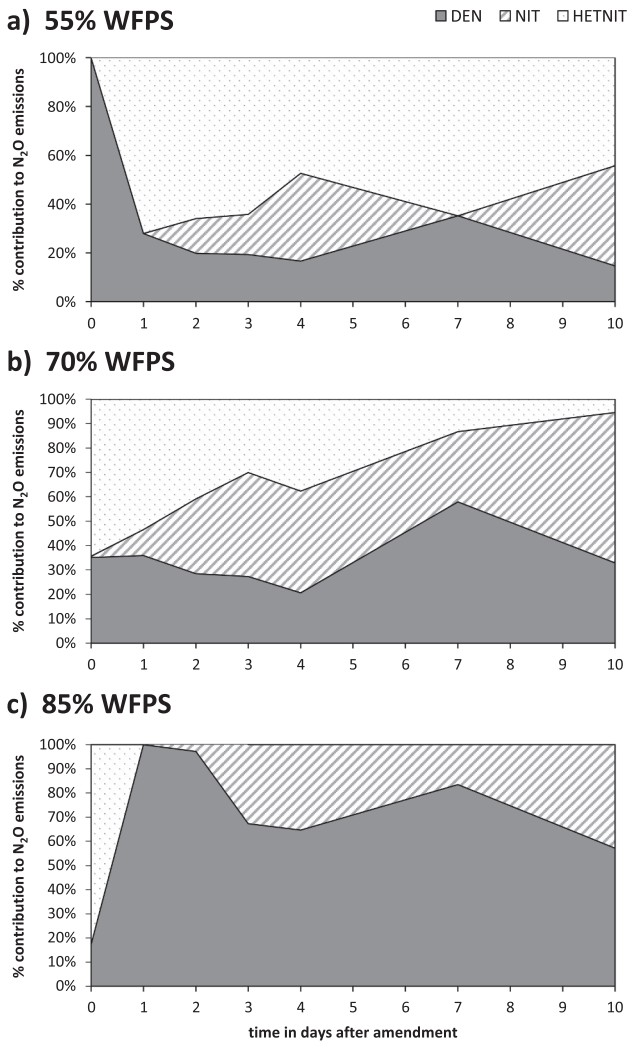


Fig. 7. Apportioning of the N_2O emission pathways for each WFPS as determined by the model by Müller et al. (2014).

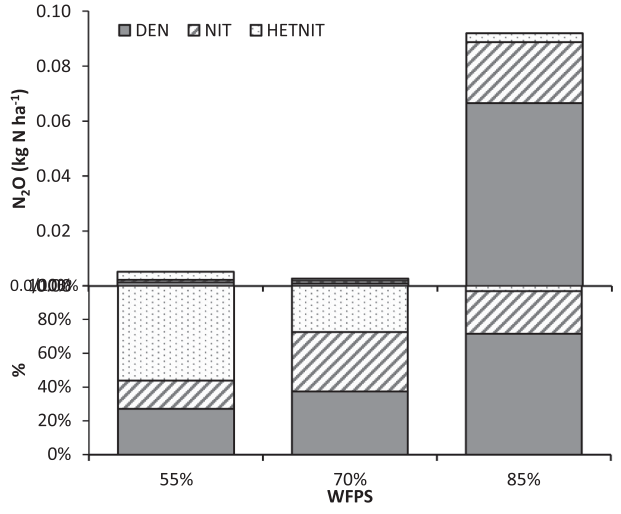


Fig. 8. N_2O emissions attributed to each of the three processes (DEN = denitrification, NIT = nitrification, HETNIT = heterotrophic nitrification) as total in kg N per ha and as percentage contribution of each of those processes to the total N_2O emissions as determined by the model by Müller et al. (2014).

indicating that most of the emitted N_2O was produced via denitrification. However, total amounts of N_2O were very small, as were CO_2 emissions (Fig. 2d), both indicating that the microbial N-transformation processes and denitrification in particular were very slow/small under these conditions.

5.1.3. *N-emission processes at 55% WFPS*

The lowest WFPS of 55% was chosen to promote nitrification. The results of the *Ntrace* analysis tool support that this was the case with nitrification and heterotrophic nitrification contributing to about 80% of N_2O emissions (Fig. 8), while denitrification only played a role at the very beginning of the incubation after amendment was applied, which would have temporarily increased the WFPS at the top of the core and promoted anaerobic, denitrifying conditions prior to the amendment solution percolating into the soil. This is supported by the ^{15}N analysis of the emitted N_2O, which initially showed a high contribution of added $^{15}NO_3^-$ to N_2O emissions, indicating denitrification being the main process producing N_2O, which quickly declined. By day 1 both, applied $^{15}NO_3^-$, as well as $^{15}NH_4^+$, contributed equally to N_2O emissions. (Fig. 3a). Considering that N_2O is not an obligatory intermediate during nitrification, but merely a potential by-product (Anderson, 1964), these results also indicate that nitrification processes dominate over denitrification under these low moisture conditions.

5.2. *Influence of WFPS on soil N-transformation processes*

Our study demonstrates the influence of WFPS on soil N-transformation processes. Generally, gross soil N transformation rates associated with both NH_4^+ and NO_3^- turnover decreased with increasing WFPS. The total contribution of nitrification to soil N transformation processes was higher at low WFPS and decreased with increasing WFPS. However, an interesting observation was that the oxidation of organic N to NO_3^- increased almost 5-fold from 70 to 85% WFPS which may support the higher denitrification rate by supplying additional electron acceptors. However, this increase was not paralleled by an increase of N_2O emitted. This may be due to an increasing reduction of N_2O to N_2 (i.e. increasing N_2:N_2O ratio or decrease in N_2O:N_2 as described earlier) under increasing anaerobicity (Butterbach-Bahl et al., 2013).

The optimal conditions for nitrification are said to occur between 30 and 60% WFPS (Medinets et al., 2015). Emissions of NO can derive from nitrification as well as denitrification, though it has been found that the rates of produced NO measured as emissions are higher under drier conditions, where a lower WFPS leaves more air-filled pores enabling NO to escape to the surface (Pilegaard, 2013). At WFPS above 65% it is believed that emissions of N_2O and N_2 increase due to an increase in denitrification. NO, however, while it is being produced to a larger extent at high soil moisture, is also reduced to N_2O due to a longer residence time decreasing the amount emitted to the surface (Pilegaard, 2013). In this study, the observed increase in NO emissions with increasing moisture levels suggests denitrification was the source. Loick et al. (2016) concluded that up to 0.67% of the added N (from a nitrate source) was emitted as NO from denitrification supporting our findings.

Our results did not confirm our first hypothesis that losses are lower at higher moisture levels for NO and N_2O. In fact, for all gases, losses were higher at the high soil moisture possibly because the soil was not saturated enough to impede gas diffusion. Our second hypothesis was partly proved, as at the high soil moisture the proportion of N_2O from nitrate containing amendments was higher. The results for the lower moisture level did not agree with our hypothesis as the proportion of N_2O from all the amendments was similar and not mainly from NH_4^+.

Overall, our results support the assumption that nitrification (autotrophic as well as heterotrophic) plays a bigger part at lower WFPS, when air filled pores increase aerobicity, while denitrification becomes more important the higher the WFPS and therefore the lower the aerobicity. With our ^{15}N tracing approach we found that heterotrophic nitrification was the dominant process at 55% WFPS disproving our

third hypothesis that nitrification and denitrification dominate at all moisture levels, its contribution quickly decreased with increasing WFPS, while nitrification contributed most at the intermediate WFPS of 70% and least at 55%. Heterotrophic nitrification has been reported in previous studies as dependent on soil pH, C:N ratio and land use and that it can contribute up to 85% of the total N_2O flux in soils with pH values between 4.2 and 8.4 (Zhang et al., 2015). This process converts organic N (although it is believed it also happens with inorganic N sources (Zhang et al., 2014)) to NO_3^-. It is believed this occurs particularly in acidic soils where autotrophic nitrification can be inhibited. The soil used in this study was of pH 5.6 (Table 1) placing it within the soils that can potentially undergo this process. Müller et al. (2014) stated that heterotrophic nitrification is a contributor to N_2O emissions in grassland soils with high organic matter contents. This further supports the finding that this process occurs in this study (organic matter content 11.7% Table 1). In the study by Rütting and Müller (2008) it was shown that heterotrophic nitrification would carry out oxidation of organic N to NO_2^- (rather than NO_3^-). We also know that microbial consortia exist where a network of metabolic activity is present (Butterbach-Bahl et al., 2013), therefore it is likely that NO_2^- originating from the organic N pool is directly reduced to N_2O (and not further oxidised to NO_3^-) by the activity of denitrifying organisms. This also explains that higher percentages of N_2O via the organic pathway occur under higher WFPS values.

At the WFPS above 70% it has been shown that N_2O is produced solely by denitrification (Bateman and Baggs, 2005). However, in our case denitrification only became dominant at 85% WFPS, and denitrification contributed about 70% of the N_2O emissions at this WFPS (Figs. 7 and 8), while overall not much activity was found at neither 50, nor 70% WFPS.

The lower N_2O emissions for the 2 lower moisture levels over the course of the experiment could be due to a slower response of the microbial community to the added N compared to the highest soil moisture treatment where nutrient availability is expected to be higher (Papendick and Camprell, 1981).

Emissions of CO_2 have been used as an indicator of microbial respiration and activity (López-Aizpún et al., 2018). In this study the results indicate that the microbial community was most active at a WFPS of 85% in agreement with the above statement, but this was followed by the driest treatment and the least active was at the intermediate WFPS of 70% coinciding with the N_2O trend. Other factors need to also be considered as N_2O production and consumption from biogenic processes as well as abiotic processes such as gas diffusion, are both dependant of moisture in soil.

5.3. Conclusions

Our results highlight the variability in the effect of WFPS on the dominance of different N transformation processes in soil. Though the general assumption, that denitrification is more important at high WFPS, is supported here, the actual percentage of WFPS attributed to the different processes was not as expected. Heterotrophic nitrification was found to be an important source of N_2O especially under drier conditions while nitrification plays a crucial role for N_2O emissions, directly but also via nitrification coupled with denitrification under medium and high WFPS.

Results obtained from the experiment performed at 85% WFPS show the importance of nitrification even under high WFPS and raise the question if and how much of the N_2O emissions could have been mitigated by preventing nitrification supplying NO_3^- for denitrification by e.g. using nitrification inhibitors (Owusu-Twum et al., 2017; Wu et al., 2017a, 2017b).

Our study was performed under controlled conditions with a clay soil that had not received any fertiliser or manure/slurry input for few years. Under these conditions, we found a relatively equal contribution of nitrification, denitrification and heterotrophic nitrification to N_2O production at 70% WFPS. At the lower WFPS of 55% the contribution of heterotrophic nitrification dominated, while at the highest WFPS of 85% denitrification contributed most of the measured N_2O. These results will not necessarily apply to other soil types, particularly extreme high or low organic matter soils. Further studies to understand how carbon quality affect the fate of N in soils are needed.

However, the process that will be supported at a certain WFPS will most likely depend on the type of soil including its natural carbon and nutrient content, its history and the microbial community present. Emissions are also influenced by abiotic factors that are also dependant on soil moisture.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anderson, J., 1964. The metabolism of hydroxylamine to nitrite by Nitrosomonas. Biochem. J. 91, 8–17.
Arah, J.R.M., 1997. Apportioning nitrous oxide fluxes between nitrification and denitrification using gas-phase mass spectrometry. Soil Biol. Biochem. 29, 1295–1299.
Attard, E., Recous, S., Chabbi, A., De Berranger, C., Guillaumaud, N., Labreuche, J., Philippot, L., Schmid, B., Le Roux, X., 2011. Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. Global Change Biol. 17, 1975–1989.
Barrat, H., Evans, J., Chadwick, D.R., Clark, I.M., LeCocq, K., Cardenas, L.M., 2020. The impact of drought and rewetting on N2O emissions from soil in temperate and Mediterranean climates. J. Soil Sci. Eur. https://doi.org/10.1111/ejss.13015.
Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. Biol. Fertility Soils 41, 379–388.
Bergstermann, A., Cárdenas, L., Bol, R., Gilliam, L., Goulding, K., Meijide, A., Scholefield, D., Vallejo, A., Well, R., 2011. Effect of antecedent soil moisture conditions on emissions and isotopologue distribution of N2O during denitrification. Soil Biol. Biochem. 43, 240–250.
Bollmann, A., Conrad, R., 1998. Influence of O2 availability on NO and N2O release by nitrification and denitrification in soils. Global Change Biol. 4, 387–396.
Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos. Trans. R. Soc. B: Biol. Sci. 368, 20130122.
Cárdenas, L.M., Hawkins, J.M.B., Chadwick, D., Scholefield, D., 2003. Biogenic gas emissions from soils measured using a new automated laboratory incubation system. Soil Biol. Biochem. 35, 867–870.
Clayden, B., Hollis, J.M., 1984. Criteria for differentiating soil series, Soil Survey Technical Monograph, No. 17, Harpenden, UK.
Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biol. Biochem. 41, 144–153.
Khalil, K., Mary, B., Renault, P., 2004. Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O2 concentration. Soil Biol. Biochem. 36, 687–699.

Kirkham, D., Bartholomew, W.V., 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci. Soc. Am. J. 18, 33–34.
Laughlin, R.J., Stevens, R.J., Zhuo, S., 1997. Determining nitrogen-15 in ammonium by producing nitrous oxide. Soil Sci. Soc. Am. J. 61, 462–465.
Loick, N., Dixon, E., Abalos, D., Vallejo, A., Matthews, P., McGeough, K., Watson, C., Baggs, E.M., Cardenas, L.M., 2017. "Hot spots" of N and C impact nitric oxide, nitrous oxide and nitrogen gas emissions from a UK grassland soil. Geoderma 305, 336–345.
Loick, N., Dixon, E.R., Abalos, D., Vallejo, A., Matthews, G.P., McGeough, K.L., Well, R., Watson, C.J., Laughlin, R.J., Cardenas, L.M., 2016. Denitrification as a source of nitric oxide emissions from incubated soil cores from a UK grassland soil. Soil Biol. Biochem. 95, 1–7.
López-Aizpún, M., Arango-Mora, C., Santamaría, C., Lasheras, E., Santamaría, J.M., Ciganda, V.S., Cárdenas, L.M., Elustondo, D., 2018. Atmospheric ammonia concentration modulates soil enzyme and microbial activity in an oak forest affecting soil microbial biomass. Soil Biol. Biochem. 116, 378–387.
Medinets, S., Skiba, U., Rennenberg, H., Butterbach-Bahl, K., 2015. A review of soil NO transformation: associated processes and possible physiological significance on organisms. Soil Biol. Biochem. 80, 92–117.
Meijide, A., Cardenas, L.M., Bol, R., Bergstermann, A., Goulding, K., Well, R., Vallejo, A., Scholefield, D., 2010. Dual isotope and isotopomer measurements for the understanding of N2O production and consumption during denitrification in an arable soil. Eur. J. Soil Sci. 61, 364–374.
Misra, A., Tyler, G., 1999. Influence of Soil Moisture on Soil Solution Chemistry and Concentrations of Minerals in the Calcicoles Phleum phleoides and Veronica spicata Grown on a Limestone Soil. Ann. Bot. 84, 401–410.
Müller, C., Clough, T., 2014. Advances in understanding nitrogen flows and transformations: gaps and research pathways. J. Agric. Sci. 152, S34–S44.
Müller, C., Laughlin, R.J., Spott, O., Rütting, T., 2014. Quantification of N2O emission pathways via a 15N tracing model. Soil Biol. Biochem. 72, 44–54.
Müller, C., Rütting, T., Kattge, J., Laughlin, R.J., Stevens, R.J., 2007. Estimation of parameters in complex 15N tracing models by Monte Carlo sampling. Soil Biol. Biochem. 39, 715–726.
Müller, C., Stevens, R.J., Laughlin, R.J., 2004. A 15N tracing model to analyse N transformations in old grassland soil. Soil Biol. Biochem. 36, 619–632.
Owusu-Twum, M.Y., Loick, N., Cardenas, L.M., Coutinho, J., Trindade, H., Fangueiro, D., 2017. Nitrogen dynamics in soils amended with slurry treated by acid or DMPP addition. Biol. Fertility Soils 53, 339–347.
Papendick, R., Camprell, G., 1981. Water potential relations in soil microbiology. In: Kral, D.M. (Ed.), Theory and Measurement of Water Potential, 1–22. Soil Science Society of America, Madison, pp. 1–22.
Pilegaard, K., 2013. Processes regulating nitric oxide emissions from soils. Philos. Trans. R. Soc. B: Biol. Sci. 368.
Rütting, T., Müller, C., 2008. Process-specific analysis of nitrite dynamics in a permanent grassland soil by using a Monte Carlo sampling technique. Eur. J. Soil Sci. 59, 208–215.
Rütting, T., Clough, T.J., Müller, C., Lieffering, M., Newton, P.C.D., 2010. Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. Global Change Biol. 16, 2530–2542.
Saggar, S., Jha, N., Deslippe, J., Bolan, N.S., Luo, J., Giltrap, D.L., Kim, D.G., Zaman, M., Tillman, R.W., 2013. Denitrification and N2O:N2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. Sci. Total Environ. 465, 173–195.
Stange, C.F., Spott, O., Müller, C., 2009. An inverse abundance approach to separate soil nitrogen pools and gaseous nitrogen fluxes into fractions related to ammonium, nitrate and soil organic nitrogen. Eur. J. Soil Sci. 60, 907–915.
Stark, J.M., 2000. Nutrient transformations. In: Sala, O.E., Jackson, R.B., Mooney, H.A., Howarth, R.W. (Eds.), Methods in Ecosystem Science. Springer, New York, New York, NY, pp. 215–234.
Stevens, R.J., Laughlin, R.J., 1994. Determining N-15 in nitrite or nitrate by producing nitrous-oxide. Soil Sci. Soc. Am. J. 58, 1108–1116.
Stevens, R.J., Laughlin, R.J., 1995. Nitrite transformations during soil extraction with potassium-chloride. Soil Sci. Soc. Am. J. 59, 933–938.
Van Hecke, K., Van Cleemput, O., Baert, L., 1990. Chemo-denitrification of nitrate-polluted water. Environ. Pollut. 63, 261–274.
WMO, 2018. Greenhouse Gas Bulletin. Global Atmosphere Watch, World Meterological Organization, p. 6.
Wu, D., Cárdenas, L.M., Calvet, S., Brüggemann, N., Loick, N., Liu, S., Bol, R., 2017a. The effect of nitrification inhibitor on N2O, NO and N2 emissions under different soil moisture levels in a permanent grassland soil. Soil Biol. Biochem. 113, 153–160.
Wu, D., Koster, J.R., Cardenas, L.M., Bruggemann, N., Lewicka-Szczebak, D., Bol, R., 2016. N2O source partitioning in soils using (15)N site preference values corrected for the N2O reduction effect. Rapid Commun. Mass Spectrom. 30, 620–626.
Wu, D., Senbayram, M., Well, R., Brüggemann, N., Pfeiffer, B., Loick, N., Stempfhuber, B., Dittert, K., Bol, R., 2017b. Nitrification inhibitors mitigate N2O emissions more effectively under straw-induced conditions favoring denitrification. Soil Biol. Biochem. 104, 197–207.
Zhang, J., Müller, C., Cai, Z., 2015. Heterotrophic nitrification of organic N and its contribution to nitrous oxide emissions in soils. Soil Biol. Biochem. 84, 199–209.
Zhang, J., Sun, W., Zhong, W., Cai, Z., 2014. The substrate is an important factor in controlling the significance of heterotrophic nitrification in acidic forest soils. Soil Biol. Biochem. 76, 143–148.
Zhu, X., Burger, M., Doane, T.A., Horwath, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2O and NO under low oxygen availability. PNAS 110, 6328–6333.