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Interactions between N application rate, CH_4 oxidation and N_2O production in soil

S. D. Acton · E. M. Baggs

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Abstract Here we report on a controlled environment experiment in which we applied ^{13}C- and ^{15}N-enrichment approaches to quantify methane oxidation rates and source partition N_2O production in a silt loam soil following application of NH_4NO_3, enabling us to look for potential interactions between methane oxidation and nitrifier-N_2O production. ^{15}N-N_2O, $^{14+15}N$-N_2O and CO_2 fluxes and mineral N concentrations were measured over a 23-day period after application of NH_4NO_3 (5 at.% excess ^{15}N) at rates of 0, 5, 10, 20, 30 and 40 g N m^{-2} to a silt loam soil. Change in $^{12/13}C$-CH_4 concentrations (as indicative of ^{13}C-CH_4 oxidation rates) and production of ^{13}C-CO_2 were monitored over the first 72 h after addition of 1.7 µl ^{13}C-CH_4 l^{-1} (10 at.% excess ^{13}C) to these N treatments. Oxidation of applied ^{13}C-CH_4 was slower in the 5, 10, 20 and 30 g N m^{-2} (5 at.% excess ^{15}N) treatments (0.24–0.32 µg ^{13}C-CH_4 l^{-1} day^{-1}) than in the control (0.40 µg ^{13}C-CH_4 l^{-1} day^{-1}), suggesting that these N loadings inhibited oxidation. N_2O production was raised after N addition, and in the 10, 20 and 30 g N m^{-2} treatments nitrification was the predominant source of N_2O accounting for 61, 83 and 57% of the total ^{15}N-N_2O produced, respectively. Our results point towards the possibility of methylotrophs switching function to oxidise ammonia in the presence of N, which may result in greater atmospheric loading of both CH_4 and N_2O.

Keywords Denitrification · Methane oxidation · Nitrification · Nitrous oxide · Soil · Stable isotopes

S. D. Acton · E. M. Baggs (✉)
Institute of Biological and Environmental Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen AB24 3UU, UK
e-mail: e.baggs@abdn.ac.uk

Introduction

Physiological, biochemical and ecological similarities between methane- and ammonia-oxidising bacteria promote competition that affects both CH_4 and NH_3 oxidation rates, and net emissions of CH_4 and N_2O from soils. Little is known of the extent and importance of this competition, particularly after inorganic N application or with N deposition, and there is controversy in the literature which requires resolution as to the effect of N on CH_4 oxidation in soil (Bodelier and Laanbroek 2004). Both CH_4 and N_2O are key greenhouse gases with high global warming potentials (Solomon et al. 2007), and N_2O is also involved in the destruction of stratospheric ozone (Ravishankara et al. 2009). Agricultural soils are a major source of atmospheric N_2O, mainly in positive response to inorganic N application (Eichner 1990; Mosier 1994; Bouwman 1996; Liu and Greaver 2009), but these soils may also act as either a net source or a sink (oxidation) for atmospheric CH_4, depending on environmental conditions, soil type and

N availability (Topp and Pattey 1997; Le Mer and Roger 2001; Khalil and Baggs 2005). Differences in N cycling are important in regulating the soils potential to act as a sink for CH_4 (Mosier et al. 1991) and may therefore have a significant impact on atmospheric loading of this greenhouse gas. Understanding the effect of varying N application rates on the extent of interactions between CH_4 oxidation and emission of N_2O has powerful implications for mitigation of both CH_4 and N_2O, as there is currently no soil management strategy that mitigates both gases.

Addition of N to soil has been reported to inhibit (Sitaula et al. 1995; Gulledge and Schimel 1998; Wang and Ineson 2003; Baggs and Blum 2004), stimulate (Cai and Mosier 2000; De Visscher et al. 2001; Veldkamp et al. 2001) or have no effect (Castro et al. 1995; Dobbie and Smith 1996; Steinkamp et al. 2001; Alluvione et al. 2009) on CH_4 oxidation. However, a recent meta-analysis by Liu and Greaver (2009) has indicated a 38% lowering in CH_4 uptake in response to N addition to terrestrial (agricultural and non-agricultural) ecosystems. The mechanism involved in any inhibition of CH_4 oxidation is uncertain and may be related to the role of NH_3 in competing for methane monoxygenase enzymes (Holmes et al. 1995), NO_2^- or hydroxylamine toxicity (King and Schnell 1994), or osmotic effects (Hütsch et al. 1996; Kravchenko et al. 2002). Discrepancies between studies are exacerbated by differences in soil types with differing native microbial populations, N availabilities and CH_4 concentrations (de Visscher et al. 2001; Bodelier and Laanbroek 2004). Any N inhibition of CH_4 oxidation is thought to result in an increase in ammonia oxidation (Hütsch 1998), meaning that there is the potential for N application to lead to a switch in function of methylotrophs away from their beneficial effect in lowering atmospheric loading of CH_4. However, the role of these bacteria in ammonia oxidation or N_2O production in soil is still uncertain.

Recent advances in stable isotope techniques have enabled identification and quantification of different microbial sources of N_2O (Baggs 2008), but still little is known about any changes in N_2O source in response to varying fertiliser-N application rates. If methylotrophic bacteria have the potential to contribute to nitrifier-N_2O production in soil (Hooper et al. 1997) then any inhibition of CH_4 oxidation, and subsequent NH_3 oxidation by methylotrophs, would be expected to increase the proportional contribution of nitrification (primarily ammonia oxidation) to N_2O emissions following N application. Conversely, conditions conducive to CH_4 oxidation would be expected to not only lower net CH_4 emissions, but to indirectly lower the contribution of nitrification to measured N_2O production.

The objective of the experiment we report here was to determine the effect of varying N availabilities (0–40 g N m^{-2} applied as NH_4NO_3) on CH_4 oxidation rates and N_2O production from soil. Stable isotope approaches (^{13}C- and ^{15}N-enrichment) were employed to quantify CH_4 oxidation rates more accurately than inference from changes in net emissions of CH_4 (Baggs and Blum 2004), and to source partition the N_2O between nitrification (ammonia oxidation and nitrifier denitrification) and denitrification (Baggs 2008). We hypothesised that increasing rates of NH_4NO_3 application would lower CH_4 oxidation in soil, due to an increased competition between NH_3 and CH_4 for the active site of the methane monooxygenase enzyme, and that this lower CH_4 oxidation would be associated with increased N_2O production, with proportionally greater nitrifier-N_2O production at higher N application rates.

Materials and methods

Experimental set-up

Soil (0–15 cm depth) was sampled from an arable field on the Imperial College London Estate at Wye. The soil was a brown earth silt loam (17% sand, 68% silt, 15% clay, total carbon 1.9%, total N 0.2%, pH (H_2O) 7.1, bulk density 1.23 g cm^{-3}) of the Coombe series classified as a Cambisol (FAO classification). Soil was air dried, sieved <2 mm and stored at 4°C for 3 weeks until establishment of the experiment. The experiment was conducted under controlled environment conditions at 21°C in the dark in 1 l Kilner jars with gas-tight lids fitted with a gas sampling port. Soils were conditioned at 40% water-filled pore space (WFPS), 21°C, for 5 days prior to experimental set-up.

Nitrogen treatments were applied (time 0) and soil water content was adjusted to 60% WFPS with distilled water. The soil WFPS was determined based

on bulk density, and a particle density of 2.65 g cm^{-3} (Khalil and Baggs 2005). N was applied in solution as $^{14}NH_4{}^{15}NO_3$ or $^{15}NH_4{}^{15}NO_3$ (5 at.% excess ^{15}N) to different replicates at rates of 0 (unfertilised control), 5, 10, 20, 30, 40 g N m^{-2}, equivalent to 0, 0.18, 0.36, 0.71, 1.1 and 1.4 mg N g $soil^{-1}$, respectively, giving six experimental treatments. Each fertiliser treatment was replicated eight times for gas sampling, and four times for destructive soil sampling. Immediately after N addition (0 h), ^{13}C-CH_4 (10 at.% excess ^{13}C) was applied at a concentration of 17 µl l^{-1} to the closed headspace of the Kilner jars of half of the replicates established for gas sampling ($n = 4$), replacing an equivalent volume of headspace gas to ensure constant pressure.

Gas sampling and analysis

Gas samples for $^{12+13}C$-CH_4 and ^{13}C-CH_4 analysis were taken at 0, 12, 24, 48 and 72 h from the closed Kilner jar headspaces to which ^{13}C-CH_4 had been applied. Samples for $^{14+15}N$-N_2O, ^{15}N-N_2O and $^{12+13}C$-CO_2 analysis were taken at 1, 3, 5, 7, 14 and 23 days from the treatment replicates to which ^{15}N but no ^{13}C-CH_4 had been applied. Samples were taken from these jars at 20, 40 and 60 min after jar closure on each sampling day and the measured flux determined by linear interpolation between these samples. To keep the headspace pressure constant during gas sampling, sample volumes removed from the headspaces were replaced with the same volume of laboratory air.

Both 12- and 125-ml gas samples were taken from each jar headspace on each sampling occasion. The 12-ml samples were stored in pre-evacuated 12-ml gas vials (Labco), and 1 ml of this gas analysed for N_2O, CH_4 and CO_2 on an Agilent 6890 gas chromatograph fitted with an electron capture detector and flame ionisation detector with methaniser (column and detector temperatures 40 and 250°C, respectively). The 125-ml samples were stored in helium-flushed pre-evacuated 125-ml gas-tight glass bottles (Supelco, UK), and analysed for the ^{13}C and ^{15}N enrichment in CH_4 and N_2O, respectively, on a SerCon 20/20 isotope ratio mass spectrometer following cryofocusing in an ANCA TGII gas preparation module. This provides precision of ±1‰ ^{13}C or ^{15}N. The measured ^{15}N-N_2O fluxes were source partitioned between nitrification and denitrification, according to Baggs et al. (2003), whereby ^{15}N-N_2O fluxes from the $^{15}NH_4{}^{15}NO_3$ replicates minus the ^{15}N-N_2O fluxes from the $^{14}NH_4{}^{15}NO_3$ replicates were attributed to nitrification, and ^{15}N-N_2O fluxes from the $^{14}NH_4{}^{15}NO_3$ replicates were attributed to denitrification. ^{15}N-N_2O fluxes from the $^{15}NH_4{}^{15}NO_3$ replicates could have been produced during nitrification, denitrification or nitrification-coupled denitrification. Nitrification-coupled denitrification had previously been found to be negligible compared to ^{15}N-N_2O denitrified from fertiliser ^{15}N-$NO_3{}^-$ applied at this rate and at.% enrichment to our soil, so it was considered unnecessary to quantify this source here. We present our measured N_2O fluxes on a per area basis as the fertiliser was applied in solution to the soil surface.

Soil sampling and analysis

Soil was destructively sampled from additional replicates of the unfertilised and N-amended treatments on days 1, 3 7, 14 and 23 for mineral N analysis and determination of pH in water. $NH_4{}^+$-N and $NO_3{}^-$-N were extracted from these soils with 1 M KCl (25 g soil to 100 ml KCl solution) and determined by colorimetric analysis on a Burkard SFA2 continuous flow analyser.

Statistical analysis

Data were tested for normality and log-transformed where appropriate (Parkin and Robinson 1993), prior to means comparisons using Independent t tests, analysis of variance and correlation, all using Genstat version 5.

Results

Methane oxidation and N_2O production over the first 72 h

Decline in ^{13}C-CH_4 concentration and increase in ^{13}C-CO_2 in the closed headspaces over time was taken as indicative of oxidation (after Baggs and Blum 2004). ^{13}C-CH_4 oxidation followed 1st order reaction kinetics in most treatments, with rate constants (k) per hour calculated as $\log_e[^{13}C\text{-}CH_4]/[^{13}C\text{-}CH_4]_0$. ^{13}C-CH_4 oxidation was biphasic in the control

and 40 g N m^{-2} treatment, for which the average k value for each phase was calculated. ^{13}C-CH_4 oxidation rates over the first 12 h were highest ($P < 0.05$) in the control and 40 g N m^{-2} treatments (Fig. 1; Table 2), with respective k values of 0.17 and 0.14 during this period. Oxidation rates were slower in the other N treatments, with k values over the entire 72 h of between 0.013 and 0.018. There was no significant difference in ^{13}C-CH_4 oxidation rates between the control and 40 g N m^{-2} treatment, suggesting that other factors than N application rate were affecting CH_4 oxidation in this soil, with a possible reduction in efficiency of N as an inhibitor above 30 g N m^{-2}.

Production of ^{13}C-CO_2 was highest in the control, with recovery of ^{13}C in CO_2 at 72 h accounting for 81% of the ^{13}C applied as ^{13}C-CH_4. This recovery only ranged from 1 to 23% in the other treatments. ^{13}C-CH_4 ($\log_e$) concentrations were negatively correlated with ^{13}C-CO_2 ($\log_e$) concentrations in the control, 5, 10 and 20 g N m^{-2} treatments ($r = -0.49$, $P < 0.05$; Fig. 2), but positively correlated in the 40 g N m^{-2}

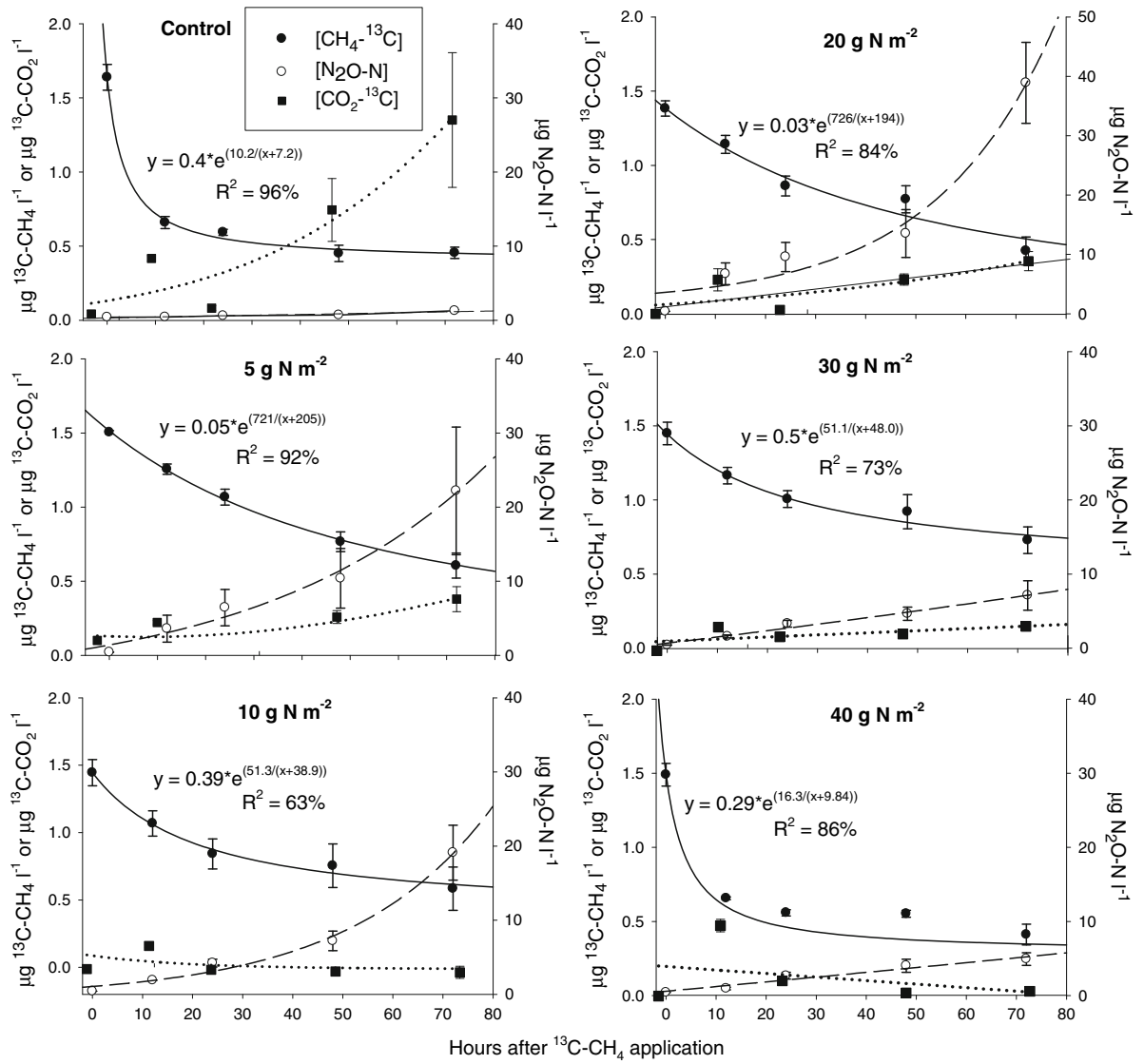

Fig. 1 Concentrations of ^{13}C-CH_4, ^{13}C-CO_2 and N_2O over 72 h following application of ^{13}C-CH_4 (1.7 μl ^{13}C-CH_4 l^{-1}; 10 at.% excess ^{13}C) and NH_4NO_3 at 0 (control), 5, 10, 20, 30 and 40 g N m^{-2} to soil. Error bars represent ±1 standard error of the mean

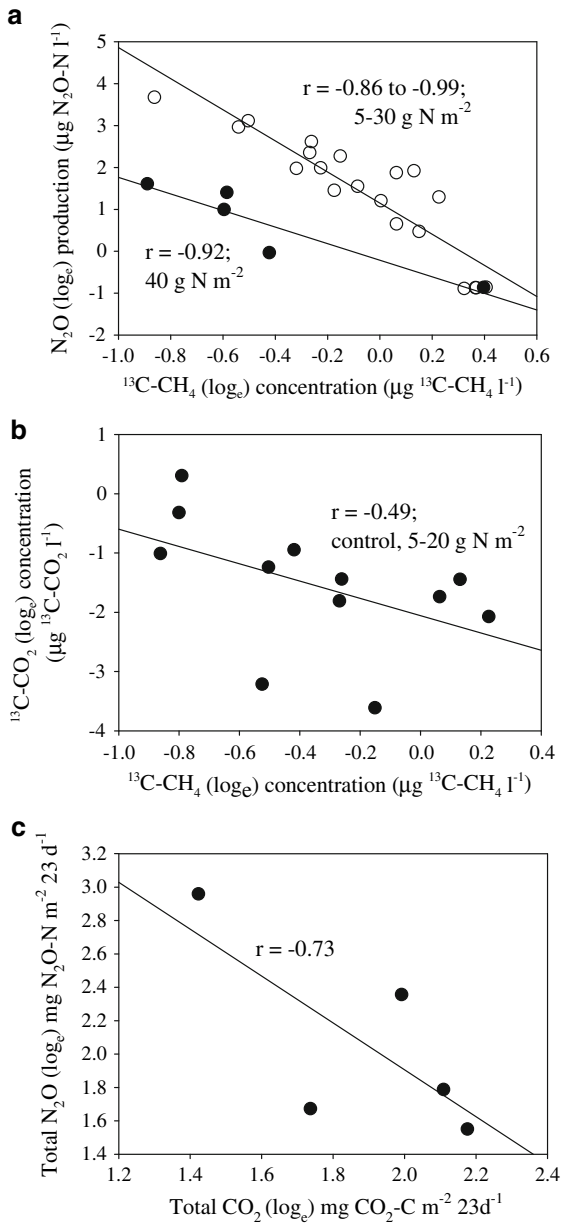

Fig. 2 Correlations between **a** ^{13}C-CH_4 (log_e) and N_2O (log_e) concentrations in the 5–40 g N m^{-2} treatments over 72 h following application of ^{13}C-CH_4 (1.7 µl ^{13}C-CH_4 l^{-1}; 10 at.% excess ^{13}C), **b** ^{13}C-CH_4 (log_e) and ^{13}C-CO_2 (log_e) concentrations in the control and 5–20 g N m^{-2} treatments over this 72 h period, and **c** total CO_2 (log_e) and total N_2O (log_e) emitted over 23 days after application of 5–40 g N m^{-2}

treatment ($r = 0.68$; $P < 0.05$). N_2O was produced in all treatments after addition of ^{13}C-CH_4, and by 72 h was highest ($P < 0.05$) in the 20 g N m^{-2} treatment (39 µg N_2O-N l^{-1}) and lowest ($P < 0.05$) in the control treatment (0.4 µg N_2O-N l^{-1}; Fig. 1). Log_e ^{13}C-CH_4 concentrations and $^{14+15}$N-N_2O (log_e) concentrations were negatively correlated in the N addition treatments ($r = -0.86$ to -0.99; $P < 0.05$; Fig. 2).

Nitrous oxide and CO_2 production over the 23-day experimental period

Production of N_2O was higher ($P < 0.05$) in the N amended treatments than in the control, with only 1.1 mg $^{14+15}$N-N_2O m^{-2} emitted from the control over 23 days (Table 1). The 17.2 mg $^{14+15}$N-N_2O m^{-2} 23 day^{-1} emitted from the 40 g N m^{-2} treatment was significantly greater ($P < 0.05$) than emissions from the other N amended treatments. Fluxes increased on day 3 after N addition, but it took until day 14 for the maximum flux of 1.3 mg N_2O-N m^{-2} h^{-1}, measured from the 40 g N m^{-2} treatment (Fig. 3). N_2O (log_e) fluxes and concentration of available NH_4^+ (log_e) were positively correlated ($r = 0.79$; $P < 0.05$) in the 5 and 10 g N m^{-2} treatments ($r = 0.69$ and 0.43, respectively; $P < 0.05$), but negatively correlated in the 40 g N m^{-2} treatment ($r = -0.83$; $P < 0.05$). N_2O (log_e) fluxes were positively correlated with NO_3^- (log_e) concentrations ($r = 0.81$ to 0.98; $P < 0.05$). CO_2 production declined throughout the experiment, and on most days was lowest in the 40 g N m^{-2} treatment (Fig. 4). Total CO_2 production over the 23 days was greatest ($P < 0.05$) in the control treatment, with 9.8 mg CO_2-C m^{-2} emitted over 23 days, and decreased with increasing N application rate, with only 4.2 mg CO_2-C m^{-2} 23 day^{-1} emitted from the 40 g N m^{-2} treatment. Total CO_2 (log_e) and total N_2O (log_e) production from the N amended treatments over 23 days were negatively correlated ($r = -0.73$; $P < 0.05$; Fig. 2).

Source partitioning of ^{15}N-N_2O

No ^{15}N-enrichment was detected in the NH_4^+ or NO_2^- pools in the $^{15}NH_4{}^{15}NO_3$ replicates at any of the sampling points, indicating that nitrate ammonification of ^{15}N-NO_3^- to ^{15}N-NO_2^- or ^{15}N-NH_4^+, or immobilisation and subsequent re-mineralisation of this ^{15}N, were negligible. This meant that denitrifier-^{15}N-N_2O could be quantified from the $^{14}NH_4{}^{15}NO_3$ replicates, and nitrifier-^{15}N-N_2O from the difference between the

Table 1 Total $^{14+15}N$-N_2O, ^{15}N-N_2O, nitrifier-^{15}N-N_2O and denitrifier-^{15}N-N_2O production and % of ^{15}N applied emitted as ^{15}N-N_2O over the 23-day experimental period

	$^{14+15}N$-N_2O (mg N_2O-N m^{-2} 23 day^{-1})	% of ^{15}N applied emitted as ^{15}N-N_2O	^{15}N-N_2O (μg ^{15}N-N_2O m^{-2} 23 day^{-1})	Nitrifier ^{15}N-N_2O (μg ^{15}N-N_2O m^{-2} 23 day^{-1})	Denitrifier ^{15}N-N_2O (μg ^{15}N-N_2O m^{-2} 23 day^{-1})
Control (0 g N m^{-2})	1.14 (±0.12)^{d}	n/a	n/a	n/a	n/a
5 g N m^{-2}	3.02 (±0.41)^{c}	0.06 (0.02)	148 (±49)^{c}	49 (±40)^{d}	95 (±43)^{b}
10 g N m^{-2}	4.86 (±0.59)^{b}	0.06 (0.01)	304 (±47)^{b}	186 (±35)^{c}	118 (±41)^{b}
20 g N m^{-2}	6.24 (±1.03)^{b}	0.06 (0.03)	613 (±253)^{a}	507 (±249)^{a}	106 (±5)^{b}
30 g N m^{-2}	4.78 (±1.02)^{b}	0.03 (0.01)	463 (±160)^{a}	263 (±57)^{b}	200 (±108)^{b}
40 g N m^{-2}	17.17 (±1.42)^{a}	0.03 (0.001)	621 (±24)^{a}	239 (±82)^{b}	382 (±83)^{a}

Values in *parentheses* are ± 1 s.e.m. *Superscript letters* indicate significant differences ($P < 0.05$) between treatments, within a column

n/a Not applicable

$^{15}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$ replicates (after Baggs et al. 2003).

Between 0.03 and 0.06% of ^{15}N applied was emitted as ^{15}N-N_2O, and between 3.6% (40 g N m^{-2} treatment) and 9.8% (20 g N m^{-2} treatment) of the total $^{14+15}N$-N_2O emitted over 23 days was ^{15}N-N_2O. Total ^{15}N-N_2O production was highest ($P < 0.05$) in the 20 g N m^{-2} treatment, with 0.51 mg ^{15}N-N_2O m^{-2} 23 day^{-1} produced during nitrification and 0.11 mg ^{15}N-N_2O m^{-2} 23 day^{-1} produced during denitrification in this treatment (Table 1). Nitrification was the predominant N_2O-producing process in the 10, 20 and 30 g N m^{-2} treatments, accounting for 61, 83 and 57% of the measured total ^{15}N-N_2O emission, respectively, at 23 days. N_2O source partitioning varied throughout the course of the experiment (Fig. 3). A maximum nitrifier-^{15}N-N_2O flux of 3.4 μg ^{15}N-N_2O m^{-2} day^{-1} was measured from the 20 g N m^{-2} treatment on day 7, which was more than double that from the other treatments on this day. The maximum nitrifier-^{15}N-N_2O flux lagged in time with increasing N application rate. Nitrifier-^{15}N-N_2O ($\log_e$) fluxes in the 30 and 40 g N m^{-2} treatments were positively correlated with NO_3^- ($\log_e$) concentrations ($r = 0.87$ and 0.81, respectively; $P < 0.05$), but negatively correlated with NH_4^+ ($\log_e$) concentrations ($r = -0.85$ and -0.77, respectively, $P < 0.05$). The greatest denitrifier-N_2O production was in the 40 g N m^{-2} treatment, with a maximum flux of 2.2 μg ^{15}N-N_2O m^{-2} day^{-1} measured on day 14, and 382 μg ^{15}N-N_2O m^{-2} emitted over 23 days from this treatment. These denitrifier-^{15}N-N_2O ($\log_e$) fluxes were positively correlated with NO_3^- ($\log_e$) concentrations in this treatment ($r = 0.82$; $P < 0.05$).

Soil mineral N

Addition of N lowered the soil pH by up to 1.2 pH units over the 23 days, and pH was lowest ($P < 0.05$) in the 40 g N m^{-2} treatment (Fig. 5). Concentrations of NO_3^- were greater in the N-amended treatments than in the unfertilised control soil on all days, but NH_4^+ concentrations in the 5 g N m^{-2} treatment were not significantly different from in the control from day 3 onwards (Fig. 5). NH_4^+ concentrations decreased over time after application, whereas NO_3^- concentrations gradually increased, being indicative of nitrification. Net nitrification rates of 66, 177, 198, 153 and 130 mg N kg $soil^{-1}$ were estimated for the 5, 10, 20, 30 and 40 g N m^{-2} treatments, respectively, over the 23 day experiment.

Discussion

CH_4 oxidation rates in response to N application

Over the first 12 h after application of 17 μl ^{13}C-CH_4 l^{-1}, ^{13}C-CH_4 oxidation was most rapid in the control and the 40 g N m^{-2} treatment, with oxidation in the other N-amended treatments being 51–76% lower than in the control. However, over the entire 72 h period of measurement CH_4 oxidation rates in the 5–30 g N m^{-2} treatments were only

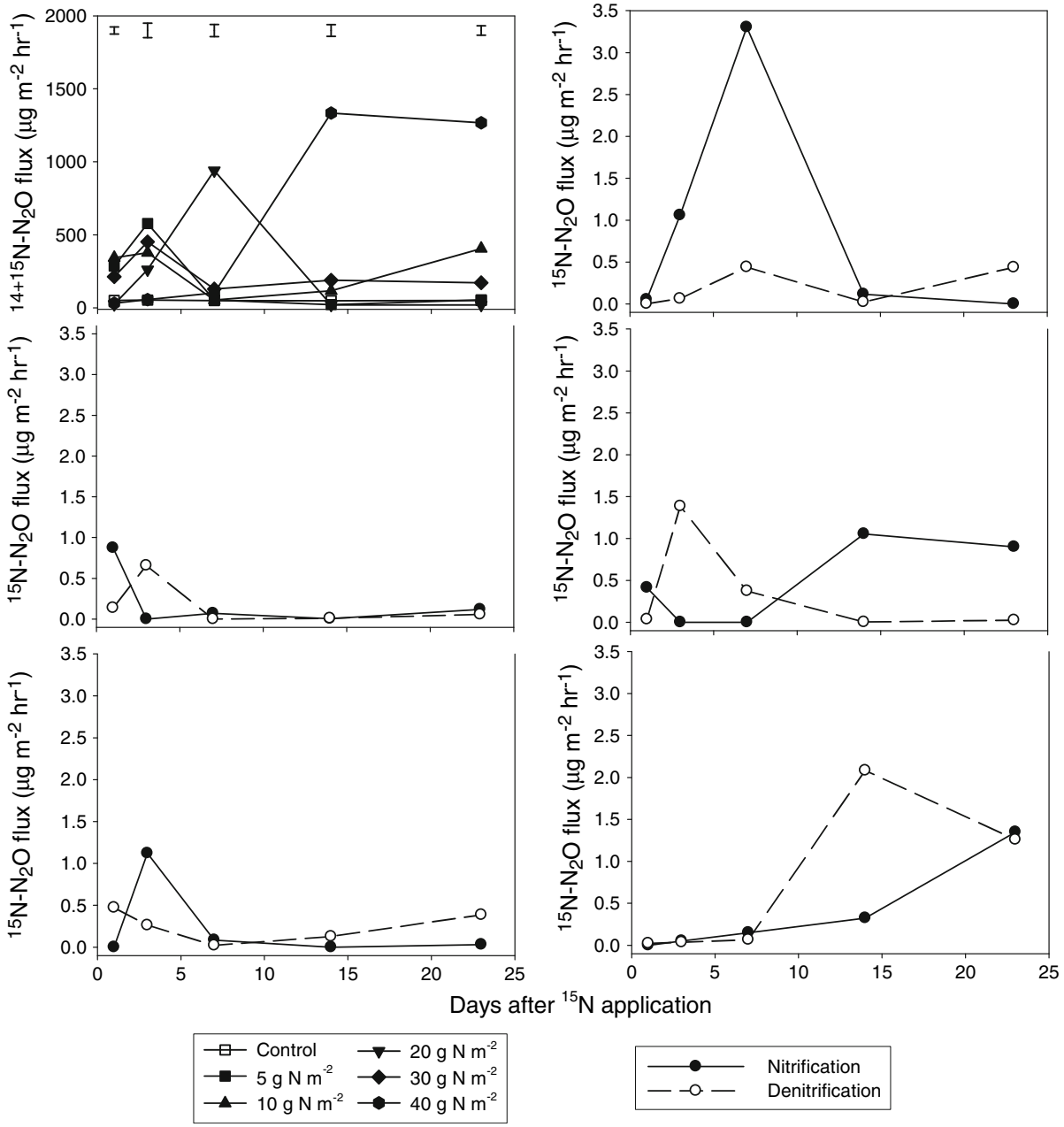

Fig. 3 Emissions of $^{14+15}N$-N_2O, denitrifier-^{15}N-N_2O and nitrifier-^{15}N-N_2O over 23 days following application of 0 (control), 5, 10, 20, 30 and 40 g N m^{-2} to soil. Error bars represent ±1 standard error of the difference

19–39% lower than in the control, reflecting the biphasic nature of oxidation in the control. This lowering of oxidation rate is most likely an inhibition in response to N availability. This is in accordance with reported inhibitions of 50–85% following application of a range of 4–19 g N m^{-2} to arable, grassland and forest soils (Hütsch 1996; Tlustos et al. 1998; Steudler et al. 1989; Baggs and Blum 2004; Gulledge et al. 2004) with soil concentrations of 40–60 mg NH_4^+ kg^{-1} being demonstrated to be sufficient to induce temporary inhibition of CH_4 oxidation (Hütsch 1998; Kravchenko et al. 2002). In our experiment, concentrations of NH_4^+ remained above this threshold during the 72 h period of

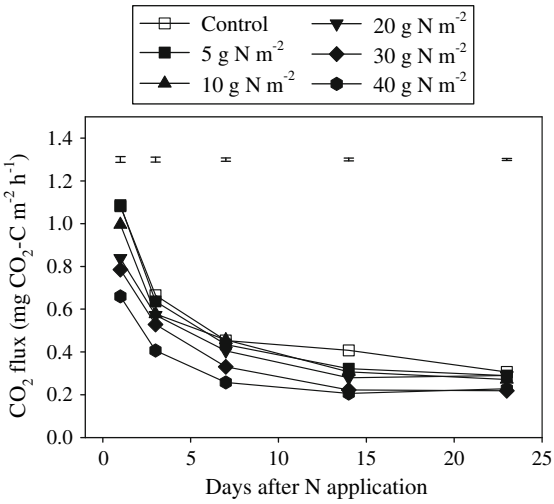

Fig. 4 Emissions of CO_2 over 23 days following application of 0 (control), 5, 10, 20, 30 and 40 g N m^{-2} to soil. Error bars represent ±1 standard error of the difference

monitoring CH_4 oxidation, in all N amended treatments except the 5 g N m^{-2} treatment in which concentrations fell from 85 to 30 mg NH_4^+ kg^{-1} between 24 and 72 h. It was not possible to determine here the exact mechanism responsible for this inhibition, but it is usually attributed to the direct effects of preferential oxidation of NH_3 over CH_4 by methane monooxygenase enzymes (Bédard and Knowles 1989), or toxicity of NH_2OH, or NO_2^- produced during NH_3 oxidation (King and Schnell 1994), and so is often related to nitrification rates (Mosier et al. 1991; Baggs and Blum 2004), here exemplified by the decrease in NH_4^+ and increase in NO_3^- concentrations, even between 1 and 72 h in the 5–30 g N m^{-2} treatments.

The reason for the more rapid CH_4 oxidation in the 40 g N m^{-2} treatment than in the other fertilised treatments is unclear, but indicates that the relationship between N application rate and CH_4 oxidation was not simply a function of mineral N concentration, but that other factors controlling availability need to be considered. The higher rate of N application was associated with the lowest pH within the 72 h period of ^{13}C-CH_4 oxidation rate determination, possibly due to proton exchange after NH_4^+ addition. The unprotonated NH_3 is the preferred form of N for ammonia oxidation as NH_4^+ requires an active transport mechanism to enter the cell and is therefore energetically inefficient (Burton and

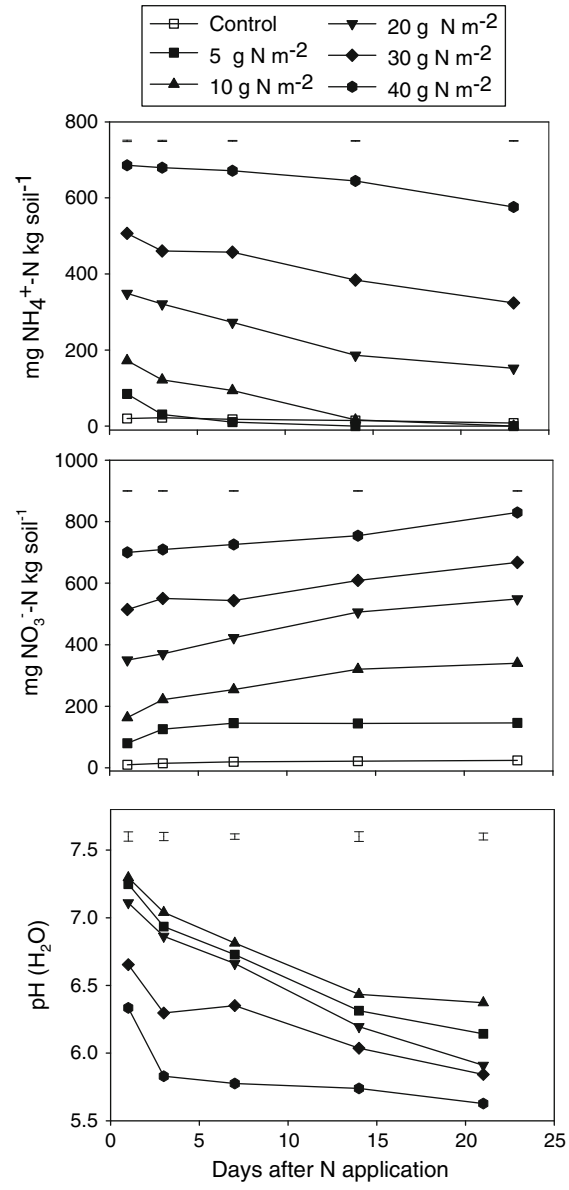

Fig. 5 Available soil N concentrations and pH (H_2O) following application of 0 (control), 5, 10, 20, 30 and 40 g N m^{-2} to soil. Error bars represent ±1 standard error of the difference

Prosser 2001). Exponential decline in NH_3 availability with lowering pH ($NH_3 + H^+ \leftrightarrow NH_4^+$; pKa = 9.25), may possibly account for less inhibition of CH_4 oxidation in the 40 g N m^{-2} treatment, despite the high N application rate. Lower pH may also be conducive to build-up of toxic NH_2OH, having a more direct effect on activity of methane oxidising bacteria. These complex interactions

Table 2 Comparison of changes in net emission, ^{13}C-CH_4, and ^{13}C-CO_2 approaches for estimating rates of CH_4 oxidation in soil over 0–12 and 12–72 h after addition of ^{13}C-CH_4 to microcosm headspaces

	Change in net [$^{12+13}C$-CH_4]		Change in [^{13}C-CH_4]		Production of [^{13}C-CO_2]	
	0–12 h $\mu g\ l^{-1}$ 0–12 h^{-1}	12–72 h $\mu g\ l^{-1}$ 60 h^{-1}	0–12 h $\mu g\ l^{-1}$ 12 h^{-1}	12–72 h $\mu g\ l^{-1}$ 60 h^{-1}	0–12 h $\mu g\ l^{-1}$ 12 h^{-1}	12–72 h $\mu g\ l^{-1}$ 60 h^{-1}
Treatment						
Control	8.37 (±0.32)	1.10 (±0.15)	0.98 (±0.10)	0.21 (±0.03)	0.39 (±0.04)	0.98 (±0.9)
5 g N m^{-2}	1.79 (±0.28)	5.14 (±0.38)	0.25 (±0.04)	0.65 (±0.05)	0.13 (±0.03)	0.15 (±0.06)
10 g N m^{-2}	1.83 (±0.30)	4.67 (±1.04)	0.38 (±0.03)	0.48 (±0.16)	0.18 (±0.02)	0.18 (±0.75)
20 g N m^{-2}	1.96 (±0.17)	6.30 (±0.42)	0.24 (±0.02)	0.72 (±0.04)	0.24 (±0.15)	0.13 (±0.03)
30 g N m^{-2}	1.51 (±0.38)	4.11 (±0.80)	0.28 (±0.06)	0.44 (±0.12)	0.16 (±0.02)	0.005 (±0.01)
40 g N m^{-2}	7.92 (±1.13)	2.06 (±0.60)	0.76 (±0.01)	0.24 (±0.06)	0.49 (±0.35)	−0.45 (±0.05)

Rates were calculated as $[^{12+13}C - \text{ or } ^{13}C - CH_4]_t - [^{12+13}C - \text{ or } ^{13}C - CH_4 \text{ or } ^{13} - CO_2]_{t_0}$. Values in *parentheses* are ±1 s.e.m

between NH_3 availability, nitrification rates and pH may in part explain reports of CH_4 oxidation at much lower pH than measured here (Steudler et al. 1989; Schnell and King 1994) and the role of CEC in regulating CH_4 oxidation rates through NH_4^+ availability (de Visscher et al. 1998).

A ^{13}C approach for determining CH_4 oxidation rates

We adopted a ^{13}C approach to estimate oxidation rates of applied ^{13}C-CH_4, as this is considered to be more reliable than changes in net emissions, as it negates the effect of any production of CH_4 during methanogenesis (Baggs and Blum, 2004; Khalil and Baggs, 2005). The ^{13}C approach estimated lower oxidation rates than relying on changes in net CH_4 emission (Table 2). The reason for this is unclear, but it is possible that some methanogenesis was occurring here, lowering the ^{13}C-enrichment of CH_4, but having a comparably minor effect on net changes in $^{12+13}C$-CH_4 concentration. Even when correcting for the typical kinetic isotope effect (fractionation factor) of $\sim$1.02 during methane oxidation (Barker and Fritz 1981; Coleman et al. 1981; Snover and Quay, 2000), this can not account for the discrepancy in estimated oxidation rates. We did not take into account the diffusion of CH_4 into the soil, but this would have overestimated oxidation rates rather than resulted in lower estimates than from changes in net emission, and any fractionation during diffusion would only be expected to be predominant when microbial activity is low (Gonzalez-Gil et al. 2008). Our comparison of approaches means that caution is required when comparing ^{13}C-CH_4 and net $^{12+13}C$-CH_4 approaches to estimate CH_4 oxidation rates, particularly where methanogenesis may significantly contribute to CH_4 flux.

We found negative correlations between ^{13}C-CH_4 ($\log_e$) and ^{13}C-CO_2 ($\log_e$) concentrations in the control, 5, 10 and 20 g N m^{-2} treatments, but we consider ^{13}C-CO_2 production to be an unreliable surrogate for methane oxidation rates on two counts. Firstly, the percent of CH_4-C assimilated (C conversion efficiency) is known to vary, with upper conversion efficiencies ranging from 31 to 54% in non-agricultural soils (Whalen et al. 1992; Roslev et al. 1997; Yawitt et al. 1990). It is also possible that any differences in % C assimilation may be exacerbated following N addition when some methane oxidisers may switch to oxidising ammonia, as C conversion efficiency may decrease after ammonia addition to soil (Roslev et al. 1997). Secondly, rapid turnover of C in soil (Rangel-Castro et al. 2005) means that ^{13}C-CO_2 production from heterotrophic activity as opposed to methane oxidation per se can not be discounted.

Microbial source of N_2O with increasing N application

Our total N_2O production accounted for up to 0.1% of N applied, with the ^{15}N-N_2O accounting for up to 0.06% of ^{15}N applied. This falls within the IPCC (Solomon et al. 2007) emission factor for fertilised soils, and our fluxes were of the same magnitude as

reported by Bateman and Baggs (2005) for this soil when fertilised with 20 g N m^{-2} as NH_4NO_3. Total N_2O emissions increased with increasing N application rate, however this relationship was not linear over time, with a temporal delay in maximum N_2O flux observed with increasing N application. This suggests that parameters other than N availability were limiting for N_2O production, particularly in the 30 and 40 g N m^{-2} treatments, over the first 2 weeks. It is possible that N_2O was further reduced to N_2 in these treatments, but ^{15}N-N_2 was not analysed for here.

A ^{15}N-enrichment approach was used to distinguish between nitrification and denitrification as microbial sources of N_2O (Baggs et al. 2003; Baggs 2008). Their respective contributions varied with N application rate and time after N application. Nitrification was the predominant source of N_2O over 23 days in the 10, 20 and 30 g N m^{-2} treatments, accounting for 61, 83 and 57%, respectively, of the total ^{15}N-N_2O emitted from these treatments. This predominance of nitrification confirms results of Bateman and Baggs (2005) for this soil at 60% WFPS, following application of 20 g N m^{-2}, providing evidence of the importance of nitrification (ammonia oxidation + nitrifier denitrification) in soil N_2O emission after addition of high rates of NH_4^+ addition. The maximum nitrifier-^{15}N-N_2O fluxes lagged in time depending on N application rate, with the peak nitrifier-^{15}N-N_2O flux in the 40 g N m^{-2} treatment only occurring at 23 days, but at 1, 3, 7 and 14 days in the 5, 10, 20 and 30 g N m^{-2} treatments, respectively. Similarly, Inubishi et al. (1996) and Stevens et al. (1997) have shown nitrifier-N_2O production to lag behind denitrifier-N_2O production. This time lag was not reflected in the soil mineral N concentrations, and so most likely reflects slower growth of ammonia oxidising bacteria. Denitrification was the predominant N_2O source in the 40 g N m^{-2} treatment. The lower CO_2 production in this treatment, and the negative correlation between CO_2 and N_2O fluxes, indicates that this was not in response to creation of sub-oxic microsites resulting from heterotrophic respiration. It is possible that nitrification was more sensitive to the lower pH in this treatment, with no contribution of methane oxidisers to N_2O production here.

Possible interactions between CH_4 oxidation and nitrifier-N_2O production

During the 72 h period of measurement of CH_4 oxidation, $^{14+15}N$-N_2O ($\log_e$) production and ^{13}C-CH_4 ($\log_e$) concentrations were negatively correlated in the N-amended treatments. This may have been a response to greater substrate availability for N_2O-producing processes, but may also have been symptomatic of the lowering in CH_4 oxidation, and interactions between the two. Kravchenko et al. (2002) showed a negative relationship between CH_4 oxidation rates and N_2O fluxes from arable soil following different rates of NH_4^+-N application (4–100 mg N kg^{-1} soil), and here we demonstrate relationships over a wider range of N application rates (0–1.4 g N kg^{-1} soil) and measured the highest nitrifier-N_2O production (over 23 days) from the 20 g N m^{-2} treatment in which CH_4 oxidation rates were lowest over the first 12 h. We hypothesise that the proportionally high nitrifier-N_2O production in this treatment may have in part been due to inhibition of CH_4 oxidation, and N_2O production during ammonia oxidation by methylotrophs as well as ammonia oxidisers (Baggs and Blum, 2004).

Our results demonstrate the potential for positive feedback between nitrification and CH_4 oxidation with these rates of fertiliser-N application of 10–30 g N m^{-2}, resulting in a more detrimental effect on the atmosphere by lowering CH_4 oxidation and increasing nitrifier-N_2O production, which has implications for the management of fertilised soils. Some methylotrophs may possess the hydroxylamine oxidoreductase enzyme necessary for N_2O production during NH_3 oxidation (Hooper et al. 1997). However, such a contribution of this functional group has yet to be verified in situ. Our results suggest that if methylotrophs are switching to oxidise ammonia instead of CH_4, then this switch occurs in our silt loam soil under a defined range of conditions that may, for example, occur in the first few days after addition of NH_4NO_3 fertiliser to an arable crop: 180–520 mg NH_4^+-N kg $soil^{-1}$, 180–550 mg NO_3^--N kg $soil^{-1}$, pH 6.8–7.3, 60% WFPS, total carbon 1.9%, total N 0.2% and bulk density 1.23 g cm^{-3}. Gulledge et al. (2004) suggests that such increased nitrification and lowered CH_4 oxidation rates may arise from changes

in the microbial community structure, although this is more likely to become apparent over longer time periods than this 23-day experiment.

Conclusions

Here we adopted an isotope enrichment approach to quantify ^{13}C-CH_4 oxidation rates and to source partition ^{15}N-N_2O production to demonstrate the effect of fertiliser-N application rates on these processes, and potential interactions between them. We provide evidence for increased nitrifier-N_2O production (by ammonia oxidising, and possibly also by methane oxidising bacteria) occurring under conditions where CH_4 oxidation was lowered, or inhibited. This suggests the possibility of methylotrophs switching function to oxidise ammonia in our 10, 20 and 30 g N m^{-2} treatments, and future research is required to verify their involvement in N_2O production, to determine the mechanisms involved, to clarify the threshold conditions required for such a switch, and the implications for atmospheric loading of CH_4 and N_2O from N-enhanced ecosystems. A greater understanding of how both CH_4 and N_2O emission can be controlled through targeted N applications, or by managing sites of high N deposition, is essential for informing policy for the combined mitigation of these greenhouse gases.

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