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High soil temperatures alter the rates of nitrification, denitrification and associated N_2O emissions

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Abstract

Purpose The responses of nitrification and denitrification are not well characterised at temperatures above 35 °C, which is the focus of our study.

Materials and methods Soils collected from two dairy pastures (Victoria, Australia) were incubated at 10 to 45 °C in the dark for 5 to 10 days following amendment with 100 μg N g^{-1} either as NH_4NO_3, $^{14}NH_4{}^{15}NO_3$ or $^{15}NH_4{}^{15}NO_3$ (10 atom% ^{15}N excess) at 50% water-filled pore space. To detect N_2O from heterotrophic nitrification, acetylene (0.01% *v/v*) was used in a subset of samples amended with $^{15}NH_4{}^{15}NO_3$. Atom% ^{15}N enrichments of NO_3^-, N_2O and N_2 were measured during the experiment to evaluate the responses of nitrification and denitrification to temperature.

Results and discussion N_2O production from the two soils increased with rising temperature and peaked between 35 and 40 °C. N_2O production from nitrification and denitrification both had similar thermal responses, which were different to N_2 production. The N_2O/N_2 ratio decreased from > 4 at 35–40 °C to 0.5 at 45 °C, due to greater N_2 than N_2O production in the Dermosol. Heterotrophic nitrifiers oxidised NH_4^+ and released N_2O at 35–40 °C, suggesting a role for heterotrophs in N cycling under warm climates. T_{opt} for nitrification was between 35 and 40 °C, which is higher than reported previously. A short-term effect of high temperatures could provide NH_4^+ for the growth of crops but may also decrease soil C pools.

Conclusions Increasing temperature above 35 °C altered the rates of nitrification, denitrification associated N_2O and N_2 production. Nitrification and denitrification peaked at 35–40 °C in the Chromosol and Dermosol. The production of N_2 increased rapidly above 40 °C, which may be related to high soil respiration rates that likely decreased O_2 availability, thus expanding the anaerobic microsites; such circumstances increased the reduction of N_2O to N_2 production from the Dermosol.

Keywords C pools · Heterotrophs · N_2O reduction · N_2O/N_2 ratios

1 Introduction

Global surface temperatures have increased recently (NASA 2018), which will impact on the nitrogen (N) cycle, including the release of nitrous oxide (N_2O), a powerful greenhouse gas that contributes to the destruction of stratospheric ozone (Crutzen 1983; Pierzynski et al. 2005; Ravishankara et al. 2009). Increased temperatures influence soil organic matter (SOM) availability, soil moisture, the growth of microorganisms and enzymatic reactions, which may alter N losses from soils as N_2O emissions. Therefore, the effects of increased surface land temperature on N_2O emissions from soils in the short-term and longer-term are of importance.

The thermal response of N_2O emissions, which arise primarily from nitrification (Schmidt 1982) and denitrification (Tiedje 1988), has rarely been documented above 35 °C. It is generally recognised that N_2O emissions from soil increase with rising temperature then peaks around 20 to 35 °C (Parton et al. 2001). The composite models of Breuer and Butterbach-Bahl (2005) use the temperature optimums (T_{opt}) to describe

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N_2O emissions from published data and supposes that nitrification and denitrification have their own separate temperature optimums. The difficulties of these models are that T_{opt} for nitrification and denitrification may vary with climatic regions. Nitrification and associated N_2O emissions had optima at 20 °C in temperate soils (Malhi and McGill 1982; Maag and Vinther 1996) but between 30 and 35 °C in soils from warmer regions (Myers 1975; Goodroad and Keeney 1984; Liu et al. 2015) as a result of the acclimation and/or adaptation of nitrifiers (Anderson et al. 1971; Stark 1996; Avrahami et al. 2003). The greatest reduction of NO_3^- by denitrifying populations was found between 40 and 60 °C in the tested soils (Malhi et al. 1990), while total denitrification (N_2O + N_2) was maximised at 35 °C in a Dermosol (Lai and Denton 2018). As denitrifying organisms can be adapted to mean annual temperatures (Powlson et al. 1988), the thermal adaptation of these microorganisms may influence their ability of conversions of NH_4^+ to NO_3^- and NO_3^- to N_2O or N_2 at high temperatures (> 35 °C), which are poorly understood. Thus, the responses of NO_3^- production, and N_2O and N_2 emissions to high temperaturesare required.

The contributions of nitrification and denitrification to N_2O emissions may vary with temperature. Nitrification was the major contributor (~ 70%) to N_2O emissions at 4 °C but ~ 30% of overall N_2O at 35 °C in a silt loam soil (Gödde and Conrad 1999). Using the same soil, Avrahami et al. (2003) showed that nitrification produced 35–50% of total N_2O emissions between 4 and 25 °C and around 10% of total N_2O at 37 °C. These findings, however, are in contrast to that reported by Liu et al. (2016) that nitrification produced 74 to 86% of total N_2O production at 35 °C in an acidic gravelly loam soil with 50 to 70% water-filled pore space (WFPS). In fact, these studies did not measure N_2O produced by heterotrophic nitrification, which could be mistakenly attributed to denitrification. Although heterotrophic nitrification accounted for 20% of total N_2O production at 21 °C (Bateman and Baggs 2005), its thermal response is not documented. Thus, the response of heterotrophic nitrification, as a source of N_2O, to high temperatures, is the focus of this study.

The influence of temperature on the reduction of N_2O to N_2 has been observed previously, but results are contradictory. Rising the temperature between 5 and 25 °C did not impact on denitrification and the N_2O/N_2 ratios (Focht 1974; Rudaz et al. 1999). However, the N_2O/N_2 ratios were reported to decline beyond 15 °C in others (Keeney et al. 1979; Avalakki et al. 1995; Maag and Vinther 1996; Dobbie and Smith 2001). Soil N losses as atmospheric N_2 are difficult to quantify in field studies due to a high natural background of N_2, so direct measurement of $^{15}N_2$ production from soil using labelled $^{15}NO_3^-$ in controlled experiments will improve our knowledge of N_2O reduction to N_2 from soils at high temperatures.

Our objective was therefore to understand the short-term impact of high soil temperatures on the reduction of NH_4^+, NO_3^- and N_2O from nitrification and denitrification. The study was conducted on the two pasture soils, a Chromosol and Dermosol, from south west Victoria, Australia. Soil samples were supplied at 100 μg N g^{-1} (unlabelled N and labelled ^{15}N) after 7 days of pre-incubation and continuously incubated at 10 to 45 °C up to 10 days. Changes in ^{15}N enrichment of NO_3^-, N_2O and N_2 were quantified to assess the thermal responses of nitrification, denitrification, associated N_2O and N_2 emissions.

2 Materials and methods

2.1 Study site and sample collection

The experimental soils (a Chromosol and a Dermosol, according to the Australian soil classification (Isbell 1996)) were collected from the upper 10-cm layer of the soil at two dairy pasture farms in southwest Victoria, Australia. Intact soil samples (4.6 cm × 10 cm) were taken to maintain the soil structure for measurements of the physical characteristics (bulk density, gravimetric moisture content and soil texture) of the two soils.

The two soils differ in soil texture (Table 1) but appear to experience a similar pattern of thermal condition (Fig. 1). Briefly, the surface soil (0–10 cm) temperature recorded from the field sites between April and November was 8–18 °C but ranged from 19 to 46 °C between the December to March summer season (Fig. 1). The two sites are not irrigated and have an average annual rainfall of 745 mm (Chromosol) and 522 mm (Dermosol).

The experimental soils were sieved to remove the > 2-mm fraction and thoroughly mixed to ensure uniformity. The sieved soils were stored at field moisture in sealed containers at 4 °C, 2 weeks before conducting the experiments. Some characteristics of the top 10 cm of the two soils are provided in Table 1.

2.2 Laboratory incubations

2.2.1 Sterilisation

To assess the release of N_2O from chemical reactions, autoclaving was used to eliminate biological activity from the two collected soils. Soil samples (~ 1.5 kg of dry soil) were autoclaved at 121 °C and 0.3 MPa for 20 min.

2.2.2 Pre-incubation

Non-sterilised soils were wetted with distilled water to achieve 40% WFPS then placed into plastic bags with holes to allow gas exchange with the atmosphere. All soil samples were pre-incubated in dark condition at 25 °C (same temperature on sampling period) for 7 days to enhance microbial activity in

Table 1 Some properties of unamended soils used in this study

	Chromosol	Dermosol
General		
Location	38° 14′ S, 142° 55′ E	38° 10′ S, 142° 58′ E
Land use	Rainfed dairy pasture	Rainfed dairy pasture
Soil		
Soil type[a]	Brown Chromosol	Black Dermosol
Texture	Light fine sandy-clay loam	Clay loam
Clay (%)	15.7	37.0
Silt (%)	24.4	21.0
Coarse sand (%)	5.9	9.0
Fine sand (%)	52.8	16.0
Gravel (%)	1.2	17
Bulk density (g/cm^3)	1.02	1.12
Moisture content (− 33 kPa) (g water/g soil)	0.38	0.37
pH_{water}	5.4	6.2
NH_4^+-N (mg N kg^{-1} dw)	16.5	12.8
NO_3^--N (mg N kg^{-1} dw)	6.2	8.5
Organic carbon (%)	4.06	5.24

Soils were sampled from 0 to 10 cm depth

[a] Australian Soil Classification (Isbell 1996)

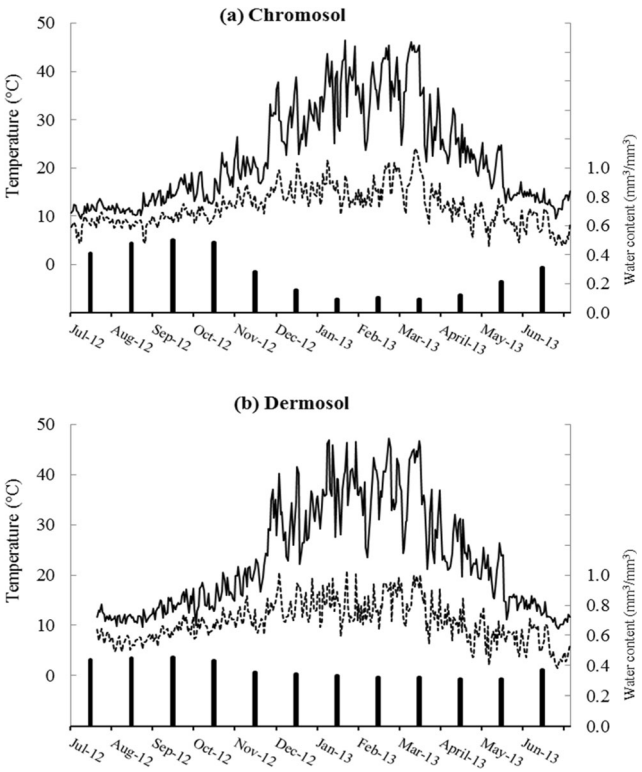

Fig. 1 The variation in temperature and water content at study sites from June 2012 until July 2013 for the **a** Chromosol and **b** Dermosol used in these experiments. Lines indicate daily soil maximum (solid line) and minimum temperature (dash line); bars present the average monthly volumetric water content

the experiments (Davidson 1991). Soil moisture was checked every day and water added, if required, to maintain 40% WFPS.

2.2.3 Incubation experiments

To study the response of overall N_2O emissions from soils to temperature, the first incubation experiment was conducted using both the Chromosol and the Dermosol. Subsamples of non-sterilised and sterilised soils (50 g dry weights equivalent) were packed into PVC cores (ø37 mm × 42 mm deep, 10.75 cm^2 surface area) to achieve the original bulk density measured at field sites (Table 1). A solution of NH_4NO_3 (2 ml of 0.09 M) was added slowly onto the top of upright cores to provide 100 μg N g^{-1} soil. This solution increased soil moisture to 50% WFPS, which reflects the average soil moisture in the field during summer (Fig. 1). All soil cores were placed into 250-ml jars equipped with a rubber septum (Subaseal#25, Sigma-Aldrich, St. Louis, MO, USA). The jars were placed into seven different incubators at constant temperatures of either 10, 20, 25, 30, 35, 40 or 45 °C in the dark for 5 days, using four replicate samples.

A second experiment was established to quantify N_2O production from nitrification and denitrification between 35 and 45 °C, the temperatures at which the greatest rates of these processes were predicted to occur (based on results of experiment 1), using the Dermosol. The Dermosol was selected in this experiment because N_2O emissions were more responsive to temperature than those the Chromosol. Also, soil pH of the

Dermosol (6.2) reduces the potential of N_2O produced by nitrifier-denitrification (Wrage et al. 2001), which was not a focus of this study. After the pre-incubation as described in the first experiment, a ^{15}N-labelled technique (Bateman and Baggs 2005) was applied to determine the rates of nitrification, N_2O produced by nitrification and denitrification. Briefly, 2 ml of nitrate solutions (0.09 M), either as $^{14}NH_4{}^{15}NO_3$ or $^{15}NH_4{}^{15}NO_3$ containing 10 atom% ^{15}N excess, was added to the soil cores to provide 100 μg N g^{-1} soil (equivalent to 50 kg N ha^{-1}). The addition of these N solutions increased soil moisture to 50% WFPS (~0.31 g water cm^{-3}). To detect N_2O produced by heterotrophic nitrification, acetylene was used in a subset of samples with $^{15}NH_4{}^{15}NO_3$ added. An additional sample of each treatment was injected with 10 Pa of acetylene to inhibit autotrophic nitrification (Yoshinari et al. 1977; Klemedtsson et al. 1988). Each soil core was placed into a 250-ml jar with a gas-tight lid equipped with a rubber septum (Suba-seal#25, Sigma-Aldrich, St Louis, MO, USA) and the jars were incubated at 35, 40 or 45 °C in the dark for 10 days, using four replicates.

2.3 Sampling and analysis

A 20-ml gas sample was taken from the headspace (228 ml) of each jar using a Hamilton 50-ml gas-tight syringe (Hamilton Company, Reno, VN, USA) at 0, 3 and 5 days after incubation (experiment 1) and at 0, 3, 7 and 10 days after incubation (experiment 2). The sample of gas was transferred into a pre-evacuated 12-ml Exetainer (Labco, Ceredigion, UK) for measurement of N_2O and CO_2 concentrations (experiment 1) and N_2O and CO_2 concentrations and ^{15}N enrichment of N_2O and N_2 concentrations (experiment 2). After each gas sample collection, some soil cores were removed for the purpose of mineral N analysis. Remaining jars were aerated by removing the lids and the headspace was refreshed using a mini-fan for 5 min. Jars were continuously incubated at the corresponding temperatures.

Soil samples for N analyses were taken from the removed cores and homogenised. Approximately 10 g of moist soil was mixed with 50 ml of KCl (2 M) then placed in a rotation shaker for 1 h. The analyses of mineral N were performed colorimetrically with an Auto-Analyser (AA3 HR, SEAL, West Midlands, UK) using the hydrazine reduction method for NO_3^- (Kamphake et al. 1967) and the nitroprusside method for NH_4^+ (Kaplan 1965). To quantify ^{15}N enrichment of the NO_3^- pools, nitrate ($^{14}NO_3^-$ + $^{15}NO_3^-$) in the extracted solution was trapped in acidic filter discs using the diffusion method (Brooks et al. 1989) and analysed on an IRMS (Hydra 20-20, Thermo Scientific, Bremen, Germany).

2.4 Gas measurements

The N_2O concentration of headspace gas samples was analysed on a Varian 450 gas chromatograph (Agilent 7890, Bremen, Germany) equipped with a ^{63}Ni electron capture detector (ECD) at 360 °C, using Pureshield Argon as the carrier gas (35 ml min^{-1}). Separation was carried out on a 1-m column (55 °C) packed with HayeSep Q 60–80 mesh (Haye Separations, Bandera, TX). Trace gas isotope ratios ($\delta^{15}/\delta^{14}N$) were determined using an isotope ratio mass spectrometer—IRMS (Hydra 20-20, Thermo Scientific, Bremen, Germany). The concentration of carbon dioxide (CO_2) in headspace gas samples was measured using a gas chromatograph (Hewlett Packard 5890, Bremen, Germany) fitted with a thermal conductivity detector (TCD) at 60 °C. The separation was performed on a 1.5-m column (80 °C) packed with Porapak Q, 50–80 mesh using grade A helium (BOC, Australia) as the carrier gas (40 ml min^{-1}).

2.5 Calculation of nitrogen transformation rates

The rate of N_2O production from soils was calculated according to Eq. (1):

$$N_2O = PV/RT \times \Delta C/\Delta t \times m/w \quad (1)$$

where N_2O is the rate of N_2O production (μg N-N_2O g^{-1} soil day^{-1}), P is the pressure of jar on sampling date (atm), V is the volume of the headspace of jar (L), R is the universal gas constant (0.082 L atm mol^{-1} K^{-1}), T is the temperature on the sampling date (K), $\Delta C/\Delta t$ is the change of N_2O concentration (μL L^{-1}) per unit of time (day^{-1}), m is the molecular weight of N_2O (44 g mol^{-1}), and w is the dry weight of soil (g).

The $^{15}N_2O$ was calculated according to Eq. (2) (Stevens et al. 1993).

$$\text{atom}\%{}^{15}N_2O = \left({}^{45}R + 2{}^{46}R\right)/2\left(1 + {}^{45}R + {}^{46}R\right) \times 100 \quad (2)$$

where atom $\%{}^{15}N_2O$ is the ^{15}N content of the N_2O in samples, ^{45}R is isotope ratio of $^{45}N_2O/^{44}N_2O$ and ^{46}R is isotope ratio of $^{46}N_2O/^{44}N_2O$.

The production of $^{15}N_2O$ was calculated using atom% excess ^{15}N and total N_2O as Eq. (3):

$$^{15}N_2O = \text{atom}\%\ {}^{15}N \text{ excess} \times N_2O \quad (3)$$

where $^{15}N_2O$ is the rate of $^{15}N_2O$ production (μg ^{15}N-N_2O g^{-1} soil day^{-1}), atom% $^{15}N_2O$ excess is the ^{15}N enrichment in samples after subtracting the natural abundance of ^{15}N in unlabelled control sample (~0.37%) from the % atom ^{15}N of the sample (%) and N_2O is the net rate of N_2O production from soil (μg N-N_2O g^{-1} soil day^{-1}).

Production of $^{15}N_2O$ from nitrification and denitrification was calculated following the approach of Bateman and Baggs (2005). Briefly, $^{15}N_2O$ derived from the $^{15}NH_4{}^{15}NO_3$ amendment is attributed to denitrification and nitrification. The

production of $^{15}N_2O$ from the $^{14}NH_4{}^{15}NO_3$ amendment is attributed to denitrification only. The difference in $^{15}N_2O$ production between $^{15}NH_4{}^{15}NO_3$ and $^{14}NH_4{}^{15}NO_3$ is attributed to nitrification. Critically, atom% $^{15}NO_3^-$ pools differed between $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$ over experimental periods, resulting in different $^{15}N_2O$ produced from denitrification between two the amendments. This potentially led to overestimation of the production of $^{15}N_2O$ from nitrification as presented in Eq. (4).

$$^{15}N_2O_{control} = A + \Delta \tag{4}$$

where $^{15}N_2O_{control}$ is $^{15}N_2O$ produced by nitrification from soil at room temperature using the approach of Bateman and Baggs (2005). A is the absolute amount of $^{15}N_2O$ production from nitrification; Δ is the error caused by difference in atom% $^{15}NO_3^-$ pools between $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$.

At different incubation temperatures (t), Eq. (4) can be expressed as:

$$^{15}N_2O_t = (A + \Delta) \times r_t \tag{5}$$

where $^{15}N_2O_t$ is the production of $^{15}N_2O$ from nitrification relative to incubation temperature (t) using the approach of Bateman and Baggs (2005) and r_t is the responsive rate of $^{15}N_2O$ from nitrification to incubation temperature (t).

When two temperature treatments are compared in relative to control value, Eq. (5) can be presented as Eq. (6):

$$\begin{aligned}\left(^{15}N_2O_{t1} - {}^{15}N_2O_{t2}\right)/{}^{15}N_2O_{control} &= (A + \Delta)(r_1 - r_2)/(A + \Delta)\\ &= (r_1 - r_2)\end{aligned} \tag{6}$$

where $t_{1,\,2}$ present different incubation temperatures and $r_{1,\,2}$ are the responsive rates of $^{15}N_2O$ from nitrification to t_1 and t_2, respectively.

The difference in $^{15}N_2O$ production between $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3 + C_2H_2$ (0.01% v/v) amendments is attributed to heterotrophic nitrification. The error caused by difference in atom% $^{15}NO_3^-$ pools between these amendments can be eliminated similarly to the calculation of $^{15}N_2O$ from nitrification (Eqs. (4)–(6)).

The calculation of $^{15}N_2$ was based on Stevens and Laughlin (1998). Briefly, molecular species with mass to charge (m/z) ratios 28, 29 and 30 were separated using IRMS. The intensity of the ion beams corresponding to m/z 28, 29 and 30 was directly related to the isotopic composition of the N_2. The ratio differences (δ) between normal and enriched atmospheres enabled the mole fractions of the NO_3^- in the soils that were denitrified ($^{15}X_N$) and the fraction proportional to the amount of ^{15}N-labelled N_2 in the headspace to be calculated using Eq. (7).

$$^{15}X_N = 2\left(\delta^{30}R/\delta^{29}R\right)/\left(1 + 2\delta^{30}R/\delta^{29}R\right) \tag{7}$$

where $\delta^{29}R$ = ($^{29}N_2/^{28}N_2$ in headspace) – ($^{29}N_2/^{28}N_2$ in ambient air) and $\delta^{30}R$ = ($^{30}N_2/^{28}N_2$ in headspace) – ($^{30}N_2/^{28}N_2$ in ambient air).

The amount of ^{15}N-labelled N_2 was then calculated using Eq. (8):

$$^{15}N_2 = \delta^{30}R/\left(^{15}X_N\right)^2 \tag{8}$$

As $^{15}N_2$ production was exclusively derived from $^{15}N_2O$ during denitrification, since the labelled source of N provided was $^{14}NH_4{}^{15}NO_3$, it was assumed that the measured ratio of $^{15}N_2O/^{15}N_2$ was equivalent to the ratio of N_2O/N_2 from denitrification, as indicated in Eq. (9):

$$^{15}N_2O/^{15}N_2 = N_2O/N_{2denitrification} \tag{9}$$

The gross rates of nitrification (n) were calculated using the equations of Kirkham and Bartholomew (1954) and Davidson et al. (1991), as indicated in Eq. (10):

$$n = \frac{M_0 - M_t}{t} \times \frac{\log\left(\frac{H_0 M_t}{H_t M_0}\right)}{\log(M_0/M_t)} \tag{10}$$

where n is nitrification rate (μg N g^{-1} soil day^{-1}), M_0 the total NO_3^- (μg N g^{-1} soil) at day 0, M_t the total NO_3^- at day t (μg N g^{-1} soil), H_0 the $^{15}NO_3^-$ (μg N g^{-1} soil) at day 0, H_t the $^{15}NO_3^-$ at day t (μg N g^{-1} soil) and t is time of incubation (days).

The percentage of N_2O as a proportion of nitrified N (P_n) were estimated using Eq. (11):

$$P_n = (N_2O_n/n) \times 100 \tag{11}$$

2.6 Soil pH

To measure pH, 5.0 g of dry soil sample was mixed with 25 ml of distilled water and shaken for 1 h. After allowing sediment to settle for 30 min, the pH of the supernatant was measured using an electrode. The measurements of pH were done on four replicates at the beginning and the end of incubation in each treatment to assess whether incubation temperature impacted on soil pH.

2.7 Statistical analysis

The pooled ANOVA for measurement over time (Gomez and Gomez 1984) was applied to determine the rate of change over time of N_2O production and mineral N concentrations. One-way ANOVA was used to determine the significant difference among temperature treatments at the end of experiment. The Tukey ($HSD_{0.05}$) test was applied post hoc to identify specific differences between treatments and to determine whether N_2O and N_2 production varied significantly with temperature. Statistical tests on ratios such as N_2O/N_2 were based on log-

transformed data. All statistical analyses were performed using Statistix 10.0 (Tallahassee, USA).

3 Results

3.1 The response of overall N_2O emissions in soils to temperature

The production of N_2O was not detected in the sterilised soils during the experiment (Fig. 2). In the presence of soil microorganisms, the production of N_2O was dependent on incubation temperatures. The N_2O production from biological processes was low at 10 to 25 °C in the two tested soils and below 0.04 μg N_2O-N g^{-1} soil over the 5 days of incubation. The production of N_2O in the two soils increased above 25 °C, particularly in the Dermosol, where the greatest increase in N_2O production was found between 25 and 30 °C. In the Chromosol, the production of N_2O increased rapidly between 35 and 40 °C. The amounts of N_2O production in both tested soils peaked between 35 and 40 °C. At 45 °C, total N_2O production declined in the two soils (Fig. 2). Generally, N_2O production was more responsive to temperature in the Dermosol than in the Chromosol (Fig. 2).

3.2 Production of $^{15}N_2O$ from nitrification and denitrification

The rates of $^{15}N_2O$ production from nitrification and denitrification ($^{15}N_2O_{ND}$) differed with temperatures between 35 and 45 °C (Fig. 3). Over the experimental period, the production of $^{15}N_2O_{ND}$ was much greater at 35 to 40 °C than at 45 °C.

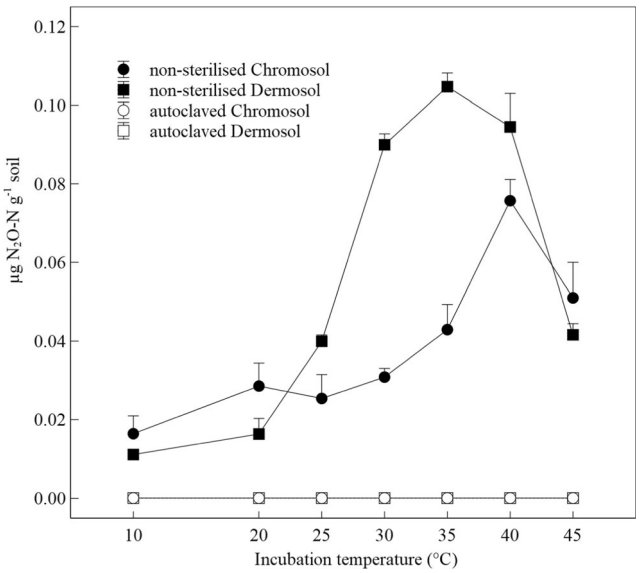

Fig. 2 The production of N_2O from soils incubated at different temperatures over 5 days following amendments of N fertiliser as NH_4NO_3 at 100 μg N g^{-1} soil. *Error bars* are + 1 SE of four replicates

Denitrification was a significant contributor to $^{15}N_2O$ emissions at 35 and 40 °C, accounting for 50–60% total $^{15}N_2O$ emissions from tested soil (Fig. 3). The highest rate of $^{15}N_2O$ production from denitrification was found at 35 °C, followed by 40 °C, and the lowest rate was measured at 45 °C at all sampling times ($P < 0.05$, Fig. 3). The rate of $^{15}N_2O$ production from nitrification was 0.9 to 1.3 ng N g^{-1} day^{-1} between 35 and 40 °C in the first 7 days of incubation, with no significant difference between these temperatures ($P > 0.05$, Fig. 3a). From days 7 to 10, the rates of $^{15}N_2O$ production from nitrification declined slightly at 35 and 40 °C (Fig. 3cc). At 45 °C, the production of $^{15}N_2O$ from nitrification was relatively low (< 0.6 ng N g^{-1} day^{-1}) during the experimental periods (Fig. 3a, b).

The differences in $^{15}N_2O$ production between $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$ + C_2H_2 (0.01% *v*/*v*) indicate that heterotrophic microorganisms nitrified $^{15}NH_4{}^+$ and released $^{15}N_2O$ emissions, since C_2H_2 inhibits autotrophic nitrifiers under these conditions (Fig. 4). Cumulative $^{15}N_2O$ production from heterotrophic nitrification, after subtracting $^{15}N_2O$ from the background ($^{14}NH_4{}^{14}NO_3$), was similar at 40 and 35 °C ($P < 0.05$, Fig. 4). At 45 °C, there was a strong decrease in $^{15}N_2O$ production from heterotrophic nitrification.

3.3 The production of $^{15}N_2$ and N_2O/N_2 ratios

The rates of $^{15}N_2$ production from denitrification were much lower than $^{15}N_2O$ production at 35 and 40 °C over 10 days (Fig. 5). The N_2O/N_2 ratios from denitrification (equivalent to $^{15}N_2O/^{15}N_2$ in the $^{14}NH_4{}^{15}NO_3$ treatment) were 6.2 and 4.2 at 35 and 40 °C, respectively. The rate of $^{15}N_2$ production increased at 45 °C, where cumulative $^{15}N_2$ production was twofold the amount of $^{15}N_2O$ production, resulting in a lower N_2O/N_2 ratio of 0.5 compared to those at 35 and 40 °C (Fig. 5).

3.4 Soil mineral N and enrichment of the $^{15}NO_3^-$

The concentration of NH_4^+ at 35 °C decreased slowly in the first 7 days then declined faster from days 7 to 10 (Fig. 6aa). At 40 °C, the NH_4^+ pool decreased slightly in the first 3 days but increased in subsequent measurements, reaching to 82 to 86 μg N g^{-1} on days 7 and 10. In particular, on days 7 and 10, NH_4^+ concentration was much higher at 45 °C than those at lower temperatures throughout the experiment. The accumulation of nitrogen to the NH_4^+ pool at 45 °C was 300 μg N g^{-1} after 10 days (Fig. 6). NO_3^- pools at 35 and 40 °C increased constantly over the experimental period, reaching 75 and 72 μg N g^{-1} after 10 days of incubation, respectively (Fig. 6bb). At 45 °C, NO_3^- concentration remained stable during the experiment (Fig. 6bb).

The enrichment of the $^{15}NO_3^-$ measured in soil following addition of $^{14}NH_4{}^{15}NO_3$ decreased marginally over the

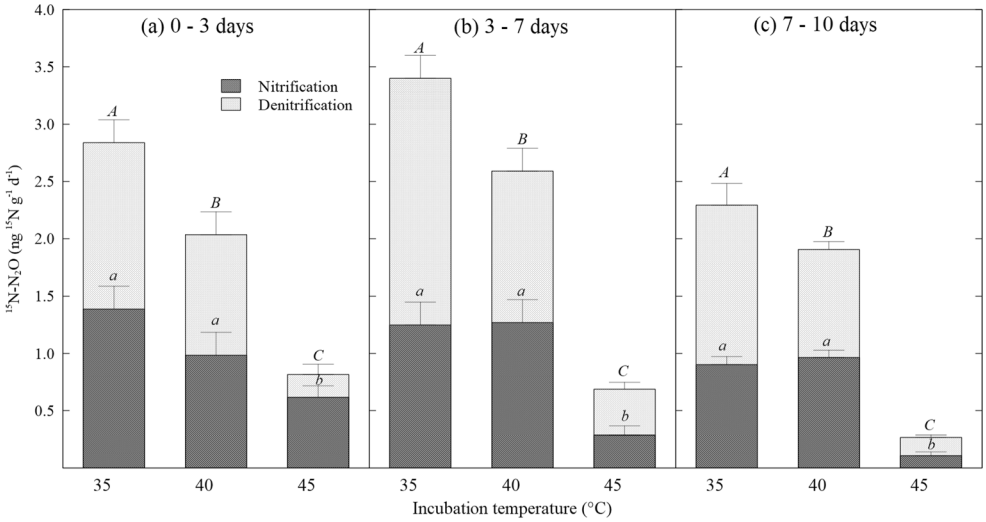

Fig. 3 The response of $^{15}N_2O$ from nitrification and denitrification to high temperatures in the Dermosol following amendments of N fertiliser at 100 μg N g^{-1} soil over experimental periods of **a** 0–3 days, **b** 3–7 days and **c** 7–10 days. Soil samples were maintained at 50% WFPS. Different letters indicate significant differences in the sources of $^{15}N_2O$ among temperatures (lower letters for nitrification; upper letters for denitrification) within each incubation period ($P < 0.05$). *Error bars* are + 1 SE of four replicates

experimental period at all temperatures (Fig. 6c), indicating the addition of $^{14}NO_3^-$ to total NO_3^- pools from nitrification. Atom% $^{15}NO_3^-$ decreased faster at 35 and 40 °C than at 45 °C (Fig. 6c). At 35 and 40 °C, $^{15}NO_3^-$ was 6.5 atom% on day 3 and 3.5 atom% on day 10. At 45 °C, $^{15}NO_3^-$ was 8.1 atom% on day 3 then decreased to 6.0 atom% from day 7 to 10 (Fig. 6c).

3.5 The rates of nitrification and soil respiration

Gross nitrification rates (NO_3^- produced from the oxidation of NH_4^+) were significantly greater at 35 and 40 °C than at 45 °C at all measurement times ($P < 0.05$, Table 2). Gross nitrification rates did not differ between 35 and 40 °C (Table 2). Rising the temperature to 45 °C decreased rapidly the gross

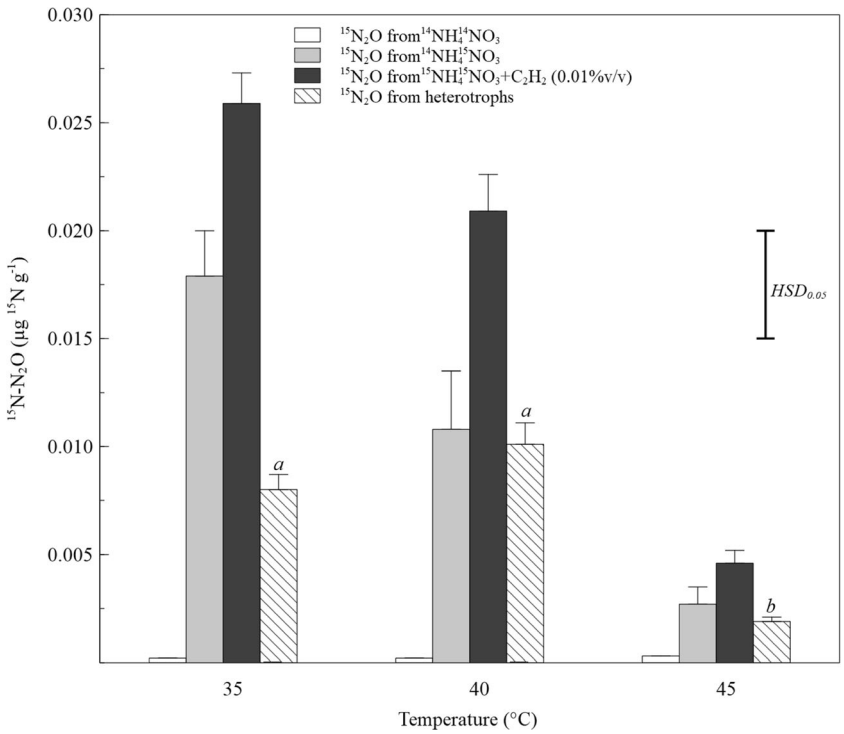

Fig. 4 Production of ^{15}N-N_2O in the Dermosol added with different forms of N solutions and C_2H_2 over 10 days of incubation at high temperatures. The differences in ^{15}N-N_2O between $^{14}NH_4^{15}NO_3$ and $^{15}NH_4^{15}NO_3$ with C_2H_2 present ^{15}N-N_2O from heterotrophic nitrification. Different letters indicate significant differences in $^{15}N_2O$ produced by heterotrophs among temperatures. *Error bars* are + 1 SE of four replicates

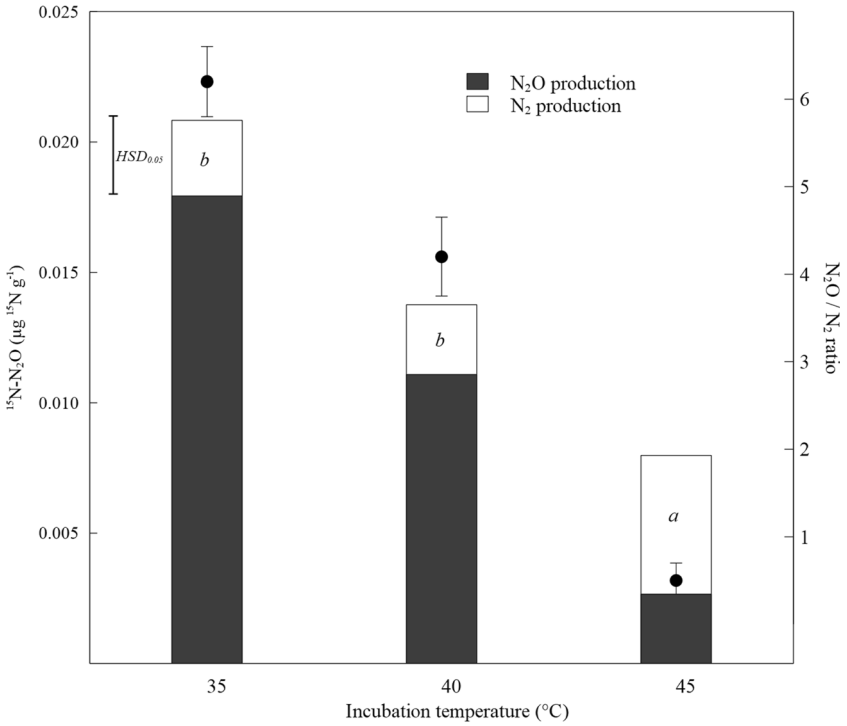

Fig. 5 The responses of $^{15}N_2O$ production and reduction (or $^{15}N_2$ production) during denitrification to high temperatures in the Dermosol, following amendments of N fertiliser at 100 μg N g^{-1} soil as $^{14}NH_4{}^{15}NO_3$ at 10 atom% excess ^{15}N (without C_2H_2). Soil samples were maintained at 50% WFPS. Different letters indicate significant differences in $^{15}N_2$ production among temperatures ($P < 0.05$). Black circles indicate the ratios of N_2O to N_2. *Error bars* are ±SE of four replicates

nitrification rate, which ranged from 2.1 to 3.4 μg N g^{-1} day^{-1} (Table 2).

The proportion of N_2O to NO_3^- (N_2O/NO_3^-) from nitrification varied from 0.3 to 0.5% with temperatures during the experiment (Table 2). The N_2O/NO_3^- ratios did not differ across sampling times (Table 2).

Soil respiration measured by carbon dioxide (CO_2) production increased with rising temperature in the tested soil (Table 2). The rate of CO_2 production in the Dermosol was much greater at 45 °C than at 35 and 40 °C over the experiment (Table 2).

3.6 Soil pH

At the beginning of the experiment, the soil pH_{water} of the Dermosol was 6.2 (± 0.006). At day 10, the pH was 6.0 (± 0.002) in the treatment at 45 °C. The pH decreased in soil incubated at 35 or 40 °C. The largest decrease in the pH was observed in the treatments at 35 °C, which had a mean value of 5.8 (± 0.004) at the end of the experiment.

4 Discussion

The production of N_2O increased with rising temperature and peaked between 35 and 40 °C from the tested soils. The formation of N_2O through abiotic reactions was not detected in the present study, although insignificant N_2O from these processes was measured previously in unamended soils (Bremner 1997; Jones et al. 2013; Heil et al. 2015). As N_2O produced by nitrifier-denitrification was reported to be negligible in soils with pH > 5 and OC > 3% (Wrage et al. 2001), N_2O emissions from the tested soils were attributed to nitrification and denitrification. The N_2O production was more responsive to rising temperature in the Dermosol than the Chromosol, which may be related to higher %clay and soil pH in the Dermosol (Table 1), thus favouring denitrification. The result of ^{15}N experiment identified that the temperature optimum (T_{opt}) for nitrification is the same as that for denitrification, around 35 and 40 °C. This finding differs to the composite function by Breuer and Butterbach-Bahl (2005) that supposes unique T_{opt} for each process. Although the composite function fitted to published N_2O production at 2 to 30 °C, this function does not allow a decrease in N_2O production at higher temperatures. Our finding suggests a significant decrease in the N_2O production from nitrification and denitrification above 40 °C. The same response function can be used to describe the production of N_2O from the two processes.

Greater production of N_2 than N_2O above 40 °C resulted in lower N_2O/N_2 ratios in the Dermosol. The thermal response of N_2O/N_2 ratios is not consistent in the literature. Increasing soil temperature from 5 to 25 °C decreased the N_2O/N_2 ratios in some studies (Keeney et al. 1979; Avalakki et al. 1995; Maag and Vinther 1996; Dobbie and Smith 2001) but had no impact in others (Focht 1974; Rudaz et al. 1999). The production of N_2 using C_2H_2 method (10 kPa) in the above studies was likely underestimated because the method often failed to completely inhibit the reduction of N_2O due to the un-even

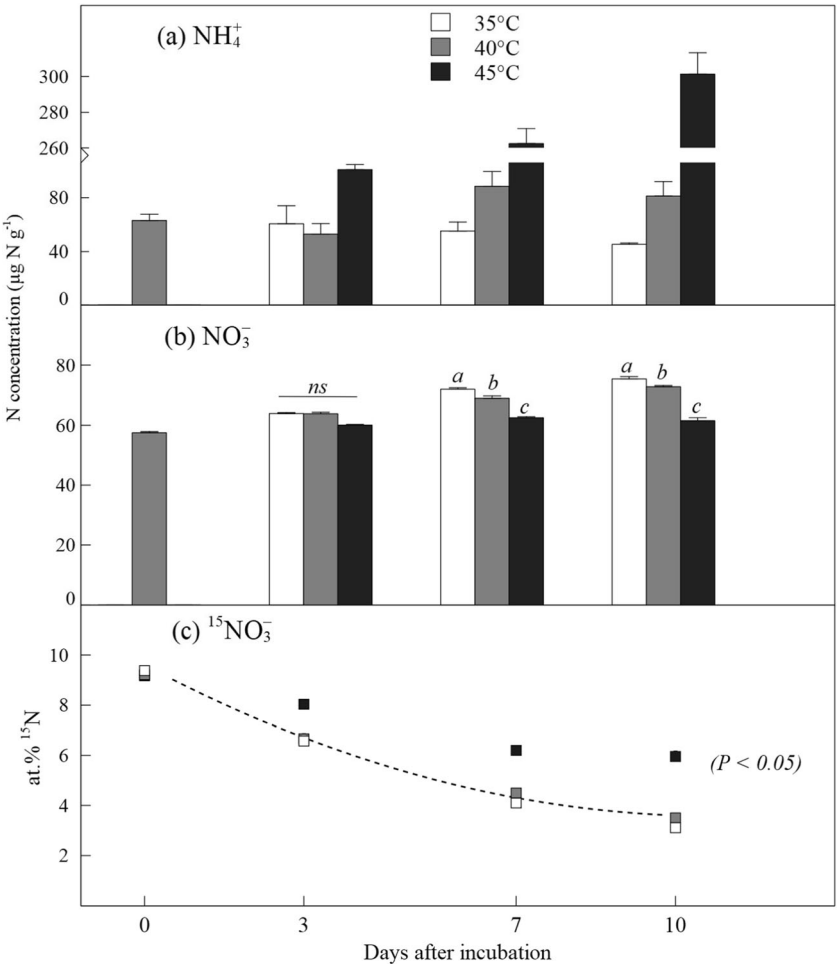

Fig. 6 The concentrations of ammonium (**a**), nitrate (**b**) and ^{15}N enrichment of the NO_3^- (**c**) at different temperatures in the Dermosol, following N fertilisation with 100 μg N g^{-1} as $^{14}NH_4{}^{15}NO_3$ (10 atom% excess ^{15}N). As the measured NH_4^+ and NO_3^- did not differ by temperatures at day 0, one bar is presented. Soil samples were maintained at 50% WFPS during the incubation time. *Error bars* are + 1 SE of four replicates

distribution of C_2H_2 to the whole soil (Mosier 1980; Knowles 1990; Malone et al. 1998; Groffman et al. 2006; Yu et al. 2010a). Using a ^{15}N approach in our study, $^{15}N_2$ production was found to increase up to 45 °C, while $^{15}N_2O$ production decreased beyond 40 °C, resulting in low the N_2O/N_2 ratios (~ 0.5). In agreement with the pattern of N_2O/N_2 ratio in response to high temperatures (Lai and Denton 2018), this result indicates a decoupling of N_2 and N_2O production to high temperatures. T_{opt} for N_2 production was higher than T_{opt} for N_2O production during denitrification. Decreased N_2O production at ≥ 40 °C may reflect a reduction of expression of nitrite reductase (*nir*K) and nitric oxide reductase (*nor*B). Schmidt (2009) reported the reduction of *nir*K and *nor*B expression with increasing NH_4^+ concentrations from 0.2 to 10 mM (equivalent to 140 μg N g^{-1}), which was also observed at 45 °C in our study (Fig. 6a). The high cumulative NH_4^+ at 45 °C appeared to become a limiting factor of N_2O produced by denitrification. Another possibility is that decreased O_2 at 45 °C coupled with low dissolved O_2 in soil water may expand the volume of anaerobic conditions, increasing the reduction of N_2O to N_2. Greater N_2 production above 35 °C suggests significant N loss as N_2 emissions at high temperatures from many Australian soils, which is seldom measured as it is difficult to quantify N_2 in the presence of a high natural background.

The gross rate of nitrification (NO_3^- production) was greatest between 35 and 40 °C, which is higher than published reports. Nitrification rates were previously estimated by the differences in NO_3^- concentrations between treatments with and without 10 Pa C_2H_2 (Martikainen et al. 1993; Maag and Vinther 1996; Garrido et al. 2002). As the C_2H_2 method does not inhibit the activity of heterotrophic nitrifiers (Yoshinari et al. 1977; Klemedtsson et al. 1988), the results of these studies neglected the contributions of NO_3^- from heterotrophs to total nitrification. Using the changes in $^{15}NO_3^-$ and total NO_3^-, the gross rate of nitrification was found to peak around 35 to 40 °C. Evidence from the literature supports the premise that nitrification potentials at these temperatures are likely due to the activity of ammonia oxidising archaea (AOA) rather than ammonia oxidising bacteria (AOB). T_{opt} for AOA have been reported between 35 and 40 °C but just around 25–31 °C for AOB (Ouyang et al. 2017; Taylor et al. 2017; Duan et al. 2018). Importantly, the climatic region is believed to determine T_{opt} for nitrification (Haynes 1986; Stark 1996) as a

Table 2 Gross nitrification rate (n: μgN g^{-1} day^{-1}) and the proportion of N_2O/NO_3^- (P_n: %) from nitrification and carbon dioxide production (CO_2: μg C g^{-1} day^{-1}) at different incubation temperatures after application of NH_4NO_3 to the Dermosol

Period		Temperature (°C)			$HSD_{0.05}$
		35	40	45	
Days 0–3	n (μg N g^{-1} day^{-1})	7.07 ± 0.6^a	6.81 ± 0.6^a	2.83 ± 0.2^b	2.69
	P_n (%)	0.41	0.41	0.50	
	CO_2 (μg C g^{-1} day^{-1})	63.4 ± 4.5^b	82.1 ± 2.9^b	125.2 ± 5.9^a	18.2
Days 3–7	n (μg N g^{-1} day^{-1})	8.02 ± 0.7^a	6.45 ± 0.5^a	3.41 ± 0.7^b	2.61
	P_n (%)	0.52	0.33	0.39	
	CO_2 (μg C g^{-1} day^{-1})	49.8 ± 3.8^b	59.6 ± 6.0^b	130.8 ± 8.2^a	13.6
Days 7–10	n (μg N g^{-1} day^{-1})	6.59 ± 0.5^a	5.96 ± 0.9^a	2.12 ± 0.2^b	0.88
	P_n (%)	0.37	0.51	0.30	
	CO_2 (μg C g^{-1} day^{-1})	43.7 ± 3.0^b	62.6 ± 3.8^b	132.5 ± 5.9^a	19.8

Different letters indicate significant differences in nitrification rates among temperatures at 5%

result of physiological adaptation of AOA and AOB to local temperature regimes (Stark 1996; Stark and Firestone 1996; Avrahami et al. 2003). This explains why T_{opt} for nitrification vary across climates, at 20 °C for temperate soils (Malhi and McGill 1982; Maag and Vinther 1996) but 30–35 °C in warmer soils (Sabey et al. 1959; Myers 1975; Liu et al. 2015). Decreased $^{15}NO_3^-$ at 35 or 40 °C (Fig. 6c) during the experiment indicates the functioning of ammonium oxidizers to transform NH_4^+ to NO_3^- at these temperatures.

Heterotrophic nitrification, which produced $^{15}N_2O$ by oxidising $^{15}NH_4^+$ supply, had the same T_{opt} as denitrification and nitrification. Inorganic N has been assumed to be less important than organic N compounds as a substrate for heterotrophic nitrifiers (Schimel et al. 1984; Islam et al. 2007). However, the substrate selection of heterotrophic nitrifiers is likely to be dependent on soil mineral N availability. In the soils with high inorganic N contents, the use of organic N by heterotrophs was inhibited (Bateman and Baggs 2005; Liu et al. 2015). Thus, the addition of 100 μg N g^{-1} in the present study may have allowed heterotrophs to oxidise the NH_4^+ supply in preference to organic N and released $^{15}N_2O$, particularly at 35 and 40 °C. The production of $^{15}N_2O$ from heterotrophic nitrification decreased at 45 °C, indicating the temperature sensitivity of N_2O produced by heterotrophic nitrification under warm climates, which has not been previously reported.

A rapid decrease in nitrification above 40 °C contributed partly to the cumulative NH_4^+ concentration in the tested soil. A positive correlation between NH_4^+ concentration and CO_2 production ($r = 0.86$, $P < 0.01$) in the present study provides the evidence for high rate of biologically mediated N mineralisation at 45 °C, which is consistent with the temperature sensitivity of NH_4^+ accumulation in previous studies (Sierra and Marban 2000; Zaman and Chang 2004; Lai and Denton 2018). The results suggest a short-term impact of high temperatures (> 40 °C) that could provide benefits for agricultural systems such as releasing NH_4^+ from SOM for the subsequent growth of crops. When soil N existing as NH_4^+ can be highly conserved in dry summer conditions (Bronson et al. 1999), this N budget will be available for the following winter crops.

High soil respiration rates above 40 °C can add more CO_2 emissions to the atmosphere and potentially decrease soil organic carbon (SOC). Our results support the acceleration of the losses of SOC with increasing temperature identified in previous studies (Kirschbaum 1993, 1995; Schlesinger and Andrews 2000; Conant et al. 2011). Although the losses of SOC could depend on net primary production, which supplies the inputs of C to soil, very high temperatures could decrease SOC rapidly, as evidenced by a large amount of CO_2 production at 45 °C in our study (Table 2), providing a positive feedback in the C cycle due to global warming.

The proportions of N_2O derived by nitrification to NO_3^- production (N_2O/NO_3^-) were less than 0.5% and unresponsive to temperature. A similar thermal response of NO_3^- and N_2O production during nitrification explained a small variation in the N_2O/NO_3^- ratio. The yields of N_2O from nitrification are consistent with the published values in the literature, indicating that temperature alone has a little impact on the N_2O/NO_3^- ratio (Table 3). It appears to be more related to soil moisture, the availability of O_2 and NH_4^+ in soils (Table 3). Reduced O_2 availability in soil microsites is considered to have the greatest impact on the N_2O/NO_3^- ratio. Data presented in Table 3 indicate that the N_2O/NO_3^- ratio is greater than 1.0% when O_2 is < 1.5 kPa as the threshold. Although O_2 availability likely decreased with increasing temperatures in our experiment, it presumably did not reach this threshold to trigger altering the yields of N_2O. Yu et al. (2010b) found that the yield of N_2O from nitrification may increase with fluctuating aerobic and anaerobic conditions in soils with high NH_4^+ concentrations,

Table 3 The proportion of nitrified N as N_2O at specified temperatures in the literature

Authorship	Soil type[a]	Method[b]	Relevant treatments		N_2O-N/nitrified N
Bremner and Blackmer (1979)	Storden, Harps, Webster, Nicollet	C_2H_2 0.1% *v/v*	Different WC	30 °C	0.04–0.2%
Goodroad and Keeney (1984)	Planosoil	Direct measure	0.2/0.3 m^3/m^3	10–30 °C	0.08–0.15%
Tortoso and Hutchinson (1990)	Agricultural soil	Direct measure	− 0.1 MPa	25 °C	0.02%
Martikainen et al. (1993)	Forest soil	C_2H_2 (2.5 kPa)	22% *w/w*	20 °C	0.03%
Maag and Vinther (1996)	Sandy loam	C_2H_2 (10 Pa)	55/70% FC	5 °C 15–20 °C	0.49/0.55% 0.18/0.33%
Ingwersen et al. (1999)	Acid forest	BaPS	70% *w/w*	5–25 °C	0.01–0.05%
Garrido et al. (2002)	Luvisol Calsisol Rendosol	C_2H_2 (0.1–10 kPa)	− 0.01 MPa	20 °C	0.08% 0.031/0.04% 1.0%
Cheng et al. (2004)	Molisol Alphiol Aridisol	Direct measure	60% WFPS	25 °C	0.06–0.23% 0.08–0.36% 0.42%
Khalil et al. (2004)	Orthic luvisol	^{15}N	20.4 kPa O_2 4.3 kPa O_2 1.5 kPa O_2 0.76 kPa O_2	20 °C	0.16% 0.42% 1.09% 1.48%
Ambus (2005)	Typic Hapludult	^{15}N	Field study		0.004–0.29%
Bateman and Baggs (2005)	Silt loam	$^{15}N + C_2H_2$	35% WFPS 50–60%WFPS	21 °C	0.53% 0.18%
Mathieu et al. (2006)	Gleyic luvisol	^{15}N	75% FC 150% FC	20 °C	0.13% 2.32%
Mørkved et al. (2006)	Molic gleysol	^{15}N	− 10 kPa	5 °C	0.27–1.0%
Mørkved et al. (2007)	Sapric histosol	^{15}N	18.4 *w/w*	20 °C	1.4–7.6%
Carter (2007)	Loamy sand	^{15}N	Field study	10 °C	0.02–0.29%
Galbally et al. (2010)	Brown sodosol	^{15}N	Field study	10–25 °C	0.01–0.05%
Chen et al. (2010)	Brown Vertosol	Direct measure	Inhibitors	5–25 °C	0.03–0.12%
Frame and Casciotti (2010)	*Nitrosomonas marina* Cultures	$^{15}N + {}^{18}O$	0.5–20% O_2	22 °C	0.4–2.2%
Zhu et al. (2013)	Sandy loam to clay loam	$^{15}N + {}^{18}O + C_2H_2$ (0.01% *v/v*)	21 kPa_{O2} 3 kPa_{O2} 0.5 $kPaO_2$	22 °C	0.08–0.11% 0.52–2.9% 6.9–8.3%
Liu et al. (2016)	Chromosol	^{15}N	85% WFPS 50–70% WFPS	25 °C 35 °C	0.61% 0.007–0.66%
Hink et al. (2018)	Sandy loam	C_2H_2/1-octyne	60% WFPS	30 °C	0.075%

C_2H_2 refers to the use of acetylene as nitrification inhibitor. ^{15}N refers to the use of ^{15}N isotopic tracers. BaPS refers to the barometric process separation method

[a] Terminology for soil type is not standardised: usage varies from author to author

[b] Method used for quantifying N_2O by nitrification

suggesting the potential interactive effect between land management (such as different tillage methods) and high temperatures, which regulate NH_4^+ and O_2 availability, on nitrification and the N_2O/NO_3^- ratio. Future studies exploring fluctuating temperatures are required to provide a better understanding of the impact of temperature on the fraction of N_2O from nitrification.

The production of N_2O from denitrification was simulated more than that from nitrification by increasing temperature. This finding supports previous studies (Gödde and Conrad 1999; Avrahami et al. 2003) in which denitrification accounted for major N_2O production at 35–37 °C in loam soils (~ 30% clay). Our results, however, differ to those of Liu et al. (2016), who found the dominant N_2O produced by nitrification at 25 and 35 °C in soil with 50% WFPS. The differences could be due to the differential O_2 availability induced by soil texture and experimental conditions between the two studies. At the same value of WFPS, the Dermosol (37% clay) repacked into PVC cores induced a higher anaerobic volume in the present study than in a Chromosol (19% clay, without repacking) in Liu et al. (2016). Furthermore, as discussed, rising temperature above 35 °C increased soil respiration rates, decreasing further O_2 concentration. This provided favourable conditions for denitrification than nitrification since more

NO_3^- could be initially encouraged to use as a TEA during denitrification (Cho et al. 1997; Strong and Fillery 2002; Gillam et al. 2008). Increased $^{15}N_2O$ production and decreased $^{15}NO_3^-$ (atom%) indicate the function of denitrifying microorganisms, which were influenced by a combination of high temperatures, soil moisture and soil texture. Increased anaerobic conditions in soil microsites also enhanced the reduction of nitrous oxide to dinitrogen ($N_2O \rightarrow N_2$) above 35 °C, since large amounts of N_2O likely acted as the electron acceptor in this reduction.

It is acknowledged that the ^{15}N labelling technique used in our study still has own its limitations in estimating N_2O production from nitrification and denitrification. This technique is not able to quantify N_2O produced by nitrifier-denitrification (Wrage et al. 2005). This limitation was resolved by using the Dermosol (pH of 6.0–6.2 and 5.2% OC) in the ^{15}N experiment since nitrifier-denitrification is apparently negligible in soils with pH > 5.0 and OC above 3% (Wrage et al. 2001). Another drawback is that atom% $^{15}NO_3^-$ pools differed between $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$ or between $^{14}NH_4{}^{15}NO_3$ and $^{15}NH_4{}^{15}NO_{3+acetylene}$ amendments, resulting in differential $^{15}N_2O$ produced by denitrification, which potentially lead to overestimation of $^{15}N_2O$ from nitrification. The error is, however, eliminated when two temperature treatments are compared relative to control values, as expressed in Eqs. (4), (5) and (6). Moreover, the gross nitrification rates (NO_3^- production) were estimated using the standard method of Davidson et al. (1991) that considers the changes of atom% $^{15}NO_3^-$ in relation to total NO_3^- pools (Eq. (10)) in the treatment with $^{14}NH_4{}^{15}NO_3$ added. Therefore, gross nitrification rates in the current study were not affected by the limitations discussed above.

5 Conclusions

The production of N_2O from the Chromosol and Dermosol increased with rising temperature and peaked between 35 and 40 °C, indicating high potential N losses as N_2O emissions in soils that experience seasonally high temperatures. The same thermal response function can be used for N_2O produced by nitrification and by denitrification, but N_2 production was best described using a different function, since N_2 production continuously increased above 40 °C, leading to lower N_2O/N_2 ratios (~ 0.5) at 45 °C in the Dermosol. The temperature sensitivity of N_2O by denitrification was greater than that by nitrification, which may relate to high respiration rates that have resulted in anaerobic microsites and encouraged the use of NO_3^- as a terminal electron acceptor during denitrification. Heterotrophic nitrifiers released N_2O by oxidising mineral N sources above 35 °C, which suggests their important role in N cycling. The optimal temperature for nitrification is around 35 and 40 °C, which is higher than previous reports. A rapid decrease in nitrification at 45 °C contributed partly to the high accumulation of NH_4^+ concentration. A short-term effect of high temperatures may have implications for agricultural systems by releasing NH_4^+ from SOM for the growth of crops. The evidence from high rate of soil respiration at 45 °C reflects the potential losses of C from soil and may add greater CO_2 emissions to the atmosphere.

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References

Ambus P (2005) Relationship between gross nitrogen cycling and nitrous oxide emission in grass-clover pasture. Nutr Cycl Agroecosyst 72: 189–199

Anderson OE, Boswell F, Harrison RM (1971) Variations in low temperature adaptability of nitrifiers in acid soils 1. Soil Sci Soc Am J 35: 68–71

Avalakki U, Strong W, Saffigna P (1995) Measurement of gaseous emissions from denitrification of applied $^{15}N_2$. Effects of temperature and added straw. Soil Res 33:89–99

Avrahami S, Liesack W, Conrad R (2003) Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. Environ Microbiol 5:691–705

Bateman E, Baggs E (2005) Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biol Fertil Soils 41:379–388

Bremner JM (1997) Sources of nitrous oxide in soils. Nutr Cycl Agroecosyst 49:7–16

Bremner J, Blackmer A (1979) Effects of acetylene and soil water content on emission of nitrous oxide from soils. Nature 280:380–381

Breuer L, Butterbach-Bahl K (2005) Local temperature optimum of N_2O production rates in tropical rain forest soils of Australia. Soil Res 43: 689–694

Bronson KF, Sparling GP, Fillery IR (1999) Short-term N dynamics following application of 15N-labeled urine to a sandy soil in summer. Soil Biol Biochem 31:1049–1057

Brooks P, Stark JM, McInteer B, Preston T (1989) Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci Soc Am J 53:1707–1711

Carter MS (2007) Contribution of nitrification and denitrification to N_2O emissions from urine patches. Soil Biol Biochem 39:2091–2102

Chen D, Suter HC, Islam A, Edis R (2010) Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea. Soil Biol Biochem 42:660–664

Cheng W, Tsuruta H, Chen G, Yagi K (2004) N_2O and NO production in various Chinese agricultural soils by nitrification. Soil Biol Biochem 36:953–963

Cho C, Burton D, Chang C (1997) Kinetic formulation of oxygen consumption and denitrification processes in soil. Can J Soil Sci 77: 253–260

Conant RT, Ryan MG, Ågren GI, Birge HE, Davidson EA, Eliasson PE, Evans SE, Frey SD, Giardina CP, Hopkins FM, Hyvönen R, Kirschbaum MUF, Lavallee JM, Leifeld J, Parton WJ, Megan Steinweg J, Wallenstein MD, Martin Wetterstedt JÅ, Bradford MA (2011) Temperature and soil organic matter decomposition rates–synthesis of current knowledge and a way forward. Glob Chang Biol 17:3392–3404

Crutzen P (1983) Atmospheric interactions. Homogeneous gas reactions of C, N, and S containing compounds. In: Bolin B, Cook R (eds) The major biogeochemical cycles and their interactions, vol SCOPE 21. Wiley, pp 67–114

Davidson EA (1991) Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers JE, Whitman BW (eds) Microbial production and consumption of greenhouse gases: methane, nitrogen oxides and halomethanes. Am. Soc. Microbiol, Washington, DC, pp 219–235

Davidson E, Hart S, Shanks C, Firestone M (1991) Measuring gross nitrogen mineralization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. J Soil Sci 42:335–349

Dobbie K, Smith K (2001) The effects of temperature, water-filled pore space and land use on N_2O emissions from an imperfectly drained gleysol. Eur J Soil Sci 52:667–673

Duan P, Wu Z, Zhang Q, Fan C, Xiong Z (2018) Thermodynamic responses of ammonia-oxidizing archaea and bacteria explain N2O production from greenhouse vegetable soils. Soil Biol Biochem 120:37–47

Focht D (1974) The effect of temperature, pH, and aeration on the production of nitrous oxide and gaseous nitrogen—a zero-order kinetic model. Soil Sci 118:173–179

Frame CH, Casciotti K (2010) Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. Biogeosciences 7:2695–2709

Galbally IE, Meyer MC, Wang Y-P, Smith CJ, Weeks IA (2010) Nitrous oxide emissions from a legume pasture and the influences of liming and urine addition. Agric Ecosyst Environ 136:262–272

Garrido F, Hénault C, Gaillard H, Perez S, Germon J (2002) N_2O and NO emissions by agricultural soils with low hydraulic potentials. Soil Biol Biochem 34:559–575

Gillam K, Zebarth B, Burton D (2008) Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. Can J Soil Sci 88:133–143

Global Temperature (2018). In: Global Climate Change. NASA. Available via https://climate.nasa.gov/Global Temperature. Accessed 15 April 2018

Gödde M, Conrad R (1999) Immediate and adaptational temperature effects on nitric oxide production and nitrous oxide release from nitrification and denitrification in two soils. Biol Fertil Soils 30:33–40

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research. Wiley, Hoboken

Goodroad L, Keeney D (1984) Nitrous oxide production in aerobic soils under varying pH, temperature and water content. Soil Biol Biochem 16:39–43

Groffman PM, Altabet MA, Böhlke JK, Butterbach-Bahl K, David MB, Firestone MK, Giblin AE, Kana TM, Nielsen LP, Voytek MA (2006) Methods for measuring denitrification: diverse approaches to a difficult problem. Ecol Appl 16:2091–2122

Haynes R (1986) Niitrification. In: Haynes R (ed) Mineral nitrogen in the plant-soil system. Academic, London, pp 127–165

Heil J, Liu S, Vereecken H, Brüggemann N (2015) Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. Soil Biol Biochem 84:107–115

Hink L, Gubry-Rangin C, Nicol GW, Prosser JI (2018) The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. ISME J 12:1084

Ingwersen J, Butterbach-Bahl K, Gasche R, Papen H, Richter O (1999) Barometric process separation: new method for quantifying nitrification, denitrification, and nitrous oxide sources in soils. Soil Sci Soc Am J 63:117–128

Isbell R (1996) The Australian soil classification. In: Australian soil and land survey handbook, vol 4. CSIRO, Melbourne

Islam A, Chen D, White R (2007) Heterotrophic and autotrophic nitrification in two acid pasture soils. Soil Biol Biochem 39:972–975

Jones L, Peters B, Lezama J, Casciotti K, Fendorf S (2013) Emission of the greenhouse gas nitrous oxide resulting from ferrous iron disturbance of denitrification. In: AGU Fall Meeting Abstracts, p 0413

Kamphake L, Hannah S, Cohen J (1967) Automated analysis for nitrate by hydrazine reduction. Water Res 1:205–216

Kaplan A (1965) Standard methods of clinical chemistry. Academic, New York

Keeney D, Marx G, Fillery I (1979) Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. Soil Sci Soc Am J 43:1124–1128

Khalil K, Mary B, Renault P (2004) Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O_2 concentration. Soil Biol Biochem 36:687–699

Kirkham D, Bartholomew W (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci Soc Am J 18:33–34

Kirschbaum MU (1993) A modelling study of the effects of changes in atmospheric CO_2 concentration, temperature and atmospheric nitrogen input on soil organic carbon storage. Tellus Ser B Chem Phys Meteorol 45:321–334

Kirschbaum MU (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol Biochem 27:753–760

Klemedtsson L, Svensson B, Rosswall T (1988) A method of selective inhibition to distinguish between nitrification and denitrification as sources of nitrous oxide in soil. Biol Fertil Soils 6:112–119

Knowles R (1990) Acetylene inhibition technique: development, advantages, and potential problems. In: Denitrification in soil and sediment. Springer, Berlin, pp 151–166

Lai TV, Denton MD (2018) N_2O and N_2 emissions from denitrification respond differently to temperature and nitrogen supply. J Soils Sediments 18:1548–1557

Liu R, Suter H, He J, Hayden H, Chen D (2015) Influence of temperature and moisture on the relative contributions of heterotrophic and autotrophic nitrification to gross nitrification in an acid cropping soil. J Soils Sediments 15:2304–2309

Liu R et al (2016) The effect of temperature and moisture on the source of N_2O and contributions from ammonia oxidizers in an agricultural soil. Biol Fertil Soils 53:141–152

Maag M, Vinther FP (1996) Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. Appl Soil Ecol 4:5–14

Malhi S, McGill W (1982) Nitrification in three Alberta soils: effect of temperature, moisture and substrate concentration. Soil Biol Biochem 14:393–399

Malhi S, McGill W, Nyborg M (1990) Nitrate losses in soils: effect of temperature, moisture and substrate concentration. Soil Biol Biochem 22:733–737

Malone J, Stevens R, Laughlin R (1998) Combining the ^{15}N and acetylene inhibition techniques to examine the effect of acetylene on denitrification. Soil Biol Biochem 30:31–37

Martikainen PJ, Lehtonen M, Lång K, De Boer W, Ferm A (1993) Nitrification and nitrous oxide production potentials in aerobic soil samples from the soil profile of a Finnish coniferous site receiving high ammonium deposition. FEMS Microbiol Ecol 13:113–121

Mathieu O, Hénault C, Lévêque J, Baujard E, Milloux M-J, Andreux F (2006) Quantifying the contribution of nitrification and

denitrification to the nitrous oxide flux using ^{15}N tracers. Environ Pollut 144:933–940

Mørkved PT, Dörsch P, Henriksen TM, Bakken LR (2006) N_2O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. Soil Biol Biochem 38:3411–3420

Mørkved PT, Dörsch P, Bakken LR (2007) The N_2O product ratio of nitrification and its dependence on long-term changes in soil pH. Soil Biol Biochem 39:2048–2057

Mosier A (1980) Acetylene inhibition of ammonium oxidation in soil. Soil Biol Biochem 12:443–444

Myers R (1975) Temperature effects on ammonification and nitrification in a tropical soil. Soil Biol Biochem 7:83–86

Ouyang Y, Norton JM, Stark JM (2017) Ammonium availability and temperature control contributions of ammonia oxidizing bacteria and archaea to nitrification in an agricultural soil. Soil Biol Biochem 113:161–172

Parton W et al (2001) Generalized model for NO_x and N_2O emissions from soils. J Geophys Res-Atmos 106:17403–17419

Pierzynski GM, Vance GF, Sims JT (2005) Soils and environmental quality. CRC Press, Taylor & Francis Group

Powlson D, Saffigna P, Kragt-Cottaar M (1988) Denitrification at suboptimal temperatures in soils from different climatic zones. Soil Biol Biochem 20:719–723

Ravishankara A, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125

Rudaz A, Wälti E, Kyburz G, Lehmann P, Fuhrer J (1999) Temporal variation in N_2O and N_2 fluxes from a permanent pasture in Switzerland in relation to management, soil water content and soil temperature. Agric Ecosyst Environ 73:83–91

Sabey BR, Frederick LR, Bartholomew WV (1959) The formation of nitrate from ammounium in soils. III. Influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. Proc Soil Sci Soc Am 23

Schimel JP, Firestone MK, Killham KS (1984) Identification of heterotrophic nitrification in a Sierran forest soil. Appl Environ Microbiol 48:802–806

Schlesinger WH, Andrews JA (2000) Soil respiration and the global carbon cycle. Biogeochemistry 48:7–20

Schmidt EL (1982) Nitrification in soil. In: Stevenson F (ed) Nitrogen in agricultural soil, vol 22. American Soc. Agronomy, Madison, pp 253–267

Schmidt I (2009) Chemoorganoheterotrophic growth of Nitrosomonas europaea and Nitrosomonas eutropha. Curr Microbiol 59:130–138

Sierra J, Marban L (2000) Nitrogen mineralization pattern of an oxisol of Guadeloupe, French West Indies. Soil Sci Soc Am J 64:2002–2010

Stark JM (1996) Modeling the temperature response of nitrification. Biogeochemistry 35:433–445

Stark JM, Firestone MK (1996) Kinetic characteristics of ammonium-oxidizer communities in a California oak woodland-annual grassland. Soil Biol Biochem 28:1307–1317

Stevens R, Laughlin R (1998) Measurement of nitrous oxide and dinitrogen emissions from agricultural soils. Nutr Cycl Agroecosyst 52:131–139

Stevens R, Laughlin R, Atkins G, Prosser S (1993) Automated determination of nitrogen-15-labeled dinitrogen and nitrous oxide by mass spectrometry. Soil Sci Soc Am J 57:981–988

Strong D, Fillery I (2002) Denitrification response to nitrate concentrations in sandy soils. Soil Biol Biochem 34:945–954

Taylor AE, Giguere AT, Zoebelein CM, Myrold DD, Bottomley PJ (2017) Modeling of soil nitrification responses to temperature reveals thermodynamic differences between ammonia-oxidizing activity of archaea and bacteria. ISME J 11:896–908

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder AJB (ed) Environmental microbiology of anaerobes, vol 717. Wiley, New York, pp 179–244

Tortoso AC, Hutchinson G (1990) Contributions of autotrophic and heterotrophic nitrifiers to soil NO and N_2O emissions. Appl Environ Microbiol 56:1799–1805

Wrage N, Velthof G, Van Beusichem M, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol Biochem 33:1723–1732

Wrage N, Groenigen JW, Oenema O, Baggs E (2005) A novel dual-isotope labelling method for distinguishing between soil sources of N_2O. Rapid Commun Mass Spectrom 19:3298–3306

Yoshinari T, Hynes R, Knowles R (1977) Acetylene inhibition of nitrous oxide reduction and measurement of denitrification and nitrogen fixation in soil. Soil Biol Biochem 9:177–183

Yu K, Seo DC, DeLaune RD (2010a) Incomplete acetylene inhibition of nitrous oxide reduction in potential denitrification assay as revealed by using ^{15}N-nitrate tracer. Commun Soil Sci Plant Anal 41:2201–2210

Yu R, Kampschreur MJ, MCv L, Chandran K (2010b) Mechanisms and specific directionality of autotrophic nitrous oxide and nitric oxide generation during transient anoxia. Environ Sci Technol 44:1313–1319

Zaman M, Chang S (2004) Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. Biol Fertil Soils 39:269–279

Zhu X, Burger M, Doane TA, Horwath WR (2013) Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. Proc Natl Acad Sci 110:6328–6333