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Nitrogen transformation rates and N_2O production pathways in two pasture soils

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Abstract

Purpose This study aimed to further understand the regulation of N_2O-related N transformation processes in pasture soils and to contribute knowledge on N fertilizer management and targeted mitigation strategy development.

Materials and methods ^{15}N tracer technique combined with C_2H_2 method was used to measure gross N mineralization, immobilization, nitrification and denitrification rates, and to distinguish the pathways of N_2O production in two Australian pasture soils. Soils were collected from Glenormiston (GN) and Terang (TR), Victoria, Australia and incubated at soil moisture content of 60% WFPS and at 20 °C.

Results and discussion The tested pasture soils were characterized by high mineralization and immobilization turnover. The average gross N nitrification rate (n_{tot}) was 7.28 mg N kg^{-1} day^{-1} in TR and 5.79 mg N kg^{-1} day^{-1} in GN soils. Heterotrophic nitrification rates (n_{h}), accounting for 50.8% and 41.9% of n_{tot} and 23.4% and 30.1% of N_2O emissions in GN and TR soils, respectively, played roles similar to that of autotrophic nitrification rates in total nitrification and N_2O emission. Denitrification rates reached 0.003–0.004 mg N kg^{-1} day^{-1} under selected incubation conditions but contributed more than 30% of N_2O emissions.

Conclusions Results showed the rapid N transformation rates of mineralization, immobilization, and nitrification of the studied pasture soils. Heterotrophic nitrification can be a valuable NO_3^-–N production transformation process in the studied pasture soils. Except for autotrophic nitrification, the roles of heterotrophic nitrification and denitrification in N_2O emission in the two pasture soils should be considered in mitigation strategy development.

Keywords Acetylene • Autotrophic nitrification • Denitrification • Heterotrophic nitrification • Immobilization • Mineralization

1 Introduction

The use of synthetic N fertilizers has increased in response to the intensification of agricultural systems (Vitousek et al. 1997) but caused highly associated N losses, such as those of ammonia (NH_3) (Bouwmeester et al. 1985), nitric oxide (NO), and nitrous oxide (N_2O) (Smith et al. 1997; Bouwman et al. 2002), from agricultural soils to the atmosphere and to water bodies (nitrate (NO_3^-)) (Di and Cameron 2002). In the last 250 years, N_2O emission partly accelerated global warming and stratospheric ozone depletion (IPCC 2007). Soils are a vital and the largest N_2O source, accounting for an estimated 65% of anthropogenic atmospheric loading of this gas (IPCC 2007).

The cattle and sheep industries substantially use N fertilizer (Lu and Tian 2017). N_2O emission from grazed pasture soils reaches approximately 1600 Gg per year, contributes 28% of the global anthropogenic N_2O emissions (IPCC 1996, 2007), and is thus considered a large contributor to global N_2O emissions (Saggar et al. 2008; Abdalla et al. 2009; Di and Cameron 2016). Pasture soils account for the principal land use of cattle and sheep industries and cover approximately 450 million ha in Australia (AGO 2010). Thorough understanding of N transformations in pasture soils bears importance in improving N fertilizer management and reducing adverse environmental costs. Knowledge on the regulation of N_2O-related N transformation processes is also valuable for developing targeted mitigation strategies.

Internal N transformation in soils involves in all processes that transform N from one chemical form to another and transports N between different N pools (Hart et al. 1994, Denk et al. 2017; Fig. 1). These processes are driven by soil microbial abundance and activity, both of which can be affected by various factors, such as substrate quality and quantity, soil pH, water content, and temperature (Hart et al. 1994, Lan et al. 2013; Lan et al. 2014; Denk et al. 2017). Mineralization of soil organic matter refers to the inorganic N production in soils and is affected separately and/or interactively by a wide range of factors, including N availability, C source, and C/N ratio (Nannipieri and Eldor 2009; Nave et al. 2009; Geisseler et al. 2010). Nitrogen immobilization and mineralization occur concurrently in soils (Fig. 1), and their balance (i.e., net N mineralization) often determines the supply or conservation of N by soils. Most organic N is mineralized to ammonium (NH_4^+) before soil uptake. This pathway is generally known as mineralization and immobilization turnover (MIT) routes (Geisseler et al. 2010). The nitrate ion is widely accepted to be produced by two pathways in soils. One pathway is the oxidation of NH_4^+ to NO_3^- by chemoautotrophic nitrifiers, whereas the other involves heterotrophic nitrification, a process driven by heterotrophic nitrifying bacteria or fungi. Autotrophic nitrification was conventionally assumed to be a two-step process involving ammonia oxidation, which is believed to be catalyzed by ammonia-oxidizing archaea and bacteria, and nitrite oxidation by nitrite-oxidizing bacteria (Morkved et al. 2007; Sahrawat 2008; Hu and He 2017; Fig. 1). However, this long-held assumption of labor division between the two functional groups was challenged by the recent unexpected discovery of complete ammonia oxidizers within the *Nitrospira* genus; single organisms of these ammonia oxidizers (comammox) can convert ammonia to nitrate (Hu and He 2017). In addition to mineralization, heterotrophic nitrification plays an important role in soil N cycling by providing a direct means of producing mineral N from organic N (Zhang et al 2013; Chen et al 2015; Chen et al 2017; Fig. 1). Evidence shown that, heterotrophic nitrification may act as the predominant pathway for producing NO_3^- in soils at low pH and high recalcitrant organic carbon primarily in grassland and forest ecosystems (De Boer and Kowalchuk 2001; Müller et al. 2004; Zhang et al. 2013; Chen et al. 2015; Liu et al. 2015a; Zhu et al. 2015). Denitrification is a pathway where reactive N in terrestrial and aquatic ecosystems is transformed back into inert N_2 gas (Galloway et al. 2004; Fig. 1). Denitrification is normally affected by soil abiotic properties, such as water-filled pore space (WFPS), NO_3^-, and available C. A high soil WFPS reduces O_2 diffusion to the pore space; along with NO_3^- and C addition, this high soil WFPS promotes denitrifying conditions (Loick et al. 2016). C availability not only supports the activity of denitrifiers but also indirectly affects soil microsite anaerobiosis because of increased respiratory demand for O_2 in such condition (Fernandez et al. 2011; Loick et al. 2016). Soil denitrification can increase N_2O and NO concentrations in the atmosphere (Russow et al. 2009; Lan et al. 2014; Loick et al. 2016). Moreover, anammoxes have been recently also demonstrated to contribute to N_2 production (Yang et al. 2012; Long et al. 2013; Shan et al. 2016). Relative strengths of these soil N transformation processes can be measured by estimating N transformation rates. Net N transformation rates are traditionally used as indicators of available plant N. However, this parameter fails to provide insights into individual N cycling processes. Thus, to gain a mechanistic understanding regarding N cycling, scholars must unravel the complexity of interdependent N transformations by exploring individual gross N transformation rates (Hart et al. 1994; Accoe et al. 2004; Booth et al. 2005).

Nitrous oxide is produced in soils through several N biological pathways, including denitrification, autotrophic or heterotrophic nitrification, nitrifier denitrification, and nonbiological processes, such as chemical decomposition of nitrite or hydroxylamine (Baggs 2011; Braker and Conrad 2011; Butterbach-Bahl et al. 2013). Among these processes, autotrophic nitrification and denitrification are the two most important N_2O pathways in the agricultural field (Zhang et al. 2016; Liu et al. 2017). However, increasing evidence showed that heterotrophic nitrification of organic N might play an important role in N_2O emission from soils, especially acidic soils (Zhang et al. 2015). Multiple pathways involved in N_2O production and N_2O consumption occur simultaneously in different microenvironments within the same soil; thus, a significant challenge exists in allocating their relative contributions. To date, several techniques have been developed to measure the contributions of these processes to soil N_2O emissions; examples of these techniques include the NO/N_2O

ratio indicator approach (del Prado et al. 2006), acetylene (C_2H_2) inhibition technique (Garrido et al. 2002), ^{15}N isotope enrichment approach (Inubushi et al. 1996; Zhang et al. 2011; Lan et al. 2013), ^{18}O–^{15}N dual-isotope labeling method (Wrage et al. 2005), and N_2O-site preference method (Wu et al. 2016; Rohe et al. 2017). Among these methods, an effective and convenient C_2H_2 inhibition technique is acceptable for distinguishing among autotrophic nitrification, heterotrophic nitrification, and denitrification (De Boer and Kowalchuk 2001; Islam et al. 2007).

In the present study, ^{15}N tracing technique along with C_2H_2 inhibition method was used to 1) estimate gross N transformation rates, including rates of mineralization, immobilization, autotrophic nitrification, heterotrophic nitrification, and denitrification, in Australian pasture soils and 2) to evaluate the relative contributions of autotrophic nitrification, heterotrophic nitrification, and denitrification to N_2O emission in pasture soils. The hypothesis of this study were:1) Heterotrophic nitrification bears importance and may dominate nitrification in pasture soils with high C availability and low soil pH. 2) Autotrophic nitrification, heterotrophic nitrification, and denitrification play equal important roles in N_2O production from pasture soils.

2 Materials and methods

2.1 Soil sampling

Surface soil samples were collected from two locations, namely, Glenormiston (GN) and (38.18° S, 142.97° E) and Terang (TR) (33.73° S, 84.43° E), in Victoria, Australia. At each site, 10 replicate top-soil (0–10 cm) samples were collected, thoroughly homogenized, and transported on ice to the laboratory. Roots and stones were removed, and fresh soil samples were sieved (<2 mm) prior to analysis and incubation experiments. Table 1 summarizes the physical and chemical properties of the soil samples. Soil moisture contents were determined by oven-drying methods (at 105 °C for 48 h) and soil pH (1:5=soil:water), along with soil texture (sieve and hydrometer procedures), soil organic carbon (SOC) (wet digestion with H_2SO_4–$K_2Cr_2O_7$), cation exchange capacity (CEC; extraction with 1 mol L^{-1} ammonium acetate), and total N (Semimicro–Kjeldahl digestion using Se, $CuSO_4$, and K_2SO_4 as catalysts).

2.2 Experimental set-up

^{15}N tracer incubation experiments were conducted using C_2H_2 to measure gross N transformation rates in pasture soils with emphasis on nitrification. Based on this information, the first step in autotrophic nitrification can be inhibited by C_2H_2 at low pressure (0.01%–0.1%), whereas C_2H_2 at high pressure (1%–10%) blocks reduction of N_2O into N_2 during denitrification (Klemedtsson et al. 1977; Okereke 1984). Therefore, gross N autotrophic and heterotrophic nitrification can be distinguished by ^{15}N tracing techniques combined with low C_2H_2 pressure. Denitrification rate ($N_2O + N_2$) can be measured as the N_2O amount produced in soil treated with high-pressure C_2H_2. Similarly, the contributions of autotrophic and heterotrophic nitrification and denitrification to N_2O emission can be quantified by different C_2H_2 pressures.

Laboratory incubation experiments were conducted in the dark at 20 °C for 10 days. For each soil sample, a series of 500 ml capped vials containing fresh soil (equal to 60 g of 105 °C-dried soil) was prepared. The samples were preincubated at 20 °C for 3 days. Afterward, 2 ml of each treatment was applied to each incubation vessel, and water was added to reach the targeted soil moisture content of 60% water-filled pore space (WFPS). The applied treatments contained 50 mg NH_4^+–N kg^{-1} soil and 50 mg NO_3^-–N kg^{-1} soil, which were added to the soils as follows: 1) $^{15}NH_4NO_3$ (T1), 2) $NH_4{}^{15}NO_3$ (T2), 3) $NH_4{}^{15}NO_3$+0.1% *v*/*v* C_2H_2 (T3), and 4) $NH_4{}^{15}NO_3$+10% *v*/*v* C_2H_2 (T4) (three replicates for each treatment). Both

NH_4^+–^{15}N and NO_3^-–^{15}N were at 10 atom% enrichment. For C_2H_2 treatments, C_2H_2 (0.1% *v*/*v* [T3] or 10% *v*/*v* [T4]) was injected using an air-tight syringe to replace the corresponding volume of headspace air in each vial. The vials were maintained airtight for 8 h for complete incorporation of C_2H_2 into the soil. The soils were aerated by removing the caps every 2 days. Soil moisture contents in the vials were then maintained every 2 days by weighing the vials, and C_2H_2 was replenished by reinjection.

2.3 Gas sampling and analysis

Headspace gas for N_2O analysis was obtained from the 500 ml vials by using gas-tight syringes at 0, 2, 4, 6, and 10 days after N fertilizer application. The three replicate gas samples (20 ml) were collected from the 500 ml vials through gas-tight syringes at 0 and 12 h at each sampling day. A preliminary test performed before the experiments determined that gas accumulated linearly over 12 h between sampling. Prior to gas sample collection, 20 ml compressed zero air was injected into the 500 ml vials to maintain pressure. Then, 20 ml gas samples were collected and transferred into pre-evacuated exetainers (Exetainer®, Labco Ltd., Lampeter, Ceredigion, UK). The samples were analyzed for N_2O concentration by a gas chromatograph (Agilent 7890, electron capture detector as detector).

2.4 Soil sampling and analysis

Measurements of mineral N (NH_4^+–N and NO_3^-–N) concentrations and their respective ^{15}N abundances were carried out on days 0, 2, 4, 6, and 10. Soil (60 g) in the sample vials (three replicates for each treatment) was extracted with 300 ml of 2 M KCl by shaking for 1 h. The extracts were filtered through a quantitative filter paper (Whatman 42) and maintained at −20 °C prior to analysis by segmented flow analyzer (Skalar, SAN^{++}). ^{15}N abundances of NH_4^+–N and NO_3^-–N were determined after microdiffusion as reported by Saghir et al. (1993)

and analyzed using isotope ratio mass spectrometry (Hydra 20–20, SerCon, Crewe, UK).

2.5 Calculations

Net mineralization (m_{net}) was calculated from the differences in NH_4^+ plus NO_3^- concentrations between the samples measured at the start and end of incubation period. The measured net nitrification (n_{net}) was the value calculated from the differences in NO_3^- concentrations. Gross rates of N mineralization (m), NH_4^+–N immobilization (i_a), and nitrification rates (n_{tot}) were calculated through the classical isotopic dilution equation developed by Kirkham and Bartholomew (1954). Given the assumption that autotrophic nitrifiers can be completely inhibited by 0.1% v/v acetylene, gross nitrification rate (n_{tot}) = nitrification rate in T2 = [autotrophic nitrification (n_a)+ heterotrophic nitrification (n_h)]; n_h=n_{tot} in T3; n_a= n_{tot} − n_h. Denitrification rate = total N_2O production in T4. Contributions of autotrophic (N_2O_a), heterotrophic nitrification (N_2O_h), and denitrification (N_2O_d) to N_2O production were respectively calculated as follows: N_2O_a = N_2O_{tot} (averaged N_2O production in T1 and T2) − N_2O produced in T3; N_2O_h = $N_2O_a \times n_h/n_a$; N_2O_d= N_2O in T3−N_2O_h.

2.6 Statistical analyses

Data were analyzed using SPSS 18.0 software for Windows, and means were compared by one-way ANOVA and least significant difference at a significance level of $P < 0.05$.

3 Results and discussion

NH_4^+–N concentrations decreased, whereas NO_3^-–N concentrations increased with incubation time in non-C_2H_2 treatments (T1 and T2) of GN soil, indicating the occurrence of nitrification (Fig. 2). By contrast, both NH_4^+–N and NO_3^-–N concentrations increased with incubation time in C_2H_2 treatments (T3 and T4) of GN soil, and both levels increased in all four treatments of TR soil (Fig. 2). Additionally, NO_3^-–N concentrations were lower, whereas NH_4^+–N concentrations were higher in the presence than in the absence of C_2H_2 for both soils. Previous research demonstrated that C_2H_2 can completely block autotrophic nitrification (Liu et al. 2015b). Therefore, we deduced the occurrence of autotrophic nitrification in the studied acidic pasture soils. Increasing NH_4^+–N concentration, which indicated NH_4^+–N consumption processes (e.g., autotrophic nitrification and NH_4^+–N immobilization), was overwhelmed by NH_4^+–N production processes (e.g., mineralization of organic N to NH_4^+ and heterotrophic nitrification of organic N to NH_4^+). The average measured net mineralization rate (m_{net}) at days 0–10 were significantly lower in the GN soil (2.29 mg N kg^{-1} day^{-1}) than in the TR soil (5.04 mg N kg^{-1} day^{-1}) ($P<0.05$, Table 2). On the other hand, at days 0–10, no statistical difference in measured net nitrification rates (n_{net}) existed between the two soils ($P>0.05$, Table 2).

In $^{15}NH_4NO_3$ applied treatment (T1), ^{15}N enrichment of NH_4^+ gradually declined, whereas that of NO_3^- increased as incubation proceeded (Fig. 3). Such results indicated the continuous input of NH_4^+ at natural abundance or low ^{15}N enrichment in the ^{15}N-labeled NH_4^+ pool and nitrification of ^{15}N–NH_4^+. Gross N mineralization rates (m), which were calculated from the changes in NH_4^+–N concentrations and the corresponding ^{15}N abundance, were relatively constant in the GN soil during incubation but fluctuated with incubation time in the TR soil (Table 2). No statistical difference was observed in average m between the GN (7.76 mg N kg^{-1} day^{-1}) and TR soils (8.52 mg N kg^{-1} day^{-1}) ($P<0.05$). The determined rates of m in the present study were higher than those in grassland soils (2.53–4.80 mg N kg^{-1} day^{-1}) reported by Cheng et al. (2013), while they were comparable to those of forest soils (2.30 to 9.20 mg N kg^{-1} day^{-1}) reported by Zhu et al (2013). However, these values lay within the range reviewed by Booth et al (2005). Similar to the high rates of m, gross NH_4^+–N immobilization rate (i_a) in the present study was high and resulted in a generally high MIT. A positive relationship between i_a and m in the two soils (r^2 = 0.725, $P<0.05$) indicates the dependence of N immobilization rate on its mineralization rate, and this condition may be due to soil microflora, which are responsible for the release and uptake of inorganic N (Booth et al. 2005). At an ecosystem scale, the C/N ratio of soil organic matter is considered a key factor determining N MIT, because soil heterotrophs usually feature a lower C/N ratio than that of the soil they inhabit (van Veen et al.1984; Verhagen and Laanbroek 1991). When cells yield a C/N ratio of 10 and respire approximately 50% of their C uptake, they may be N-limited above a soil C/N ratio of 20 and C-limited below (Tate et al. 1995; Bengtsson et al. 2003). Soils with C/N ratios higher than 20 may then be characterized by rapid N immobilization; by contrast, soils with C/N ratios less than 20 may be characterized by slow N immobilization and a surplus of available NH_4^+ derived from organic C deamination (Tate et al. 1995; Bengtsson et al. 2003). In this study, C/N ratios of the two pasture soils were approximately 10. Therefore, N immobilization may be overwhelmed by mineralization. Additionally, previous studies have shown that N mineralization can be modified by the ecophysiology of soil microbial communities. According to Bengtsson et al. (2003), N mineralization and immobilization in forest soils are more related to microbial community activity than to soil C/N ratio. Therefore, further investigations must be conducted to gain mechanistic insights into the effects of microbial community activity on high MIT in pasture soils.

In the applied $NH_4{}^{15}NO_3$ treatment (T2, T3, and T4), ^{15}N enrichment in the NO_3^- pool declined during days 0–4 and 6–10, regardless of the presence or absence of C_2H_2 (Fig. 3). This observation indicates the input of NO_3^- at natural abundance or low ^{15}N enrichment in the ^{15}N-labeled NO_3^- pool. Herrmann et al. (2007) and Liu et al. (2015b) demonstrated that C_2H_2 completely inhibited autotrophic nitrification in acidic crop soils. Therefore, the enlarged decline in ^{15}N enrichment and concentration in the NO_3^- pool with C_2H_2 addition suggests the occurrence of heterotrophic nitrification in the GN and TR soils. Gross nitrification rates (n_{tot}), which were calculated by changes in NO_3^-–N concentrations and the corresponding ^{15}N abundance, increased from day 0 to day 4 but decreased afterward in the GN and TR soils (Table 2). On the contrary, no statistical difference was observed in average n_{tot} between the TR (7.28 mg N kg^{-1} day^{-1}) and GN soils (5.79 mg N kg^{-1} day^{-1}) ($P>0.05$, Table 2). This finding implies that soil property difference between the two soils may not lead to differences in their N nitrification (Table 1, 2). The n_{tot} measured in this study was comparable to the rates reported for other pasture sites (Islam et al. 2007; Liu et al. 2015c). Average autotrophic nitrification rates (n_a) at days 0–10 reached 2.85 and 4.03 mg N kg^{-1} day^{-1}, accounting for 49.2% and 58.1% n_{tot} in the GN and TR soils, respectively. Average heterotrophic nitrification rates (n_h) totaled 2.95 and 3.05 mg N kg^{-1} day^{-1}, which corresponded to 50.8% and 41.9% of n_{tot} in the GN and TR soils, respectively. Soil pH strongly regulates autotrophic nitrification and heterotrophic nitrification (De Boer and Kowalchuk 2001). Sahrawat (2008) demonstrated that autotrophic nitrification could occur in a wide range of arable soils, and autotrophic nitrification rate increases with pH, with pH 8.5 as the optimum level. By contrast, heterotrophic nitrification is more prominent in soils with low pH and high recalcitrant organic C (De Boer and Kowalchuk 2001; Zhang et al. 2015). Consistently, in this study, the pH of the two tested soils reached less than 6, and their soil organic C contents were high (Table 1). Heterotrophic nitrification was demonstrated as important as autotrophic nitrification that produces NO_3^-, which will be central in further recognizing the potential of NO_3^- buildup in pasture soils. Similarly, Liu et al. (2015a) demonstrated that NO_3^- production is mainly heterotrophic in acid dairy soils with high organic content in Australia. However, Islam et al. (2007) observed that heterotrophic nitrification only accounted for 7%–19% of total nitrification in two acid pasture soils. Heterotrophic nitrification has been reported to mainly occur in grassland and forest soils

(Müller et al. 2004; Nelissen et al. 2012; Zhang et al. 2015) but was recently observed in croplands (Chen et al. 2015; Liu et al. 2015c; Chen et al. 2017). In a literature synthesis of available data on heterotrophic nitrification, Chen et al. (2015) summarized that heterotrophic nitrification rate is not only positively related to soil organic C and C/N but also highly inversely related to pH and soil bulk density. According to Cai et al. (2010), heterotrophic microorganisms may carry out nitrification in environments unfavorable (low temperature and low moisture) for autotrophic nitrifying bacteria. Given the importance of NO_3^- to plant growth and its environmental effects, heterotrophic nitrification should be considered when studying N transformation processes in pasture soils. The underlying mechanisms also merit further investigation.

In contrast to high nitrification rate, in the present study, constant denitrification rates were observed during incubation, and they reached as low as 0.003–0.004 mg N kg^{-1} day^{-1} in the two tested soils under the selected incubation conditions (Table 2). Consistent with previous studies, denitrification rate was negligible under aerobic conditions and soil moisture content below 60% WFPS (Amha and Bohne 2011; Cheng et al. 2012). This effect can be ascribed to the proteins, which are only produced under anoxic conditions, required for denitrification; if anaerobically grown cells are exposed to oxygen, protein activities become inhibited (Fernandez et al. 2011).

Fig. 4 shows the dynamics of N_2O production rates in GN and TR soils. N_2O production rates increased at 0–2 days in T3 treatment and 0–4 days in T1, T2, and T4 treatments. Afterward, the rates decreased in the GN soil. N_2O production rates fluctuated with incubation time in the TR soil. No statistical difference was observed in N_2O production among T1, T2, and T4 treatments in both soils (P>0.05). However, the rates in T1, T2, and T4 treatments were significantly higher than that in T3 treatment (Fig. 4). Assuming that N_2O was only produced through autotrophic (N_2O_a) and heterotrophic (N_2O_h) nitrification and denitrification (N_2O_d), relative contributions of these processes to N_2O production were estimated (Fig. 5). In the GN soil, the contribution of denitrification to N_2O emission decreased, whereas that from nitrification increased with incubation time. By contrast, N_2O was mainly produced from denitrification on days 0–2 (67.5%) and 6–10 (60.7%) in the TR soil, whereas nitrification was the main N_2O pathway on days 2–6 (Fig. 5). On average, N_2O_a, N_2O_h, and N_2O_d accounted for 29.1%, 23.4%, and 47.5% of total N_2O emissions in the GN soil and 37.3%, 30.1%, and 32.7% in the TR soil, respectively (Fig. 6). N_2O_a and N_2O_h values were similar between the two soils, whereas the GN soil yielded a higher percentage of N_2O_d than TR soil (Fig. 6).

Considering the soil aerobic conditions under incubation (60% WFPS), denitrification was negligible in the two tested soils (Table 2) but significantly contributed to N_2O emission (Fig. 5). Inconsistent with previous results, the contribution of denitrification to N_2O emission was <30% from agricultural soils under aerobic conditions (soil moisture content < 60%) (Stevens et al. 1997; Müller et al. 2014). However, some researchers reported the important role of denitrification in N_2O emission under aerobic conditions. For example, Zhu et al. (2011) observed that denitrification accounted for 22.5%–57.7% of N_2O production in intensively farmed vegetable fields under a soil moisture content of 50% water holding capacity. Zhang et al. (2011) showed that denitrification was the primary source of N_2O emission in subtropical acid forest soils and contributed more than 50% of N_2O production under the soil moisture content of 40%–50% WFPS. A possible explanation for denitrification under aerobic conditions is the presence of anaerobic microsites created by either microbial growth or water saturation within soil aggregates after addition of labeling solution (Renault and Stengel 1994). Additionally, the high SOC content in two soils may also contribute to denitrification (Fernandez et al. 2011; Loick et al. 2016).

In the present study, autotrophic and heterotrophic nitrifications played equal roles in

N_2O emission (Fig. 5). In a review, Zhang et al. (2015) summarized that the contribution of heterotrophic nitrification of organic N to total N_2O emissions depends on soil pH, C/N ratio, and land use. However, numerous bacteria and fungi can also carry out heterotrophic nitrification by using NH_4^+ and organic N compounds as substrates (Zhang et al. 2014; Medinets et al., 2015). Nevertheless, fungi are considered the most efficient microorganisms for heterotrophic nitrification (Pedersen et al. 1999). Jirout et al. (2013) and Jirout (2015) demonstrated that N_2O-producing fungi are the common constituents of fungal communities in pasture soils influenced by overwintering cattle. Consequently, we argue that high soil organic C and low pH may be factors that facilitate N_2O production from fungus-mediated heterotrophic nitrification in the tested pasture soils.

4 Conclusions

In conclusion, two tested pasture soils were characterized by high MIT under selected conditions. However, further investigations are needed to gain mechanistic insights into the effects of microbial community activity on high MIT in pasture soils. High gross N nitrification rates were observed in both pasture soils. Heterotrophic nitrification played a role similar to that of autotrophic nitrification in total nitrification and N_2O emission. We argue that in the tested pasture soils, high SOC and low pH may be factors facilitating heterotrophic nitrification by fungi. Denitrification rates in the two tested soils were negligible but contributed more than 30% of N_2O emission under selected aerobic conditions. Therefore, except for autotrophic nitrification, the roles of heterotrophic nitrification and denitrification in N_2O emission in the two pasture soils should be considered in mitigation strategy development Given the limited number of soil samples/sites and the specific conditions applied, further studies are needed to confirm our results. Specifically, realistic environmental conditions for N transformation processes and N_2O pathways should be adopted. Additional sampling sites at different pasture regions should be selected to definitively establish the underlying mechanisms and factors influencing gross N transformation rates and N_2O production from different N transformation processes.

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Table 1 Soil physical and chemical properties of the studied soils

Soil	Organic matter (%)	Organic C (%)	Total N (%)	C/N	pH (1:5 water)	CEC (c mol kg^{-1})	Clay (<2 μm, %)	Silt (2–60 μm, %)	Sand (60–2000 μm, %)	Soil texture	NH_4^+–N mg kg^{-1}	NO_3^-–N mg kg^{-1}
Terang (TR)	7.9	4.60	0.5	9.3	5.50	7.67	8	63	29	Sandy Loam	12.3	6.90
Glenormiston (GN)	10.0	5.90	0.6	9.8	6.00	24.0	11	53	36	Sandy Loam	12.2	14.9

Table 2 Nitrogen transformation rates in the two tested pasture soils (mg N kg^{-1} day^{-1})

Soil[a]	Time period (day)	m_{net}[b]	n_{net}	m	i_a	n_{tot}	n_{a}	n_{h}	d
GN	0–2	8.95 (0.13)[c]	8.50 (0.75)	8.29 (1.35)	2.13 (0.32)	2.64 (0.34)	0.33 (0.10)	2.36 (0.24)	0.003 (0.000)
	2–4	−3.60 (0.80)	2.10 (0.01)	7.80 (2.26)	2.59 (0.87)	12.1 (1.01)	10.7 (0.00)	1.41 (1.01)	0.004 (0.000)
	4–6	3.50 (0.42)	6.30 (0.90)	6.51 (1.22)	3.01 (1.52)	4.10 (1.66)	0.55 (0.59)	3.62 (1.07)	0.004 (0.000)
	6–10	1.30 (0.48)	4.58 (1.08)	8.10 (1.41)	0.99 (0.43)	5.04 (0.12)	1.33 (0.11)	3.75 (0.22)	0.004 (0.000)
	0–10	2.29 (0.46)	5.21 (0.76)	7.76 (1.53)	1.94 (0.83)	5.79 (0.65)	2.85 (0.18)	2.96 (0.55)	0.004 (0.000)
TR	0–2	15.6 (1.16)	14.0 (0.74)	7.06 (1.14)	2.56 (1.55)	1.76 (0.40)	0.64 (0.52)	1.12 (0.30)	0.003 (0.001)
	2–4	4.90 (1.55)	2.20 (1.10)	1.66 (1.22)	0.49 (0.12)	11.4 (2.48)	8.15 (0.96)	2.24 (1.51)	0.004 (0.001)
	4–6	−2.66 (1.56)	−0.86 (0.87)	4.10 (1.08)	1.31 (0.48)	2.80 (2.02)	1.11 (0.40)	1.69 (0.42)	0.004 (0.000)
	6–10	3.69 (0.33)	2.44 (0.19)	14.9 (1.10)	6.32 (1.09)	10.2 (1.50)	5.13 (1.13)	5.10 (0.40)	0.004 (0.000)
	0–10	5.04 (0.98)	4.04 (0.62)	8.52 (1.13)	3.40 (0.92)	7.28 (1.58)	4.03 (0.83)	3.25 (0.60)	0.004 (0.000)

[a], GN: pasture soil from Glenormiston, Victoria, Australia. TR: pasture soil from Terang, Victoria, Australia.

[b], m is gross N mineralization of organic N to NH_4^+; i_a is gross NH_4^+ immobilization rate; n_{tot} is total gross N nitrification rate; n_{a} is autotrophic nitrification rate; n_{h} is heterotrophic nitrification rate; d is denitrification rate; m_{net} is net mineralization rate, and n_{net} is the measured net nitrification rate.

[c], Values in brackets are standard deviations ($n = 3$)

Fig. 1 Processes involved in soil N transformation: mineralization (m), autotrophic nitrification (n_a), autotrophic nitrification (n_h), denitrification (d), immobilization (i), volatilization (v), biological N_2 fixation (f), dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonia oxidation (an) (Denk et al. 2017).

Fig. 2 Dynamics of NH_4^+–N and NO_3^-–N under different treatments (T1: $^{15}NH_4NO_3$, T2: $NH_4{}^{15}NO_3$, T3: $NH_4{}^{15}NO_3$ + 0.1% C_2H_2, and T4: $NH_4{}^{15}NO_3$ + 10% C_2H_2) in GN and TR soils after 10 days of incubation. Vertical bars indicate the standard errors of three replicates.

Fig. 3 ^{15}N enrichment of NH_4^+ and NO_3^- pools in GN and TR soils from different treatments (T1: $^{15}NH_4NO_3$, T2: $NH_4{}^{15}NO_3$, T3: $NH_4{}^{15}NO_3$ + 0.1% C_2H_2, and T4: $NH_4{}^{15}NO_3$ + 10% C_2H_2) after 10 days of incubation. Vertical bars indicate the standard deviations of three replicates.

Fig. 4 Dynamics of NH_4^+–N and NO_3^-–N under different treatments (T1: $^{15}NH_4NO_3$, T2: $NH_4{}^{15}NO_3$, T3: $NH_4{}^{15}NO_3$ + 0.1% C_2H_2, T4: $NH_4{}^{15}NO_3$ + 10% C_2H_2) in GN and TR soils after 10 days of incubation. Vertical bars indicate the standard errors of three replicates.

Fig. 5 Contribution percentages of autotrophic nitrification (N_2O_a), heterotrophic nitrification (N_2O_h), and denitrification (N_2O_d) to N_2O production in GN and TR soils after 10 days of incubation

Fig. 6 Average contribution percentages of autotrophic nitrification (N_2O_a), heterotrophic nitrification (N_2O_h), and denitrification (N_2O_d) to N_2O production in GN and TR soils. Different capital letters indicate significant difference at $P<0.05$ among the N_2O production pathways within the same soil; different lowercase letters indicate significant difference at $P<0.05$ of one production pathway between the two soils.

Fig. 1

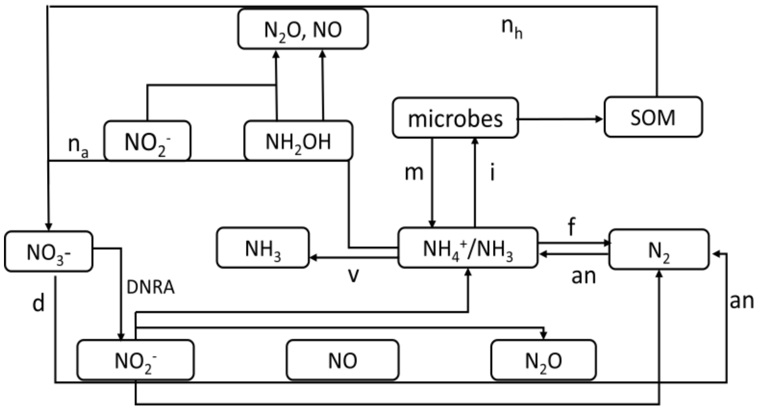

N$_2$O, NO
n$_h$
microbes
SOM
n$_a$
NO$_2^-$
NH$_2$OH
m
i
f
NO$_3$-
NH$_3$
NH$_4^+$/NH$_3$
N$_2$
v
an
DNRA
d
an
NO$_2^-$
NO
N$_2$O

Fig. 2

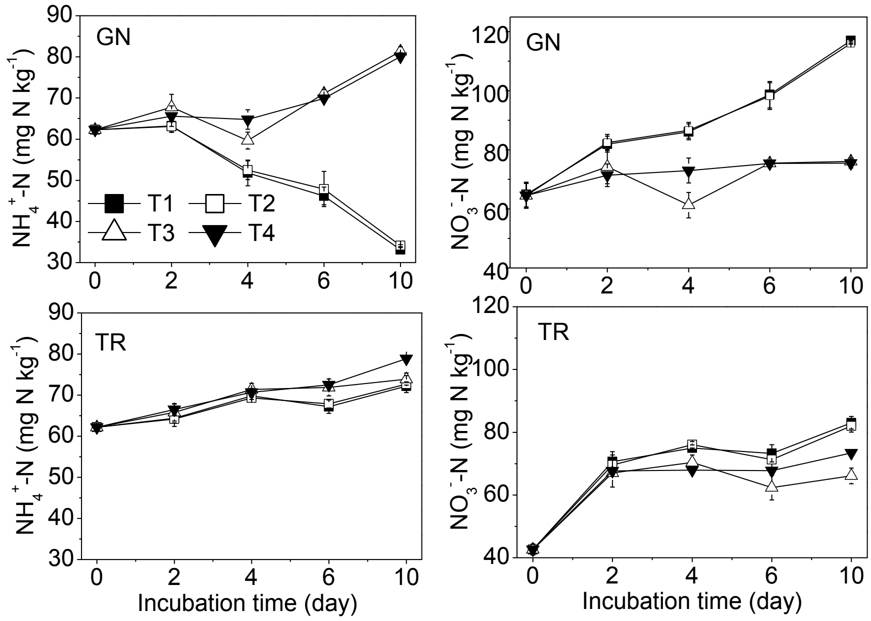

GN
GN
TR
TR
T1
T2
T3
T4
NH_4^+-N (mg N kg^{-1})
NO_3^--N (mg N kg^{-1})
30
40
50
60
70
80
90
100
120
0
2
4
6
10
Incubation time (day)
Incubation time (day)

Fig. 3

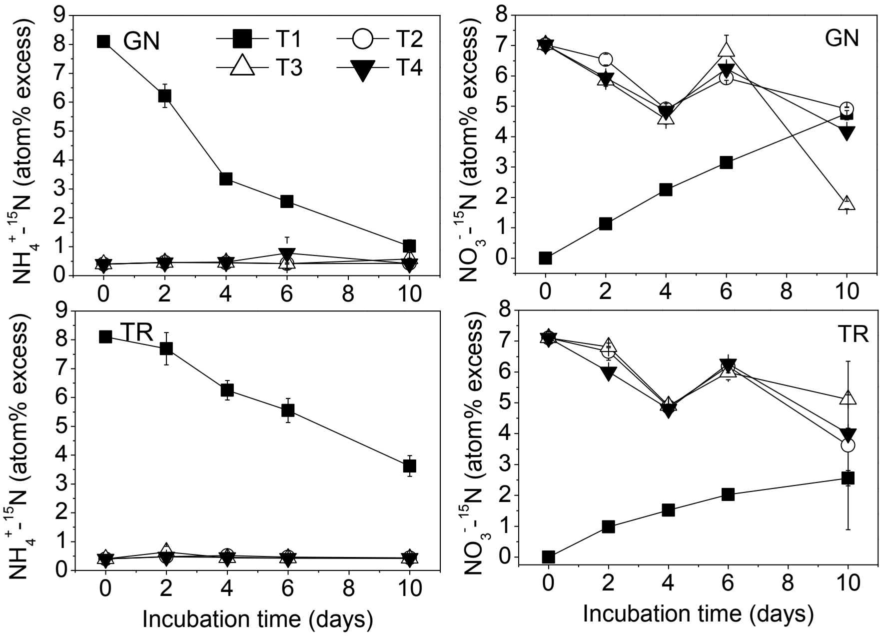

GN
T1
T2
T3
T4
NH$_4^+$-^{15}N (atom% excess)
NO$_3^-$-^{15}N (atom% excess)
TR
Incubation time (days)
0
2
4
6
8
10
1
3
5
7
9

Fig. 4

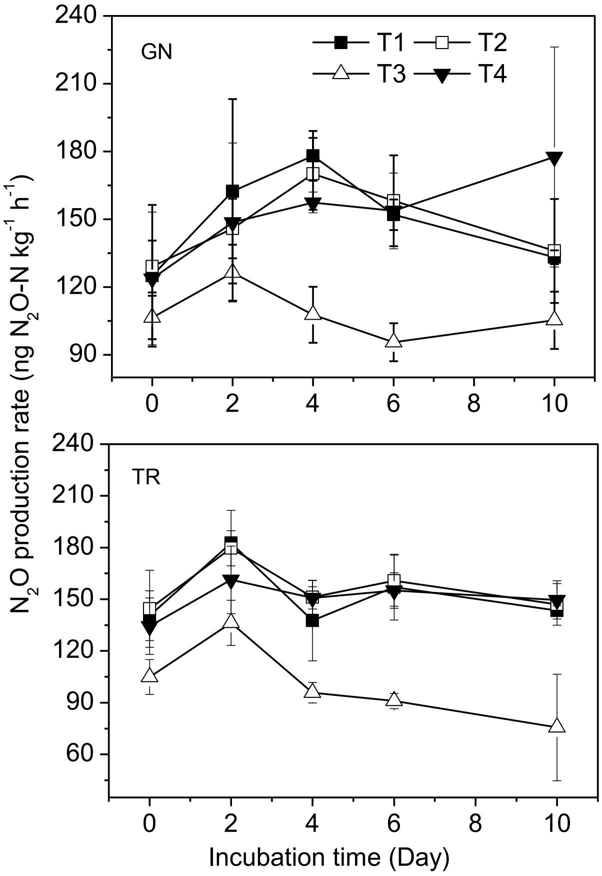

GN
TR
T1
T2
T3
T4
N$_2$O production rate (ng N$_2$O-N kg^{-1} h^{-1})
240
210
180
150
120
90
60
0
2
4
6
8
10
Incubation time (Day)

Fig. 5

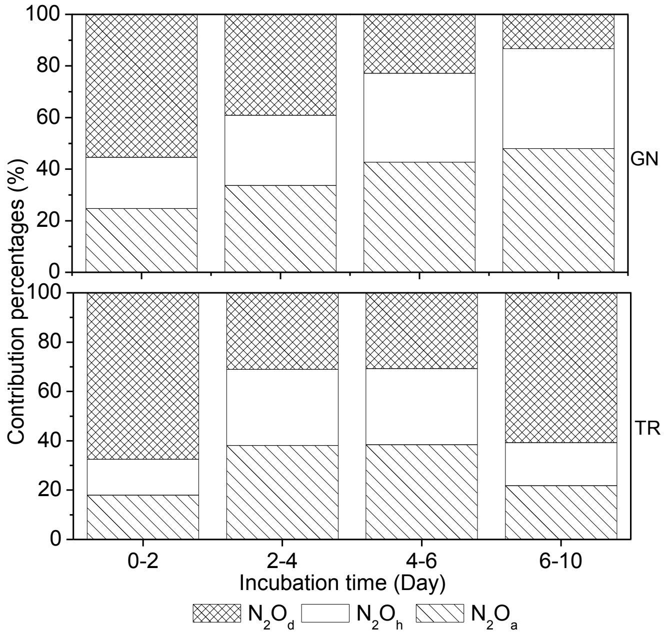
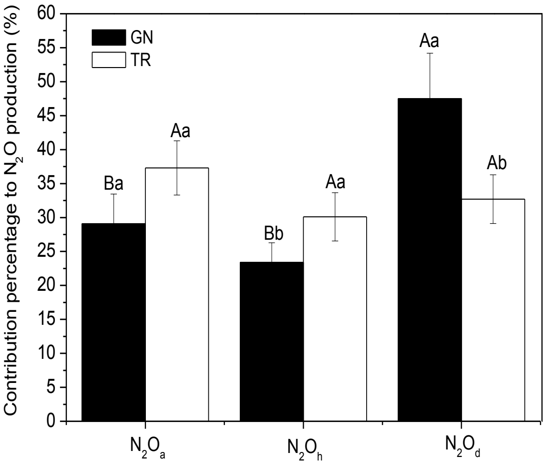

Contribution percentages (%)
100
80
60
40
20
0
GN
TR
0-2
2-4
4-6
6-10
Incubation time (Day)
N2Od
N2Oh
N2Oa

Fig. 6


Contribution percentage to N_2O production (%)
60
55
50
45
40
35
30
25
20
15
10
5
0
GN
TR
Ba
Aa
Bb
Aa
Aa
Ab
N_2O_a
N_2O_h
N_2O_d