Nitrification is a primary driver of nitrous oxide production in agricultural soils

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Abstract
The continuous increase of the greenhouse gas nitrous oxide (N_2O) in the atmosphere due to increasing anthropogenic nitrogen input in agriculture has become a global concern. In recent years, identification of the microbial sources responsible for soil N_2O production has substantially advanced with the development of isotope enrichment techniques and the discovery of specific nitrogen-cycling functional genes. However, little information is available to effectively quantify the N_2O produced from different microbial pathways (i.e. nitrification and denitrification). ^{15}N-tracing incubation experiments were conducted, using soil from different land-uses, under controlled laboratory conditions to quantify nitrification-sourced N_2O production. Nitrification was found to be the main contributor to N_2O production, contributing to 96.7% of the N_2O emissions in the sugarcane soil followed by 70.9% in the cereal cropping soil and 70.9% in the dairy pasture soil, while only around 20.0% of N_2O was produced from nitrification in vegetable soil. The greatest contribution from nitrification was observed at 50% and 70% WFPS regardless of soil temperature. At 50%, 70% and 85% WFPS, nitrification contributed 87%, 80% and 53% of total N_2O production, respectively at 25°C, and 86%, 74% and 33% of total N_2O production, respectively at 35°C. These findings can be used to develop better models for simulating N_2O from nitrification to inform soil management practices for improved N use efficiency.

Key Words
^{15}N isotope technique, land-use soil types, soil temperature, soil moisture, nitrification, N_2O production

Introduction
N_2O is a potent greenhouse gas (GHG) greatly contributing to global climate change, with a 300-fold higher global warming potential than CO_2 (IPCC, 2007). Global N_2O concentrations are likely to continue to rise in the coming decades due to increasing application of nitrogen (N) fertilizer for food production to feed a growing population (Reay et al., 2012). Soil ecosystems are the largest source of N_2O, accounting for an estimated 65% of the atmospheric loading of this gas (IPCC, 2007). In Australia, agriculture accounts for nearly 80% of the total N_2O released (Australia Greenhouse Office, 2001), with 32% of this estimated to be derived from N fertilizer (Dalal et al., 2003).

The major pathways of N_2O production in soils include nitrification and denitrification, which can occur independently or simultaneously. However, the current N_2O emission measurements from field sites, either by small chambers or large scale micro-meteorological techniques, do not distinguish between N_2O sources. Furthermore, agricultural practice, climatic conditions and soil properties all influence N_2O emission (Jørgensen and Elberling, 2012). There is high uncertainty in estimation of N_2O derived from soils and many process-oriented biogeochemical models, such as CENTURY, DAYCENT and DNDC, estimate the contribution of nitrification to N_2O emission using a fixed ratio due to lack of available data (Chen et al., 2008).

Therefore, to improve modelling capability and understanding of N_2O emissions in order to develop more effective mitigation strategies to counteract the steady increase in N_2O emissions, it is essential to quantify how much N_2O comes from nitrification and what are the factors affecting N_2O emission from this process.

Methods
^{15}N tracer laboratory microcosm incubation under controlled conditions
Surface soil (0-10 cm) was collected from Hamilton, Victoria, Australia (38.32° S, 142.07° E). The soil contained 0.52% total N, 5.2% organic C, and the soil pH $_{(H2O)}$ was 4.5. Soil (60g oven-dry equivalent) was placed into 500 ml incubation vials. Distilled water was added to soil to just under the final moisture content (50%, 70% and 85% WFPS) and the microcosms were pre-incubated at 25°C and 35°C for 3 weeks. After pre-incubation, treatments were applied to each vessel. The treatments contained 100 mg N kg^{-1} as NH_4^+-N and 50 mg N kg^{-1} as NO_3^--N, which were added to the soil in 2 ml solution to achieve a final moisture

content of 50%, 70% and 85% WFPS as 1) $^{15}NH_4Cl$ (10 atom% ^{15}N excess) + KNO_3 and 2) NH_4Cl + $K^{15}NO_3$ (10 atom% ^{15}N excess). The soil samples were aerated and water was replenished every 3 days. Gas samples were collected for N_2O (20 ml) and ^{15}N-N_2O (70 ml) analysis on days 0, 4, 7, 12, 15 and 21 after treatment application, and were analysed by gas chromatography (Agilent 7890A) using an ECD detector and Isotope Ratio Mass Spectrometry (IRMS) (Hydra 20–20, SerCon, Crewe, UK) respectively. Triplicate samples were extracted with 2 M KCl (soil-to-solution ratio 1:5) on days 0, 7 and 15 by shaking for 1 h. The soil extracts were filtered through Whatman number 42 filter papers and analysed for mineral N (NH_4^+ and NO_3^-), using a segmented-flow analyzer (Skalar, SAN++), and analysed for ^{15}N abundance in mineral N (Saghir et al., 1993) by the IRMS (Hydra 20–20, SerCon, Crewe, UK).

^{15}N tracer laboratory microcosm incubation with four soils from different land-uses

Soil samples (0-10 cm) were collected from four agricultural sites across Australia: sugarcane at Bundaberg, QLD (24°57'S, 152°20'E), vegetable at Boneo, VIC (38°24'S, 144°53'E), dairy pasture at Longworry, VIC (38°08'S, 145°43'E) and cereal cropping at Hamilton, VIC (38°19'S, 142°42'E). Soil moisture contents were determined by oven-drying three subsamples (10 g of air-dried soil) at 105°C for 48 h. Soil pH (1:5 soil/water), texture (sieve and hydrometer procedures), total carbon (Dumas method) and other soil properties are demonstrated in Table 1. The design of the soil microcosm incubation was as described above with samples incubated only at 25°C and 50% water-filled pore space (WFPS). Gas and soil samples were collected for N_2O, mineral N and ^{15}N enrichment measurements.

Table 1 Field site description and basic characteristics of soils used in this study

Land-use	Site name	Climate	Texture	Clay	Sand	Silt	pH	NH_4-N	NO_3-N	TC	TN
					%		(H_2O)	mg N kg^{-1} soil		%	%
Sugarcane	Bundaberg, QLD	Subtropical	Sand	5	90	5	6.0	2.6	8.8	1.2	0.06
Vegetable	Beneo, VIC	Temperate	Sand	1	91	8	7.8	1.1	19	0.8	0.08
Dairy pasture	Longworry, QLD	Tropical	Clay loam	4	75	21	4.8	16	47	9.3	0.8
Cropping	Hamilton, VIC	Temperate	loam	10	61	29	7.0	5.1	10	ND	ND

Statistical analyses

Data were analysed using SPSS 19 and means were compared using one-way ANOVA between treatments to test the variance with a level of significance of $P < 0.05$. Significant differences in soil properties and microbial gene abundance levels over time and between treatments were examined by ANOVA. Relationships between measures were assessed by correlation analysis, using Pearson's r if data were normally distributed and Spearman's rho if data were not normally distributed (normality assessed by Shapiro-Wilk's W), in both Stata12/SE and the statistical software package PAST (v2.17; Hammer et al. 2001).

Results

N_2O emission under different environmental conditions

N_2O flux rates increased over the first 7 days and then decreased before stabilising (Figure 1). N_2O production reached a peak flux on day 4 at 70% and 85% WFPS at 25°C, and on day 3 at 35°C for all moisture treatments (Figure 1). However, at 25°C and 50% WFPS, N_2O production reached a peak flux on day 7. The largest N_2O flux (48.92 mg N_2O-N kg^{-1} d^{-1}) was detected at 85% WFPS and 25°C. A significantly ($P < 0.05$) higher level of N_2O was emitted at each temperature when the soil moisture content was 70% or 85% WFPS than 50% WFPS. The N_2O flux rates were significantly higher ($P < 0.05$) when the soil temperature was 35°C compared to 25°C, except for the 85% WFPS treatments (Figure 1).

N_2O emission in different land-use soils

The N_2O production rates were found to be highly variable across different land-use in agricultural soils (Figure 2). The highest N_2O production rate was recorded in the cereal cropping soil (average 1.16 mg N_2O-N kg^{-1} d^{-1}), which was significantly higher than those in the sugarcane soil (0.07 mg N_2O-N kg^{-1} d^{-1}), vegetable soil (0.12 mg N_2O-N kg^{-1} d^{-1}), and dairy pasture soil (0.28 mg N_2O-N kg^{-1} d^{-1}). The N_2O flux continuously decreased throughout the incubation period in the cereal cropping soil, while in the sugarcane,

dairy pasture, and vegetable soils, N_2O production rates stabilized after 7 days of incubation (Figure 2). The soils with higher total N contents tended to have higher N_2O production rates (Figure 2).

N_2O sources under different environmental conditions

The contribution of nitrification (C_n) to N_2O production decreased with increasing temperature and moisture, (Table 2). Nitrification contributed more to N_2O emission than denitrification when soil moisture content was 50% and 70% WFPS. However at 85% WFPS the contribution of denitrification was approximately equal to (35°C) or less than (25°C) that from nitrification. The nitrification-derived N_2O (N_2O_n) was significantly ($P < 0.05$) influenced by soil moisture and soil temperature (Table 2). N_2O from nitrification (N_2O_n) increased as the soil moisture content increased from 50% to 85% WFPS, with the greatest flux detected at 25°C and 85% WFPS in the first week.

N_2O sources in different land-use soils

Denitrification was responsible for only 3.3% of N_2O production in the sugarcane soil (Table 2), however, in the vegetable soil, denitrification was the predominant pathway of N_2O emission, responsible for 76.3% of N_2O production (Table 3). The nitrification-derived N_2O peak from the cereal cropping soil was 25.12 mg N_2O-N kg^{-1} d^{-1} (Table 3), which was strikingly higher than that in the sugarcane soil (0.15 mg N_2O-N kg^{-1} d^{-1}) although the C_n of the sugarcane soil was higher than that of the cereal cropping soil. In the acidic soils (pH<6), the C_n was higher than that of denitrification (C_d) (Table 3), and followed the order sugarcane soil > cereal cropping soil > dairy pasture soil. The proportion of nitrified N emitted as N_2O (P_{N2O}) over 7 days varied between soils (Table 3). The gross nitrification rates for the four different land-use types followed the order of cereal cropping > vegetable > dairy pasture > sugarcane, whilst P_{N2O} followed the order of cereal cropping > dairy pasture > sugarcane > vegetable.

Conclusion

Nitrification was the main contributor of N_2O emissions in acidic sugarcane, dairy pasture and cereal cropping soils. Compared to the cereal cropping, sugarcane and dairy pasture soils, more nitrification-sourced N_2O was emitted from the sugarcane soil (C_n 96.7%). Nitrification was found to be the main contributor to N_2O production, with the greatest effect observed at 50% and 70% WFPS regardless of soil temperature. Soil temperature and moisture influenced nitrification-sourced N_2O emission.

Table 2. Gross nitrification rate, the relative contribution of denitrification (C_d) and nitrification (C_n) to N_2O production, N_2O derived from denitrification (N_2O_d) and nitrification (N_2O_n), and the proportion of N_2O to gross nitrification (P_{N2O}) under the different environmental conditions. Values in brackets are standard deviations.

Temperature	Moisture	Gross nitrification rate mg N kg^{-1} d^{-1}	Relative contribution %		N_2O_d [c] mg N_2O-N kg^{-1} d^{-1}	N_2O_n [d] mg N_2O-N kg^{-1} d^{-1}	P_{N2O} %[e]
			C_d [a]	C_n [b]			
25°C	50%WFPS	1.97 (0.32)	10.68 (1.22)	89.32 (1.98)	0.0002 (2.3E-5)	0.0017 (2.3E-5)	0.0015 (0.0004)
	70%WFPS	8.32 (0.69)	13.08 (2.45)	86.92 (1.68)	0.048 (0.009)	0.317 (0.009)	0.249 (0.103)
	85%WFPS	3.84 (0.78)	29.09 (4.050	70.91 (6.98)	10.979 (1.53)	26.76 (1.53)	18.101 (5.22)
35°C	50%WFPS	5.39 (0.16)	11.14 (2.11)	88.86 (3.46)	0.0024 (0.0005)	0.019 (0.0005)	0.0161 (0.013)
	70%WFPS	7.05 (0.33)	16.21 (2.58)	83.79 (8.99)	0.320 (0.024)	1.952 (0.039)	1.970 (1.93)
	85%WFPS	11.17 (2.24)	50.10 (4.20)	49.90 (6.78)	3.152 (0.264)	3.139 (0.109)	2.343 (0.19)

Table 3. Gross nitrification rates and the ratios of N_2O production to nitrification in the studied agricultural soils. Values in brackets are standard deviations.

Land-use	Gross nitrification rate	Relative contribution %		N_2O_d [c]	N_2O_n [d]	P_{N2O} %[e]
	mg N kg^{-1} d^{-1}	C_d [a]	C_n [b]	mg N_2O-N kg^{-1} d^{-1}		
Sugarcane	1.70 (0.50)	3.30 (0.45)	96.67 (6.8)	0.15 (0.03)	4.38 (0.34)	0.030 (0.0016)
Vegetable	5.42 (0.43)	76.36 (9.2)	23.64 (3.91)	11.31 (1.03)	3.11 (0.30)	0.024 (0.0011)

Dairy Pasture	3.84 (0.78)	29.09 (4.1)	70.90 (4.97)	3.78 (0.22)	9.32 (1.34)	0.033 (0.0026)
Cereal cropping	9.88 (2.30)	28.74 (8.6)	71.26 (1.82)	25.12 (2.06)	62.54 (6.63)	0.260 (0.0189)

[a] The relative contribution by denitrification (C_d) to N_2O production
[b] The relative contribution by nitrification (C_n) to N_2O production
[c] N_2O production from denitrification (N_2O_d)
[d] N_2O production from nitrification (N_2O_n)
[e] The proportion of nitrified N emitted as N_2O (P_{N2O})

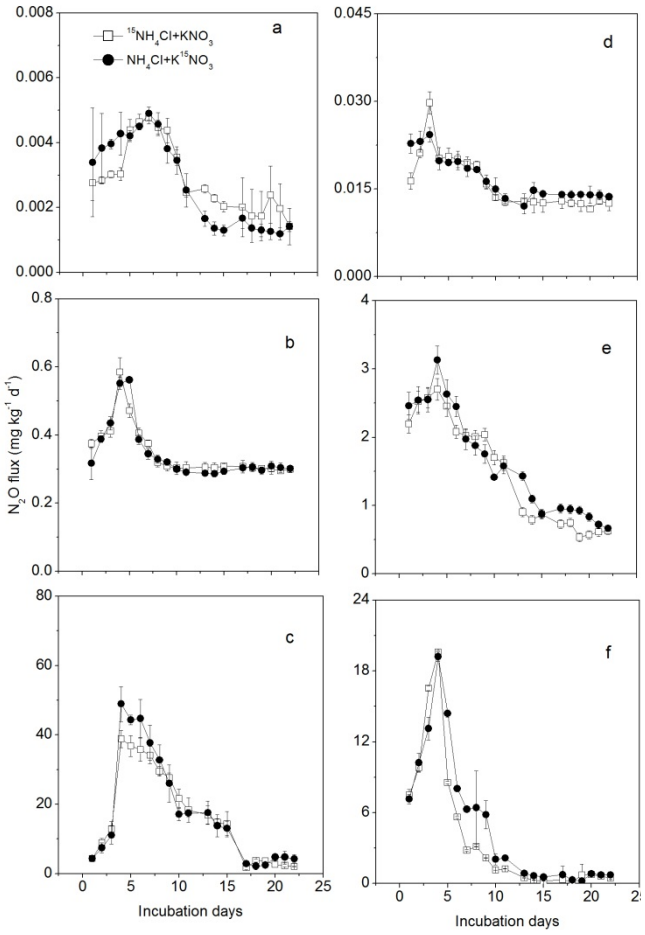

Figure 1. N_2O production rate from the Hamilton soil under different incubation conditions: 25°C + 50% WFPS (a), 25°C + 70% WFPS (b), 25°C + 85% WFPS (c), 35°C + 50% WFPS (d), 35°C + 70% WFPS (e), and 35°C + 85% WFPS (f). Error bars are the standard deviation of four replicates.

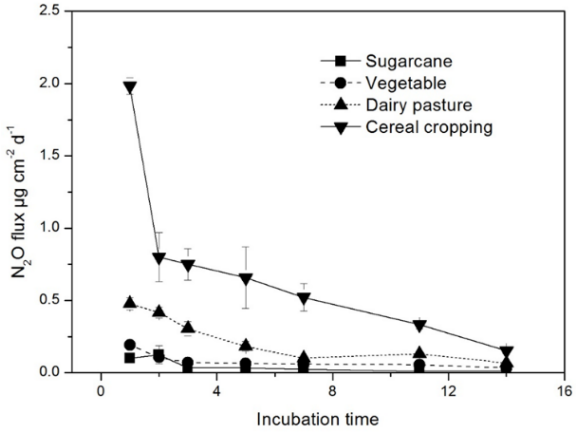

Figure 2. Soil N_2O production rates from different land-use types in agricultural soils. Error bars represent standard error.

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