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Nitrification rates and associated nitrous oxide emissions from agricultural soils – a synopsis

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Abstract. Laboratory incubations were performed to estimate nitrification rates and the associated nitrous oxide (N_2O) emissions under aerobic conditions on a range of soils from National Agricultural Nitrous Oxide Research Program field sites. Significant site-to-site variability in nitrification rates and associated N_2O emissions was observed under standardised conditions, indicating the need for site-specific model parameterisation. Generally, nitrification rates and N_2O emissions increased with higher water content, ammonium concentration and temperature, although there were exceptions. It is recommended that site-specific model parameterisation be informed by such data. Importantly, the ratio of N_2O emitted to net nitrified N under aerobic conditions was small (<0.2% for the majority of measurements) but did vary from 0.03% to 1%. Some models now include variation in the proportion of nitrified N emitted as N_2O as a function of water content; however, strong support for this was not found across all of our experiments, and the results demonstrate a potential role of pH and ammonium availability. Further research into fluctuating oxygen availability and the coupling of biotic and abiotic processes will be required to progress the process understanding of N_2O emissions from nitrification.

Additional keywords: agriculture, ammonia oxidation, modelling, nitrogen.

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Introduction

Nitrous oxide (N_2O) emissions from agricultural systems are thought to result principally from the microbial-mediated processes of nitrification (the oxidation of ammonium to nitrate) and denitrification (the reduction of nitrate to N_2O and dinitrogen) in soil, although other processes and combinations thereof exist (Butterbach-Bahl *et al*. 2013). Most agro-ecological models (e.g. DayCent, APSIM, DNDC, WNMM, NOE) explicitly deal with each of these processes with varying degrees of process understanding and empiricism (Li *et al*. 1992; Parton *et al*. 1998; Hénault *et al*. 2005; Li *et al*. 2007; Thorburn *et al*. 2010). By necessity, these processes are often simplistically modelled using potential process rates that then are modified by drivers such as substrate availability, water content, pH and temperature (Fig. 1) following the 'hole-in-the-pipe' schema of Firestone and Davidson (1989). Although this approach may be suitable for a particular site in a given year, such models may not be portable because the true mechanisms, which can vary over space and time, have not been captured.

Nitrification in agricultural systems is thought to be mostly carried out by autotrophic bacteria (Jia and Conrad 2009), although some autotrophic archaea and heterotrophs can also nitrify. The mechanism of N_2O production from nitrification has not been systematically determined (Khalil *et al*. 2004; Shaw *et al*. 2006). The oxidative decomposition of hydroxylamine (Bremner *et al*. 1980; Hooper and Terry 1979) and nitrifier denitrification (Poth and Focht 1985; Zhu *et al*. 2013*a*) (i.e. denitrification by ammonia oxidising bacteria, as proposed by Baggs (2011)) have been implicated, with a key role for nitrite suggested (Mørkved *et al*. 2007). The roles of abiotic factors such as metal ion species, including iron III and manganese II, in the oxidation of hydroxylamine (Hooper and Terry 1979; Bremner *et al*. 1980; Zhu *et al*. 2013*b*; Heil *et al*. 2015), chemical or thermal decomposition of hydroxylamine, and processes such as nitrifier denitrification under conditions of oxygen stress have not been widely acknowledged or included in models. Consequently, most models adopt a grey-box approach to predicting N_2O from nitrification, allocating a set proportion of nitrified N to N_2O emission (P_n in Fig. 1). Some exceptions to the use of fixed proportions exist. For example, the empirically based NOE model (Bessou *et al*. 2010) now incorporates a function based on water content as a rudimentary proxy for the influence of oxygen availability on the proportion of nitrified N emitted as N_2O (P_n), as observed by Khalil *et al*. (2004), and the *ecosys* model explicitly represents the role of nitrite in the generation of N_2O (Grant 1995). However, there remains a paucity of data to support these approaches across a range of soils.

Few studies have explicitly reported nitrification rates and the associated N_2O emissions. Of the reports we could find in the literature (Table 1), the varying methodologies used, range of experimental systems involved and dearth of data preclude a meta-analysis to determine which factors influence the proportion or ratio of N_2O produced to nitrified N across a range of soils. Studies have used pure and mixed cultures of various nitrifiers, soil slurries, loose soil incubations, repacked cores, intact cores and *in situ* techniques. Measurements have relied on the use of inhibitors or strictly aerobic conditions to

prevent denitrification, and isotopic labels, but rarely has it been clearly stated whether the calculations were based on net or gross rates, which may account for some of the variance in observed product ratios. Of the factors examined, a negative relationship between oxygen availability and P_n was the most common observation, with the most decisive work being done by using cultures (Goreau *et al*. 1980; Lipschultz *et al*. 1981; Hynes and Knowles 1984; Kester *et al*. 1997) with varying oxygen contents, although cell density was also important (Remde and Conrad 1990; Frame and Casciotti 2010). In addition, oxygen availability has been shown to be important in soil, either directly (Goodroad and Keeney 1984; Khalil *et al*. 2004; Mørkved *et al*. 2006; Zhu *et al*. 2013*a*) or via water content (Maag and Vinther 1996; Garrido *et al*. 2002; Ambus 2005; Mathieu *et al*. 2006; Chen *et al*. 2010), although some studies have found conflicting responses (Bateman and Baggs 2005). Interestingly, Hynes and Knowles (1978) found that the addition of sterile soil to a culture increased N_2O emission 10-fold and broadened the range of oxygen concentrations over which high N_2O emissions occurred. There were conflicting responses of P_n to temperature (Goodroad and Keeney 1984; Maag and Vinther 1996; Ingwersen *et al*. 1999). Martikainen (1985) found an exponential increase in P_n as pH declined from 4.7 to 4.1 in some acidic forest soils, and Mørkved *et al*. (2007) reported N_2O emissions two orders of magnitude higher in soils of pH <5, possibly due to chemodenitrification. However, other studies were less conclusive about the influence of pH (Goodroad and Keeney 1984; Mørkved *et al*. 2006). Jiang and Bakken (1999) noted that P_n increased when conditions were unfavourable to nitrification, i.e. because of acid and low ammonium availability. The source of nitrogen also seems to be important, with urine and urea giving higher P_n values than ammonium (Carter 2007; Zhu *et al*. 2013*a*). Recent work has again highlighted other abiotic factors such as metal species (Zhu *et al*. 2013*b*; Heil *et al*. 2015) as being important determinants of N_2O emissions from nitrification. In addition,

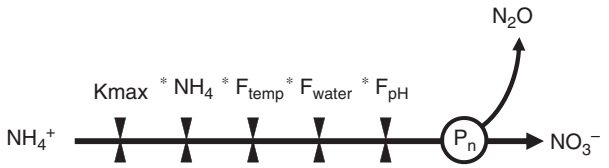

Fig. 1. A general model of nitrous oxide (N_2O) emissions from nitrification. In most agroecosystem models, the nitrification rate is a function of the potential nitrification rate (Kmax), the soil ammonium content (NH_4^+), and functions of temperature, water and pH. Then a set proportion (P_n) of the nitrified N is emitted as N_2O with the remainder going to nitrate. Some models now have dynamic proportionality constants (P_n). Note that the actual proposed mechanisms of N_2O production from nitrification (oxidation of hydroxylamine and nitrifier denitrification) are not explicitly modelled.

Table 1. Nitrous oxide emissions as a proportion of nitrified N, as reported in the literature (in chronological order)

Authorship	Experiment type	N_2O-N/nitrified N	Relevant treatments
Yoshida and Alexander 1970	*Nitrosomonas europea* culture	0–95%	Time, $[NH_4]$, $[NH_2OH]$, $[PO_4]$
Bremner and Blackmer 1979	Soil incubation (C_2H_2 inhibition)	0.04–0.2%	Soil types
Freney *et al*. 1979	Soil incubation (aerobic)	0.1%	
Goreau *et al*. 1980	Cultures (various nitrifiers)	0.3–8%	pO_2, microorganism
Lipschultz *et al*. 1981	*Nitrosomonas europea* culture	0.15–2.5%	pO_2
Goodroad and Keeney 1984	Soil incubation	0.1–1.1%	pH, pO_2 and temperature
Hynes and Knowles 1984	*Nitrosomonas europea* culture	0.06–12.26%	NH_4, pO_2
Jørgensen *et al*. 1984	Marine sediment incubation (N-serve inhibition)	Up to 25%	pO_2
Martikainen 1985	Forest soil incubations (inhibitors)	Up to 20%	pH
Tortoso and Hutchinson 1990	Soil incubation (inhibitors)	0.02%	
Remde and Conrad 1990	*Nitrosomonas europea* culture	0.1–3.9%	Cell density
Martikainen and de Boer 1993	Forest soil slurry incubation (C_2H_2 inhibition)	Up to 1%	pH
Maag and Vinther 1996	Soil incubations (C_2H_2 inhibition)	0.17–0.93%	H_2O and temperature
Kester *et al*. 1997	*Nitrosomonas europea* chemostat culture	0.04–0.78%	Air saturation, *N. winogradskyi*
Jiang and Bakken 1999	Cultures (various nitrifiers)	0.07–5%	Strains, pH buffering, NH_4 limitation
Ingwersen *et al*. 1999	Intact soil cores (barometric pressure separation)	0.01–0.055%[A]	Temperature 5−25°C
Garrido *et al*. 2002	Soil incubations (C_2H_2 inhibition)	<0.001–1%	Soil types, H_2O
Khalil *et al*. 2004	Soil incubation (^{15}N)	0.16–1.48%[A]	pO_2
Cheng *et al*. 2004	Soil incubations	0.01–0.22%	Time
Ambus 2005	Grass-clover pasture monoliths (^{15}N)	0.004–0.29%[A]	Water, time
Bateman and Baggs 2005	Soil incubation (^{15}N, C_2H_2 inhibition)	0.17–0.53%[A]	Water
Mathieu *et al*. 2006	Batch experiment (^{15}N)	0.13–2.32%[A]	Water
Mørkved *et al*. 2006	Soil incubations (^{15}N)	<0.1–27%	pO_2
Mørkved *et al*. 2007	Soil slurries (^{15}N)	0.02–7.6%	pH
Carter 2007	Grass sward (^{15}N microplots)	0.02–0.29%[A]	N source
Chen *et al*. 2010	Soil incubation (inhibitors)	0.03–0.12%	Water, temperature
Galbally *et al*. 2010	Legume pasture (^{15}N microplots)	0.01–0.05%[A]	
Frame and Casciotti 2010	Marine (*Nitrosomonas marina*) cultures (site preference)	0.4–2.2%	Cell density, pO_2, $[NO_2^-]$
Zhu *et al*. 2013*a*	Soil incubation (^{15}N-^{18}O isotopes and 0.1% C_2H_2)	0.09–8.3%[A]	O_2, N source

[A]Gross nitrification rates specified.

the role of ammonia sorption in moderating the inhibitory effect of ammonia on nitrite oxidation and hence N_2O from nitrification was recently identified (Venterea *et al*. 2015).

It has been suggested that, in many Australian environments, nitrification may be a significant contributor to N_2O emissions because much of our broadacre agriculture occurs in a semi-arid climate and soil fertility is relatively low (e.g. Barton *et al*. 2008, 2010; 2011). However, few studies attribute N_2O emissions to the actual processes that produce them, notwithstanding the diversity in soils, climate and management practices. Without this knowledge, estimates of the contribution of nitrification *v.* denitrification to total N_2O fluxes can vary widely, and the ability to develop robust mitigation strategies remains compromised.

This study used laboratory-based incubations to estimate nitrification rates and associated N_2O emissions under standard conditions in a range of soils from field sites within the National Agricultural Nitrous Oxide Research Program (see papers from this special journal issue for details). The objectives of the study were to provide evidence for site-specific parameterisation of models and to test model assumptions. In addition, incubation conditions were modified to explore whether any factors result in changes to the proportion of nitrified N emitted as N_2O, which for many models has been a fixed value with limited experimental evidence. The goal remains to derive new algorithms where sufficient information is available so that models can be modified to account for the known variability in potential nitrification rates and the proportion of nitrified N emitted as N_2O. Such models will be crucial for developing mitigation strategies and performing life-cycle analyses and are likely to have a role in improving inventories of N_2O emissions for the agricultural sector.

Materials and methods

A simple incubation technique was used to allow an approximation of potential nitrification rates and the associated N_2O emissions in standardised laboratory incubations, adopting similar methods to Garrido *et al*. (2002) and Freney *et al*. (1979), who conducted extensive method testing. The method relies on maintaining aerobic conditions in which case denitrification does not occur. Assuming that no nitrate is immobilised and abiotic production of N_2O is non-existent, the increase in nitrate is used to calculate the nitrification rate, and all N_2O produced can be attributed to nitrification. Although net nitrification rates were measured, we anticipate that the net nitrification rates approximate the gross rates under the conditions of the experiments. To test whether the assumption of no denitrification holds, acetylene at 10 kPa was added to a subset of samples representative of each treatment within each experiment to inhibit both nitrification and the reduction of N_2O to N_2 by denitrifiers (Davidson *et al*. 1986; Klemedtsson *et al*. 1988). This test allowed the detection of denitrification for each experimental treatment to give some confidence in the validity of the results. A pre-incubation period allowed the soil biota to resuscitate after soil sampling, transport and preparation, as well as avoiding flushes of activity on wetting up.

Soils and incubation

Soil sampling

Soils were sampled from each of the sites in Table 2. Soil samples from all sites were collected at 0–10 cm depth, and an additional sample at the Camden site was collected at 0–2 cm depth. Samples were air-dried at 40°C in a draught oven (except for Lucaston where field-moist samples were immediately transported on ice to preserve the temperature sensitivity of the microbial community) before transportation to the laboratory.

Soil preparation

Air-dried soil was sieved to ≤5 mm (the Hamilton soil was also sieved to ≤2 mm) to exclude large chunks of root material and gravel. The gravimetric water content of the sieved soil at

Table 2. Sampling location and some characteristics of the soils used in this study
CEC, Cation exchange capacity; SF fine sand; SC, course sand; θ_{10kPa}, water content at 10 kPa suction

Site	Lat., long.	Land use	pH 1 : 5 Water	pH 1 : 5 $CaCl_2$	NH_4^+-N (initial; mg kg^{-1})	NO_3^--N (initial; mg kg^{-1})	Org. C (% w/w)	Fe^A (mg kg^{-1})	Mn^A (mg kg^{-1})	CEC (cmol kg^{-1})	Clay (%)	Silt (%)	SF (%)	SC (%)	θ_{10kPa} (% w/w)
Hamilton (Vic.)	–37.6, 142.1	Cropping	5.8	5.2	16	12	3.1	296	8.5	11.4	25	26	40	4	42
Mundiwa clay loam (NSW)	–34.6, 146.4	Cropping	5.4	4.7	15	3	2.0	340	27	9.6	31	20	45	3	27
Hanwood loam (NSW)	–34.3, 146.1	Cropping	7.4	6.6	23	1	1.2	24	9	16.2	23	8	52	18	26
Banna sand (NSW)	–34.3, 146.1	Cropping	7.5	6.5	24	11	1.1	16	13	7.9	6	5	77	12	14
Wagga Wagga (NSW)	–35.0, 147.3	Cropping	6.4	5.8	17	34	1.6	61	27	8.5	14	23	53	11	23
Tamworth (NSW)	–31.2, 151.0	Cropping	7.3	6.3	17	4	1.0	15	19	43.6	39	24	33	4	53
Kingsthorpe (Qld)	–29.3, 152.5	Cropping	7.9	6.9	16	2	1.3	17	21	60.4	60	19	21	0	60
Kingaroy legume (Qld)	–26.6, 151.9	Cropping	6.5	5.6	17	2	1.7	45	78	13.1	49	15	20	16	29
Kingaroy grass (Qld)	–26.6, 151.9	Cropping	6.6	5.8	17	2	1.8	40	66	14.6	51	16	24	9	29
Camden (NSW)	–34.1, 150.7	Dairy pasture	6.3	5.9	12	12	2.6	140	64	11.5	10	23	63	5	28
Camden 0–2 cm (NSW)	–34.1, 150.7	Dairy pasture			20	31									33
Noorat (Glenormiston) (Vic.)	–38.2, 143.0	Dairy pasture	6	5.4	7	9	5.9	140	64	34.9	11	36	49	3	49
Terang (Vic.)	–38.3, 142.9	Dairy pasture	5.5	4.8	25	9	4.6	490	6.9	11	8	29	50	14	47
Lucaston (Tas.)	–43.0, 147.0	Horticulture	7	6.3	12	30	3.5	37	2.1	19.1	20	17	56	7	35

[A]DTPA extraction.

100 cm of water suction or 10 kPa (denoted θ_{10kPa}) was measured by using suction plates (Table 2).

Experimental units were prepared by weighing 7 g (oven-dry equivalent) of soil into 50-mL centrifuge tubes and then tapping the tube gently on a workbench to aid settling of the soil and give an even surface. The experiments are therefore best described as loose soil incubations, although some settling to a natural bulk density occurred, but was not quantified.

Pre-incubation

Soils were pre-incubated to allow microbial activity to recover after sampling and processing, and to avoid flushes of activity on wetting up. Soils were pre-incubated by wetting up to a defined water content with Milli-Q water, which allowed the addition of 250 µL substrate at the commencement of the incubation proper (see below). Moisture and air exchange were maintained for 2–3 weeks at 22°C in a constant-temperature room. In some experiments, additional treatments involving the pre-incubation phase (e.g. no pre-incubation to examine initial flushes of activity and thermal acclimation) were included.

Incubation proper

At the start of the incubation proper, water contents were adjusted and substrate added, giving the final water content as defined in Table 3 as a percentage of θ_{10kPa} and 60% θ_{10kPa} for the Kingaroy and Lucaston experiments. For most experiments, ammonium sulfate at 100 µg N g^{-1} dry soil was added, but ammonium concentrations were altered in some experiments as per Table 3. All solutions were added by pipetting evenly onto the soil surface. Tubes were immediately capped with Suba-Seals (Sigma-Aldrich, St Louis, MO, USA) then equilibrated to atmospheric pressure by using an open-ended needle. Soils were incubated for 2–4 days. Various treatments were imposed at time zero, usually in a balanced factorial design with four replicates.

The consumption of oxygen was quantified in preliminary experiments using CO_2 measurements and found to be well below the oxygen available in the headspace, although it is acknowledged that localised anaerobiosis in microsites is possible. Hence, acetylene was used in a subset of samples to detect denitrification. Within each replicate an additional sample of each treatment was injected with 10 kPa of acetylene (10% of an atmosphere), with the accumulation of N_2O being indicative of denitrification, because nitrous oxide reductase activity was blocked (Yoshinari and Knowles 1976) simultaneously with ammonia oxidase (Hynes and Knowles 1978; Bremner and Blackmer 1979; Walter *et al*. 1979).

Time zero samples were sampled immediately as described below. Remaining samples were incubated at 22°C in a constant temperature room, except for the Lucaston experiment in which water baths were used to modify the temperatures (see Table 3).

Incubation sampling

Prior to gas sampling, the soil atmosphere was mixed with the headspace by vigorously bashing each tube on the bench. Tubes were sampled by taking gas samples in excess of 20 mL through the Suba-Seal using a Hamilton 50-mL gas-tight syringe (Hamilton Company, Reno, NV, USA) equipped with a valve and side-port taper needles. The use of an oversized syringe and negative pressure also assisted with mixing the soil and headspace atmospheres. Gas (20 mL at atmospheric pressure) was then injected into evacuated glass 12-mL Exetainers (Labco, Lampeter, UK). Extracts of the soil (1 : 5 KCl) were performed by adding 35 mL of 2 M KCl directly to the tubes immediately after gas sampling and shaking for 2 h. The tubes were centrifuged and the supernatant was filtered before freezing until further analysis.

Analyses

The gas samples were analysed for N_2O, CO_2 and CH_4 on an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) fitted with an electron capture detector (ECD), thermal conductivity detector and flame ionisation detector and equipped with HayeSep packed columns (HayeSeparations Inc., Bandera, TX, USA) using helium carrier gas with 10% CH_4 in argon as a makeup gas for the ECD. KCl extracts were analysed for NH_4-N and NO_x-N by flow injection colourimetry on a Lachat QuikChem 8500 series 2 (Lachat, Loveland, CO, USA). Nitrate was reduced to nitrite by Cd reduction, and the resultant nitrite was reacted with N-1-naphthylethylenediamine dihydrochloride with sulphanilamide; ammonium was determined after reaction with sodium salicylate and dichloro-isocyanurate as per Rayment and Lyons (2011). A subset of samples was analysed for nitrite alone, but levels were negligible so all NO_x-N was assumed to be NO_3-N.

Calculations

The method used assumes that no denitrification occurred and a check was performed using acetylene to block both nitrification and the reduction of N_2O to N_2 by denitrifiers. No increase in N_2O concentration over time in tubes with acetylene for a particular treatment provided confidence that no denitrification occurred within that treatment.

Where no denitrification occurred, the increase in nitrate from time zero approximates the nitrification rate because the nitrate was not consumed by denitrification, and NO loss and NO_2^- accumulation (tested in preliminary experiments) were considered minimal. Immobilisation of nitrate was also considered minimal given the pre-incubation period and this was supported by measurements of mineral N at each stage of the experiment. The increase in headspace N_2O from time zero was considered to be the N_2O produced via nitrification, because no denitrification occurred and N_2O production from other sources was assumed to be non-existent.

Both the nitrate accumulation (nitrification) and associated N_2O emissions are presented as a mass of nitrogen transformed g^{-1} soil day^{-1}.

In addition to N_2O in the soil atmosphere and headspace, dissolved N_2O was calculated by assuming equilibration with the headspace and using the Henry's law constant, the concentration of N_2O in the headspace and the temperature of the sample (Hudson 2004). Thus, the total amounts of N_2O produced (soil atmosphere and headspace plus dissolved N_2O) are presented.

Table 3. Potential nitrification rates, associated nitrous oxide emissions and product ratio by soil and treatments

The water content for Kingaroy and Lucaston was 60% of the gravimetric water content at 10 kPa of suction (θ_{10kPa}). Values in parentheses are the standard deviations (s.d.) of up to four replicates

Site (land use)	Treatment 1	Treatment 2	Nitrification rate ($\mu g\ N\ g^{-1}$ soil day^{-1})	N_2O from nitrification ($\eta g\ N\ g^{-1}$ soil day^{-1})	P_n (%)
Hamilton Cropping	2-mm sieved	40% θ_{10kPa}	2.51 (0.59)	0.94 (3.76)	0.040 (0.019)
		60% θ_{10kPa}	3.72 (1.14)	1.16 (0.42)	0.031 (0.003)
		80% θ_{10kPa}	Denitrification present		
	5-mm sieved	40% θ_{10kPa}	3.37 (2.83)	1.19 (1.64)	0.047 (0.028)
		60% θ_{10kPa}	4.56 (2.60)	1.37 (0.63)	0.033 (0.011)
		80% θ_{10kPa}	Denitrification present		
Griffith lysimeters Irrigated cropping	Mundiwa clay loam	60% θ_{10kPa}	2.16 (0.93)	2.40 (0.73)	0.131 (0.064)
		70% θ_{10kPa}	2.85 (0.89)	2.47 (0.57)	0.096 (0.041)
		80% θ_{10kPa}	2.72 (0.43)	2.50 (0.46)	0.095 (0.029)
		90% θ_{10kPa}	2.73 (0.90)	3.15 (1.47)	0.122 (0.061)
	Hanwood loam	60% θ_{10kPa}	5.24 (2.02)	4.41 (1.06)	0.089 (0.017)
		70% θ_{10kPa}	6.26 (2.35)	3.83 (2.69)	0.092 (0.043)
		80% θ_{10kPa}	5.76 (1.35)	3.49 (1.10)	0.061 (0.016)
		90% θ_{10kPa}	7.76 (2.02)	4.57 (1.41)	0.059 (0.010)
	Banna sand	60% θ_{10kPa}	2.05 (1.11)	1.21 (0.41)	0.065 (0.018)
		70% θ_{10kPa}	1.34 (0.47)	1.11 (0.09)	0.087 (0.024)
		80% θ_{10kPa}	2.79 (1.04)	1.06 (0.16)	0.040 (0.010)
		90% θ_{10kPa}	1.67 (0.82)	1.39 (0.36)	0.102 (0.052)
Wagga Wagga Cropping	pH 5.5	60% θ_{10kPa}	3.38 (1.61)	1.13 (0.19)	0.039 (0.020)
		80% θ_{10kPa}	2.57 (0.90)	0.96 (0.17)	0.041 (0.013)
	pH 7.5	60% θ_{10kPa}	2.74 (0.61)	1.46 (0.22)	0.056 (0.016)
		80% θ_{10kPa}	3.30 (0.17)	2.15 (0.33)	0.065 (0.011)
Tamworth Cropping	40% θ_{10kPa}	NH_4-N 10 $\mu g\ g^{-1}$	2.95 (1.83)	3.21 (2.07)	0.115 (0.019)
		NH_4-N 20 $\mu g\ g^{-1}$	4.20 (0.18)	5.46 (0.59)	0.130 (0.012)
		NH_4-N 50 $\mu g\ g^{-1}$	4.53 (0.36)	5.42 (0.59)	0.120 (0.011)
		NH_4-N 100 $\mu g\ g^{-1}$	4.67 (0.23)	5.76 (1.22)	0.123 (0.021)
	60% θ_{10kPa}	NH_4-N 0 $\mu g\ g^{-1}$	2.42 (0.03)	3.27 (1.82)	0.135 (0.076)
		NH_4-N 10 $\mu g\ g^{-1}$	6.85 (0.42)	21.24 (13.77)	0.317 (0.224)
		NH_4-N 20 $\mu g\ g^{-1}$	7.31 (1.90)	14.73 (8.12)	0.188 (0.071)
		NH_4-N 50 $\mu g\ g^{-1}$	9.92 (0.70)	27.87 (6.30)	0.279 (0.046)
		NH_4-N 100 $\mu g\ g^{-1}$	9.97 (0.84)	52.50 (35.98)	0.532 (0.369)
	80% θ_{10kPa}	NH_4-N 100 $\mu g\ g^{-1}$	Denitrification present		
Kingsthorpe Cropping	40% θ_{10kPa}	NH_4-N 10 $\mu g\ g^{-1}$	0.95 (0.13)	0.80 (0.28)	0.086 (0.037)
		NH_4-N 20 $\mu g\ g^{-1}$	0.72 (0.40)	0.95 (0.60)	0.115 (0.053)
		NH_4-N 50 $\mu g\ g^{-1}$	1.10 (0.08)	1.18 (0.15)	0.107 (0.015)
		NH_4-N 100 $\mu g\ g^{-1}$	1.31 (0.49)	1.22 (0.18)	0.102 (0.037)
	50% θ_{10kPa}	NH_4-N 10 $\mu g\ g^{-1}$	2.53 (0.04)	5.11 (0.40)	0.202 (0.015)
		NH_4-N 20 $\mu g\ g^{-1}$	2.81 (0.21)	5.42 (1.17)	0.192 (0.034)
		NH_4-N 50 $\mu g\ g^{-1}$	2.89 (0.16)	6.11 (1.59)	0.210 (0.047)
		NH_4-N 100 $\mu g\ g^{-1}$	3.28 (0.44)	7.79 (1.50)	0.239 (0.050)
Kingaroy grass Cropping	No pre-incubation	Nil NH_4-N	0.66 (0.17)	2.87 (0.27)	0.447 (0.081)
		NH_4-N 100 $\mu g\ g^{-1}$	0.53 (0.32)	3.47 (0.20)	0.881 (0.499)
	Pre-incubated	Nil NH_4-N	1.97 (0.22)	5.78 (0.63)	0.295 (0.042)
		NH_4-N 100 $\mu g\ g^{-1}$	1.79 (0.13)	6.79 (0.66)	0.382 (0.046)
Kingaroy legume Cropping	No pre-incubation	Nil NH_4-N	0.94 (0.14)	4.19 (0.87)	0.446 (0.055)
		NH_4-N 100 $\mu g\ g^{-1}$	1.77 (0.81)	5.87 (0.54)	0.363 (0.136)
	Pre-incubated	Nil NH_4-N	2.06 (0.19)	8.18 (1.84)	0.403 (0.113)
		NH_4-N 100 $\mu g\ g^{-1}$	1.88 (0.16)	10.71 (1.40)	0.575 (0.107)
Camden Dairy pasture	0–10 cm soil	40% θ_{10kPa}	9.03 (2.60)	8.21 (2.72)	0.092 (0.016)
		50% θ_{10kPa}	13.61 (1.97)	11.07 (0.97)	0.084 (0.021)
		60% θ_{10kPa}	16.26 (1.38)	15.78 (2.25)	0.098 (0.019)
		70% θ_{10kPa}	17.14 (1.88)	15.59 (3.86)	0.092 (0.023)
		80% θ_{10kPa}	18.31 (0.46)	12.69 (0.89)	0.069 (0.006)
	0–2 cm soil	50% θ_{10kPa}	23.78 (6.11)	19.63 (4.75)	0.084 (0.017)
		60% θ_{10kPa}	28.05 (5.82)	18.53 (2.73)	0.067 (0.005)
		70% θ_{10kPa}	32.75 (3.35)	22.76 (1.26)	0.070 (0.006)

(*continued next page*)

Table 3. (*continued*)

Site (land use)	Treatment 1	Treatment 2	Nitrification rate ($\mu g\ N\ g^{-1}$ soil day^{-1})	N_2O from nitrification ($\eta g\ N\ g^{-1}$ soil day^{-1})	P_n (%)
Noorat	42% θ_{10kPa}		3.40 (0.38)	11.81 (3.87)	0.342 (0.091)
Dairy pasture	54% θ_{10kPa}		4.83 (1.47)	22.55 (5.89)	0.500 (0.196)
	65% θ_{10kPa}		5.28 (1.33)	18.48 (6.03)	0.381 (0.179)
	75% θ_{10kPa}		6.46 (1.41)	24.17 (5.19)	0.376 (0.032)
	87% θ_{10kPa}		Denitrification present		
Terang	40% θ_{10kPa}		2.29 (0.18)	2.58 (1.68)	0.114 (0.081)
Dairy pasture	60% θ_{10kPa}		2.40 (0.28)	2.63 (1.08)	0.110 (0.042)
	80% θ_{10kPa}		Possible denitrification		
Lucaston	30°C		1.75 (0.52)	4.38 (0.99)	0.257 (0.044)
Horticulture	35°C		4.05 (1.07)	4.68 (1.62)	0.127 (0.059)
	40°C		2.49 (0.20)	4.22 (2.92)	0.225 (0.022)
	45°C		0.92 (0.80)	5.66 (0.65)	01.076 (0.771)

Finally, the N_2O from nitrification was normalised by dividing the N_2O production rate by the apparent nitrification rate. Most publications express N_2O as a proportion of nitrified N; however, this may be misleading in situations where only net nitrification rates were measured. This metric should be reserved for experiments where the gross nitrification rate was measured. Regardless, in our work, there should be only a minor variance between the ratio and the proportion because the production of N_2O from nitrification was generally three orders of magnitude lower than the nitrification rate, and gross nitrification should be reasonably close to the net nitrification given the conditions of the experiment.

Statistical analyses

All statistical analysis was done using GENSTAT 16 (VSN International, Hemel Hempstead, UK). Each experiment was analysed separately by using a balanced design and a 1-, 2- or 3-factor analysis of variance, depending on the number of treatments. Data were checked for homogeneity of variance and normality. In some experiments, the treatments were non-orthogonal, in which case subsets of treatments were tested separately. In some experiments, obvious outliers were identified and with additional evidence were omitted from the analysis. In these cases, an unbalanced analysis of variance was performed.

Results

Potential nitrification rates ranged from <1 to 33 $\mu g\ N\ g^{-1}$ soil day^{-1}, with the majority of cases being <6 $\mu g\ N\ g^{-1}$ soil day^{-1}. The potential nitrification rate varied by site and by treatments such as soil depth, water content, temperature and ammonium concentration.

The N_2O emissions associated with nitrification ranged from <1 to 50 $\eta g\ N\ g^{-1}$ soil day^{-1}, although the upper values were subject to large within-replicate variability. Generally, when potential nitrification rates were high, so too were the associated N_2O emissions, although there were some discrepancies resulting in variation in the ratio of N_2O produced to the nitrification rate.

The ratio of N_2O to nitrification rate ranged from 0.03% to 1%, although the high values were associated with greater variability within replicates and could be considered an overestimate. There was significant site-to-site variation in the ratio (see Table 3), and factors such as water content, ammonium availability, pH and temperature also affected it, but often in interactions with other treatments and sometimes inconsistently across experiments. Relationships between the nitrification rate and N_2O grouped by site can be seen in Fig. 2.

Water content

In general, the nitrification rates increased with water content, as did the associated N_2O emissions. In some cases (Tamworth, Kingsthorpe), the nitrification-derived N_2O increased more than the nitrification rate itself with increasing water content, so the ratio of the N_2O produced to the nitrification rate (i.e. P_n) was greater at higher water contents. However, the increase in P_n with water content was not widespread as demonstrated by the Camden, Griffith lysimeters soils, Terang and Noorat experiments.

Ammonium availability

In the Tamworth experiment, increasing ammonium availability up to 50 $\mu g\ N\ g^{-1}$ soil did result in higher nitrification rates for soil at 60% θ_{10kPa} but not at 40% θ_{10kPa}. The N_2O associated with nitrification and the ratio of N_2O produced to the nitrification rate appeared to increase with N addition, although there was significant variability in these data. There was also an increase in N_2O from nitrification with increasing NH_4 addition in the Kingsthorpe soil at 50% θ_{10kPa}.

The addition of NH_4-N at 100 $\mu g\ N\ g^{-1}$ soil had minimal effects on nitrification rates compared with no additional ammonium in the Kingaroy experiment; however, higher N_2O emissions and proportions of nitrified N emitted as N_2O were evident with N addition, particularly on the soil with a legume history.

Soil depth

Potential nitrification rates were higher in the surface 0–2 cm than the 0–10 cm soil from the Camden site. Similarly, the rate of N_2O production associated with nitrification was higher in the surface soil. Accordingly, there was little variance in the proportion of nitrified N emitted as N_2O by depth.

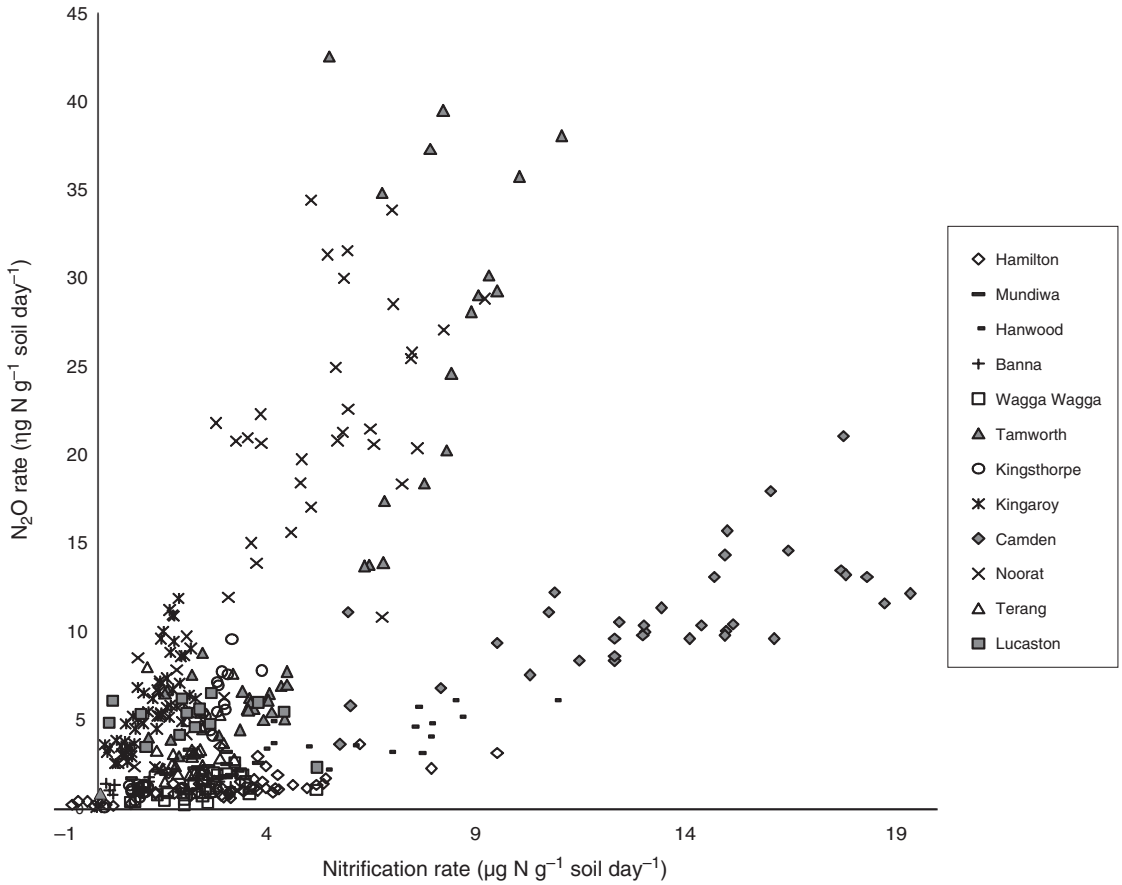

Fig. 2. Scatterplot of nitrous oxide (N_2O) emissions vs nitrification rate across all soils, grouped by soil.

Aggregate size

In the Hamilton experiment, the soil was sieved to $\leq$5 mm as in other experiments, and to $\leq$2 mm in an attempt to reduce variability thought to be associated with aggregates and particulate organic matter that was observed in a preliminary experiment. In this experiment, the sieve size had little effect on nitrification rates and associated N_2O emissions.

pH

The pH of the Wagga Wagga soil was manipulated by the addition of calcium hydroxide, raising the pH from 5.5 to 7.5 during the 3-week pre-incubation period. The nitrification rate was only marginally higher in the higher pH soil, whereas the N_2O from nitrification was significantly higher in the higher pH soil at 80% θ_{10kPa} but not at 60% θ_{10kPa}. The proportion of nitrified N emitted as N_2O was on average higher in the higher pH soil (0.056% *v.* 0.035%), but these values were still much lower than in other soils.

History

In the Kingaroy experiment, soils with a legume or grass history were compared. Nitrification rates were higher in the soil with a legume history and this soil responded to added N compared with the soil with a grass history, which did not respond to added N.

Temperature

The Lucaston experiment demonstrated a peak in the nitrification rate at 35°C and very low nitrification rates at 45°C. By contrast, the associated N_2O emissions were relatively consistent with the highest emission coming from the 45°C treatment. Accordingly, we calculated that 1% of the nitrified N was emitted as N_2O at 45°C but acknowledge the variability in that treatment. The possibility of thermal decomposition of hydroxylamine to N_2O at these high temperatures was noted.

Texture

The three soils of different texture (sand, loam and clay loam) from the Griffith lysimeters were compared within the same experiment, but water contents were set as proportions of water content at 10 kPa of suction to provide some semblance of attainable water contents in the field rather than on a volumetric or gravimetric basis. At similar proportions of θ_{10kPa}, the loam soil had the highest nitrification rates, which increased with water content, but also the lowest proportion of nitrified N emitted as N_2O. The high nitrification rates in the loam were

consistent with lysimeter studies where this soil had higher mineralisation rates and accumulated more nitrate (Jamali *et al*. 2016).

Discussion

The contribution of nitrification to N_2O emissions under aerobic conditions is likely to be small, with ratios of N_2O emitted to nitrate accumulation generally peaking at 0.5% for some sites, but being <0.1% for most sites and conditions. Evidently, using a fixed value for the proportion of nitrified N emitted as N_2O in models is not correct, although for some soils (e.g. Camden) the value calculated was reasonably consistent across treatments. The values we obtained for the ratio of N_2O emitted to nitrate accumulated are more constrained in range than published proportions, which range from 0.01% to >20% (Table 1). It should be noted, though, that the values obtained in the present work are associated with aerobic conditions, and it is probable that the proportion of nitrified N emitted as N_2O will increase with fluctuating aerobic and anaerobic conditions. In particular, Yu *et al*. (2010), using pure cultures, showed that N_2O production from nitrifiers occurs on the transition from anaerobic to aerobic conditions and is related to the accumulation of ammonium during the anaerobic phase.

The nitrification rates estimated here are intended to set an upper limit on the rate of conversion of ammonium to nitrate for each soil at a standard temperature of 22°C. Although it is not possible to extrapolate directly from laboratory-based assays to rates in the field, such data can be used to modify potential nitrification rates in models in a relative sense and provide an evidence-based means to do so when consistent methodology is used. The site-to-site variation in potential nitrification rates demonstrates the need to adjust the models for each site, but to do so without evidence risks developing models that provide reasonable simulations for the wrong reasons and can limit the portability of such models through both space and time. Simple incubation methods such as used in this work provide independent evidence for site-specific calibration instead of relying purely on mathematical or non-objective means of calibrating models, and have been employed in some modelling strategies such as NOE (Hénault *et al*. 2005), where empirical evidence is used to parameterise the model.

Overall, between-site variation in potential nitrification rates and associated N_2O emissions was greater than within-site variation, demonstrating the need to characterise and parameterise each site individually. Further analysis is required to determine which properties govern the site-to-site variation; however, from the results a few candidates emerge.

The Camden soil had higher potential nitrification rates across a range of water contents than other soils, indicative of rapid nitrogen cycling, which, if uncoupled from nitrogen uptake by plants or microorganisms (e.g. from land-use change), might result in high fluxes of N_2O. This scenario can be seen from the field-scale measurements at the Hamilton site where cropping has been introduced into productive pasture (Belyaeva *et al*. 2016).

With the exception of Noorat, the cropping sites appear to have higher proportions of nitrified N emitted as N_2O than the dairy pasture sites. Until a firm mechanism for N_2O production via nitrification is identified, it is very difficult to determine why. Possible explanations may be related to soil type and longer term management such as soil disturbance, nitrogen addition or efficiency of plant uptake. The Kingaroy site in particular had a high proportion of nitrified N emitted as N_2O. In this soil, the nitrification rate was not very sensitive to the addition of ammonium, with added ammonium at 0 and 100 $\mu g\,N\,g^{-1}$ soil having similar but low rates of nitrification. However, the N_2O from nitrification was sensitive to the addition of ammonium, especially in the soil from the legume site. We hypothesise that the presence of legumes altered the soil microbial community, resulting in the observed differences. The Tamworth site was notable for the relatively high N_2O emissions associated with nitrification that was responsive to the addition of ammonium. The consistent presence of denitrification in the 80% θ_{10kPa} treatment makes the Tamworth soil suitable for further studies, including examining water relations, oxygen availability and thresholds for the presence of denitrification.

Nitrification rates increased with increasing water content, even at water contents generally thought to inhibit aerobic processes (e.g. the 90% θ_{10kPa} treatment in the Hanwood loam). Indeed, water content is widely used as a proxy for the aerobicity of soil even though water can contain significant amounts of dissolved oxygen, and despite decoupling of the actual drivers and water content having been demonstrated previously (e.g. Hall *et al*. 2013). Water also affects the diffusion of solutes, not just gases, so the capture of these multiple effects is challenging.

Water content was not as strong a predictor of the proportion of nitrified N emitted as N_2O as anticipated. For example, in the Camden soil, the nitrification rate increased with water content, as did the N_2O from nitrification. Hence, the proportion of nitrified N emitted as N_2O remained constant with water content at that site. However, in other experiments, the proportion of nitrified N emitted as N_2O was greater at higher water contents, which is consistent with other reports (Table 1).

In some soils, a clear threshold of water content was evident for denitrification being present. These soils were from Hamilton, Tamworth, Terang and Noorat, each at 80% θ_{10kPa}. This study was not aimed at identifying thresholds for denitrification, although it would seem that acetylene at 10 kPa in laboratory incubations is useful for that purpose. Indeed, comparing the response of processes to water content across different soils is difficult because different metrics can be used, and water content is usually a proxy for several factors that influence process rates. With respect to N_2O emissions and soil water metrics, some progress has been made since Farquharson and Baldock (2008) highlighted this issue, with metrics such as volumetric water content and relative diffusivity providing improvements in explaining N_2O emissions (Castellano *et al*. 2010; van der Weerden *et al*. 2012; Balaine *et al*. 2013; Klefoth *et al*. 2015; Jamali *et al*. 2016). Ultimately, the modelling of oxygen demand and transport (Cook *et al*. 2013) is likely to provide the best prospects for improving simulations of N_2O dynamics.

Apart from response functions based on water content and temperature, most models do not account for several abiotic

interactions. At a basic level, some models invoke, for example, a physical protection of organic matter as a function of clay content, but to our knowledge, no model explicitly accounts for thermal decomposition of hydroxylamine or the catalysis of reactions by metal species, although DNDC purportedly tracks oxidation–reduction reactions over a range of potentials. The Lucaston experiment provided some evidence for a role of abiotic processes in producing N_2O. In work yet to be published (T. Lai, M. Denton, R. Farquharson, unpubl. data), we have consistently observed a decline in nitrification rates above a certain temperature (usually ~35°C), whereas N_2O emissions continue or decline to a lesser extent. Further investigation is required to unravel responses at high temperatures, considering that surface-soil temperatures >35°C are possible for much of the continent. Indeed, many models are built on data from temperate systems, resulting in poor model functions at the higher temperatures that can occur in Australian soils. There is also debate around thermal acclimation and adaptation, which is particularly pertinent when attempting to predict the influence of climate change on biogeochemical cycling.

Bulk soil pH has long been used to adjust potential nitrification rates in models, based on the observation that autotrophic nitrification rates tend to be lower on acidic soils. Indeed, nitrification and N_2O emissions were low from the Wagga Wagga soil with a pH_{CaCl} of 5.8; however, adjusting the pH to 7.5 over 3 weeks did not increase nitrification rates significantly. Like many soil properties, including redox potential, there are significant challenges in studying the influence of pH on microbial processes. Microsite pH where processes are active can be quite different from bulk soil pH and is in fact modified by the very processes we are interested in (Strong *et al*. 1997). In addition, issues of adaptation and buffering need to be considered over varying lengths of time (Bramley and White 1989). We did observe some differences with higher N_2O emissions on higher pH soil from Wagga Wagga, but we also observed large changes in ammonia availability in tubes with acetylene, indicating significant loss or immobilisation of ammonium-N in the acidic soil and significant gain (most likely due to mineralisation of organic N) in the higher pH soil. This can have indirect effects on nitrification (specifically nitrite oxidation) and deserves further attention (Venterea *et al*. 2015).

Several model assumptions were tested during the course of this work and the outcomes are summarised below:

- The proportion of nitrified N emitted as N_2O (P_n in Fig. 1) is not constant as assumed in many models.
- The proportion of nitrified N emitted as N_2O can vary as a function of water content (as now captured in some models, e.g. NOE; Bessou *et al*. 2010) but not always.
- Potential nitrification rates (Kmax in Fig. 1) do vary across sites. Potential nitrification rates should be parameterised using independent evidence because the cyclical nature of nitrogen dynamics makes it extremely difficult to resolve such parameters mathematically.
- Potential nitrification rates do vary with depth, with the surface soil being more active than deeper soil.
- Nitrification rates and N_2O emissions can increase with ammonium addition, but can also be unresponsive in some soils. Use of ammonium concentration as a multiplicative factor in models (e.g. Fig. 1) may need to be reconsidered, especially where saturation kinetics are not adopted. The sorption of ammonium related to cation exchange capacity (Venterea *et al*. 2015), pH changes, mineralisation–immobilisation dynamics and other limiting factors may need to be taken into account.
- The pH functions for nitrification rates in models are likely being misused given microsite issues, the existence of empirically derived potential nitrification rates, which would account for the differences, and the potential for soil microbial communities and functions to adapt.
- Water functions are not universal. Water functions are problematic because they attempt to account for multiple processes or mechanisms simultaneously (Hall *et al*. 2013). As a solution, explicit modelling of oxygen transport and consumption (Cook *et al*. 2013), for example, could be implemented in models.
- Microsites are prevalent in soils, with hotspots and hot moments potentially contributing significantly to the overall flux of N_2O from soil (Parkin 1987; Nielsen and Revsbech 1998; Groffman *et al*. 2009). Modelling soils in homogenous layers, although common, can be problematic. Hotspots are not a simple function of a single driver, so capturing the interactions and recognising heterogeneity in soil is important (Korsaeth *et al*. 2001). Averaging is the antithesis of capturing heterogeneity and may result in erroneous predictions, especially in non-linear systems with interacting factors, as is common for biogeochemical cycling.
- Many models have not been properly tested for responses at higher temperatures. We observed a thermal optimum for nitrification at ~35°C in one temperate soil, but we also note the possibility that thermal acclimation or adaptation can occur (Avrahami and Bohannan 2009; Gödde and Conrad 1999).
- The proportion of nitrified N emitted as N_2O appears to uncouple at high soil temperatures, which may be due to abiotic processes such as the decomposition of hydroxylamine to N_2O.
- Abiotic processes and some abiotic factors may be important and are not yet captured in most models. For example, the decomposition of hydroxylamine may be more rapid at high soil temperatures, which could explain the increase in P_n in the Lucaston experiment. As well, the role of metal ion species in the chemical oxidation of hydroxylamine (Heil *et al*. 2015) and the role of cation exchange capacity on ammonium sorption and nitrite oxidation (Venterea *et al*. 2015) require further investigation.
- Land-use history might be important in governing nitrogen cycling and, in addition to the above factors, is probably linked to changes in soil biota in response to more active nitrogen cycling, and longer term legacy effects related to organic matter dynamics.

In some of the experiments, considerable heterogeneity was observed. Experimental studies aim to reduce heterogeneity; however, when it comes to N_2O emissions, the heterogeneity itself might be an important determinant of process rates and hence emissions. The juxtaposition of nitrification and denitrification sites in soil is one example where heterogeneity

itself is important. Yet, models and experimental methods either do not explicitly recognise or attempt to minimise heterogeneity, when instead it needs to be accounted for at a range of scales (Turner *et al*. 2008).

To explore further the production of N_2O from nitrification when conditions simultaneously suit denitrification, alternative methods, including the use of stable isotopes, are required. We also suggest that some measurement of redox potential (e.g. Vorenhout *et al*. 2004) could be informative about which processes are active within a sample (e.g. Hernandez-Ramirez *et al*. 2009). Although microsite issues would remain (as indeed they do for measurements of pH), routine measurements of redox potential could provide valuable insights into which processes are responsible for the observations made (Husson 2013), as well as inform management practices to minimise emissions of N_2O and methane (Yu and Patrick 2003). In the medium term, it is important that standardised techniques are used and additional data collected to enable relationships to be elucidated across a range of soils by using appropriate statistical techniques.

Although observations in field experiments are useful, the resulting information can only be used to inform basic empirical models because the multiple processes and drivers, underlain by soil heterogeneity, are still poorly understood and rarely quantified. Only by understanding such complexity can truly mechanistic models be built. These very models will be crucial to developing robust and tailored mitigation strategies.

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References

Ambus P (2005) Relationship between gross nitrogen cycling and nitrous oxide emission in grass–clover pasture. *Nutrient Cycling in Agroecosystems* **72**, 189–199. doi:10.1007/s10705-005-1269-4

Avrahami S, Bohannan BJM (2009) N_2O emission rates in a California meadow soil are influenced by fertilizer level, soil moisture and the community structure of ammonia-oxidizing bacteria. *Global Change Biology* **15**, 643–655. doi:10.1111/j.1365-2486.2008.01727.x

Baggs E (2011) Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. *Current Opinion in Environmental Sustainability* **3**, 321–327. doi:10.1016/j.cosust.2011.08.011

Balaine N, Clough TJ, Beare MH, Thomas SM, Meenken ED, Ross JG (2013) Changes in relative gas diffusivity explain soil nitrous oxide flux dynamics. *Soil Science Society of America Journal* **77**, 1496–1505. doi:10.2136/sssaj2013.04.0141

Barton L, Kiese R, Gatter D, Butterbach-Bahl K, Buck R, Hinz C, Murphy DV (2008) Nitrous oxide emissions from a cropped soil in a semi-arid climate. *Global Change Biology* **14**, 177–192.

Barton L, Murphy DV, Kiese R, Butterbach-Bahl K (2010) Soil nitrous oxide and methane fluxes are low from a bioenergy crop (canola) grown in a semi-arid climate. *Global Change Biology. Bioenergy* **2**, 1–15. doi:10.1111/j.1757-1707.2010.01034.x

Barton L, Butterbach-Bahl K, Kiese R, Murphy DV (2011) Nitrous oxide fluxes from a grain–legume crop (narrow-leafed lupin) grown in a semiarid climate. *Global Change Biology* **17**, 1153–1166. doi:10.1111/j.1365-2486.2010.02260.x

Bateman EJ, Baggs EM (2005) Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* **41**, 379–388. doi:10.1007/s00374-005-0858-3

Belyaeva ON, Officer SJ, Armstrong RD, Harris RH, Wallace A, Partington DL, Fogarty K, Phelan AJ (2016) Use of the agricultural practice of pasture termination in reducing soil N_2O emissions in high-rainfall cropping systems of south-eastern Australia. *Soil Research* **54**, 585–597. doi:10.1071/SR15307

Bessou C, Mary B, Léonard J, Roussel M, Gréhan E, Gabrielle B (2010) Modelling soil compaction impacts on nitrous oxide emissions in arable fields. *European Journal of Soil Science* **61**, 348–363. doi:10.1111/j.1365-2389.2010.01243.x

Bramley RGV, White RE (1989) The effect of pH, liming, moisture and temperature on the activity of nitrifiers in a soil under pasture. *Australian Journal of Soil Research* **27**, 711–724. doi:10.1071/SR9890711

Bremner JM, Blackmer AM (1979) Effects of acetylene and soil water content on emission of nitrous oxide from soils. *Nature* **280**, 380–381. doi:10.1038/280380a0

Bremner JM, Blackmer AM, Waring SA (1980) Formation of nitrous oxide and dinitrogen by chemical decomposition of hydroxylamine in soils. *Soil Biology & Biochemistry* **12**, 263–269. doi:10.1016/0038-0717(80)90072-3

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **368**, 20130122. doi:10.1098/rstb.2013.0122

Carter MS (2007) Contribution of nitrification and denitrification to N_2O emissions from urine patches. *Soil Biology & Biochemistry* **39**, 2091–2102. doi:10.1016/j.soilbio.2007.03.013

Castellano MJ, Schmidt JP, Kaye JP, Walker C, Graham CB, Lin H, Dell CJ (2010) Hydrological and biogeochemical controls on the timing and magnitude of nitrous oxide flux across an agricultural landscape. *Global Change Biology* **16**, 2711–2720. doi:10.1111/j.1365-2486.2009.02116.x

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 Biology & Biochemistry* **42**, 660–664. doi:10.1016/j.soilbio.2009.12.014

Cheng W, Tsuruta H, Chen G, Yagi K (2004) N_2O and NO production in various Chinese agricultural soils by nitrification. *Soil Biology & Biochemistry* **36**, 953–963. doi:10.1016/j.soilbio.2004.02.012

Cook FJ, Knight JH, Kelliher FM (2013) Modelling oxygen transport in soil with plant root and microbial oxygen consumption: depth of oxygen penetration. *Soil Research* **51**, 539–553. doi:10.1071/SR13223

Davidson EA, Swank WT, Perry TO (1986) Distinguishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. *Applied and Environmental Microbiology* **52**, 1280–1286.

Farquharson R, Baldock J (2008) Concepts in modelling N_2O emissions from land use. *Plant and Soil* **309**, 147–167. doi:10.1007/s11104-007-9485-0

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. In 'Exchange of trace gases between terrestrial ecosystems and the atmosphere'. (Eds MO Andreae, DS Schimel) pp. 7–21. (John Wiley & Sons Ltd: Chichester, New York)

Frame CH, Casciotti KL (2010) Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. *Biogeosciences* **7**, 2695–2709. doi:10.5194/bg-7-2695-2010

Freney JR, Denmead OT, Simpson JR (1979) Nitrous oxide emission from soils at low moisture contents. *Soil Biology & Biochemistry* **11**, 167–173. doi:10.1016/0038-0717(79)90096-8

Galbally IE, Meyer MCP, Wang YP, Smith CJ, Weeks IA (2010) Nitrous oxide emissions from a legume pasture and the influences of liming and urine addition. *Agriculture, Ecosystems & Environment* **136**, 262–272. doi:10.1016/j.agee.2009.10.013

Garrido F, Hénault C, Gaillard H, Pérez S, Germon JC (2002) N_2O and NO emissions by agricultural soils with low hydraulic potentials. *Soil Biology & Biochemistry* **34**, 559–575. doi:10.1016/S0038-0717(01)00172-9

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. *Biology and Fertility of Soils* **30**, 33–40. doi:10.1007/s003740050584

Goodroad LL, Keeney DR (1984) Nitrous oxide production in aerobic soils under varying pH, temperature and water content. *Soil Biology & Biochemistry* **16**, 39–43. doi:10.1016/0038-0717(84)90123-8

Goreau TJ, Kaplan WA, Wofsy SC, McElroy MB, Valois FW, Watson SW (1980) Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. *Applied and Environmental Microbiology* **40**, 526–532.

Grant RF (1995) Mathematical modelling of nitrous oxide evolution during nitrification. *Soil Biology & Biochemistry* **27**, 1117–1125. doi:10.1016/0038-0717(95)00038-G

Groffman PM, Butterbach-Bahl K, Fulweiler RW, Gold AJ, Morse JL, Stander EK, Tague C, Tonitto C, Vidon P (2009) Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* **93**, 49–77. doi:10.1007/s10533-008-9277-5

Hall S, McDowell W, Silver W (2013) When wet gets wetter: decoupling of moisture, redox biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. *Ecosystems* **16**, 576–589. doi:10.1007/s10021-012-9631-2

Heil J, Liu SR, Vereecken H, Brüggemann N (2015) Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. *Soil Biology & Biochemistry* **84**, 107–115. doi:10.1016/j.soilbio.2015.02.022

Hénault C, Bizouarn F, Laville P, Gabrielle B, Nicoullaud B, Germon JC, Cellier P (2005) Predicting *in situ* soil N_2O emission using NOE algorithm and soil database. *Global Change Biology* **11**, 115–127. doi:10.1111/j.1365-2486.2004.00879.x

Hernandez-Ramirez G, Brouder SM, Smith DR, Van Scoyoc GE, Michalski G (2009) Nitrous oxide production in an Eastern corn belt soil: sources and redox range. *Soil Science Society of America Journal* **73**, 1182–1191. doi:10.2136/sssaj2008.0183

Hooper AB, Terry KR (1979) Hydroxylamine oxidoreductase of *Nitrosomonas* production of nitric-oxide from hydroxylamine. *Biochimica et Biophysica Acta* **571**, 12–20. doi:10.1016/0005-2744(79)90220-1

Hudson F (2004) 'Sample preparation and calculation for dissolved gas analysis in water samples using a GC headspace equilibration technique.' (US Environmental Protection Agency: Washington, DC)

Husson O (2013) Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: a transdisciplinary overview pointing to integrative opportunities for agronomy. *Plant and Soil* **362**, 389–417. doi:10.1007/s11104-012-1429-7

Hynes RK, Knowles R (1978) Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*. *FEMS Microbiology Letters* **4**, 319–321. doi:10.1111/j.1574-6968.1978.tb02889.x

Hynes RK, Knowles R (1984) Production of nitrous oxide by *Nitrosomonas europaea*: effects of acetylene, pH, and oxygen. *Canadian Journal of Microbiology* **30**, 1397–1404. doi:10.1139/m84-222

Ingwersen J, Butterbach-Bahl K, Gasche R, Richter O, Papen H (1999) Barometric process separation: new method for quantifying nitrification, denitrification, and nitrous oxide sources in soils. *Soil Science Society of America Journal* **63**, 117–128. doi:10.2136/sssaj1999.03615995006300010018x

Jamali H, Quayle W, Scheer C, Rowlings D, Baldock J (2016) Effect of soil texture and wheat plants on N_2O fluxes: A lysimeter study. *Agricultural and Forest Meteorology* **223**, 17–29. doi:10.1016/j.agrformet.2016.03.022

Jia Z, Conrad R (2009) *Bacteria* rather than *Archaea* dominate microbial ammonia oxidation in an agricultural soil. *Environmental Microbiology* **11**, 1658–1671. doi:10.1111/j.1462-2920.2009.01891.x

Jiang Q, Bakken LR (1999) Nitrous oxide production and methane oxidation by different ammonia-oxidizing bacteria. *Applied and Environmental Microbiology* **65**, 2679–2684.

Jørgensen KS, Jensen HB, Sørensen J (1984) Nitrous oxide production from nitrification and denitrification in marine sediment at low oxygen concentrations. *Canadian Journal of Microbiology* **30**, 1073–1078. doi:10.1139/m84-167

Kester RA, De Boer W, Laanbroek HJ (1997) Production of NO and N_2O by pure cultures of nitrifying and denitrifying bacteria during changes in aeration. *Applied and Environmental Microbiology* **63**, 3872–3877.

Khalil K, Mary B, Renault P (2004) Nitrous oxide production by nitrification and denitrification in soil aggregates as affected by O_2 concentration. *Soil Biology & Biochemistry* **36**, 687–699. doi:10.1016/j.soilbio.2004.01.004

Klefoth RR, Clough TJ, Oenema O, Van Groenigen JW (2015) Soil bulk density and moisture content influence relative gas diffusivity and the reduction of nitrogen-15 nitrous oxide. *Vadose Zone Journal* **13**.

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

Korsaeth A, Molstad L, Bakken LR (2001) Modelling the competition for nitrogen between plants and microflora as a function of soil heterogeneity. *Soil Biology & Biochemistry* **33**, 215–226. doi:10.1016/S0038-0717(00)00132-2

Li C, Frolking S, Frolking TA (1992) A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity. *Journal of Geophysical Research* **97**, 9759–9776. doi:10.1029/92JD00509

Li Y, White R, Chen D, Zhang J, Li B, Zhang Y, Huang Y, Edis R (2007) A spatially referenced water and nitrogen management model (WNMM) for (irrigated) intensive cropping systems in the North China Plain. *Ecological Modelling* **203**, 395–423. doi:10.1016/j.ecolmodel.2006.12.011

Lipschultz F, Zafiriou OC, Wofsy SC, McElroy MB, Valois FW, Watson SW (1981) Production of NO and N_2O by soil nitrifying bacteria. *Nature* **294**, 641–643. doi:10.1038/294641a0

Maag M, Vinther FP (1996) Nitrous oxide emission by nitrifiation and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology* **4**, 5–14. doi:10.1016/0929-1393(96)00106-0

Martikainen PJ (1985) Nitrous oxide emission associated with autotrophic ammonium oxidation in acid coniferous forest soil. *Applied and Environmental Microbiology* **50**, 1519–1525.

Martikainen PJ, de Boer W (1993) Nitrous oxide production and nitrification in acidic soil from a dutch coniferous forest. *Soil Biology & Biochemistry* **25**, 343–347. doi:10.1016/0038-0717(93)90133-V

Mathieu O, Hénault C, Leveque J, Baujard E, Milloux MJ, Andreux F (2006) Quantifying the contributions of nitrification and denitrification to the nitrous oxide flux using ^{15}N tracers. *Environmental Pollution* **144**, 933–940. doi:10.1016/j.envpol.2006.02.005

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 Biology & Biochemistry* **38**, 3411–3420. doi:10.1016/j.soilbio.2006.05.015

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 Biology & Biochemistry* **39**, 2048–2057. doi:10.1016/j.soilbio.2007.03.006

Nielsen TH, Revsbech NP (1998) Nitrification, denitrification, and N-liberation associated with two types of organic hotspots in soil. *Soil Biology & Biochemistry* **30**, 611–619. doi:10.1016/S0038-0717(97)00211-3

Parkin TB (1987) Soil microsites as a source of denitrification variability. *Soil Science Society of America Journal* **51**, 1194–1199. doi:10.2136/sssaj1987.03615995005100050019x

Parton WJ, Hartman M, Ojima D, Schimel D (1998) DAYCENT and its land surface submodel: description and testing. *Global and Planetary Change* **19**, 35–48. doi:10.1016/S0921-8181(98)00040-X

Poth M, Focht DD (1985) ^{15}N kinetic analysis of N_2O production by *Nitrosomonas europaea*: an examination of nitrifier denitrification. *Applied and Environmental Microbiology* **49**, 1134–1141.

Rayment GE, Lyons DJ (2011) 'Soil chemical methods: Australasia.' (CSIRO Publishing: Melbourne)

Remde A, Conrad R (1990) Production of nitric oxide in *Nitrosomonas europaea* by reduction of nitrite. *Archives of Microbiology* **154**, 187–191. doi:10.1007/BF00423331

Shaw LJ, Nicol GW, Smith Z, Fear J, Prosser JI, Baggs EM (2006) *Nitrosospira* spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environmental Microbiology* **8**, 214–222. doi:10.1111/j.1462-2920.2005.00882.x

Strong DT, Sale PWG, Helyar KR (1997) Initial soil pH affects the pH at which nitrification ceases due to self-induced acidification of microbial microsites. *Australian Journal of Soil Research* **35**, 565–570. doi:10.1071/S96055

Thorburn PJ, Biggs JS, Collins K, Probert ME (2010) Using the APSIM model to estimate nitrous oxide emissions from diverse Australian sugarcane production systems. *Agriculture, Ecosystems & Environment* **136**, 343–350. doi:10.1016/j.agee.2009.12.014

Tortoso AC, Hutchinson GL (1990) Contributions of autotrophic and heterotrophic nitrifiers to soil NO and N_2O emissions. *Applied and Environmental Microbiology* **56**, 1799–1805.

Turner DA, Chen D, Galbally IE, Leuning R, Edis RB, Li Y, Kelly K, Phillips F (2008) Spatial variability of nitrous oxide emissions from an Australian irrigated dairy pasture. *Plant and Soil* **309**, 77–88. doi:10.1007/s11104-008-9639-8

van der Weerden TJ, Kelliher FM, de Klein CAM (2012) Influence of pore size distribution and soil water content on nitrous oxide emissions. *Soil Research* **50**, 125–135.

Venterea RT, Clough TJ, Coulter JA, Breuillin-Sessoms F (2015) Ammonium sorption and ammonia inhibition of nitrite-oxidizing bacteria explain contrasting soil N_2O production. *Scientific Reports* **5**, 12153. doi:10.1038/srep12153

Vorenhout M, van der Geest HG, van Marum D, Wattel K, Eijsackers HJP (2004) Automated and continuous redox potential measurements in soil. *Journal of Environmental Quality* **33**, 1562–1567. doi:10.2134/jeq2004.1562

Walter HM, Keeney DR, Fillery IR (1979) Inhibition of nitrification by acetylene. *Soil Science Society of America Journal* **43**, 195–196. doi:10.2136/sssaj1979.03615995004300010038x

Yoshida T, Alexander M (1970) Nitrous Oxide Formation by Nitrosomonas Europaea and Heterotrophie Microorganisms. *Soil Science Society of America Journal* **34**(6), 880–882. doi:10.2136/sssaj1970.03615995003400060020x

Yoshinari T, Knowles R (1976) Acetylene inhibition of nitrous oxide reduction by denitrifying bacteria. *Biochemical and Biophysical Research Communications* **69**, 705–710. doi:10.1016/0006-291X(76)90932-3

Yu K, Patrick WH (2003) Redox range with minimum nitrous oxide and methane production in a rice soil under different pH. *Soil Science Society of America Journal* **67**, 1952–1958. doi:10.2136/sssaj2003.1952

Yu R, Kampschreur MJ, van Loosdrecht MCM, Chandran K (2010) Mechanisms and specific directionality of autotrophic nitrous oxide and nitric oxide generation during transient anoxia. *Environmental Science & Technology* **44**, 1313–1319. doi:10.1021/es902794a

Zhu X, Burger M, Doane TA, Horwath WR (2013*a*) Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 6328–6333. doi:10.1073/pnas.1219993110

Zhu X, Silva LCR, Doane TA, Horwath WR (2013*b*) Iron: the forgotten driver of nitrous oxide production in agricultural soil. *PLoS One* **8**, e60146. doi:10.1371/journal.pone.0060146