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SHORT COMMUNICATION

Temperature effects on N_2O and N_2 denitrification end-products for a New Zealand pasture soil

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Denitrification results in the formation of two gaseous end-products: radiatively active greenhouse gas nitrous oxide (N_2O) and radiatively inert di-nitrogen (N_2). Measuring N_2 produced by soil microorganisms has historically been difficult because of interference by high background concentrations (780,840 ppm) of atmospheric N_2. Here, we applied a new automated technique for simultaneous measurement of the denitrification end-products N_2O and N_2. We tested the hypothesis that the temperature response function (defined here as Q_{10}) for N_2O was greater than for N_2 under anaerobic conditions in the laboratory. The Q_{10} for N_2O was 2.0 and the Q_{10} for N_2 was 1.4, indicating differences in sensitivity to temperature. Denitrified-N shifted towards N_2O as temperature increased. While moisture strongly controls the relative abundance of denitrification end-products, additional experiments are needed to determine how a wider range of temperatures may affect N_2O and N_2. Results could enhance understanding of temperature controls on N_2O production and consumption processes.

Keywords: anaerobiosis; biogeochemistry; nitrification; nitrogen; soil temperature

Introduction

Temperature, along with moisture, is one of the most influential environmental factors affecting rates of nutrient cycling and production of greenhouse gases in soil (Kirschbaum 1995; Smith 1997). Emissions of the greenhouse gas nitrous oxide (N_2O) and inert di-nitrogen (N_2) are predicted to rise in New Zealand as a result of continued dairy expansion and application of nitrogen (N) fertilizers. However, few comprehensive quantitative data are available indicating effects of temperature on denitrification for New Zealand pasture soil. Both N_2O and N_2 are gaseous products of denitrification that may increase as soil temperatures rise under climate change (Smith et al. 2003), yet it is not known if the two major products of denitrification (N_2O and N_2) will both increase similarly when soil is exposed to higher temperatures.

Quantification of temperature effects on N_2O and N_2 production can be problematic when factors such as aeration status are not controlled due to multiple source pathways leading to N_2O emissions (Castaldi 2000; Butterbach-Bahl et al. 2002). Controlled studies are needed to understand and model the effect of temperature changes on specific processes contributing to production of N_2O and N_2 (Wallenstein et al. 2006). Different microbial processes that lead to N_2O emissions respond to temperature increases in different ways (Smith 1997). Anaerobic processes, such as denitrification, need to be measured separately from aerobic processes such as nitrification (Seitzinger et al. 2006). Exogenous N amendment temporarily enhances rates of denitrification and/or nitrification, such as after fertilization or urine deposition, but these processes also occur as part of the endogenous

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N-cycle. Here, we aimed to determine in the laboratory how temperature alone (in the absence of N amendment) affects N_2O and N_2 denitrification end-products under controlled, anaerobic conditions for a silt-loam New Zealand pastoral soil.

At low oxygen and high moisture, denitrification commonly results in emissions of N_2O and N_2. Bacterial reduction of N_2O to N_2, however, requires a specific enzyme (N_2O reductase), which is inhibited at low pH and high oxygen levels (Čuhel et al. 2010). Induction of this enzyme, however, is not expressed immediately following the onset of anaerobiosis (Firestone & Tiedje 1979). Consequently, production of N_2 initially lags behind N_2O. Denitrification by heterotrophic bacteria to N_2O and N_2 has been well studied, yet fungal archaean denitrification pathways also lead to production of N_2O and/or N_2 (Shoun et al. 2012; Long et al. 2013). Under highly acidic conditions, chemodenitrification (when NO_2^- accumulates and reacts with organic compounds to produce N_2O and N_2) may also occur (Stevens & Laughlin 1998).

Temperature response curves have been reported for N_2O and N_2 under variable field and laboratory conditions using the acetylene-block technique (Smith 1997; Holtan-Hartwig et al. 2002), which entails chemical inhibition of the N_2O reductase enzyme. Recently, direct measurements of N_2 (in the absence of chemical inhibition) were compared with N_2 measurements using the acetylene-block technique, and substantive biases were reported (Qin et al. 2013). Butterbach-Bahl et al. (2002) reported temperature-response curves for N_2O and N_2 in the absence of acetylene inhibition of N_2O reductase for forest soils, but temperature effects were difficult to ascertain with incubation methods using intact soil cores. In this study, we chose to use sieved soil at a moisture content known to stimulate denitrification and N_2O and N_2 end-products (Schindlbacher et al. 2004; Castellano et al. 2010).

Simultaneous measurements of N_2O and N_2 indicate how N_2O produced in an anaerobic headspace might link to N_2 production, and how this relationship changes with temperature (Butterbach-Bahl et al. 2002). The ratio of N_2O produced relative to N_2 may decrease with increasing temperature (Smith 1997), and this relationship is supported by a modelling study where climate scenarios driven by Photosynthesis-Evapotranspiration-Model-Denitrification-Decomposition-Model (PnET-N-DNDC) indicated a lowering of N_2O from European forests in response to warming (Kesik et al. 2006). Pasture is the dominant land use in New Zealand, but it is unknown if denitrification end-products in soil change as conditions warm. Changes in soil pH (common following exogenous N addition), often affect the denitrification end-product ratio by inhibiting production of N_2 to a greater extent than N_2O (Dannenmann et al. 2008). However, it is not clear if increasing soil temperature inhibits production of N_2 to a greater extent than N_2O. We hypothesized that the temperature response for net N_2O production (measured as Q_{10}) would be steeper than for net N_2 production for the upper end of soil temperatures (from 19 to 35 °C). By applying new, automated techniques for N_2O and N_2 measurement (Molstad et al. 2007), we tracked how temperature alone affected net emissions of N_2O and N_2, and the ratio of N emitted as N_2O relative to total N emissions.

Methods

A pasture historically seeded with ryegrass (*Lolium perenne*) and clover (*Trifolium repens*) and grazed by sheep near Palmerston North, New Zealand (40° 23′ 1″S; 175° 36′ 36″E), was selected for this study. Classification by New Zealand soil survey indicates these are weathered, fluvial, recent soils (Hewitt 1998), while the US Department of Agriculture indicates these are Dystric Fluventic Eutrochrept soils (Soil Survey Division Staff 1993). These soils are common in New Zealand and are referred to as Manawatu silt-loams. Climate records (over 30 years) indicate an annual mean rainfall of 980 mm and mean monthly air temperatures ranging from 9 °C in July to 18 °C in February. The pasture was fenced to exclude grazing in winter 2013, and preliminary soil cores were collected on 20 August 2013 for determination of soil physical properties. Six cores (10 cm in diameter, 10 cm depth) were analysed for bulk density, particle size distribution, particle density and intact-core

moisture release at multiple matric potentials (Gradwell 1972). On 15 April 2014, 20 additional soil cores were collected, composited and sieved (2 mm mesh size). Subsamples of sieved soil were used to determine nitrate and ammonium by cadmium reduction using a continuous-flow analyser (Lachat Quick-Chem FIA 8000 Series, Lachat Instruments, Loveland, CO, USA). Moisture release at multiple matric potentials was also determined on sieved soil. A total of 20 g equivalent dry weight was measured into 20, 125 mL serum bottles from the bulked soil sample (Sigma Aldrich, Part No. 98334, Milwaukee, WI, USA). A total of 20 bottles were prepared and stored at 4 °C for 3 days before initiating incubations. On the day of incubation, five bottles were randomly selected and wetted to 83% water-filled pore space (% WFPS), then sealed with a butyl rubber/PTFE lined septum and aluminium crimp seal (Grace Discovery, Part No. 95584). Soils were allowed to equilibrate for 1 h before anaerobiosis was induced (Qin et al. 2014) by evacuating and flushing with ultra-pure helium (He). Each day a fresh set of five bottles were incubated at a different temperature, starting at 19 °C on day 1 and ending with 35 °C on day 4.

Anaerobiosis was achieved by evacuating bottles for 10 min and flushing with ultra-high purity (99.999%) He; this procedure was repeated three times. The vials were placed on a rack and immersed in a temperature-controlled water bath under a Gilson GX-271 Liquid Handler (Gilson, UK). Immediately after anaerobiosis was induced, measurements were made using a robotized incubation system similar to that described by Molstad et al. (2007). Briefly, the system monitors the headspace concentrations of relevant gases (N_2O, N_2, carbon dioxide [CO_2] and oxygen [O_2]) by repeated gas sampling through septa of the serum bottles. The gas samples were withdrawn by a peristaltic pump, which returned an equal volume of He to maintain atmospheric pressure. Headspace dilution sources were taken into account when calculating headspace concentration at each time point for repeated measurements (Butterbach-Bahl et al. 2002; Molstad et al. 2007). The gas chromatograph (model GC-2010, Shimadzu, Kyoto, Japan) was equipped with an electron capture detector (ECD) for N_2O and CO_2, a flame ionization detector (FID) for methane (CH_4), and a thermal conductivity detector (TCD) for N_2 and O_2. Headspace samples were injected into the GC every 1.1 h over the 9 to 10 h incubation.

Calibration of N_2 was performed using known standard concentrations of 500, 2000, 5000 and 10,000 ppm. The coefficient of variation on replicate N_2 standards (4.5%) was calculated by running 15 replicate standards (500 ppm N_2). The coefficient of variation on replicate N_2O standards (1%) was calculated by running 12 replicate standards (1 ppm N_2O). Gas concentration data, for both headspace and dissolved gases (Wilhelm et al. 1977), were used to calculate fluxes based on the linear portion of the time course ($R^2 > 0.9$). Oxygen data confirmed that conditions remained anaerobic throughout the time-course incubation. Temperature response was estimated from flux (calculated from gas concentration data) as the dependent variable in an exponential function using Proc NLIN, and the effect of temperature on flux was evaluated in an analysis of variance using Proc GLM (SAS Institute, Cary, NC, USA) for each gas species. Temperature response was expressed as a Q_{10} function (van't Hoff 1989), which is a convenient tool for summarising observed responses (Kirschbaum 1995) and used to describe N_2O fluxes in previous work (Phillips et al. 2012). Concentration data at each temperature were plotted to evaluate temporal changes in N_2O reduction and N_2 production over the 10 h time-course.

Results

Soil particle size distribution was 34% sand, 47% silt and 19% clay, with a pH of 5.7 and bulk density of 1.08 g cm^{-3}. Mineral N at the 2014 soil sampling was 7 mg NH_4-N kg^{-1} and 5 mg NO_3-N kg^{-1}. Mean volumetric water content at saturation for intact soil cores (100% WFPS) was 0.58 cm^3 cm^{-3}. Mean volumetric water content at saturation (100% WFPS) for sieved soils (<2 mm) was 0.68 cm^3 cm^{-3}. Since sieved soil was used in these incubations, %WFPS was calculated according to the

moisture release curves constructed on sieved soil data.

Mean fluxes for N_2O, N_2 and CO_2 tended to increase between 19 °C and 35 °C (Table 1), which were calculated based on the linear portion of each time course. For N_2, fluxes between 30 °C and 35 °C did not incrementally increase to the same degree as fluxes between 19 °C and 30 °C (Table 1), which contrasts with temperature responses for N_2O and CO_2. Differences in the temperature response functions between N_2O and N_2 affected the amount of N emitted as N_2O relative to total N. The N_2O product ratio, defined as $N_2O/(N_2O+N_2)$, ranged from 0.65 at 19 °C to 0.76 at 35 °C.

The statistical model indicated that temperature significantly affected flux for all gas species tested: N_2O ($F = 89.79$; $P < 0.0001$); N_2 ($F = 5.64$; $P < 0.01$); CO_2 ($F = 508.99$; $P < 0.0001$). Similarly, the N_2O product ratio was also significantly affected by temperature ($F = 5.15$; $P < 0.05$). Calculated Q_{10} values (95% confidence intervals) were 2.0 (1.8 to 2.2) for N_2O, 1.4 (1.1 to 1.6) for N_2 and 1.6 (1.5 to 1.7) for CO_2. Applying the fitted model to each species, the coefficient of determination for predicted versus observed mean flux was 0.94 for N_2O, 0.51 for N_2 and 0.99 for CO_2.

Concentrations of N_2O and N_2 in the headspace followed distinct patterns at each incubation temperature. The rise in headspace N_2O concentration to peak values occurred sooner than for N_2. As temperature increased, the time needed to reach peak N_2O and complete reduction of N_2O to N_2 was shortened (Fig. 1). The increase in N_2 was approximately linear between 3 h and 7 h at 19 °C and 25 °C and between 1 h and 3 h at 30 °C and 35 °C. Peak headspace concentration was achieved more quickly at the higher temperatures for both N_2O and N_2.

There appeared to be a synchrony between complete consumption of headspace N_2O (where N_2O was zero) and a levelling-off in N_2 headspace concentration (Fig. 1). When headspace N_2O was near zero, N_2 concentration plateaued near 5 mg N_2-N kg^{-1} at all temperatures. Net N_2 production was no longer evident when N_2O was no longer available in the headspace at 19 °C and 25 °C. At 30 °C and 35 °C, N_2 concentration increased again after 8 h, suggesting (a) production of N_2O was balanced by rapid reduction to N_2 and/or (b) an alternative N_2 production pathway (e.g. co-denitrification) was invoked.

Discussion

Staggered synthesis of denitrification end-products occurred in response to anoxia, resulting in initial production of N_2O followed by consumption of N_2O, in accordance with earlier studies (Firestone & Tiedje 1979; Holtan-Hartwig et al. 2002). Declines in N_2O concentration after a period of anaerobiosis suggest an increase in N_2O reductase activity (Qin et al. 2014). Following onset of anaerobiosis, rates of N_2O production exceeded N_2O reduction. Later in the time course, rates of N_2O reduction exceeded production, as shown by increased headspace N_2 without N_2O accumulation (Fig. 1). Since denitrification is a heterotrophic process, mineralization of organic matter at higher temperatures could explain why protracted increases in N_2 concentration were more apparent at 35 °C than at 19 °C. Alternatively, enhanced mineralization could have also fuelled

Table 1 Mean (± standard error of the mean) flux of three gaseous products from pasture soils at different temperatures.

	Net flux (μg gdw^{-1} h^{-1})			
Gas	19 °C	25 °C	30 °C	35 °C
N_2O-N	0.43 (0.06)	0.92 (0.07)	1.14 (0.07)	1.63 (0.20)
N_2-N	0.38 (0.10)	0.55 (0.16)	0.73 (0.18)	0.14 (0.14)
CO_2	2.71 (0.04)	4.06 (0.15)	6.49 (0.20)	8.03 (0.40)

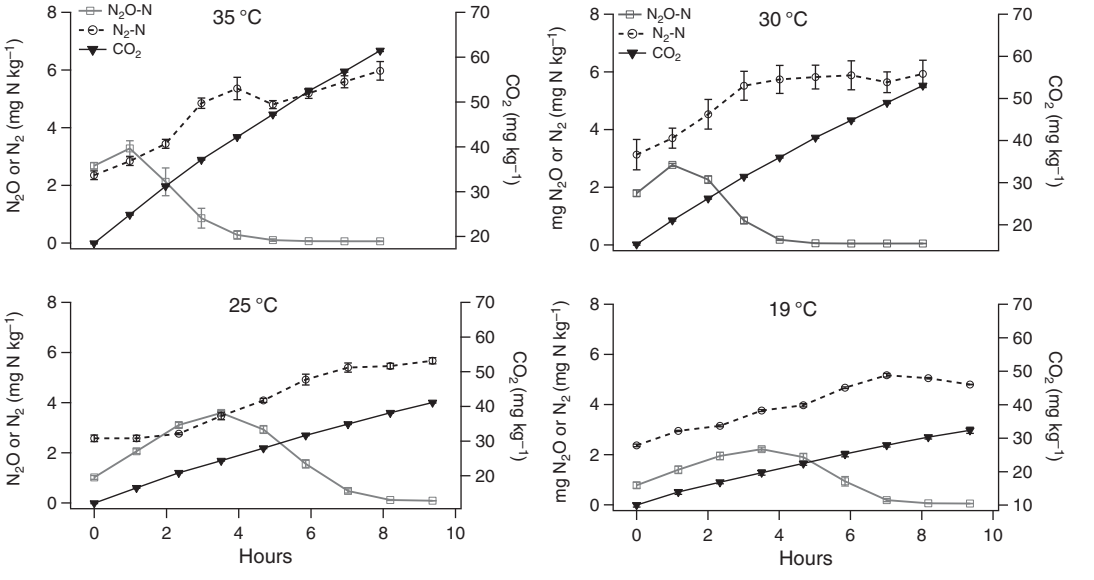

Figure 1 Mean concentrations of N_2 and N_2O during the time-course incubation. Each panel represents a unique set of samples at a specific incubation temperature. Error bars represent ± standard error of the mean.

other anaerobic N_2-production pathways that require organic matter and produce N_2, such as co-denitrification (Long et al. 2013).

A fundamental difference between this work and other N_2 reports is the incubation conditions. Here, we used fresh, un-amended soil that was not completely saturated with water, which contrasts with soil slurry incubations. Further, conditions were not optimized to induce maximum rates of N_2O-reductase activity. For example, our results contrast with Qin et al. (2014), where soil slurries were amended with N substrate and conditions known to maximize rates of N_2O reduction. Consequently, our actual rates of N_2O-reductase activity, reported as μg N_2-N g^{-1} h^{-1} (Table 1), are considerably lower than potential rates (approximately 2.2 μg N_2-N g^{-1} h^{-1}) reported by Qin et al. (2014).

The fraction of N gas emitted as N_2O relative to total N_2O + N_2 is likely to be highly variable when nitrification and denitrification are occurring at the same time (Stevens & Laughlin 1998). Like Qin et al. (2014) and Qu et al. (2014), our results represent anaerobic conditions only. Anaerobic incubation conditions help to isolate aerobic from anaerobic processes, but are limiting with respect to field interpretation. Changes after the onset of anaerobiosis reported here would be expected to occur in newly created or growing anaerobic zones in soil, as compared with emissions at the surface comprised of both aerobic and anaerobic processes (Smith 1997).

A review of laboratory incubations in this temperature range using sieved soil from pasture and arable lands indicates Q_{10} values for N_2O ranging from 1.7 to 4.8 (Goodroad & Keeney 1984), but higher values have been reported (Castaldi 2000). McMillan et al. (2014) used anaerobic incubations and found fluxes were dominated by N_2, with little or no N_2O, but these incubations were longer and measurements less frequent. Similar to Qin et al. (2014), we found most N_2O was produced in the first 2 h after onset of anaerobiosis. If measurements had been delayed beyond 6 h, N_2O would not have been evident in the headspace. Product ratios need to be considered carefully (van Cleemput 1998), as they are highly dependent on measurement timing.

We report initial results using a novel robotic technique without N amendment to avoid influencing

end-products through changes in soil pH. We also selected a moisture content conducive to production of both N_2O and N_2 (Schindlbacher et al. 2004) and temperatures at the upper end of those reported in New Zealand. The Q_{10} values reported for denitrification are known to vary with soil moisture content and the range of incubation temperatures, with greater Q_{10} values at higher moisture and lower temperatures (Maag & Vinther 1996; Phillips et al. 2012). Substantive N_2O emissions after rainfall occur during summer in New Zealand, even in the absence of N amendment (Luo et al. 2000), but a broader range of temperatures need to be evaluated in future studies.

Mathematical models aim to predict ecosystem N_2O flux, but do not always include exponential temperature response functions for denitrification (Parton et al. 1996). For the Denitrification Decomposition (DNDC) model, the Q_{10} values for N_2O and N_2 are both 2 (Li et al. 1992). Future scenarios using the PnET-N-DNDC model predicted a shift towards lower N_2O:N_2 ratios at elevated temperatures (Kesik et al. 2006). One rationale for this prediction is that fewer electron acceptors would be available, as anaerobiosis proceeds more quickly at elevated temperature (resource depletion), which might force denitrifiers to express the full chain of denitrification enzymes (Butterbach-Bahl & Dannenmann 2011). Here, anoxic conditions prevailed among all temperature groups.

This work indicates that Q_{10} values for N_2O (2.0) and N_2 (1.4) may not be equal under anaerobic conditions. Ideally, a generalized model for denitrification would include predictive equations with respect to temperature that are relevant under both anaerobic and aerobic conditions, with model-based estimates for the fractions of aerobic versus anaerobic soil zones. Experiments aimed at evaluating oxygen and microsite contributions to N_2O and N_2 production (Wick et al. 2012) may assist with spatially modelling the effects of temperature. Additional studies into the factors controlling denitrification end-products are needed to address open questions regarding effects of climate change on nitrogen cycling in agricultural systems (Butterbach-Bahl & Dannenmann 2011).

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References

Butterbach-Bahl K, Dannenmann M 2011. Denitrification and associated soil N_2O emissions due to agricultural activities in a changing climate. Current Opinion in Environmental Sustainability 3: 389–395.

Butterbach-Bahl K, Willibald G, Papen H 2002. Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. Plant and Soil 240: 105–116.

Castaldi S 2000. Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment. Biology and Fertility of Soils 32: 67–72.

Castellano MJ, Schmidt JP, Kaye JP, Walker C, Graham CB, Lin H et al. 2010. Hydrological and biogeochemical controls on the timing and magnitude of nitrous oxide flux across an agricultural landscape. Global Change Biology 16: 2711–2720.

Čuhel J, Šimek M, Laughlin RJ, Bru D, Cheneby D, Watson CJ et al. 2010. Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity. Applied and Environmental Microbiology 76: 1870–1878.

Dannenmann M, Butterbach-Bahl K, Gasche R, Willibald G, Papen H 2008. Dinitrogen emissions and the N_2:N_2O emission ratio of a Rendzic Leptosol as influenced by pH and forest thinning. Soil Biology and Biochemistry 40: 2317–2323.

Firestone MK, Tiedje JM 1979. Temporal change in nitrous oxide and dinitrogen from denitrification following onset of anaerobiosis. Applied and Environmental Microbiology 38: 673–679. http://aem.asm.org/content/38/4/673 (accessed 23 October 2014).

Gradwell MW 1972. Methods for physical analysis of soils. New Zealand Soil Bureau Scientific Report 10C. Wellington, Department of Scientific and Industrial Research.

Goodroad LL, Keeney DR 1984. Nitrous oxide emission from forest, marsh, and prairie ecosystems. Journal of Environmental Quality 13: 448–452.

Hewitt AE 1998. New Zealand soil classification. 2nd edition. Landcare Research Science Series 1. Lincoln, Manaaki Whenua.

Holtan-Hartwig L, Dorsch P, Bakken LR 2002. Low temperature control of soil denitrifying communities:

kinetics of N_2O production and reduction. Soil Biology & Biochemistry 34: 1797–1806.

Kesik M, Brüggemann N, Forkel R, Kiese R, Knoche R, Li C et al. 2006. Future scenarios of N_2O and NO emissions from European forest soils. Journal of Geophysical Research: Biogeosciences 111: G02018.

Kirschbaum MUF 1995. The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and Biochemistry 27: 753–760.

Li C, Frolking S, Frolking TA 1992. A model of nitrous oxide evolution from soil driven by rainfall events: 2. Model applications. Journal of Geophysical Research: Atmospheres 97: 9777–9783.

Long A, Heitman J, Tobias C, Philips R, Song B 2013. Co-occurring anammox, denitrification, and codenitrification in agricultural soils. Applied and Environmental Microbiology 79: 168–176.

Luo J, Tilman RW, Ball PR 2000. Nitrogen loss through denitrification in a soil under pasture in New Zealand. Soil Biology and Biochemistry 32: 497–509.

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

McMillan A, Phillips R, Berbin P, Palmada T, Jha N, Saggar S 2014. Automated N_2O/N_2 analysis – a new tool for studying denitrification dynamics and testing mitigation strategies. In: Currie LD, Christensen CL eds. Nutrient management for the farm, catchment and community. Occasional Report 27. Palmerston North, Fertilizer and Lime Research Centre, Massey University. 14 p. http://flrc.massey.ac.nz/publications.html (accessed 23 October 2014).

Molstad L, Dörsch P, Bakken LR 2007. Robotized incubation system for monitoring gases (O_2, NO, N_2O N_2) in denitrifying cultures. Journal of Microbiological Methods 71: 202–211.

Parton WJ, Mosier AR, Ojima DW, Schimel DS, Weier K, Kulmalla AE 1996. Generalized model for N_2 and N_2O production from nitrification and denitrification. Global Biogeochemical Cycles 10: 401–412.

Phillips RL, Wick AF, Liebig MA, West M, Daniels LW 2012. Biogenic emissions of CO_2 and N_2O at multiple depths increase exponentially during a simulated soil thaw for a northern prairie Mollisol. Soil Biology and Biochemistry 46: 14–22.

Qin S, Yuan H, Dong W, Hu C, Oenema O, Zhang Y 2013. Relationship between soil properties and the bias of N_2O reduction by acetylene inhibition technique for analyzing soil denitrification potential. Soil Biology and Biochemistry 66: 182–187.

Qin S, Yuan H, Hu C, Oenema O, Zhang Y, Li X 2014. Determination of potential N_2O-reductase activity in soil. Soil Biology and Biochemistry 70: 205–210.

Qu Z, Wang J, Almøy T, Bakken LR 2014. Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. Global Change Biology 20: 1685–1698.

Schindlbacher A, Zechmeister-Boltenstern S, Butterbach-Bahl K 2004. Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils. Journal of Geophysical Research: Atmospheres 109: D17302.

Seitzinger S, Harrison JA, Böhlke JK, Bouwman AF, Lowrance R, Peterson B et al. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecological Applications 16: 2064–2090.

Shoun H, Fushinobu S, Jiang L, Kim S, Wakagi T 2012. Fungal denitrification and nitric oxide reductase cytochrome P450nor. Philosophical Transactions of the Royal Society B: Biological Sciences 367: 1186–1194.

Smith K 1997. The potential for feedback effects induced by global warming on emissions of nitrous oxide by soils. Global Change Biology 3: 327–338.

Smith KA, Ball T, Conen F, Dobbie KE, Massheder J, Rey A 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. European Journal of Soil Science 54: 779–791.

Soil Survey Division Staff 1993. Soil survey manual. USDA Handbook No. 18. Washington, DC, US Government Printing Office.

Stevens RJ, Laughlin RJ 1998. Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils. Nutrient Cycling in Agroecosystems 52: 131–139.

van Cleemput O 1998. Subsoils: chemo-and biological denitrification, N_2O and N_2 emissions. Nutrient Cycling in Agroecosystems 52: 187–194.

van't Hoff JH 1989. Lectures on theoretical and physical chemistry. London, UK, Edward Arnold.

Wallenstein MD, Myrold DD, Firestone M, Voytek M 2006. Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. Ecological Applications 16: 2143–2152.

Wick AF, Phillips RL, Liebig MA, West M, Daniels WL 2012. Linkages between soil micro-site properties and CO_2 and N_2O emissions during a simulated thaw for a northern prairie Mollisol. Soil Biology and Biochemistry 50: 118–125.

Wilhelm E, Battino R, Wilcock RJ 1977. Low-pressure solubility of gases in liquid water. Chemical Reviews 77: 219–262.