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Grassland plant species and cultivar effects on nitrous oxide emissions after urine application

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ABSTRACT

We tested whether plant species identity influenced emissions of nitrous oxide (N_2O) in response to the addition of urine at a rate of 530 kg nitrogen (N) ha^{-1}. The plants tested are all used in temperate pastoral agriculture and comprised 11 C_3 grasses, 3 forbs and 2 legumes with controls of bare ground and a standard ryegrass/white clover mixture used in the region. Total N_2O emitted over 6 weeks after the application of cattle urine was highest in the bare ground. Plant identity was important with emissions ranging from an average of 0.67 kg N_2O-N ha^{-1} for the Italian ryegrass Grasslands Moata (*Lolium multiflorum*) to 3.20 kg N_2O-N ha^{-1} for the upland brome Grasslands Gala (*Bromus stamineus*). The 4 perennial ryegrass (*Lolium perenne*) cultivars had generally low emissions (average of 1.02 kg N_2O-N ha^{-1}) and the white clover (*Trifolium repens*) cultivars high emissions (average of 2.86 kg N_2O-N ha^{-1}). Plants with high N uptake rates had low N_2O emissions. However, this was not always due to a high N uptake rate per se as during the first 2 weeks after urine was applied, when N_2O emissions were at their highest, soil mineral N was in excess of plant N demand. It seems that some traits related to a high plant N uptake, such as low soil nitrification potential, might be important in driving differences in emissions associated with different plant species.

1. Introduction

Agriculture is the source of about 60% of anthropogenic nitrous oxide (N_2O) emissions most of which are a result of N_2O production in soils (Reay et al., 2012). Consequently, agriculture represents the greatest opportunity for mitigation of anthropogenic N_2O (Reay et al., 2012) and the management of factors that influence soil emissions has the greatest potential.

In grazed grassland, nitrogen (N) is taken up by plants from the soil and then recycled back to the soil in part through the action of grazing animals that harvest N and return it in their excrement (Haynes and Williams, 1993; Parsons et al., 1991). The return of N in urine, especially, creates an environment of high N concentration (up to 2000 kg N ha^{-1} in some circumstances) (Selbie et al., 2015); the N load is in excess of the capacity of plants to take up and, as a result, N can be lost from the system by volatilization, by leaching and through emissions of N_2O and dinitrogen (N_2) (Ball et al., 1979; Soussana and Lemaire, 2014). About 20% of global agricultural N_2O emissions come from urine and dung deposited by animals grazing managed temperate grasslands (Oenema et al., 2005).

The development of mitigation strategies for N_2O is handicapped by an incomplete understanding of the underlying processes despite many years of, largely, soil focussed research (Butterbach-Bahl et al., 2013; Van Groenigen et al., 2015). This is well demonstrated by the difficulty there is in producing ecosystem models capable of simulating N_2O fluxes in the field (Farquharson and Baldock, 2008; Giltrap et al., 2015). It is now becoming clear that a wider view is required and a greater emphasis on the influence of plants and plant/microbe interactions have been identified as a key to better understanding of N cycling and N_2O production (Butterbach-Bahl et al., 2013; Van Groenigen et al., 2015) and thus a way in which better mitigation strategies might be identified.

The role of plants in N_2O production from grasslands has received scant attention although plants are known to strongly modify nitrogen (N) cycling processes in soils (Cantarel et al., 2015; Chapman et al., 2006; Knops et al., 2002; Personeni et al., 2005; Waksman and Tenney, 1927). Some evidence for a plant effect on N_2O production is now accumulating (Abalos et al., 2014; Baggs et al., 2003; Bowatte et al.,

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2014; Dijkstra et al., 2010; Niklaus et al., 2016) but how this plant effect is mediated remains poorly understood (Abalos et al., 2018).

From the limited literature it can be said that legumes appear to have high emissions relative to other species (Baggs et al., 2003; Niklaus et al., 2016), that significant differences between species in monocultures are apparent but the effects are not necessarily additive in mixtures (Abalos et al., 2014) and that species composition alters the timing of the peak of N_2O production after urine application (Hoeft et al., 2012). Plant biomass production i.e. competitiveness for N with microbes (Abalos et al., 2014; Abalos et al., 2018) and plant effects on soil moisture (Niklaus et al., 2016) are two possible mechanisms causing differences in N_2O emissions but these appear to be incomplete explanations as noted in these and other studies (Dijkstra et al., 2010).

If plant identity has an influence on N_2O emissions from grazed temperate pastures this could provide a new opportunity for mitigation. To examine this possibility we measured N_2O emissions from monoculture plots of a range of temperate grass species and cultivars after urine was applied. The species tested were selected to represent a wide range of influence on soil nitrification potential (Bowatte et al., 2016), and thus hopefully N_2O emissions, as nitrification inhibition is known to reduce N_2O emissions (Byrnes et al., 2017; Di et al., 2010; Subbarao et al., 2012). Our objective was to see whether emissions differed depending on plant identity, and, if so, develop hypotheses on the mechanisms driving these differences and identify potentially interesting comparisons for further study.

2. Materials and methods

The field trial site was located at AgResearch Grasslands in Palmerston North, New Zealand (latitude 40°S, longitude 175°E). The trial was conducted within a 20 × 20 m area of a 1 ha sheep-grazed paddock containing a *Lolium perenne* L./*Trifolium repens* L. (ryegrass/clover) mixed pasture. The soil at the site is classified as a Karapoti silt loam (Dystric Eutrochrept) (Cowie, 1972); soil characteristics measured before the trial began were; pH 5.1 (1:2.1 v/v water slurry: electrode determination); carbon (C) 3.95 g 100 g dry soil^{-1} (chromium trioxide wet oxidation, colorimetric determination); N 0.35 g 100 g dry soil^{-1} (combustion elemental analyser: thermal conductivity detection); phosphorus (P) 37.8 μg mL soil extraction^{-1} (Olsen extraction: colorimetry) and potassium 8 MAF QT (K) (QT = (μg mL^{-1})/18.2) (ammonium acetate extraction: Flame Emission Spectroscopy) (Eurofins, Ruakura Research Centre, Hamilton, New Zealand).

In February 2014 (late-summer) the trial area was marked out, sprayed with a broad spectrum herbicide (5 L ha^{-1} of glyphosate at 360 g L^{-1}; product Roundup G2) left fallow for 2 weeks and then cultivated by ploughing and powered harrowing. The spray, fallow and cultivation routine was repeated twice more at 2–3 weekly intervals. In early April 2014, 100 plots (1 × 1 m) were marked out in 5 blocks, with 20 plots in each block and a 2 m guard area between blocks. Sixteen plant species/cultivars (Table 1) were selected for this experiment. We also included a bare soil treatment as a control and a standard ryegrass/white clover pasture mix as a further control or benchmark. Seed was sourced from the Margot Forde Germplasm Centre, AgResearch, Palmerston North or from commercial seed companies (sources available on request from the corresponding author). All the endophyte infected seed was from commercial companies; we made no independent check of endophyte status but assumed the status was as described by the relevant company.

The seeds of selected plant species/cultivars were sown at four times the commercially recommended rates in mid-April 2014. The plots were watered during the establishment phase but not thereafter. Weeds were removed by hand to ensure that the plots remained as monocultures. In order to ameliorate the low soil pH (5.1) at the site a fine quality granulated limestone ($CaCO_3$; Rapid Lime NZ®) was applied on one occasion 4 weeks post seedling emergence. Through winter and spring the plants were periodically cut to 5 cm above ground level, with cut herbage removed from the plots.

A 6 week N_2O measurement experiment was conducted in late-spring/early-summer 2014 (30 October–12 December) 6 months after sowing when all species were fully established. This duration was chosen as Luo et al. (2008) in a study over different seasons found the effects of cattle urine on N_2O were observed for up to 6 weeks. The experiment included a cattle urine treatment and a water treatment, both of which were applied to each plot. Cattle urine was collected from dairy cattle grazing a standard ryegrass/clover mixed pasture. The urine was collected in buckets while animals voluntarily urinated during milking time at the Massey University's No 1 Dairy Farm, Palmerston North and stored at 4 °C for 5 days before application to the plots. The water treatment consisted of tap water supplied to the Grasslands site by the Palmerston North City Council, and is lightly chlorinated (0.5–0.6 ppm to comply with Drinking Water Standards of New Zealand). Fluoride is also added to a concentration of 0.7–1.0 ppm.

Four days before the urine and water treatments were applied three root zone soil samples (25 mm diameter × 75 mm depth cores) were collected from each plot. The three soil samples were bulked for each plot and a short term nitrification potential (SNP) assay was conducted on the bulked samples. Soil (5 g) was added to 50 mL of 1 mM $(NH_4)_2SO4$ in separate centrifuge tubes and shaken using an end-over-end shaker at approximately 30 rpm for 1 h. After shaking, 10 mL of supernatant was sampled and 25 mL of phenylmercuric acetate was added to stop microbial activity before centrifuging for 10 min at 3000 rpm. The solutions were filtered (Whatman #42 filter paper) and NO_3—N concentration analysed using a FIAstar 5000 Analyser (Foss Tecator, Hoganas, Sweden). We determined NO_3^--N in the soil solution after 1 h and then, after further shaking, at 16 h. The difference in the two values provided the nitrification potential i.e. NO_3^--N production over 16 h from the supplied NH_4^+-N by the existing nitrifier population (Sarathchandra, 1978).

A static soil chamber technique was used to measure N_2O emissions. The chamber dimensions were 250 mm diameter, 130 mm height which was inserted 30 mm into the soil. Further details of the gas sampling methodology can be found in De Klein et al. (2003). Briefly, the week before the gas measurements commenced two chamber bases were installed in each plot. On 30 October 2014, the plants inside each chamber base area were cut to 5 cm above ground level and the herbage removed. The exception was for the forage rape species, where plants were cut to 10 cm above ground level, ensuring optimal conditions for vegetative plant growth for that species. The urine and water treatments were both applied at a rate equivalent to a standard dairy cow urine patch (10 L m^{-2}) (Haynes and Williams, 1993). The N concentration of the applied urine was 0.53%, giving an N loading rate of 530 kg N ha^{-1}. The urine and water treatments were applied slowly and evenly to the chamber base areas on 4 November 2014.

Gas samples were taken 1 week before treatment application, 2 h after treatment application, then 2 days after treatment application. Thereafter, twice weekly gas samples were taken for an additional 5 weeks. On each sampling day, N_2O measurements were carried out between 12 noon and 2 p.m. (De Klein et al., 2003). On each occasion the chamber base covers were secured by clips onto the bases to give an airtight seal and three headspace gas samples were taken during a cover period of 60 min at times 0, 30 and 60 min. Headspace gas samples were taken using a 20 mL polypropylene syringe and 12 mL was injected into a 6 mL septum-sealed screw-capped pre-evacuated glass vial (Exetainer, Labco Ltd., High Wycombe, UK).

The N_2O concentration of the gas samples was analysed by gas chromatography (Kelliher et al., 2012) at the National Centre for Nitrous Oxide Measurement, Lincoln University, Christchurch, New Zealand. The hourly N_2O emissions were calculated for each chamber from the linear increase in head space N_2O concentrations over the sampling time. From regressions of the three sample times 91% of these were considered linear ($r^2 > 0.9$). Hourly N_2O emissions (mg N m^{-2} h^{-1}) were calculated as follows (De Klein et al., 2003):