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Is liming soil a strategy for mitigating nitrous oxide emissions from semi-arid soils?

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ABSTRACT

Nitrous oxide (N_2O) emissions in semi-arid regions are often greater following summer rainfall events when the soil is fallow, than in response to N fertiliser applications during crop growth. Nitrogen fertiliser management strategies are therefore likely to be ineffective at mitigating N_2O emissions from these cropped agricultural soils. Here we examined the influence of raising soil pH on N_2O emissions, nitrification rates, and both nitrifier and denitrifier populations following simulated summer rainfall events. The soil pH was raised by applying lime to a field site 12 months before conducting the laboratory experiment, resulting in soil of contrasting pH (4.21 or 6.34). Nitrous oxide emissions ranged from 0 when the soil was dry to 0.065 μg N_2O–N g dry soil^{-1} h^{-1} following soil wetting; which was attributed to both denitrification and nitrification. Increasing soil pH only decreased N_2O emissions when losses were associated with nitrification, and increased *amoA* gene copy numbers. We propose increasing soil pH as a strategy for decreasing soil N_2O emissions from acidic soils following summer rainfall in semi-arid regions when emissions result from nitrification.

1. Introduction

Semi-arid soils constitute one fifth of the global land area (Leemans and Kleidon, 2002; Lal, 2004) with the area predicted to increase with climate change (IPCC, 2007). These regions are widely used for agricultural production and contain one fifth of the world's population (Galbally et al., 2008). Agricultural soils are a source of trace gas emissions, including nitrous oxide (N_2O), a potent greenhouse gas (Crutzen, 1981). Nitrous oxide concentrations in the earth's atmosphere have increased since the industrial revolution due to greater use of synthetic N fertilisers, and the expansion of agricultural soils, enhancing soil microbial production of N_2O (IPCC, 2001; Davidson, 2009; Smith et al., 2012).

Recent studies in semi-arid regions indicate N_2O emissions are greater following summer rainfall than in response to N fertiliser applications (Barton et al., 2008, 2010, 2011). Indeed, N_2O emissions following summer rainfall accounted for approximately 50% of the annual emissions in these studies. This contrasts with findings from temperate climates where the greatest N_2O emissions often coincide with the application of N fertiliser (Stehfest and Bouwman, 2006; Van Groenigen et al., 2010; Thomson et al., 2012). Traditionally it is thought soil N_2O emissions are best mitigated by improving N fertiliser management via improved synchrony between plant N uptake and N fertiliser application (Millar et al., 2010; Van Groenigen et al., 2010; Thomson et al., 2012). This approach is unlikely to be effective in semi-arid regions where the greatest N_2O emissions are not in direct response to N inputs. Instead, new approaches are required for developing strategies for minimising N_2O emissions from agricultural soils in semi-arid environments.

Nitrification, rather than denitrification, is argued to be the main source of N_2O emissions in semi-arid regions as soils are rarely sufficiently anaerobic to invoke denitrification (Barton et al., 2008; Galbally et al., 2008). Nitrification is the oxidation of ammonia (NH_3) to NO_3^- via hydroxylamine (NH_2OH) and nitrite (NO_2^-) in a two-step process (Wrage et al., 2001). Ammonia oxidation, the conversion of NH_3 to hydroxylamine, is the first and rate limiting step in nitrification, and can be performed by both ammonia oxidising bacteria (AOB) and ammonia oxidising archaea (AOA). Despite both AOB and AOA possessing the *amoA* gene that encodes a subunit of the ammonia monooxygenase enzyme (AMO) there are distinct differences between the AOB and AOA oxidation processes.

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The intermediate product of bacterial ammonia oxidation is hydroxylamine (Kowalchuk and Stephen, 2001), while the intermediate for archaeal ammonia oxidation remains unclear as both hydroxylamine and nitroxyl (HNO) are proposed intermediaries (Walker et al., 2010). Furthermore the AMO structure has been reported to differ between AOB and AOA (Konneke et al., 2005; Walker et al., 2010), with archaeal AMO displaying greater substrate affinity than bacterial AMO (Martens-Habbena et al., 2009; Martens-Habbena and Stahl, 2011). Nitrification, and its regulation, appears to differ between AOB and AOA which could be due to niche separation based on substrate availability and prevailing soil conditions (e.g., soil pH, which has a direct effect on the availability of NH_3) (Di et al., 2009).

The contribution of denitrification to N_2O emissions following soil re-wetting cannot be entirely dismissed as denitrifying enzymes have been shown to survive drought and to reactivate on wetting (Peterjohn, 1991). Denitrification is the reduction of nitrate (NO_3^-) to di-nitrogen gas (N_2) under anaerobic conditions, with N_2O and nitric oxide (NO) intermediary gaseous products (Wrage et al., 2001). The step-wise process is catalysed by a series of enzymes: nitrate reductase (encoded by *narG* and *napA*), nitrite reductase (encoded by *nirK/S*), nitric oxide reductase (encoded by *norB*) and nitrous oxide reductase (encoded by *nosZ*) (Bakken et al., 2012, and references therein), and is conducted predominately by heterotrophic bacteria (Knowles, 1982). A wide range of taxonomic groups have the capacity to carry out denitrification, consequently the genes encoding the catalytic sub-unit of the different denitrification reductases are often used as molecular markers when studying the denitrification process (Baggs and Philippot, 2010; Bru et al., 2011).

Soil pH can be considered a major variable controlling bacterial populations across many soil types (Frostegård et al., 1993; Fierer and Jackson, 2006). It is known to alter the activity and diversity of both AOB and AOA (De Boer and Kowalchuk, 2001; Nicol et al., 2008) as well as soil denitrifiers (Enwall et al., 2005; Čuhel et al., 2010) although the effect is not consistent. For example, increasing soil pH has increased AOA abundance, but had no effect or decreased AOB abundance (Nicol et al., 2008; Pereira e Silva et al., 2012). Ammonia oxidising archaea have been reported to dominate AOB in soils where N availability is low (<15 μg NH_4^+–N per g soil), whereas AOB seems to become more competitive at higher N availability (Erguder et al., 2009; Di et al., 2010; Xia et al., 2011); although this is not consistent as other studies have suggested that substrate concentration does not influence archaeal ammonia oxidation (Stopnisek et al., 2010; Verhamme et al., 2011). For denitrifiers most, but not all, produce nitrous oxide reductase and are therefore able to reduce N_2O to N_2 (Rösch et al., 2002; Philippot et al., 2011). However, the nitrous oxide reductase of bacteria has been reported to be sensitive to low pH (Knowles, 1982) suggesting the production, rather than the activity, of the N_2O reductase enzyme is limited at low pH (Bergaust et al., 2010; Liu et al., 2010).

The net effect of soil pH on N_2O emissions is also difficult to predict (Page et al., 2009), and may vary depending on the source of the N_2O emission (Clough et al., 2004). There are examples where N_2O emissions resulting from nitrification have decreased in response to liming (Feng et al., 2003; Clough et al., 2004). For example, Feng et al. (2003) found increasing the soil pH of an arable soil via liming led to a more rapid conversion of NO_2^- to NO_3^-, restricting the availability of NO_2^- for reduction to N_2O. By contrast increasing soil pH tends to increase denitrification activity and N_2O emissions; although the ratio of N_2O to N_2 often also decreases with increasing pH (Šimek and Cooper, 2002; Clough et al., 2004; Zaman et al., 2008). Investigating the effectiveness of raising soil pH as a means of lowering N_2O emissions from semi-arid soils following summer rainfall events, and factors regulating these emissions, warrants examination if nitrification is hypothesised to be the main source of the emissions.

The aim of this research was to investigate the relationship between soil pH and N_2O emissions following a simulated rainfall event, in a semi-arid soil collected from a field site that had been limed 12 months prior to commencing the laboratory-based study. We sought to meet this aim by (1) measuring soil N_2O emissions with time after wetting the soil of contrasting pH, (2) quantifying bacterial *amoA*, archaeal *amoA* plus the denitrification genes N_2O reductase (*nosZ*) and nitric oxide reductase (*norB*), and (3) determining the nitrification rates at each soil pH and relating these to soil N_2O emissions and nitrifier and denitrifier population size. Although numerous studies have investigated the effect of soil pH on N_2O emissions (Page et al., 2009), often the soils are maintained at constant soil water content, have been incubated with lime for a limited period (e.g., <1 month) prior to commencing the study, and receive additional N inputs. Here we investigated the effect of soil pH on N_2O emissions following a simulated rainfall event, utilising soil collected from the field site where lime had been applied 12 months prior to commencing the laboratory experiment.

2. Material and methods

2.1. Soil

Bulk soil samples (surface 50 mm) were collected from an experimental site in the central grainbelt of south-western Australia (Wongan Hills; 30°51′S, 116°44′E) that included a non-limed and limed free-draining sand (Typic Quartzipsamment, USDA, 1992) (Barton et al., 2013). Soil was collected in summer (22 March 2010), after an extended period (92 days) since rain, which is typical for the region. Lime (3.5 t ha^{-1}) had been applied to the limed treatment site approximately 12 months (18 March 2009) before collecting soil for the present study. Prior to liming, the surface horizon (0–100 mm) had a pH of 4.39 (1:5 soil: 0.01 M $CaCl_2$ extract), electrical conductivity of 49 μS cm^{-1} (1:5 soil: water extract), cation exchange capacity of 1.93 cmol kg^{-1}, total C concentration of 10.2 mg g^{-1}, total N concentration of 0.93 mg g^{-1}, bulk density of 1.38 g cm^{-3}, and contained 89.5% sand, 2.8% silt, and 7.7% clay. Twelve months after applying the lime, soil pH had increased to 6.34. Soil pH in the surface 50 mm was measured every four weeks for 9 months before commencing the study, and had stabilised by the time of soil collection (Barton et al., 2013). The collected soil was sieved (<4 mm) before being used in this study. Soil pH of the collected soil was measured by adding 20 mL of 0.01 M $CaCl_2$ to 4 g of sieved (<2 mm) air-dried and shaking for 1 h. The extractant was allowed to settle for 30 min before measuring pH with an electronic probe (Rayment and Higginson, 1992).

2.2. Experimental design and approach

The influence of soil pH on soil N_2O emissions, and associated microbial populations, following the wetting of a dry soil was investigated in the laboratory using a factorial design. The experiment included two soil pH treatments [4.21 ('low', non-limed), 6.34 ('high', limed)], eleven destructive soil sampling times (0, 2, 6, 24, 30, 72, 146, 152, 174, 192 and 216 h) and six replicates. The treatment destructively sampled on the final sampling date (216 h) was used to measure N_2O, $^{15}N_2O$ and $^{15}N_2$ fluxes throughout the experiment.

Soil samples (50 g) were packed into unsealed, polyethylene vials (42 mm in diameter), to the same bulk density as the soil at the field site (1.38 g cm^{-3}). All treatments received 10.5 mL of water (equivalent to 6.0 mm rainfall event) at 0 h, and then again at 144 h. The first application of water (0 h) contained 0.179 mM of