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Effects of the nitrification inhibitor acetylene on nitrous oxide emissions and ammonia-oxidizing microorganisms of different agricultural soils under laboratory incubation conditions

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ABSTRACT

Acetylene (C_2H_2) is an effective nitrification inhibitor targeting autotrophic ammonia oxidizers, and has shown promise for improving nitrogen use efficiency by mitigating greenhouse gas nitrous oxide (N_2O) emissions and reducing nitrate leaching. Its efficacy, however, varies considerably with edaphic and environmental conditions and remains largely less studied in dryland agricultural soils. Here we conducted two laboratory microcosm incubations to explore the efficacy of C_2H_2 across various agricultural soils and under different conditions. The first incubation was with four agricultural soils at 25 °C and 60% water-filled pore space (WFPS), and the second incubation included one cropping soil under a range of conditions (15 °C, 25 °C, 35 °C and 50%, 70% WFPS). Our results showed that incubation of soil with 1% v/v C_2H_2 resulted in complete or partial inhibition of nitrification, N_2O emission, and AOA or AOB growth under the experimental conditions. Acetylene can totally inhibit nitrification in acidic cropping and dairy pasture soils through retarding both AOA and AOB growth, while C_2H_2 partly inhibited nitrification and N_2O emission in the alkaline vegetable soil through impeding only AOB growth. The highest inhibition effect of C_2H_2 was achieved at 25 °C and 50% WFPS, while there was no inhibitory effect of C_2H_2 when soil was incubated at 15 °C and 50% WFPS suggesting soil temperature may have a significant influence on C_2H_2 effectiveness. The inhibition of C_2H_2 on cumulative N_2O emission increased with increasing temperature at 50% WFPS. In contrast, at 70% WFPS, the inhibition of C_2H_2 on cumulative N_2O emission decreased with increasing temperature. Since the effect of C_2H_2 varied with soils and environmental conditions, this highlights the assumption that N_2O production and nitrification can be affected by low concentrations of C_2H_2 may be not appropriate in some occasions.

1. Introduction

Nitrogen (N) is an essential nutrient for food production, but the amount of applied fertilizer N used by crops rarely exceeds 40% (Chen et al., 2008), and can be as low as 20% in vegetable production systems in Australia (Suter et al., 2014). The majority of applied N is lost from agro-ecosystems through ammonia (NH_3) volatilization, gaseous emission of nitrous oxide (N_2O) and di-nitrogen (N_2) and nitrate (NO_3^-) leaching. Nitrous oxide is a potent greenhouse gas contributing significantly to global climate change with a 300-fold higher global warming potential than CO_2 (IPCC, 2007). It is also involved in the destruction of the protective ozone layer (Ravishankara et al., 2009). Soil ecosystems are the largest source of N_2O, accounting for approximately 65% of the atmospheric N_2O loading (IPCC, 2007). The major pathways of N_2O production in soils include microbial-mediated nitrification and denitrification (Hu et al., 2015; Zhang et al., 2015). Microorganisms, such as ammonia oxidizers and bacterial denitrifiers, involved in the N cycle can directly regulate N_2O production and consumption from soils, and increased abundance and activity of these microorganisms may increase N_2O emissions (Burger et al., 2005).

Nitrification inhibitors (NIs) can decelerate the rate of soil nitrification by deactivating the enzyme ammonia monooxygenase (AMO) which catalyses ammonia oxidation, the first and rate-limiting step of nitrification which is encoded by the *amo*A gene within ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (Zhang et al., 2012). NIs can block the growth of AOA and AOB (Di et al., 2010; Hink et al., 2016), and are widely used to improve N fertiliser efficiency, mitigate N_2O emissions and reduce NO_3^- leaching in agricultural systems (Chen et al., 2008, 2010; Kelly et al., 2008; Di et al., 2010; Zhang et al., 2012; Hu et al., 2015). Acetylene (C_2H_2) is an

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

Land use	Site name	Texture	pH (H_2O)	Clay	Sand	Slit	NH_4-N	NO_3-N	CEC	OC
				%			mg N/kg soil		Cmolc/kg	%
Cropping	Hamilton	Loam	4.5	19	44	38	13	93	9.42	6.2
Sugarcane	Bundaberg	Sand	6.0	5	90	5	2.6	8.8	2.6	1.2
Dairy pasture	Glenormiston	Loam	6.2	10	56	34	11	5.8	24	5.6
Vegetable	Boneo	Sand	7.8	1	91	8	1.1	19	6.9	0.8

effective inhibitor of bacterial ammonia oxidation (Offre et al., 2009), acting with AMO as a suicide substrate. It is usually used as an autotrophic nitrification inhibitor in experimental studies (De de Boer and Kowalchuk, 2001) and can inhibit nitrification at a low concentration (e.g.10 Pa) in most soils under aerobic conditions (Hyman and Wood, 1985; Schmidt and Bock, 1998; De Boer et al., 1991; Offre et al., 2009). In contrast, C_2H_2 does not efficiently inhibit ammonia oxidation by heterotrophic nitrifiers (Moir et al., 1996; Daum et al., 1998). Higher concentrations of C_2H_2 (1–20 kPa) will inhibit the N_2O reductase of denitrifying microorganisms (Davidson et al., 1986; Klemedtsson et al., 1988). As a result of this C_2H_2 has been used as a routine method to distinguish nitrification-related N_2O and denitrification-related N_2O in soils experiments based on the inhibition of soil ammonia oxidation (Bateman and Baggs, 2005; Butterbach-Bahl et al., 2013).

It is generally believed that low concentrations of C_2H_2 (0.1–10 Pa) totally inhibit nitrification (Hynes and Knowles, 1978; Berg et al., 1982) by forming a reactive epoxide which then irreversibly inactivates the AMO enzyme (Hyman and Wood, 1985), however, this does not always occur and its efficacy is considerably varied. While it was previously reported that 10 Pa of C_2H_2 totally inhibited nitrification (Wrage et al., 2004), Bremner and Blackmer (1978) found that10 Pa only partially inhibited soil nitrification.

Acetylene was also widely used to determine the community compositions of nitrifiers in soils through laboratory work (Boyle-Yarwood et al., 2008; Scheer et al., 2014). Recent studies have shown however, that AOA and AOB may have a variety of responses to C_2H_2 application. Gubry-Rangin et al. (2010) and Offre et al. (2009) revealed that AOA growth was inhibited in C_2H_2-containing microcosms but not AOB growth. Liu et al. (2015a) demonstrated that both AOA and AOB were inhibited by C_2H_2 in three Australian agricultural soils. However, these results contrast with those of Jia and Conrad (2009), who found changes in the abundance of AOB *amo*A genes correlated best with nitrification rate rather than AOA *amoA*, and bacterial growth occurred only in actively nitrifying microcosms with added C_2H_2. Considering the various responses of AOA and AOB to C_2H_2 addition, it is therefore necessary to find out how soil factors influence the response of AOA and AOB to C_2H_2 addition and what are the key factors affecting the response of AOA and AOB to C_2H_2.

This study was designed to determine the impact of C_2H_2 on N_2O emissions, nitrification rates and the abundance of ammonia oxidizers in different agricultural soils under laboratory conditions. Microcosm incubation experiments were established under a set of controlled environment conditions with the following objectives: (i) to examine the effects of C_2H_2 on N_2O emissions and the abundances of AOA and AOB from different agricultural soils and (ii) to investigate the effects of C_2H_2 on N_2O emissions and the abundances of AOA and AOB under different temperature and soil water contents in one agricultural soil. We hypothesized that (i) C_2H_2 would have significant inhibitory effects on nitrification and N_2O production from soils with different physicochemical traits, and (ii) AOA and AOB would exhibit distinctly different responses to C_2H_2. This study represents comprehensive efforts to examine C_2H_2 efficacy from different soils under controlled conditions, and the findings from the study can improve our understanding of the interactions between soil microbial communities and the nitrification inhibitor C_2H_2 in different agricultural soils under laboratory controlled conditions.

2. Materials and methods

2.1. Site description and soil sampling

The soils used in this study were collected from four agricultural sites in Australia: vegetable soil at Boneo, VIC (38.3°S, 144.9°E), sugarcane soil at Bundaberg, QLD (24.8°S, 152.3°E), dairy pasture soil at Glenormiston, VIC (38.2°S, 143°E), and cereal cropping soil at Hamilton, VIC (38.3°S, 142.7°E). At each site, 10 replicate samples of the top soil (0–10 cm) were collected, thoroughly homogenized, and transported on ice to the laboratory. Fresh soils were sieved through a 2.0 mm mesh, and root and leaf residues were removed with tweezers prior to the establishment of microcosms. Soil moisture contents were determined by oven-drying three subsamples (10 g of fresh soil) at 105 °C for 48 h. Soil texture (sieve and hydrometer procedures), pH (1:5 soil/water), total carbon (Dumas method) and other soil properties were determined and are shown in Table 1.

2.2. Soil microcosm incubations

2.2.1. The laboratory incubation with different agricultural soils

Soil microcosms were established in 500 ml vials containing 60 g of soils (oven-dry equivalent). Distilled water was added to soil to just under the final moisture content (60% water-filled pore space, WFPS) and the microcosms were pre-incubated at 25 °C for three weeks to stabilise soil microbial communities and minimise priming effects associated with wetting events. After pre-incubation, treatment was applied to each incubation vial to reach 60% WFPS (Linn and Doran, 1984). The treatments contained 100 mg N kg^{-1} soil as exchangeable NH_4^+-N and 50 mg N kg^{-1} soil as NO_3^--N, which were added to the soil as 1) NH_4Cl + KNO_3; and 2) NH_4Cl + KNO_3 + C_2H_2. Five ml of C_2H_2 (1% v/v) was injected into the headspace of the vials using an airtight syringe. Aerobic conditions, soil moisture and C_2H_2 contents in the vials were maintained every three days by opening microcosms and replenishing. Soil microcosms were incubated at 25 °C in the dark for three weeks.

2.2.1.1. Gas sampling and analysis. Gas samples were collected on days 0, 4, 8, 12, 16 and 20 after fertilizer application. Gas samples (20 ml) for N_2O analysis were taken from the 500 ml vials using gas-tight syringes. Prior to collection of gas samples, the vials were opened to ensure that N_2O concentration in the headspace was at ambient levels. During each sampling, gas samples were collected at 0, 8, 16, 24, 48 and 72 h after vials closure. Before gas collection, 20 ml compressed zero air was injected into 500 ml vials to maintain the pressure in the vials and then 20 ml gas samples were collected into a pre-evacuated 12 ml exetainer (Exetainer®, Labco Ltd., Lampeter, Ceredigion, UK). Samples were analysed for N_2O concentration by a gas chromatograph (Agilent 7890A) using an ECD (N_2O) detector.

2.2.1.2. Soil sampling and analysis. Soils were destructively sampled for soil mineral N analysis on days 0, 7, 14 and 21 immediately after gas sampling. There were four replicates at each sampling day. A subsample