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Title: 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.

Keywords: Acetylene; nitrification inhibitor; AOA; AOB; N_2O emission; temperature; moisture

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; Kelly et al., 2008; Chen et al., 2010; Di et al., 2010; Zhang et al., 2012; Hu et al., 2015). Acetylene (C_2H_2) is an 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 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 (1979) 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 air-tight 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 hours 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 (2 g) of soil was taken from each vial for molecular analysis and stored in a -80°C freezer before DNA extraction. The remaining 50 g of soil in the vials was shaken with 250 ml of 2 M KCl for 1 h at 200 rpm at room temperature, and the extract filtered through a qualitative filter paper (Whatman 42). The extracts (30 ml) were stored at -20°C prior to measurement of NH_4^+ and NO_3^- concentrations on a segmented-flow analyzer (Skalar, SAN++).

2.2.1.3 Soil DNA extraction and quantitative PCR (qPCR)

The Power Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) was used for DNA extraction following the manufacturer's instructions. Extracted DNA was quantified using a NanoDrop ND2000c spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and the quality of extracted DNA was checked on a 1% agarose gel. The archaeal and bacterial *amo*A gene copy numbers were quantified from triplicate samples using qPCR. The primer sets were Arch-amoAF/Arch-amoAR (Francis et al., 2005) and amoA1F/amoA2R (Rotthauwe et al., 1997), respectively. Each qPCR reaction for the

archaeal *amo*A gene was performed in a 20 µl volume including 0.5 µM of each primer, 10 µl SensiFAST SYBR No-ROX reagent (Bioline, Sydney, Australia), and 2 µl of 10-fold dilution DNA template (1–10 ng). Each qPCR reaction for the bacterial *amo*A gene was performed in a 10 µl volume containing 0.6 µM of each primer, 5 µl iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, USA), and 2 µl of 10-fold dilution DNA template (1–10 ng). Amplification programs for both AOA and AOB were as follows: 3 mins at 95°C, 40 cycles of 5 s at 95°C, 30 s at 60°C, and 72°C for 45 s. A known copy number of plasmid DNA was used to create a standard curve for each AOA or AOB assay. For all assays, qPCR efficiency was 92.7–98.4% and r^2 was 0.96–0.99.

2.2.2 Soil microcosm incubation under different temperature and water contents with one agricultural soil

The cropping soil used for the second experiment was chosen from one of the four sites and collected from a cropping paddock in Hamilton, VIC (38.3°S, 142.7°E) (Table 1). The study site is in a high rainfall zone (688 mm per annum), and was chosen because the soil was subjected to two different land management practices, cropping and pasture. Field studies have shown that high N_2O emissions occurred when the soil was converted from pasture to cropping. The laboratory soil incubation was carried out in 500 ml vials containing 60 g of soils (oven dry weight equivalent) to investigate the effects of C_2H_2 on N_2O emissions and the abundances of *amo*A genes under different soil temperature and moisture contents. The same treatments were established as the first experiment, though after the pre-incubation the samples were incubated at three temperatures (15°C, 25°C, 35°C) and two soil moisture levels (50% and 70% WFPS). During the 21-day incubation, soil, gas samples and *amo*A genes abundance were analysed as well.

2.3 Calculations

191 The equation developed by Persson and Wirén (1995) was used to calculate the net
192 nitrification rates over the incubation time (21 days)

193 $n = [(NO_3^-\text{-}N)_{d21} - (NO_3^-\text{-}N)_{d0}]/21$

194 where $(NO_3^-\text{-}N)_{d0}$ and $(NO_3^-\text{-}N)_{d21}$ are the NO_3^--N concentrations in the soil on days 0 and 21,
195 respectively.

196 **2.4 Statistical Analyses**

197 Data were analysed using SPSS 19 and means were compared using one-way analysis of
198 variance (ANOVA) between treatments to test the variance with a level of significance of $P <$
199 0.05. Spearman correlation analysis was performed to test the relationships between N_2O, the
200 abundances of AOA and AOB under different conditions.

201

202 **3. Results**

203 **3.1 Soil microcosm incubation with different land-use agricultural soils**

204 The physical and chemical properties of the soil samples were highly variable across the
205 different sampling sites (Table 1). Briefly, all soils except the vegetable soil were acidic (pH
206 $\leq$ 6.2). The cropping soil had the highest organic C content (6.2%), while the vegetable soil
207 had the lowest (0.8%). Nitrate-N (NO_3^-N) was the dominant inorganic nitrogen ranging from
208 8.8 to 93 mg kg^{-1} soil, with the highest value recorded in the cropping soil. Sugarcane and
209 vegetable soils had a sandy texture, but cropping and dairy pasture soils were loam. The
210 cropping soil has the highest clay content at 19%.

211 The highest cumulative N_2O emission was 3204.5 (±52.5) mg N kg^{-1} soil in the cropping soil,
212 compared to 122.4 (±5.1) mg N kg^{-1} soil in the sugarcane soil, which was the lowest emission
213 from fertilizer treatments (Table 2). Acetylene addition significantly reduced the cumulative

214 N_2O emissions by 28.6% to 54.6% (Table 2). The efficacy of C_2H_2 on reducing cumulative
215 N_2O emissions was ordered by dairy pasture (28.6%) < sugarcane (37.6%) < vegetable
216 (44.9%) < cropping (54.6%).

217 Changes in the NO_3^--N concentrations of the four soils during the incubation period are
218 shown in Figure 1. The NO_3^--N concentrations showed an increasing trend in all soils from
219 fertilized treatments. While C_2H_2 addition significantly reduced the NO_3^--N concentrations in
220 all the soils ($P < 0.05$), the efficacy of C_2H_2 varied among soils. For example, the average net
221 nitrification rates over 21 days in the dairy pasture soil were 1.8 (±0.1) mg NO_3-N kg^{-1} soil
222 day^{-1} for the fertilized treatment and 0 mg NO_3-N kg^{-1} soil day^{-1} for the fertilizer plus C_2H_2
223 treatment, while in the vegetable soil they were 2.1 (±0.4) mg NO_3^--N kg^{-1} soil day^{-1} for the
224 fertilized treatment and 1.5 (±0.3) mg NO_3^--N kg^{-1} soil day^{-1} for the fertilizer plus C_2H_2
225 treatment (Table 2).

226 The abundance of AOB before adding fertilizer varied greatly from 2.7×10^5 copies g^{-1} soil
227 in the dairy pasture soil to 2.6×10^6 copies g^{-1} soil in the vegetable soil, and was evidently
228 higher in the vegetable soil than in other soils (Figure 2). The addition of fertilizer
229 significantly increased AOB abundance in all soils on day 7, while C_2H_2 significantly
230 decreased the AOB abundance in cropping, sugarcane and vegetable soils on day 7. In the
231 dairy pasture soil, the abundance of AOB significantly decreased compared to the fertilizer
232 treatment on day 14 ($P < 0.05$). Before fertilizer addition, the AOA abundance was highest in
233 the vegetable soil up to 1.5×10^8 copies g^{-1} soil and much lower in the cropping, sugarcane,
234 and dairy pasture soils ranging between 5.8×10^6 and 1.1×10^7 copies g^{-1} soil (Figure 3).
235 Addition of fertilizer significantly increased AOA abundance in all soils except the vegetable
236 soil. There was a decreasing trend in AOA abundance during incubation in the vegetable soil.
237 AOA growth was inhibited by C_2H_2 addition in cropping, sugarcane and dairy pasture soils,

however, there was no significant difference in the AOA abundance between treatments during the incubation in the vegetable soil (Figure 3).

3.2 Soil microcosm incubation under different temperature and soil water contents

The NO_3^--N concentrations in fertilized treatments increased under 50% WFPS with increasing soil temperature except at 35°C (Figure 4). At 70% WFPS, the soil NO_3^--N concentrations decreased with increasing temperature over the incubation period. The concentrations of NO_3^--N remained largely unchanged in the fertilizer plus C_2H_2 treatments at different conditions except at 35°C with 70% WFPS. Under 35°C with 70% WFPS, NO_3^--N concentration significantly ($P < 0.05$) decreased after day 14 in the fertilizer plus C_2H_2 treatment. Acetylene addition substantially reduced the nitrification rate by 53% - 100%, suggesting C_2H_2 was capable of inhibiting nitrification under all conditions (Table 3), with the highest inhibition effect found at 25°C and 50% WFPS. The efficacy of C_2H_2 on nitrification decreased with increasing soil temperature and moisture except at 15°C.

Total N_2O emission increased with increasing temperature and moisture (Table 3). When C_2H_2 was applied into soil microcosms, cumulative N_2O emission was reduced by 0–86.7%. There was no difference in cumulative N_2O emission between the fertilizer and fertilizer plus C_2H_2 treatments at 15°C and 50% WFPS. The inhibition of C_2H_2 on cumulative N_2O emission increased with increasing temperature at 50% WFPS. In contrast, when soil was wetted to 70% WFPS, the inhibition of C_2H_2 on cumulative N_2O emission decreased with increasing temperature. At the same soil temperature, the efficacy of C_2H_2 on total N_2O emission decreased with increasing moisture except at 15°C.

The changes in abundances of AOB and AOA *amo*A genes are shown in Figures 5 and 6. In the fertilized treatment, AOA and AOB *amo*A gene copy numbers ranged from 1.0×10^7 to 3.3×10^8 and 1.8×10^6 to 1.7×10^7 copies g^{-1} soil, respectively, and increased with

increasing soil moisture. However, both AOA and AOB abundance decreased with increasing soil temperature. AOA *amo*A genes at different soil temperatures and moistures were 3.3-107 times more abundant than AOB *amo*A genes. AOA *amo*A gene abundance comprised 93–96% and 76–92% of the total *amo*A gene abundance at 15°C to 35°C, respectively. The ratio of AOA to AOB decreased significantly with increasing soil temperature ($P < 0.05$). Acetylene application significantly ($P < 0.05$) decreased AOA abundance by 48% (15°C and 70% WFPS) and reduced AOB abundance by 93% (25°C and 70% WFPS) relative to that in the fertilizer treatments on day 21. However, no significant effect of C_2H_2 addition on AOA or AOB *amo*A gene abundances was observed at 35°C, 50% WFPS and 15°C, 70% WFPS, respectively.

4. Discussion

This study investigated the changes in N_2O production, nitrification rates, and abundance of ammonia oxidizers after addition of the nitrification inhibitor C_2H_2 at various conditions. Our experiments 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 in all conditions.

4.1 Inhibitory effects of C_2H_2 in different soils.

The results demonstrate that C_2H_2 has different efficacy in inhibiting nitrification and N_2O emission in different soils. Nitrification can be inhibited by C_2H_2, implying AMO-dependent (and presumably autotrophic) nitrification, as frequently observed in many acidic soils (De Boer and Kowalchuk, 2001). In this study, C_2H_2 was much more effective in inhibiting nitrification and cumulative N_2O emission in acidic soils than the alkaline soil, and the acidic cropping soil had the highest inhibitory effect among the four soils. Soil pH might be a key factor in C_2H_2 inhibitory effects because pH is a strong environmental determinant of AOA

and AOB abundance. A number of studies have shown niche separation based on pH with AOA favoured in acidic soils and AOB in alkaline. The abundances of AOA and AOB in our acidic and alkaline soils supported this observation (as reviewed in He et al., 2012). Our findings are consistent with these previous studies, and supported the clear niche separation between AOA and AOB shaped by soil pH. We suggest that in the alkaline soil, nitrifiers can be protected within microenvironments due to the high amounts of carbonates present in the alkaline condition.

Besides soil pH, other soil physicochemical traits may also have the potential effects on C_2H_2 inhibitory efficacy. The dairy pasture soil had the lowest inhibition by C_2H_2 on cumulative N_2O emission (28.6%) while there was a complete inhibition (100%) on nitrification. It is possibly that because the dairy pasture soil had the highest ratio of C to NO_3^-, that there might be a loss in inhibitory efficiency on N_2O production. Previous studies have shown that the use of C_2H_2 to inhibit N_2O production and reduction has been problematic for soils with a very high ratio of C to NO_3^- (Davidson et al., 1986). Another potential explanation for low inhibition of C_2H_2 on cumulative N_2O emission is that the release of N_2O was due to processes other than nitrification, such as denitrification and heterotrophic nitrification, rendering the C_2H_2 unable to effect N_2O emitted from either denitrification or heterotrophic nitrification. The dairy pasture soil had a high organic carbon content which might result in high denitrification-related N_2O because of the high availability of soil organic C for denitrifiers. Wan et al. (2009) also demonstrated that more N_2O was released from denitrification than from nitrification in a soil with high organic carbon. Regarding the highest inhibitory effect on nitrification in dairy pasture soil, this may be due to nitrification in the dairy pasture soil being largely autotrophic. This can be supported by our previous study (Liu et al., 2015b) where we indicated that nitrification was primarily autotrophic with heterotrophic nitrification accounting for only 20%. In the alkaline sandy soil, C_2H_2

311 inhibition of NO_3^- production was only 29%, thus 1% v/v of C_2H_2 may be insufficient to
312 inhibit nitrification completely in this soil, nevertheless, most studies have indicated that this
313 partial pressure of C_2H_2 is clearly sufficient for a complete inhibition. This result contrasts
314 with our previous study (Liu et al., 2015a) where we found that C_2H_2 addition produced
315 100% nitrification inhibition in an alkaline clay loam soil. The difference between these two
316 studies may be because the two alkaline soils differed in soil texture.

317 Overall, the four soils chosen from different land uses had very differing physicochemical
318 properties. Of the properties characterised for the four soils, soil pH, texture, organic C and
319 NO_3^- content might be the key factors influencing the effectiveness of C_2H_2 on nitrification
320 and cumulative N_2O emission. Many recent studies also showed that soil physicochemical
321 properties affected the efficacy of other NIs, such as DMPP and DCD, with their efficacy
322 diminished with the addition of soil organic matter (Fisk et al., 2015) and decreased with
323 higher clay content (Marsden et al., 2016). Although it is not possible to clearly discern the
324 effects of land use from this experimental design, we speculate that land use may affect the
325 efficacy of C_2H_2 to inhibit nitrification and N_2O emissions. However, a multiple regression
326 including more soil physicochemical properties is still necessary.

327 C_2H_2 addition was able to inhibit nitrification and N_2O emission in all soils, albeit to varying
328 degrees. It is unclear however if this reduction in N_2O emission and nitrification is linked to
329 soil microbial communities due to adding C_2H_2 and further investigation is needed to
330 examine if C_2H_2 stimulates changes in microbial community population and activity.

331 As observed previously (He et al., 2007; Shen et al., 2008; Levicˇnik-Höfferle et al., 2012;
332 Liu et al., 2015a), AOA grow better in acidic soils, while AOB thrive better in alkaline soils,
333 which was supported by our study. In our study, the acidic sugarcane soil had the highest
334 AOA abundance, while the highest AOB population was found in the alkaline vegetable soil.

Growth of AOB, and not of AOA, has been linked to soil nitrification activity with high levels of ammonium (Di et al., 2009; Jia and Conrad, 2009). In contrast, growth of AOA is associated with nitrification in soils with a continual supply of ammonia at low concentration through the mineralisation of organic matter (Offre et al., 2009). The difference in substrate preferences could affect the distribution of AOA and AOB in soils and further affect the efficacy of C_2H_2 across different soils. Our study indicated that the application of C_2H_2 could block AOA and AOB growth but to different extents. In acidic cropping, sugarcane and dairy pasture soils, C_2H_2 addition significantly decreased AOA and AOB abundance, suggesting both AOA and AOB mediated nitrification in these soils and were sensitive to C_2H_2. The observed significant decrease in N_2O emission and nitrification by the addition of C_2H_2 in the cropping and sugarcane soils is mostly likely caused by the inhibitory effect of C_2H_2 on both AOA and AOB growth. However, there was no inhibitory effect on AOA in vegetable soil and AOB on day 7 in dairy pasture soil, suggesting that AOB rather than AOA were involved in nitrification in the alkaline vegetable soil while in the dairy pasture soil AOB might be less important in nitrification than AOA. Moreover, it is possible that C_2H_2 application may also change active strains of ammonia oxidizers and different effectiveness possibly due to different sensitive strains appearing in soils (Belser et al., 1980), and this needs further investigation.

4.2 Inhibitory effects of C_2H_2 at different incubation conditions.

From both previous studies (Kool et al., 2010; Liu et al., 2017) and the current study, soil water content and temperature were the predominant factors regulating N_2O emission from soils. The cumulative N_2O emissions increased with increasing WFPS and temperature in the cropping soil (Table 3). The significant increase in emissions at 35°C between 50% and 70% WFPS is probably because these incubation conditions favoured nitrifying and denitrifying enzyme synthesis (Liu et al., 2017), and 35°C at 70% WFPS is the most suitable condition for

360 microbial activity. The degree of inhibition of cumulative N_2O emission and nitrification by
361 C_2H_2 was shown to vary across the different incubation conditions, indicating that
362 temperature and moisture content significantly affect C_2H_2 efficacy. At lower soil
363 temperature (15°C) and in drier soil (50% WFPS), C_2H_2 addition had no inhibitory effect on
364 the total N_2O emissions, suggesting that nitrification may be heterotrophic under these
365 conditions in this soil. This result is supported by Liu et al. (2015c) where under 15°C and
366 50% WFPS incubation, nitrification was found to be predominantly heterotrophic. The low
367 efficacy of C_2H_2 to inhibit N_2O emission under wetter soils (70% WFPS) is probably because
368 more N_2O was produced from denitrification. Another possibility for the low efficacy of
369 C_2H_2 at the lower soil temperature of 15°C is that C_2H_2 may be unable to form a reactive
370 epoxide to inactivate the AMO enzyme (Hynes and Knowles, 1978).

371 Compared to 70% WFPS, the inhibitory effect of C_2H_2 was much greatest at inhibiting
372 nitrification and cumulative N_2O emission at 50% WFPS except at 15°C. This is possibly
373 because 25°C and 35°C with 50% WFPS are more suitable for nitrification to occur (Garrido
374 et al., 2002; Huang et al., 2014). During incubation, the nitrification rates in the fertilizer
375 treatments were similar at low water content (50% WFPS) regardless of soil temperature,
376 while the effectiveness of C_2H_2 on inhibiting nitrification was different (Table 3). This
377 possibly indicates that concentrations of C_2H_2 may need to be adjusted for optimal inhibition
378 under different soil temperature and moisture.

379 AOA and AOB reduced in abundance with varying degrees after C_2H_2 addition under
380 different environmental conditions, which might be attributed to the differential sensitivity of
381 AOA and AOB to C_2H_2 under different conditions. Under 15°C, 50% and 70% WFPS, C_2H_2
382 showed no effect on AOB populations. One possible explanation is that under lower soil
383 temperature (15°C), the active strains of AOB were less abundant than under higher
384 temperature resulting in less sensitivity to C_2H_2 addition. Another possible explanation is

AOA may prefer lower a soil temperature and be more involved in nitrification at 15°C than AOB.

The underlying mechanism of how C_2H_2 targets AOA remains largely unknown. Acetylene might suppress the growth of *amo*A-containing archaea by inactivating the archaeal AMO protein, as demonstrated in AOB (Hyman and Wood 1985). However, Offre et al. (2009) demonstrated that the enzymes and metabolic pathways of AOA might differ significantly from those of AOB, in which C_2H_2 interferes with the AMO protein. Future studies based on soil RNA and pure cultures are therefore necessary to elucidate the mechanism by which C_2H_2 blocks the growth of AOA.

In the first incubation, we found that different soils have different C_2H_2 efficacy. Therefore, we cannot ascertain whether the findings of the incubation study at different temperatures and moistures can be extended to other soils. Future work with alkaline soils or a larger range of soil properties is definitely needed to clarify this question.

5. Conclusions

In conclusion, C_2H_2 was more effective in inhibiting nitrification and N_2O emissions in acidic soils and under drier condition (50% WFPS). Soil pH, C content, texture, temperature, moisture and land use might be important factors affecting the efficacy of C_2H_2. The various C_2H_2 inhibitory effects on nitrification and N_2O production were also linked to different responses of the ammonia oxidizers to C_2H_2. Therefore, since the effect of C_2H_2 varied with soils and environmental conditions, the assumption that N_2O production and nitrification can be affected by low concentrations of C_2H_2 may need verification for specific soils and conditions of interest.

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