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Manipulating interactions between plant stress responses and soil methane oxidation rates

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Abstract

It has recently been hypothesised that ethylene, released into soil by stressed plants, reduces the oxidation of methane by methanotroph. To test this, a field trial was established in which maize plants were grown with and without soil moisture stress, and the effects of addition aminoethoxyvinylglycine (AVG; an ethylene biosynthesis inhibitor), and biochar (increases soil water holding capacity and reduces plant stress) were determined following the static incubation of soil samples. AVG increased methane oxidation rates by 50% (P=0.039), but only in the absence of irrigation. No other treatment effects were observed. This result provides evidence for a positive feedback system between plant stress, ethylene production, and impacts on methanotrophic activity.

Keywords methane oxidation; plant stress; ethylene inhibition; methanotroph; positive feedback

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1 Introduction

The atmospheric concentration of methane (CH_4) has almost tripled over the past 150 years, making a substantial contribution to climate change (Forster et al., 2007). Aerobic soils provide an important habitat for methanotrophic bacteria, and provide the only significant biological sink for atmospheric CH_4 (20-45 Tg CH_4 yr^{-1}) (Forster et al., 2007). However, CH_4 uptake by these soil ecosystems can be impacted by environmental stress (Kolb, 2009). A common plant physiological response to ecological stress, such as drought, is the production of ethylene (Morgan and Drew, 1997). In soils, however, ethylene maybe inhibitory to methanotrophic activity (Jäckel et al., 2004; Pierek et al., 2006; Zhou et al., 2013), and thereby reduce CH_4 oxidation. This potential interaction needs to be understood, as it may constitute an important positive feedback loop between climate disruption, soil ecosystem disturbance, and reduced methane removal from the atmosphere (Bousquet et al., 2006; Zhou et al., 2013).

This study tested the hypothesis that drought stress on plants can result in reduction of soil methanotrophic activity by adding the ethylene biosynthesis inhibitor aminoethoxyvinylglycine ([S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride; hereafter AVG) (Boller et al., 1979) to soils with and without water stress. In addition, the study tested the hypothesis that addition of biochar to soils may result in increased water holding capacity, reducing drought stress and thereby acting as a potential tool to maintain CH_4 oxidation (Karhu et al., 2011). This is illustrated conceptually in Fig. 1, in which the application of irrigation (IR) and biochar (BC) are able to maintain rates of CH_4 oxidation by reducing moisture stress and therefore ethylene production, whereas AVG prevents the production of ethylene after the plant experiences stress.

2 Material and methods

2.1 Study site

The study site was located in the Bjelke-Petersen Research Station at Kingaroy (26.53° S, 151.83° E) in the South Burnett Region of Queensland, Australia. Precipitation averages 789 mm per annum with erratic summer droughts frequent in the region. Soil at the field trial site soil is an acidic red ferrosol (pH 5.5) with high cation exchange capacity (Isbell, 1993). The site has a long history of cultivation, supporting peanut and maize rotations with winter fallows.

2.2 Experiment design and management

A full factorial, split plot design field trial was established as follows: two IR treatments (IR and no IR) × two BC treatments (BC at 9.2 t ha^{-1} and no BC) × two ethylene suppression treatments (AVG and no AVG). Each treatment had five replicates, producing a total of 40 plots. Due to practical concerns regarding application and maintenance, the IR treatments were established in two discrete areas that were spanned by five blocks. A schematic of the trial site is given in the supplementary information (Fig. S1).

The BC treatment was established through application of peanut shell BC to the surface of the planting zone (~450 mm wide strip each row) in early 2013. The BC was incorporated into the soil with a rotary hoe to a depth of 200 mm. The chemical properties of the peanut shell BC are provided in the supplementary information (Table S1).

The site was machine planted with maize cultivar Pioneer 32p55 (Dupont Pioneer Australia) at a density of approximately 4 plants m^{-2} in late January, 2014. Compound fertiliser ($N:P_2O_5:K_2O$ 11.9:14.1:9.9) at 180 kg ha^{-1}, and urea at 100 kg ha^{-1}, were applied at sowing. Trickle tapes, installed into plots receiving IR, were used to distribute water equivalent to ~50 mm of rainfall whenever there was a continuous dry spell for two weeks throughout the growing season (late January to late June).

To reduce the *in planta* production of ethylene, the commercial plant growth regulator ReTain (containing 15% AVG, Valent Bioscience Cooperation, Walnut Creek, CA, USA) was sprayed onto the crop four times from mid-April to mid-June (the peak maize growth window) at intervals of three weeks. During each event, the treated rows of maize received approximately 750 ml of ReTain solution (prepared at the label rate of 1 g ReTain l^{-1} water) directly to the surface of the plants.

2.3 Sample collection and analysis

In late June 2014, six soil cores from 0 - 100 mm depth were collected from the maize rooting zone of each plot using a 30 mm diameter soil auger. All samples were collected from the two middle rows of maize in each plot, and the six soil cores from within each plot bulked to a single plot sample. After sieving to 2 mm, a 50 g (fresh mass) subsample of each sample was set aside for CH_4 oxidation rate measurements and the remaining material dried at 105°C for 48 hours to determine soil moisture content.

Soil CH_4 oxidation rates were determined by the static incubation technique. Briefly, 50 g soil subsamples were incubated in glass jars at ambient atmospheric CH_4 concentration (assumed to be 1.8 ppm) for two weeks in the dark at 25°C. Headspace gas samples were collected through a rubber septum in the jar lid, and concentrations of CH_4 determined using GC-FID (GC-2010 Plus, Shimadzu, Japan). The oxidation rate in each chamber was calculated from changes in the headspace CH_4 concentration over the incubation time (Zhou et al., 2008), and adjusted to soil dry weight. Standards were measured once every 10 samples; the coefficient of variation in CH_4 oxidation rate was less than 5% and control jars had ambient CH_4 concentrations.

2.4 Statistical analysis

Statistical analysis was carried out in R-3.2.3 (Zhou et al., 2017) using a multi-factor ANOVA model incorporating an error structure accounting for the split-plot design associated with the non-random assignment of the IR treatment. The multi-comparison analysis methods provided in the "easyanova 4.0" R package was used to test for treatment interactions.

3 Results

Over the course of the field trial, five dry spells occurred. Irrigation to the IR-plots resulted in delivery of 250 mm more water to this treatment than the controls. This resulted in significantly increased soil moisture ($P<0.001$) in IR soils (18.9%) compared with the non-irrigated soils (15.4%) at sampling time. Neither the AVG or BC treatments had any effect ($P>0.05$) on soil moisture.

No significant main effects were observed, but a significant interaction between irrigation and AVG application was detected (Table 1). Exploration of this interaction with multi-comparison analysis determined that CH_4 oxidation rates were increased by 50% following AVG application ($P=0.039$), but only in the absence of IR (Fig. 2). The addition of biochar had no effect on CH_4 oxidation rates either as a main or interactive effect.

4 Discussion

The increase in CH_4 methane oxidation with the AVG treatment either alone or in combination with the BC treatment aligns with past studies assessing the effect of increased ethylene concentrations on soil CH_4 oxidation rates (Jäckel et al., 2004; Xu et al., 2008). This response also supports the hypothesis that *in planta* ethylene production in response to stress decreases the capacity

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of soil to support methanotrophic activity (Zhou et al., 2013).

The lack of effect of BC on CH_4 oxidation is at odds with the results of previous work (e.g. Karhu et al., 2011; Kim et al., 2017). However, BC added in this study had no influence on soil moisture content, and this is proposed to be a key mechanism for BC to support CH_4 oxidation in drought conditions (Karhu et al., 2011). The reason why BC addition did not result in increased soil moisture in this case is unclear, but may be related to differences in BC chemistry or amounts applied as these vary from study to study (e.g. Kim et al., 2017).

The significant interaction between the AVG and IR treatments is more difficult to reconcile. The IR treatment was intended to significantly increase soil moisture content compared to the no IR treatment, reducing water stress and likely *in planta* ethylene production. It was noted that increased soil moisture content can directly influence methanotrophic activity, as water-driven increases in microbial activity can enhance methanotroph, whereas water content that exceed field capacity can rapidly decrease methane oxidation rates by reducing gas mobility through soil pores (Le Mer and Roger., 2001). Given the initial soil water content and scale of the increase with the IR treatment, direct stimulation of methane oxidation was considered the most likely outcome when considering plant-independent effects. Consequently, it was anticipated that any effect of AVG on CH_4 oxidation (putatively via reductions in ethylene production) would only manifest without IR, as the IR treatment would make the AVG treatment redundant. However, CH_4 oxidation rates in plots treated with the either IR or IR and AVG in combination were not significantly greater than untreated control plots. It is possible that the moisture addition associated with the IR treatment was insufficient to substantially alleviate plant drought stress, driving an increase in ethylene production, which could then account for the numerical difference between the AVG and IR treatments (Fig. 2). The water addition may have also been insufficient to meaningfully directly stimulate methanotroph activity. However, it would be expected that the combination of IR and AVG would produce CH_4 oxidation rates either the same, or potentially greater than, those observed for AVG alone. This was not the case, and the explanation for the significant interaction remains unknown.

The lack of data explicitly describing ethylene release into soil in response to the treatments is a limitation to this trial. However, the quantification of ethylene in soil is not trivial, particularly when

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conducted over time (i.e. continuous) and was outside the resources available for this study. However, given the findings of this study, and considering treatments were field-based, further investigations of the interactions between AVG, plant stress, and CH_4 oxidation should be conducted. In these studies, consideration should be given to collection and integration of ethylene data, particularly given that this data may help shed light on the nature of any interactions between treatments.

Overall, the findings of this study indicate that application of an ethylene biosynthesis inhibitor to plant tissue can cause a measurable increase in the capability of soil to oxidise methane under moisture stressed conditions. This supports the hypothesis that the stress-induced production of ethylene by plants can disrupt the activity of methanotrophs, as well as identifying a potential management pathway to help retain, or even enhance, the methanotrophic capability of soils in productive systems. Given the global importance of a positive feedback between environmental stress, plant ethylene production, and lowered microbial CH_4 oxidation activity, further work in this area is needed. In addition, methods to moderate impacts on the methanotrophic community, such as use of alternative forms or rates of biochar application, require investigation to enable provision of important ecosystem services.

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Table 1. Analysis of treatment effects on methane oxidation rates, accounting for the split-plot design of the trial.

Block	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
Residuals	4	25.27	6.318		
Block:IR	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
IR	1	0.057	0.057	0.01	0.925
Residuals	4	22.785	5.696		
Block:IR:BC	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
BC	1	0.946	0.946	0.245	0.634
IR:BC	1	0.815	0.815	0.211	0.658
Residuals	8	30.924	3.865		
Block:IR:BC:AVG	Df	Sum Sq	Mean Sq	F Value	Pr(>F)
AVG	1	4.77	4.768	1.796	0.199
IR:AVG	1	17.38	17.384	6.549	**0.021**
BC:AVG	1	6.19	6.186	2.33	0.146
IR:BC:AVG	1	0.42	0.422	0.159	0.695
Residuals	16	42.47	2.654		

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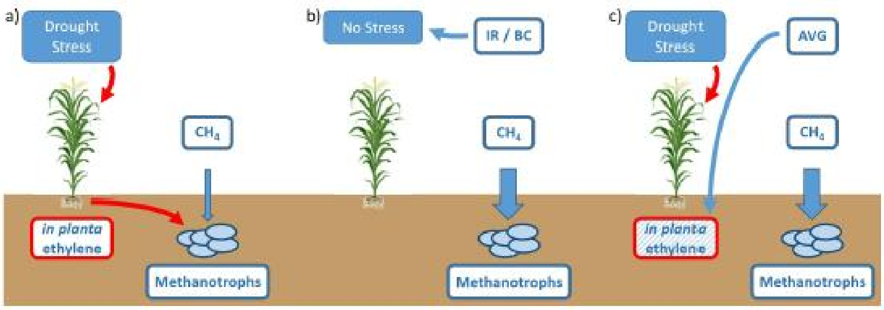

Fig. 1. Conceptual outline of the proposed relationships between soil CH_4 oxidation rates and aboveground plant biomass with regard to the anticipated effects of the treatments applied in this study. a) Under environmental stress, *in planta* ethylene production is stimulated, resulting in ethylene exudation into the soil atmosphere and the inhibition of soil CH_4 oxidation by methanotrophs. b) The application of irrigation (IR) increases soil moisture while the application of biochar (BC) increases soil moisture holding capacity, both acting to reduce plant stress and prevent ethylene exudation into the soil atmosphere. c) The application of AVG disrupts ethylene production, and limiting or preventing the inhibition of CH_4 oxidation by the stressed plant.

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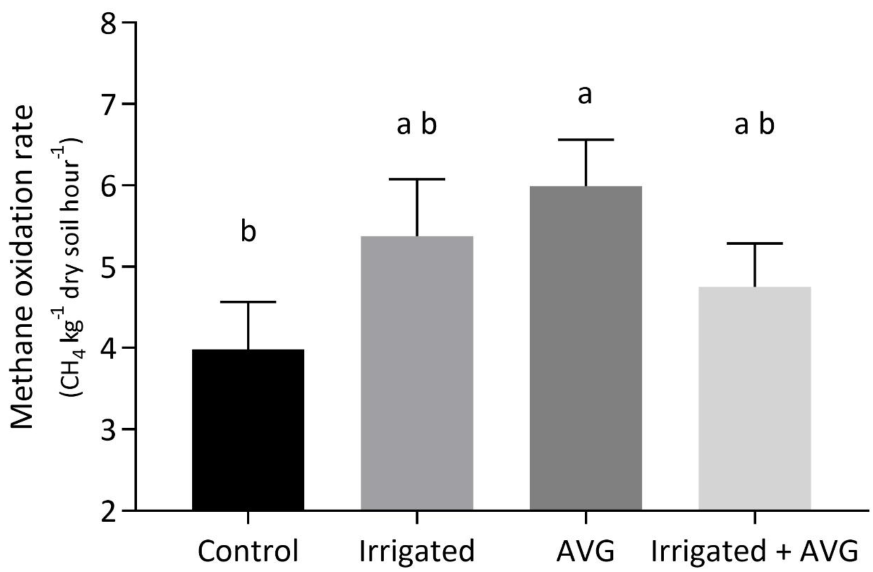

Fig. 2. Response of soil CH_4 oxidation rates to treatment with irrigation and AVG under maize plants. Letter groupings indicate significant differences at $P<0.05$; error bars are standard error of the mean. The biochar treatment did not influence results, so the data presented are the means of both biochar and no biochar treatments.