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Manipulation of soil methane oxidation under drought stress

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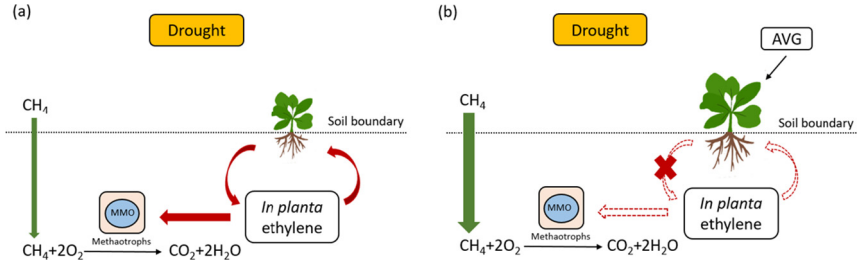
HIGHLIGHTS

- Drought influences soil CH_4 oxidation through different pathways.
- Drought induced higher *in planta* C_2H_4 production.
- High C_2H_4 production inhibits soil CH_4 oxidation.
- Reducing *in planta* C_2H_4 production increased soil CH_4 oxidation.
- We can manipulate *in planta* C_2H_4 production *via* inoculation of PGPR.

GRAPHICAL ABSTRACT

Putative pathway for ethylene produced by drought-stressed plants to inhibit soil methane oxidation.



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ABSTRACT

Drought events are predicted to occur more frequently, but comprehensive knowledge of their effects on methane (CH_4) oxidation by soil methanotrophs in upland ecosystems remains elusive. Here, we put forward a new conceptual model in which drought influences soil CH_4 oxidation through a direct pathway (*i.e.*, positive effects of soil CH_4 oxidation *via* increasing soil aeration) and through an indirect pathway (*i.e.*, negative effects of *in planta* ethylene (C_2H_4) production on soil CH_4 oxidation). Through measuring soil CH_4 efflux along a gradient of drought stress, we found that drought increases soil CH_4 oxidation, as the former outweighs the latter on soil CH_4 oxidation, based on a mesocosm experiment employing distinct levels of watering and a long-term drought field trial created by rainfall exclusion in a subtropical evergreen forest. Moreover, we used aminoethoxyvinylglycine (AVG), a C_2H_4 biosynthesis inhibitor, to reduce *in planta* C_2H_4 production under drought, and found that reducing *in planta* C_2H_4 production increased soil CH_4 oxidation under drought. To confirm these findings, we found that inoculation of plant growth-promoting rhizobacteria containing the 1-aminocyclopropane-1-carboxylate deaminase alleviated the negative effects of drought-induced *in planta* C_2H_4, thus increasing soil CH_4 oxidation rates. All these results provide strong evidence for the hypothesis that *in planta* C_2H_4 production inhibits soil CH_4 oxidation under drought. To our knowledge, this is the first study to manipulate the negative feedback between C_2H_4 production and CH_4 oxidation under drought stress. Given the current widespread extent of arid and semiarid regions in the world, combined with the projected increased frequency of drought stress in future climate scenarios, we provide a reliable means for increasing soil CH_4 oxidation in the context of global warming.

1. Introduction

It has been predicted that drought events will become more and more frequent and have longer duration by the end of this century (IPCC, 2013). Drought has dramatic impacts on ecosystem functions like soil methane (CH_4) uptake and oxidation by soil microbes in

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terrestrial ecosystems (Wang et al., 2014; Tate, 2015; Ni and Groffman, 2018). Upland ecosystems such as forests and grasslands can oxidise ~20–45 Tg CH_4 $year^{-1}$ from the atmosphere and act as the largest biological sink worldwide (Dutaur and Verchot, 2007). Given that CH_4 is the second most important greenhouse gas after carbon dioxide (CO_2) and contributes about 25–30% to global warming (IPCC, 2013), CH_4 oxidation and the underlying mechanisms controlling this have received much attention.

The oxidation of CH_4 in upland soils is mediated by a specific group of microorganisms known as methanotrophs (Hanson and Hanson, 1996; Kolb, 2009). Methanotrophs are Gram-negative bacteria within the Verrucomicrobia, Proteobacteria and the novel phylum NC10 that use CH_4 as their sole source of carbon and energy (Kolb, 2009; Bowman, 2011; Tate, 2015). The key step in soil CH_4 oxidation is catalysed by CH_4 monooxygenase (MMO), which converts CH_4 into methanol *via* the CH_4 oxidation pathway (Fig. 1) (Kolb, 2009). Previous studies have demonstrated that similar gases such as ethylene (C_2H_4) compete with CH_4 for the active site of MMO, thus inhibiting soil CH_4 oxidation (Jackel et al., 2004; Zhou et al., 2013; Bu et al., 2019).

Based on the literature, it is clear that the capacity of methanotrophs to oxidise CH_4 in the soil gas atmosphere is influenced by various factors but the predominant driver of this activity is soil moisture content (Kolb, 2009; Fest et al., 2015; Tate, 2015). In general, soil CH_4 oxidation rates in upland ecosystems exhibit a unimodal response to changes in soil moisture contents: in a particular environment, soil CH_4 oxidation rates can reach a peak at optimal soil moisture contents but they tend to decrease with increasing or decreasing soil moisture contents (Zhou et al., 2014; Tate, 2015). When soil moisture is higher than optimal water holding capacity, soil forms anaerobic environment, thus inhibiting methanotrophic activity and stimulating methanogenic activity (Le Mer and Roger, 2001). The mechanisms for reduced soil CH_4 oxidation capacity under drought are still unclear. Our previous work was the first study to put forward a hypothesis that drought-induced *in planta* C_2H_4 production can inhibit methanotrophic activity (Jackel et al., 2004; Pierik et al., 2006; Zhou et al., 2013). This potential interaction needs to be understood, as methanotrophic activity could support the development of a positive feedback loop linking climate disruption, plant stress and reduced CH_4 removal from the atmosphere (Bousquet et al., 2006; Zhou et al., 2013).

Drought, considered here as an ongoing reduction in soil moisture availability, can greatly influence CH_4 uptake and oxidation by soil methanotrophs through two pathways, namely direct and indirect pathways in upland ecosystems (see Fig. 1). The direct pathway has positive effects, as drought can increase soil aeration, thereby increasing the availability of CH_4 in the soil gas atmosphere, enabling greater CH_4 uptake and oxidation (Fest et al., 2015). However, the indirect pathway has negative effects, as drought can increase *in planta* C_2H_4 production and release into the soil environment, thereby inhibiting CH_4 oxidation through competition for MMO (Jackel et al., 2004; Zhou et al., 2013; Crombie and Murrell, 2014; Bu et al., 2019). Compared with the direct pathway, the impact of the indirect pathway remains relatively unknown. If the indirect pathway is of significance, application of C_2H_4 biosynthesis inhibitors such as aminoethoxyvinylglycine ([*S*]-*trans*-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) (AVG) (Boller et al., 1979), or inoculation of plant growth-promoting rhizobacteria (PGPR) containing the 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Zhou et al., 2013), may alleviate the negative effects of drought-induced *in planta* C_2H_4, thus increasing soil CH_4 oxidation capacity (Fig. 1b) (Question 1). Moreover, to our knowledge, few studies have investigated the negative effects of *in planta* C_2H_4 under drought in natural upland ecosystems and to what extent these effects occur (Question 2).

Here, to address these questions, we carried out a mesocosm experiment using laboratory incubations (Experiment 1 for answering Question 1) and the *in situ* effects of drought manipulation on soil CH_4 oxidation (Experiment 2 for answering Question 2). For Experiment 1, we established a series of mesocosms supporting *Arabidopsis thaliana* (L.) Heynh. plants across a drought gradient with and without the application of the C_2H_4 biosynthesis inhibitor of AVG (Boller et al., 1979). For Experiment 2, we selected a long-term *in situ* extreme drought platform by reducing rainfall by 70% in a subtropical forest (Bu et al., 2018). These two experiments allowed us to investigate the effects of *in situ* C_2H_4 concentrations on soil CH_4 oxidation and determine the magnitude of this effect and thus test the hypothesis that drought-induced *in planta* C_2H_4 can inhibit soil CH_4 oxidation. We acknowledge that we did not consider the effects of C_2H_4 on methanogenic activity, as past research indicates that there is negligible CH_4 production under drought conditions (Zhou et al., 2008a; Nazaries et al., 2011; Tate, 2015).

2. Materials and methods

2.1. Experimental design

For Experiment 1, 40 sealed mesocosms were prepared by placing approximately 200 mL of a dry mixture in a 250-mL pot and then placing that pot into the mesocosm chamber. The mixture comprised vermiculite and a loam-based compost in a 1:7 ratio by mass. The final mixture characteristics were as follows: total carbon, 41.6 ± 1.63 g kg^{-1}; total nitrogen, 1.48 ± 0.14 g kg^{-1}; NH_4^+–nitrogen, 25.9 ± 3.5 mg kg^{-1}; NO_3^-–nitrogen, 4.21 ± 0.26 mg kg^{-1}; pH 5.85 ±

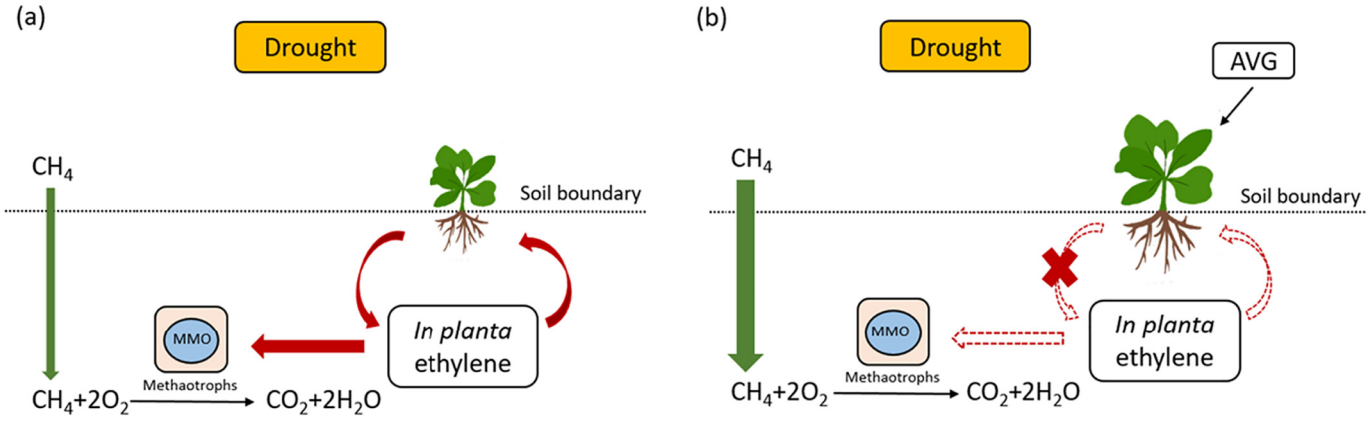

Fig. 1. Putative pathway for ethylene produced by drought-stressed plants to inhibit soil methane oxidation. Methanotrophs in aerobic conditions can convert methane to carbon dioxide (CO_2), enabling the soil to act as a net methane sink. These harbour the key enzyme of methane monooxygenase (MMO). Drought stress increases endogenous ethylene production and exudation from plant roots. Ethylene inhibits methane oxidation *via* competition for MMO (a). The application of aminoethoxyvinylglycine (AVG) disrupts ethylene production, as shown by the dotted lines, thus increasing soil methane oxidation and plant growth under drought (b).

0.08. The test plant was *A. thaliana*. This species was selected for use because of the wide body of knowledge relating to the responses of *A. thaliana* to drought (Taji et al., 2002; Huang et al., 2008; Jin et al., 2011). Seeds of *A. thaliana* were selected from a pool on the basis of maintaining seed weight homogeneity, then surface-disinfected with 95% ethanol for 5 min and 20% sodium hypochlorite for 7 min. After washing with sterile distilled water, the seeds were stratified at 4 °C for 3 days, then planted into the mesocosm soil. After a single seed had been planted, all mesocosms were initially maintained at well-watered conditions (*i.e.* 80% water-holding capacity (WHC)) in a plant-growth incubator in order to enhance initial plant growth. The environment in the incubator was maintained at a photosynthetic photon flux density of 200 μmol m^{-2} s^{-1}, a 16:8 h light: dark photoperiod and a constant temperature of 22 °C. The WHC of the soil was determined as described in Werner (1997). After 1 month, the growth of the seedlings in the mescosoms was assessed and 30 were selected for inclusion in the experiment, on the basis of their uniformity and the vigour of plant growth. These mesocosms were randomly divided into three groups consisting of 10 replicates each.

Each group was randomly allocated to a watering regime designed to maintain soil moisture content at 30%, 50% and 80% of WHC, representing drought, normal and well-watered conditions. To account for evapotranspirational losses, the mass of each mesocosm system was monitored and lost moisture was replaced daily. To enable the impact of drought on methanotrophic activity to be assessed with reduced interference by any putative effects from plant-produced C_2H_4, the C_2H_4 biosynthesis inhibitor AVG (Sigma, Shanghai, China) was applied to a subset of mesocosms at the same time the watering regimes commenced. Each water regime group was subdivided into two sets of five mesocosms, one of which was treated with 2 mL of an AVG solution applied to the plant foliage (1 g AVG L^{-1} distilled water); plants not receiving AVG treatment received the same volume of distilled water. This design produced a total of six treatments, comprising three levels of watering combined with two levels of AVG application, each replicated five times. The application of AVG was targeted specifically to the leaves of the plants to ensure that any effects on CH_4 oxidation would be derived from alterations to *in planta* C_2H_4 production and not from any potential effect on methanotrophic activity in the soil. At the end of the experiment, the plants were harvested and the biomass was determined by drying the plant material at 70 °C to a constant weight.

For Experiment 2, we used an existing rainfall manipulation experiment site that was established in July 2013 at Tiantong National Forest Park, Ningbo, Zhejiang Province, Eastern China (29°52′N, 121°39′E, 200 m above sea level). The region experiences a typical subtropical monsoon climate, with a mean annual temperature of 16.2 °C. The mean annual precipitation is 1383.94 mm (1953–2012). The dominant tree species at the study site are *Castanopsis fargesii* Franch., *Schima superba* Gardner and Champ., *Castanopsis carlesii* (Hemsl.) Hayata and *Lithocarpus glaber* (Thunb.) Nakai (Bu et al., 2018).

There were three treatments: a 70% rainfall reduction to simulate an extreme drought scenario in the future (hereafter referred to as the "drought" treatment) by using large plastic plates, a shade treatment to account for the effect of plates in the drought treatment (hereafter referred to as the "disturbance" treatment) and an ambient control treatment. Each treatment had three replicates, resulting in nine plots in total. The size of each plot was 25 m × 25 m, with at least 5-m spacing between adjacent plots. To minimise the effects of disturbance, buffer regions with a 2.5-m width were set around each plot. Detailed information about these experimental sites and soil properties have been given in Table S1 and in previous studies (Bu et al., 2018).

2.2. Measurements of CH_4 and C_2H_4 concentrations and plant biomass in Experiment 1

Soil CH_4 efflux was determined by the static incubation technique 1 and 2 weeks after the treatments commenced. Each mesocosm was placed into a 1-L glass jar and topped with lids modified by drilling a hole in the centre of the lid, which was then sealed with a rubber septum for gas sampling. Mesocosms were incubated at ambient atmospheric CH_4 concentrations for 5 h in the dark at 22 °C. At the beginning and end of the incubation, gas samples were collected from the headspace of jars with two 30-mL syringes for determining CH_4 and C_2H_4 concentrations.

The concentration of CH_4 was determined with a gas chromatograph equipped with a flame ionisation detector (7890B GC, Agilent, USA). The CH_4 efflux for each mesocosm was calculated from changes in the headspace CH_4 concentrations over the incubation time (Zhou et al., 2008a; Bu et al., 2019), then standardised to account for soil dry mass and plant biomass in the mesocosm. Standards were measured once every 10 samples to monitor the accuracy of the analytical equipment. The coefficient of variation in CH_4 efflux was less than 5% and control jars containing ambient air were processed *via* the same protocol as the control for checking gas leakage. The soil CH_4 efflux was expressed as μg kg^{-1} dry soil h^{-1} for the first week's samples, though it was expressed as μg kg^{-1} dry soil h^{-1} g^{-1} plant biomass for the second week's samples.

In addition, concentrations of C_2H_4 were determined on a gas chromatograph with a flame ionisation detector, using a GDX-502 column and an injection mode (Varian GC9800, Shanghai, China) (Bu et al., 2019). The parameters for C_2H_4 measurements were set as follows: the temperatures of the column, injection pool and detector were 80 °C, 100 °C and 120 °C respectively; the flow rate of the carrier gas (N_2) was 40 mL min^{-1}. The C_2H_4 production rates for each mesocosm were calculated from changes in the headspace C_2H_4 concentration over the incubation time and expressed as μg kg^{-1} dry soil h^{-1} for the first week's samples and as μg kg^{-1} dry soil h^{-1} g^{-1} plant biomass for the second week's samples.

To support the mesocosm study, a greenhouse trial was established with an eggplant crop. We carried out this trial in a plastic greenhouse (width: 25 m; length: 30 m; height: 2.5 m) located at a crop cultivation farm in Fengxian District, Shanghai, southeastern China (30°52′N, 121°34′E). There was a typical subtropical monsoon climate in this region, with a hot, humid summer and a drier, cold winter. According to long-term meteorological data records, the mean annual temperature is 15.8 °C and the mean annual precipitation is 1149 mm. Detailed site information and soil properties have been described before in other studies (Xue et al., 2020). Within the greenhouse, nine plots measuring 1 m × 2 m were randomly selected in early February of 2019. Each plot was at least 2 m away from the others. After 2 and 6 weeks of eggplant seedling growth, we applied AVG and a pure strain of FX-1 (Fig. S2), a PGPR containing ACC deaminase, into the seedlings. Each treatment had three replicates. AVG was applied at a rate of 1 g AVG L^{-1} distilled water; 500 mL of PGPR inoculum with 10^8 cells mL^{-1} was applied into the eggplant seedlings in an area of m^2; plants not receiving treatments received the same volume of distilled water. After harvest in late June 2019, we collected plant residues for measuring aboveground biomass and soil samples for measuring CH_4 oxidation rates (Zhou et al., 2008a; Bu et al., 2019).

2.3. Measurements of *in situ* CH_4 and C_2H_4 concentrations in Experiment 2

We collected gas samples to measure *in situ* CH_4 and *in situ* C_2H_4 concentrations across all drought plots at the four sampling times in the subtropical evergreen forest, namely in late April 2017, in early July 2017, in early July 2018 and in late October 2019 (Bu et al., 2018). The changes in soil temperature and moisture content in the forests have been given in Fig. S3. We used the static chamber approach to collect gas samples (Zhou et al., 2008a; Stiles et al., 2018). Briefly, we first made these chambers from polyethylene. They consisted of removable cover boxes (30 cm in diameter and 40 cm high without a bottom) equipped with a three-way sampling port and a cylindrical collar. Three polyethylene cylinder collars with a total height of 8 cm were permanently installed 3 cm deep into the soil in each of the plots. For

sampling, gas samples (~10 mL) were taken with a 30-mL polypropylene syringe at 10-min intervals over 40 min after deployment. The samples were then transferred to evacuated 10-mL glass vials with Chromacol butyl septa. Samples in the glass vials were transported to the laboratory and subsequently analysed to determine CH_4 and C_2H_4 concentrations as previously described. The *in situ* CH_4 flux was calculated from the slopes of linear regressions between gas concentrations and sampling time (Zhou et al., 2008a; Stiles et al., 2018).

2.4. Determination of the effects of C_2H_4 on soil CH_4 oxidation rates along a gradient of concentrations in Experiment 2

We used soil samples from the control plots collected in February 2017 to examine the effects of C_2H_4 addition on soil CH_4 oxidation rates *via* a laboratory incubation. Briefly, approximately 10 g (dry weight equivalent) of field-moist soil was incubated in a 1-L sealed flask under ambient air conditions for measuring CH_4 oxidation rates in the dark at 22 °C for 7 days. The effects of C_2H_4 on CH_4 oxidation rates were determined by the addition of different C_2H_4 concentrations (0, 3, 5, 10, 15, 20, 30, and 50 ppm) to the flask's ambient headspace. The CH_4 oxidation rates for each soil sample were calculated from changes in the headspace CH_4 concentration over the incubation time as described above (Bu et al., 2019).

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine the effects of drought and AVG application on C_2H_4 production rates, CH_4 efflux and plant biomass in Experiment 1, and to determine the effects of drought and sampling time on C_2H_4 concentrations and *in situ* CH_4 efflux in Experiment 2. Plant biomass data was log-transformed to meet assumptions of normality for exploration *via* ANOVA. As plant biomass data were only collected at harvest (2 weeks after treatment), the CH_4 efflux data collected 1 week after treatment were used to assess temporal variations in the response of CH_4 efflux to the treatments independently of plant biomass. We used a non-linear fitting to quantify the negative effects of C_2H_4 on soil CH_4 oxidation rates, as we set soil the CH_4 oxidation rates in the ambient control as 100% CH_4 oxidation capacity. Given that there were large variations in CH_4 efflux and C_2H_4 efflux, we used generalized least models with varIdent functions to evaluate differences among the treatments (Zuur et al., 2009). After statistical analysis *via* ANOVA in the "stats" R package (R Foundation for Statistical Computing, Vienna, Austria), Tukey's HSD test was used to compare the significant differences among the treatments. Statistical analysis was carried out in R version 3.5.3 (R Development Core Team, 2019). Significant differences were considered at $P < 0.05$.

3. Results

In Experiment 1, the watering regime and AVG application both significantly affected CH_4 efflux after 2 weeks (Fig. 2). Mesocosms maintained at 30% water-holding capacity (WHC) exhibited a significant increase in CH_4 oxidation compared with mesocosms maintained at 50% WHC ($P < 0.001$) and 80% WHC ($P < 0.01$), whereas AVG application increased CH_4 uptake ($P < 0.05$) at all moisture levels (Fig. 2a). Examination of the CH_4 efflux rates calculated independently of plant biomass indicated that the response to the WHC treatment was unaffected by the time of sampling (both $P < 0.001$) (Table 1). The response to AVG was not significant after 1 week ($P = 0.07$) but it became significant by Week 2 ($P < 0.01$); this difference was driven by greater CH_4 uptake with AVG and a reduction in the extent of variation in Week 2's measurements (Table 1).

The response of C_2H_4 production varied considerably within treatment combinations, resulting in relatively large error values compared with mean values (Fig. 2b). In the absence of AVG application, C_2H_4 production was lower in mesocosms maintained at 80% WHC than in mesocosms maintained at 30% WHC ($P < 0.05$) and 50% WHC ($P < 0.01$). The application of AVG numerically reduced C_2H_4 production but this effect was only statistically significant in mesocosms

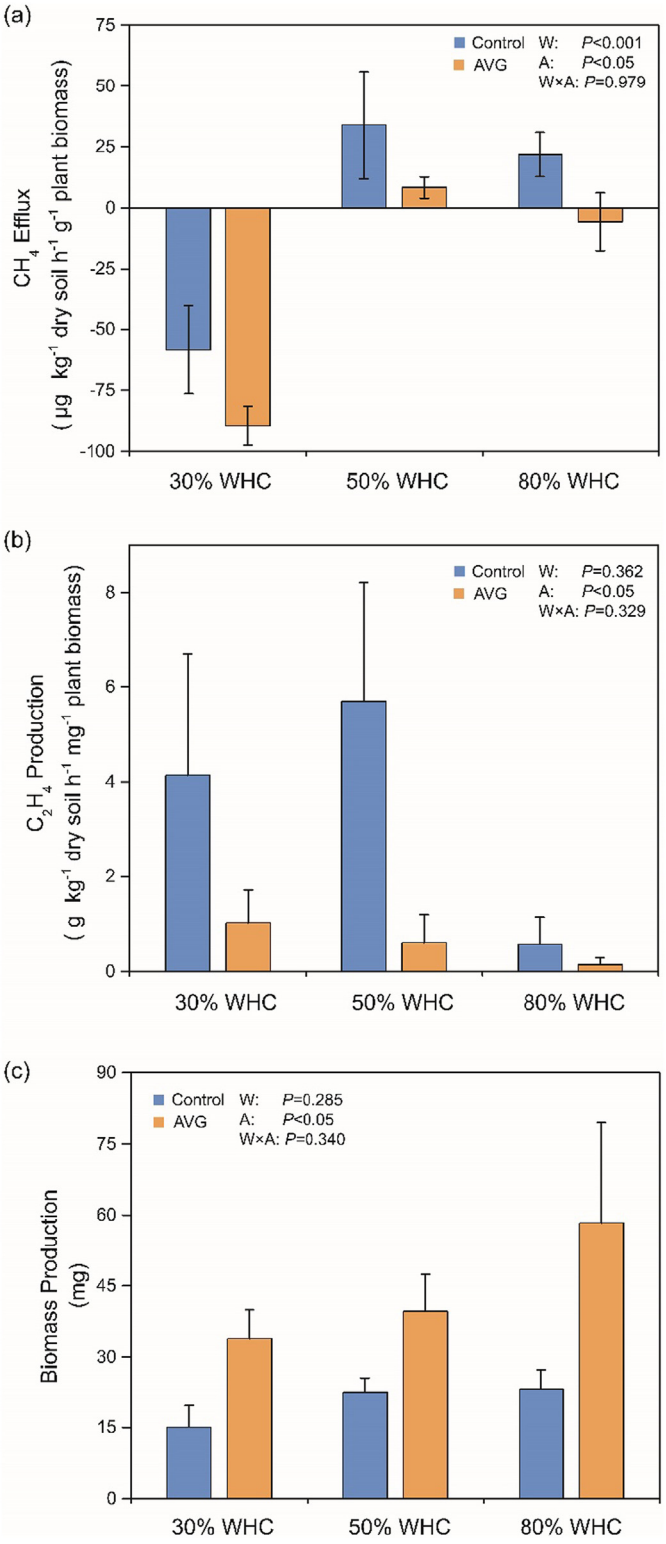

Fig. 2. Variations in soil methane (CH_4) efflux (a), ethylene (C_2H_4) production rates (b) and *A. thaliana* biomass (c) 2 weeks after foliar treatment with the ethylene inhibitor aminoethoxyvinylglycine (AVG) along a gradient of soil water-holding capacities (WHC), based on a mesocosm experiment. Negative values indicate soil CH_4 uptake. Data represent the mean values for measurements ($n = 5$); error bars indicate the standard error of the mean. W, watering; A, AVG; W × A, watering × AVG.

Table 1
Mean values showing the effects of watering regime and application of the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) addition on soil CH_4 efflux after 1 and 2 weeks, calculated independently of plant biomass.

Treatment	Week 1		Week 2	
	CH_4 efflux ($\mu g\ kg^{-1}\ h^{-1}$)		CH_4 efflux ($\mu g\ kg^{-1}\ h^{-1}$)	
AVG				
No	0.1 (0.5)	a	0.1 (0.3)	a
Yes	−1.0 (0.6)	a	−1.2 (0.5)	b
WHC				
30%	−2.6 (0.7)	a	−2.1 (0.3)	a
50%	0.8 (0.4)	b	0.6 (0.6)	b
80%	0.3 (0.5)	b	−0.1 (0.3)	b

Values in parentheses indicate the standard error of the mean. Different letters within each group indicate significant differences at $P < 0.05$.

maintained at 50% WHC ($P < 0.05$) (Table 1). Plant biomass production tended to increase with greater water availability but this response was not significant (Fig. 2c). The application of AVG increased biomass production by 109% on average across the three watering regimes ($P < 0.01$). Images illustrating the effect of AVG on plant growth rates are provided in the Supplementary Material (Fig. S1). No significant interaction terms were detected (Fig. 3).

These results were supported by our field study, showing that application of AVG increased aboveground eggplant (*Solanum melongena* L.) biomass by 5.98% in a subtropical agricultural farm under normal water conditions (Fig. 3). We found that inoculation with PGPR expressing ACC deaminase significantly increased soil CH_4 oxidation rates, and it increased aboveground eggplant biomass by 12.4% (Fig. 3). A conceptual overview of the processes underpinning our findings is presented in Fig. S4. Further studies are needed to investigate the relative changes in CH_4 production by methanogens and in CH_4 oxidation by methanotrophs in upland ecosystems.

In Experiment 2 we found that drought significantly increased both soil CH_4 uptake and C_2H_4 production compared with the ambient control treatment (both $P < 0.05$) (Fig. 4). As the sampling time had no significant effect on *in situ* C_2H_4 concentrations across the treatments (Fig. 4b), we combined all *in situ* C_2H_4 concentrations together for each treatment and found that drought significantly increased *in situ* C_2H_4 concentrations (1.93 ± 0.66 parts per million (ppm)) relative to the control (0.21 ± 0.08 ppm) ($P < 0.05$) as well, but the disturbance treatment had no significant effect.

To test the inhibitory effects of C_2H_4 on soil CH_4 oxidation, we manipulated a gradient of C_2H_4 concentrations *via* laboratory incubations. We found that at 3 ppm of C_2H_4 in the headspace, soil CH_4 oxidation rates reduced by nearly 40%; at 10 ppm, they were reduced by ~80%; at 50 ppm, oxidation was completely inhibited (Fig. 4c). These data allowed the construction of a function explaining the inhibitory effects of C_2H_4 on soil CH_4 oxidation, from which we estimated that *in situ* C_2H_4 concentrations under drought could drive a reduction of 1.2–29.8% (mean value of 16%) in CH_4 uptake, although the net effect of drought was still increased CH_4 oxidation.

4. Discussion

Our results provide strong evidence for the hypothesis that plant-derived C_2H_4 production can inhibit soil CH_4 oxidation under drought. To address our first question, we found that reducing *in planta* C_2H_4 production increased soil CH_4 oxidation in the mesocosm experiment. To address the second question, we found that long-term drought increased *in situ* C_2H_4 concentrations, which had a markedly negative influence on soil CH_4 oxidation and accounted for large variations in soil CH_4 oxidation in the subtropical evergreen forest. To our knowledge, this is the first study to investigate the relationships between *in situ* C_2H_4 production and *in situ* CH_4 oxidation in natural upland ecosystems.

4.1. Soil CH_4 oxidation under drought

As discussed above, drought can influence methanotrophic activities and thus CH_4 uptake *via* two pathways: a direct pathway and an indirect pathway, as shown in Fig. 1. The enhancement in soil methanotrophic activity associated with the direct pathway is driven by primarily by increased soil aeration, allowing greater movement of CH_4 throughout the soil gas atmosphere and concomitant increases in CH_4 uptake by methanotrophs (Zhou et al., 2014; Fest et al., 2015). Here we have shown that the reduction in soil methanotrophic activity associated with the indirect pathway can be substantial.

As predicted, C_2H_4 had a strong inhibitory effect on CH_4 oxidation rates during incubation, which was consistent with previous findings in forest soils under laboratory incubation (Jackel et al., 2004; Xu and Inubushi, 2009). It was noted that drought had higher *in situ* C_2H_4 concentrations, with greater potential to inhibit soil methanotrophic activity compared with the control treatments. Overall, however, the drought treatment produced significantly greater soil CH_4 oxidation in both the mesocosm study and in the subtropical forest, indicating that the positive effects of soil aeration overcame the negative effects of

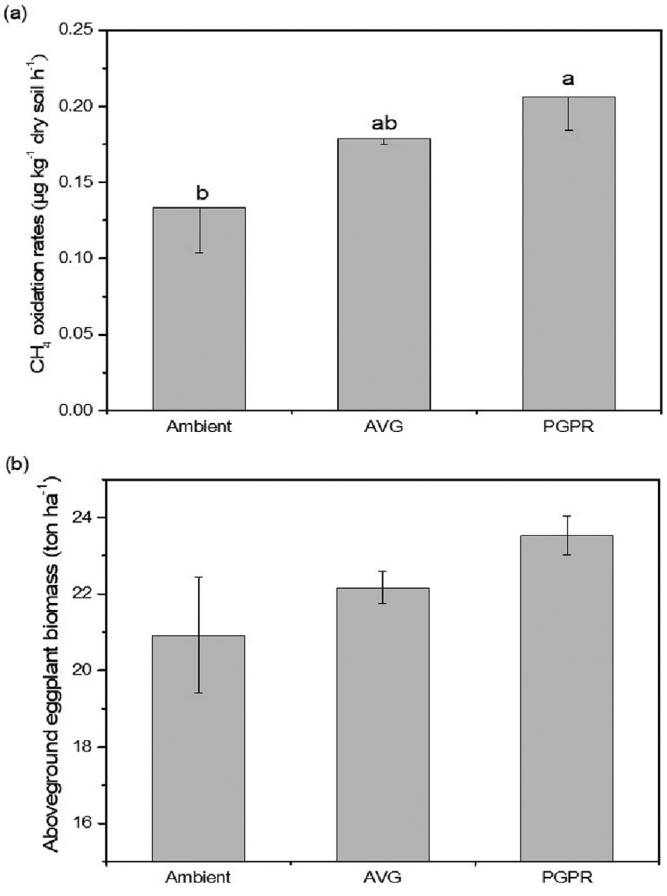

Fig. 3. We applied the ethylene inhibitor aminoethoxyvinylglycine (AVG) and inoculated eggplant specimens with plant growth-promoting bacteria (PGPR) containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase during the growth period between February 2019 and June 2019. After harvest, we collected soil samples to measure (a) soil CH_4 oxidation rates *via* laboratory incubations and (b) aboveground eggplant biomass (stem + leaves) under different treatments.

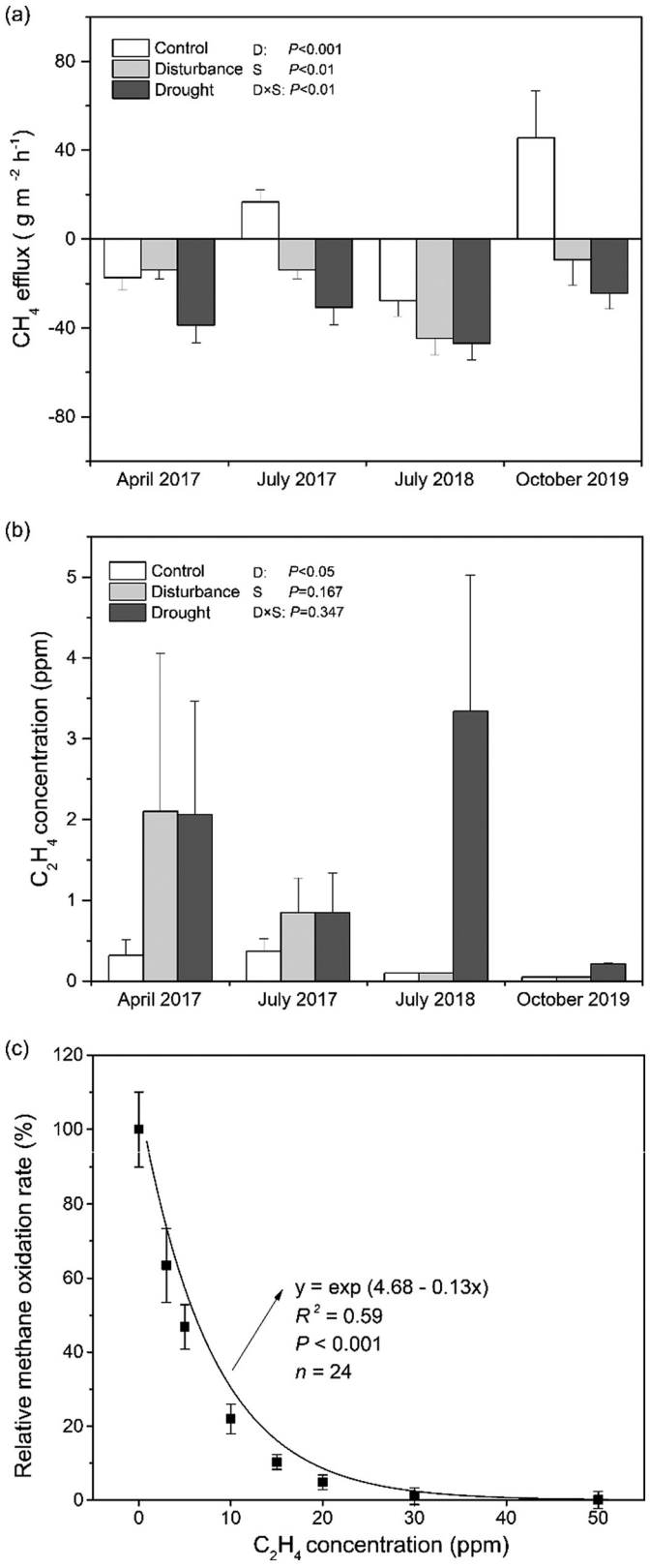

Fig. 4. Variations in *in situ* CH_4 efflux (a) and the corresponding *in situ* ethylene (C_2H_4) concentrations (b) between April 2017 and October 2019, and changes in relative soil CH_4 oxidation rates (c) produced *via* laboratory incubation in response to a gradient of C_2H_4 concentrations from 0 parts per million (ppm) to 50 ppm in the soils of the control plots collected in February 2017 from a subtropical evergreen forest. The trend line indicates the fitted relationship. D, drought treatment; S, sampling time; D × S, drought × sampling time.

drought-induced C_2H_4 on methanotrophic activity, resulting in net soil CH_4 oxidation.

Moreover, we noted that there were large variations in C_2H_4 concentrations across all plots. Our results might also provide a reasonable explanation for the large variation of *in situ* soil CH_4 oxidation. The C_2H_4 concentrations in the drought plots might reduce *in situ* CH_4 oxidation by 1.2–29.8%, according to the laboratory incubation assay, indicating that soil CH_4 oxidation could potentially reach 30% more than that seen without C_2H_4 production under drought. These values are markedly larger than what we expected. More research is needed to test if we can extrapolate this finding to other terrestrial ecosystems, although environmental stressors like drought are common in the field (Zhou et al., 2013). The response of the soil microbial community to drought (or different levels of moisture availability, as established in the mesocosm experiment) should also be considered, as this has the potential to create further indirect effects on *in planta* C_2H_4 production through plant-microbe signalling or other interactions (*e.g.* Zolla et al., 2013).

Previous research reviewing the influences on methanotrophic activity have identified various abiotic factors such as soil moisture content (Hanson and Hanson, 1996; Kolb, 2009; Tate, 2015) but do not mention the effects of C_2H_4 on soil methanotrophic activity. The reasons for this could be twofold. First, C_2H_4 is normally produced at low concentrations by plants to regulate various physiological and developmental processes (Glick et al., 1998; Pierik et al., 2006). Second, the release of substantial volumes of C_2H_4 from drought-stressed plants may only be transitory (Morgan and Drew, 1997). However, in this study, we detected enhanced C_2H_4 concentrations for a period that exceeded our expectations, suggesting that plant responses to drought play an important role in regulating CH_4 oxidation in the field. We strongly suggest that plant–soil interactions and C_2H_4 production should be given more attention when considering soil CH_4 oxidation.

4.2. Manipulation of soil CH_4 oxidation under drought

The hypothesis that reducing drought-induced *in planta* C_2H_4 production can increase soil CH_4 oxidation rates was supported by an earlier pilot study, showing that the application of AVG to maize (*Zea mays* L.) (grown with and without soil moisture stress) increased soil CH_4 oxidation rates (Zhou et al., 2018). The work presented here significantly extends this previous work by including extensive *in situ* C_2H_4 data, providing definitive confirmation of the hypothesis.

It is interesting to note that the application of AVG also decreased soil CH_4 emissions at 50% WHC and at 80% WHC. It is known that compared with negligible CH_4 production under oxic conditions (Zhou et al., 2008b; Nazaries et al., 2011), CH_4 production by methanogens occurs under anoxic conditions but most of the CH_4 is oxidised by methanotrophs before it can be released into the atmosphere (Le Mer and Roger, 2001). A possible mechanism is that the application of AVG can alleviate the negative effects of C_2H_4 production on soil CH_4 oxidation under environmental stresses like drying–rewetting or flooding conditions. This speculation was supported by lower C_2H_4 production with AVG application within the mesocosms maintained at 50% WHC and 80% WHC (Fig. 2b). The consistent positive response of plant biomass to AVG application regardless of the watering regime (Fig. 2c) suggests that some additional factor(s) beyond moisture availability may have been stressing plants in the mesocosms. Significant production (and exudation) of C_2H_4 only occurs when concentrations rise in response to environmental stresses such as drought or flooding (Pierik et al., 2006). Increased *in planta* C_2H_4 production can, in turn, reduce plant growth and biomass in the short term (Czarny et al., 2006). The positive biomass response to AVG, even in the mesocosms maintained at 80% WHC, which produced comparatively little C_2H_4, confirmed the sensitivity of plant growth to this phytohormone.

As shown in Fig. 2b, the production of C_2H_4 was generally highly variable, and as such no significant effect of WHC level was observed

despite the marked reduction at 80% WHC. This variation occurred despite efforts to produce standardised conditions *via* the use of a homogenised media and controlled atmosphere growth cabinets. This outcome strongly suggests that there are factors influential to ethylene production that were not adequately accounted for. A potential driver could be variations in the activity of the soil microbial community, as discussed above, but this is only speculation and no mechanism can be identified at this time.

The application of AVG has been studied as an option to manage aspects of fruit production (Greene and Stover, 2005) but it is likely that the cost of AVG may be prohibitive. Another option to manipulate C_2H_4 production in response to environmental stress may be the use of PGPR containing ACC deaminase (Zhou et al., 2013). Various studies have shown inoculation with PGPR containing ACC deaminase can effectively reduce *in planta* C_2H_4 production under drought stress (Glick et al., 1998). Use of these bacteria has also been shown to increase plant yield under drought stress, similar to the results of AVG application observed by Belimov et al. (2009) and in our field study. Other studies also reported that inoculation with PGPR containing ACC deaminase increased tomato (*Solanum lycopersicum* L.) and wheat (*Triticum aestivum* L.) yields under drought stress (Mayak et al., 2004; Gontia-Mishra et al., 2016). Similar to these results, we applied PGPR containing ACC deaminase into an eggplant crop in a subtropical agricultural farm and found that it increased soil CH_4 oxidation and eggplant aboveground biomass as well. All these results suggest that *in planta* C_2H_4 production can be manipulated to increase soil CH_4 uptake under drought.

5. Conclusions

These results provide strong evidence for the hypothesis that reducing *in planta* C_2H_4 production increases soil CH_4 oxidation and plant biomass under drought stress. To our knowledge, this is the first study to manipulate the negative feedback between C_2H_4 production and CH_4 oxidation under drought stress. Given the widespread arid and semiarid regions in the world and the higher frequency of drought stress in future global climate scenarios (IPCC, 2013), we provide a reliable means for increasing CH_4 oxidation in the context of global warming. Moreover, failure to take account of this feedback will enable the development of a self-reinforcing cycle linking intensifying climate change, enhanced plant stress and greater CH_4 emissions from the soil.

CRediT authorship contribution statement

XZ, SJS and PWC conceived the idea. XZ designed the research. XZ and XG performed the experiment. XZ and SJS analysed the data and wrote the manuscript. All authors contributed to the discussion of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144089.

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