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Reducing plant-derived ethylene concentrations increases the resistance of temperate grassland to drought

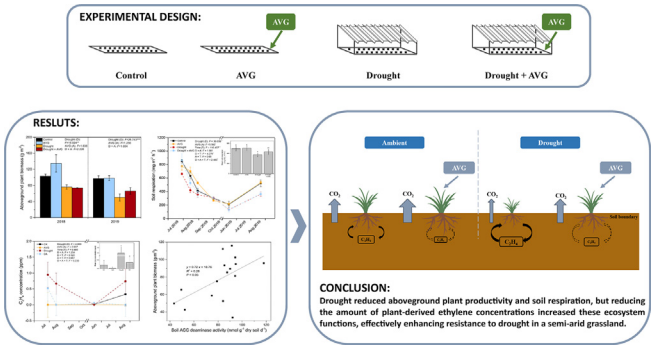
Xinyun Gu [a], Simeon J. Smaill [b], Bo Wang [a], Zhaoying Liu [a], Xingliang Xu [c], Yanbin Hao [d], Paul Kardol [e], Xiaoqi Zhou [a,*]

[a] Zhejiang Tiantong Forest Ecosystem National Observation and Research Station, Institute of Eco-Chongming, School of Ecological and Environmental Sciences, East China Normal University, Shanghai 200241, China
[b] Scion, PO Box 29237, Riccarton, Christchurch 8440, New Zealand
[c] Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China
[d] School of Life Sciences, University of Chinese Academy of Sciences, Yuquan Road, Beijing 100049, China
[e] Swedish University of Agricultural Science, Department of Forest Ecology & Management, Umea, Sweden

HIGHLIGHTS

- Drought reduced aboveground plant productivity and soil respiration in grassland.
- Drought induced higher plant-derived ethylene production.
- We manipulated ethylene production *via* the addition of AVG.
- Reducing ethylene concentrations enhanced the grassland resistance to drought.
- There were higher ACC deaminase activity and associated gene abundances under AVG.

GRAPHICAL ABSTRACT



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ABSTRACT

Model predictions indicate that extreme drought events will occur more frequently by the end of this century, with major implications for terrestrial ecosystem functions such as plant productivity and soil respiration. Previous studies have shown that drought-induced ethylene produced by plants is a key factor affecting plant growth and development, but the impact of drought-induced ethylene on ecosystem functions in natural settings has not yet been tested. Here, we reduced the amount of plant-derived ethylene concentrations by adding the ethylene inhibitor aminoethoxyvinylglycine (AVG), and investigated *in situ* plant productivity, soil respiration and ethylene concentrations for two years in a semi-arid temperate grassland in Inner Mongolia, China. Drought significantly reduced plant productivity and soil respiration, but the application of AVG reduced ethylene concentrations and significantly increased aboveground plant productivity and soil respiration, effectively enhancing resistance to drought. The reason for this could be that AVG application increased the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and abundance of the *acdS* gene (the key gene for ACC deaminase), facilitating reduced ACC concentrations in the plant tissue and reduced *in planta* ethylene synthesis. In addition, there was a significant correlation between soil ACC deaminase activity and plant productivity. Given the global distribution of arid and semi-arid areas, and the expected increases in the frequency and intensity of drought stress, this is a significant concern. These results provide novel evidence of the impact of drought-induced plant ethylene production on ecosystem functions in semi-arid temperate grassland ecosystems.

* Corresponding author.
E-mail address: xqzhou@des.ecnu.edu.cn (X. Zhou).

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1. Introduction

Grassland ecosystems occupy nearly one-third of the Earth's surface, providing many important ecosystem services and functions, such as pasture production, while also supporting 30 % of the total global soil carbon storage (Eswaran et al., 1993). Grassland ecosystems are predominantly found in arid and semi-arid environments, and are generally more vulnerable to drought stress than other ecosystems (Hoover et al., 2014; Stuart-Haëntjens et al., 2018). Model predictions indicate that drought frequency and severity will increase substantially by the end of this century (IPCC, 2021), with the potential to significantly affect terrestrial ecosystems. Despite the sensitivity and importance of grasslands, we still have a limited understanding of the mechanisms by which drought affects the functions of grassland ecosystems.

Grassland productivity is closely related to local rainfall patterns (Pei et al., 2013). Many studies have shown that drought reduces the productivity of grassland plants and the influence increases with the extent of drought (Tilman and El Haddi, 1992; Zhao and Running, 2010); for example, severe drought can result in plant death over a wide area (Hodgkinson and Müller, 2005). The extent to which a grassland ecosystem can resist the effects of drought and maintain its function is variable (Grime et al., 2008; Vogel et al., 2012; Craine et al., 2013). For example, across a given location, different plant communities can exhibit different levels of resistance to drought stress (Wang et al., 2019). In general, the higher the plant diversity, the stronger the ecosystem's resistance to drought (Isbell et al., 2015). However, the underlying mechanisms of drought stress on grasslands' ecosystem function are poorly understood (Wolf et al., 2013), but various studies suggest that hormones (such as ethylene) produced by drought-stressed plants could play an important role in this process.

Ethylene has an important impact on plant growth and development (Dubois et al., 2018). As part of their physiological response to drought stress, plants can rapidly produce a large amount of ethylene, part of which is released into the soil environment (Glick et al., 2007; Belimov et al., 2009; Zhou et al., 2021a). The sudden increase in ethylene concentration puts the plant into a “survival state” with the intention of protecting itself against the stress, but also has a negative effect on plant growth and will lead to a decline in plant productivity (Czarny et al., 2006). The *in planta* production of ethylene can be moderated by soil microbes that produce the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which cleaves the ethylene precursor ACC into α-ketobutyrate and ammonia (Glick et al., 1998). As the pool of ACC in the soil is broken down, more ACC is exuded by the plant to maintain equilibrium, diminishing the pool within the plant that is available to produce ethylene. Our current understanding of the wider effects of ethylene production by drought-stressed plants is predominantly based on lab or glasshouse experiments and with individual plants (Gupta et al., 2020; Zhou et al., 2021a). The effects on ecosystem functions in natural settings, however, have not yet been tested.

To address this gap in knowledge, we selected an area of semi-arid temperate grassland in Inner Mongolia for an *in situ* study of the effects of drought on plant productivity and ethylene production over a period of two years. We also monitored soil respiration, yet another important ecosystem function (Hopkins et al., 2013; Burri et al., 2018). The application of an ethylene synthesis inhibitor was integrated into the study to manipulate ethylene production. Further, as ethylene concentrations are closely related to the activity of ACC deaminase in soils (Glick et al., 2007; Zhou et al., 2013), we analyzed soil ACC deaminase activity and the abundance of the *acdS* gene that encodes for this enzyme by using metagenomics as an indicator of the impact of ethylene produced by drought-stressed plants. The aim of our study was to (1) test the effects of drought and ethylene inhibitors on plant productivity and soil respiration in a semi-arid temperate grassland; (2) test the underlying mechanism through which ethylene production influences the resistance of grassland ecosystems against drought stress. We hypothesized that drought would decrease ecosystem functions, whereas ethylene inhibitors would increase the resistance of grassland ecosystems (Fig. 1).

2. Materials and methods

2.1. Study site

We established a rainfall reduction manipulation experiment (Fig. S1) in June 2017 at the Inner Mongolia Grassland Ecosystem Research Station in the Xilin River watershed, a semi-arid temperate grassland in Xilinhot City, Inner Mongolia, northern China (43 °20′N, 116 °40′E, 1200 m a.s.l). The mean annual temperature at the site is 2.5 °C, and the mean annual precipitation is 281 mm, with about 86 % of the total precipitation historically received during the period from May to September. The soil is classified as dark chestnut in the Chinese classification or as a Haplic Calcisol according to the Food and Agricultural Organization of the United Nations classification, with 60 % sand, 21 % clay and 19 % silt (Li et al., 2020). The plant

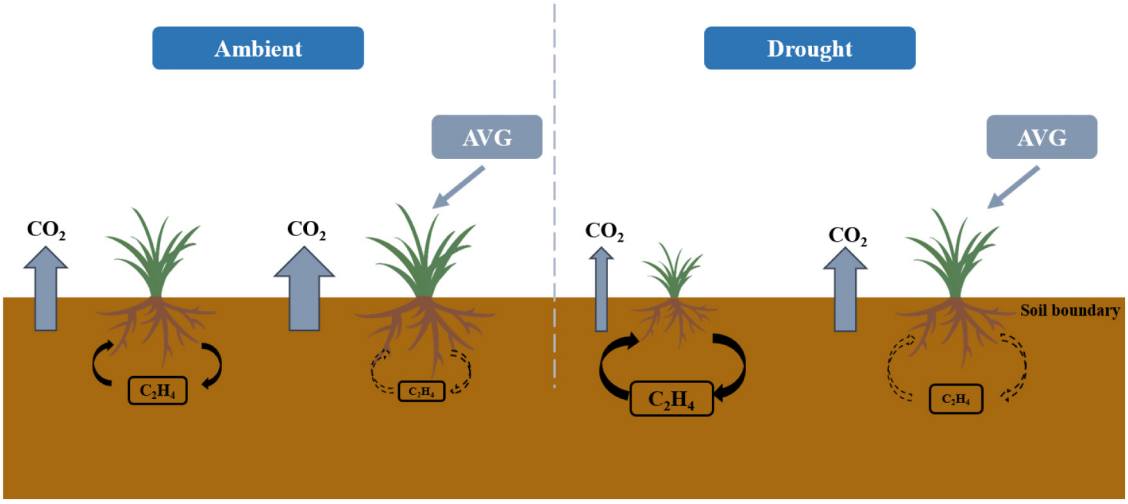

Fig. 1. Conceptual diagram showing how we used the ethylene inhibitor aminoethoxyvinylglycine (AVG) to manipulate plant-derived ethylene (C_2H_4) concentrations in aboveground plant biomass and soil respiration under drought in a semi-arid temperate grassland. Drought stress increases plant-derived ethylene production and, therefore, the concentration of ethylene in the soil environment, which can inhibit plant root growth, thus reducing aboveground plant biomass and soil respiration. Continuous and dashed black arrows indicate the control and decreased ethylene concentrations, respectively. The application of AVG can alleviate the effects of ethylene concentrations under drought. The width of the grey-blue arrows indicates the hypothesized soil respiration.

community in this study site is dominated by the grasses *Leymus chinensis*, *Stipa grandis*, *Achnatherum sibiricum* and *Agropyron cristatum* (Li et al., 2020).

2.2. Experimental design

The experiment consisted of 16 plots, 2.5 m × 2.5 m each, with 2-m spacing between them. Two precipitation levels were applied: an ambient control treatment and a 60 % rainfall reduction to simulate a drought scenario (Fig. S1). Steel-framed rainout shelters were covered with clear fiberglass-reinforced polyester roofs (0.8 mm thick; 90 % light transmission) were installed at a height of 1.5–3.5 m above the ground to produce the rainfall reduction effect (see Fig. S1). The 60 % reduction in rainfall (*i.e.*, about 112 mm of mean annual precipitation) falls within the range of extreme probability for mean annual precipitation, based on the normal distribution (Fig. S2). To explore the impact of plant-derived ethylene under drought, treatments with or without a spray solution of the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) (5 μM) (Sigma, Shanghai, China) were applied. In AVG-treated plots, the AVG solution was sprayed onto the plant foliage; plots not receiving AVG received the same volume of distilled water. The AVG solution was applied twice during the growing season every year: once in late June, then two weeks later in mid-July. We used a randomized plot design to implement a complete factorial combination of drought and AVG application, with four treatments and four replicates for each treatment: (1) ambient control, (2) AVG, (3) drought and (4) drought plus AVG. Daily mean air temperature and precipitation data were collected by the local meteorological station (Fig. S2a). Soil moisture content was measured by 5TM soil volumetric water content sensors (METER Group, USA). The sensors were connected to a CR300 data logger (Campbell Scientific, UK), which recorded data every 30 min (Fig. S2b).

Further, to confirm the effect of AVG application on plant-derived ethylene concentrations, we selected the model plant species *Brassica pekinensis* and carried out a microcosm experiment with and without the application of AVG (detailed information has been given in Appendix A and in Fig. S3). At the end of the experiment, we collected gas samples from the microcosms to measure ethylene concentrations and we measured plant biomass. We found that AVG application reduced plant-derived ethylene production rates (Fig. S3a). At the same time, AVG application increased the biomass of the *B. pekinensis* seedlings by about 59.8 % (Fig. S3b).

2.3. Measurements of aboveground plant biomass, in situ soil respiration and ethylene concentrations under the drought and AVG treatments

Plant aboveground biomass was quantified in 0.25-m^2 quadrats in each plot at the end of the growing season in late August of 2018 and 2019. Different locations were chosen to prevent resampling the same quadrat from year to year. All plants were clipped at ground level and then oven-dried at 70 °C to a constant weight for biomass measurements in the laboratory (Hao et al., 2017). Community composition, plant community coverage and plant height were recorded before the aboveground biomass was clipped, and these data were used for calculating species richness and the Shannon–Wiener index.

In situ soil respiration and ethylene concentrations were measured with a static chamber method once a month during the growing season (Stiles et al., 2018; Zhou et al., 2021a). Metal chambers consisting of boxes with a removable cover (50 cm × 50 cm × 50 cm) and a square metal frame (50 cm × 50 cm in area and 10 cm in height) were installed to a depth of 7 cm in each plot. Each frame had an open groove at the top, which was filled with water prior to chamber deployment to ensure an airtight connection. The air in the chamber was mixed by pulling and pushing the plunger of the syringe before sampling. For each efflux sampling, gas samples (~30 mL) were taken with a 30-mL polypropylene syringe at 30-minute intervals. The samples were transferred into 10-mL glass vials equipped with Chromacol butyl septa for determining the CO_2 concentration. Subsequently, gas samples were taken for measuring the ethylene concentration. A detailed description of the methodology is given in Zhou et al. (2021a).

The concentration of carbon dioxide (CO_2) was determined with a gas chromatograph equipped with a flame ionisation detector (7890B GC, Agilent, USA). The *in situ* CO_2 flux was calculated from the slope of the linear regression between gas concentrations and sampling time (Stiles et al., 2018). Standards were measured once every 10 samples to monitor the accuracy of the analytical equipment. The coefficient of variation in CO_2 efflux was <5 % and control jars containing ambient air were processed *via* the same protocol as a control for checking gas leakage. In addition, the resistance index of soil respiration ($Resistance_{SR}$) was determined using the following equation, as described by Orwin and Wardle (2004):

$$Resistance_{SR} = 1 - \frac{2\left|SR_e - \overline{SR_C}\right|}{\left(SR_C + \left|SR_e - \overline{SR_C}\right|\right)}$$

where $\overline{SR_C}$ indicates the mean value of soil respiration in the ambient control treatment and SR_e indicates soil respiration in the drought treatment. Similarly, the resistance index of soil respiration ($Resistance_{SR\text{-}AVG}$) under AVG application treatments was determined using the following equation:

$$Resistance_{SR-AVG} = 1 - \frac{2\left|SR_{DA} - \overline{SR_{AVG}}\right|}{\left(SR_{AVG} + \left|SR_{DA} - \overline{SR_{AVG}}\right|\right)}$$

where $\overline{SR_{AVG}}$ indicates the mean value of soil respiration in AVG treatment and SR_{DA} indicates soil respiration in the drought plus AVG treatment.

Soil ethylene was measured in 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 the ethylene 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}.

2.4. Measurements of soil ACC deaminase activity and acdS functional gene abundance based on metagenomic analysis

Soil samples were collected from all plots in August 2019 by following a diagonal sampling pattern with a soil auger (2.5 cm in diameter) at a depth of 0–10 cm. The soil cores were mixed immediately and passed through a 2-mm sieve before temporary storage in a cooler at 4 °C. Multiple sub-samples from each sample were then taken for various analyses. One set of sub-samples was stored at −20 °C for DNA extraction. Soil moisture contents were determined after another set of sub-samples was oven-dried at 105 °C overnight. Sub-samples were further used to determine soil pH at a 1:2.5 dry soil/water ratio, and other sub-samples were air-dried and then finely ground for determining the total carbon content using a Vario MICRO cube elemental analyzer (Elementar, Germany). Soil nitrogen in the form of ammonium (NH_4^+–N) and ammonia (NO_3–N), extractable organic carbon and extractable organic nitrogen contents were determined by hot water extraction (Gu et al., 2019; Wang et al., 2020). Soil ACC deaminase activity was determined for a set of sub-samples by the production of alpha ketobutyrate from ACC on a Cytation5 imaging reader (BioTek, USA) (Penrose and Glick, 2003).

For measurements of the functional gene abundance, soil genomic DNA was extracted from stored samples according to procedures described previously (Bu et al., 2018; Gu et al., 2019; Zhou et al., 2021b). The total DNA extracted from the soil samples of the control and drought plots in 2019 was sequenced with an Illumina HiSeq 4000 sequencer. The high-quality reads were assembled *de novo* into contigs with Megahit version 1.0.6 (Li et al., 2015), with the default parameters for all samples. Contigs with lengths of >500 bp in MetaGeneMark (version 3.26) were used, and a nonredundant gene catalog was constructed by CD-HIT with a sequence identity cutoff of 0.95 and a minimum coverage cutoff of 0.9 for the contigs (Li and Godzik, 2006). Gene taxonomy classifications in our catalog were determined by searching against the NCBI-NR database (June 2017 version) (Xue et al., 2020). Functional annotation of the protein sequences of the predicted genes were determined by searching against the Kyoto

Encyclopedia of Genes and Genomes (KEGG) database with DIAMOND Blastp and an e-value threshold of 10^{-5} (Buchfink et al., 2014).

2.5. Statistical analyses

Two-way analysis of variance was used to determine the single and interactive effects of drought and AVG applications on the soil pH and contents of total carbon, extractable organic carbon, extractable organic nitrogen, NH_4^+–N and NO_3^-–N, *in situ* C_2H_4 concentration, soil ACC deaminase activity and relative abundance of the *acdS* gene (Zhou et al., 2021b). Repeated-measures analysis of variance was conducted to test the effects of drought and AVG on plant aboveground biomass, plant community coverage and *in situ* soil respiration, as well as the effects of AVG on the resistance index of soil respiration throughout the experiment with the "agricolae" package. Differences among the treatments were compared by Tukey's honestly significant difference (HSD) test and were considered significant at $P < 0.05$. All statistical analyses were performed in R (Team, 2019).

3. Results

3.1. Effects of drought on plants' aboveground productivity and soil respiration

Drought significantly reduced the aboveground biomass of plants in the temperate grassland, and there were significant inter-annual differences in plant community characteristics (Fig. 2a). The aboveground plant biomass under drought was only 74.8 % of that in the ambient control. In 2019, the aboveground biomass of plants in the drought plots was significantly lower than that in the control plots, decreasing by 48.3 % (Fig. 2a). The plant community coverage displayed a similar trend, with drought reducing plant coverage by 12.2 % (not significant) in 2018 and by 30.7 % (significant) in 2019 (Fig. 2b).

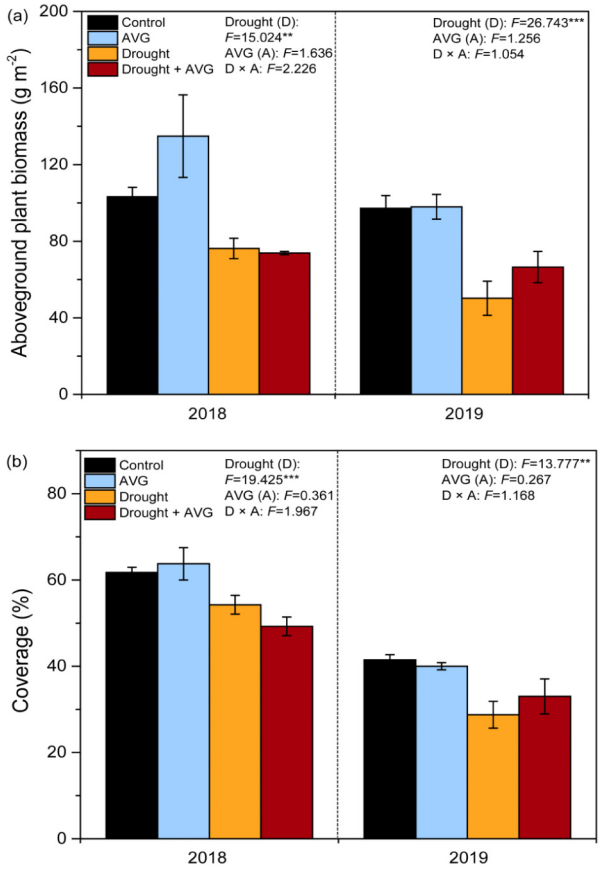

Fig. 2. Aboveground plant biomass (a) and plant community coverage (b) under combinations of drought and the ethylene inhibitor aminoethoxyvinylglycine (AVG) in July 2018 and August 2019 in a semi-arid temperate grassland. Data shown are means and standard errors ($n = 4$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Soil respiration varied substantially over the 2-year experimental period, ranging from 304 ± 16 to 848 ± 28 CO_2 mg m^{-2} h^{-1} in 2018 and from 210 ± 49 to 524 ± 47 CO_2 mg m^{-2} h^{-1} in 2019 (Fig. 3a). The drought treatment significantly reduced soil respiration, with annual mean values reducing by 53 % in 2018 and 37 % in 201.9 (Fig. 3a). According to the difference in soil respiration between the drought and control treatments, we found that the resistance of soil respiration to drought ranged from 0.51 ± 0.12 to 0.78 ± 0.24 (Fig. 3b).

Drought and the AVG treatment had no significant effects on pH, total carbon, TN, extractable organic carbon, extractable organic nitrogen, NH_4^+–N and NO_3^-–N (Tables 1 and S1).

3.2. Effects of AVG application on plants' aboveground biomass and soil respiration

The AVG treatment significantly increased the aboveground biomass of plants (134.82 ± 21.55 g m^{-2} *vs.* 103.33 ± 4.82 g m^{-2} in the ambient control plot) in 2018, but the effect was not marked under drought conditions. In 2019, the AVG treatment increased the aboveground biomass of plants in the drought plots by up to 32.4 % (Fig. 2a). However, the effect of AVG treatment on plant community coverage was not marked, although it shows an increasing trend (Fig. 2b). There were no interactive effects of drought and AVG application on aboveground plant biomass (Fig. 2a).

Overall, the AVG treatment significantly increased soil respiration in the semi-arid temperate grassland. The AVG treatment significantly increased soil respiration by up to 28.4 % in 2018; under drought conditions, the effect of AVG on soil respiration was even more obvious, reaching up to 31.2 %. The AVG treatment still promoted soil respiration in 2019, but to a lesser extent (Fig. 3a). Moreover, the resistance of soil respiration to drought under the AVG treatment was higher than in the ambient control (Fig. 3b). However, there were no interactive effects of drought and AVG application on soil respiration (Fig. 3a).

3.3. Effects of AVG application on in situ ethylene concentrations and acdS genes in soils

The drought treatment increased soil ethylene concentrations from 0.08 to 0.59 ppm, and the AVG treatment significantly reduced the soil ethylene concentration to 14.7 % of the control; under drought conditions, the AVG treatment reduced soil ethylene concentrations to 24.8 % of that in the drought plots (Fig. 4a).

We found that the drought treatment significantly reduced soil ACC deaminase activity by up to 26.9 %, whereas the AVG treatment had a certain recovery effect on soil ACC deaminase activity by up to 24.3 % (Fig. 4b). Although the abundance of the *acdS* gene was unaffected by the treatments (Fig. 4c), there was a positive correlation between soil ACC deaminase activity and aboveground plant biomass (Fig. 4d).

4. Discussion

The extent of the susceptibility of semi-arid grasslands to drought is both contentious and highly relevant, given the future scenario of more frequent and more severe drought events. We found that drought did not increase soil ethylene concentrations, suggesting that the plant community in this semi-arid grassland might adapt to this level of drought, as it did not exhibit the expected stress response of increased ethylene production and release. However, the application of AVG enhanced vegetation growth through inhibiting ethylene production, strongly suggesting that plant growth may be constantly limited by ethylene, potentially as an adaptation to the dry conditions at the site. To our knowledge, this is the first study showing that plant-derived ethylene concentrations under drought stress play a role in regulating ecosystem functions in natural grassland ecosystems.

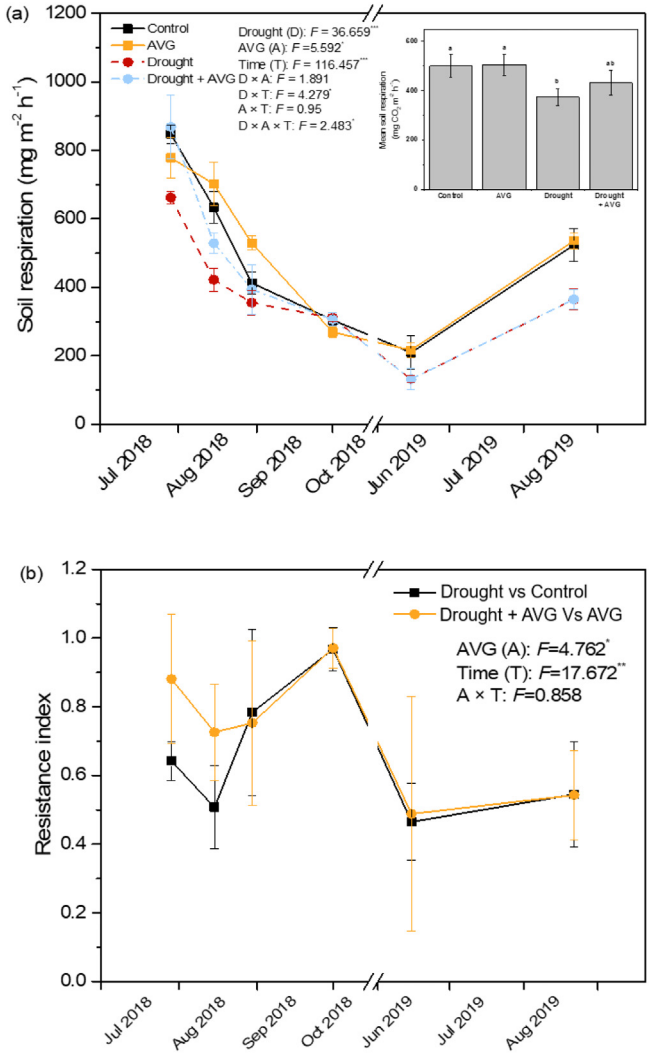

Fig. 3. Soil respiration (a) and the resistance index (b) under drought and the ethylene inhibitor aminoethoxyvinylglycine (AVG) from July2018 to August 2019 (b) in a semi-arid temperate grassland. Data shown are means and standard errors ($n = 4$). Different lowercase letters indicate significant differences at $P < 0.05$ among the treatments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

4.1. Effects of drought on the functions of temperate grassland ecosystems

Grassland ecosystems in northern China are an important part of the Eurasian continent, and water is one of the main factors influencing ecosystem functions in this region (Bai et al., 2004). This area is currently facing an increased risk of drought, and this situation is expected to continue for the next few decades (Hessl et al., 2018).

The annual mean aboveground productivity of plants from 1981 to 2000 in the *Leymus chinensis*-dominated temperate grassland was 186.3 g m^{-2} (Hao et al., 2017). In our study, we found that the aboveground productivity of plants was lower than the multi-year average, but it was closest to the 2016 observations (Li et al., 2020), suggesting that productivity in this area is threatened by drought, on the basis of the generally negative impact of drought on grassland productivity (Tilman and El Haddi, 1992; Zhao and Running, 2010).

Soil respiration is the second largest carbon flux between terrestrial ecosystems and the atmosphere (Bond-Lamberty and Thomson, 2010), and there is a strong association between plants' aboveground productivity and soil respiration (Reichstein et al., 2003; Chen et al., 2014). This aligns with our observation of significant reductions in soil respiration under drought in the temperate grassland, potentially driven by water-based limitations on the microbial metabolic processes related to soil respiration (Li et al., 2020) and the reduced autotrophic respiration (which usually accounts for about 40 % of soil respiration in semi-arid temperate grasslands) as a consequence of lower plant productivity (Li et al., 2018; Zhou et al., 2019). We found that soil microbial respiration, *i.e.*, heterotrophic respiration, was unaffected by drought (Fig. S5); therefore, it was concluded that decreased soil respiration under drought was predominantly caused by a reduction in the availability of root exudates, which may suppress autotrophic respiration (Hopkins et al., 2013; Burri et al., 2018).

Surprisingly, the drought and AVG treatments did not significantly affect soil pH, total C and N contents, and extractable organic C and N contents in the semi-arid grassland (Table 1). The most likely explanation for this lack of an effect is that not enough time passed for the treatments' effects on the plants to elicit significant alterations in soil properties. However, this requires longer-term investigation.

Table 1
Soil physico-chemical properties (pH, total carbon (TC) and total nitrogen (TN) contents, extractable organic carbon (EOC) and extractable organic nitrogen (EON) contents, and NH_4^+–N and NO_3^-–N contents) as affected by drought and the ethylene inhibitor aminoethoxyvinylglycine (AVG) in a semi-arid temperate grassland. Data shown are means ± standard errors ($n = 4$).

Soil properties	Control	AVG	Drought	Drought + AVG
pH	6.95 ± 0.05	7.37 ± 0.24	7.15 ± 0.04	7.00 ± 0.14
TC (g kg^{-1})	30.33 ± 1.07	32.97 ± 0.87	32.01 ± 1.93	32.00 ± 1.59
TN (g kg^{-1})	2.00 ± 0.18	2.03 ± 0.21	2.17 ± 0.05	1.98 ± 0.28
EOC (mg kg^{-1})	711.59 ± 26.62	704.25 ± 18.19	743.69 ± 57.09	784.83 ± 35.89
EON (mg kg^{-1})	16.35 ± 1.54	13.50 ± 1.49	16.92 ± 1.76	17.11 ± 1.51
NH_4^+–N (mg kg^{-1})	24.74 ± 1.83	25.73 ± 2.15	23.73 ± 1.97	26.17 ± 1.43
NO_3^-–N (mg kg^{-1})	1.20 ± 0.42	0.93 ± 0.19	1.46 ± 0.52	2.07 ± 0.41

4.2. Reducing plant-derived ethylene concentrations to increase ecosystem function under drought

The observation of enhanced soil ethylene concentrations under drought (Fig. 4a) is in line with previous research. Stressed plants increase the *in planta* synthesis of ethylene, a proportion of which is released from root tissues to the soil, thereby increasing the ethylene concentrations in the soil gas atmosphere (Barnawal et al., 2013; Chandra et al., 2018). Increased ethylene production under drought stress has an inhibitory effect on plant root development, reducing plant productivity and even causing vegetation death (Czarny et al., 2006). The application of AVG to reduce plant-derived ethylene concentrations under drought stress can effectively alleviate the inhibitory effect of soil ethylene on plant root development, thereby increasing plant productivity (Glick et al., 1998; Liu et al., 2019).

Our results indicate that the reduction in ethylene concentration associated with AVG application may occur *via* two mechanisms. Firstly, AVG treatment reduces ethylene production in plant roots, decreasing ethylene release to the soil environment. An indoor soilless seedling culture experiment confirmed that the AVG treatment reduced the ethylene production of plants (Fig. S3; Zhou et al., 2021a). Secondly, the AVG treatment was found to increase soil ACC deaminase activity (Fig. S6), facilitating reduced ACC concentrations in the plant tissue and reduced *in planta* ethylene synthesis; therefore, less ethylene was released to the soil (Liu et al., 2019). This process was supported by the observed increase in the abundance of the *acdS* gene (the key gene for ACC deaminase) under the AVG treatments (Fig. 4b, c). Our results are also supported by previous findings that *acdS* genes are strongly related to the ACC deaminase activity and the expression of *acdS* genes enhances the stress tolerance in plants (Singh et al., 2022). These results show that reducing the soil ethylene concentrations can effectively increase soil ACC deaminase, plant aboveground productivity and soil respiration under drought (Figs. 2, 3 and 4), which was supported by the significantly positive correlation between plant aboveground productivity and soil ACC deaminase activity (Fig. 4d).

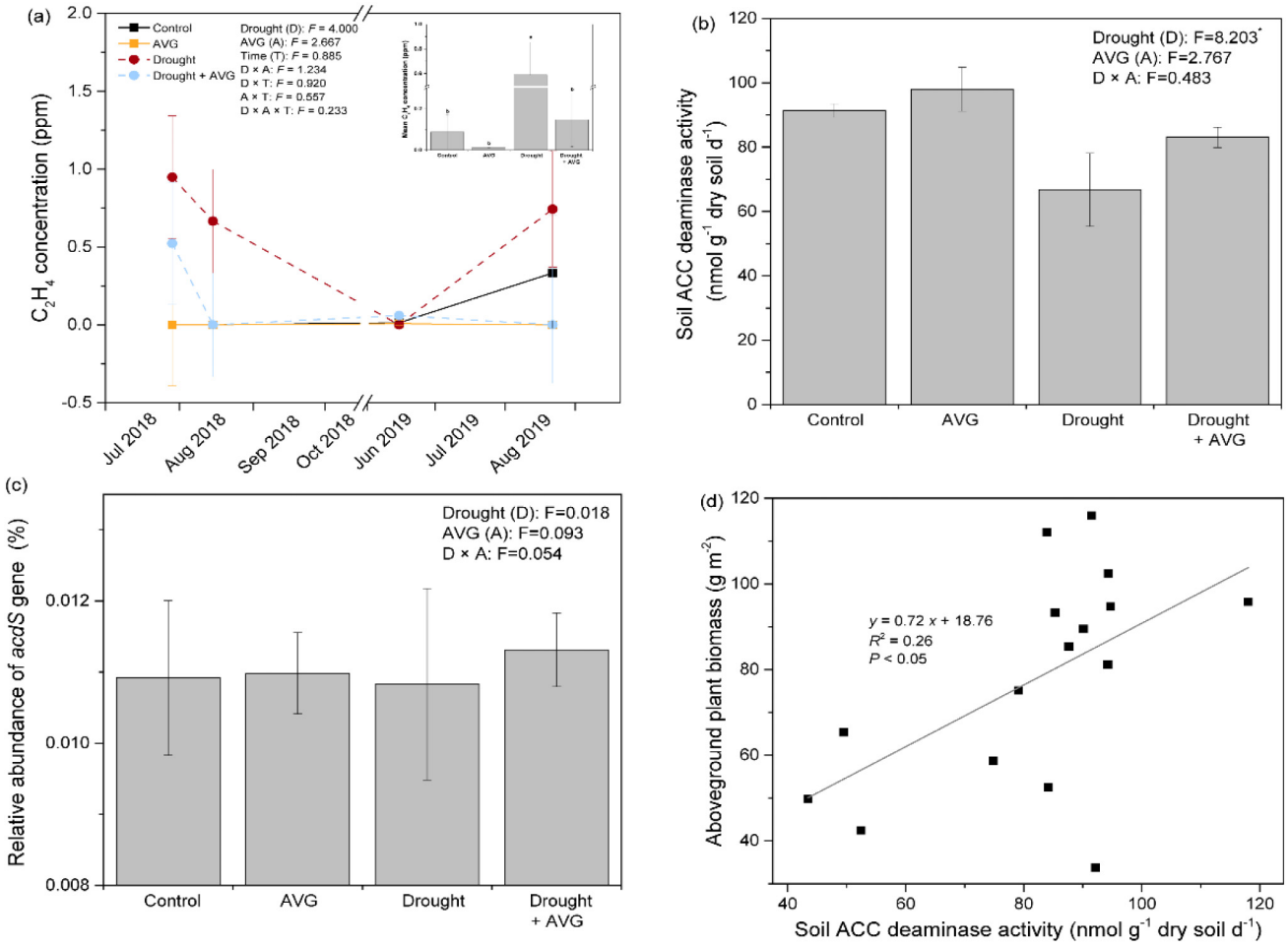

Fig. 4. Changes in ethylene (C_2H_4) concentrations (a), soil 1-aminocylopropane-1-carboxylic acid (ACC) deaminase activity (b), the relative abundance of the *acdS* gene (c), and the relationship between soil ACC deaminase activity and aboveground plant biomass (d) under combinations of drought and treatment with the ethylene inhibitor aminoethoxyvinylglycine (AVG) in a semi-arid temperate grassland. Data shown are means and standard errors ($n = 4$). Different lowercase letters indicate significant differences at $P < 0.05$ among the treatments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

5. Conclusion

In semi-arid temperate grassland ecosystems, drought can significantly reduce aboveground plant productivity and soil respiration through induced ethylene production, whereas reduced soil ethylene concentrations under drought conditions can promote aboveground plant productivity and soil respiration. Our study indicates that this effect was driven by the increased ACC deaminase activity and higher expression of the associated functional gene that encodes for this enzyme in the presence of the ethylene inhibitor AVG. To the best of our knowledge, this is the first report demonstrating that soil ethylene concentrations play an important role in affecting ecosystem functions under drought stress in a natural grassland ecosystem.

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Author contributions

XZ designed the experiment; XZ, XG, BW and ZL performed the experiments; XZ contributed the materials and agents; XG and XZ wrote the manuscript; SS, XX and PK made large contributions to the revision of the manuscript and all authors contributed to discussions on the manuscript.

Data and materials availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

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.

Appendix A. Supplementary data

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