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Title: Ethylene-regulated leaf lifespan explains divergent responses of plant productivity to warming among three hydrologically different growing seasons

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Abstract

Leaf senescence is known to be regulated by the plant hormone ethylene, but how leaf lifespan responds to global environmental change and links to ecosystem-level responses remains largely unexplored. Here we investigated the effects of climate warming and nitrogen addition on plant functional traits, plant hormone ethylene and net primary production in a 13-year field experiment in a desert steppe. Across the last three years of the experiment (2016–2018), plant productivity increased under warming only in 2016, when there was above normal precipitation, but consistently increased with nitrogen addition. Warming enhanced net photosynthesis, leaf nitrogen and ethylene production and reduced leaf lifespan in 2016 (a wet year), but not in 2017 (a drought year); the effect of warming in 2018 (a year with normal precipitation) was opposite to 2016, likely due to the below-normal precipitation in the mid-growing season in 2018. Nitrogen addition led to increases in leaf nitrogen, ethylene production and net photosynthesis, and declines in leaf lifespan in 2016 and 2018, but not in 2017. The ethylene-regulated lifespan was further evidenced by the addition of CoCl_2 (an ethylene biosynthesis inhibitor) that reduced ethylene production and prolonged lifespan. Structural equation modeling showed that leaf lifespan had a negative effect on plant productivity, both directly and indirectly via its negative effect on net photosynthesis, across all three years. Our results demonstrate the divergent responses of leaf lifespan and, in turn, plant productivity to warming under inter-annual and intra-annual precipitation variation, thus linking plant hormone production, functional traits and ecosystem functioning in the face of global environmental change.

KEYWORDS

Climate warming, leaf longevity, nitrogen deposition, photosynthetic rate, plant biomass, plant hormone, precipitation change

1 | INTRODUCTION

Global warming has resulted in an increase of 1.0°C in Earth's surface temperature above pre-industrial levels, and a further increase of 1.5°C is projected by the mid-21st century (IPCC, 2018). In parallel, human activities have doubled the amount of bio-available inorganic nitrogen (N) in the biosphere since 1860s, and significantly altered global N cycles (Ackerman, Millet, & Chen, 2019). These anthropogenic environmental changes are known to have widespread

ecological consequences, including changes in biodiversity, species composition and ecosystem functioning (Isbell et al., 2013; Melillo et al., 2017; Parmesan, 2006). Given the importance of plant productivity of natural and managed ecosystems for humanity, considerable effort has been undertaken to understand how plant productivity responds to warming and N deposition (e.g., Ackerman, Millet, & Chen, 2019; Lin, Xia, & Wan, 2010). Various effects of warming on plant productivity have been reported: positive (e.g., Fernández-Martínez et al., 2019), negative (e.g., Klein, Harte, & Zhao, 2007), and neutral (e.g., Wang et al., 2020). Increases in N deposition enhance the availability of soil N, which is often in limited supply, and thus often result in increased primary productivity, although little to no effect of N addition on plant productivity has also been reported (e.g., Tang, Deng, An, Yan, & Shangguan, 2017; Wang et al., 2010). Global meta-analyses indicate that mean annual temperature (MAT) and precipitation, plant functional type and evolutionary history (a proxy of phylogenetically conserved plant functional traits) could account for more than half of the total variance in plant productivity responses to warming (Lin et al., 2010; Shao et al., 2019), and that N supply and soil pH could explain approximately a quarter of the variation in plant productivity response to N deposition at the global scale (Stevens et al., 2015). A mechanistic understanding of these effects may be improved by explicitly considering plant functional traits, which have been recognized to be important for predicting plant responses to warming and N deposition (Diaz & Cabido, 2001; Gornish & Prather, 2014; Xu et al., 2018).

Leaf lifespan, a trait known to modulate carbon gain (Chabot & Hicks, 1982), nutrient utilization and remobilization (Achat, Pousse, Nicolas, & Augusto, 2018), is closely associated with a number of important plant structural and functional characteristics, including leaf mass per area and photosynthetic rate (Gornish & Prather, 2014; Reich, Walters, & Ellsworth, 1992; Wright et al., 2004; Yu et al., 2019). Leaf lifespan has been found to be negatively correlated with mean annual temperature (MAT) for evergreen species (Kikuzawa, Onoda, Wright, & Reich, 2013; Li, Krauchi, & Dobberty, 2006; Zhang, Luo, Zhu, Daly, & Deng, 2010), but positively correlated with MAT for deciduous species (Wright et al., 2005). This contrasting pattern is explained by the carbon budget theory that leaves need to assimilate enough carbon during the growing season to ensure positive plant growth (Kikuzawa et al., 2013). Therefore, evergreen species need to prolong their leaf lifespan to compensate for the shorter photosynthetic period and/or rate in low MAT environments, whereas deciduous species tend to have leaf lifespan similar to the length of favorable periods for growth, which is longer at sites with higher MAT (Kikuzawa et al., 2013).

However, whether these patterns of long-term acclimation of leaf lifespan to temperature can be applied to the short-term or instantaneous response of leaf lifespan to warming remains poorly understood. Moreover, leaf lifespan has long been hypothesized to decrease with increasing soil N supply (Chabot & Hicks, 1982), as the trade-off between carbon gain and cost can cause leaf lifespan to decrease with increased photosynthetic rate under higher N supply. However, current evidence indicates no consistent leaf lifespan response to N amendment (Oikawa, Suno, & Osada, 2017; Pornon, Marty, Winterton, & Lamaze, 2011). In addition, it is largely unknown whether the response of leaf lifespan to warming and N would depend on precipitation variability, which is also projected to increase (IPCC, 2018). Overall, our understanding of how leaf lifespan responds to warming and increased N deposition, the two important components of global environmental change, and links to plant productivity under global change remains extremely limited.

Plant hormone influences a diverse array of plant growth and developmental processes (e.g., Pandey et al., 2021; Sun, Yu, Shugart, & Wang, 2016), and in particular, ethylene is known to promote leaf senescence (Abeles, Morgan, & Saltveit, 2012). Climate warming and increased N deposition may have the potential to alter plant ethylene, and in turn, leaf lifespan. The response of ethylene production to warming, however, remains ambiguous. For example, increased temperature promoted ethylene production and consequently accelerated leaf senescence in soybean (Djanaguiraman & Prasad, 2010), whereas it inhibited ethylene production and delayed leaf senescence in wheat (Arshad & Frankenberger, 2002). Although high N supply has been found to increase ethylene production from plant leaves (Ren et al., 2013) and roots (Tian, Sun, & Zhang, 2009), it remains unclear whether the inconsistent responses of leaf lifespan to N addition (Oikawa, Suno, & Osada, 2017; Pornon, Marty, Winterton, & Lamaze, 2011) would suggest inconsistent responses of ethylene production to increased N supply. The variation in ethylene production can be explained by the fact that the expression of genes encoding ACS (1-aminocyclopropane-1-carboxylic acid synthase) and ACO (1-aminocyclopropane-1-carboxylic acid oxidase), the two key enzymes responsible for ethylene synthesis, is affected by high temperature and N availability (Tian, Sun, & Zhang, 2009; Hyodo 2018). However, little is known about the linkage between ethylene production, leaf lifespan and plant productivity under global change scenarios.

Here, we report on a 13-year field manipulation experiment, conducted in a temperate desert

steppe in northern China, to explore the effects of warming and N addition on plant hormone ethylene, plant functional traits and net primary production. As an ecologically and economically important ecosystem stretching across the Eurasian continent, the temperate desert steppe is currently experiencing significant anthropogenic environmental changes including climate warming, increased N deposition and altered precipitation patterns (Kang, Han, Zhang, & Sun, 2007; Wu et al., 2020), thus necessitating a thorough understanding of their ecological consequence. Our experiment aimed to address three main questions. First, how do warming and N addition affect leaf lifespan under inter-annual and intra-annual variation in precipitation? Second, how do changes in leaf lifespan, in response to increased air temperature and N input, contribute to the changes in net primary production? Third, does ethylene play an important role in modulating leaf lifespan and plant productivity responses to warming and N addition?

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

The experiment was carried out in a temperate grassland located at Siziwang Banner (111°53' E, 41°46' N, elevation 1456 m a.s.l.) in Inner Mongolia, China. The climate has warm summers and cold winters with monthly mean temperature of 20.7 °C in July and –15.7 °C in January. Mean annual temperature is approximately 3.7 °C. Mean annual precipitation (2006–2018) is approximately 228 mm, about 85% of which falls during the growing season from May to September. Precipitation varied substantially across years, particularly during the last three years (2016–2018) of our experiment. Annual precipitation was 337 mm in 2016 (48% above average), 185 mm in 2017 (19% below average), and 274 mm in 2018 (20% above average). Moreover, the monthly distribution of precipitation also differed substantially across the three years: two thirds of the annual precipitation fell in June and July in 2016; precipitation was roughly evenly distributed throughout the growing season from May to September in 2017, but occurred little prior to July in 2018 (Figure S1).

The soil at our experimental site, which has a sandy loam texture, is classified as Kastanozem according to the Food and Agriculture Organization classification system. The soil (0–10 cm depth) has a soil bulk density of 1.3 g cm⁻³, a total N of 1.7 g kg⁻¹, a total carbon of 16.9 g kg⁻¹ and pH of

8.0. Plant communities at the study site are dominated by two perennial grasses, *Stipa breviflora* and *Cleistogenes songorica*, and one perennial semi-shrub, *Artemisia frigida*.

The experiment was established in May 2006 using a split-plot design, with warming as the whole-plot factor and N as the sub-plot factor. There were six pairs (blocks) of 3 m × 4 m main plots comprising naturally assembled communities in a homogenous, flat field. One main plot in each pair was randomly assigned to the warming treatment and the other assigned to the ambient temperature treatment. The distance between any two adjacent main plots was 3 m. Each main plot was split into two sub-plots of 3 m × 2 m each, which were randomly assigned to the non-N addition and N addition treatments. Therefore, the four treatments included the control, warming, N addition, and the combination of warming and N addition, each of which had six replicates. Warming was implemented continuously throughout the year using a MSR-2420 infrared radiator (Kalglo Electronics Inc. Bethlehem, PA, USA) suspended 2.25 m above the ground surface in each plot, with an electrical power output of 2,000 W for each heater. Across the 13 years of the experiment, we achieved an average warming magnitude of +1.4 °C during the growing season (May–September) and of +1.2 °C during the non-growing season (October–April) at a height of 0–2 cm above ground surface. To account for the potential effects of shading, we also installed a dummy heater of the same shape and size in each of the ambient temperature plots. One of the limiting resources of the studied grassland is N (natural atmospheric N deposition, 1.2 g N m⁻² yr⁻¹; Jia et al., 2014). N addition rate was 10 g N m⁻² yr⁻¹, which has been estimated to be the ecosystem saturation rate of N deposition for Inner Mongolian grasslands (Bai et al., 2010); N was added in the form of NH₄NO₃ in late June each year.

On this long-term warming and N addition experimental platform, we conducted an ethylene manipulation experiment to uncover the role of the plant hormone ethylene in determining variation in leaf lifespan. This was done by adding an inhibitor of ethylene biosynthesis (cobalt chloride, CoCl₂) during the growing season to three plant species in the control plots in the last three years of the long-term experiment (2016–2018). The three manipulated species included two dominant species, *S. breviflora* and *C. songorica*, and one other common species, *Convolvulus ammannii*, which together made up approximately 60% of net primary production in the controls (Figure S2). Eight randomly selected individuals of each of the three studied species were marked in each control plot; four individuals of each species were randomly selected and treated with the

addition of CoCl₂ and the other four were left as controls. A CoCl₂ solution of 10 µM was injected into the soil at four locations equally spaced around, and approximately 2 cm from the stem of each individual to a depth of 10 cm using a syringe with stainless needle; CoCl₂ addition was repeated every two weeks. Equal amount of water without CoCl₂ was added at the same time to the control plants.

2.2 | Measurements of leaf lifespan

We determined the average leaf lifespan with a protocol modified from Craine & Reich (2001), and their definition of leaf area-days was used to estimate leaf lifespan. Leaf census was made in the field every ten days from May to September. Leaf lifespan was calculated by dividing the accumulated leaf area-days of all the leaves monitored in a plot by the accumulated area of those leaves that were born and died over the census period according to the following equations (Craine & Reich, 2001; Ren et al., 2013).

$$\text{Leaf lifespan} = (A - B - C - D) / (S_j - LA_i)$$

$$A = \left\{ \sum_{n=i}^j \left(\frac{LA_n + LA_{n+1}}{2} \right) \times K_n \right\} \quad \text{Eqn 1A}$$

$$B = \left\{ \sum_{n=i}^j \left(\frac{S_n + S_{n+1}}{2} \right) \times K_n \right\} \quad \text{Eqn 1B}$$

$$C = \left\{ \left[(n_s - n_i) \times LA_i \right] - \left[\sum_{n=i}^{n_s} \left(\frac{S_n + S_{n+1}}{2} \right) \times K_n \right] \right\} \quad \text{Eqn 1C}$$

$$D = \left\{ \left[\sum_{n=n_l}^j \left(\frac{LA_n + LA_{n+1}}{2} \right) \times K_n \right] - \left[(n_j - n_l) \times S_j \right] \right\} \quad \text{Eqn 1D}$$

where i is the starting census date; j , the ending census date; LA_n and S_n , the cumulative area of leaves produced (LA_n) and senesced (S_n) by the census date n ; K_n , the time interval between census date n and $n+1$; n_s , the date at which the amount of leaf area senesced is equivalent to the amount of leaf area at the first census ($S = LA_i$); and n_l , the date at which the cumulative leaf area produced is equal to the amount of leaf area senesced by the last census date ($LA = S_j$). n_l and n_s were calculated by linear interpolation.

Eqn 1A and Eqn 1B represent the cumulative amount of leaf area-days for both green and senesced leaves, respectively, and their difference is the total unsenesced leaf area-days over the entire census period. Eqn 1C represents the amount of leaf area-days for leaves present at the beginning of the census period, and Eqn 1D represents the leaf area-days of leaves that had not senesced by the end of the census period.

This leaf lifespan calculation model considered the leaves with various ages, including leaves with unknown birthdate, leaves with unknown death date, and leaves that were born and senesced within the experimental period (Craine & Reich, 2001). Therefore, this calculation of leaf lifespan is not influenced by variations in leaf age.

2.3 | Measurements of leaf ethylene, functional traits, biomass and soil characteristics

Plant leaves were harvested 4 h after CoCl_2 addition to measure ethylene production and N concentration. The leaves collected from different individuals within each sub-plot were pooled and placed in 5 ml vials for 30 minutes, after which the vials were sealed using the gas-tight stopper. One millilitre gas was taken from each vial after 1 h under dark conditions and injected into a gas chromatograph with a photoionization detector and a packed Teflon column (GC-4400; East & West Analytical Company, Beijing, China), to measure ethylene production. Leaf samples were then dried at 65 °C and ground to measure N concentration with an elementary analyzer (Elementar vario MICRO cube; Elementar, Langenselbold, Germany).

Leaf net photosynthetic rate was measured every two weeks during the peak of growing season (July and August), with a Li-Cor 6400 photosynthesis system (Li-Cor, Lincoln, NE, USA) at a saturated photosynthetic photon flux density of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ using an LED light source for the three studied species. Area and dry mass of leaves used to measure net photosynthesis were later measured for leaf mass per area (LMA). Aboveground net primary production (ANPP) was harvested by clipping a 50 cm \times 50 cm quadrat just above the soil surface in late August. All living vascular plants were sorted by species, dried, and weighed. Below-ground net primary production (BNPP) was estimated by the root in-growth method. Specifically, in early May of each year, we excavated one 30-cm-deep cylindrical hole using an 8-cm diameter soil core

sampler in each plot, removed roots in the soil sample via 2-mm sieves, and replaced the soil in the same location of the plot. We retrieved root in-growth samples in late September by using a 7-cm diameter soil core sampler at the centre of the original root-in-growth holes. Samples were rinsed over a 0.5 mm sieve in the lab. Root fragments on the sieves were collected and oven-dried at 70 °C for 48 h, and weighed to determine their biomass. Ecosystem net primary production was estimated as the sum of ANPP and BNPP.

Soil samples (0–10 cm) were collected by mixing two 3 cm diameter cores in each plot in mid-August. Then a 10 g of fresh soil sample was sieved to pass through a 2 mm-mesh and extracted with 50 mL of 2 mol L⁻¹ KCl. The extracts were measured for inorganic N concentration with a continuous flow spectrophotometer (FIAstar 5000 Analyzer; Foss Tecator, Hillerød, Denmark). The air-dry soil samples and the form of N applied (NH₄NO₃) were measured for $\delta^{15}\text{N}$ using an Isotope-Ratio Mass Spectrometer (IRMS, Isoprime 100, Isoprime Ltd., Stockport, UK) coupled with an Elementary Analyzer (Elementar vario MICRO cube). Soil temperature and moisture (0–10 cm) were monitored by water content and temperature sensors (ECH₂O-TE/TM, Decagon Devices, Inc. USA) throughout the experimental period, and data were logged every two hours and downloaded to a computer each week.

2.4 | Data analyses

We used linear mixed-effect models to test for the effects of warming and N addition on leaf ethylene production, leaf lifespan, other plant functional traits, plant productivity and soil characteristics. We treated warming, N treatment, species and year as fixed factors, and the six blocks as random factors. Linear regressions were used to assess the relationships among hormone ethylene, plant functional traits, soil characteristics and plant productivity. We performed structural equation modeling (SEM) to analyze causal pathways through which warming and N addition affected leaf lifespan in relation to plant productivity. We first considered a full model that included all possible pathways, and then sequentially eliminated non-significant pathways until we attained the final model. Separate SEM was built for each of the three years (2016–2018) as the effect of warming differed substantially across the years. We used the chi-square test, RMSEA (root mean square error of approximation) and CFI (comparative fit index) to judge the fit of the model. The model has a good fit when $0 \leq \chi^2 \leq 2$, $0.05 < P \leq 1$, $0 \leq \text{RMSEA} \leq 0.05$ and

0.97<CFI<1.00. All SEM analyses were performed using the software AMOS 21.0 (IBM SPSS Inc, Chicago, IL). Other statistical analyses were conducted in R 3.5.1 (R Core Team 2018).

3 | RESULTS

3.1 | Leaf lifespan and ethylene responses to warming, N and CoCl₂ addition

Significant negative leaf lifespan responses to warming were observed in 2016 (Figure 1a-c, Table S1a), with lifespan reduction of 3.3 days for *S. breviflora*, 5.0 days for *C. songorica* and 4.8 days for *C. ammannii* observed in the heated plots relative to the controls. Leaf lifespan was unaffected by warming in 2017 (Table S1b). By contrast, in 2018, lifespan increased by 5.0 days for *S. breviflora*, 5.5 days for *C. songorica* and 5.0 days for *C. ammannii* in the heated plots than in the control plots (Figure 1). N addition reduced leaf lifespan by 4.4 days for *S. breviflora*, 4.4 days for *C. songorica* and 7.2 days for *C. ammannii* on average across 2016 and 2018, but showed little change in 2017. A non-additive effect of N and warming on leaf lifespan emerged in 2018 (Table S1c), where the positive warming effect disappeared under N addition, which also resulted in a significant warming \times N \times year interactive effect on leaf lifespan (Table S2).

Opposite to leaf lifespan responses, leaf ethylene production of the three species increased in 2016, decreased in 2018, and was comparable to the controls in 2017 in response to warming (Figure 1d-f, Table S1). N addition increased leaf ethylene production in 2016 and 2018, but not in 2017, for all the three species. The warming \times N interaction was significant, indicative of a generally sub-additive effect, in 2016 and 2018 ($P < 0.001$), but was non-significant in 2017 (Table S1).

The addition of CoCl₂, an ethylene biosynthesis inhibitor, significantly reduced ethylene production, and correspondingly enhanced the leaf lifespan of all three species in both 2016 and 2018, compared to the control (Figure 1). Adding CoCl₂ did not affect leaf ethylene production or lifespan in 2017.

3.2 | Effects of warming and N addition on leaf N, net photosynthesis, LMA, plant

productivity and soil characteristics

Both leaf N concentration and light-saturated net photosynthesis in all the studied species showed significant increases in 2016, no change in 2017, and decreases in 2018 in response to warming (Figure 2, Table S1). Under N addition, leaf N and photosynthetic rate in all species were greater in 2016 and 2018, but did not differ from the controls in 2017 (Figure 2, Table S1). Warming led to significant decreases of LMA for all the species in 2016, no change in 2017, and increases in 2018 (Figure S3, Table S2). N addition reduced LMA of the three species in both 2016 and 2018, but had no effect in 2017. Warming increased net primary production in 2016, but reduced productivity in 2017 and 2018, whereas N addition consistently increased plant productivity across the three years (Figure 3, Table S2). Similar patterns were found when above- and below-ground productivity were examined separately (Figure S4, Table S2).

Warming significantly elevated soil temperature but did not change soil moisture over the three years of the experiment (Figure S5a-b, Table S3). Warming had positive and negative effects on soil inorganic N in 2016 and 2018, respectively, but did not affect soil inorganic N in 2017 (Figure S5c, Table S3). N addition did not alter soil temperature or moisture, but increased soil inorganic N in 2016 and 2018 (Figure S5a-c, Table S3). Both warming and N addition did not change the $\delta^{15}\text{N}$ of soil total N in 2016, but significantly enhanced soil $\delta^{15}\text{N}$ in 2017 and 2018 (Figure S5d, Table S3).

3.3 | Pathways through which experimental treatments influenced plant productivity

When using SEM to disentangle the contributions of the direct and indirect effects of warming and N addition on leaf lifespan and net primary production, we found that leaf lifespan had a consistently negative effect on plant productivity in each of the three years and that the pathways through which warming and N addition affected productivity were similar across years (Figure 4). In 2016 (a wet year), warming and N addition increased total plant productivity by enhancing soil N availability, leaf N concentration and ethylene production, which in turn, reduced leaf lifespan (Figure 4a). In 2017 (a drought year), soil moisture, which was unaffected by warming and N addition, largely controlled plant productivity by regulating soil and leaf N concentration, leaf ethylene and, in turn, leaf lifespan (Figure 4b). In 2018 (a year with relatively normal

precipitation, but little precipitation in June), warming decreased soil N availability and ethylene production, increased leaf lifespan, and consequently reduced plant productivity (Figure 4c). N addition, on the other hand, resulted in increased soil N availability and ethylene production, reduced leaf lifespan, and consequently increased plant productivity (Figure 4c).

4 | DISCUSSION

Changes in plant phenology under climate change have been frequently reported. For example, recent studies indicate that climate warming has led to advances in the budburst date for subboreal tree species (Montgomery, Rice, Stefanski, Rich & Reich, 2020), and resulted in earlier leaf senescence in the Northern Hemisphere (Wu et al., 2018). Nevertheless, how leaf lifespan responds to climate warming against a background of inter-annual and intra-annual climate variation remains poorly understood. The few available studies on this topic have shown that as MAT increases, evergreen plants tend to decline in their leaf lifespan (Li et al., 2006; Zhang et al., 2010), whereas deciduous plants tend to increase in their leaf lifespan (Wright et al., 2005). Our study explored how long-term warming influenced leaf lifespan and plant productivity in a temperate desert steppe, documenting a previously unrecognized divergent responses of leaf lifespan to warming over three consecutive years. Specifically, warming resulted in substantial leaf lifespan reduction in 2016 (a wet year), followed by the lack of warming effect on leaf lifespan in 2017 (a drought year), then by warming enhancing leaf lifespan in 2018 (a year with relatively normal annual precipitation, but higher temperature). The mirroring responses of ethylene to warming, coupled with the result of CoCl_2 addition experiment (i.e., the addition of this ethylene biosynthesis inhibitor decreased ethylene production and prolonged leaf lifespan), suggest that variation in ethylene hormone production across years under warming may have underlain the temporal changes in leaf lifespan in response to warming. To our knowledge, our study is the first to report temporal variation in leaf lifespan of the same study system across years of different climate conditions. Importantly, these warming-induced changes in leaf lifespan translated into observed changes in plant productivity, providing the rare evidence that environmental change can alter ecosystem functioning via modifying plant functional traits.

An important question is what caused the variation in the warming effect on ethylene production and leaf lifespan between the three years of the experiment. The results of SEM

analysis indicated that this variation could be attributed to different effects of warming on soil inorganic N availability, which is critical for determining ethylene production and leaf lifespan (Figure 4a). In 2016, when there was abundant rainfall, warming resulted in increased soil inorganic N concentration, which was likely the consequence of warming-induced higher N mineralization rate (Liu et al., 2017; Wang, Wan, Xing, Zhang, & Han, 2006). Such increases in mineralization under elevated temperature have been interpreted as the result of higher depolymerization rate of organic matter due to elevated exocellular enzyme activities, generating organic N-containing monomers that may be utilized by plants and microbes (Guntinas, Leiros, Trasar-Cepeda, & Gil-Sotres, 2012; Schimel & Bennett, 2004). The greater soil inorganic N concentration then led to increased leaf N concentration, which, in turn, resulted in higher ethylene production and shorter leaf lifespan (Figure 4a). In 2017, when precipitation was below normal, the desert steppe was strongly limited by water availability. In this drought year, soil moisture was likely of most importance in regulating microbial N mineralization. Therefore, soil moisture, not warming, largely determined soil inorganic N concentration, and, eventually, leaf lifespan. These results contrast sharply with those in 2018, when warming resulted in reduced soil inorganic N content, and lower ethylene production and longer leaf lifespan. While overall precipitation was slightly above normal in 2018, precipitation was almost zero in the active growth month of June, and ambient air temperature during the growing season in 2018 was on average 2.1°C higher than in 2016 (Figure S1). It is thus likely that the warming treatment may have induced thermal stress altering N cycles. To further explore this idea, we measured $\delta^{15}\text{N}$ of soil total N, where higher values indicate a more open N cycling and greater N losses (Robinson, 2001). We found that warming did not alter soil $\delta^{15}\text{N}$ in 2016, but increased soil $\delta^{15}\text{N}$ in 2017 and 2018 (Figure S5), thus suggesting the negative effect of warming on N availability in 2018 was at least, in part, attributed to increased N loss. Likewise, a seven-year warming experiment triggered large N losses in subarctic soils (Maranon-Jimenez et al., 2019). Leaching and gaseous N emissions from the processes of nitrification and denitrification are the two pathways for ecosystem N loss. For instance, previous work has shown that warming may influence N leaching losses by enhancing NO_3^- availability and the frequency of soil freeze-thaw cycles over winter (Turner & Henry, 2010).

More generally, our finding in 2018, where the total amount of precipitation was near normal but had a highly skewed distribution across the growing season (i.e., little precipitation in June),

highlights the importance of intra-annual precipitation variation for modulating plant responses to other environmental changes. Climate change not only involves increases in the frequency and intensity of extreme precipitation events, but also changes in the seasonal distribution of precipitation events, such as the projected increases in precipitation seasonality in China (Deng et al., 2019). It has been reported that annual and seasonal changes in precipitation can alter the effects of warming on ecosystem carbon exchange (Xia, Niu, & Wan, 2009) and N and phosphorus cycling (Ren, Kang, Yuan, Xu, & Han, 2018) in temperate grasslands. Our work thus adds to an increasing body of literature reporting interactive effects of different dimensions of global environmental change on plant productivity and other ecosystem properties.

Carbon cost-benefit analysis suggests that leaf lifespan should decline with increasing photosynthetic rate or decreasing construction cost (Chabot & Hicks, 1982; Kikuzawa et al., 2013; Zani, Crowther, Mo, Renner, & Zohner, 2020). LMA, which measures the leaf dry-mass investment per unit of light-intercepting leaf area, is a common indicator of leaf construction cost. Plants with higher LMA tend to have thicker leaf blades and/or denser tissues, indicative of higher leaf construction cost (Wright et al., 2004). In parallel with leaf lifespan, we also found that warming reduced LMA in 2016, had no effect on LMA in 2017, and increased LMA in 2018 (Figure S3). This congruence between theoretical prediction and experimental data supports the robustness of our results.

Our results show that N addition reduced leaf lifespan in 2016 (a wet year) and 2018 (a year with normal precipitation), but did not affect leaf lifespan in 2017 (a drought year). SEM showed that the N effect on leaf lifespan in 2016 and 2018 was modulated by the positive response of soil N availability to N addition. High N supply has been shown to transcriptionally up-regulate the expression of genes encoding the two key enzymes responsible for ethylene synthesis (1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid oxidase), thereby increasing ethylene production (Tian, Sun, & Zhang, 2009). In parallel, N addition also resulted in reduced LMA in 2016 and 2018 (Figure S3), suggesting that from a carbon budget perspective, the leaves needed short lifespan to pay back the construction cost and high photosynthetic rate to ensure positive plant growth. N addition, which often leads to increased aboveground plant biomass and thus reduced light availability, could also contribute to the senescence of older leaves by shading (Oikawa, Hikosaka, & Hirose, 2005). However, this

mechanism appears unlikely to operate in our experiment where shading among the studied species rarely occurs, because of their tiny leaves and loose canopy structure in the desert steppe (e.g., Ren et al., 2013). Finally, the lack of the leaf lifespan response to N addition in 2017 arose probably because the increased soil N loss rate, indicated by elevated soil $\delta^{15}\text{N}$ (Figure S5), offset increased N input in this drought year.

Current models of ecosystem response to global changes have focused on shifts in general biogeochemical and ecological processes (e.g., Bouskill, Riley, & Tang, 2014; Piao et al., 2019; Wu, Dijkstra, Koch, & Hungate, 2012), without considering plant hormones known to be linked to plant functional traits. Our results, however, emphasize the importance of plant hormone production and key leaf traits for regulating grassland productivity under climate change. In the wet year (2016), leaf lifespan decreased, but net photosynthesis and plant productivity increased under warming, suggesting that plant species tended to grow faster under the warmer environment. In 2018 with relatively normal annual precipitation but higher temperature, warming induced heat stress for plants, leading to reduced leaf ethylene production, prolonged leaf lifespan, and decreased plant productivity, indicating that here plant species grew more slowly under warming. Plants consistently grew faster and attained greater productivity under N addition in 2016 and 2018. A caveat of note is that our study focused on plant net productivity, but did not examine plant respiration or mortality. Future studies should thus examine the responses of plant respiration and mortality (Yu et al., 2019), in addition to net productivity, to gain a more comprehensive picture of ecological consequences of environmental change in plant-based ecosystems. Another caveat is that our study focused on examining three common species, without measuring ethylene production and leaf lifespan of other species in the community. Future investigations should consider quantifying these traits for most, if not all, plant species, for a more complete understanding of plant trait responses at the community level. Also note that our study only examined data from the last three years of the 13-year experiment, because this reduces the possibility that community and ecosystem properties are strongly influenced by transient dynamics, which tend to be more important during the earlier stages of ecological experiments (e.g., Reich, Hobbie, Lee, & Pastore, 2018), and because the CoCl_2 treatment was implemented only for the last three years of our experiment.

Our results indicate that plant hormone ethylene and leaf lifespan played important roles in

modulating plant productivity responses to global environmental change, emphasizing the need for land surface models to incorporate changes in plant hormones, which regulate plant functional traits, for a more accurate prediction of ecosystem-level responses to global change. Note that our study is the first, to our knowledge, to identify the role of ethylene in regulating plant leaf lifespan, and in turn, net primary production in the face of global environmental change. The generality of our results would need to be evaluated in other ecosystems and under different global change scenarios. Finally, our finding of the divergent response of leaf lifespan and plant productivity to warming under precipitation variability highlights the challenge to predict plant traits and ecosystem functions across space and time, until a thorough understanding of ecological and physiological mechanisms underlying plant hormone and trait responses to multiple dimensions of environmental change is obtained.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

FIGURE 1 Responses of (a-c) leaf lifespan and (d-f) ethylene production to warming, N and CoCl_2 (an ethylene biosynthesis inhibitor) addition, for two dominant species (*Stipa breviflora* and *Cleistogenes songorica*) and one other common species (*Convolvulus ammannii*), in the eleventh (2016), twelfth (2017) and thirteenth (2018) year of a long-term grassland experiment. Different letters indicate significant differences among different treatments (multiple comparisons, $P < 0.05$). Error bars show one standard error of the mean.

FIGURE 2 Responses of (a-c) leaf N concentration and (d-f) net photosynthesis to warming and N addition over three years of the long-term experiment in a desert steppe. Different letters indicate significant differences among different treatments (multiple comparisons, $P < 0.05$). Error bars show one standard error of the mean.

FIGURE 3 Response of net primary production (aboveground + 0 to 30 cm belowground) to warming and N addition in the eleventh (2016), twelfth (2017) and thirteenth (2018) year of a long-term grassland experiment. Error bars show one standard error of the mean.

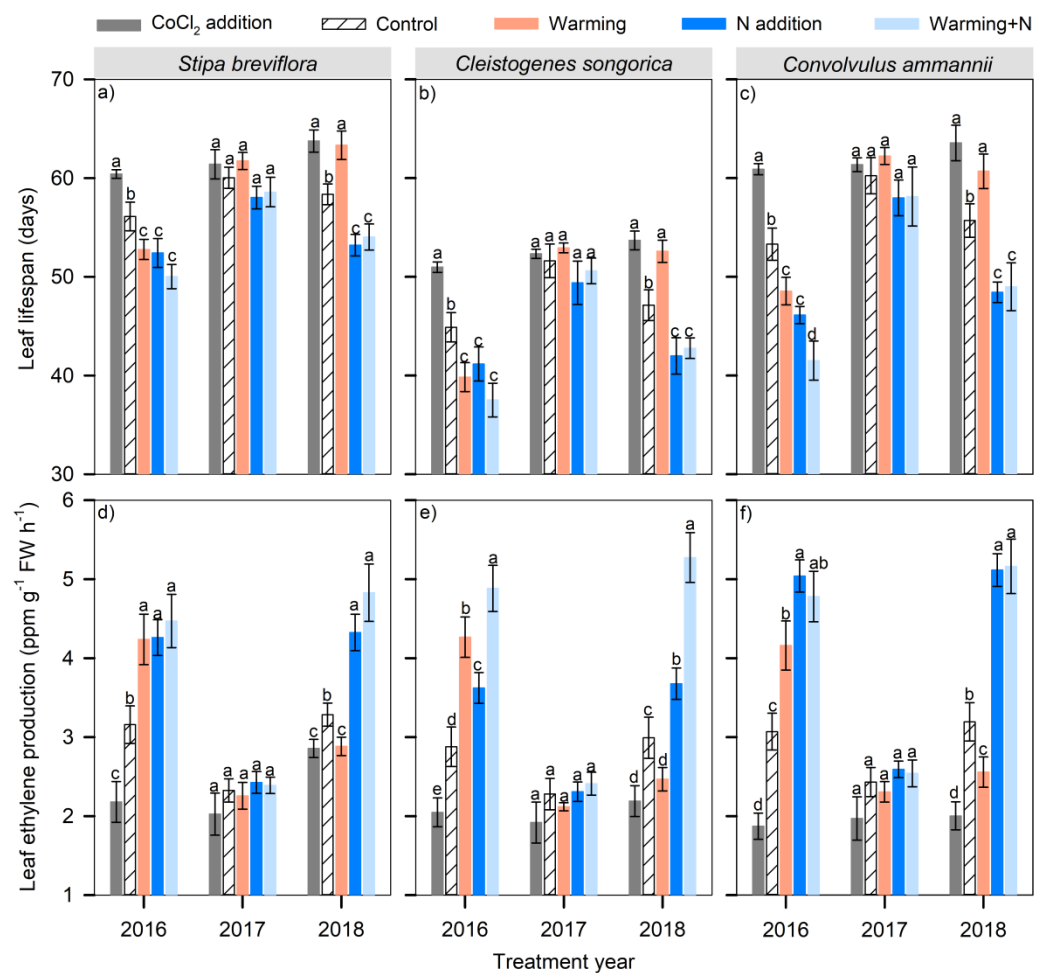
FIGURE 4 Structural equation model analysis showing the pathways through which warming and N addition influence leaf lifespan and net primary production. Results of model fitting: (2016) $\chi^2 = 34.933$, $P = 0.229$, d.f. = 20; RMSEA = 0, $P = 0.212$; CFI = 0.980; (2017) $\chi^2 = 37.779$, $P = 0.128$, d.f. = 20; RMSEA = 0, $P = 0.139$; CFI = 0.990; (2018) $\chi^2 = 39.084$, $P = 0.155$, d.f. = 20; RMSEA = 0, $P = 0.121$; CFI = 0.982. The proportion of variation explained is given as r^2 . Bold arrows represent significantly positive (solid) and negative (dotted) pathways ($P < 0.05$), and grey arrows indicate nonsignificant pathways. Numbers adjacent to the arrows are standardized path coefficients.

SUPPORTING INFORMATION

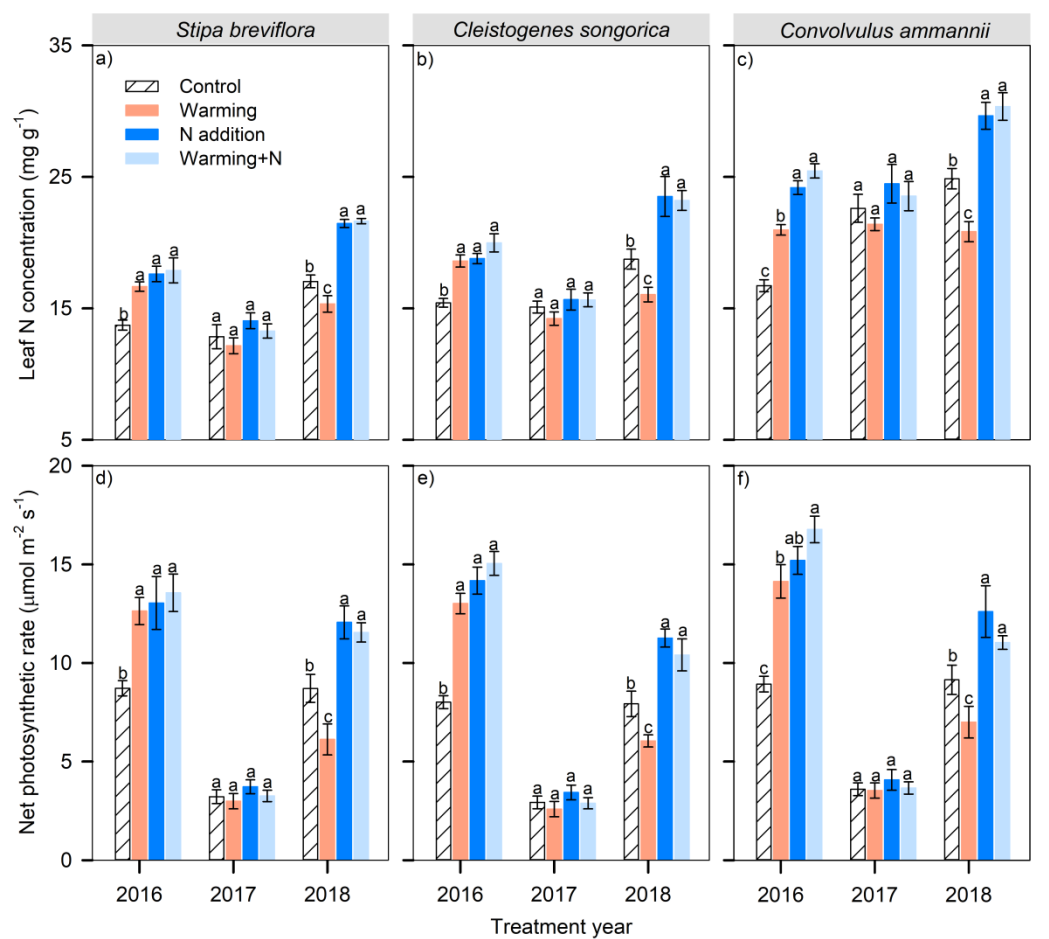
Additional supporting information may be found online in the Supporting Information section.

TABLES S1 to S3

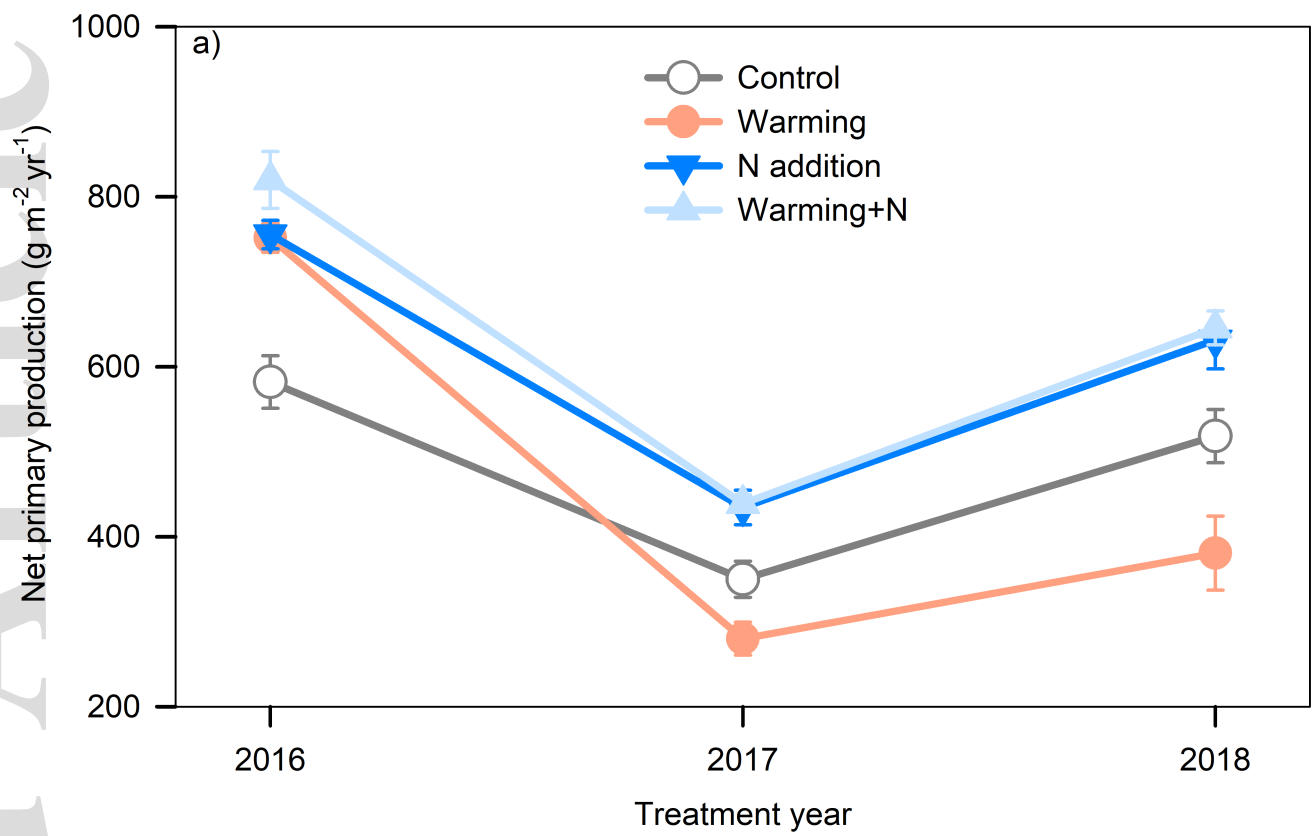
FIGURES S1 to S5



gcb_15718_f1.png

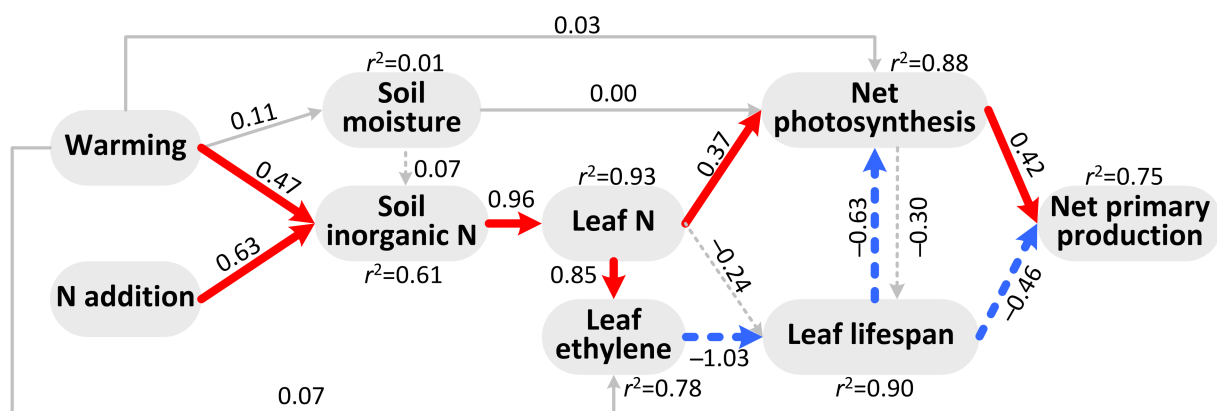


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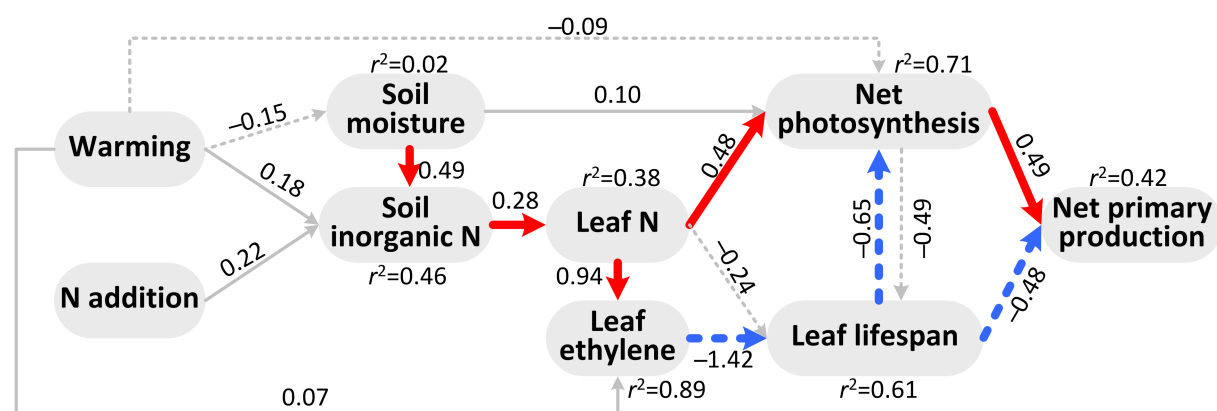


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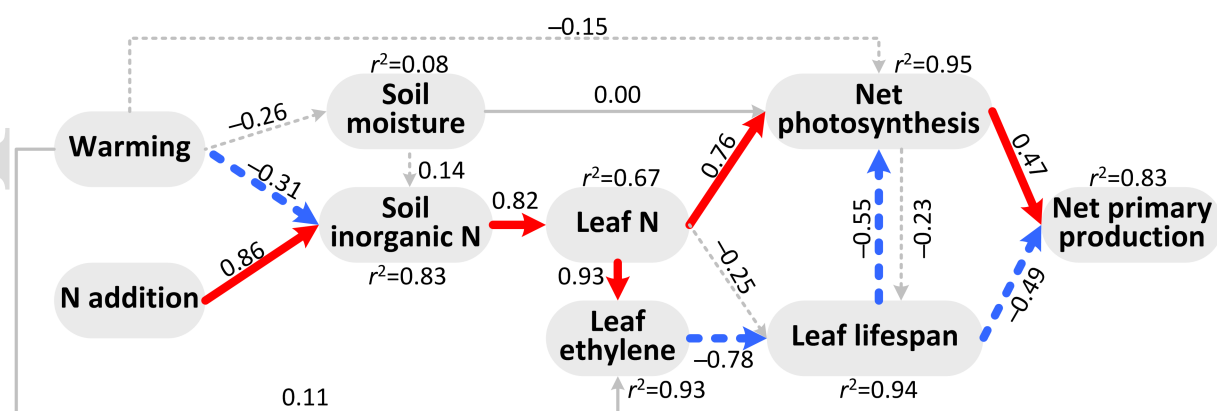
a) 2016 (a wet year)



b) 2017 (a dry year)



c) 2018 (a year with normal precipitation)



gcb_15718_f4.png