Climate Change and Legume Performance in Grassland Agroecosystems

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Abstract

We reviewed the literature to assess the effect of climate change factors on forage legumes. Whether growing in monoculture or mixtures with grasses, exposing legumes to elevated CO_2 (eCO_2) generally leads to sustained increases in forage accumulation (FA) and N fixation, but elevated temperature (eT) in conjunction with eCO_2 usually reduces magnitude of these responses. In legumes, nodules represent large C sinks, precluding photosynthetic acclimation to eCO_2 observed in non-N fixing plants. Greater N fixation in legume-grass mixtures exposed to eCO_2 is due to greater percentage of legume N derived from symbiotic fixation and often an increase in legume proportion in mixtures. Herbage nutritive value (NV) responses to eCO_2 are less pronounced than FA, but lesser herbage N and greater non-structural carbohydrate (NSC) concentrations are common with eCO_2. Drought effects on legume NV are inconsistent, but eT usually decreases NV. Data from one legume species suggest eCO_2 and eT negatively affect pollen grain morphology and viability, but they increase flower number and nectar sugar concentration. Under eT, flowers opened earlier in the day causing earlier pollinator visits, but when combined with water stress, eT reduced pollinator visits. Though there is variation in the literature for some responses, we conclude that eCO_2 generally increases legume FA, N fixation, and tissue NSC concentration, while reducing herbage N concentration. Drought reduces FA, but drought effects on NV are not consistent. Elevated temperature has a negative effect on legume NV, and, when combined with eCO_2, can reduce the magnitude of the positive FA and N fixation response to eCO_2. Climate change factors can affect legume pollen viability and pollinator behavior, potentially influencing plant reproductive success. Overall, effects of climate change factors on forage legumes can be generalized, but interactions among change factors and site-specific soil and climate conditions may cause variation from expected responses.

Introduction

Climate change is defined as a change in mean or variability of climate that persists for a decade or longer (IPCC 2014). Anthropogenic activities, particularly greenhouse gas emissions, are drivers of climate change (Arias et al. 2021). Projected changes in climate by 2100 include an increase in atmospheric CO_2 concentration up to 1100 μL CO_2 L^{-1}, an increase in global surface temperatures of up to 3.3-5.7°C, and a reduction in terrestrial water stores (Arias et al. 2021; Pokhrel et al. 2021). Legume inclusion in grasslands may mitigate potential negative impacts of grassland agroecosystems on climate by reducing greenhouse gas emissions from animal enteric fermentation, substituting biological N fixation for N fertilizer produced through the Haber-Bosch reaction, and improving production efficiency (Jensen et al. 2012). Hence, it is crucial to understand how climate change may affect legume responses in grassland agroecosystems. Our objective is to review and synthesize literature addressing effects of climate change factors on forage legume responses.

Methods

We focus solely on response of forage legumes to climate change factors including atmospheric CO_2 concentration, air temperature, and soil moisture. References cited include both warm- and temperate-climate legumes growing in monoculture or mixtures. Search stream keywords included the generic "climate change and forage legumes" as well as streams linked to specific responses to climate change.

Results and Discussion

Content is organized by category of forage legume response to climate change factors. Responses include plant physiological mechanisms, FA, symbiotic N fixation, NV, and flowering and seed production.

Legume Physiological Responses

Many C_3 plants increase photosynthesis with short-term exposure to eCO_2, but they acclimate to eCO_2 and decrease photosynthetic capacity after long-term exposure (Irigoyen et al. 2014). This response is termed photosynthetic acclimation and can be due to decreases in CO_2 stomatal and/or mesophyll conductance or to decreases in Rubisco carboxylation and/or ribulose 1,5-bisphosphate regeneration. Balance between sources and sinks also determines whether acclimation occurs (Erice et al. 2006). For legumes, nodule development represents a large C sink that precludes acclimation to eCO_2. Additionally, nodule maintenance via exchange of plant C for N with the N_2-fixing symbionts at the nodule level creates an extra C sink that minimizes the occurrence of photosynthetic acclimation (Irigoyen et al. 2014). For example, greater photosynthetic rate of alfalfa under eCO_2 increased translocation of sugars from leaves to roots, favoring nodule development, which in turn further enhanced the rate of photosynthesis due to the increased sink:source ratio associated with C costs of N_2 fixation (Baslam et al. 2014). The general consensus is unlike other C_3 plants, legumes do not acclimate to elevated CO_2, except when other growth-limiting factors are present (Irigoyen et al. 2014).

Legumes under eCO_2 generally have greater total leaf area and altered leaf net photosynthetic rates, stomatal conductance, and water-use efficiency (Erice et al. 2006). The effect of eCO_2 (600 μL CO_2 L^{-1}) and eT (+2°C) on stomatal properties and their impact on the plant were assessed for *Stylosanthes capitata* Vogel (Habermann et al. 2019). The eCO_2 treatment alone reduced stomatal density, stomatal index, and stomatal conductance, resulting in reduced transpiration, increased leaf temperature, and greater maintenance of soil moisture. Greater CO_2 concentration inside leaves stimulated photosynthesis, starch concentration, water-use efficiency, and photosynthetic photosystem II performance. At eT, leaves had smaller stomata; however, there were no effects of warming on stomatal conductance, transpiration, or leaf water status. Authors concluded that eCO_2 was the main driver of changes in stomatal opening control and anatomy, while eT had more pronounced effects on photosynthetic photosystem II performance and antioxidant defenses.

Legume breeding programs have identified white clover (*Trifolium repens* L.) plants with superior drought tolerance. They had greater net photosynthetic rates, relative water content, and leaf water potential under drought-stressed conditions (Jiang et al. 2010). Changes in response to drought of tolerant plants included lesser stomatal density, greater root growth following water withholding, and greater accumulation of osmolytes (e.g., raffinose and galactinol) and flavonoid antioxidants (Araújo et al. 2015).

Legume Forage Accumulation and Proportion in Mixtures

There are numerous published studies assessing legume FA response to eCO_2 (presented as an absolute value) and eT (presented as amount of increase above ambient) in monocultures and in mixed swards.

A C_3 grassland community, consisting of various perennial grasses and white clover, was exposed to eCO_2 (600 μL CO_2 L^{-1}) for 3 yr and harvested 3 or 6 times annually (Picon-Cochard et al. 2004). The eCO_2 did not affect FA in the first year, but in Year 2 there was a positive eCO_2 effect on both mixture FA (+26%) and N harvested (+30). Greater legume FA response and symbiotic N_2 fixation under eCO_2 were associated with increased ecosystem-scale N demand. In another study, white clover was grown in monoculture or in mixture with perennial ryegrass (*Lolium perenne* L.) at season-long ambient and eCO_2 (380 and 670 μL CO_2 L^{-1}) concentrations in open-top chambers (Schenk et al. 1997a). Total-season FA increased 16–38% for legume monocultures and 12–29% for mixtures at eCO_2. White clover proportion in mixtures increased with eCO_2. Similar white clover responses to eCO_2 were observed in mixed swards with perennial ryegrass (Hebeisen et al. 1997; Newton et al. 1996). Monocultures and mixtures of subterranean clover (*Trifolium subterraneum* L.) and a C_3 grass were assessed in southeastern Australia at ambient and eCO_2 (690 μL CO_2 L^{-1}) and ambient and eT (+3.4°C) (Lilley et al. 2001a). The eCO_2 increased clover FA in the monoculture by 19% and by 31% in the mixture. Warming reduced clover monoculture FA at ambient CO_2 by 28% and reduced the level of growth enhancement at $eCO2$ to +8%.

In monoculture, rhizoma peanut (*Arachis glabrata* Benth.), a warm-climate legume, averaged 43, 74, 19, and 80% greater leaf, stem, root, and new rhizome mass, respectively, at eCO_2 (640 µL CO_2 L^{-1}) compared with ambient (Fritschi et al. 1999b). In monoculture and mixtures, if key nutrients are not limiting growth and N_2 fixation, legumes typically benefit from eCO_2 to a greater level than non-fixing species (Hebeisen et al. 1997; Lüscher et al. 2014).

The Integrated Farm System Model was used to predict FA for alfalfa (*Medicago sativa* L.) and a C_3 grass grown alone or in mixture in eastern Canada (Thivierge et al., 2016). Under projected future climate with no harvest management adaptation, annual FA of alfalfa and the mixture increased in colder areas and decreased in warmer areas. Annual alfalfa FA and percentage in the mixture increased, with greater FA of the first cutting due to faster accumulation of growing degree days, but greater water and temperature stress reduced FA of subsequent harvests.

Legume Nitrogen Fixation

The ability of legumes to fix atmospheric N is integral to their response to climate change factors. Symbiotic N fixation was quantified in field tunnels for pure and mixed swards of subterranean clover with a C_3 grass at ambient and eCO_2 (690 µL CO_2 L^{-1}) and ambient and eT (+3.4°C) (Lilley et al. 2001b). Elevated CO_2 stimulated greater N_2 fixation by clover and an 8% increase in total N harvested for the monoculture and 16% for the mixture. The proportion of clover N derived from N_2 fixation increased 12% under elevated CO_2, but it decreased by 6% under warming. White clover was grown at ambient and eCO_2 (600 µL CO_2 L^{-1}) in monoculture or in mixture with a C3 grass and harvested 3-4 times per year in Switzerland (Zanetti and Hartwig 1997). In monoculture with eCO_2, percentage of white clover N derived from symbiotic N fixation increased from 59 to 66% due to increased symbiotic N_2 fixation and an increase in proportion of clover in mixtures. The mechanism for greater symbiotic N_2 fixation in white clover under eCO_2 was assessed in a pot study at two levels of soil N (Zanetti et al. 1998). Greater N_2 fixation was due to a greater number of nodules, a response to increased plant N-sink strength, rather than greater N_2 fixed per unit of root dry weight. Sink strength was also enhanced by the association of a legume with a grass in mixtures. Similar patterns of nodule response to eCO_2 were observed by Baslam et al. (2014).

Legume Nutritive Value

Nutritive value responses to eCO_2 are less pronounced than FA, but most studies report a decrease in herbage N concentration (and therefore crude protein [CP]) and an increase in non-structural carbohydrates (NSC), with some exceptions (Fritschi et al. 1999a; Baslam et al. 2014). A meta-analysis of Mediterranean data showed white clover herbage N was 7% less under eCO_2 than ambient (Dumont et al. 2015), while in Switzerland, white clover CP concentrations were 288 and 252 g kg^{-1} at ambient and eCO_2 (600 µL CO_2 L^{-1}), respectively (Frehner et al. 1997). Likewise, white clover starch concentrations increased, while N decreased under eCO_2 (690 µL CO_2 L^{-1}) in southeastern Australia (Lilley et al. 2001b). Rhizoma peanut had 50% greater leaf NSC under eCO_2 (640 µL CO_2 L^{-1}) than ambient, but leaf N concentration was 6% less (Fritschi et al. 1999a). In contrast, when grown in field soils under ambient and eT (+1.5-4.5°C) and ambient and eCO_2 (700 µL CO_2 L^{-1}), rhizoma peanut showed little effect of eCO_2 on digestibility or fiber concentrations (Newman et al. 2005). For alfalfa, eCO_2 generally reduced leaf soluble protein and N concentration and increased leaf saccharides or starch (Irigoyen et al. 2014). In general, eCO_2 reduces N and CP concentrations, but increases concentration of carbohydrates.

Similar to eCO_2, drought effects on legume NV are typically less pronounced than on FA (Kuchenmeister et al. 2013; Staniak and Harasim 2018), and they are often inconsistent, perhaps in part because definition of drought stress is not consistent across studies. Moderate drought stress was applied in a greenhouse to 6 temperate legumes (Kuchenmeister et al. 2013). It had negligible effect on NV, but strong stress decreased concentrations of CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) and increased water-soluble carbohydrates. Drought-stressed alfalfa had lesser crude fiber, but in contrast to Kuchenmeister et al. (2013), CP tended to increase (Staniak and Harasim 2018). There are other studies showing increased alfalfa herbage NDF and ADF with severe drought stress (Liu et al. 2018). For *S. capitata*, however, the combination of drought stress and warming (+2°C) increased ADF

(18%) and NDF (13%), but it decreased CP (27%), and in vitro digestibility (11%) compared with a well-watered control at ambient temperature (Habermann et al. 2021). In Germany, 4 temperate legumes grown under drought stress had lesser dry matter degradability than the control, but CP, NDF, and ADF degradability responses were not consistent (Riede et al. 2019). Thus, the effect of drought stress on legume NV cannot be generalized.

There was no effect of temperature (ambient or eT of +1.5-4.5°C) on NDF concentration of rhizoma peanut leaves or stems during the year of planting in Florida USA, but at the end of the year, leaf N concentration was less for eT (Fritschi et al. 1999a). Established rhizoma peanut was exposed to the temperatures used in Fritschi et al. (1999a) and harvested every 6-8 wk (Newman et al. 2005). Leaf NV was not affected by eT, but stem digestibility declined from 570 to 532 g kg^{-1}, and stem NDF, ADF, and lignin increased from 538 to 545 g kg^{-1}, from 412 to 418 g kg^{-1}, and from 80 to 93 g kg^{-1}, respectively, with temperature increase from ambient to +4.5°C. Three genotypes of birdsfoot trefoil (*Lotus corniculatus* L.) were grown at temperatures from 10-30°C in pots in growth chambers (Morris et al. 2021a, 2021b). Plants grown at 25°C had the greatest digestibility, lignin concentration was greatest at eT, and total NSC was greater at 10 than 25°C. Warming (+2°C) increased lignin concentration of *S. capitata* (Habermann et al. 2021) and decreased starch concentration in white clover (+3.4°C), but it had no effect on N concentration (Lilley et al., 2001b). In general, eT decreases forage NV.

Legume Flowering and Pollination
Studies of climate change effects on reproductive responses of forage legumes are rare, with the exception of *S. capitata*. The legume was exposed to eCO$_2$ (600 μL CO$_2$ L^{-1}) at ambient temperature, ambient CO$_2$ at eT (+2°C), combined eCO$_2$+eT, and control ambient CO$_2$ and temperature (Alzate-Marin et al. 2021b). Morphological abnormalities were associated with eCO$_2$ and eT and decreased viability of pollen grains from 71% for the ambient control to 64% for eCO2+eT. Thus, pollen sacs and pollen grains of *S. capitata* are vulnerable to warming, eCO$_2$, and their combined effects. The same treatments were imposed on *S. capitata* to assess flowering and pollinator behavior (Alzate-Marin et al. 2021a). Warming alone increased flower number by 62% vs. ambient, while the increase for eCO$_2$+eT was 137%. The latter was associated with an 83% increase in bee floral visits during greatest flower production. Under eT, flowers began opening 1 h earlier than the control, but duration of flower opening was not affected. Timing of pollinator visits shifted to earlier in the day for eT plots, likely because nectar temperature influences pollinator preference. Thus, mechanisms driving plant-pollinator relationships seem to adapt to eT.

In a subsequent study, the effects of eT (+2°C) and soil water status were assessed on time of flower opening and closing, sugar concentration in the nectar, and plant–flower visitor interactions (Maluf et al. 2022). Elevated temperature caused earlier *S. capitata* flower opening compared with ambient, and nectar sugar concentration for eT was 39% greater than ambient. Others have shown that plants subjected to eT combined with water stress can flower earlier, produce fewer flowers, begin flowering without producing nectar, or limit the proportion of flowers with nectar as a drought mitigation strategy (Phillips et al. 2018). This can suppress visits by pollinators, suggesting when water stress is associated with eT, the combination might compromise the intricate interactions between organisms.

Conclusions and Implications
Though there is variation in the literature for some responses, we conclude that eCO$_2$ generally increases legume FA, N fixation, and tissue NSC concentration, while reducing herbage N concentration. Drought reduces FA, but drought effects on NV are not consistent. Elevated temperature has a negative effect on legume NV, and, when combined with eCO$_2$, can reduce the magnitude of the positive FA and N fixation response to eCO$_2$. Climate change factors can also affect legume pollen viability and pollinator behavior, potentially influencing plant reproductive success. Overall, effects of climate change factors on forage legumes can be generalized to an extent, but interactions among eCO$_2$, eT, soil moisture status, and local climate and soil attributes may cause variation from expected responses.

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