



# Reduced water-availability lowers the strength of negative plant–soil feedbacks of two *Asclepias* species

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## Abstract

Negative plant–soil feedbacks can serve as a mechanism for plant species coexistence. Despite predicted changes in precipitation patterns due to climate change, little is known as to how the strength and direction of feedbacks change under differing soil moisture regimes. We performed a fully reciprocal greenhouse experiment where seedlings of two co-occurring *Asclepias* spp. (milkweed) were grown either with their own or the other species' microbial communities under high or low watering treatments. We found that seedlings of each species were smaller when exposed to conspecific relative to heterospecific soil biota, perhaps due to a build-up of specific soil pathogens. Importantly, this negative feedback diminished under reduced water-availability, and also in the absence of live soil organisms. Our findings suggest that the ability for plants to coexist may be fundamentally altered in areas that face increased drought.

**Keywords** Drought · Stress · Belowground interactions · Plant–microbial interactions · Plant–soil feedback

## Introduction

Plants interact with a multitude of microorganisms that affect plant abundance, fitness, and coexistence (e.g., Packer and Clay 2000; Bever et al. 2015; Mangan et al. 2010) and there is increasing evidence that these plant–microbial interactions lead to feedbacks (e.g., Bever et al. 1997; Janzen 1970; Connell 1971; Klironomos et al. 2011). These feedbacks may be critical to patterns of species coexistence with dominant soil organisms, affecting the ability for plants to coexist by altering the strength and direction (positive or negative) of plant–soil feedbacks (Bever et al. 2006). However, most studies on feedbacks are restricted to a single

set of environmental conditions despite known variance in climate throughout a species range (Bauer et al. 2015; Bever et al. 2015). Additionally, increasing variability in environmental factors due to climate change may fundamentally alter plant–microbial interactions thereby also altering plant fitness and community dynamics (Rudgers et al. 2015). By examining plant–microbial interactions under differing environmental factors we may better understand the secondary effects of climate change on plant community composition.

Microbial communities in a given location are a product of both plant-mediated accumulations of microbes and the environmental conditions experienced by both the plants and the microbes (Rudgers et al. 2015). Thus, the strength and direction of plant–soil feedbacks will depend on both the types of microbial communities accumulated and the environmental conditions experienced. Pathogens and the resulting negative feedbacks can maintain local plant diversity by suppressing conspecific recruitment (Klironomos 2002; Mangan et al. 2010) while positive feedbacks between beneficial and species-specific organisms [e.g., arbuscular mycorrhizal fungi (AMF), nitrogen-fixing bacteria] promote the fitness of conspecifics relative to heterospecifics (e.g. Mangan et al. 2010; Vannette et al. 2013) resulting in lower species richness. While microbial communities can accumulate based on plant diversity, climatic changes such as reduced precipitation can alter microbial community composition

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as well as a plants willingness to associate with microbes. By restricting microbial access to necessary water (Waring and Hawkes 2014), or by changing nutrient availabilities by changing decomposition times or rates of nutrient runoff (Fierer and Gabet 2002; Nearing et al. 2004) microbial associations with plants may shift relative to climate.

Although much research has focused on the effects of drought on plants or microbes independently (ex: Blankinship et al. 2011; Manzoni et al. 2012), fewer studies have examined the effect of drought on feedbacks formed between plants and their soil-borne microbes (but see Meijer et al. 2011; Al-Karaki and Al-Raddad 1997; Kaisermann et al. 2017; Fry et al. 2018). This is despite evidence that negative feedbacks may be partially dependent on abiotic conditions (Smith and Reynolds 2015; van der Putten et al. 2016). Consequently, under climate change, drought may release plants from pathogens due to the higher impact of drought on fast-growing organisms and the high moisture-specificity of some pathogenic bacteria (van der Putten et al. 2016). Understanding how interactions between plants and their soil biota are affected by environmental factors, such as drought, is especially important to improve our predictive ability of community responses under a changing climate.

While studies have speculated on the importance of climate change factors such as drought on plant–soil interactions (Afkhami et al. 2014), the effect of changing moisture conditions on the strength and direction of plant–soil feedbacks is understudied. Accordingly, the goal of our study was to test whether the strength and direction of plant–soil interactions differ depending on soil moisture conditions in two closely related species of *Asclepias* (Milkweed) that can

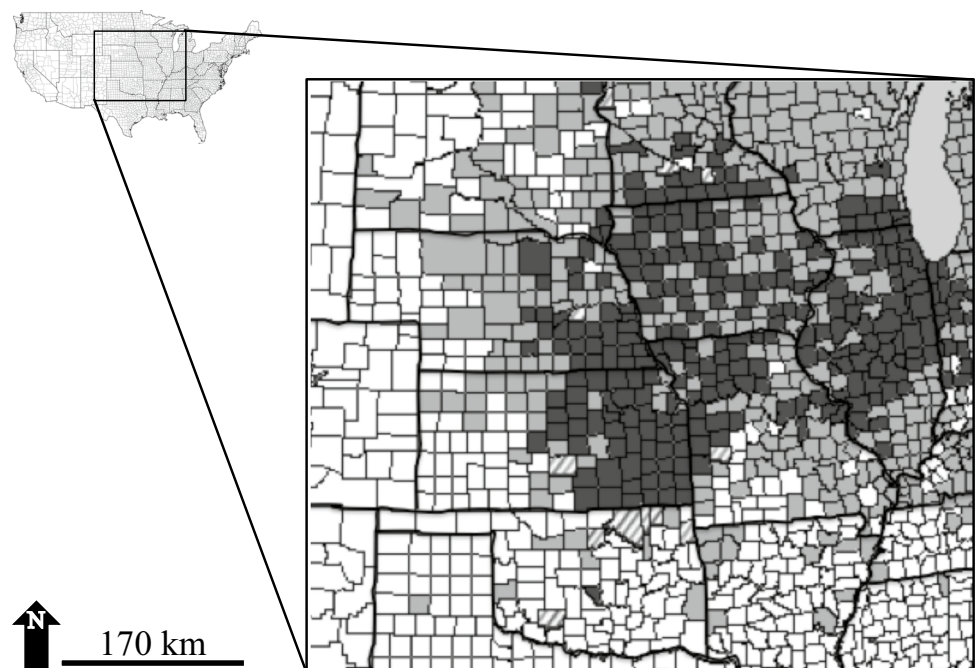
co-occur locally. To do so, we grew seedlings of these two plant species in either their own soil biota or the biota from the other species under two soil moisture conditions to test whether plant–soil biota interactions are species-specific and drive plant–soil feedback. We expected each plant species would grow more poorly with their conspecific soil due to the suppressive effects of species-specific soil-borne microbes such as pathogenic fungi or bacteria. We also predicted that lower soil moisture (i.e., drought) would mitigate the negative impact of conspecific soil biota on plant growth.

## Methods

### Study species

*Asclepias* is a genus of perennial herbaceous plants whose members show strong growth responses to their soil biota (Vannette et al. 2013) including both arbuscular mycorrhizal fungi and pathogens (Wilson and Hartnett 1998; Klironomos 2002; Bauer et al. 2003). We examined the effect of soil moisture on plant–soil feedbacks formed between two closely related species in the genus *Asclepias* (Agrawal et al. 2009). *Asclepias sullivantii* (Prairie Milkweed) and *Asclepias syriaca* (Common Milkweed), herein referred to by their common names to avoid confusion, overlap in range from eastern Michigan and Ohio to the southern edge of Kansas and up to the southern tip of North Dakota (Fig. 1) (Kartesz 2015). However, outside of this range Prairie Milkweed is primarily found in moist prairies (Klips and Culley 2004) and ranges from as far south as Oklahoma up to

**Fig. 1** Map of area showing the overlap for the ranges of Prairie Milkweed (striped) and its overlap (black) with Common Milkweed (grey), in the United States by county. Adapted from Kartesz (2015)



southern Ontario, and from Nebraska east to Ohio (Woodson 1954; Kartesz 2015) while Common Milkweed is a habitat generalist found from as far north as Newfoundland to Saskatchewan and has been successfully introduced from Georgia to Texas and in Oregon (Kartesz 2015; Woodson 1954). Despite opportunities for cross-fertilization due to range overlap (Brotherson 1983) and morphological similarities, it is uncommon for them to hybridize in the wild (Klips and Culley 2004).

## Experiment

### Soil culturing phase

We established a fully factorial greenhouse experiment to examine the impact of water availability on the performance of Prairie Milkweed and Common Milkweed when grown with conspecific and heterospecific soil biotic communities. To prepare these biotic communities, live soil was collected from five separate locations of well-established Prairie Milkweed and Common Milkweed individuals (5–10 individuals each) naturally occurring within 10 km of Urbana, Illinois (10 locations total). Each site was separated by at least 300 m to ensure that soil biotic communities originated from distinct plant populations. From these individuals, soil cores (30.5 cm deep) were taken near the rooting zone and were thoroughly mixed within species and within a site.

We then amplified soil biotic communities in the greenhouse by inoculating seedlings of Prairie Milkweed and Common Milkweed with their respective soil communities separately in a total of ten 1.9 L pots in the greenhouse (Bauer et al. 2015). Seeds of each species were obtained from Prairie Moon Nursery (Winona County, Minnesota, USA), and surface-sterilized (70% ethanol for 30-s, 30% sodium hypochlorite for 2 m) and rinsed three times in deionized water. Seeds were cold-stratified for 1 month at 4 °C and then germinated in flats containing twice-autoclaved soil to ensure soil sterility (Bauer et al. 2012). Eighteen days after germination we filled each of the ten 1.9 L pot with 1.0 L of sterilized field soil, transplanted ten seedlings of either Prairie Milkweed or Common Milkweed, and inoculated each with 100 mL of their own field-collected inoculum, keeping each collection site separate. Soil microbial communities were allowed to amplify for 5 weeks before being used in the factorial experiment. To prepare soil for use in the factorial experiment all above and belowground plant material was removed and soils were homogenized within a species (Fry et al. 2018).

### Factorial experiment

The 1.9 L pots were filled with 600 mL of twice-autoclaved sandy field soil. Pots were then inoculated with 100 mL

of live or twice-sterilized soil originating from pots of the amplification stage of either Prairie Milkweed or Common Milkweed (Vannette and Hunter 2011). Finally, we capped all pots with an additional 400 mL of sterilized background soil to reduce the possibility of cross contamination. Seeds were surface sterilized and germinated in sterile soil as mentioned above, and young seedlings (~7-day old) of each *Asclepias* species were transplanted into 1.9 L pots. In the sterile controls, 100 mL of sterilized inoculum were added in the place of live inoculum. To minimize transplant shock, all seedlings were well watered for the first week after transplant. No seedlings died within the first 2 weeks of the experiment.

One week following the transplant, we initiated the drought treatment by providing well-watered pots with 200 mL of water, while drought pots received 75 mL of water. This watering level was experimentally determined beforehand to ensure well-watered treatments would neither experience water-stress nor be over-watered, whereas seedlings, where water was reduced, exhibited visual signs of water stress, but no mortality. Pots were watered when the top centimeter of soil appeared visually dry in 90% of the well-watered pots (approximately every 3–4 days).

This factorial experiment consisted of two plant species, two inoculum types (conspecific versus heterospecific), and two watering levels. All treatment combinations containing live inoculum were replicated 20 times, with a single replicate of each combination arranged in randomized blocks in the greenhouse (160 pots in total). Due to space constraints, only four replicates of pots with sterilized inoculum were used as controls for each treatment (32 pots total). After 60 days, total biomass was harvested, dried, and weighed. To confirm that we adequately reduced AMF in sterilized treatments, we stained roots with Trypan Blue to distinguish structures of AMF colonization (Kozioł and Bever 2015). The relative abundance of internal hyphae, arbuscules, vesicles, coils, and external hyphae were all separately quantified, as were non-AMF structures and necrosis (Online Resource 1).

### Statistical analysis

We used a linear model to examine the effects of species, soil biota origin (conspecific or heterospecific), water availability, block, and all interactions as fixed effects on plant growth where initial plant height was included as a covariate. Due to the difference in replicate size, we analyzed sterile and live treatments separately. Because of the variation in size of plant at death and the amount of time individuals took to die, only individuals remaining when the experiment ended were included in the analysis. All analyses were done in R Studio (RStudio, Version 0.99.903, RStudio Inc., Boston, Massachusetts, USA) using the software R (R version

3.3.3, 2017-03-06) and the package stats (version 3.3.3, 2017-03-17).

## Results

Arbuscular mycorrhizal colonization, including arbuscules, were significantly lower in sterile soil treatments compared to live treatments (Table 1). This indicates that sterilization of soil and seeds were successful and results should be due to purposeful inoculation and the experimental design. We similarly found that our drought treatment successfully reduced total plant biomass for both species (water availability live:  $F_{1,114} = 183.289$ ,  $P < 0.001$ ; water availability sterile:  $P < 0.001$ ,  $F_{1,17} = 85.687$ ,  $P < 0.001$ ).

For plants inoculated with live biota, there was a trend towards a differential response between Prairie Milkweed and Common Milkweed in response to drought (watering: plant species:  $F_{1,114} = 2.939$ ,  $P = 0.0892$ ) (Table 2). Under drought conditions, Common Milkweed trended towards a greater reduction in biomass than Prairie Milkweed, although this was smaller than the reduction in biomass in sterile drought conditions (watering: plant species:  $F_{1,17} = 2.939$ ,  $P = 0.013$ ) (Table 1).

Seedling growth of each species depended on the origin of the soil biota (species  $\times$  biota origin:  $F_{1,114} = 5.472$ ,  $P = 0.0211$ ), where both *Asclepias* species tended to grow worse with their own soil biota than with heterospecific biota. This was especially true for Common Milkweed when exposed to well-watered conditions (Fig. 2a). However, the strength of this interaction weakened and was not significant under reduced water availability (Fig. 2b). This resulted in a significant interaction between plant species, soil origin, and watering treatment ( $F_{1,114} = 4.414$ ,  $P = 0.0378$ ). We found no evidence for feedback interactions between plants inoculated

**Table 2** The effects of soil inoculum, plant species, watering treatment and their interactions on total biomass in live soil using a three-way ANOVA, with initial height as a covariate and all other variables as fixed factors

Effect	df	F	P
Live soil inoculum			
Initial height	1	4.023	0.047
Species	1	24.407	<0.001
Soil inoculum	1	2.675	0.105
Water-availability	1	183.289	<0.001
Species $\times$ soil inoculum	1	5.472	0.021
Species $\times$ water-availability	1	2.939	0.089
Soil inoculum $\times$ water-availability	1	0.036	0.849
Species $\times$ soil inoculum $\times$ Water-availability	1	4.414	0.038
Block	19	1.196	0.273
Residuals	114		

with sterilized biota of either species (Fig. 3) ( $F_{1,17} = 0.004$ ,  $P = 0.9519$ ) or for a three-way interaction between watering treatment, plant species, and soil origin when sterilized inoculum was used ( $F_{1,17} = 0.135$ ,  $P = 0.7179$ ). However, we did find an interaction between soil inoculum ( $F_{1,17} = 4.991$ ,  $P = 0.039$ ) and inoculum and water in sterilized soil ( $F_{1,17} = 6.483$ ,  $P = 0.021$ ).

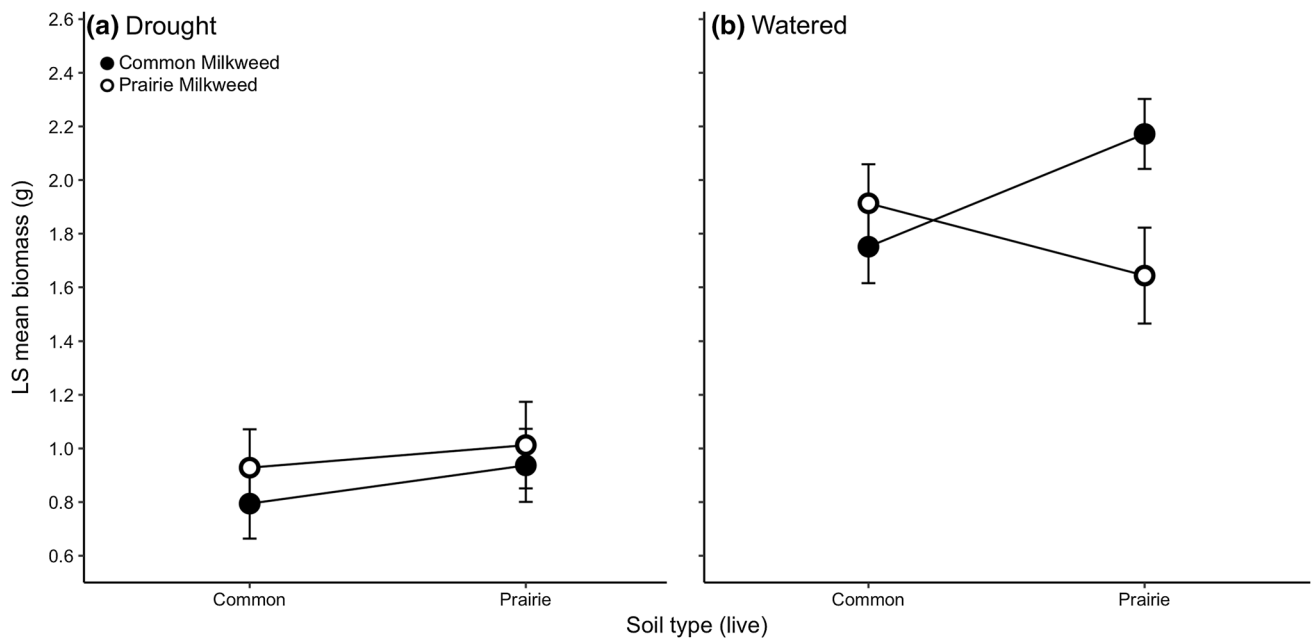
In addition to effects on total biomass, we also found high mortality for Prairie Milkweed seedlings when grown with their own soil biota. In total, fifteen individuals died throughout the duration of the experiment. All individuals that died were Prairie Milkweed, and twelve of these were in live Prairie Milkweed conditioned soil. Six were in the low water treatment and six were in the well-watered treatment.

## Discussion

In light of the projected expectations of significant climatic shifts, understanding how plant–soil feedbacks may interact with abiotic conditions to affect species growth, which contributes to species diversity and persistence, may be critical. This work found water availability significantly influences the degree to which soil communities exert species-specific effects on plant growth. In well-watered soils, both Common Milkweed and Prairie Milkweed grew more poorly with conspecific soil biota than with heterospecific soil biota, resulting in significant negative feedback. However, when plants experienced drought, the reciprocal responses across soil biota origin vanished, resulting in the diminishing strength of negative feedback. Although it is now well established that plant communities are dominated by negative plant–soil feedbacks (Kulmatiski et al. 2008; Mangan et al. 2010; van der Putten et al. 2013; Munzbergova and Surinova 2015),

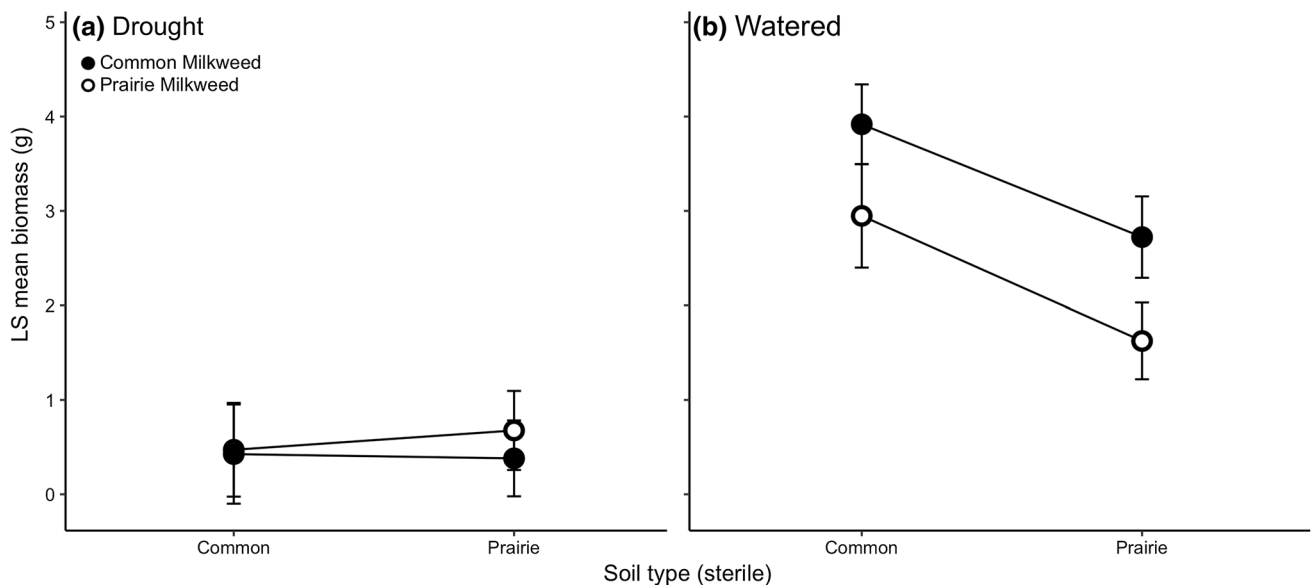
**Table 1** The effects of soil inoculum, plant species, watering treatment and their interactions on total biomass in sterile soil using a three-way ANOVA, with initial height as a covariate and all other variables as fixed factors

Effect	df	F	P
Sterile soil inoculum			
Initial height	1	0.760	0.395
Species	1	3.387	0.083
Soil inoculum	1	4.991	0.039
Water-availability	1	85.687	0.001
Species $\times$ soil inoculum	1	0.004	0.952
Species $\times$ water-availability	1	7.608	0.013
Soil inoculum $\times$ water-availability	1	6.483	0.021
Species $\times$ soil inoculum $\times$ water-availability	1	0.135	0.718
Block	3	0.199	0.895
Residuals	17		



**Fig. 2** Effects of heterospecific and conspecific live soil biota on total biomass of two species of milkweed (Common: *Asclepias syriaca*, Prairie: *Asclepias sullivantii*) under two watering treatments **a**

drought and **b** watered. Points are the LS mean of total biomass and the bars are SE for LS means



**Fig. 3** Effects of sterile soil biota (heterospecific and conspecific) on total biomass of two species of milkweed (Common: *Asclepias syriaca*, Prairie: *Asclepias sullivantii*) under two watering treatments **a**

drought, and **b** watered. Points are the LS mean of total biomass and the bars are SE for LS means

our study adds to a new branch of experimental demonstrations that strengths of these negative interactions can be mediated by abiotic factors such as water availability (Kaisermann et al. 2017; Fry et al. 2018). However, our study did homogenize soil from our cultures, potentially reducing variation that could have otherwise been seen across

treatments (Reinhart and Rinella 2016; Rinella and Reinhart 2017, 2018).

Differences in soil moisture likely influenced the strength of feedback through direct impacts on the abundance and activities of specialized soil biota. The negative effects of conspecific biota on plant growth in well-watered soils



suggest that the effects of antagonistic microbes such as fungal pathogens likely dominated the microbial community. Previous studies have shown that activities of fungal pathogens are moisture dependent, with increased levels of dormancy or unviability when faced with drought (Huber and Gillespie 1992; Schimel et al. 2007; Jones and Lennon 2010). The large number of deaths in conspecific soil further suggests that species-specific pathogens may be a driving factor for mortality in addition to growth. This further suggests changes in plant–soil feedbacks could alter community composition.

The lack of feedback for plants with sterilized biota indicates both that our sterilization was successful and that feedbacks seen in the live soil is due to live biota present in the soil rather than nutrient or allelopathic differences caused by each species conditioning the soil. For surviving individuals, the larger suppression of Common Milkweed growth by conspecific biota in live soil suggests that different communities of soil organisms associate with each *Asclepias* species. The removal of roots in the 100 mL of inoculum, and the potential dilution of phytotoxicity by mixing inoculum with 1800 mL of sterile soil per pot, as well as the absence of plant–soil interactions in sterile soil reduces the likelihood that allelopathy was the cause of these results. However, the impact of soil inoculum in sterile treatments does indicate that allelopathy may have played a role in our results. This may be due to second and third order impacts from the presence of these chemicals rather than from a change in nutrient content. In addition, different relative abundances of soil biota, rather than entirely different community composition could lead to the observed patterns.

Inoculated soil has previously been found to negatively impact congeneric species (ex: Munzbergova and Surinova 2015; Burns et al. 2015; Brandt et al. 2013). However, research demonstrating that congeneric impacts of soil communities are reduced compared to conspecific impacts are much rarer and may become of increasing importance with climate change. Regardless of the mechanism, understanding the response of plants and their soil communities to changing abiotic conditions is of increasing importance. As climate change intensifies, shifting climatic patterns will result in drier summers throughout the range of the study species and much of the US that limit physiological capabilities for growth (Leung and Gustafson 2005; IPCC 2014).

Negative plant–soil feedbacks help maintain diversity and promote species coexistence (Mangan et al. 2010) and our work shows that climate change may alter the strength of plant–soil feedbacks thereby changing coexistence and dominance in communities. If coexistence between Common Milkweed and Prairie Milkweed depends on wetter conditions, the predicted drier summers may reduce the strength of conspecific plant–soil feedbacks on Common Milkweed, allowing it to increase in its dominance. Alternatively, wetter

winters may increase the pathogen load of these species and allow for the Prairie Milkweed to increase its range. Changes in plant growth and community composition can have significant effects on species that feed on milkweeds, such as Monarchs (*Danaus plexippus*) which have increased mortality on certain species of milkweeds (Pocius et al. 2017) and whose population size is limited by milkweed availability and competition for resources (Flockhart et al. 2012). Climate change, and the ensuing changes in precipitation may thus indirectly impact plant community assembly and higher trophic levels through plant–soil feedbacks. As climate change progresses, understanding the interspecific interactions between soil communities and their host species in altered abiotic conditions may become essential to predicting future plant ranges and community compositions.

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**Author contribution statement** AES and ANHT conceived and designed the experiment. AES and ANHT performed the experiment. AES analyzed the data. AES and ANHT wrote the manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare they have no conflict of interest.

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