Connecting plant–soil feedbacks to long-term stability in a desert grassland

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Abstract. Temporal fluctuations in plant species coexistence are key to understanding ecosystem state transitions and long-term maintenance of species diversity. Although plant microbiomes can alter plant competition in short-term experiments, their relevance to natural temporal patterns in plant communities is unresolved. In a semiarid grassland, the frequency and magnitude of change in plant species composition through time varied from relatively static to highly dynamic among patches across the landscape. We field tested whether these alternative successional trajectories correlated with alternative plant–soil interactions. In temporally stable patches, we found negative plant–soil feedbacks, where plants grew worse with conspecific than heterospecific soil biota—a mechanism that maintains stability in mathematical models. In contrast, feedbacks in temporally dynamic patches were neutral to positive. Importantly, the magnitude of feedbacks depended on plant frequency, enabling plant species to increase in cover when rare, which theory predicts will promote long-term, stable coexistence. Although our study does not determine the direction of causality, our results reveal a novel link between plant—microbe interactions and temporal stability of plant species coexistence and help to explain 20+ yr of plant abundance dynamics at the patch-to-landscape scales.

Key words: Bouteloua eriopoda; Bouteloua gracilis; *coexistence; plant–microbe interactions; plant–soil feedback; semiarid grassland; stability.*

Introduction

Ecologists have puzzled over the drivers of species coexistence for decades because it is key to understanding the maintenance of species diversity, and to predicting future community trajectories under global environmental change (e.g., Tilman and Downing 1994, Knapp and Smith 2001, Hallett et al. 2014). Past explanations have focused primarily on fluctuating abiotic conditions, such as climate or resource availability (e.g., Beisner et al. 2003, Smith et al. 2009, Letten et al. 2018), modified by interspecific competition as the dominant biotic driver (e.g., Tilman 1994, Kilpatrick and Ives 2003). However, in plants, fitness, abiotic tolerances, and competitive ability all can be altered by plant-associated soil biota, which could be as important as other studied mechanisms in mediating species coexistence (Reynolds et al. 2003, Kivlin et al. 2013, Lekberg et al. 2018). Although the possibility that soil biota influence species coexistence is increasingly recognized, experimental tests have been few (e.g., Bever et al. 1997,

Pendergast et al. 2013, Chung and Rudgers 2016). Furthermore, experiments are typically conducted in artificial environments, which are disconnected from the dynamics of species in natural landscapes (but see Burns and Brandt 2014, Pellkofer et al. 2016).

Evidence supports the potential for plant microbiomes to alter coexistence outcomes among plant competitors, and thus to shape the temporal trajectory of plant community composition. For example, as a plant species increases in abundance, it can accumulate a unique microbial community in roots and nearby soil that is more detrimental to itself than to co-occurring plant species, causing a negative plant–soil feedback (Bever et al. 1997). Such feedbacks could increase the magnitude of negative intraspecific interactions relative to interspecific interactions, potentially stabilizing species coexistence and slowing successional change (Burns and Brandt 2014, Chung and Rudgers 2016). Alternatively, plant–soil feedbacks could promote directional change in plant community composition, if feedbacks favor the establishment of later-successional species (Kardol et al. 2013). Finally, mathematical models predict that negative plant–soil feedbacks can promote stable coexistence in situations where one plant would otherwise competitively exclude another by regulating the amplitude of oscillations in the relative abundances of two species

(Bonanomi et al. 2005, Revilla et al. 2013). However, a link between the strength of plant–soil feedbacks and the long-term, stable coexistence of competing plant species has not, to our knowledge, been documented for any plant community in the field.

Determining the importance of plant–soil feedbacks to the temporal trajectories of natural ecosystems requires long-term data on community dynamics and the ability to test feedbacks at field-relevant temporal and spatial scales that capture the legacy of historical plant community dynamics. We conducted a field experiment to link the strength of plant–soil feedbacks to long-term plant community dynamics in a semiarid grassland. We first quantified 26 yr of abundance dynamics for the dominant grass species (blue grama, *Bouteloua gracilis*, and black grama, *Bouteloua eriopoda*) to identify landscape patches that differed in the rates of temporal change in vegetation composition (Methods; Collins and Xia 2015). Dynamic patches had frequent changes in species relative abundances, whereas static patches had more stable plant species composition through time. Like historical abiotic conditions (e.g., Hawkes et al. 2017), these alternative histories of plant dynamics may leave legacies in the composition of soil biota. Next, we examined the soil abiotic environment and found that abiotic variables could not explain the spatial variation in temporal stability of plant dynamics (see Methods and Appendix S1: Supplemental results). We then conducted a plant–soil feedback field experiment to measure the fitness of each plant species in its own (conspecific) and the other's (heterospecific) soils in both static and dynamic patches (Fig. 1). Finally, we evaluated whether plant–soil feedbacks in static or dynamic patches would stabilize plant coexistence (negative feedback) or destabilize coexistence (positive feedback; Bever et al. 1997, Bever 2003).

We asked: (1) Do the strength and direction of plant–soil feedbacks differ between temporally dynamic vs. static patches? Theory predicts mechanisms that increase negative intraspecific interactions will slow down competitive exclusion and stabilize coexistence (Volterra 1926, Lotka 1978, Chesson 2000). Therefore, we predicted that negative feedbacks would occur in static patches of high community stability, whereas dynamic patches would have weak or neutral feedbacks. (2) In static patches, does plant–soil feedback strength reflect the frequency of the plant species in the community? The ecological impacts of host-specific microbes should scale with the relative frequency of its host species in a patch (Bever et al. 1997). Therefore, in static patches with stable coexistence, the relative benefit of growing in heterospecific soil compared to conspecific soil should be largest when a species is rare because of the high frequency of heterospecific soil biota in that patch. This leads to more negative feedbacks that promote the establishment of a plant species most strongly when it is rare (invasion criterion for coexistence), thus promoting its ability to stably persist (Chesson 2000, Yenni et al. 2012).

METHODS

Study site and species

This study was conducted in a northern Chihuahuan Desert grassland co-dominated by black grama (*B. eriopoda*) and blue grama (*B. gracilis*) at the Sevilleta National Wildlife Refuge, New Mexico, USA. Both are long-lived perennial C_4 grasses but differ in growth form. Blue grama is a caespitose grass that forms rings (Ravi et al. 2008), whereas black grama expands via stolons and occasional seed reproduction (Peters and Yao 2012). Field observational and manipulative experiments at Sevilleta have shown that blue grama is a superior competitor to black grama (Peters and Yao 2012, Thomey et al. 2015), has higher seed viability (Peters 2002), and is more resistant to fire and herbivory (Gosz and Gosz 1996). We used high-spatial-resolution (1 cm), long-term species abundance data from a permanent line-intercept transect (Deep Well, 34°21′32″ N, 106°41′16″ W; Collins 2016), which stretches 400 m and has been continuously monitored in the spring and fall since 1989 by the Sevilleta Long Term Ecological Research (LTER) program (http://sev.lternet.edu/data/sev-4). Past work revealed spatial variation in the temporal dynamics of the dominant grasses on this transect (Collins and Xia 2015). Dynamic patches showed frequent changes in species relative abundances, whereas static patches showed more stable species composition through time (Appendix S1: Figs. S1 and S2). Past greenhouse work revealed that negative plant-soil feedbacks increased negative frequency dependence of blue grama and facilitated coexistence between blue and black grama (Chung and Rudgers 2016), motivating the current study.

Magnitude and frequency of temporal dynamics

Plant cover data from spring censuses 1989–2012 were binned into 4-m patches by year. Patch size was chosen to target neighborhood-scale plant–plant and plant–soil interactions (individual plants can reach ~1 m in diameter) and to average over small interannual variation due to observation error. Within each 4-m patch and for each year, we calculated total plant cover and the absolute and relative cover of blue and black grama. Throughout the record, blue and black grama combined made up 78% ($\pm 0.5\%$ SE) of total patch vegetation cover on average. Only patches with mean total plant cover >35% across years, and for which both species (combined) represented >50% of total plant cover were considered for the experiment. These cutoffs maximized sample size, controlled for spatial heterogeneity, and allowed us to include 77 of the 100 total patches.

To quantify the *magnitude* of change in relative percentage cover of blue grama and black grama for each patch, we summed the Euclidean distances in the two-dimensional species-space defined by blue grama cover

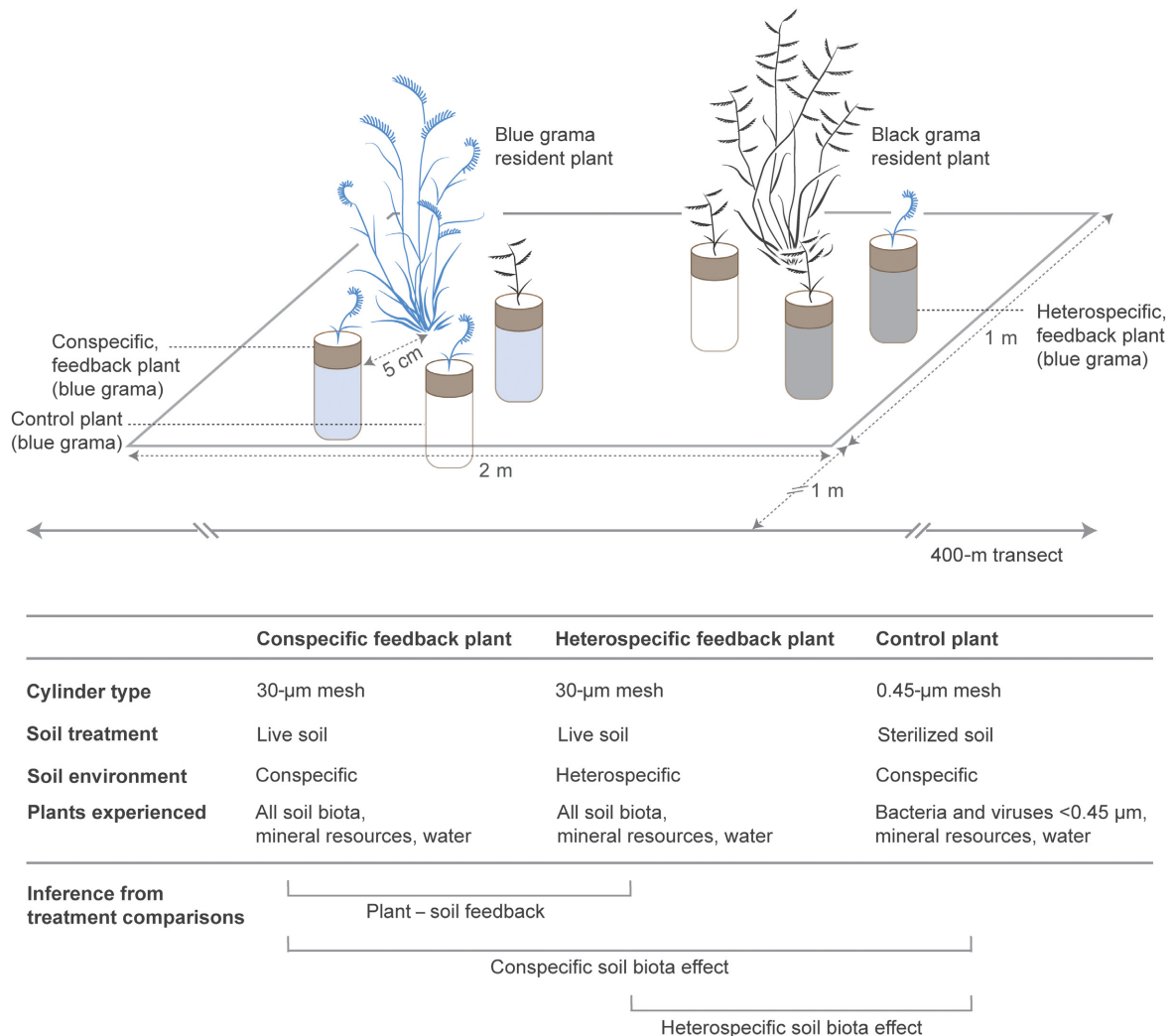|	Conspecific feedback plant	Heterospecific feedback plant	Control plant
Cylinder type	30-μm mesh	30-μm mesh	0.45-μm mesh
Soil treatment	Live soil	Live soil	Sterilized soil
Soil environment	Conspecific	Heterospecific	Conspecific
Plants experienced	All soil biota, mineral resources, water	All soil biota, mineral resources, water	Bacteria and viruses <0.45 μm, mineral resources, water
Inference from treatment comparisons	Plant – soil feedback		
	Conspecific soil biota effect		
		Heterospecific soil biota effect	

FIG. 1. Field plant–soil feedback experimental design. Resident black grama and blue grama plants were identified in each plot, and experimental transplants planted around the resident plants in cylinders that either allowed soil biota colonization to test feedbacks (colored cylinders) or excluded organisms >0.45 μm with a mesh barrier containing sterilized soil (white cylinders, "Control plants"). Feedback plants experienced the soil environment of the resident plant, indicated by the color (blue or black) of each cylinder. Conspecific feedback plants grew in their own soil with host-specific soil biota (e.g., blue grama next to blue grama resident plant). Heterospecific feedback plants grew in soil conditioned by the competitor plant species (e.g., blue grama next to black grama resident plant). Other natural vegetation in the plot is omitted for clarity. Art by Daisy Chung.

and black grama cover for each pair of years in the time series (large summed Euclidean distances = large change in relative abundance of a species). For example, a change from (10%, 60%) to (60%, 10%) in (%blue grama, %black grama) space calculated as the Euclidean distance between those points would be larger than a change from (40%, 50%) to (50%, 40%). To quantify the *frequency* of change, we counted the number of times that dominance (defined as >5% higher cover than the competitor) switched between the grass species. In the context of this study, dominance indicates greater cover of one species over another. We chose 5% difference to ensure that the cover difference fell outside the 95%

confidence interval around mean percentage of change $(1.4 \pm 2.4\%)$ for dominance switches in the historical data. The metrics quantifying *frequency* and *magnitude* of change were positively correlated ($t = 7.5$, df = 75, $P < 0.0001$, $R = 0.66$). Therefore, we combined them into a single index ("dynamic score") by transforming both metrics to a standard normal distribution and adding them together. Conservatively, patches with scores of <0 were considered static candidates, and those >1 were dynamic (Appendix S1: Fig. S2). From these candidates, we then chose 10 spatially paired dynamic and static patches (20 patches total; pairs were 4–12 m apart) to account for abiotic gradients along the transect. Paired

patches represented a single block. Care was taken that chosen patches classified as "dynamic" were not due to switches in dominance following a fire in 2009 by individually plotting and examining the temporal dynamics of each patch (Collins 2016, Appendix S1: Figs. S3 and S4). Each patch included the presence of both focal species, with the static patches including five patches that were dominated by black grama and five dominated by blue grama in the long-term data. In the field, we established one 1 × 2 m experimental plot within each patch and <2 m from the transect (N = 20 plots). Two naturally established "resident" plants, one of each grama species, were identified in each plot to act as "soil biota donors" in the field experiment described below (Fig. 1).

Abiotic covariates

To evaluate potential abiotic drivers, we measured soil texture, chemistry, temperature, and moisture at each resident plant in each experimental plot. Soil moisture and temperature were measured using an *Aquaterr T-350* probe (Aquaterr Instruments & Automation, Costa Mesa, California) at 10–15-cm depth in June 2014, and following precipitation in July 2014. Soil texture was determined using a hydrometer (Bouyoucos 1962). Soil chemistry and nutrients (soil N, Ca, Mg, K, P, Fe, Mn, S, Al) during June–July 2014 (total duration = 40 d) were measured using plant-root simulators with ion resin exchange membranes (PRS® probes, Western Ag Innovations, Saskatoon, Canada; Drohan et al. 2005). We investigated effects of spatial block, plant species, community stability type (dynamic vs. static), and their interactions on all measured abiotic covariates together in a multivariate analysis of variance (MANOVA). We then further evaluated each covariate in a separate ANOVA to clarify which drove MANOVA results. There was a significant spatial gradient in soil chemistry and moisture along the transect, which we accounted for by blocking our field experiment. However, there were no significant differences in soil abiotic properties between blue and black grama in static or dynamic patches (Appendix S1: Supplemental results).

Field plant–soil feedback transplant experiment

To determine if the direction or strength of plant–soil feedback differed between static and dynamic patches, we transplanted seedlings into each patch in late July 2014 (Fig. 1). Two-month-old seedlings of each species (see Appendix S1: Supplemental methods) were planted next to a resident conspecific or heterospecific plant to experience the belowground biotic environment of the resident plant (Fig. 1 "feedback plants"; four per plot, two each species, 80 plants total). In this ecosystem, bare interspaces between naturally occurring resident plants are common (Pockman and Small 2010). By installing experimental seedlings close to resident plants (5 cm from base), we ensured that transplants experienced soil

conditions predominantly influenced by the chosen resident plant and not by other neighbors.

Transplants were enclosed in 30-μm Nitex® mesh cylinders (7.6 cm diam., 30 cm tall; modified from Reed and Martiny 2007). This mesh size allowed ambient colonization of bacteria, fungi, and microfauna from the rhizosphere of the resident plant, but excluded direct root competition. In the field, a 10 cm diam. × 30 cm deep soil core was taken from each planting location and passed through a 2 mm sieve to remove rocks. The mesh cylinder was inserted into the ground and filled with the sieved field soil. Seedlings were germinated and grown in the greenhouse in sterile soil for 2 months (Appendix S1: Supplemental methods). Seedlings were transplanted directly into installed cylinders and immediately watered (35 mL). To protect against mortality under drought, transplants were watered 30 mL each (equivalent to a 6.6-mm rain event) during the growing season (May–October) when rain had not occurred for more than two consecutive weeks. The frequency, magnitude, and total amount of additional watering each year did not substantially increase above-average historical monsoon season (June–September) precipitation (Appendix S1: Supplemental methods).

Our original design included an "exclusion" control treatment (Fig. 1 "control plants"; two per plot, one each species, 40 plants total). In each plot, a seedling was planted next to its conspecific resident in a cylinder filled with sterilized (autoclaved 3 h gravity at 121°C) soil in cylinders made of 0.45-μm nylon mesh (Ultracruz® transfer membrane, Santa Cruz Biotech, Dallas, Texas, USA), pores of which allowed free solute flow but excluded all but the smallest bacteria. Previous work in this ecosystem showed that autoclaving did not significantly alter the chemistry of the soils at this site, which are nutrient-poor and low in organic matter (Chung and Rudgers 2016). The small mesh size was implemented to identify whether the net effect of resident soil biota was mutualistic (e.g., plant performance: conspecific soil > exclusion) or pathogenic (conspecific soil < exclusion). The control successfully reduced fungal colonization. Even after three growing seasons, root fungal colonization (percentage of views colonized) was 32% lower in controls (61.3% ± 8.0 SE) than in live soils (88.3% ± 5.3 SE; $F_{1,32}$ = 15.68, $P < 0.001$). However, the amount of colonization in control plants was sufficiently high to make it difficult to partition differences due exclusively to soil biota. Thus, we focused on the main feedback between conspecific and heterospecific soil environments (see Appendix S1: Figs. S5–S8 for results including the "exclusion" plants).

Field plant–soil feedback response variables

Nondestructive.— During 2014–2016, we censused transplants every 2 weeks, June–October. We recorded survival, number of tillers, basal area diameter (2015–2016 only), and height of the tallest tiller. Only one individual

reproduced. In June 2016, we added 10 conspecific seeds to each field experimental cylinder to investigate effects on recruitment. Seedling emergence was recorded once a month. We conducted an additional seedling emergence experiment in soils collected from under resident plants in the growth chamber to insure against low field germination (Appendix S1: Supplemental methods). As survival and seedling emergence did not differ among treatments, we focus on the growth/biomass results from the field experiment in the main text and provide those results in the Supplement (Appendix S1: Supplemental results, Figs. S6–S8).

Harvest.—On 13–15 July 2016, all live/recently dead plants were harvested for above and belowground biomass (38 plants remaining of 120). Aboveground and belowground biomass was dried at 60°C for 1 week and weighed. As final survival was low, we used final harvest biomass and plant size variables to fit allometric equations to estimate plant biomass throughout the experiment to make use of the full experimental design (Appendix S1: Supplemental methods). Additional analyses showed similar results using tiller number or plant height as response variables (Appendix S1: Table S1), thus we report allometric biomass here as the most comprehensive measure of plant fitness. No plants were root-bound at harvest.

Plant–soil feedback.—We used estimated allometric biomass to calculate single-species plant–soil feedbacks as ln(mass in conspecific soil/mass in heterospecific soil) for live plant pairs in each experimental plot (Brinkman et al. 2010). This metric directly reflects whether each focal plant species does worse in its own soil environment (negative feedback) or better in a heterospecific environment (positive feedback). We calculated the interaction strength metric (I_s) as the sum of single species plant–soil feedbacks for blue and black grama in each plot following Bever et al. (1997). This metric is calculated using responses from both plant species and is a quality of a species pair. It considers the effects of plant–soil feedback for both plant species to determine whether net pairwise feedbacks will result in stable coexistence between the species pair (negative I_s).

Data analysis

To determine if the strength and/or direction of plant–soil feedback differed between dynamic and static patches, we used repeated-measures analyses. For the single species ln-ratio response, plant species were analyzed separately, whereas net pairwise feedbacks (or interaction strength I_s) included the responses of both species. The general repeated-measures model included community stability (dynamic vs. static) and census date as fixed effects, and experimental plot and spatial block as random effects to account for spatial gradients and temporal nonindependence (lmer

function in package lme4 [Bates et al. 2016]). Additionally, to determine whether each feedback response significantly differed from zero, we used means parameterization in a model with community stability as a fixed effect, and experimental plot and spatial block as random effects (lme function in package nlme [Pinheiro et al. 2018]).

To compare plant growth in heterospecific and conspecific soil environments directly, we examined ln-transformed estimated allometric biomass for each species separately throughout the experiment in repeated-measures analysis. We used a mixed model with the fixed effects of feedback environment (heterospecific or conspecific), community stability, their interaction, and census number, as well as plant ID and block as random effects (lmer function in package lme4 [Bates et al. 2016]). Within each community stability type, we tested pairwise differences between feedback environments using package lsmeans, with Tukey adjustment (Lenth 2016). Seedling emergence and transplant survival were similarly assessed using generalized linear mixed models. As these responses did not show strong feedbacks, we do not report the results in the main text (Appendix S1: Figs. S6–S8).

Next, we tested whether feedback effects scaled with plant frequency. For each plant species, ln-ratio feedback was modeled with conspecific frequency (rare vs. common) as a fixed effect, and plant identity and block as random effects. Contrasts against zero were conducted as above. Allometric transplant biomass was modeled with feedback environment, conspecific frequency (rare vs. common), and their interaction as fixed effects, and plant identity and block as random effects. Pairwise comparisons were conducted as above. Conspecific frequency category (rare/common) for each static patch was identified prior to the experiment based on abundance dynamics in the long-term data (see Magnitude and frequency of temporal dynamics). We also attempted post hoc analyses using continuous measures of conspecific cover (absolute and ratio of total; contemporary and historical mean) to predict soil effects. These analyses yielded qualitatively similar trends as the planned categorical analyses, but with heteroscedasticity issues that could not be remedied with transformations. Thus we do not report those results here.

RESULTS

Do the strength and direction of plant–soil feedbacks differ between temporally dynamic vs. static patches?

Negative feedbacks occurred in static patches, consistent with stable coexistence, but did not occur in dynamic patches (Fig. 2). Feedbacks were primarily driven by blue grama grass, which, in static patches, grew less in conspecific than in heterospecific soils. We measured plant–soil feedback between blue grama and black grama using two metrics (see Methods: Plant–soil
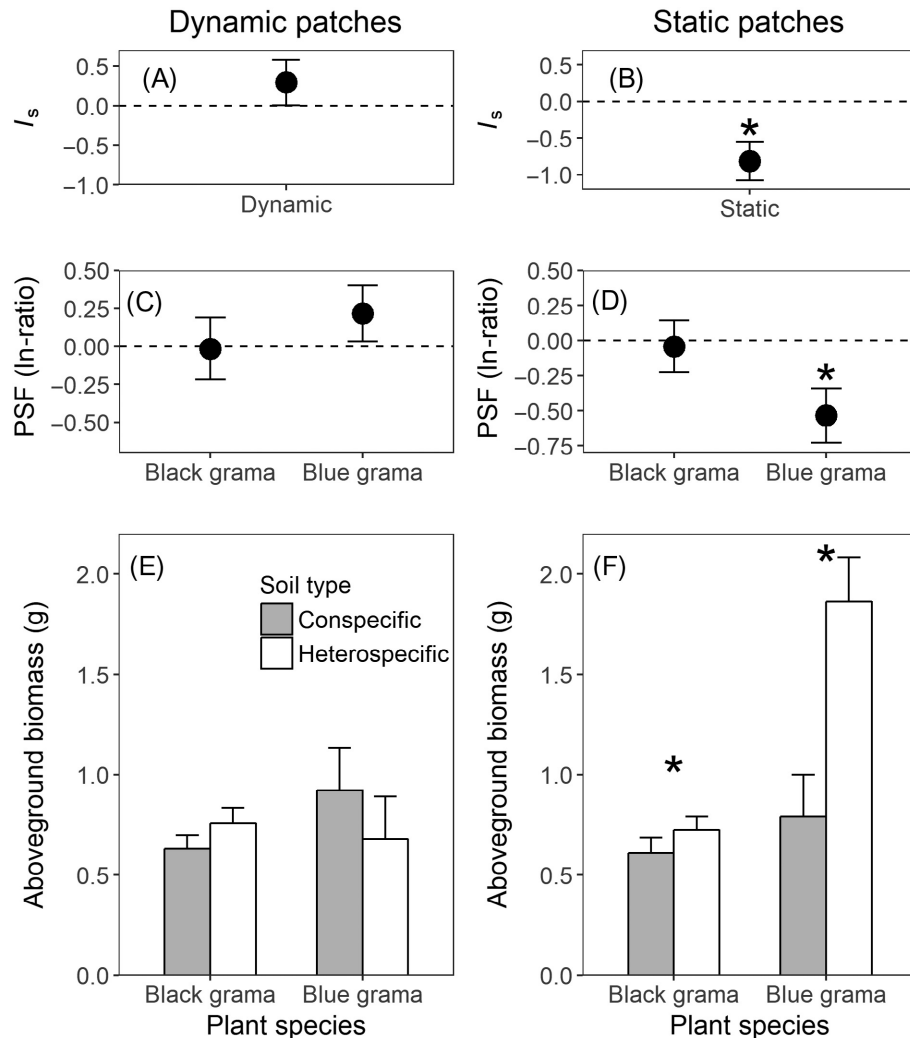
FIG. 2. Plant–soil feedback between blue and black grama in dynamic (left column) and static (right column) patches. The top row shows net pairwise feedback, or interaction strength (I_s), between blue and black grama (A), (B). The middle row depicts feedback for each plant species [ln-ratio; (C), (D)], and the bottom row shows the soil environment effects on biomass of individual plants (E), (F). Error bars are fitted SE, and asterisks indicate feedbacks that are significantly different from zero (A)–(D), or significantly different pairwise comparisons in repeated-measures analyses (E), (F).

feedback): (1) single species comparisons (using ln-ratio) of growth in conspecific vs. heterospecific soils, where negative feedback values indicate potential for coexistence, and (2) interaction strength (I_s; Bever et al. 1997), which sums the single species comparison for species in a community, with negative feedbacks indicating stable coexistence. We present the results in a top-down approach, starting with I_s, then decomposing each metric into its component parts.

Overall, net pairwise plant–soil feedbacks were neutral in dynamic patches (I_s; $t = 1.64$, df = 9, $P = 0.14$; Fig. 2A) and negative in static patches ($t = -2.70$, df = 9, $P = 0.02$; Fig. 2B). In dynamic patches, blue grama and black grama both had neutral plant–soil feedback ($P = 0.32$ and 0.57, respectively; Fig. 2C).

This occurred because in dynamic patches, each plant species performed similarly in conspecific and heterospecific soils over the duration of the field experiment ($P = 0.23$ and 0.76, respectively; Fig. 2E). In static patches, blue grama had negative plant–soil feedback ($t = -2.30$, df = 17, $P = 0.03$; Fig. 2D), and plants grew 135% larger in heterospecific soils than in conspecific soils (pairwise comparison $t = 2.67$, df = 26.4, $P = 0.01$; Fig. 2F). In static patches, black grama plants also grew ~19% larger in heterospecific soils than in conspecific soils (pairwise comparison $t = 2.16$, df = 21.1, $P = 0.04$; Fig. 2F); however, this growth difference was not large enough to generate significant negative feedback for black grama in static patches ($t = -1.56$, df = 9, $P = 0.15$, Fig. 2D).

In static patches, does plant–soil feedback strength reflect the frequency of the plant species in the community?

In static patches, single-species plant–soil feedbacks were 3–4 times stronger for both grama species when the species was rare than when it was common (Fig. 3A, B), demonstrating that feedbacks fulfill both the qualitative and quantitative requirements for stabilizing coexistence. In static patches where blue grama was rare, it gained 145% more biomass in heterospecific soils than in its own soils (pairwise comparison $t = 2.79$, df $= 8.4$, $P = 0.02$; Fig. 3C)—a strong negative feedback. When blue grama was common, the advantage of heterospecific soil was ~40% weaker than when it was rare, with no significant difference in growth between heterospecific and conspecific soils (pairwise comparison $t = 0.56$, df $= 7.4$, $P = 0.59$; Fig. 3D). In static patches when black grama was rare, its growth was larger in heterospecific soils than conspecific soils (63% increase; $t = 3.64$, df $= 8.9$, $P = 0.006$; Fig. 3C), showing moderately negative feedback compared to blue grama. When black grama was common, like blue grama, it did not respond significantly to soil treatments ($P = 0.91$; Fig. 3D).

DISCUSSION

Negative plant–soil feedbacks dominated patches of stable species coexistence

Theory predicts that stable coexistence occurs when a species limits itself more than it does its competitor (Volterra 1926, Lotka 1978). Results from our multiyear field experiment point to plant–soil feedbacks as key drivers of stable coexistence in a semiarid grassland. Stabilizing, negative feedbacks occurred in static patches, where blue and black grama grew worse in conspecific soils than in heterospecific soils—evidence of self-limitation. In contrast, feedbacks were neutral trending positive in dynamic patches, suggesting that abundance fluctuations in these patches could be partially driven by directional feedbacks in combination with other mechanisms.

What were the causes of feedback and their heterogeneity in this ecosystem? Plant–soil feedbacks can be driven by biotic factors, such as soil microbes, and/or by abiotic factors, such as soil physical or chemical properties that develop in association with a plant species
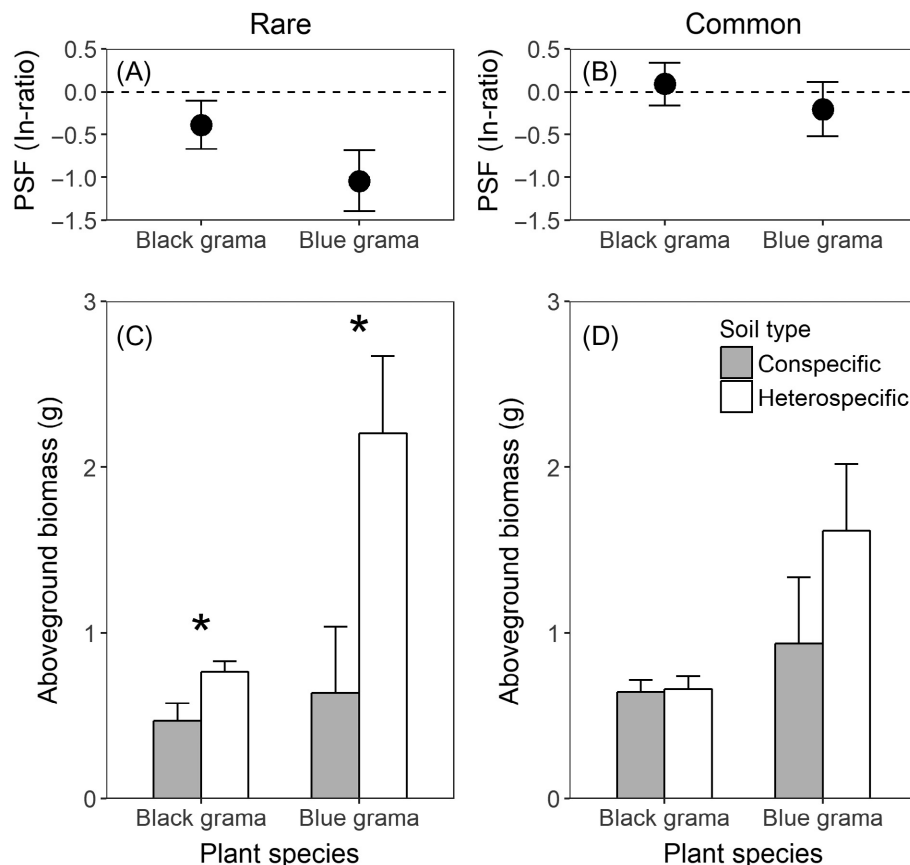

FIG. 3. Plant–soil feedback between blue and black grama in static patches when each species was rare (left column) or common (right column). The top row shows the feedback for each plant [ln-ratio; (A), (B)]. The bottom row depicts soil environment effects on aboveground biomass of individual plants (C), (D). Errors are fitted SE, and asterisks indicate feedbacks that were significantly different from zero (A), (B), or significant pairwise differences in repeated-measures analyses (E), (F).

(Ehrenfeld et al. 2005, Bezemer et al. 2006, Hendriks et al. 2015). Variation in community temporal dynamics at patch scales, as seen in this grassland (Collins and Xia 2015), has been documented in other long-term studies and is commonly attributed to spatial variation in abiotic conditions (Spotswood et al. 2015, Ratajczak et al. 2017). For example, in an oak savannah, large shifts in species composition of understory plants were associated with variation in soil clay content (Spotswood et al. 2015). In this study, we found no evidence that soils associated with blue grama or black grama differed between dynamic and static patches in their abiotic variables, including soil moisture, chemistry, temperature, or texture (Appendix S1: Supplemental results).

Resource competition is another source of self-limitation that could potentially create the feedbacks observed in this study. Our design minimized this factor by preventing direct root competition for belowground nutrients between seedlings and resident plants. In addition, light is unlikely a limiting resource in this open, dryland ecosystem where ~40% of surface area is unvegetated. Resource competition is also unlikely to explain differences in feedback between static and dynamic patches. We found no evidence that plant-available nutrients or moisture differed in the soils around blue and black grama (which would have indicated resource partitioning) or between patch types, which would have suggested that resource competition underlies differences in feedbacks. In addition, if resource competition were the key driver, we would expect seedlings to perform worst under conditions where they experience the strongest intraspecific competition. For example, we would expect blue grama seedlings to perform the worst in static patches where blue grama was common and paired with a conspecific adult plant, which was not the case (Fig. 3C, D).

Instead, several lines of evidence suggested that plant–soil feedbacks were driven by biotic factors, possibly root-associated fungi. First, the negative feedbacks in this field study paralleled results from a previous greenhouse experiment using the same plant species to examine the effects of plant–soil feedbacks on competition (Chung and Rudgers 2016). Inoculation with live, conspecific soil increased the negative intraspecific effects for blue grama beyond the background competition for abiotic resources, supporting a key role for living soil biota in causing feedbacks in this ecosystem. Second, we directly sequenced root fungi of resident and seedling plants of this study and found significantly different fungal communities for blue grama vs. black grama soils, as well as in dynamic vs. static patches (Chung et al. 2018). Specifically, the composition of Glomeromycota taxa significantly differed between dynamic and static patches. On the individual fungal taxon level, the abundance of two Glomeromycota taxa significantly correlated with the magnitude of positive feedback, and the abundance of one known plant pathogen, *Fusarium redolens*, significantly correlated with the strength of

negative feedback. Using molecular tools and inoculation experiments to open the black box of microbial interactions is at the forefront of plant–soil feedback research. Although we have so far focused on soil fungi, it is possible that other members of the soil biota, such as nematodes or bacteria, could drive the observed dynamics. Our work frames the context for future studies that could isolate specific belowground players in plant–soil feedbacks using direct inoculations. Future work may also incorporate reciprocal transplants of whole soil cores, combine resource additions with feedback treatments, or consider plant nutrient acquisition strategies (Teste et al. 2017) to help distinguish resource-mediated vs. microbially mediated soil effects on plant–plant interactions.

Although we have documented a novel relationship between plant–soil feedbacks and temporal patch dynamics, long-term experiments are needed to determine the direction of causality: Plant–soil feedbacks could cause temporal stability, or stability could cause the accumulation of feedbacks. In our study, a combination of abiotic covariates and stochasticity likely structure the soil environment, defining the range of possible plant–soil feedbacks and patterns of plant community stability. For example, work in other ecosystems uncovered variable root fungal communities in a single host plant at spatial scales <10 m (Ettema and Wardle 2002, Grünig et al. 2002, Pickles et al. 2010, Rasmussen et al. 2018); this variation could create spatially variable feedbacks that drive aboveground dynamics. Our complementary sequencing work found that plants in dynamic patches hosted more diverse root mycobiomes than plants in static patches (Chung et al. 2018). This could reflect different soil legacies in static vs. dynamic patches because of the stability and persistence of host plant presence (Hawkes et al. 2013). Alternatively, diverse microbial communities could drive plant community turnover. More than likely, the patterns observed reflect a combination of these mechanisms, supporting our hypothesis that interactions between plants and soil biota are key to understanding temporal variation in plant communities.

Feedback effects were frequency-dependent

One key component of evaluating stable coexistence is the mutual invasibility criterion, where competitors are each more advantaged (and thus have positive population growth) when rare (Chesson and Ellner 1989). For both grama species, increased growth in heterospecific soils was stronger when the focal species was rare and its heterospecific competitor was common. The observed pattern is expected if host-associated soil biota increase monotonically with greater host plant abundance, as assumed in plant–soil feedback theory (Bever et al. 1997, Bever 2003). Heterospecific soil should be most beneficial compared to conspecific soil when conspecific plant frequency is low; the benefits get progressively

diluted by conspecific soil biota as the frequency of the focal plant species increases. Future work to link individual plant growth to population growth rates would provide further evidence for the invasibility criterion.

Our results also support a growing literature demonstrating that strong intraspecific interactions, rather than interspecific interactions, serve as a major structuring force in plant communities (Wills et al. 1997, Comita et al. 2010, Chu and Adler 2015, Adler et al. 2018). For example, across forests worldwide, the magnitude of intraspecific density dependence correlated positively with tree species diversity, consistent with the observed latitudinal gradient in diversity (LaManna et al. 2017). Studies that investigated the strength and direction of plant–soil feedbacks have correlated more negative plant–soil feedbacks with greater plant rarity, measured at a single time point in the successional trajectory (Klironomos 2002, Mangan et al. 2010, but see Maron et al. 2016). This pattern suggests that strong host-specific pathogens could drive plants to rarity in a community, and negative feedbacks keep these rare species in stable coexistence at low abundances by favoring establishment and reducing extinction probability (Yenni et al. 2012, 2017). Our findings support this pattern at the patch-level within a landscape, demonstrating stronger feedbacks for the rarer species in static patches.

Temporal trajectories of grassland community dynamics

Although our study focused on contrasting patch-scale dynamics, it also sheds light on perplexing transect-wide spatial dynamics. For example, experimental work demonstrated that blue grama was a stronger competitor and was more resilient to disturbance than black grama (Gosz and Gosz 1996, Peters and Yao 2012, Chung and Rudgers 2016). Yet despite its weaker competitive abilities, black grama cover has increased more than blue grama over a 20-yr period at the landscape scale (Collins and Xia 2015). Differential growth forms could be a factor, as stoloniferous grasses, like black grama, can respond faster to resource pulses than caespitose growth forms, such as blue grama (Humphrey and Pyke 1998). However, our work suggests that stronger negative plant–soil feedbacks for blue grama may underlie its slower spread relative to black grama. The predominantly vegetative proliferation strategy of these two grasses means that new growth is likely to encounter conspecific soils close to the parent plant. Negative plant–soil feedbacks could act as a stabilizing coexistence mechanism to overcome the large fitness difference between the two competitors (Lekberg et al. 2018). Similarly, recent work revealed that rhizobia-generated plant–soil feedbacks can predict the puzzling long-term coexistence of remarkably similar clover species in the field (Siefert et al. 2019). Plant–soil feedbacks provide an additional dimension along which plant species can partition niche space, and its explicit consideration can illuminate previously unexplained patterns in nature.

Our work showed that the long-term temporal dynamics of plant communities are inextricably linked to plant interactions with belowground communities. Thus, as we move toward forecasting the effects of global change on plant communities, the role of belowground soil biota cannot be overlooked. For example, future climate predictions in this ecosystem indicate increased aridity along with amplified variability in interannual precipitation (Gutzler and Robbins 2011, Cook et al. 2015), conditions that long-term observations predict will affect blue grama more negatively than black grama (Rudgers et al. 2018). Whether plant–soil feedbacks amplify or dampen the effects of increased environmental stochasticity and aridity on the dynamics of plant populations will be key to predicting the fate of these communities in the future (van der Putten et al. 2016).

Conclusion

Solving current environmental issues requires a better understanding of the drivers of long-term ecological change. Our results point to a key role for plant–soil feedbacks in controlling the temporal dynamics of plant communities: more negative plant–soil feedbacks corresponded to plant communities with more stable coexistence. Additionally, the magnitude of feedback depended on plant species frequency in precisely the direction predicted by theory to promote long-term species coexistence. Our work revealed that plant–soil feedbacks cannot be overlooked as drivers of temporal dynamics in vegetation at the patch to landscape scales.

Literature Cited

Adler, P. B., D. Smull, K. H. Beard, R. T. Choi, T. Furniss, A. Kulmatiski, J. M. Meiners, A. T. Tredennick, and K. E. Veblen. 2018. Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. Ecology Letters 21:1319–1329.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Beisner, B., D. Haydon, and K. Cuddington. 2003. Alternative stable states in ecology. Frontiers in Ecology and the Environment 1:376–382.

Bever, J. D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist 157:465–473.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology 85:561–573.

Bezemer, T. M., C. S. Lawson, K. Hedlund, A. R. Edwards, A. J. Brook, J. M. Igual, S. R. Mortimer, and W. H. V. D. Putten. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grasslands. Journal of Ecology 94:893–904.

Bonanomi, G., F. Giannino, and S. Mazzoleni. 2005. Negative plant–soil feedback and species coexistence. Oikos 111:311–321.

Bouyoucos, G. J. 1962. Hydrometer method improved for making particle size analyses of soils. Agronomy Journal 54:464–465.

Brinkman, E. P., W. H. van der Putten, E.-J. Bakker, and K. J. F. Verhoeven. 2010. Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. Journal of Ecology 98:1063–1073.

Burns, J. H., and A. J. Brandt. 2014. Heterogeneity in plant–soil feedbacks and resident population dynamics affect mutual invasibility. Journal of Ecology 102:1048–1057.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343.

Chesson, P. L., and S. Ellner. 1989. Invasibility and stochastic boundedness in monotonic competition models. Journal of Mathematical Biology 27:117–138.

Chu, C., and P. B. Adler. 2015. Large niche differences emerge at the recruitment stage to stabilize grassland coexistence. Ecological Monographs 85:373–392.

Chung, Y. A., and J. A. Rudgers. 2016. Plant–soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. Proceedings of the Royal Society B 283:20160608.

Chung, Y. A., A. Jumpponen, and J. A. Rudgers. 2018. Divergence in diversity and composition of root-associated fungi between greenhouse and field studies in a semiarid grassland. Microbial Ecology https://doi.org/10.1007/s00248-018-1277-y

Collins, S. L. 2016. Grassland vegetation line-intercept transects at the Sevilleta National Wildlife Refuge, New Mexico (1989–present). Environmental Data Initiative.

Collins, S. L., and Y. Xia. 2015. Long-term dynamics and hotspots of change in a desert grassland plant community. American Naturalist 185:E30–E43.

Comita, L. S., H. C. Muller-Landau, S. Aguilar, and S. P. Hubbell. 2010. Asymmetric density dependence shapes species abundances in a tropical tree community. Science 329:330–332.

Cook, B. I., T. R. Ault, and J. E. Smerdon. 2015. Unprecedented 21st century drought risk in the American Southwest and Central Plains. Science Advances 1:e1400082.

Drohan, P. J., D. J. Merkler, and B. J. Buck. 2005. Suitability of the plant root simulator probe for use in the Mojave desert. Soil Science Society of America Journal 69:1482–1491.

Ehrenfeld, J. G., B. Ravit, and K. Elgersma. 2005. Feedback in the plant–soil system. Annual Review of Environment and Resources 30:75–115.

Ettema, C. H., and D. A. Wardle. 2002. Spatial soil ecology. Trends in Ecology and Evolution 17:177–183.

Gosz, R. J., and J. R. Gosz. 1996. Species interactions on the biome transition zone in New Mexico: response of blue grama (*Bouteloua gracilis*) and black grama (*Bouteloua eripoda*) to fire and herbivory. Journal of Arid Environments 34:101–114.

Grünig, C. R., T. N. Sieber, S. O. Rogers, and O. Holdenrieder. 2002. Spatial distribution of dark septate endophytes in a confined forest plot. Mycological Research 106:832–840.

Gutzler, D. S., and T. O. Robbins. 2011. Climate variability and projected change in the western United States: regional downscaling and drought statistics. Climate Dynamics 37:835–849.

Hallett, L. M., et al. 2014. Biotic mechanisms of community stability shift along a precipitation gradient. Ecology 95:1693–1700.

Hawkes, C. V., S. N. Kivlin, J. Du, and V. T. Eviner. 2013. The temporal development and additivity of plant–soil feedback in perennial grasses. Plant and Soil 369:141–150.

Hawkes, C. V., B. G. Waring, J. D. Rocca, and S. N. Kivlin. 2017. Historical climate controls soil respiration responses to current soil moisture. Proceedings of the National Academy of Sciences 114:6322–6327.

Hendriks, M., E. J. W. Visser, I. G. S. Visschers, B. H. J. Aarts, H. de Caluwe, A. E. Smit-Tiekstra, W. H. van der Putten, H. de Kroon, and L. Mommer. 2015. Root responses of grassland species to spatial heterogeneity of plant–soil feedback. Functional Ecology 29:177–186.

Humphrey, L. D., and D. A. Pyke. 1998. Demographic and growth responses of a guerrilla and a phalanx perennial grass in competitive mixtures. Journal of Ecology 86:854–865.

Kardol, P., G. B. De Deyn, E. Laliberté, P. Mariotte, and C. V. Hawkes. 2013. Biotic plant–soil feedbacks across temporal scales. Journal of Ecology 101:309–315.

Kilpatrick, A. M., and A. R. Ives. 2003. Species interactions can explain Taylor's power law for ecological time series. Nature 422:65–68.

Kivlin, S. N., S. M. Emery, and J. A. Rudgers. 2013. Fungal symbionts alter plant responses to global change. American Journal of Botany 100:1445–1457.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70.

Knapp, A. K., and M. D. Smith. 2001. Variation among biomes in temporal dynamics of aboveground primary production. Science 291:481–484.

LaManna, J. A. et al. 2017. Plant diversity increases with the strength of negative density dependence at the global scale. Science 356:1389–1392.

Lekberg, Y. et al. 2018. Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. Ecology Letters 21:1268–1281.

Lenth, R. V. 2016. Least-squares means: the R package lsmeans. Journal of Statistical Software 69:1–33.

Letten, A. D., M. K. Dhami, P.-J. Ke, and T. Fukami. 2018. Species coexistence through simultaneous fluctuation-dependent mechanisms. Proceedings of the National Academy of Sciences 115:6745.

Lotka, A. J. 1978. The growth of mixed populations: Two species competing for a common food supply. Pages 1923–1940. The Golden Age of Theoretical Ecology: Lecture Notes in Biomathematics, Vol 22. Springer, Berlin, Heidelberg.

Mangan, S. A., S. A. Schnitzer, E. A. Herre, K. M. L. Mack, M. C. Valencia, E. I. Sanchez, and J. D. Bever. 2010. Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752–755.

Maron, J. L., A. Laney Smith, Y. K. Ortega, D. E. Pearson, and R. M. Callaway. 2016. Negative plant–soil feedbacks increase with plant abundance, and are unchanged by competition. Ecology 97:2055–2063.

Pellkofer, S., M. G. A. van der Heijden, B. Schmid, and C. Wagg. 2016. Soil communities promote temporal stability

and species asynchrony in experimental grassland communities. PLoS ONE 11:e0148015.

Pendergast, T. H., D. J. Burke, and W. P. Carson. 2013. Belowground biotic complexity drives aboveground dynamics: a test of the soil community feedback model. New Phytologist 197:1300–1310.

Peters, D. P. C. 2002. Recruitment potential of two perennial grasses with different growth forms at a semiarid–arid transition zone. American Journal of Botany 89:1616–1623.

Peters, D. P. C., and J. Yao. 2012. Long-term experimental loss of foundation species: consequences for dynamics at ecotones across heterogeneous landscapes. Ecosphere 3:1–23.

Pickles, B. J., D. R. Genney, J. M. Potts, J. J. Lennon, I. C. Anderson, and I. J. Alexander. 2010. Spatial and temporal ecology of Scots pine ectomycorrhizas. New Phytologist 186:755–768.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-140. https://CRAN.R-project.org/package=nlme.

Pockman, W. T., and E. E. Small. 2010. The influence of spatial patterns of soil moisture on the grass and shrub responses to a summer rainstorm in a Chihuahuan Desert ecotone. Ecosystems 13:511–525.

Rasmussen, P. U., L. W. Hugerth, F. G. Blanchet, A. F. Andersson, B. D. Lindahl, and A. J. M. Tack. 2018. Multiscale patterns and drivers of arbuscular mycorrhizal fungal communities in the roots and root-associated soil of a wild perennial herb. New Phytologist 220:1248–1261.

Ratajczak, Z., P. D'Odorico, J. B. Nippert, S. L. Collins, N. A. Brunsell, and S. Ravi. 2017. Changes in spatial variance during a grassland to shrubland state transition. Journal of Ecology 105:750–760.

Ravi, S., P. D'Odorico, L. Wang, and S. Collins. 2008. Form and function of grass ring patterns in arid grasslands: the role of abiotic controls. Oecologia 158:545–555.

Reed, H. E., and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. FEMS Microbiology Ecology 62:161–170.

Revilla, T. A., G. F. Veen, M. B. Eppinga, and F. J. Weissing. 2013. Plant–soil feedbacks and the coexistence of competing plants. Theoretical Ecology 6:99–113.

Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. Ecology 84:2281–2291.

Rudgers, J. A., Y. A. Chung, G. Maurer, D. Moore, E. Muldavin, M. Litvak, and S. L. Collins. 2018. Climate sensitivity functions and net primary production: a framework for incorporating climate mean and variability. Ecology 99:576–582.

Siefert, A., K. W. Zillig, M. L. Friesen, and S. Y. Strauss. 2019. Mutualists stabilize the coexistence of congeneric legumes. American Naturalist 193:200–212.

Smith, M. D., A. K. Knapp, and S. L. Collins. 2009. A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change. Ecology 90:3279–3289.

Spotswood, E. N., J. W. Bartolome, and B. Allen-Diaz. 2015. Hotspots of community change: temporal dynamics are spatially variable in understory plant composition of a California oak woodland. PLoS ONE 10:e0133501.

Teste, F. P., P. Kardol, B. L. Turner, D. A. Wardle, G. Zemunik, M. Renton, and E. Laliberté. 2017. Plant–soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. Science 355:173–176.

Thomey, M. L., S. L. Collins, M. T. Friggens, R. F. Brown, and W. T. Pockman. 2014. Effects of monsoon precipitation variability on the physiological response of two dominant C4 grasses across a semiarid ecotone. Oecologia 176:751–762.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. Ecology 75:2–16.

Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. Nature 367:363–365.

van der Putten, W. H., M. A. Bradford, E. Pernilla Brinkman, T. F. J. van de Voorde, and G. F. Veen. 2016. Where, when and how plant–soil feedback matters in a changing world. Functional Ecology 30:1109–1121.

Volterra, V. 1926. Variations and fluctuations of the number of individuals in an animal species living together. Pages 409–448 in R. N. Chapman, editor. Animal ecology. McGraw Hill, New York.

Wills, C., R. Condit, R. B. Foster, and S. P. Hubbell. 1997. Strong density- and diversity-related effects help to maintain tree species diversity in a neotropical forest. Proceedings of the National Academy of Sciences 94:1252–1257.

Yenni, G., P. B. Adler, and S. K. M. Ernest. 2012. Strong self-limitation promotes the persistence of rare species. Ecology 93:456–461.

Yenni, G., P. B. Adler, and S. K. M. Ernest. 2017. Do persistent rare species experience stronger negative frequency dependence than common species? Global Ecology and Biogeography 26:513–523.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2756/suppinfo

DATA AVAILABILITY

All data and code necessary for analyses in this manuscript can be found on Zenodo at https://doi.org/10.5281/zenodo.2644344. Data are also archived in the EDI data repository at https://doi.org/10.6073/pasta/6f99d77fced924c6329c6b0f9fc3a728.