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Plant legacies and soil microbial community dynamics control soil respiration

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ABSTRACT

Through litter and root inputs, plants modulate soil properties and the soil microbial communities around them. In turn, these changes in soil properties and microbial community composition can impact plant performance (i.e., plant-soil feedbacks). Many studies have focused on how plant-soil feedbacks affect plant performance and successional patterns, but studies on the impact of plant-soil feedbacks on ecosystem processes are rare. Our research focused on the potential of species-specific plant-soil feedbacks to affect rates of soil organic matter (SOM) decomposition. We conducted a "home vs. away" plant-soil feedback greenhouse experiment using two C_3 grass species (*Bromus inermis* and *Pascopyrum smithii*) grown in C_4 tallgrass prairie soil. We used a closed-circuit CO_2 trapping method and isotopic analysis to differentiate between root-derived and SOM-derived CO_2 production. Contrary to our predictions, plant-soil feedbacks on plant biomass were independent of the effects of plant-soil interactions on SOM-derived CO_2 production, but we did detect a significant legacy of conditioning by *B. inermis* on subsequent total belowground respiration (i.e., total belowground respiration was higher in soils originally conditioned by *B. inermis* regardless of which plant species was currently growing in those soils). We attribute these results to the differential effects of these plant species on soil chemistry and soil microbes during the original conditioning phase. This is supported by the observation that differences in soil chemistry and bacterial community composition persisted in soils conditioned by different plant species throughout the entire experiment. Together these results suggest that plant-soil history is important for soil respiration and that differences in soil microbial communities induced by conditioning with different plant species may have lasting effects on ecosystem processes.

1. Introduction

Plants are a critical link between aboveground and belowground processes. Through surface litter and root inputs, plants modulate the physical, chemical, and biological soil properties around them. In turn, these changes in soil properties impact plant performance (i.e., plant-soil feedbacks; Ehrenfeld et al. 2005; Van der Putten et al. 2013). Plant-soil feedbacks (PSFs) are considered to be positive if plant-mediated changes to the soil environment increases the growth rate or survival of that plant species (Bever, 1994). Whether PSFs are negative or positive may explain why some plants are rare while others are dominant (Klironomos, 2002). Multiple negative PSFs within a community have been posited to promote species coexistence, while multiple positive PSFs may lead to a single species dominating a community (Bever, 2003; Bonanomi et al., 2005). Although PSFs can vary temporally and may switch from positive to negative (Bever et al., 1997; Diez et al., 2010), plants that are associated with beneficial soil microbes or are not susceptible to local pathogens are more likely to become dominant (Ehrenfeld et al., 2005; Jeschke, 2014). Through this association with beneficial or harmful microbes, plants can create legacy effects on soil properties and soil microbial communities that persist even after the plants are no longer present, which can influence future plant success (Bartelt-Ryser et al., 2005; Hamman and Hawkes, 2013).

On average, plants allocate 17% of their total photosynthate belowground as rhizodeposits (Nguyen, 2003), but since rhizodeposition is positively related to plant biomass (Van der Krift et al., 2001) changes in plant performance due to PSFs may change the amount of carbon (C) that plants can allocate belowground. Microorganisms use rhizodeposits and root exudates as an energy source to support cell maintenance, growth, and the extracellular enzyme production that

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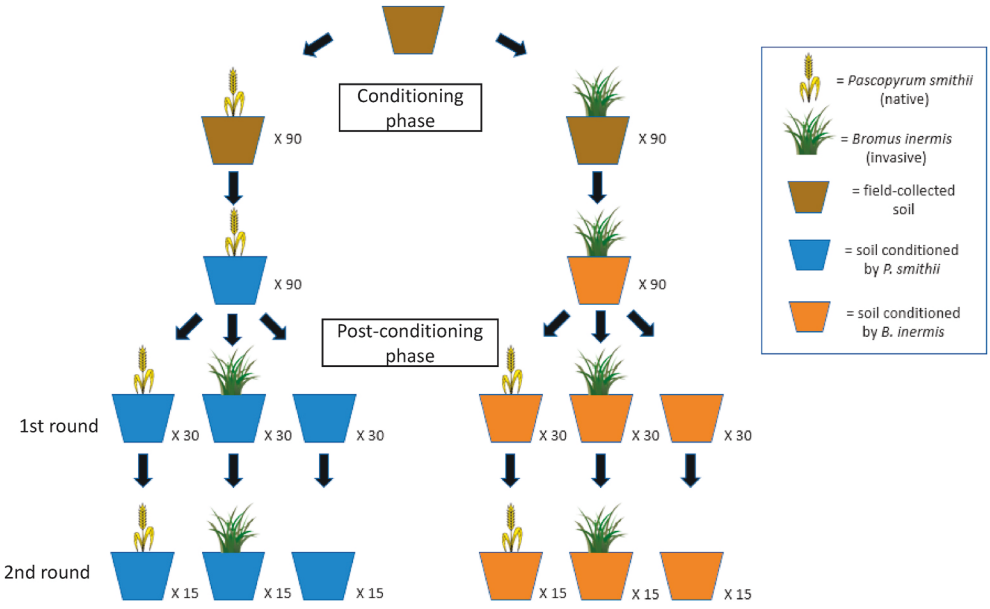

Fig. 1. The overall experimental design. We let two C_3 grasses (*Bromus inermis* and *Pascopyrum smithii*) condition the same field-collected soil from a tallgrass prairie site for ten weeks. After ten weeks, we let a second individual of the same plant species condition the soil for an additional ten weeks such that the total conditioning phase lasted twenty weeks. After the conditioning phase, we grew a new individual of each plant species in its home soil (i.e., conditioned by a conspecific) and in its away soil (i.e., conditioned by a heterospecific) as well as leaving a no-plant treatment. The first post-conditioning round of the greenhouse experiment lasted 12 weeks. After the first post-conditioning round concluded, we planted a new individual of each species in the same pattern as we did in the first post-conditioning round. The second post-conditioning round of the greenhouse experiment lasted another 12 weeks. During the post-conditioning phase of the experiment, 30 pots were destructively sampled every four weeks to measure soil respiration, plant biomass, soil chemistry, and soil microbial properties.

decomposes soil organic matter (SOM) (Dakora and Phillips, 2002; de Vries et al., 2019; Kaštovská et al., 2015). In return, microbes can assist plants by enhancing nutrient availability, assisting with nutrient acquisition, and by protecting against herbivores and pathogens (Philippot et al., 2013). Rhizodeposits vary in chemical composition and quality among plant species (Badri and Vivanco, 2009). Since plant species vary in root traits and belowground C inputs, plants have the potential to "cultivate" microbes that can best use the specific substrates released from their roots (Berg and Smalla, 2009; Grayston et al., 1998).

Many studies have focused on how PSFs affect plant performance, interspecific competition, and plant succession (Bever, 1994, 2003; Kardol et al., 2013), yet studies on the impact of plant-soil feedbacks on ecosystem processes are rare. Since rhizosphere processes are an important local control on C and nutrient cycles (Cardon and Whitbeck, 2007; Finzi et al., 2015; Singh et al., 2004), plant-induced changes in soil microbial communities may play previously unappreciated roles in ecosystem processes (Weathers et al., 2016). Soil microbes are responsible for many transformations of nitrogen (N) and C and mediate soil nutrient cycling (Docherty and Gutknecht, 2012). Therefore, plant-mediated changes in soil microbial communities likely alter critical soil N and C transformations. In addition, recent technical and methodological developments now allow a more detailed assessment of the identities and function of microbes previously considered hidden within the "black box" of soil biodiversity (Tiedje et al., 1999; Zimmerman et al., 2014). Since SOM is the largest pool of terrestrial C (Stockmann et al., 2013) and interactions between plant roots and soil microbes modulate its decomposition rate (Huo et al., 2017; Kuzyakov, 2002), we aimed to assess how changes in plant performance and soil microbial communities due to PSFs affect SOM decomposition.

Ecosystem processes are affected by both contemporary plant-soil interactions, and also by the legacies of previous plant communities on soil biotic and abiotic properties (Elgersma et al., 2011). The legacy effects of invasive plants are of particular concern. Shifts in the microbial community associated with invasive plants can persist in invader-conditioned soil and negatively impact subsequent native plant establishment, even if the invasive plant is no longer present (Jordan et al., 2008). For example, *Bromus inermis,* a Eurasian C_3 grass introduced to North America in the 1880s for forage and erosion control, has spread throughout the continent and is the second-most common exotic plant species in remnant tallgrass prairies behind *Poa pratensis* (Cully et al., 2003). It has been posited that *B. inermis* may impede efforts to restore native grasslands through allelopathy (Chung and Miller, 1995) and changes in soil microbial communities (Perkins and Hatfield, 2016; Piper et al., 2015). What is less understood is the impact of such legacy effects on C fluxes in soil.

We conducted a greenhouse experiment to investigate PSFs of two species of C_3 grass, one native to tallgrass prairie (*Pascopyrum smithii*) and the other an exotic species generally considered to be invasive (*B. inermis*), by growing each species either in "home" soil (i.e., in soil conditioned by a conspecific) or "away" soil (i.e., in soil conditioned by a heterospecific). In addition to characterizing potential legacy effects on plant performance, soil respiration, and soil bacterial communities, we evaluated two hypotheses: 1) The magnitude of SOM decomposition will be related to PSF effects on plant biomass (i.e., positive PSFs will increase SOM decomposition). 2) Species-specific plant-mediated changes to soil chemistry and the soil bacterial community may persist after plant conditioning and influence the subsequent magnitude of SOM decomposition.

2. Methods

2.1. Soil collection

All soil used in the greenhouse study was collected in 2015 from the upper 15 cm of an area of native tallgrass prairie dominated by C_4 grasses at the Konza Prairie Biological Station, Manhattan, Kansas, USA. The soil was a silty clay loam (fine, mixed mesic Pachic Argiustoll) classified by the USDA Soil Survey as part of the Dwight-Irwin complex. After collection, all soil was passed through a 6 mm mesh sieve, coarsely hand-picked to remove roots, and stored air-dried in barrels until the greenhouse experiment began the following year.

2.2. Soil conditioning phase

In June 2016, the stored soil was distributed into 180 pots (10 cm diameter x 25 cm deep) constructed from PVC pipe with airtight bottom caps. To allow better drainage and water accumulation below the soil, a 0.5 kg nylon sandbag (~5 cm depth) was placed at the bottom of each pot. To inoculate the stored soil with a fresh microbial community, a small amount of freshly collected soil from the field site was mixed into

each pot (3% fresh soil in each pot). Commercially available seeds of the native C_3 grass *Pascopyrum smithii* and the invasive C_3 grass *Bromus inermis* (Stock Seed Farms, Murdock, NE, USA) were germinated in potting soil in the greenhouse. One week after germination, individual seedlings were transplanted into each PVC pot so that half of the soils would be conditioned by *P. smithii* and half of the soils would be conditioned by *B. inermis* (Fig. 1). After ten weeks of growth, the plants were harvested, and coarse root biomass was removed. Fine root biomass was picked from the soil and removed as much as possible. Ten weeks of soil conditioning has been shown to be sufficient for plants to significantly change their soil environment (Klironomos, 2002). However, to reduce the amount of variability in soil conditioning due to intraspecific variation of individual plants, we chose to condition the soil for a second ten-week period, as specified below.

Soils conditioned by the same plant species were pooled, homogenized, and redistributed into new pots. Newly germinated seedlings of *P. smithii* and *B. inermis* were transplanted into pots with soil that had been conditioned by the same species for the second round of soil conditioning. After ten weeks, the aboveground and belowground biomass of the plants was harvested. Fine root biomass was removed from the soil as much as possible. Again, soils that were conditioned by the same plant species were pooled and homogenized before setting up the greenhouse experiment.

2.3. Post-conditioning phase

For the post-conditioning portion of this greenhouse study, the 180 pots were split into two 'Plant Legacy' treatments based on the conditioning phase described above: 1) soil conditioned by *B. inermis* or 2) soil conditioned by *P. smithii*. Each pot was also assigned one of the three 'Current Plant' treatments: 1) one *B. inermis* seedling or 2) one *P. smithii* seedling, as well as 3) a no plant control.

The greenhouse experiment was conducted over two post-conditioning rounds or growing periods (Fig. 1) to assess the potential for legacy effects of the original conditioning phase on SOM decomposition or microbial properties to persist in pots with the opposite plant species growing in them. Thirty pots, 5 of each Plant Legacy x Current Plant treatment, were destructively sampled every four weeks over a 3-month growing period, so that after 12 weeks, 90 of the pots were sampled (Post-conditioning Round 1). For the remaining 90 pots, aboveground and belowground biomass was harvested at the end of the first post-conditioning round, fine roots were removed from the soil as much as possible, and soils belonging to the same treatment were pooled, homogenized, and redistributed in preparation for a second post-conditioning round of growth and sampling. A new seedling of either *P. smithii* or *B. inermis* was transplanted into each pot in the same distribution as the first post-conditioning round of the experiment and allowed to grow for a second 12-week period. This second post-conditioning round allowed us to detect whether legacy effects from the original conditioning phase persisted in soils that had the opposite plant growing in them during the first post-conditioning round. We destructively sampled every four weeks throughout the post-conditioning phase of the experiment in order to track bacterial community dynamics, soil properties, and belowground respiration as the plants developed.

Throughout the conditioning and post-conditioning phases of the study, soils were kept at 60% water-filled pore space. Pots were watered with DI-H_2O via a 50 cc syringe attached to a 15 cm perforated tube inserted into the soil at the time the pots were filled. This allowed water to be distributed evenly through the soil. Additionally, to prevent anoxia, the soils were aerated for 1 h every day via a vacuum pump connected to each pot to draw air through the soil. Pots also were rotated within the greenhouse every 2 weeks to avoid potential artefacts of location.

2.4. CO_2 trapping and destructive sampling

A closed-circuit CO_2 trapping method (Cheng et al., 2003) was used to collect belowground CO_2 efflux after 4, 8, and 12 weeks of growth during each post-conditioning round of the experiment. Thirty pots were randomly selected for measurement on each trapping date (2 Plant Legacy x 3 Current Plant x 5 replicates). Briefly, liquid silicone rubber (Silicones-Inc., High Point, NC, USA) was spread over the surface soil of each pot to form an airtight seal separating aboveground and belowground portions of the pots and plants, which allowed sampling of CO_2 released from soil and intact plant roots. After allowing the silicone rubber to cure for 16–18 h, each pot was connected to a soda lime column, and the soil atmosphere was scrubbed for 40 min with the closed-circuit system to ensure that we were only trapping CO_2 produced during the measurement period. For 24 h, all the CO_2 produced belowground in each pot was trapped by bubbling air via air stones in the trapping circuit through bottles containing 300 ml of 0.25 M NaOH. After trapping was completed, the silicone rubber was removed, and the pots and plants were destructively sampled. Two subsamples of soil were collected from each pot. One subsample was stored at 4 °C for subsequent nutrient and microbial biomass analysis, and the other (collected from the rhizosphere) was stored at −20 °C for subsequent bacterial community analysis. In pots that contained plants, aboveground and belowground biomass was collected. After rinsing the belowground biomass with DI-H_2O, all plant biomass was dried at 60 °C for 48 h and weighed. SOM-derived CO_2 production rates were standardized by soil C content while root-derived CO_2 production rates were standardized at the pot level in order to avoid the inflation of root-specific CO_2 production rates when root biomass was very low.

2.5. Microbial biomass analysis

Soil microbial biomass C (MBC) was determined using a fumigation-extraction method (Jenkinson and Powlson, 1976) and calculated as the difference between fumigated and unfumigated samples. For each unfumigated sample, ~15 g of moist soil was extracted with 75 ml of 0.5 M K_2SO_4 on a shaker table at 200 rpm for 1 h. Extracts were passed through a 0.4 μm polycarbonate filter and stored at −20 °C. Another set of soil samples was placed into a vacuum desiccator and fumigated with chloroform under a vacuum for 48 h. Following fumigation, the beaker of chloroform was removed, and residual chloroform was removed from soil samples by repeatedly applying a vacuum and opening the chambers. Total organic C in the extracts was measured with a Shimadzu TOC-L dissolved carbon analyzer (Shimadzu, Kyoto, Japan).

2.6. Soil chemistry

We measured total %C and total %N of soil using a coupled combustion-gas chromatography Flash EA 1112 C/N autoanalyzer. Also, approximately 12 g of moist soil was extracted with 50 ml of 2 N KCl on a shaker table at 200 rpm for 1 h. Extracts were passed through a 0.4 μm polycarbonate filter and frozen for later analysis. Extractable inorganic nitrogen (NH_4^+ and NO_3^-) was determined colorimetrically at the Soil Testing Lab at Kansas State University (Manhattan, KS, USA).

2.7. Determining SOM-derived CO_2

Since the SOM from the collected tallgrass prairie soil originated from C_4 plants and we grew C_3 grasses in our experiment, we were able to use differences in the natural abundance of the ^{13}C isotope in organic matter derived from C_3 and C_4 plants to determine how much trapped CO_2 was SOM-derived versus plant root-derived. Total CO_2 respired from soil and plant roots was determined from the inorganic C content of the NaOH traps, measured on a Shimadzu TOC-L dissolved carbon analyzer (Shimadzu, Kyoto, Japan). To determine $\delta^{13}C$ of the respired C, trapped CO_2 was precipitated as $SrCO_3$ by adding excess 1 M $SrCl_2$ to a

subsample of the NaOH traps. The precipitate was rinsed with DI-H_2O once every 24 h for 7 days to neutralize pH, and then dried at 105 °C for 24 h. The $\delta^{13}C$ of the $SrCO_3$ was measured using a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Chesire, UK) at the UC Davis Stable Isotope Facility (Davis, CA, USA). The proportion of CO_2 derived from SOM was calculated according to the following isotope mixing model equation:

$$\%SOM_{CO2} = [(\delta_t - \delta_p) / (\delta_s - \delta_p)] * 100$$

Where δ_t represents the $\delta^{13}C$ value of the trapped CO_2. The δ_p represents the $\delta^{13}C$ value of the plants. In this study, we used a value of −27.5‰ for δ_p for both plants based on the average $\delta^{13}C$ value of C_3 plant biomass (Peterson and Fry, 1987). The δ_s represents the $\delta^{13}C$ value of the soil. We used a value of −16.24‰ for δ_s based on analysis of the collected bulk soil.

2.8. Calculating feedbacks

Feedback effects for plant biomass, cumulative soil respiration, cumulative SOM-derived CO_2, and cumulative root-derived CO_2 were calculated for both *B. inermis* and *P. smithii* using the following formula:

$$\ln(x_{home}/x_{away})$$

Where x_{home} represents the value of the response variable when a plant is grown in its "home" soil (i.e., conditioned by a conspecific) while x_{away} represents the value of the response variable when a plant is grown in the "away" soil (i.e., not conditioned by a conspecific). The *boot* package was used to calculate the bias-corrected and accelerated (BCa) bootstrap 95% confidence interval (CI) for each feedback metric for each plant species using 999 permutations (Canty and Ripley, 2019; Davison and Hinkley, 1997). The feedback effect was considered significant if the BCa bootstrap 95% CI did not cross zero.

2.9. 16S rRNA gene sequencing

Genomic DNA (gDNA) was extracted from soils using a MoBio PowerSoil Extraction kit (QIAGEN, Carlsbad, CA, USA) according to the manufacturer's instructions. Successful gDNA extraction was confirmed using a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The bacterial and archaeal 16S rRNA gene was targeted using universal bacterial primers (515F/806R) and amplified using PCR according to Earth Microbiome Project protocols (Caporaso et al., 2012) with a few exceptions. First, we added 2 μl of 1% Bovine Serum Albumin, 0.25 μl of $MgCl_2$ and double the amount of gDNA to each reaction well. Additionally, PCR was only run for 25 cycles instead of 35. Each sample was amplified in triplicate, and amplification was confirmed using gel electrophoresis. Each sample was normalized by DNA concentration and combined into a single amplicon library. The combined library was cleaned using a QIAquick Gel Extraction Kit (QIAGEN, Carlsbad, CA, USA) according to included instructions. The library was sequenced using a 2 × 150 paired-end Illumina MiSeq run, with v2 reagents and a 10% PhiX spike, in the Integrated Genomics Facility at Kansas State University (Manhattan, KS, USA).

2.10. Processing sequencing data

Raw sequence data were initially processed with the QIIME1 software (Caporaso et al., 2010b). Sequences were quality filtered, joined and demultiplexed, and assigned to operational taxonomic units (OTUs) at 97% sequence similarity. OTUs were aligned to the GreenGenes v. 12_10 16S rRNA gene reference database, and taxonomy was assigned using the RDP classifier (Caporaso et al., 2010a; DeSantis et al., 2006; McDonald et al., 2012; Wang et al., 2007). Chimeras were identified with CHIMERASLAYER and removed from further analysis (Haas et al., 2011). From this point forward, the data were exported for further processing using the *phyloseq* package within the R statistical software (McMurdie and Holmes, 2013). After ensuring that the DNA extraction and PCR blanks contained a low OTU richness, the *decontam* package (Davis et al., 2017) identified and removed 168 likely contaminant sequences using a X^2 analysis (Supplementary File S1). Any sample that retained fewer than 15,000 reads at this point was removed from further analysis. Next, we filtered the taxa so that the data only included OTUs from the kingdom Bacteria. Additionally, we excluded any OTUs associated with mitochondria or chloroplasts. Finally, we removed OTUs that occurred fewer than 3 times across all samples (e.g., Zeglin et al., 2016). Alpha diversity, evenness, and phylum-level response to soil conditioning were estimated using these data. OTU richness and evenness were estimated by rarefying each sample to have the same number of reads as the sample with the least number of reads (14,611 reads). This process was repeated for 100 permutations to calculate a mean richness and evenness for each sample. For our multivariate analyses, we normalized OTU relative abundances by the median number of reads across samples (McKnight et al., 2019). The final dataset included 11,152,440 reads in 140 total samples that were affiliated with 38,831 unique OTUs. Raw sequence data are available from the NCBI GenBank SRA at accession number PRJNA674621.

2.11. Statistical analyses

Two-way analysis of variance (ANOVA) was used to analyze the effects of Plant Legacy, Current Plant, and their interaction on two different aspects of plant biomass (total and root:shoot ratio). Since we were interested in how these variables affected cumulative plant performance, only the data from the final month of each post-conditioning round were used.

For the analyses of MBC, total soil C, total soil N, and total inorganic N, the data were subset to include only samples from pots with plants growing in them at the time of measurement and then further subset by post-conditioning round. To address our prediction that plants can induce legacy effects on soil chemistry, we used linear mixed models to determine the fixed effects of Plant Legacy, Current Plant, and their interaction using the formula:

$$\text{Response} \sim \text{Plant Legacy} * \text{Current Plant} + (1|\text{Month})$$

To test our prediction that plants can induce legacy effects on bacterial community composition, we used permutational analysis of variance (PERMANOVA) to assess the effects of Plant Legacy, Current Plant, Month, and their interactions on bacterial community composition. Principal Coordinates Analysis (PCoA) was used to visualize differences between treatments. We used ANOVA to determine the effects of Plant Legacy, Current Plant, Month, and all possible interactions on OTU richness and evenness. Significant phyla-level responses to conditioning by *B. inermis* was determined using the *DESeq2* package (Love et al., 2014). If a response was positive, then that phylum was significantly more abundant in soils originally conditioned by *B. inermis*.

To explore how soil bacterial community correlated with the soil properties, plant biomass, and ecosystem processes measurements we collected each month, we extracted the scores from PCoA axes 1 and 2 (PCoA1 and PCoA2). We then used linear mixed models to determine the fixed effects of PCoA1 and PCoA2 on total soil C, total soil N, soil NO_3^-, soil NH_4^+, MBC, soil respiration rates, SOM-derived CO_2 production rates, and root-derived CO_2 production rates using the formula:

$$\text{Response} \sim \text{PCoA1} + \text{PCoA2} + (1|\text{Month})$$

To account for plant species-level differences in biomass production of the Current Plant, we used a different formula to test the fixed effects of PCoA1 and PCoA2 on total plant biomass, aboveground biomass, and belowground biomass:

$$\text{Response} \sim \text{PCoA1} + \text{PCoA2} + (1|\text{Month/Current Plant})$$

Table 1
Results from models estimating the effects of Plant Legacy (L), Current Plant (P), and their interaction on plant biomass, soil chemistry (total C, total N, and inorganic N), and microbial biomass C at the end of both post-conditioning rounds of the experiment. Plant Legacy was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis* or *P. smithii*). Two-way ANOVA was used in analyses of plant biomass (n = 20). Linear mixed models were used for analyses of soil chemistry and microbial biomass C (n = 60). A Kruskal-Wallis test was used instead of ANOVA for testing the effects of Plant Legacy and Current Plant on root:shoot ratio during the first post-conditioning round.

	Post-conditioning Round 1		Post-conditioning Round 2	
Variable	F	p	F	p
Total biomass				
Plant Legacy	18.3	**<0.001**	0.687	0.419
Current Plant	19.8	**<0.001**	29.9	**<0.001**
L * P	1.29	0.272	0.629	0.439
Root:shoot ratio				
Plant Legacy	0.571	0.450	2.59	0.127
Current Plant	5.14	**0.023**	34.3	**<0.001**
L * P	–	–	0.798	0.385
Microbial biomass C				
Plant Legacy	57.0	**<0.001**	31.6	**<0.001**
Current Plant	9.87	**0.002**	1.23	0.268
L * P	0.0950	0.758	0.650	0.420
Total C				
Plant Legacy	404	**<0.001**	256	**<0.001**
Current Plant	0.0710	0.789	0.549	0.459
L * P	0.134	0.714	0.898	0.344
Total N				
Plant Legacy	213	**<0.001**	169	**<0.001**
Current Plant	0.0240	0.878	0.494	0.482
L * P	1.25	0.264	0.122	0.727
Soil inorganic N				
Plant Legacy	120	**<0.001**	89.8	**<0.001**
Current Plant	0.123	0.726	79.7	**<0.001**
L * P	0.0370	0.847	8.68	**0.003**

All analyses were performed within the R statistical platform (R Core Team, 2019) using the packages *lme4* (Bates et al., 2015), *car* (Fox and Weisberg, 2019), and *vegan* (Oksanen et al., 2019) in addition to any packages mentioned above. Vectors corresponding to soil C and nutrient variables that were significantly correlated with PCoA2 were added to our PCoA using the *envfit* function from the *vegan* package. We used the packages *tidyverse* (Wickham et al., 2019) and *cowplot* (Wilke, 2019) to produce all figures in the manuscript.

3. Results

3.1. PSF effects on plant biomass were not related to feedback effects on soil respiration

3.1.1. Plant biomass

At the end of the first post-conditioning round, the total biomass of *B. inermis* was 170% higher when grown in its home soil (i.e., soil originally conditioned by a conspecific) than when grown in away soil (i.e., soil that was not originally conditioned by a conspecific). On the other hand, the total biomass of *P. smithii* was 44% lower when grown in its home soil than when grown in away soil. The effect of both Plant Legacy and Current Plant on total plant biomass was significant (Table 1). Total plant biomass was 133% greater, on average across both species, if soil was originally conditioned by *B. inermis*. Additionally, *B. inermis* biomass was 141% greater than *P. smithii* biomass across both Plant Legacy treatments. Overall, the root:shoot ratio for *B. inermis* was 88% higher than *P. smithii* across Plant Legacy at the end of the first post-conditioning round (Table S1).

At the end of the second post-conditioning round, total biomass of *B. inermis* was the same whether grown in its home soil or in its away soil. Total biomass of *P. smithii* was 27% lower when grown in its home soil than when grown in away soil. At the end of the second post-conditioning round, the only significant effect on total plant biomass was Current Plant species (Table 1), with *B. inermis* biomass being 109% higher, on average, than *P. smithii* biomass across Plant Legacy. Overall, the root:shoot ratio for *B. inermis* was 266% higher than *P. smithii* across Plant Legacy treatments by the end of the second post-conditioning

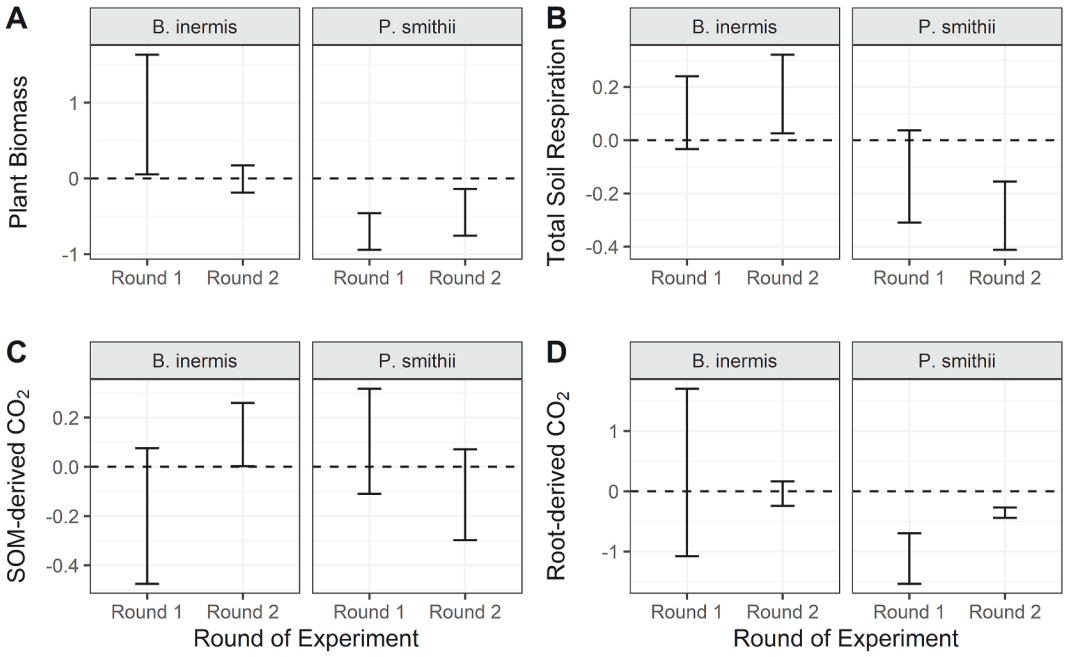

Fig. 2. The 95% CI for feedbacks on (A) plant biomass, (B) total soil respiration, (C) SOM-derived CO_2, and (D) root-derived CO_2 at the ends of the first and second post-conditioning rounds of the experiment. Feedbacks are considered significant if the 95% CI does not cross zero. If the 95% CI is positive, then that particular response is higher when a plant is grown in its home soil. If it is negative, then that particular response is lower when a plant is grown in its home soil.

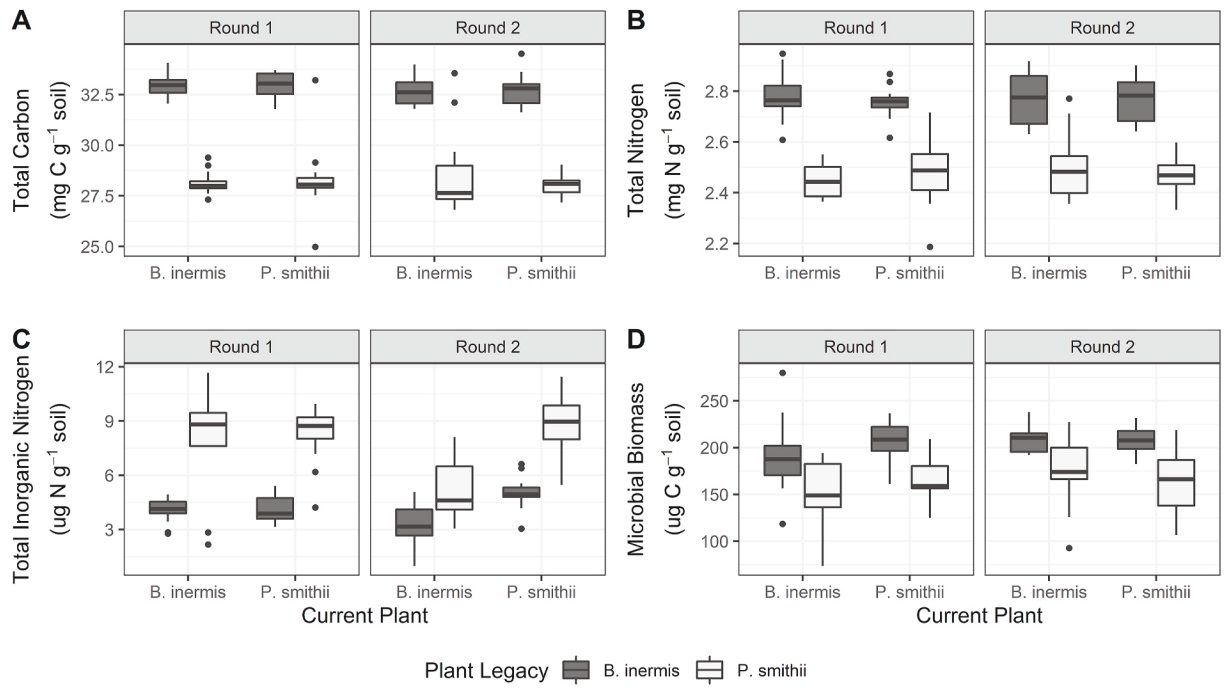

Fig. 3. Boxplots of soil chemistry [(A) total C, (B) total N, and (C) inorganic N] and (D) microbial biomass C measured throughout the first and second post-conditioning rounds of the experiment. The dark line represents the median and the box represents the first and third quartiles. Whiskers extend to the maximum and minimum of the data, excepting outliers (represented by points). The color of the box represents Plant Legacy.

round (Table S1).

3.1.2. Feedbacks

Based on total plant biomass of each species at the end of a round of growth, *B. inermis* experienced significantly positive PSFs at the end of the first post-conditioning round of the experiment (Fig. 2A, Table S1), indicating that *B. inermis* performed better in its home soils than away soils. However, there were no significant PSFs for *B. inermis* at the end of the second post-conditioning round. In contrast, PSFs for *P. smithii* were significantly negative at the end of both post-conditioning rounds indicating that *P. smithii* consistently performed worse in its home soils.

We found no significant feedbacks of Plant Legacy on total cumulative soil respiration for either species during the first post-conditioning round. During the second post-conditioning round, there was a positive feedback on total cumulative soil respiration for *B. inermis* (i.e., total cumulative soil respiration was higher when *B. inermis* was grown in its home soil) but a negative feedback for *P. smithii* (i.e., total cumulative soil respiration was lower when *P. smithii* was grown in its home soil). Therefore, across all soils, total cumulative soil respiration was significantly higher if the soil was originally conditioned by *B. inermis* regardless of which species was growing in the soil at the time of measurement (Fig. 2B, Table S2). Feedback effects on cumulative SOM-derived CO_2 production were not consistent with PSFs on biomass during either post-conditioning round of the experiment. There was no significant feedback on cumulative SOM-derived CO_2 production during the first post-conditioning round for *B. inermis* or during either post-conditioning round for *P. smithii*. However, there was a positive feedback on cumulative SOM-derived CO_2 production for *B. inermis* during the second post-conditioning round of the greenhouse experiment (Fig. 2C, Table S2) potentially indicating increased nutrient mineralization (see Discussion).

In contrast to cumulative SOM-derived CO_2 production, feedbacks on cumulative root-derived CO_2 production were always consistent with PSFs on biomass for *P. smithii*. That is, cumulative root-derived CO_2 production was lower when *P. smithii* was growing in its home soil throughout both post-conditioning rounds of the experiment (Fig. 2D, Table S2). There were no significant feedbacks on cumulative root-derived CO_2 production for *B. inermis* during either post-conditioning round of the experiment.

3.2. Species-specific legacy effects on soil chemistry and microbial communities persisted after the initial conditioning phase

3.2.1. Legacy effects on soil chemistry and MBC

Soils conditioned with *B. inermis* or *P. smithii* developed divergent total soil C and N concentrations that persisted throughout the study (Fig. 3A and B; Table 1). Across both post-conditioning rounds of the experiment, soils originally conditioned by *B. inermis* had an average C concentration of 32.6 ± 0.1 mg C g^{-1} soil, or about 15% more C than in soils conditioned by *P. smithii* (28.3 ± 0.1 mg C g^{-1} soil). Average total N concentration was also 11% greater in soils originally conditioned by *B. inermis* (2.76 ± 0.01 mg N g^{-1} soil) than soils originally conditioned by *P. smithii* (2.48 ± 0.01 mg N g^{-1} soil). In contrast, inorganic N concentrations were significantly greater in soils originally conditioned by *P. smithii* during both post-conditioning rounds of the experiment (Fig. 3C; Table 1). In addition, extractable inorganic N concentrations were lower in soils of either Plant Legacy treatment that had *B. inermis* growing in them during the second post-conditioning round. Also, during the second post-conditioning round, inorganic N was lowest when *B. inermis* was grown in its home soil, and it was highest when *P. smithii* was grown in its home soil. Concentrations of inorganic N were intermediate when either species was grown in its away soil.

During the first post-conditioning round, there were significant effects of both Plant Legacy and Current Plant on MBC (Table 1). Across both plant species, soil that was originally conditioned by *B. inermis* had 24% higher MBC, on average, than soil originally conditioned by *P. smithii*. Across Plant Legacy treatments, MBC was 9% lower, on average, when *B. inermis* was the Current Plant than when *P. smithii* was the Current Plant (Fig. 3D). During the second post-conditioning round, only Plant Legacy significantly influenced MBC (Table 1). Across both plant species, soil originally conditioned by *B. inermis* supported 22% more MBC, on average, than soil originally conditioned by *P. smithii*

Table 2
PERMANOVA results for the effects of Plant Legacy (L), Current Plant (P), Month (M), and all their interactions on soil bacterial community composition (n = 140). Plant Legacy was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis*, *P. smithii*, or no plant).

	F	R^2	p-value
Plant Legacy	19.2	0.088	**0.001**
Current Plant	1.65	0.015	**0.042**
Month	11.1	0.255	**0.001**
L * P	0.993	0.009	0.379
L * M	1.52	0.035	**0.018**
P * M	1.56	0.072	**0.001**
L * P *M	1.06	0.049	0.285
Residuals		0.477	

(Fig. 3D).

3.2.2. Bacterial community composition, richness, and evenness

According to PERMANOVA, soil bacterial community composition was significantly influenced by Plant Legacy, Current Plant, and Month (Table 2). Additionally, there were significant interactive effects of Current Plant * Month and Plant Legacy * Month. Of all the model factors, Month explained the most variation in bacterial community composition followed by Plant Legacy (Table 2, Fig. 4).

Throughout the post-conditioning phase of the experiment, OTU evenness steadily increased over time, from 0.809 ± 0.002 in the first month of the first post-conditioning round to 0.869 ± 0.004 in the final month of the second post-conditioning round. OTU richness also significantly varied across time, but there was no clear temporal directionality. Averaged across months within each round, OTU richness was significantly higher in soil originally conditioned by *B. inermis* (3006 ± 23 OTUs) compared to soil originally conditioned by *P. smithii* (2869 ± 26 OTUs; Table 3, Fig. S1A). Evenness was also significantly higher in soil that was originally conditioned by *B. inermis* (0.854 ± 0.003) than in soil conditioned by *P. smithii* (0.841 ± 0.003; Table 3, Fig. S1B). On average, soils without plants at the time of measurement had lower richness (2898 ± 30 OTUs) than soils with either *B. inermis* or *P. smithii* growing in them (2973 ± 34 OTUs and 2963 ± 28 OTUs, respectively). There was no effect of Current Plant on OTU evenness, but there was a significant effect of the interaction of Current Plant and Month on evenness (Table 3).

3.2.3. Soil bacterial taxonomic response to conditioning treatment

Proteobacteria and *Actinobacteria* were the two most abundant phyla across all samples. The most abundant families were *Chthoniobacteriaceae*, *Gaiellaceae*, and *Oxalobacteraceae*. The phyla *FCPU426*, *AD3*, *TM6*, and *Nitrospirae* were significantly less abundant in soils originally conditioned by *B. inermis* (Fig. 5). The phyla *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Cyanobacteria*, *FBP*, *Chlamydiae*, *WPS-2*, ZB3, *BHI80-139, and WS4* were significantly more abundant in soils originally conditioned by *B. inermis* (Fig. 5).

3.2.4. Correlations between soil bacterial community composition and soil properties, plant properties, and soil respiration

PCoA1 explained 24.5% of the variation in soil bacterial community, and PCoA2 explained 9.8% of the variation in soil bacterial community (Fig. 4). There was no significant correlation between PCoA1 and any of the soil properties, plant properties, or components of soil respiration that we measured (Table 4). However, PCoA2 was significantly and positively correlated with total soil C (Fig. 4, Fig. S2A) and total soil N (Fig. 4, Fig. S2B), but negatively correlated with soil NO_3^- (Fig. 4, S2C). PCoA2 was also positively correlated with MBC (Fig. 4, S2D), total plant biomass (Fig. S2E), and aboveground biomass (Fig. S2F). There were no significant correlations between PCoA2 and any of the components of soil respiration that we measured (Table 4).

4. Discussion

In this study, we used a plant-soil feedback approach to compare the legacies of two plant species: *B. inermis*, a weedy species exotic to tallgrass prairie, and *P. smithii*, a native C_3 grass, both grown in the same

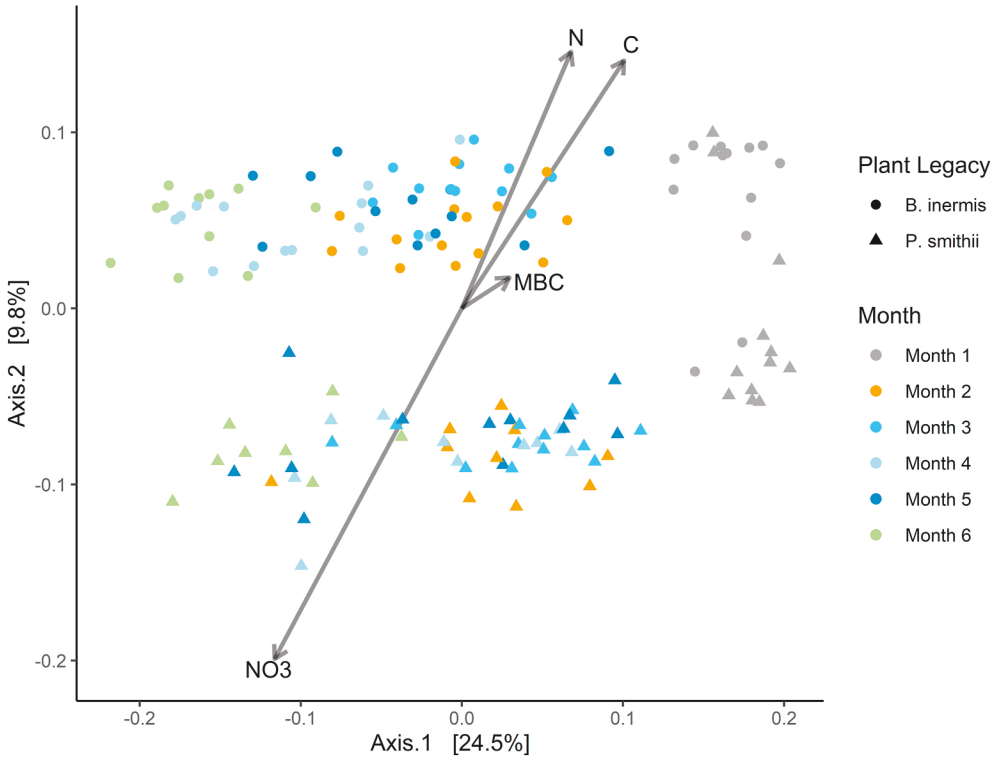

Fig. 4. PCoA ordination of soil bacterial community composition using Bray-Curtis distance. The shape of each point represents Plant Legacy. The color of each point represents Month. Environmental vectors that are significantly correlated with PCoA axis 2 according to our linear mixed models are also displayed (C = Total soil C, N = Total soil N, MBC = Microbial biomass carbon, NO3 = soil NO_3^-). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Two-way ANOVA results for the effects of Plant Legacy (L), Current Plant (P), Month (M), and all their interactions on soil bacterial richness and evenness (n = 140). Plant Legacy was determined by the identity of the plant that originally conditioned the soil (*B. inermis* or *P. smithii*). Current Plant was determined by the identity of the plant growing in the pot at the time of measurement (*B. inermis*, *P. smithii*, or no plant).

Variable	F	p-value
Richness		
Plant Legacy	38.9	**<0.001**
Current Plant	4.45	**0.014**
Month	36.7	**<0.001**
L * P	1.44	0.241
L * M	0.911	0.477
P * M	1.84	0.062
L * P *M	1.06	0.397
Evenness		
Plant Legacy	28.0	**<0.001**
Current Plant	2.99	0.055
Month	49.9	**<0.001**
L * P	0.265	0.768
L * M	0.511	0.767
P * M	2.04	**0.037**
L * P *M	1.34	0.220

field-collected prairie soil, on plant performance, soil respiration, soil properties, and soil bacterial communities. Overall, we found that plant species-specific legacy effects impacted all of these variables in some way, indicating that plant conditioning of soil microbial communities and nutrient pools can occur over relatively short (20 weeks) time scales.

Plant species-specific differences in root allocation within their "home" soils could suggest an explanation for the source of legacy effects in our study. In addition to producing more total biomass, *B. inermis* allocated proportionally more biomass belowground than *P. smithii* (Table 1). We suggest that the higher allocation belowground allowed *B. inermis* to have a greater impact on soil chemistry by increasing soil C through root inputs and converting soil inorganic N to plant organic N (Fig. 3). Bacterial community composition was correlated with soil C and N (Fig. 4, Fig. S2A and S2B). Therefore, greater or different belowground inputs by *B. inermis* during the conditioning period may underly effects on soil bacterial community structure and diversity (Fig. 4 and 5, S1) and increased microbial biomass (Fig. 3D).

The apparent consequences of integrated chemical and microbial legacy responses for plant performance and soil respiration were significant but variable: Six months after the conditioning phase, total soil respiration was higher in soil with a Plant Legacy of *B. inermis*, and biomass and root-derived CO_2 production were lower when *P. smithii* was grown in soil with its own legacy rather than a legacy of *B. inermis* (Fig. 2). This suggests that the soil chemical and microbiological changes made by *B. inermis* can have long-lasting effects on ecosystem processes and subsequent plant performance. For example, soils with a Plant Legacy of *B. inermis* had higher soil C, N, and MBC (Fig. 3A, 3B, 3D) and a bacterial community composition associated with greater biomass production (Fig. S2E). These conditions may have improved the performance of *P. smithii* such that it increased its biomass and associated with a greater abundance of microbes that metabolized its root-derived inputs. Therefore, our results demonstrate that historical plant-soil interactions as well as contemporary plant-soil interactions impact PSFs and ecosystem processes.

In the following sections, we discuss the role of plant species-specific legacy effects on SOM decomposition in the context of our original hypotheses.

4.1. Conclusion 1: PSF effects on plant biomass are not related to PSF effects on SOM-derived CO_2 production

Contrary to our hypothesis, we did not find evidence that PSF effects on plant biomass helped explain the observed differences in SOM mineralization in our study since PSFs and feedbacks on cumulative SOM-derived CO_2 production were not consistent with one another

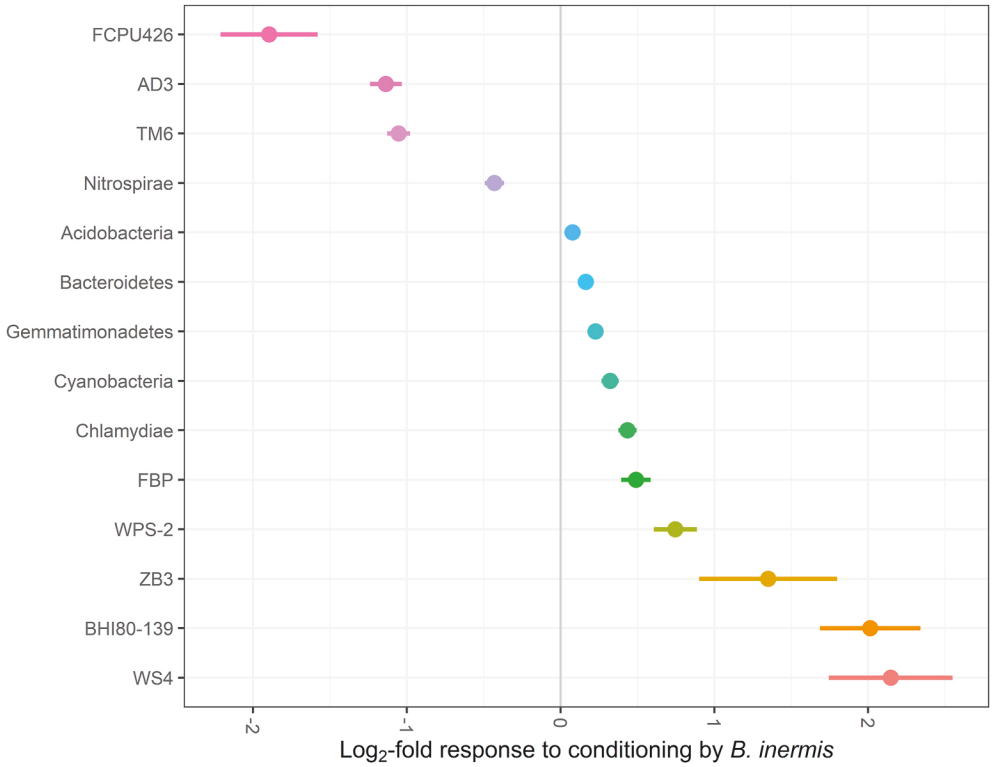

Fig. 5. Bacteria phylum-level response to Plant Legacy. Positive values indicate that a phylum was significantly more abundant in soils originally conditioned by *B. inermis*. Negative values indicate that a phylum was significantly more abundant in soils conditioned by *P. smithii*.

Table 4
Linear mixed-effects model results for the effects of PCoA1 and PCoA2 on soil properties [Total C, Total N, NO_3^-, NH_4^+, Microbial biomass C (n = 140)], plant properties [Total plant biomass, Aboveground biomass (n = 73)], and ecosystem processes [Total soil respiration (n = 140), SOM-derived CO_2 (n = 140), and Root-derived CO_2 (n = 73).

Variable	X^2	p
Total C		
PCoA1	3.71	0.054
PCoA2	231	**<0.001**
Total N		
PCoA1	0.0928	0.761
PCoA2	150	**<0.001**
NO_3^-		
PCoA1	1.96	0.161
PCoA2	49.7	**<0.001**
NH_4^+		
PCoA1	0.0202	0.887
PCoA2	0.0106	0.918
Microbial biomass C		
PCoA1	1.15	0.284
PCoA2	**50.8**	**<0.001**
Total plant biomass		
PCoA1	1.23	0.268
PCoA2	6.51	**0.011**
Aboveground biomass		
PCoA1	0.937	0.333
PCoA2	9.08	**0.003**
Belowground biomass		
PCoA1	1.36	0.243
PCoA2	1.49	0.222
Total soil respiration		
PCoA1	0.488	0.485
PCoA2	1.53	0.216
SOM-derived CO_2		
PCoA1	0.0293	0.864
PCoA2	1.42	0.233
Root-derived CO_2		
PCoA1	0.602	0.438
PCoA2	0.424	0.515

(Fig. 2). This is somewhat surprising because we expected that when plants produced less biomass due to negative PSFs, they also might not allocate as much C belowground (Van der Krift et al., 2001). Microbial priming of soil organic matter decomposition is positively correlated to root exudation rates (Bengtson et al., 2012). However, root biomass is not necessarily correlated with fine root turnover or root exudation rates (Eisenhauer et al., 2017), so it is likely that factors other than plant biomass (such as other root traits (Kardol et al., 2015), exudate quality (de Vries et al., 2019), home-field advantage effects (Austin et al., 2014), or other members of the soil microbial community such as fungi) were stronger controls of SOM decomposition in our study. For example, *B. inermis* can decrease its belowground C allocation to mycorrhizal fungi when it is less advantageous to associate with them (Grman, 2012).

Temporal variation in our feedback results could be due to changes in soil microbial community composition and diversity across the duration of the greenhouse experiment (Fig. 4 and 5, Fig. S1). If the direction and magnitude of PSFs were determined by the composition of the soil microbial community, then the feedbacks we measured would change as the soil microbial community changed. Understanding the temporal variability of PSFs is important because it can help explain the development of plant communities (Kardol et al., 2006), and our results further support other studies that temporal variability in PSFs can occur over relatively short time scales (Wubs and Bezemer, 2018). PSFs can vary through time because plants are not affected similarly by all members of a dynamic soil community (Hawkes et al., 2013). The results of our study suggest that this concept can be extended to feedbacks that affect ecosystem processes, such as soil respiration, and that predicting feedback effects is difficult if the soil microbial community changes over time. Overall, the lack of a link between PSFs and feedbacks on soil respiration and SOM decomposition suggests that the mechanisms underlying PSFs that drive plant growth and subsequent population and community-level responses are likely to be different from the mechanisms that drive ecosystem-level responses. More research dedicated to understanding PSFs on ecosystem processes within a temporal context is clearly needed (Kardol et al., 2013).

4.2. Conclusion 2: B. inermis and P. smithii leave legacy effects on soil chemistry and microbial communities which affect rates of SOM decomposition

We originally predicted that each plant species would alter soil chemical and microbiological properties in unique ways and that these changes might influence SOM decomposition. Consistent with our hypothesis, Plant Legacy was an important factor that affected bacterial community composition and diversity, soil chemistry, and soil respiration in this study. It seems that the original conditioning phase of the greenhouse experiment established unique bacterial communities that changed over time, but with different trajectories that never converged (Fig. 4). Bacterial community composition also shifted within treatments over the course of the experiment, such that the bacterial communities at the end of the greenhouse experiment were distinct from the communities at the beginning. These results further support the idea that initial changes in the soil microbial community driven by a particular plant species can leave long-lasting legacies on microbial community dynamics and function (Wubs et al., 2019).

Legacy effects can persist even after invasive plant removal (Corbin and D'Antonio, 2012; Hamman and Hawkes, 2013), and our results demonstrate that invasive plants such as *B. inermis* leave lasting impacts on ecosystem properties even in soils from which it was removed six months prior. At the end of the experiment, soil C, soil N, MBC, and total soil respiration were all higher in soils with a Plant Legacy of *B. inermis* (Figs. 2 and 3). Additionally, SOM decomposition was greater when *B. inermis* grew in its home soil. The legacy effect of *B. inermis* conditioning may contribute to its ability to act as an invasive species (Gibbons et al., 2017). By increasing the rate of SOM decomposition, *B. inermis* might increase nutrient release from SOM. Increasing nutrient availability is one potential strategy that invasive plants use to succeed in novel environments (Sardans et al., 2017; Zhou and Staver, 2019).

While we did not find a relationship between bacterial community composition and soil respiration (Table 4), the abundance of functionally important bacterial phyla was affected by Plant Legacy (Fig. 5). For example, *Acidobacteria* and *Bacteroidetes* were more abundant in soil with a Plant Legacy of *B. inermis*. *Acidobacteria* have been identified as "keystone taxa" for SOM decomposition (Banerjee et al., 2018), and changes in C mineralization in forest soils could be predicted by changes in *Bacteroidetes* abundance (Fierer et al., 2007). On the other hand, the phylum *Nitrospirae* was less abundant in soils with a Plant Legacy of *B. inermis*. Since there was less inorganic N available in soil that had *B. inermis* growing in it at the end of the second post-conditioning round (Fig. 3), this could indicate there was less nitrifier activity in soils with a Plant Legacy of *B. inermis,* or that *B. inermis* may have taken up more inorganic N than *P. smithii* during this round of the experiment. However, we do not have the data to evaluate either of these hypotheses. Clearly, understanding the influence of this invasive grass on C and N cycling warrants further investigation since our results have demonstrated that a legacy of *B. inermis* changes important soil properties (Fig. 3), potentially through changes in bacterial communities (Fig. 4, S2).

Overall, our study suggests that the impact of plant-soil interactions on ecosystem processes depends on legacies left behind by previous plant communities. Specifically, we showed that feedbacks between plants and soils that affect performance (PSFs), ecosystem processes (soil respiration), and soil bacterial community composition can be temporally variable. Finally, through their impacts on soil microbial and chemical properties, plants can have long lasting effects on SOM

decomposition that persist even after their removal. This study suggests that a full understanding of the ecosystem processes occurring at a point in time requires an understanding of the history of that ecosystem in addition to contemporary community dynamics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108350.

Data availability

All data associated with this manuscript are freely available at the Konza Prairie LTER website (Connell et al., 2020). Raw sequence data are available from the NCBI GenBank SRA at accession number PRJNA674621.

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