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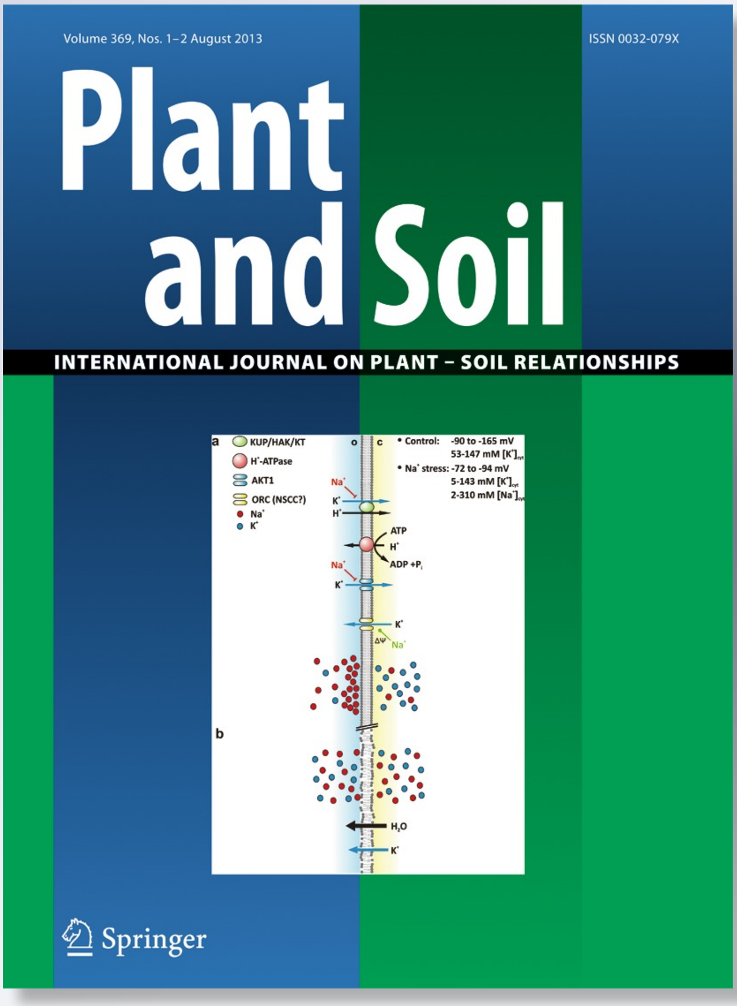

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REGULAR ARTICLE

The temporal development and additivity of plant-soil feedback in perennial grasses

Christine V. Hawkes · Stephanie N. Kivlin ·
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Abstract
Background Current knowledge of plant-soil feedback is based largely on single end point studies with soils conditioned by monocultures, but accounting for variability in the ecological impacts of feedback effects may require understanding how feedback develops over time and in multi-species plant communities.
Methods To examine temporal development and additivity of feedback, two pairs of native and non-native congeneric grasses were grown alone or in mixtures to create six soil conditioning treatments. We measured plant growth and feedback on the soils over 19 months and addressed whether plant biomass was additive or non-additive between soils treated by mixtures and their constituent monocultures.
Results For native grasses, plant-soil feedback either became progressively more negative through time or switched from neutral to negative. Feedback to non-native grasses was variably neutral to positive. Final biomass of the grasses growing on soils conditioned by mixtures was generally an additive function of growth on soils conditioned by the component monocultures, except native grasses growing in soils conditioned by their own congener mixtures, which were non-additive.
Conclusions Temporal variation and non-additivity in feedback suggest that extrapolation to communities may be complex. More work is needed to assess the generality of temporal and scaling effects.

Keywords *Bothriochloa* · Exotic · Native · *Panicum* · Plant-soil feedback · Temporal variation

Introduction

Plant-soil feedback has been proposed as a mechanism underlying plant community dynamics, including the maintenance of diversity, relative abundance, successional turnover, and species invasions (e.g., Bever 2003; Klironomos 2002; Mangan et al. 2010; Mills and Bever 1998; Reynolds et al. 2003). Negative feedback is generally expected to result in species replacement and increased community diversity, while positive feedback should predominantly reinforce plant dominance and homogenize communities (but see Molofsky et al. 2001). In a recent meta-analysis of 45 studies, negative feedback was common, comprising 70 % of cases and including annual to perennial life cycles, herbaceous and woody growth forms,

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C. V. Hawkes (✉) · S. N. Kivlin · J. Du
Section of Integrative Biology,
University of Texas at Austin,
1 University Station C0930,
Austin, TX 78712, USA
e-mail: chawkes@austin.utexas.edu

V. T. Eviner
Department of Plant Sciences, University of California,
One Shields Avenue,
Davis, CA 95616, USA

and native and non-native origins; positive feedback was also found in 28 % of cases (Kulmatiski et al. 2008).

While the prevalence and importance of plant-soil feedback have been well documented, predicting feedback effects is a challenge. Many cases of plant-soil feedback are highly species-specific (e.g., Bever 1994; Bezemer et al. 2006; Casper and Castelli 2007; Harrison and Bardgett 2010; Klironomos 2002; Manning et al. 2008; van de Voorde et al. 2011; van der Putten et al. 1993). For example, when *Jacobea vulgaris* was grown on soils conditioned by 30 different species from old field communities, 13 created negative feedback and 17 had no effect on *J. vulgaris* biomass (van de Voorde et al. 2011). In addition to species-specific feedback patterns, there can be variability in plant-soil feedback even for the same species using soils from the same sites (e.g., Casper et al. 2008). Some of the observed variability both within and across species may stem from the development of soil feedback over time leading to experiment-specific responses. Alternatively, the nature and magnitude feedback for a given plant species may change in response to different neighbors, leading to non-additivity between soils conditioned by monocultures and those conditioned by plant communities in the field.

Temporal variability in feedback may occur if plants ‘cultivate’ different microbial communities over time or become more or less responsive to the same pathogens and symbionts (Bardgett et al. 2005; Casper and Castelli 2007; van der Heijden and Horton 2009; van der Putten et al. 1993). For instance, there is nearly complete turnover of the mycorrhizal fungi found in some tropical trees within 1 year of germination, with initially rare fungal taxa replacing initially common ones (Husband et al. 2002a, b). Temporal changes in feedback can scale up to successional processes, with early- and late-successional plants experiencing different soil feedback (Kardol et al. 2006; Reynolds et al. 2003). Similarly, plant species colonizing new ranges often experience some degree of enemy release, with negative soil interactions increasing over time (e.g., Diez et al. 2010). Yet current feedback paradigms are based heavily on single end-point studies, with time spans ranging from 1.5 months to 6 years (Kulmatiski and Kardol 2008). If the direction or magnitude of plant-soil feedback changes over time, this may create large variation in feedback outcomes and partially explain why short-term greenhouse experiments do not always predict field performance of perennial plants (e.g., Casper and Castelli 2007). Understanding temporal variation in feedback development will affect how we interpret feedback studies and their contribution to the overall feedback paradigm.

Another source of variation in feedback effects may be differences in the degree of additivity, where plant growth in soils conditioned by plant species in mixtures will not necessarily reflect growth in soils conditioned by monocultures of the constituent species. Species-specific feedback may be ameliorated or enhanced in mixtures, when neighboring plants affect, for example, belowground competition, soil resources, soil microbial communities, or root mycorrhizal communities (Casper and Jackson 1997; Eisenhauer et al. 2010; Hausmann and Hawkes 2009; Hawkes et al. 2005). Non-additivity is commonly observed as overyielding in diversity-productivity studies, where growth in mixtures is not necessarily an additive function of growth in single-species monocultures (Hooper and Dukes 2004). Whether soil feedback is generally additive or non-additive is fundamental to the development of a predictive framework for plant-soil feedback.

We addressed (1) how plant-soil feedback varied with time when measured at four time points over 19 months and (2) whether feedback was additive in soil conditioning treatments based on monocultures and mixtures. In an attempt to understand the mechanisms that might underlie such variation, we created a range of potential responses over time by using plant species with different origins (native or non-native), but with similar ecology to avoid strong life history differences that might confound the findings. We focused on two congeneric pairs of native and non-native grasses and generated six soil conditioning treatments from monocultures and mixtures of the congeneric pairs.

Because the development of plant-soil feedback is assumed to affect plant growth in a linear manner (Bever 2003; Bever et al. 1997), we expected at a minimum to observe a difference in the magnitude of feedback between early and later time points regardless of plant species, origin, or soil treatment. However, feedback development over time was expected to be different for the native and non-native grasses. For example, feedback to native species might become negative more rapidly over time than feedback to non-native species if the latter experienced enemy release in these soils (e.g., Batten et al. 2008; Callaway et al. 2004; Klironomos 2002; Reinhart et al. 2003; van der Putten et al. 2007; van Grunsven et al. 2007). Given the broad variability in biomass that is generally detected when plant species are grown in monocultures vs. mixtures (e.g., Hooper and Dukes 2004), we anticipated finding both additive and non-additive effects on plant growth that were likely to be species-specific and unrelated to native status when considering soils conditioned by congener mixtures. Among the soil treatments, we further hypothesized that the non-natives might be more generalist in their interactions (Moora et al. 2011) and therefore have minimal soil-specific feedback. We had little power to directly address the effects of phylogeny vs. origin with this design and do not attempt to analyze these patterns here.

Materials and methods

Conditioning phase Soils used for the experiment were stony clays (Crawford series) collected in January 2007 from native grassland at the UT Ladybird Johnson Wildflower Center (Austin, TX), sieved to 2 mm, and mixed 3:1 with autoclaved sand in 1,000-ml pots. The soils thus initially contained the microbial community naturally present in the soil, albeit somewhat diluted by the disturbance and sand. Soil collections were made from a broad area of grassland dominated by *Aristida purpurea* to avoid historical impacts of any one of the focal plant species. To condition the soil for the feedback trial, we used four perennial C4 grass species found co-occurring in Texas grasslands, including two natives, *Bothriochloa laguroides* (DC.) Herter and *Panicum virgatum* L. and their non-native congeners, *Bothriochloa ischaemum* (L.) Keng and *Panicum coloratum* L. (Selection 75). To avoid a history of local interactions between specific plant populations and soils, seeds of the two exotics were purchased from Douglass King Seeds (San Antonio, TX) and seeds of the natives were purchased from Native American Seed Co (Junction, TX). We chose congeneric pairs as a conservative approach to minimize the confounding effects of plant life history and plant origin. The non-natives were deliberately introduced to Texas for forage, revegetation, and erosion control. *Bothriochloa ischaemum* is an 'Old World bluestem' native to Europe and Asia and introduced to Texas in the 1930s; it has been widely planted on private lands and along highways and has spread from planted areas (Gabbard and Fowler 2007). *Panicum coloratum*, or Kleingrass, was introduced from South Africa and planted throughout Texas beginning in the 1950s, with the improved variety, Selection 75, released by the USDA-SCS and Texas Agriculture Experiment Station in 1968 (Cox et al. 1988).

There were six soil conditioning treatments: monocultures of each plant species and mixtures of the two congeneric pairs. Two plants were grown in each pot, with two individuals of the same species in monocultures and one individual each of the native and non-native species in the mixtures. The conditioning treatments were replicated 60 times each for a total of 360 pots; more pots than required for the feedback experiment were used as insurance against plant mortality during the conditioning phase. Plants were grown for 9 months (January to September 2007) to create the soil treatments for the next phase. Mortality was recorded and dead plants were replaced with pre-germinated seedlings every 2 weeks to maintain the treatments. All plants were maintained in greenhouses at the University of Texas at Austin with regular watering and pot positions randomized monthly. At the end of the conditioning period, plants were measured for height, number of leaves, number of stems, number of flowering stems, and basal width in order to identify the best non-destructive measures of biomass and allow periodic measurements in the feedback phase of the experiment (see below). Plants were then harvested from the pots, with roots cleaned of remaining soil by brief immersion in water. All plant materials were dried to constant mass and weighed for final biomass. Soils from all pots within a treatment were combined, air dried, and sieved to 2 mm, to provide the soils for the six soil feedback treatments.

Feedback phase For the feedback experiment, each of the four grass species was exposed to each of the six soil treatments (1,000-ml pots) in a fully factorial design. Single individuals were planted per pot in January 2008 as germinated seeds. The 24 plant-soil treatment combinations were each replicated seven times for a total of 168 pots. To ensure adequate replicates for assessing feedback on growth of established plants, dead seedlings were replaced by germinated seeds through March 2008, at which point temperatures warmed and the C4 grasses began to grow beyond the seedling stage. In 87 % of all pots, seedlings were replaced at least once, which likely reflected our attempt to start the pots outside their normal growing season. Thus, we considered April 2008 as our time zero for the feedback study. At four dates, September 2008, January 2009, May 2009 and October 2009, plants were measured for height, number of leaves, number of stems, number of flowering stems, and basal width. At the final date (October 2009), plants were harvested, dried and weighed. The biomass and morphology measures taken both at the end of the conditioning and feedback phases were used to generate allometric relationships to estimate plant shoot biomass for each species throughout the feedback phase. The allometric relationships were determined with stepwise multiple regression, and for each species plant height was the best predictor of biomass (r^2=69–81 %; P<0.001). These relationships were used to estimate biomass in September 2008, January 2009, and May 2009.

Feedback calculations Individual feedback for each species was calculated at each time point as the ln ratio of biomass of plants grown in soils previously conditioned by conspecifics to the biomass of plants grown in soils previously conditioned by heterospecifics (Petermann et al. 2008). This calculation has the advantage of symmetrical feedback scores around zero (Pernilla Brinkman et al. 2010). Using this index, positive feedback reflects better plant growth in soils conditioned by conspecifics compared to soils conditioned by other species, whereas negative feedback occurs when plants grow more in soils conditioned by other species compared to conspecific soils. The heterospecifics included both congeners and non-congeners, which we did not distinguish in the feedback calculations.

We also calculated net pairwise feedback to assess the likely outcome of species interactions, either species coexistence (net negative feedback) or dominance (net positive feedback). Net pairwise feedback was calculated for each pair of species by summing the biomass of both species on their home soils and subtracting the sum of their biomass in each other's soils (Bever et al. 1997). Pooled standard deviations were calculated for each comparison.

Additivity calculations Whether final biomass of plants grown in soil treatments conditioned by mixtures was an additive or a non-additive function of growth in soil treatments representing the component monocultures was evaluated using the proportional deviation of observed and expected growth (Loreau 1998). We calculated this as $D_i = \frac{(O_i - E_i)}{E_i}$, where for each of the i species, O is the biomass observed on soils conditioned by mixtures and E is the biomass expected from growth on soils conditioned by each component monoculture (here calculated as the sum of biomass in the monoculture-conditioned soils divided by two). Values greater or less than zero indicate non-additive effects of soils conditioned by mixtures based on the expectations from soils conditioned by monocultures.

Statistics Final harvest biomass, root to shoot ratio, and number of flowering stems were analyzed as a function of plant species and soil conditioning treatment with univariate ANOVAs and posthoc REGW-F tests. When there were interactions of plant species and soil treatment, separate ANOVAs were run for each species. Plant mortality at the final harvest was analyzed with non-parametric Kruskal-Wallis tests. Individual feedback was analyzed as a function of plant species and soil treatment across dates using repeated measures ANOVA; because there was a significant interaction of plant species and date, we ran separate repeated measures ANOVAs for each plant species by soil treatment across dates. Where the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. Individual biomass feedback and D_i were evaluated for significant deviations from zero with t-tests. The significance of net pairwise feedback was evaluated with linear contrasts using Dunn-Sidak sequential alpha corrections for multiple comparisons (Bever 1994). All statistics were performed in SPSS v. 17.0.0 (SPSS, Inc., Chicago, IL, USA).

Results

Final plant biomass, reproduction, and survival Final biomass of the natives, *B. laguroides* and *P. virgatum*, varied with soil conditioning treatment, while biomass of the non-natives, *B. ischaemum* and *P. coloratum*, did not (Table 1, Fig. 1). The largest *B. laguroides* plants grew in soils conditioned by congener monocultures and the smallest were found in soils conditioned by conspecifics, *Bothriochloa* mixtures, and *Panicum* mixtures, with intermediate growth in non-congener monocultures (Fig. 1). Final biomass of *P. virgatum* was greatest when grown in soils conditioned by *B.*

Table 1 ANOVA for final plant biomass of each plant species as a function of soil conditioning treatment (soil)

Source	*B. laguroides*				*B. ischaemum*				*P. virgatum*				*P. coloratum*			
	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*
Soil	5	6.608	4.271	**0.004**	5	1.351	0.497	0.776	5	7.777	3.724	**0.010**	5	2.554	0.820	0.545
Error	37	1.547			34	2.719			29	2.088			30	3.115		

The boldface type indicates $P<0.05$

ischaemum monocultures and both non-congener and congener mixtures, smallest on soils conditioned by conspecifics, and intermediate in congener and *B. laguroides* monocultures (Fig. 1).

How biomass was allocated at the final harvest also varied among species, but not soil treatments. Allocation to belowground vs. aboveground biomass varied among species ($F_{3,122}=56.602$, MSE=0.949, $P<0.001$), with the root to shoot ratio four times larger in *P. virgatum* compared to the other species. The plants also differed in allocation to reproduction ($F_{3,127}=17.425$, MSE=0.926, $P<0.001$), with at least ten times more flowering stems produced by the *Bothriochloa* species compared to the *Panicum* species.

Mortality after seedling establishment differed among species ($F_{3,127}=17.425$, MSE=0.926, $P<0.001$), with lower mortality for *B. ischaemum* (4.8±3.0 %) and *B. laguroides* (2.4±2.3 %), and higher for *P. coloratum* (16.7±5.7 %) and *P. virgatum* (28.6±6.4 %). Mortality did not differ among soil conditioning treatments ($F_{5,127}=1.524$, MSE=0.926, $P=0.187$), nor was there an interaction of plant species and soil treatment ($F_{15,127}=0.750$, MSE=0.926, $P=0.730$).

Feedback to soil conditioning treatments The magnitude of individual feedback differed among soil conditioning treatments for *B. laguroides* (Table 2), which grew better in soils conditioned by *B. ischaemum* and slightly worse in *Panicum* mixture soils compared to conspecific soils (feedback to soil treatments not shown). Soil conditioning treatment affected individual feedback in *P. virgatum* variably across dates (Table 2); in posthoc tests this interaction was driven only by soils conditioned with *B. ischaemum*, which had stronger effects at the earlier two dates compared to later dates. Individual feedback was unaffected by soil conditioning treatment in the non-natives (Table 2).

Temporal feedback development The development of individual biomass feedback over the time course of the experiment was species-specific ($P<0.001$, Fig. 2), with significant variation across dates in all species

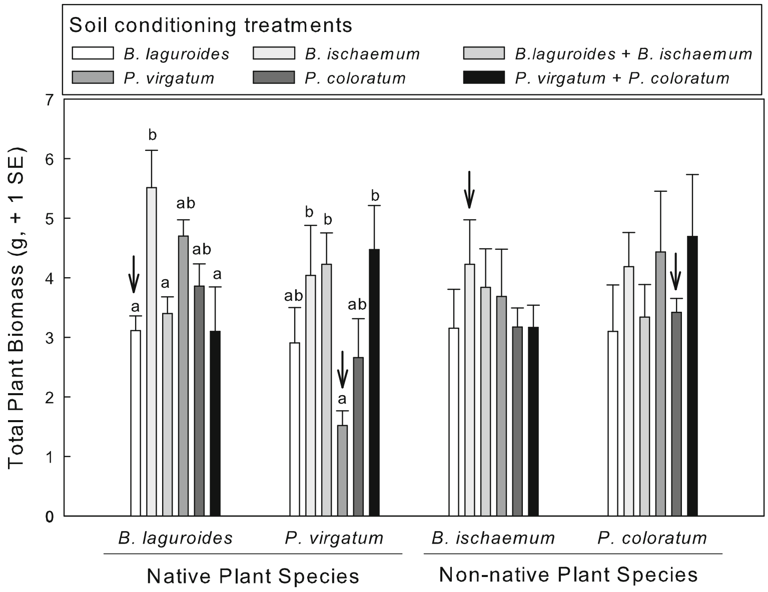

Fig. 1 Total plant biomass at the final harvest of the four plant species grown in six soil treatments. Soil treatments are indicated by the *bar colors*, with species represented by the first letters of their genus and species names in the legend. *Arrows* indicate conspecific soil conditioning treatments for each plant species. Significant differences among soil treatments in posthoc tests are indicated by different letters; biomass of the non-natives did not vary with soil treatment. Results of planned comparisons among congener monocultures and mixtures are reported in the text

Table 2 Repeated measures ANOVAs for individual feedback of each species as a function of soil conditioning treatment (soil), date, and their interaction

Source	*B. laguroides*				*B. ischaemum*				*P. virgatum*				*P. coloratum*			
	df	MS	*F*	*P*	df	MS	*F*	*P**	df	MS	*F*	*P*	df	MS	*F*	*P**
Between subjects																
Soil	4	1.863	3.265	**0.023**	4	0.176	0.815	0.527	4	1.299	2.791	0.056	4	0.144	0.307	0.870
Error	33	0.571			28	0.217			19	0.466			23	0.468		
Within subjects																
Date	3	2.549	17.545	**<0.001**	3	0.630	3.764	**0.014**	3	0.793	4.549	**0.006**	3	0.304	1.678	0.180
Date × soil	12	0.150	1.030	0.428	12	0.097	0.577	0.855	12	0.363	2.082	**0.033**	12	0.122	0.672	0.772
Error(Date)	99	0.145			84	0.167			57	0.174			69	0.181		

*Greenhouse-Geisser adjustments to *P*-values should be used where sphericity was violated (*B. ischaemum* $\varepsilon=0.754$; *P. coloratum* $\varepsilon=0.561$). The boldface type indicates $P<0.05$

except *P. coloratum* (Table 2). For the natives, individual feedback to *B. laguroides* was initially positive, switched to neutral, and was negative at the final harvest, whereas *P. virgatum* individual feedback was neutral (with a negative trend) until the final harvest where it became strongly negative and different from zero (Fig. 2). Feedback was generally smaller and more positive in the non-natives. In *B. ischaemum*, individual feedback was neutral until it became significantly positive at the final date (Fig. 2). In *P. coloratum*, individual feedback did not differ significantly across dates (Table 2) and did not differ from zero except at the third date when it was positive (Fig. 2).

Additivity in mixtures Final biomass of the two natives in soils conditioned by their congener mixtures was a non-additive function of biomass in soils conditioned by the component monocultures, with smaller than expected plants for *B. laguroides* ($D_i=1.170\pm0.357$ (SE), $T=-2.793$, $P=0.031$) and larger than expected plants for *P. virgatum* ($D_i=-0.187\pm0.067$ (SE), $T=-3.281$, $P=0.030$). In contrast, the proportional deviation of final biomass of the natives in soils conditioned by non-congener mixtures and the two non-natives in soils conditioned by all mixtures compared to monocultures did not differ significantly from zero and thus were considered additive.

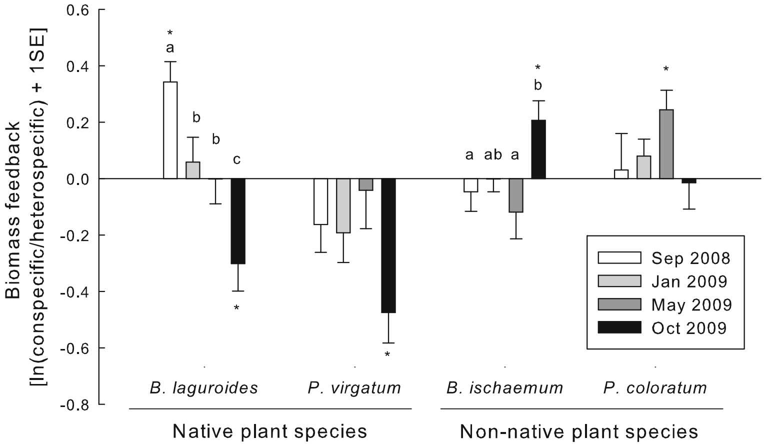

Fig. 2 Individual feedback for each species at the four sampling dates, calculated as the ln ratio of plant biomass in soils previously conditioned by conspecifics vs. heterospecifics. Data are averaged across all soil treatments at each date. The four dates represent 6, 10, 14, and 19 months of growth and feedback in the experiment. Different letters indicate significant differences among dates in posthoc comparisons; asterisks indicate feedback significantly different from zero

Net pairwise feedback Net pairwise feedback was negative for *B. laguroides* paired with *P. virgatum* ($P=0.008$) and *B. laguroides* paired with *B. ischaemum* ($P=0.012$) at the final harvest, although the latter pair was not significant after correction for multiple comparisons (Fig. 3). Other comparisons did not differ significantly from zero.

Discussion

For some plant species, the magnitude of feedback may depend heavily on the duration of growth in conditioned soils and thus may primarily reflect the time period of the experiment. Such effects can be observed in the short-term (Grman and Suding 2010; van der Stoel et al. 2002), but can also take far longer to develop (Bonanomi et al. 2005; Kulmatiski and Beard 2011). Here, the magnitude of feedback differed for all four species and the sign of feedback differed for three species depending on whether the plants were measured after growing for 6, 10, 14, or 19 months. Single end-point studies may not be sufficient to capture feedback effects beyond the life stage at which the feedback was evaluated. Casper and Castelli (2007) similarly found that plant-soil feedback was differentially expressed at grass seedling and adult stages, suggesting that the effects of feedback on the outcome of competition may vary with time. The grasses in the current study were all long-lived perennials, so the temporal variation in feedback observed over 19 months likely reflects their contribution to establishment rather than long-term plant fitness. The optimal time frame for examining plant-soil feedback may depend on both the time scale over which feedback varies and the life stages at which feedback is most important to plant fitness.

The impacts of plant-soil feedback are likely to depend on their rate of development and variability through time. For example, the magnitude of plant-soil feedback has been linked to the relative abundance of species (Klironomos 2002; Kulmatiski et al. 2011; Mangan et al. 2010), but this may only be the case if feedback develops directionally and reaches a sufficient magnitude. Directional feedback development was observed here only for *B. laguroides*, and the magnitude of feedback only became large enough to affect coexistence at the final harvest for *B. laguroides* paired with either *B. ischaemum* or *P. virgatum*. All three species had similar final biomass when grown in monoculture and can be found at some of the same local field sites, but as the non-native *B. ischaemum* invades it can reach high densities and reduce local diversity by 30 % (Gabbard and Fowler 2007). Coexistence thus may be temporary and will likely be affected by factors other than soil feedback, such as seedling mortality or competition (Eppstein and Molofsky 2007; Yelenik and Levine 2011). We found high seedling mortality of *P. virgatum* in the greenhouse, a species for which early survival and establishment is known to be sensitive to environmental factors such as temperature, soil moisture, and soil pH (McLaughlin and Adams Kszos 2005). We did not examine feedback together with competition and thus cannot evaluate their relative importance or interaction; however, others have found that intra- and

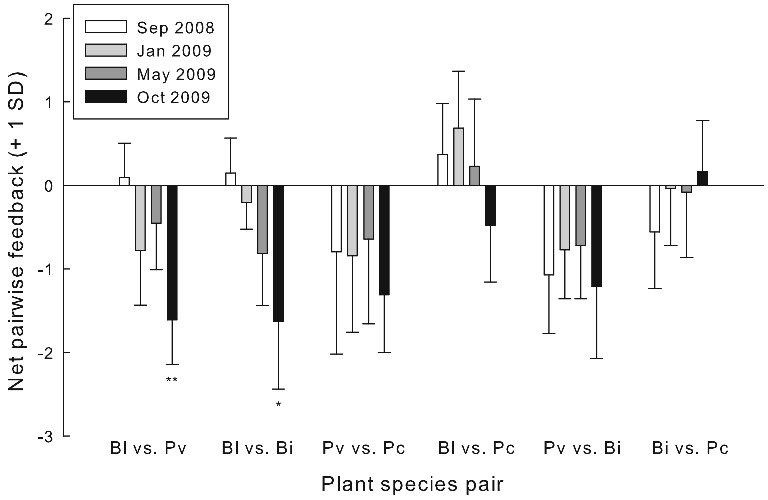

Fig. 3 Net pairwise feedback calculated across dates for biomass on conspecific vs. heterospecific soil treatments for each possible pair of plant species. Plant species are indicated by the first letters of their genus and species names. The four dates represent 6, 10, 14, and 19 months of growth and feedback in the experiment. *Error bars* are 1 pooled SD. *Asterisks* indicate $^{**}P<0.001$ and $^{*}P=0.012$ (before correction)

interspecific competition can strongly interact with plant-soil feedback (e.g., Bonanomi and Mazzoleni 2005; van der Putten and Peters 1997).

How plant-soil feedback develops over time also has implications for the ecological relevance of feedback effects for species invasions. Levine et al. (2006), for example, predicted that local feedback would affect the density of invasive species, but not their spread, based on a lag in feedback development at the invasion front. The non-native grasses *P. coloratum* and *B. ischaemum* were largely indifferent to prior soil conditioning, although positive feedback was observed at 14 and 19 months, respectively. While this may represent a lag in feedback development for *B. ischaemum*, in *P. coloratum*, the positive feedback did not persist to the next time point. The natives cultivated more negative feedback, but in both species this was not significantly different from zero until the final harvest. While these patterns may be broadly consistent with the idea that soil feedback can provide an advantage to non-native species, that conclusion depends heavily on the time point at which the assessment was made. Over even longer time scales, soil feedback was more negative with time since establishment for non-native taxa introduced to New Zealand across a 250-year span (Diez et al. 2010). The time frame required for soil conditioning and the consistency of that conditioning may contribute to the degree of invasiveness, assuming the feedback affects population growth rates.

Non-additive plant-soil feedback has been predicted for communities of coexisting species, with soils from communities expected to support faster growth of individual species than soils from constituent monocultures (Kulmatiski and Kardol 2008). We observed non-additive feedback in the two native grasses, where the effects of soils conditioned by congener mixtures on final biomass were smaller or larger than expected based on the component monocultures. There may have been complementarity between species in their soil conditioning (*Panicum*) or interference where one species may have dominated the soil conditioning (*Bothriochloa*). In all other cases, feedback appeared to be additive and therefore could be more predictable. It is possible that the natives experience non-congener soils as essentially naïve, and the non-natives may respond to all soils this way, perhaps due to enemy release (Reinhart et al. 2003; van Grunsven et al. 2007). Soil conditioning effects could alternatively be proportional to the relative size or abundance of each species, rather than simply their presence as we assumed here. In congener mixtures planted to condition the soils, the non-native grasses were ~50 % larger than the natives on average (data not shown), but the feedback to mixture-conditioned soils was not consistently in the direction of the non-native monocultures.

Others have examined feedback in soils conditioned by communities compared to monocultures, but without pairwise comparisons to explicitly test for additivity (e.g., Bartelt-Ryser et al. 2005; Brandt et al. 2009; Kardol et al. 2006; Kardol et al. 2007). Using soils from a diversity experiment, for example, Bartelt-Ryser et al. (2005) reported that soil feedback was affected for a longer period of time by individual species from the original communities than by community diversity. The soil microbial community is likely to play a large role in non-additivity; arbuscular mycorrhizal fungi, for instance, can have selection and complementarity effects that increase plant biomass by >80 % in mixture compared to growth in fungal monocultures (Wagg et al. 2011). If non-additive effects are common in plant-soil feedback, then predicting their influence at the community level will require us to move beyond monocultures and even two-species mixtures in empirical tests.

The results presented here have limitations. The mechanisms underlying the observed temporal variation in plant-soil feedback are not clear. Microbial communities can be the direct driver of feedback effects (e.g., Batten et al. 2008; Elgersma et al. 2012), as can soil nutrients (e.g., Casper et al. 2008; Harrison and Bardgett 2010). We did not quantify either microbial community composition or nutrients through time, and it is likely that they both contributed to the observed results. A second potential limitation of this study was the use of allometric relationships for biomass during the first three time points, which may limit the results to some degree. While this estimation introduced a level of uncertainty in the data that we cannot quantify, the alternative of destructive harvests of independent replicates over time would also introduce variability that could not conclusively be attributed to treatments vs. intrinsic variation in individual size due to factors such as initial seed size variation. Thus, the allometric approach was preferred in order to track the same individual plants through time.

We demonstrate that feedback can vary temporally within a single individual and that this development is not necessarily linear. In addition, feedback did not always scale additively from monocultures to mixtures. Owing to these intricacies, understanding and predicting feedback may be remarkably difficult. Including greater complexity in feedback models has clarified the conditions under which invasiveness may occur (Eppstein et al. 2006; Eppstein and Molofsky 2007) and the addition of spatial scale to feedback models supports the idea that even positive feedback can engender greater plant species richness at large spatial scales (Molofsky et al. 2001). Incorporating temporal variation and multi-species interactions into the feedback paradigm may make the framework more realistic and perhaps allow it to provide greater explanatory value for how plant-soil interactions affect plant community dynamics.

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