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Phylogenetic relatedness, phenotypic similarity, and plant-soil feedbacks

Connor R. Fitzpatrick^{1,2*}, Laura Gehant³, Peter M. Kotanen^{1,2}, Marc T. J. Johnson^{1,2}

Address of authors:

¹*Department of Biology, University of Toronto Mississauga, Mississauga, Ontario L5L 1C6, Canada*

²*Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario M5S 3B2, Canada*

³*AgroSup Dijon, Dijon 21000, Burgundy, France*

C. R. Fitzpatrick - connor.fitzpatrick@mail.utoronto.ca

L. Gehant – laura.gehant@gmail.com

P. M. Kotanen – peter.kotanen@utoronto.ca

M. T. J. Johnson - marc.johnson@utoronto.ca

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Correspondence: Connor Fitzpatrick tel:1 416 834 8270
Laboratory 2014 William G. Davis Building
3349 Mississauga Road, Mississauga ON,
Canada, L5L 1C6
connor.fitzpatrick@mail.utoronto.ca

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Summary

1. Plant-soil feedbacks contribute to species invasions, the maintenance of biodiversity, and climate change impacts on terrestrial ecosystems. Despite their far-reaching importance, we lack a general understanding of the ecological and evolutionary determinants of plant-soil feedbacks.
2. We conducted a large-scale plant-soil feedback experiment using 49 co-occurring plant species from southern Ontario, Canada, representing a wide phylogenetic range. We tested whether effects of soil conditioning varies among these species, and whether different focal species respond similarly to the same soil conditioning. Next, we asked whether plant traits and soil feedbacks depend upon phylogenetic similarity, and which plant traits affect plant-soil feedbacks between pairs of plant species. Finally we used our experimental results to test whether soil feedbacks affect co-occurrence of species in the field.
3. We found evidence of both strong positive and negative soil feedbacks between pairs of plant species. Our soil conditioning treatment explained nearly 20% of the variation in focal species performance.
4. Phylogenetic relatedness and phenotypic similarity between plant species were unrelated to the strength of their soil feedback. However, numerous plant traits of the conditioning species influenced the strength of soil feedbacks on focal species, including specific leaf area and total aboveground productivity. Trait differences between species were also predictive of plant-soil feedbacks, though for some pairs of species, increased trait differences were associated with positive plant-soil feedbacks and for others trait differences were associated with negative plant-soil feedbacks.
5. Plant species co-occurrence in the field was related to their experimentally determined soil feedbacks but only for particular plant species.
6. *Synthesis.* Our results illustrate how evolutionary history and phenotypic variation shape plant-soil feedbacks and highlight the need for trait-based studies. Due to the unique evolutionary history of individual traits and the variability in their importance across all possible interacting species, we show that indices of overall phenotypic and phylogenetic relatedness are poor predictors of plant-soil feedbacks at large phylogenetic scales. We conclude that a detailed trait-based approach can be used to predict plant-soil feedbacks, and laboratory measures of soil feedbacks can explain patterns of co-occurrence in nature.

Introduction

Land plants have modified the abiotic and biotic environment since their invasion of terrestrial ecosystems approximately 450 million years ago (Wellman, Osterloff & Mohiuddin 2003). Plant-soil feedbacks, whereby soil conditioned by one plant influences the growth of another, are one such modification that has important consequences in contemporary ecosystems

(Bever *et al.* 2010; Hodge & Fitter 2012; van der Putten *et al.* 2013). Feedbacks between land plants and the soil environment contribute to the maintenance of diversity and the succession of plant communities (Klironomos 2002; Kardol, Bezemer & van der Putten 2006), the invasion success of exotic plants (Lankau 2012), the productivity of terrestrial ecosystems (Schnitzer *et al.* 2011), and plant community responses to climate change (Fischer *et al.* 2013). Despite these far-reaching consequences we lack a general understanding of the underlying ecological and evolutionary determinants of plant-soil feedbacks (van der Putten *et al.* 2013).

Plants change the chemical composition of soil, including pH levels, nutrient and water availability (Bezemer *et al.* 2006; Orwin *et al.* 2010; Waring *et al.* 2015), and the composition of soil microbial communities (Berg & Smalla 2009; Lundberg *et al.* 2013). Soil alterations caused by one plant can feed back to affect the performance of a second plant depending on their particular resource and habitat requirements and their propensity to form mutualistic and parasitic relationships with members of the newly altered soil community. Therefore, the outcome of a soil feedback between two plants should depend on their ecological similarity as it relates to their soil resource requirements and acquisition strategies, life histories, and their herbivores, pathogens, and mutualists (Brandt, Seabloom & Hosseini 2009; Burns & Strauss 2011).

Since Darwin ecologists have understood that ecological similarity among organisms influences the outcome of species interactions (Darwin 1859). However, identifying and measuring the traits underlying species interactions is a formidable task. As a proxy for ecological similarity, biologists have recently used phylogenetic relatedness among species in an

attempt to predict the outcome of species interactions (Cahill *et al.* 2008; Violle *et al.* 2011), including the resultant community and ecosystem dynamics that emerge (Webb *et al.* 2002; Cavender-Bares *et al.* 2004; Srivastava *et al.* 2012). A growing body of work suggests that while phylogenetic relatedness can sometimes predict interactions between species (Schnitzer *et al.* 2011; Violle *et al.* 2011), including emergent ecosystem processes (Cadotte 2013), there are many instances where it does not (Cahill *et al.* 2008; Narwani *et al.* 2013; Godoy, Kraft & Levine 2014). Understanding the role of phylogenetic relatedness in contemporary ecological dynamics requires careful consideration of the traits mediating species interactions and accurate modeling of how these traits have evolved over macroevolutionary time (Best & Stachowicz 2014; Nuismer & Harmon 2015).

How phylogenetic relatedness among plant species affects soil feedbacks remains uncertain. Anacker *et al.* (2014) found that within-species soil-feedbacks (the performance of a plant species in soil conditioned by itself), exhibit significant phylogenetic signal, but how this might affect the soil feedback occurring between different plant species is unclear. When phylogenetic relatedness is correlated with ecologically relevant plant traits, then close relatives may share soil mutualists, parasites (herbivores and pathogens), and overlap in resource requirements more than distant relatives (e.g. Gilbert & Webb 2007; Burns & Strauss 2011; Tedersoo *et al.* 2013). In these circumstances the relative importance of these individual components will determine whether the net effect of a soil feedback is either positive (Burns & Strauss 2011) or negative (Liu *et al.* 2011). Alternatively when the phenotypic traits affecting plant-soil feedbacks evolve rapidly or exhibit high plasticity, they are unlikely to correlate with

phylogenetic relatedness among species (Losos 2008), resulting in no pattern between phylogenetic relatedness and soil feedbacks.

Phenotypic traits that mediate the interactions between plants and soil should be important in governing plant-soil feedbacks. These traits can influence both sides of the feedback: the effect of plant growth on soil and the response of plants grown in conditioned soil (hereafter referred to as "effects" and "responses"). Numerous plant traits related to the quality and quantity of resources that plants supply to soil ecosystems have been linked to changes in abiotic and biotic soil characteristics (Wardle *et al.* 1998; Cornelissen *et al.* 2001; Orwin *et al.* 2010). For example, plant traits that interact directly with soil such as root morphology, physiology and architecture can have strong effects on both the abiotic and biotic soil environment and will likely be important in governing plant effects and responses in soil feedbacks (Bardgett, Mommer & de Vries 2014). Functional traits, such as specific leaf area and leaf dry matter content, may also govern plant effects on, and responses to, the soil environment if these traits correlate with plant resource consumption and growth (Wardle *et al.* 1998; Orwin *et al.* 2010). Additionally, the outcome of a soil feedback between plant species may be driven by differences in their traits. For example, competitive outcomes between plant species' pairs are, in part, driven by trait differences that determine niche overlap and competitive hierarchy (Kraft, Godoy & Levine 2015). Trait differences between plant species may also determine soil resource overlap and shared symbioses with soil microorganisms.

In this experiment we sought to understand how plant traits, phylogenetic relatedness and phenotypic similarity affect soil feedbacks among plant species pairs. We experimentally measured the performance of 9 focal plant species when grown in soil conditioned by 48 heterospecific plant species and soil conditioned by conspecific plants. We asked four specific research questions:

- 1) Do soil treatments differ in their effect on focal plant performance and does this effect vary with focal species? Answering this question is a necessary first step to understanding the evolutionary and phenotypic mechanisms of soil feedbacks.
- 2) Does the phylogenetic relatedness and phenotypic similarity between plant species predict their plant-soil feedback? If phylogenetic relatedness between plant species influences soil feedbacks then we predict the strength of feedbacks will exhibit significant phylogenetic signal, with more closely related species showing stronger plant-soil feedbacks than more distantly related species. If phenotypic similarity correlates with plant-soil feedbacks, we expect species with the most similar phenotypic traits to exhibit stronger plant-soil feedbacks.
- 3) Which plant traits predict plant-soil feedback strength? Understanding the importance of plant traits will lead to a more predictive framework for plant-soil feedback effects and responses across species and communities.
- 4) Does the strength of plant-soil feedbacks in the lab predict co-occurrence of plant species in the field? Recent studies indicate greenhouse experiments may overestimate the importance of plant-soil feedbacks (Kulmatiski *et al.* 2008). We measured the co-occurrence of our species in the field and related it to our greenhouse-measured plant-soil

feedback metric. We predicted that species exhibiting stronger positive soil feedbacks are more likely to co-occur whereas those that exhibit negative feedbacks will co-occur less.

Methods

Study system

We selected 49 plant species that commonly occur in old-field and wetland habitats at the 3.5 km² Koffler Scientific Reserve (KSR), 50 km north of Toronto, ON (Fig. 1, Table S1). We selected species to span the evolutionary breadth of angiosperms found at KSR: our species include 19 plant families that represent approximately 140 MY of evolutionary history since their last common ancestor. Thirty-four (69%) species are exotic, having invaded regionally following European colonization and 15 (31%) are native (National Plant Germplasm System (<https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch.aspx>); however, since initial tests found no effect of native/exotic status on soil feedbacks we do not discuss the issue further. The seeds used in our experiments had been previously collected in southern Ontario during the past 15 years and stored frozen prior to germination. Seed collections of each species were made from multiple plants within single open-pollinated populations.

Experimental design

Plant-soil feedback experiments take place in two phases (Bever 1994). In Phase 1, plants are grown in soil to condition the biotic and abiotic soil environment. In Phase 2, focal plant species are grown in soil harvested from the first phase. The impact of the first plant on the second via soil alteration is the plant-soil feedback.

Phase 1- We grew 49 species from seed concurrently in a common environment in the summer of 2013. We refer to these species as “soil-conditioning species”. We first surface sterilized seeds using the following protocol: we placed seeds for 1 min in 70% ethanol with 0.1% tween, then 12 min in 10% bleach with 0.1% tween, then we rinsed the seeds 3x with sterile water and plated them on 1% agar media (Sigma Aldrich A1296) with half-strength MS nutrients (Sigma Aldrich M5119). We staggered our seed treatment so that all species germinated over a one-week period. We transplanted seedlings at the cotyledon stage into 1 L pots containing soil; each pot received one seedling of a single species

Pots were filled with a combination of sterilized soil and live inoculum collected from KSR. The sterilized soil was a mixture of potting soil and sand (2:3 V/V) to facilitate root harvesting. After autoclaving, 800 mL of this sterilized mixture was added to each pot. We then added 200 mL (1/5 pot volume) of live inoculum to serve as the source of the soil biota. The live soil inoculum for Phase 1 was initially collected in equal amounts (10 L/location) from 12 locations across KSR. These locations were representative of the breadth of habitats across the reserve, which includes sand, loam, and clay soil types, low-lying wetland, hardwood forests, meadow, and old-field sites. We sieved the collected soil to 2mm and thoroughly homogenized it to make a single soil inoculum. We kept the plants in a growth chamber for two weeks set to 25°C and 55% humidity, with a 16 h : 8 h, light : dark cycle (CAN-TROL Environmental Chamber, Markham, Canada) under well-watered conditions to increase seedling survival. We then arranged the plants in a randomized block design outside in full sun on a rooftop at the University of Toronto Mississauga. The plants were watered daily *ad libitum* without fertilizer for the course of the experiment (June-September 2013). During this phase we measured a suite of phenotypic traits on individual plants (see *Phenotypic measurements*). After 12 weeks of

growth we preserved all soil from each pot at -20°C, carefully avoiding cross-contamination. We pooled soil from 5 individuals for each of our 49 species for a total of 4 L of soil for each species. This method of pooling allowed us to compare species' effects and responses to soil feedbacks using what is effectively the average soil substrate across a number of individuals per species. It has recently been argued that such practices may overestimate the strength of plant-soil feedbacks, and although we recognize this potential limitation, accounting for such variation in soil conditioning was beyond the scope of our study (Reinhart & Rinella 2016; Cahill *et al.* 2016).

Phase 2 – We selected 9 common focal species to span the evolutionary and phenotypic breadth of the 49 species from Phase 1: 1) *Oenothera biennis* (Onagraceae), 2) *Rumex crispus* (Polygonaceae), 3) *Plantago rugelii* (Plantaginaceae), 4) *Phleum pratense* (Poaceae), 5) *Sporobolus neglectus* (Poaceae), 6) *Lepidium densiflorum* (Brassicaceae), 7) *Hedeoma hispida* (Lamiaceae), 8) *Trifolium pratense* (Fabaceae), and 9) *Geum canadense* (Rosaceae). Note, these 9 species were also used as conditioning species. We germinated the focal species in the same manner as above and planted seedlings singly into 500 mL pots. We planted each of our 9 focal species into unsterilized soil conditioned by each of the 49 species (including soil conditioned by the focal species themselves) from Phase 1, plus a sterile potting mix treatment, for a total of 450 unique focal plant x soil treatment combinations with 4 replicate pots per combination (1800 plants). We mixed live soil inoculum with a sterile potting soil and sand mix in the same ratio as Phase 1. Additionally we conducted a smaller experiment in which we grew two of our focal species (*Rumex crispus* and *Sporobolus neglectus*) in each of the 49 conditioned soils after the soil had been sterilized by autoclaving, with 3 replicate pots per treatment combination (294

plants). This smaller experiment was performed to determine the relative importance of the abiotic versus biotic alterations across our soil treatments. We grew all plants from Phase 2 in two identical growth chambers (Conviron CMP6050, Winnipeg, Canada) in a randomized block design. Pots were placed in plastic portion cups to prevent the transfer of water, soil material, and microorganisms, and were then placed in hard plastic trays, which we rotated within a growth chamber shelf weekly to homogenize any microenvironmental gradients. Plants were unfertilized and watered *ad libitum*. We programmed the chambers to simulate the daily and weekly temperature fluctuations during the months of May-August in Toronto, ON (Table S2). After 8 weeks we harvested all above and belowground tissue from each pot and oven-dried tissue at 60 °C for 72 hours and weighed it to the nearest 0.1 mg. We used these measurements to calculate our plant-soil feedback metric.

Plant-soil feedback metric

To compare soil feedbacks across focal species we normally standardized our raw biomass data (mean = 0, sd = 1) before calculating our feedback metric. Next we removed the effect of spatial blocks in Phase 2 from our biomass data by fitting a linear model with only block included as a predictor variable and we saved the residuals from this model as our new response variable. We then calculated the feedback metric for each focal species x soil conditioning combination as: $\ln(\text{mean biomass of focal species}_x \text{ in soil conditioned by species}_y / \text{mean biomass of focal species}_x \text{ in soil conditioned by species}_x)$. A variety of feedback metrics exist (see Pernilla Brinkman *et al.* 2010), and we chose this particular measure because it is best suited for investigating plant-soil feedbacks among multiple plant species (Klironomos 2002; Petermann *et al.* 2008). We calculated this feedback metric separately for total, above-, and belowground biomass. Positive

values indicate that a focal species performed better in soil conditioned by a heterospecific relative to a conspecific plant, whereas negative values indicate that a focal species performed worse in soil conditioned by a heterospecific relative to a conspecific plant. Our feedback metric is symmetrical which means positive and negative values are directly comparable.

Phylogeny

We used Bayesian inference to estimate phylogenetic relatedness among species. First we downloaded accessions of 3 genes (2 plastid and 1 nuclear) for each species from GenBank: ribulose-bisphosphate carboxylase (*rbcL*); maturase K (*matK*); and internal transcribed spacer (ITS) adjacent to the 5.8S ribosomal RNA gene (Table S3). We aligned sequences in MEGA v. 6.0 (Tamura *et al.* 2013) using MUSCLE (Edgar 2004) with default parameters, followed by manually checking alignments. We then built a Bayesian phylogenetic tree using BEAST v. 2.1.3 (Drummond *et al.* 2012) implemented using a standard general time-reversible model (GTR + I + Γ) for each locus and an uncorrelated lognormal clock (UCLN) to determine the rate of nucleotide change. Using BEAUTi (Drummond *et al.* 2012) we constrained the topology and major clade ages (Table S4) of the tree based on a well-resolved recently published plant phylogeny (Bell *et al.* 2010). Our Markov chain Monte Carlo simulation ran for 100 million generations sampled every 10,000 generations, which resulted in 9000 post burn-in trees. We examined stationarity and effective sample sizes of parameter estimates (all ESS > 200) using Tracer v1.6 (<http://beast.bio.ed.ac.uk/Tracer>). We constructed a consensus tree with mean node heights from the posterior distribution using Tree Annotator v1.6 (Fig. 1). We calculated the phylogenetic relatedness (patristic distance) among all pairs of species using the ‘cophenetic’

function in R and used these measures in our statistical analyses. Phylogenetic relatedness between plants ranged from 0 (conspecifics) to 260 million years.

Phenotypic measurements

We measured traits on five individuals for each of our 49 species during Phase 1, except for root hair density, which was measured in an identical fashion during a previous experiment. We measured eight phenotypic traits: i) total aboveground biomass, ii) total belowground biomass, iii) length of longest root, iv) rooting angle; v) leaf dry matter content (LDMC); vi) specific leaf area (SLA); vii) root hair density; and viii) specific root length (SRL). We chose these traits because of their potential relevance for plant effects on soil ecosystems, and because collectively they contribute to the functional similarity among plant species in the context of plant-soil feedbacks. Biomass traits have well documented effects on soil ecosystems and are highly correlated with plant fitness (Cornelissen *et al.* 2001; Bardgett & Wardle 2010). Physiological and morphological traits of leaves and roots affect interspecific plant competition and are also relevant for soil resource consumption (Orwin *et al.* 2010; Kraft *et al.* 2015). For example, SLA is an important trait that describes the broad resource acquisition strategy of a plant, scaling positively with relative growth rate and negatively with interspecific competition (Westoby 1998; Kraft *et al.* 2015; Kunstler *et al.* 2016). Finally, root morphological traits can have enormous consequences on soil ecosystems by influencing the physical attributes of surrounding soil and the colonization of particular soil microbes (Bardgett *et al.* 2014). We measured phenotypic traits on each individual following a standardized protocol (Cornelissen *et al.* 2003). After 8 weeks of growth we removed a leaf portion of equal area (1.54 cm²) from each individual and measured wet weight to the nearest 0.1 µg on a microbalance (XP2U, Mettler Toledo,

Mississauga, Canada). We then dried the leaf discs at 72°C for 3 days and weighed them. We calculated LDMC as the dry weight divided by the wet weight. To calculate SLA we divided the area of the leaf portion by its dry mass. At the end of phase 1 of the feedback experiment we cut each plant at the base of the stem where it met the soil surface. We placed all of this aboveground tissue in a paper bag and dried it for 3 days at 72° C. We gently removed the loose soil from the belowground tissue followed by washing roots using a sieve and water to remove all soil particles. We placed all washed belowground tissue in a paper bag and dried it for 3 days at 72° C. We then weighed all tissue to the nearest 0.1g to determine aboveground, belowground and total dry biomass. We measured the length of the longest root from each individual plant. We used a string to trace the length of the root before drying from the tip to its attachment to the main aboveground stem and measured the length of the string. For root angle we measured the angle of the first three lateral roots before drying relative to the taproot using a protractor. For species that exhibited no taproot we measured the angle of the first three roots emerging from the aboveground stem. This measurement captures the degree of lateral versus vertical root growth. We removed the distal 5 cm of the first 3 roots emerging from the taproot or stem. We took a photograph of each root before drying and measured the area using ImageJ. We dried these samples at 72°C for 3 days and weighed them to the nearest 0.1 µg on a microbalance. We calculated SRL by dividing the root area by dry mass. We used an identical sampling protocol on plants grown in the previous year to measure root hair density. We used a dissecting microscope to count the number of lateral roots emerging from 3 root portions (5 cm) per individual to calculate root hair density. As our results indicate, this level of replication was adequate to detect effects of individual traits on plant-soil feedbacks. To create our metric of phenotypic similarity we normally standardized each trait and from the species' mean calculated the multi-trait

Euclidean distance between species (visually represented as a dendrogram in Fig. 1). We used distances between pairs of species in this matrix as our measure of phenotypic similarity.

Field surveys

To test whether our plant-soil feedback results predict species co-occurrence in the field, we conducted field surveys at KSR in August 2015. We identified five populations of each of the nine focal species separated by at least 50 m (300m, average). At each population we identified all soil-conditioning species within a 10 m radius of the centre of the focal population. We recorded the distance of the nearest three individuals of each soil-conditioning species to an individual focal plant in the centre of the population.

Statistical analyses

All data manipulations and statistical analyses were performed in R (R Core Team v. 3.1.0).

Do soil treatments differ in their effect on plant performance and does this effect vary with focal species?

We used mixed effects models (function ‘lmer’ from the lme4 package v. 1.1-8 (Bates *et al.* 2015)) to test whether our conditioned soil treatments from Phase 1 significantly altered plant performance measured as biomass production in Phase 2. We had four replicates of each of our 9 focal species x 49 soil-conditioning treatment combinations. Focal species, soil treatment, and their interaction were all coded as random effects since focal and conditioning species represented a sample of all possible species we could have studied. We tested whether focal plant performance in conditioned soils was significantly different from performance in sterile soil and conspecific soil using planned contrasts (function ‘glht’ from the multcomp package v. 1.4-0

(Hothorn *et al.* 2008)). We performed separate analyses for total, above-, and belowground biomass.

Is there an effect of phylogenetic relatedness and phenotypic similarity on plant-soil feedbacks?

We used mixed effects models to test for an effect of phylogenetic relatedness and phenotypic similarity between each focal species and the soil feedback response in each heterospecific species' soil. Phylogenetic relatedness and phenotypic similarity were modeled as quantitative fixed effects. We acknowledge that this method does not take into account the non-independence among pairs of species, however our data are inappropriate to perform a Mantel test on the phylogenetic distance and plant-soil feedback matrices. To test whether this relationship varied across focal species we modeled the phylogenetic relatedness x focal species and phenotypic similarity x focal species interactions as random effects. Visual assessments indicated non-uniform variance in feedback strength across either phylogenetic relatedness or phenotypic similarity. We used quantile regression (function 'rq' from the package quantreg v. 5.11 (Koenker 2009)) to test whether specific quantiles of our PSF response variable exhibited a relationship with either phylogenetic relatedness or phenotypic similarity. Quantile regression tests whether an independent variable places either a maximum or minimum bound on a response variable and thus it can identify factors that constrain ecological responses (Thomson *et al.* 1996). We used the 5%, 25%, 50% and 90% quantiles. Additionally, to test the global significance of phylogenetic relatedness and phenotypic similarity on plant-soil feedbacks we performed Fisher's combined probability test using the formula $-2 \sum_{i=1}^k \ln(p_i)$, where we sum the log p-value (p_i) for the i^{th} individual test across all k-tests. This method combines p-values to generate a statistic that approximates a χ^2 distribution (d.f. =2k) and can be used to test whether the same null hypothesis holds for a number of independent tests. We combined the p-values

obtained from testing the significance of phylogenetic relatedness and phenotypic similarity on plant-soil feedbacks using each of our focal species as an independent test.

Is there phylogenetic signal in plant traits and soil-feedback?

We quantified phylogenetic signal, the tendency for close relatives to resemble each other, in phenotypic traits and in the soil feedback measured across our soil-conditioning species. We used Blomberg's K, a measure of phylogenetic signal relative to expectations under a model of constant Brownian motion evolution across the phylogeny, which is the expectation under genetic drift (Blomberg, Garland & Ives 2003). A K of 1 indicates that the trait distribution across a phylogeny corresponds to a Brownian motion model of evolution, whereas an increase or decrease from 1 indicates evolution has caused close relatives to resemble one another more or less, respectively, than expected due to constant genetic drift. We used the function 'phylosig' from the package phytools v. 0.4-60 to calculate K (Revell 2011), which accounts for within-species variation as per Ives et al (Ives, Midford & Garland 2007). To test the significance of K we performed a randomization test whereby tip data are randomized across the phylogeny repeatedly while K is re-calculated each time to give the expected distribution of K if there were no phylogenetic signal (Blomberg *et al.* 2003). The observed value of K is then compared to this distribution to obtain a P-value. Additionally we simulated trait data across our phylogeny under a Brownian motion model of evolution to obtain a distribution of K with a mean of 1. By comparing our observed value of K to this distribution we could determine whether it was significantly different from 1.

Which plant traits predict feedback strength?

We analyzed how individual traits influenced our plant-soil feedback metric through both soil-conditioning plant effects and focal plant responses. We used phylogenetic generalized least

squares regression (PGLS) to account for the evolutionary non-independence among our plant species. We analyzed how individual traits of our 49 soil conditioning species influenced their soil feedback effect averaged across the 9 focal species ($n = 4$ for each focal species x soil conditioning species combination). We also performed the same analyses using the feedback calculated from each focal species separately. To analyze how individual traits across our 9 focal species influences their soil feedback response, we modelled how their feedback response, averaged across the 49 soil conditioning treatments, was influenced by individual plant traits. Due to our low sample size ($n=9$) we calculated phylogenetic independent contrasts for the average feedback response and each of our traits (Felsenstein 1985). We then performed linear regressions between each trait PIC and the average feedback response PIC. Additionally, to understand how phenotypic variation might be acting between species pairs (including conspecifics) to influence their soil feedback, we used the absolute trait difference between our focal and soil-conditioning species to predict variation in feedback strength. Trait differences are important in determining competitive outcomes between plant species and ultimately underlie the mechanisms promoting co-existence (Chesson 2000; Kraft *et al.* 2015). To understand how trait differences might affect soil feedbacks between plants, we calculated the absolute difference between our focal and soil-conditioning species for each of our phenotypic traits and used PGLS models to test whether trait differences were positively or negatively related to plant-soil feedbacks.

For each multiple regression PGLS model we determined whether the data fit an error structure corresponding to a Brownian motion, adaptive optimum (Orstein-Uhlenbeck), or a star phylogeny (i.e. no phylogenetic signal) model of evolution. This method accounts for non-independence occurring across our response variables due to shared evolutionary history

between species. We then performed a series of likelihood ratio tests on nested models to determine the significance of individual traits in predicting feedback strength. We built separate multiple regression models for our feedback metrics based on total biomass, above-, and belowground biomass. We used the function ‘pgls’ and ‘pic’ from the package ape v. 3.3 (Paradis, Claude & Strimmer 2004). Additionally, we tested the global significance of each phenotypic trait on plant-soil feedback effects using Fisher’s combined probability test. We combined the p-values obtained from testing the significance of each trait measured on plant-soil feedbacks using each of our focal species as an independent test.

Do measures of plant-soil feedbacks predict patterns of plant co-occurrence?

We tested whether our measured plant-soil feedback strength between a given pair of soil-conditioning and focal plant species predicts their co-occurrence in the field. We used a linear mixed effects model with average distance between a given pair of soil-conditioning and focal plant species as our response variable. We modelled feedback strength as a fixed effect, and population, focal species, and the interaction between focal species and feedback strength as random effects. We then performed a series of likelihood ratio tests on nested models to determine the significance of each of our effects. We performed separate analyses for total, above-, and belowground biomass.

Results

Do soil treatments differ in their effect on plant performance?

We detected large positive and negative plant-soil feedbacks between our soil conditioning and focal plant species (Fig. 2; Table S5, LRT: total biomass $\chi^2=104.74$, df = 1, P < 0.001). This effect explained 18% of the variation in total biomass produced by focal species (Table S5).

Even after autoclaving the soil harvested from phase 1, soil treatment explained 12% of the variation in focal plant performance in our small control experiment (see *Experimental Design* total biomass $\chi^2=14.1$, $df = 1$, $P < 0.001$). This result indicates that a portion of our measured soil feedback was due to abiotic soil conditioning. On average there was a 2.28-fold difference in aboveground biomass and 2.69 fold difference in belowground biomass across our soil treatments. For the belowground biomass we also found a significant interaction between soil treatment and focal species indicating a non-uniform response of our focal species to our soil treatments (Table S5, LRT: $\chi^2=3.5$, $df = 1$, $P = 0.05$). Our planned contrasts showed that focal species performed significantly worse in plant-conditioned soil versus sterile soil (Table S5, planned contrast: total biomass $df =46$, $t=6.00$, $P<0.001$). Additionally, 8 of 9 focal species showed no significant difference in performance when grown in conspecific versus heterospecific soil treatments (*P. pratense* performed significantly worse in conspecific soil, $df =46$, $t=3.28$, $P<0.001$ Table S5. These contrasts show that our experimental inoculation significantly altered the performance of focal species relative to sterile soil and that soil conditioning by heterospecifics led to both positive and negative effects on focal plant performance relative to conspecific soil (Fig. 2).

Is there an effect of phylogenetic relatedness and phenotypic similarity on plant-soil feedbacks?

Despite finding consistently strong feedbacks on focal species caused by many different conditioning species, we found no effect of either phylogenetic relatedness or phenotypic similarity between the focal and soil conditioning species on their soil feedback (Fig. 3, Fig. S1, Table S6). We also found no evidence of an interaction of either phylogenetic relatedness or

phenotypic similarity with focal species indicating that the effect of phylogenetic relatedness and phenotypic similarity does not depend on focal species identity (Fig. S2, Table S6). Based on the recommendations (Letten & Cornwell 2014), we also performed a square root transformation on our phylogenetic relatedness matrix, which yielded qualitatively similar results. Additionally, our global test using the combined p-values from each of our focal species failed to find any evidence for an effect of either phylogenetic relatedness or phenotypic similarity (Table S6). We did find that particular quantiles of our feedback data were significantly related to phenotypic similarity. Feedbacks were positively related with decreasing phenotypic similarity for the lower quantiles (Fig. 3b, Table S7), implying that plant performance may have been constrained at the upper limit as a result of our growth conditions and lower quantiles are better able to capture the relationship with phenotypic similarity.

Is there phylogenetic signal in plant traits and the strength of soil feedbacks?

We found significant phylogenetic signal in all of our measured traits except root angle and specific leaf area (range of K 0.37-1.21; Table S8) and phylogenetic distance and phenotypic similarity were significantly correlated (Mantel test: $r = 0.16$, $P < 0.01$, Fig. 1). We also found phylogenetic signal in the average soil feedback effect measured for each of our soil-conditioning species (Fig. 2, $K = 0.30$, $P = 0.05$) and individually for the focal species *O. biennis* (Table S8, $K = 0.33$, $P = 0.03$). These results indicate that close plant relatives cause similar effects, whether positive or negative, on the performance of other plant species via soil-feedbacks. Thus across our plant species most traits evolved in a manner consistent with Brownian motion or genetic drift. Additionally, of the traits that exhibited significant phylogenetic signal, only the feedback metric measured for each of our soil-conditioning species

averaged across all focal species and individually for *O. biennis* was significantly different from 1 (Table S8 overall: $K = 0.30$, $P < 0.001$; *O. biennis*: $K = 0.33$, $P = 0.003$). Thus, at least for one of our focal species, *O. biennis*, close plant relatives cause more similar soil feedback effects than expected under a Brownian motion model of evolution.

What individual plant traits predict feedback strength?

We found that soil-conditioning species with increasing SLA produced increasingly negative effects on the total biomass averaged across our focal species (Fig. 4, Table S9, LRT: $\chi^2 = 6.8$, $df = 1$, $P < 0.01$). Whereas soil-conditioning species with higher belowground biomass produced increasingly positive effects on the total biomass averaged across our focal species (Fig. 4, Table S9, LRT: $\chi^2 = 5.67$, $df = 1$, $P = 0.01$). Total aboveground biomass of the soil-conditioning species was also negatively related to the performance across all focal species, but this effect was marginally non-significant (Table S9, LRT: $\chi^2 = 2.97$, $df = 1$, $P = 0.07$). Our global test confirmed the importance of SLA and belowground biomass on plant-soil feedbacks (Tables S9). The performance of individual focal species was related in variable ways to the traits expressed by soil-conditioning species (Fig. 4). Different focal species, and even the aboveground and belowground components to the soil feedback within a focal species, responded uniquely to plant traits expressed by the soil conditioning species (Fig. S3, S4). For example, the aboveground biomass feedback of *O. biennis* exhibited a negative relationship with increasing SLA of the conditioning species. By contrast, the belowground biomass feedback exhibited a negative relationship with SLA and root angle but a positive relationship with SRL and belowground biomass (Fig. S3, S4).

Specific leaf area was the only plant trait that influenced the response of our focal species to soil feedbacks (Fig. 5a, Table S9). Focal species with low SLA exhibited the most negative soil feedback responses whereas species with high SLA exhibited relatively positive soil feedback responses (Fig. 5a, Table S9, PIC, $r^2 = 0.51$, $P = 0.01$). Notably, the results from a simple linear regression of the average soil feedback responses and effects across our focal species (since all 9 were used as both soil-conditioning and focal plant species) indicated a strong negative relationship (Fig. 5b, PIC, $r^2 = 0.38$, $P = 0.04$). This means that plant species with large soil feedback effects tend to exhibit only modest soil feedback responses, while plant species that exhibit strong negative responses to soil feedbacks have little soil feedback effect (Fig. 5b). However we caution that these analyses are based on low sample sizes.

We also found that trait differences significantly affected plant-soil feedbacks between species (Fig. S5, Table S9). For some traits (e.g., SLA), focal species exhibited increased performance with increasing difference from soil-conditioning species (Fig. S5, Table S9), while for other traits (e.g., LDMC) focal species exhibited reduced performance with increasing difference from soil-conditioning species (Fig. S5, Table S9).

Do measures of plant-soil feedbacks predict patterns of plant co-occurrence?

The main effect of our measured soil feedbacks between pairs of plant species was unrelated to their co-occurrence in the field (Table S10). However, we found a significant interaction between soil feedback and focal species on co-occurrence (Table S10; LRT $\chi^2 = 4.58$, $df = 1$, $P = 0.03$). For certain focal species strong negative soil feedback was correlated with reduced co-occurrence while strong positive feedback was correlated with increased co-occurrence. For example, our soil feedback measure explained 10% of the variation in distance between *T*.

pratense populations and heterospecific plant species (linear regression $t = -2.26$, $df = 35$, $P = 0.03$). This implies that the strength of soil feedback can be a determinant of co-occurrence for some plant species.

Discussion

In this study we sought to understand the role of evolution, phenotypic similarity, and plant traits in shaping plant-soil feedbacks. Several key results are most important in answering our research questions. First, we observed strong plant-soil feedbacks on the aboveground and belowground biomass of focal species. Second, we found no effect of phylogenetic relatedness or overall phenotypic similarity of the conditioning species on plant-soil feedbacks. Third, individual plant traits of conditioning species strongly influenced soil feedbacks. The most important traits seemed to be those related to plant resource consumption (SLA) and belowground interactions (BGB). Finally, we found some evidence that our measured plant-soil feedback is playing a role in structuring species co-occurrence in natural communities, though we acknowledge that numerous additional factors beyond soil feedbacks are also driving the patterns of co-occurrence that we measured.

Plant-soil feedbacks across species

We found that in just three months, soil conditioning by 49 plant species significantly affected the performance of focal individuals (Fig. 2). Conditioning by plant species lead to both strong positive and negative soil-feedbacks relative to the performance of focal species in conspecific soil (Fig. 2). For some soil conditioning species these effects were consistent across our 9 focal

species, while for others their effect via soil feedback was inconsistent or weak (Fig. 2).

Furthermore, we found evidence that our measured soil-feedbacks were influencing the co-occurrence of plant species in natural communities (Table S10). These results indicate that soil feedbacks produced by some plant species might act generally to increase or reduce the performance of neighbouring plants while others are more species specific with consequences for entire plant communities. Soil feedback specificity is thought to be important in driving succession in plant communities and maintaining biodiversity (Klironomos 2002; Kardol *et al.* 2006; Pendergast, Burke & Carson 2013). More work is needed to understand the causes and consequences of soil feedback specificity. After sterilization the effect of soil conditioning species on focal plant performance was still significant. Although sterilizing soil does not completely disentangle abiotic from biotic effects, this result suggests that the effect of our soil conditioning species was not solely due to changes in soil biota; 66% of our experimentally generated soil feedback remained even after soil sterilization.

Phylogenetic relatedness, phenotypic similarity and plant-soil feedbacks

Phylogenetic relatedness and phenotypic similarity between plant species were poor predictors of soil feedbacks (Fig. 3). Interestingly, evolutionary divergence does indeed capture some degree of ecological similarity (Fig. 1), however in our case it was unimportant for plant-soil feedbacks. Our conclusions may depend on the phylogenetic scale used in our study and with greater sampling of more closely related clades might yield stronger effect of phylogenetic relatedness on soil feedbacks (e.g. Brandt *et al.* 2009). Although recent work shows that trait differences that mediate interactions between plant competitors can saturate rapidly over evolutionary time

(Germain, Weir & Gilbert 2016). Few other studies have experimentally tested how evolutionary relatedness affects plant-soil feedbacks among plants and they report conflicting results. Burns & Strauss (2011) found that early life performance of plants was positively correlated with the phylogenetic relatedness of soil-conditioning species. In contrast, Liu *et al.* (2011) found that within a dipterocarp forest, focal plant performance was negatively correlated with the phylogenetic relatedness of a soil-conditioning species. We found that soil-conditioning plant species exhibited significant, though weak, phylogenetic signal in their soil feedback (Fig. 2). However, this did not translate into an overall effect of phylogenetic relatedness on the soil feedback between soil-conditioning and focal plant species (Fig. 3).

Plant traits and soil feedbacks

Above- and below-ground biomass and specific leaf area of soil-conditioning plant species had strong effects on the performance of numerous focal species via soil feedbacks (Fig. 4). The effect of increasing aboveground biomass in soil-conditioning plant species on focal plant performance was negative, potentially due to the depletion of soil resources required for increased growth. Though increased belowground biomass should result in a reduction of soil resources, potentially leading to a negative soil feedback, we actually detected a positive effect of increasing belowground biomass in soil-conditioning species. Roots actively and passively release many compounds (e.g. organic acids, amino acids, polysaccharides) into the soil potentially acting to increase the availability of limiting soil nutrients (Bertin, Yang & Weston 2003). Increased chemical deposition in soil associated with greater belowground biomass could also promote the establishment of beneficial microorganisms and stimulate their activity (Bertin,

Yang & Weston 2003). This finding indicates that although above and belowground biomass are both influenced by overall growth rate and resource acquisition, they may have different effects on the soil environment with opposing consequences for soil feedbacks. Specific leaf area of our soil conditioning species was negatively correlated with total biomass across all focal species (Fig. 4). Plants with greater SLA typically have higher resource use and relative growth rates (Grime *et al.* 1997; Westoby 1998); therefore, plant species with high SLA may deplete soil resources, potentially reducing the performance of individuals growing in the same soil. A previous study that found soil conditioned by fast-growing plant species tended to have positive soil feedback effects (Baxendale *et al.* 2014). This suggests that while of general importance, the soil feedback effect of plant growth strategies may vary according to any number of ecological factors including the particular abiotic and biotic soil environment and the species identity of the plants involved. Interestingly, focal species with high SLA were also the least sensitive to the negative effects of soil conditioners (Fig. 5a), which presents the intriguing hypothesis that SLA may be shaping both plant species effects on, and responses, to soil feedbacks (Fig. 5). Our results suggest that plant species with strong negative effects via soil feedbacks also respond with reduced sensitivity to the negative consequences of heterospecific soil-conditioning (Fig. 5b). This is in contrast to other studies which have found no correlation in the effect and response among plants under competition (Cahill, Kembel & Gustafson 2005). More work is needed to understand the mechanisms underlying the influence of these traits on effects and responses in plant-soil feedbacks.

Trait differences between soil-conditioning and focal plant species led to both positive and negative soil feedbacks, depending on the trait and species identity (Fig. S5, Table S9).

Baxendale *et al.* (2014) also found that trait differences between soil-conditioning and focal plant

species could lead to both positive and negative soil feedbacks. The idea that trait differences among species govern ecological dynamics is pervasive in ecology (Ricklefs & Travis 1980; Kraft, Valencia & Ackerly 2008; Ingram & Shurin 2009). Yet demonstrations of how trait differences between interacting species can affect the outcome of interactions across numerous species and traits are rare (but see Kraft *et al.* 2015). Trait differences that lead to complementary soil resource partitioning or the recruitment of beneficial soil biota would result in a positive effect on the soil feedback between interacting plant species, whereas trait differences that lead to unequal resource acquisition or the recruitment of harmful soil biota would result in a negative soil feedback (Bever, Westover & Antonovics 1997). Although these are only speculative mechanisms, our data are consistent with both scenarios and show that even for the same trait, differences between soil conditioning and focal species can lead to both positive and negative effects depending on species identity (Fig. S5). We also note that our measure of trait difference excludes phenotypic plasticity expressed by our focal species in response to conditioned soil, which could be important in determining the soil feedback outcome (Burns & Strauss 2012; Baxendale *et al.* 2014). Trait differences between plant species contribute to the mechanisms that promote and constrain coexistence (Adler *et al.* 2013; Kraft *et al.* 2015). Trait differences may act through soil feedbacks on plant performance to both promote and constrain coexistence between plant species (Casper & Castelli 2007; Baxendale *et al.* 2014; Chung & Rudgers 2016). Our finding that measured plant-soil feedbacks can scale up to influence plant species co-occurrence in natural communities broadly supports this idea. Understanding how traits differences can yield positive and negative outcomes for interacting plants via soil feedbacks is an important avenue for future research.

Our experiment links plant trait variation and soil feedbacks. Recent meta-analyses show that different plant functional groups (grasses, forbs, shrubs, and trees) exhibited varying effects on, and responses to, soil communities (Kulmatiski *et al.* 2008; Meisner *et al.* 2014). However, explicitly incorporating traits into plant-soil feedbacks has not been a focus of past study (but see Baxendale *et al.* 2014). Numerous studies have documented the species-specific effect of plants on both biotic and abiotic soil properties (Bever *et al.* 1997; Klironomos 2002; Kardol *et al.* 2006; Bezemer *et al.* 2006; Berg & Smalla 2009; van de Voorde, van der Putten & Martijn Bezemer 2011) but few link these to plant traits (but see Wardle *et al.* 1998; Orwin *et al.* 2010). Experiments undertaken to understand the role of plant phenotypic variation on ecosystem variables such as litter decomposition (Grime *et al.* 1996), nutrient cycling (Wedin & Tilman 1990), and primary productivity (Hobbie 2015) have contributed to our understanding of ecosystem ecology. Similar experiments that comprehensively test the role of phenotypic traits across plant species in driving soil change and linking these to feedbacks on plant performance are needed to build a more complete understanding of terrestrial plant ecology.

Synthesis

We found that plant-soil feedbacks are ubiquitous and can influence plant species co-occurrence in natural communities but their strength and direction can vary across plant species (Fig. 2). We found that plant-soil feedbacks are not determined by phylogenetic relatedness or measures of overall phenotypic similarity at a broad range of phylogenetic scales (Fig. 3). Instead individual traits of both soil-conditioning and focal plant species strongly shape the outcome of soil feedbacks (Fig. 4, 5). The ability of phylogenetic relatedness or phenotypic similarity to predict

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plant-soil feedbacks may vary among species for several reasons (Fig. 6). First, measures of phenotypic similarity are limited by the traits used to generate them. Although some of the traits measured affected soil feedbacks, there are likely other equally important traits that we did not measure. Second, the plant traits responsible for generating soil feedbacks may exhibit varying degrees of phylogenetic signal (Fig. 6b). Third, separate trait differences between soil-conditioning and focal species can have opposing effects on their soil feedback, depending on the trait (Fig. S5). If two ecologically relevant traits, both with phylogenetic signal, influence soil feedbacks but with opposing effects, then the overall effect of phylogenetic relatedness and phenotypic similarity will be reduced (Fig. 6c). Our results are consistent with a growing body of studies that find no pattern in the strength of interaction between pairs of species and phylogenetic relatedness (Cahill *et al.* 2008; Best, Caulk & Stachowicz 2012; Narwani *et al.* 2013; Godoy *et al.* 2014). The role of evolution in shaping contemporary ecological dynamics becomes more nuanced when species interactions are shaped by multiple traits with unique evolutionary histories (Mayfield & Levine 2010), as shown in the current study. Overall, indices such as phylogenetic relatedness and phenotypic similarity may offer limited ability to predict soil feedbacks, at least at large phylogenetic scales. This is not to say that macroevolutionary processes have little impact on contemporary interactions between plants and soil. On the contrary, the processes underlying trait variation are ultimately the cause of any ecological interaction driven by phenotypes. Instead, continued investigation of the plant traits involved in soil feedbacks and their underlying mechanisms and evolutionary history will lead to a better understanding of plant-soil feedbacks and their influence on plant communities.

Author's contributions

CRF, PMK, and MTJJ conceived the ideas and designed methodology; CRF and LG performed the experiment and collected the data; CRF analysed the data; CRF led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Plant-soil feedback data, trait measurements, field surveys, and phylogenetic tree available from the Dryad data repository (Fitzpatrick 2016).

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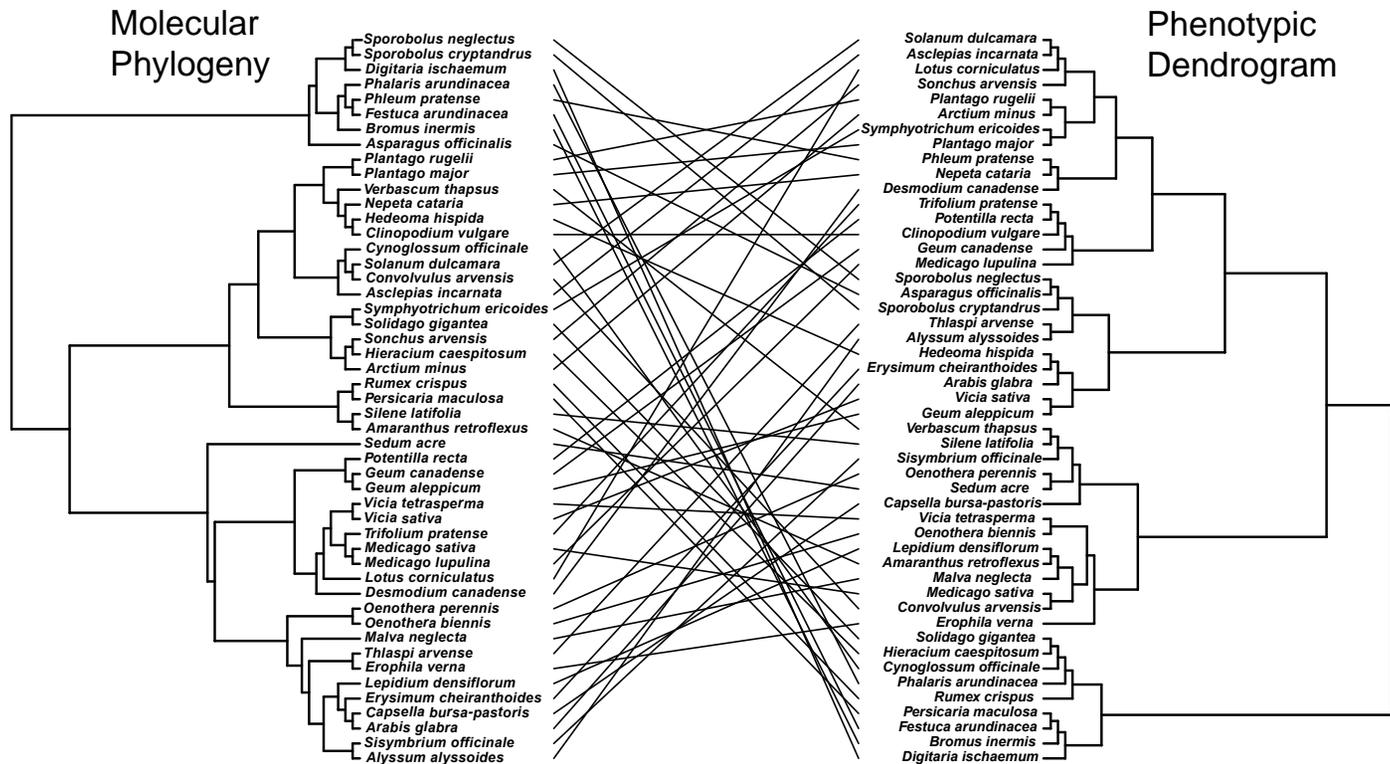


Fig. 1| Phylogenetic relatedness and phenotypic similarity are positively associated (Mantel test $r = 0.16$, $P < 0.01$). We used Bayesian inference to estimate phylogenetic relatedness among our species using three nuclear and plastid genes. We quantified phenotypic similarity between species using the Euclidean distance across all measured traits. For visualization purposes we have displayed phenotypic similarity as a dendrogram using cluster analysis with average Euclidean distance used to define clusters. We measured 8 phenotypic traits that influence and respond to changes in the soil environment. Plant species are connected between the phylogeny and phenotypic dendrogram by lines. We optimized the congruence between the two trees in R using the package ‘ape’ (Paradis *et al.* 2004).

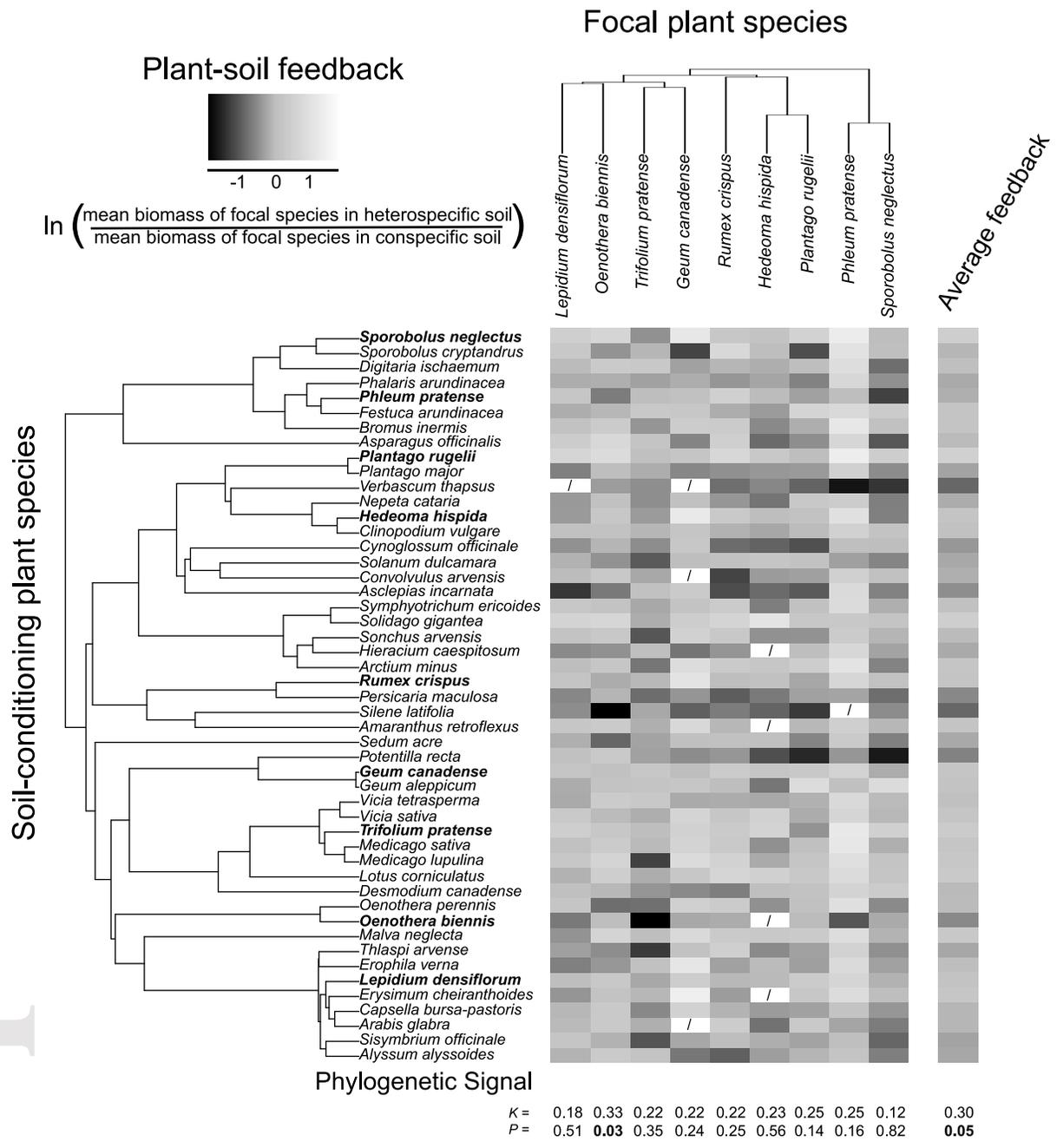


Fig. 2 | Plant-soil feedback caused by each of the 49 conditioning species across each of the 9 focal species plotted against phylogenetic structure (bolded taxa = focal species). Cells with slashes represent combinations without data. The last column in the heatmap is the soil feedback averaged across our 9 focal species. The average soil feedback and the soil feedback measured for *Oenothera biennis* exhibit significant phylogenetic structure represented by Blomberg's K.

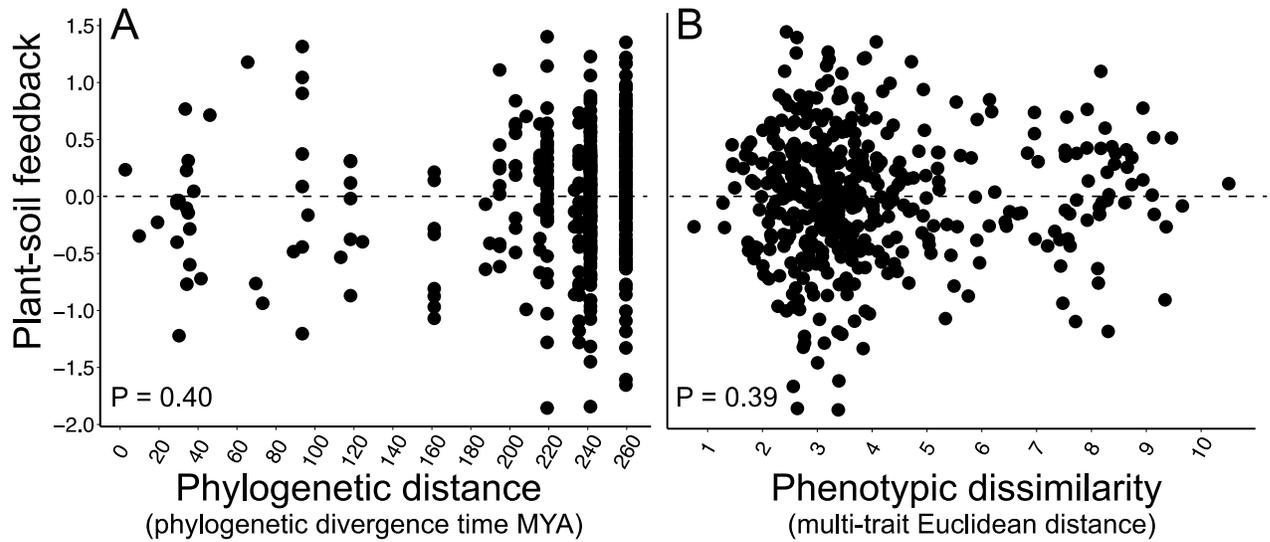


Fig. 3 | Relationship between plant-soil feedback measured with total biomass and (A) phylogenetic relatedness or (B) phenotypic similarity. There is no relationship between plant-soil feedback and either phylogenetic or phenotypic similarity despite significant phylogenetic signal in the plant-soil feedback. Each point represents the average plant-soil feedback experienced by a focal species when grown in soil conditioned by another species. Increasing values along the x-axis represent increasingly distant relatives (A), or phenotypically dissimilar plants (B). We did find significant relationships between phenotypic similarity and particular quantiles of soil-feedback (see *Results*). We found qualitatively similar patterns when we analyzed aboveground and belowground biomass components to the plant-soil feedback separately (Fig. S4).

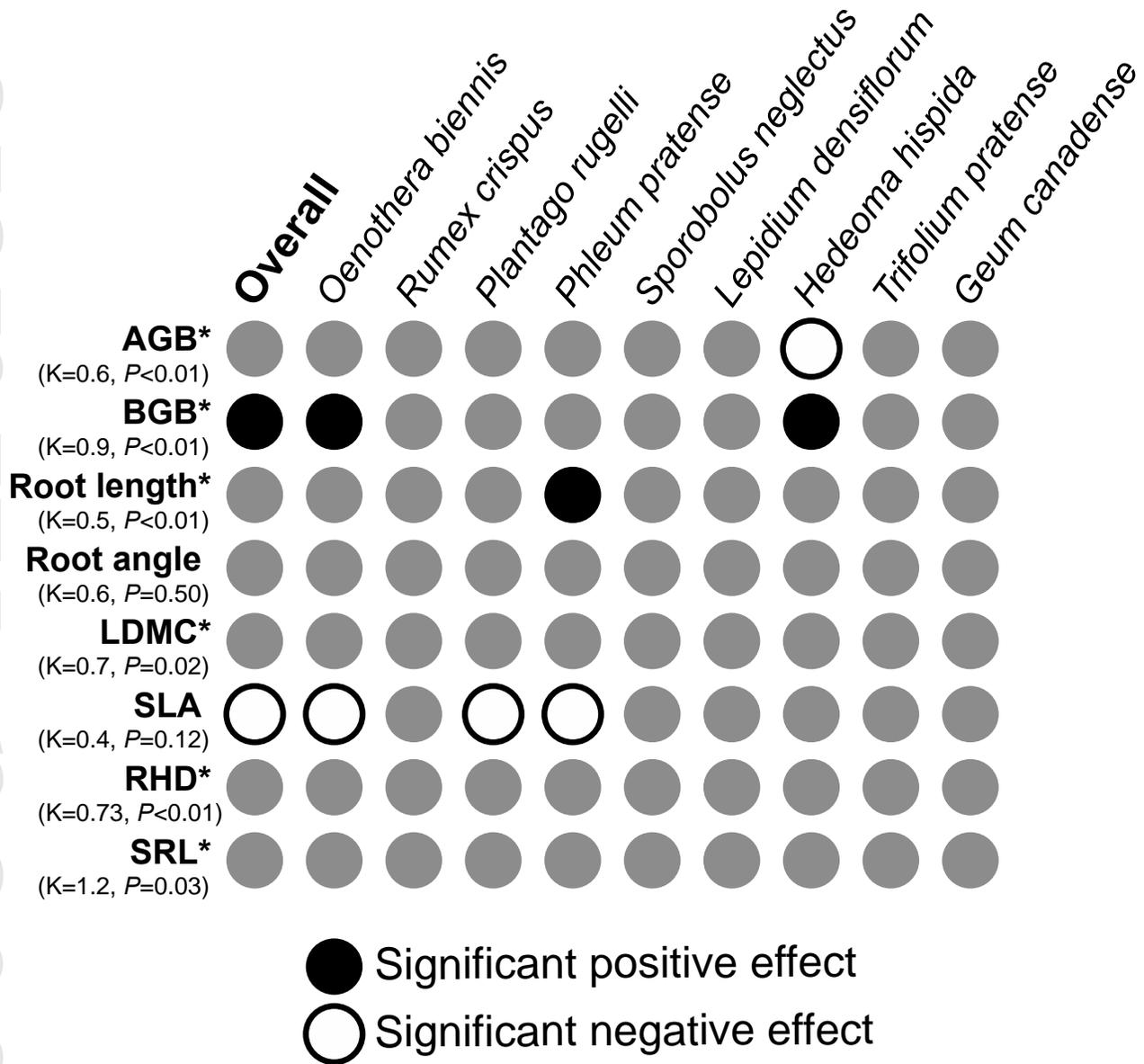


Fig. 4 | The effect of plant traits of conditioning species on the performance of focal species measured as total biomass (significance, $P < 0.05$). We used phylogenetic generalized least squares multiple regression to calculate the effect of individual traits of soil-conditioning species on the plant-soil feedback averaged across all focal species (overall) and each focal species individually (Table S6). We calculated K, accounting for within-species variation, to quantify phylogenetic signal in each of our measured traits, where * indicates significant phylogenetic signal ($P < 0.05$). Traits: AGB, aboveground biomass; BGB, belowground biomass; LDMC, leaf dry matter content; SLA, specific leaf area; RHD, root hair density; SRL, specific root length. We observed similar trends when we analyzed above- and belowground biomass components of the plant-soil feedback separately (Fig. S1, S2).

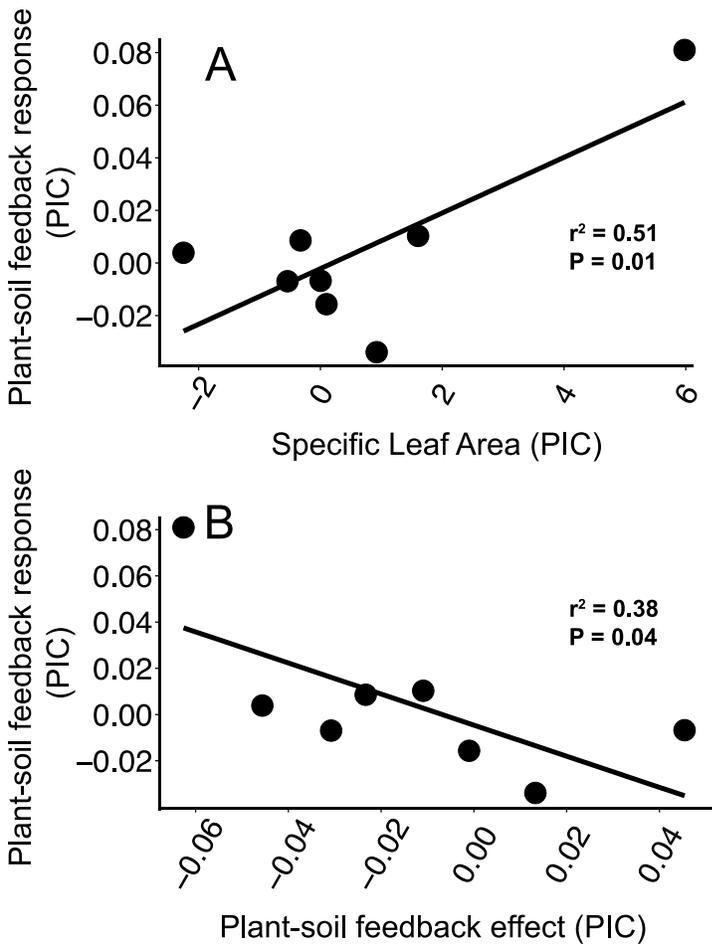


Figure 5 | Focal species with higher SLA exhibit increasingly positive overall responses to soil feedbacks (A). Plant-soil feedback responses are negatively correlated with effects (B), i.e. plant species that exhibit negative effects via soil feedbacks also exhibit more positive responses to the effect of heterospecific soil-conditioning. In panel A the x-axis displays the phylogenetic independent contrasts of SLA across our 9 focal species and in panel B the x-axis displays the phylogenetic independent contrasts of soil-feedback effects across our 9 focal species. In both panels the y-axis displays the phylogenetic independent contrasts of soil-feedback responses across our 9 focal species.

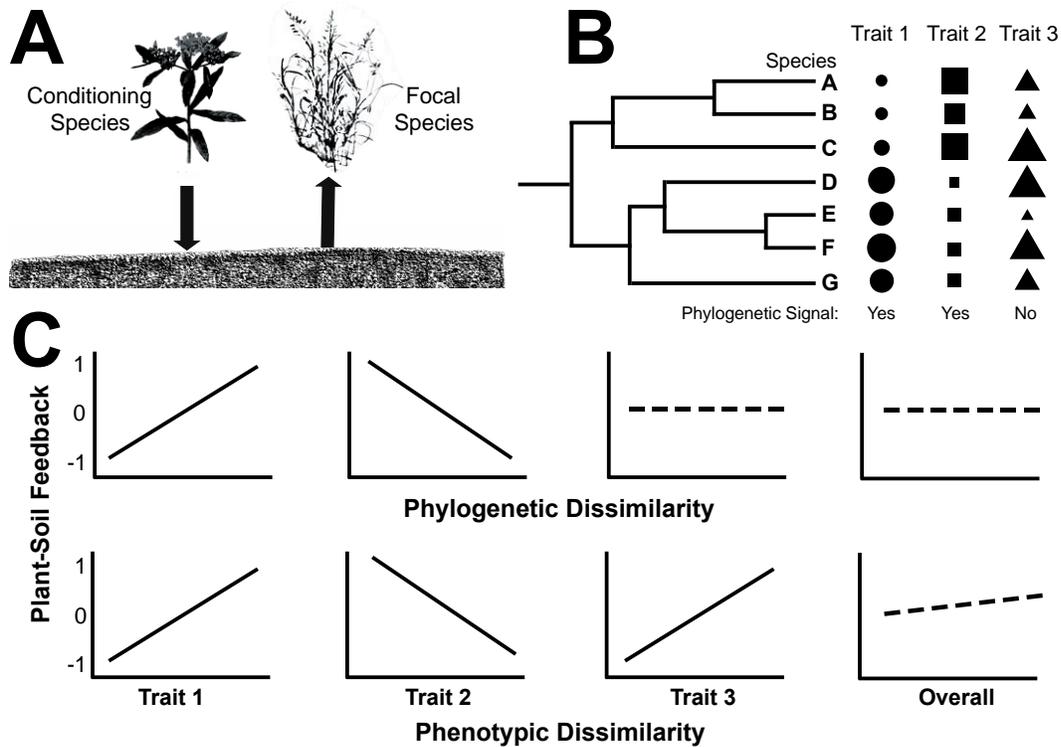


Figure 6 | A conceptual figure demonstrating how phylogenetic relatedness and phenotypic similarity can have no overall effect on a species interaction despite there being significant phylogenetic signal in traits that predict the interaction. (A) Soil feedbacks occur when a soil conditioning species alters the soil environment, which then affects the performance of a focal species. (B) Plant-soil feedbacks are mediated by evolving phenotypic traits, which may exhibit varying degrees of phylogenetic signal. (C) The effect of phylogenetic relatedness and phenotypic similarity on plant-soil feedbacks under 4 models. Trait 1: the trait exhibits significant phylogenetic signal and individuals with similar trait values experience a more negative interaction (positive effect of trait difference). As an example, species with a dissimilar resource acquisition trait may exhibit a positive soil feedback because they are complementary. This yields a positive relationship between phylogenetic distance, phenotypic dissimilarity and soil feedback. Trait 2: the trait exhibits significant phylogenetic signal and individuals with different trait values experience a more negative interaction (positive effect of trait matching). As an example, species with similar values for a trait that recruit and/or maintains specific soil mutualists may exhibit a positive soil feedback, relative to species that do not share these traits. This yields a negative relationship between phylogenetic distance, phenotypic dissimilarity and feedback. Trait 3: the trait exhibits no phylogenetic signal and regardless of the mode of action (trait matching or difference) there is no relationship between phylogenetic relatedness and feedback despite a relationship between phenotypic similarity and feedback. Overall: if these traits are acting simultaneously to influence a plant-soil feedback between species pairs than the overall effect of phylogenetic relatedness and phenotypic similarity will disappear. Varying phylogenetic signal, opposing effects of trait differences, and the simultaneous action of multiple traits potentially varying across species will all act to erode the overall effect of phylogenetic relatedness and phenotypic similarity.