Phylogenetic trait conservatism predicts patterns of plant-soil feedback

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Abstract. Plant-soil feedbacks (PSFs) are important drivers of plant community structure and diversity, with species varying in the way they both condition soils and respond to them. While plant phylogenetic relationships alone can predict this variation in some instances, trait conservatism across phylogenies may provide more reliable predictions. Using integrated common garden and glasshouse inoculation experiments including 13 *Eucalyptus* species across two subgenera, we specifically investigated soil microbial conditioning and root chemical traits as underlying drivers of phylogenetic differences in PSF. We found that eucalypt species responded variably to soils conditioned by closely related species, depending on their phylogenetic lineage, which was further related to root terpene concentrations and the presence/absence of specific fungal taxa in conditioned soils. Overall, these findings show that trait conservatism in root chemical traits and the subsequent conditioning of soil microbial communities can explain whether or not plants show phylogenetic patterns in PSF.

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INTRODUCTION

Interspecific variation in plant-soil feedback (PSF) is a major contributor to vegetation community structure, diversity, and dynamics across a broad range of ecological settings (van der Putten et al. 2013), but there is currently little predictive power to understand variation in PSF (but see Kulmatiski et al. 2008, Anacker et al. 2014, Bennett et al. 2017). Plant ecological interactions often exhibit phylogenetic signal, where closely related taxa tend to interact with their biotic and abiotic environments more similarly than expected at random (Hill and Kotanen 2011,

Reinhart et al. 2012, Senior et al. 2013, Wooliver et al. 2016). Evidence also suggests that plant evolutionary history can shape PSF, and this can occur in two ways. First, closely related species with shared evolutionary history (i.e., phylogenetic lineages) can exhibit similar feedbacks to growth within conspecific conditioned soils relative to heterospecific conditioned soils (Anacker et al. 2014). Second, the degree of phylogenetic distance between a species experiencing a conditioned soil and the soil conditioning species can also influence the magnitude of feedbacks due to variation in conserved traits with increasing phylogenetic distance. Recent studies suggest that

such an effect may contribute to observed patterns of phylogenetic overdispersion in plant communities (Liu et al. 2012, Kempel et al. 2018), where neighboring species are less phylogenetically related than expected by chance (Liu et al. 2012, Zhu et al. 2015). In such cases, the accumulation of pathogens in the soils of adults could reduce the survival of conspecific seedlings and even those of closely related species. For instance, Liu et al. (2012) found that the relative survival of eight subtropical rainforest species grown in soils collected from beneath *Castanopsis fissa* trees increased with their increasing phylogenetic distance to *C. fissa*.

While studies have begun to uncover the variable role of evolutionary history in influencing PSF (Anacker et al. 2014, Mehrabi et al. 2015, Fitzpatrick et al. 2017), a better understanding of the mechanisms underlying phylogenetic patterns in PSF is critical to advancing the field. Recent work suggests that evolutionary history can sometimes predict variable PSF (Liu et al. 2012, Anacker et al. 2014, Münzbergová and Šurinová 2015, Kempel et al. 2018), but there are also instances where it may not (Mehrabi and Tuck 2015, Mehrabi et al. 2015, Fitzpatrick et al. 2017). Such differences likely relate to varying degrees of phylogenetic trait conservatism (the tendency of lineages to retain traits through speciation events; Crisp and Cook 2012) in plant traits related to PSF. For instance, if trait differences increase with phylogenetic distance between focal and conditioning species (i.e., traits are phylogenetically conserved), feedbacks should show a pattern of phylogenetic conservatism. However, if trait differences do not vary with phylogenetic distance between focal and conditioning species (i.e., traits are not phylogenetically conserved), feedbacks should not show a pattern of phylogenetic conservatism. Thus, an understanding of the plant traits and mechanisms driving PSF are required to better predict PSF.

Studies that use inoculation and sterilization procedures are increasingly identifying the conditioning of soil microorganisms as the predominant mechanism driving PSF (Kulmatiski et al. 2008, Brinkman et al. 2010), which in conjunction with plant traits may guide phylogenetic patterns in PSF. For plant phylogenetic signal to occur in plant-microbial feedbacks, plant lineages must both distinctly condition and respond to conditioned communities. Field experiments suggest that plant phylogenetic lineages can condition distinct soil microbial communities (Tedersoo et al. 2013, Burns et al. 2015), which likely reflects underlying variation in ecologically important traits such as plant growth strategy and chemistry (van Nuland et al. 2016). Plant secondary chemistry has been implicated as an underlying driver of many plant ecological interactions aboveground (Johnson et al. 2014, Carrillo-Gavilán et al. 2015), that may also extend to belowground interactions (Schweitzer et al. 2008). For example, plant roots contain a range of plant secondary metabolites implicated in plant resistance to herbivores and pathogens (Rasmann and Agrawal 2008). Previous work has shown significant phylogenetic signal in the presence and concentrations of root secondary metabolites (Vannette and Rasmann 2012, Senior et al. 2016), which may lead to variation in resistance to host-specific pathogens, and consequently, differing direction or magnitude of pathogen-mediated PSF.

Australian trees within the genus *Eucalyptus* represent an ideal system to investigate the mechanistic basis of phylogenetic signal in PSF. On the island of Tasmania, Australia, eucalypt species typically coexist in mixed stands that tend to include at least one species from each of the two larger eucalypt subgenera, *Eucalyptus* and *Symphyomyrtus* (Austin et al. 1983, Duff et al. 1983). Many key ecological differences between the subgenera are suggested to maintain this coexistence, including differences in germination, growth, biomass allocation and susceptibility to mammalian herbivores, insect pests, and fungal pathogens (Davidson and Reid 1980, Stone et al. 1998, Eschler et al. 2000). The subgenera differ in root chemistry (Senior et al. 2016), and there is also evidence that they differ in their relationships with soil pathogens and mycorrhizae (Podger and Batini 1971). This suggests that phylogenetic differences in PSF should also be considered as a potential driver of eucalypt community dynamics and coexistence.

Utilizing 14 eucalypt species representing the subgenera *Eucalyptus* and *Symphyomyrtus*, we conducted a fully factorial experiment assessing the survival and growth responses of each species grown in potting soil inoculated with soils previously conditioned by each species. We used an

inoculation-based method to exclude the influence of conditioning effects on soil chemical properties (Brinkman et al. 2010). We hypothesized that (1) eucalypt species exhibit variable responses to increasing phylogenetic distance between themselves and soil conditioning species, (2) closely related species would respond similarly to increasing phylogenetic distance between themselves and the soil conditioning species (i.e., exhibit phylogenetic signal), (3) the responses of species to heterospecific vs. conspecific conditioned soils are related to host-specific soil microbial organisms, and (4) the abundance of host-specific soil organisms in conditioned soils is related to the root chemistry of soil conditioning species. Herein, we show that eucalypt species can condition soil microbial communities and respond to these communities variably depending on their phylogenetic lineage. These patterns were related to phylogenetic conservatism in root chemistry and the conditioning of soil fungi. By incorporating phylogenetically conserved traits and next generation sequencing into our PSF study, we provide important insight into why phylogenetic relatedness can predict PSF in some plant groups, but not others.

Methods

Soil conditioning experiment

To determine whether eucalypt species differentially condition soil microbial communities, soils were sampled from a replicated and randomized common pot experiment. In 2010, the open-pollinated seed (obtained from one to six individual maternal trees from a single locality) of the 14 species used in this study was purchased from Forestry Tasmania (http://www.forestrytas.com.au/), surface-sterilized and cold-stratified in damp paper towel for 30 d at 4°C. Seeds were then germinated for three weeks in commercial potting soil consisting of eight parts composted pine bark and three parts coarse river sand with added macro- and micronutrients from Osmocote For Natives low-phosphorus, slow-release fertilizer (Scotts Australia Pty Ltd, Baulkham Hills, New South Wales, Australia); this potting soil was consistently used throughout all aspects of the experiment. The potting soil included approximately 1.92, 0.16, 1.09 g/kg dry mass of nitrogen (N), phosphorus (P) and

potassium (K), respectively, that was slow-released over approximately six months. No additional fertilizer was applied during any part of the experiment. We did not sterilize the potting soil because we suspected either steam sterilization or autoclaving would release the slow-release fertilizer, potentially affecting the conditioning of soils or causing an increased growth response that would mask seedling responses to conditioned soils in the feedback phase of the experiment. However, being a highly artificial substrate, it likely contained only a very basic and consistent community of generalist microorganisms (e.g., saprotrophs). Twelve seedlings of each species were then transplanted separately into sterile 200-mL pots filled with potting soil. After 6 months, six individuals of each species were planted, spaced evenly apart, in each of two replicate sterile 33-L pots. These pots were organized into a completely randomized design and watered via a sprinkler system as required. The soil conditioning experiment was located in a fenced area adjacent to a patch of native eucalypt forest dominated by *Eucalyptus pulchella* (subgenus *Eucalyptus*) and *Eucalyptus globulus* and *Eucalyptus viminalis* (subgenus *Symphyomyrtus*) and thus was likely exposed to colonization by specialist microorganisms (i.e., eucalypt pathogens and mutualists). After two years growth, when some pots were beginning to show signs of becoming pot bound, samples of conditioned soils were obtained from two replicate pots from five species belonging to subgenus *Eucalyptus* and nine species belonging to subgenus *Symphyomyrtus*. Specifically, three soil cores (2×15 cm) were taken from each pot and pooled. These 28 samples were then sieved (2 mm) in order to homogenize and remove any root material. Each sample was divided into two portions: one for DNA extraction and the other for NO_3^- and NH_4^+ quantification (see "Soil NO_3^- and NH_4^+ assays"). Portions intended for NO_3^- and NH_4^+ assays were refrigerated at 4°C for <48 h before quantification, while portions intended for DNA extraction were stored at −80°C. Shortly after sampling, the same pots were harvested to provide inoculum for the PSF experiment.

DNA extraction and pyrosequencing

To determine whether eucalypt species condition different soil microbial communities, soil

microbial DNA was extracted from each sample of conditioned soil and purified using the MoBio PowerSoil DNA Isolation Kit according to the manufacturer's instructions (MoBio, Solana, California, USA). DNA extracts were sent to Molecular Research LP (Mr DNA), Shallowwater, Texas, USA, for PCR amplification and sequencing. PCR amplification of the bacterial 16S rRNA genes was carried out using the 27F-519R primer pair (Lane 1991), and the amplification of the fungal ITS region was conducted using the ITS1-ITS4 primer pair (Gardes and Bruns 1993). For both primer sets, a single-step 30-cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, California, USA) was performed under the following conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min. The 16S and ITS PCR products were then sequenced separately using the 454 FLX titanium platform (Roche, Branford, Connecticut, USA), according to the company protocols. Raw data were provided in the form of standard flowgram format (sff). Sequence analyses were performed on 16S rRNA gene and the ITS region separately using MOTHUR version 1.22.0 (Schloss et al. 2009) following the adapted sequence quality control pipeline analysis described in detail in Schloss et al. (2011). Briefly, sequences were error-checked using the using shhh.flows command, the MOTHUR implementation of the PyroNoise algorithm (Quince et al. 2009). The resulting sequences were then trimmed to remove barcodes and primers, and sequences with ambiguous characters or more than eight homopolymers were discarded. This was followed by dereplication for ease of computation. Chimera checking with the UCHIME algorithm (Edgar et al. 2011) via the chimera.uchime command allowed the removal of any other erroneous sequences, and sequences were preclustered at 1% to account for 454's titanium instrument error rate (Huse et al. 2010). Remaining sequences were then clustered into operational taxomomic units (OTUs) of 97% sequence similarity using USEARCH (Edgar 2010). This generated an average of 6942 ± 1714 (median value of 6691) fungal OTUs and an average of 4700 ± 1018 (median value of 4505) bacterial OTUs per conditioned soil. Operational taxomomic units were classified against Green Genes (DeSantis et al. 2006) for 16S and UNITE (Kõljalg et al. 2005) for fungal ITS using the RDP classifier (Wang et al. 2007) as implemented in MOTHUR with a 60% sequence similarity threshold.

Soil NO_3^- and NH_4^+ assays

We quantified the NO_3^- and NH_4^+ concentrations of conditioned soils to determine whether variable microbial conditioning was related to variation in soil nutrient content. Eight grams of each soil sample was weighed into 50-mL centrifuge tubes and combined with 40 mL of 2 mol/L KCl solution. The centrifuge tubes were agitated for one hour on an orbital shaker and centrifuged at $2500 \times g$ for three minutes to precipitate particulate matter from the extracts. Five milliliters of each extract was then drawn with separate syringes and filtered through 25-mm nylon (0.45 μm pore size) syringe filters into collection containers. The NO_3^- and NH_4^+ content of extracts was quantified using a Smartchem discrete analyzer (Westco Scientific Instruments, Brookfield, Connecticut, USA), using the Smartchem 200 methods 375-100E-1 and 210-200B for NO_3^- and NH_3^+, respectively.

Feedback experiment

For the PSF experiment, eucalypt seedlings were grown from the same seed purchased from Forestry Tasmania that was used in the conditioning experiment, meaning that the genetic material originated from the same localities. The seed of each focal species was cold-stratified before sowing to enhance germination, as described above. Seed was then sprinkled over trays containing vermiculite and grown for approximately 5 weeks until individuals of each focal species had developed the first pair of true leaves.

To test the responses of focal species to conditioned soils, seedlings from each of the 14 focal species were grown in potting soil inoculated with each conditioned soil in a fully factorial greenhouse experiment. The design consisted of 28 soils (14 conditioning species × two replicate pots) and 14 focal species by three seedlings of each focal species (which were ultimately averaged to provide better estimates of the effects of inocula on each species), totaling to 1176 seedlings. For each of the 28 conditioned soils, forty-two 200-mL forestry tubes (14 focal species × 3 seedlings) were filled three quarters full with

commercial potting soil; the top quarter of each forestry tube was left empty to prevent cross-contamination between tubes during watering. The same two-year-old soil conditioning pots that were used to test for conditioning were harvested at random over a period of one week. The soil of each pot was sieved through a 1-cm^2 mesh so that fine root fragments could pass and root-associated microbes were included in the soils. Each soil was then homogenized to create a uniform mixture for the inoculation of seedlings. Two teaspoons of inoculum soil (approximately 5% potting soil volume) was then spread over the soil surface of each of the 42 forestry tubes. Three seedlings of each focal species were transplanted directly into the inoculum of each conditioning pot, each within a separate forestry tube. Forestry tubes were organized into a randomized complete block design consisting of three replicates, with each conditioned soil by focal species combination represented once in each block. During the following week, dead seedlings were replaced. Thereafter, replacement ceased, as deaths may have been due to treatment effects. The height of seedlings was then recorded every three weeks until the conclusion of the experiment and converted to growth rates using the slope of the regression of age and height. The experiment received water as required and a natural photoperiod (9–10.5 h). We estimate the greenhouse temperature ranged between 7°C and 20°C over the duration of the experiment.

After four months of growth (before seedlings became pot bound), the surviving seedlings were harvested to test for biomass feedbacks. Seedlings were carefully removed from their forestry tubes, with soil gently shaken and massaged off the roots. The roots were rinsed to wash off any remaining soil. Seedlings were dried at 60°C for 48 h and then weighed.

Statistical analysis

To address the hypothesis that (1) eucalypt species exhibit variable responses to increasing phylogenetic distance between themselves and soil conditioning species, we examined the influence of phylogenetic distance between focal and soil conditioning species on PSF ratios calculated for each focal species. We found that variation in the depth of potting soil within forestry tubes, resulting from variation among planters (e.g.,

how firmly soil was pressed around seedlings), had a significant effect on seedling survival and growth traits (data not shown). We accounted for this by using the residuals of each seedling trait from a regression on soil depths in our calculations of response ratios. After first detecting a significant soil conditioning species by focal species interaction in the performance of seedlings in linear mixed effects models with pot included as a random term (growth rate data; $F_{179, 621} = 2.9$, $P = 0.001$), we calculated feedback ratios in a fully factorial manner comparing the performance (survival, growth rate, and total biomass) of each of the 14 focal species growing in potting soils inoculated with each of the 13 heterospecific conditioned soils. Specifically, we calculated feedback ratios as the average performance value of a focal species growing in potting soil inoculated with a different species' conditioned soils (averaged across both conditioned soils and three seedlings per conditioned soil) divided by the average performance value of that focal species growing in potting soil inoculated with its own conditioned soils (averaged across both conditioned soils and three seedlings per conditioned soil). Thus, values >1 implied negative PSF, while values <1 implied positive PSF. We calculated PSF ratios in this simple manner so that it would be easier to interpret relationships between the feedback ratios of each eucalypt species in different conditioned soils and increasing phylogenetic distance between themselves and soil conditioning species (first hypothesis). This led to a total of 13 feedback ratios for each of the 14 focal species and performance traits. For each individual focal species and trait, we then regressed the 13 feedback ratios against phylogenetic distance (values obtained from a previously published tree in Senior et al. 2016) between the focal and each soil conditioning species (ranging from 0.08 between *Eucalyptus cordata* and *Eucalyptus urnigera* up to 1.99 between *Eucalyptus amygdalina* and *Eucalyptus dalrympleana*) to obtain the slope (β) and significance of each relationship. A positive slope is interpreted as a species performing better as phylogenetic distance between itself and soil conditioning species increases, while a negative slope is interpreted as a species performing more poorly as phylogenetic distance between itself and soil conditioning species increases. These regression

coefficients were later used to test whether closely related species respond similarly to increasing phylogenetic distance between themselves and the soil conditioning species. While the phylogenetic distances were clustered due to long branch lengths between *Eucalyptus* and *Symphyomyrtus* subgenera, the slope of a linear model was the most intuitive metric to quantify the response of each species to increasing phylogenetic distance to soil conditioning species. *Eucalyptus perriniana* seedlings were removed from this analysis due to high mortality and thus low sample size, during the experiment. Above- and belowground biomass relationships were not included in the analyses separately as they exhibited similar trends to total biomass. All regressions were implemented as linear models in the statistical package R (R Core Team 2018; v. 3.5.0) using the package stats, which is included as part of R.

To address the hypothesis that (2) closely related species would respond similarly to increasing phylogenetic distance between themselves and the soil conditioning species (i.e., exhibit phylogenetic signal), we mapped the regression slopes of relationships between the survival, growth rate, and total biomass feedback ratios and phylogenetic distance to conditioning species for each species onto a previously published molecular phylogeny of the Tasmanian eucalypts (Senior et al. 2016) and calculated Blomberg's *K* (Blomberg et al. 2003) using the function phylosig within the R package phytools (Revell 2012, v. 0.6-44). The *K* statistic is a measure of phylogenetic signal or the tendency of closely related species to resemble each other more than they resemble species drawn at random from a phylogenetic tree (Blomberg et al. 2003). Values <1 imply that close relatives resemble each other less than expected under Brownian motion (random) evolution, while values >1 indicate that closely related species are more similar than expected under this model (i.e., phylogenetic trait conservatism; Losos 2008). In our calculations of *K*, we incorporated the standard errors of the linear regression coefficients to account for measurement error and/or within species variation in our estimates of plant biomass, growth rate, and survival (Ives et al. 2007). Phylogenetic signal in the feedbacks was presented by mapping species regression slopes

onto the eucalypt phylogeny using the function plot.phylo within the package ape (Paradis et al. 2004; v. 5.1).

We determined whether soil microbial communities differed between subgenera through multivariate analyses using the software PRIMER v.6 with the PERMANOVA+ add on (Plymouth Marine Laboratory, Plymouth, UK). Multivariate analyses of fungal and bacterial communities were conducted at the taxonomic levels of OTU and family. Both fungal and bacterial community datasets were converted to presence/absence data so that all microbial taxa had equal weighting and subsequent analyses would be more likely to detect host-specific soil microorganisms potentially driving PSFs (i.e., pathogens/mutualists), rather than identifying highly abundant microbial organisms (e.g., saprotrophs) that contribute greatly to quantitative variation in microbial community composition, but do not necessarily convey any significance for PSFs. All models were performed on Sorensen similarity matrices of pot-level data. Permutational multivariate analysis of variance (PERMANOVA) was used to test for the effect of subgenus on fungal and bacterial community composition at both OTU and family levels. Models included subgenus as a fixed effect and the random effect species within subgenus, which was used to test for subgenus effects. Ammonium was fitted as a covariate in all models, because even though eucalypt species did not differentially affect soil NO_3^- or NH_4^+ (ANOVA; $P > 0.05$), the soil NH_4^+ concentration of pots did significantly influence fungal and bacterial community composition (distance-based linear models, $P < 0.05$); NO_3^- was not included as a covariate as it strongly correlated with NH_4^+ ($F = 150$, $P = <0.001$, $R^2 = 0.93$), and thus was redundant. Models were performed with 999 permutations using type 1 sums of squares, which accounted for variation due to the soil NH_4^+ before testing for subgenus effects. Significant effects of NH_4^+ and subgenus on community data were visualized by plotting data against NH_4^+ and subgenus in a constrained canonical analysis of principal coordinates (CAP).

After first testing whether the different eucalypt subgenera conditioned distinct microbial communities, we next identified the microbial families contributing most to dissimilarity in

fungal community composition between the subgenera using the similarity percentages (SIMPER) function implemented in PRIMER, also using the Sorensen similarity matrix derived from pot-level data. Generalized mixed effects models were fitted in R to confirm whether the presence of these microbial families significantly differed between soils conditioned by species of each subgenus using the function glmer within the package lme4 (Bates et al. 2014; v. 1.1-17). These models included subgenus as a fixed effect and the random effect species within subgenus, which was used to test for subgenus effects. Models were implemented using a binomial error distribution with a logit link function and included the covariate NH_4^+.

To test the hypotheses that (3) the responses of species to heterospecific vs. conspecific conditioned soils are related to host-specific soil microbial organisms (as determined by analyses above), linear models were fitted in R using the package stats to test whether the abundance of fungal taxa (averaged across two conditioned soils per species) in the conditioned soils of different eucalypt species significantly affected their responses (survival, growth rate, and total biomass) to inoculation with heterospecific vs. conspecific conditioned soils (13 feedback ratios averaged for each species and trait). A positive relationship indicated that a greater abundance of a fungal taxa in a species' conditioned soils was associated with that species performing better in heterospecific soils (i.e., host-specific pathogen), while a negative relationships indicated that a greater abundance of a fungal taxa in a species' conditioned soils was associated with that species performing more poorly in heterospecific soils (i.e., host-specific mutualist). Soil microorganisms that significantly correlated with feedback ratios were mapped onto the eucalypt phylogeny.

To test our final hypothesis that (4) the abundance of host-specific soil organisms in conditioned soils is related to the root chemistry of soil conditioning species, non-parametric Kendall's rank correlations were fitted in R using the function Kendall from the package Kendall (McLeod 2005, v. 2.2). Models tested for significant correlations between the abundance of fungal taxa in soils conditioned by different eucalypt species (averaged across two conditioned soils per species) and the concentrations of defensive compounds in the fine roots of species (averaged across trees in two conditioning pots soils per species). We used non-parametric models as the assumptions of a linear model were not met. Root chemical data originating from the same conditioning trees in the present study were obtained from data published in Senior et al. (2016) and included total phenolics (Prussian blue assay), condensed tannins (acid butanol assay), and terpenes (ultra-performance liquid chromatography), each exhibiting significant phylogenetic signal. These compounds were also of particular interest since they are known to influence plant interactions with soil microorganisms (Huang et al. 2014, van Dam and Bouwmeester 2016).

RESULTS

In support of the first hypothesis, eucalypt species exhibited variable responses to increasing phylogenetic distance between themselves and the soil conditioning species (Table 1). Only three of the 13 focal species displayed significant relationships between survival feedback ratios (i.e., survival in different heterospecific/conspecific soils) and phylogenetic distance to conditioning species. These relationships were all positive, where the relative survival of seedlings increased with phylogenetic distance between focal and soil conditioning species. For instance, the survival feedback ratios of *Eucalyptus amygdalina* increased from 1.18 to 1.31 to 1.56 when inoculated with soils conditioned by a close relative (*Eucalyptus radiata*), a moderate relative (*Eucalyptus pauciflora*), and a distant relative (*Eucalyptus subcrenulata*), respectively, compared to its own soils. For the surviving seedlings, five focal species displayed significant relationships between growth rate feedback ratios and phylogenetic distance to conditioning species, where one was negative and four were positive. For instance, the growth rate feedback ratios of *Eucalyptus obliqua* increased from 1.01 to 1.04 to 1.2 when inoculated with soils conditioned by a close relative (*E. pauciflora*), a moderate relative (*E. amygdalina*), and a distant relative (*Eucalyptus dalrympleana*), respectively, compared to its own soils. Similarly, the total biomass of three focal species displayed significant responses to increasing

Table 1. Species display variable responses to soils conditioned by species of increasing phylogenetic distance.

Subgenus and species	n	Survival		Growth rate		Total biomass	
		β	SE	β	SE	β	SE
Eucalyptus							
Eucalyptus obliqua	66	**0.06***	0.02	**0.06****	0.02	**0.11***	0.04
Eucalyptus pauciflora	69	0.03	0.03	**0.07*****	0.01	**0.10****	0.03
Eucalyptus nitida	67	**0.07***	0.02	**0.07****	0.02	**0.13*****	0.03
Eucalyptus radiata	63	0.05	0.03	**0.07***	0.03	0.07	0.04
Eucalyptus amygdalina	64	**0.07***	0.02	0.03	0.02	0.04	0.04
Symphyomyrtus							
Eucalyptus rodwayi	54	0.01	0.05	−0.05	0.02	−0.05	0.04
Eucalyptus ovata	52	<0.01	0.04	**−0.05***	0.02	−0.07	0.05
Eucalyptus barberi	56	0.01	0.09	−0.07	0.04	−0.09	0.06
Eucalyptus urnigera	57	−0.01	0.04	−0.02	0.02	−0.05	0.03
Eucalyptus cordata	62	−0.01	0.03	−0.04	0.05	−0.06	0.07
Eucalyptus subcrenulata	55	0.05	0.03	0.03	0.02	0.02	0.04
Eucalyptus globulus	71	0.04	0.03	0.02	0.03	0.02	0.03
Eucalyptus dalrympleana	68	0.04	0.03	−0.02	0.05	−0.03	0.05

Notes: The number of surviving seedlings of each species at the conclusion of the experiment (n) as well as the slopes (β) and standard error (SE) of the linear relationships between the survival, growth rate, and total biomass feedback ratios (performance in different heterospecific/conspecific soils) of each eucalypt species and phylogenetic distance between focal and conditioning species. A positive slope is interpreted as a species performing better as phylogenetic distance between itself and soil conditioning species increases, while a negative slope is interpreted as a species performing more poorly as phylogenetic distance between itself and soil conditioning species increases. Bold values represent significance, where *P = <0.05, **P = <0.01, and ***P = <0.001.

phylogenetic distance to conditioning species, all of which were positive.

In support of the second hypothesis, when we mapped the feedback regression slopes onto the eucalypt phylogeny, we identified strong phylogenetic signal in the manner in which species responded to increasing phylogenetic distance between themselves and soil conditioning species (Fig. 1). The survival, growth rate, and total biomass feedback regression slopes were all phylogenetically conserved ($K = 1.0$, $P = 0.005$; $K = 1.6$, $P = 0.002$; $K = 1.9$, $P = 0.001$, respectively). That is, more closely related species tended to have more similar feedback regression slopes than expected under Brownian motion (random) evolution. These phylogenetic signals were mainly driven by differences between subgenera, but within-subgenus tests of phylogenetic signal did reveal a single significant ($K = 0.92$, $P = 0.04$) phylogenetic signal in the total biomass feedback regression slopes of *Symphyomyrtus* species. Specifically, subgenus *Eucalyptus* species uniformly displayed strong positive relationships between their relative performance in heterospecific compared to conspecific conditioned soils and increasing phylogenetic distance to soil conditioning species. In contrast,

subgenus *Symphyomyrtus* species generally displayed no significant relationships.

In support of our third hypothesis, the responses of species to heterospecific vs. conspecific conditioned soils were related to host-specific soil microbial organisms. After accounting for the significant effects of NH_4^+, subgenus significantly influenced fungal community composition at both the OTU and family levels (PERMANOVA; Pseudo-$F_{1,13} = 1.6$, $P = 0.018$ and Pseudo-$F_{1,13} = 1.7$, $P = 0.034$, respectively), but not bacterial community composition ($P > 0.05$). Subgenus explained 5.6% and 6.0% of variation in fungal community composition fungal OTUs and families, respectively. Further, canonical analysis of principal coordinates (CAP) successfully separated fungal communities by subgenus at each taxonomic level (Fig. 2). Analysis of similarities found that the first 10% of variation in fungal community composition between the subgenera was driven by ten fungal families (Table 2). Further, generalized mixed models detected significant differences in the presence/absence of three families in soils conditioned by each subgenus. These families were Fistulinaceae, an unidentified family(s) belonging to the order Hysterangiales, and Davidiellaceae. A
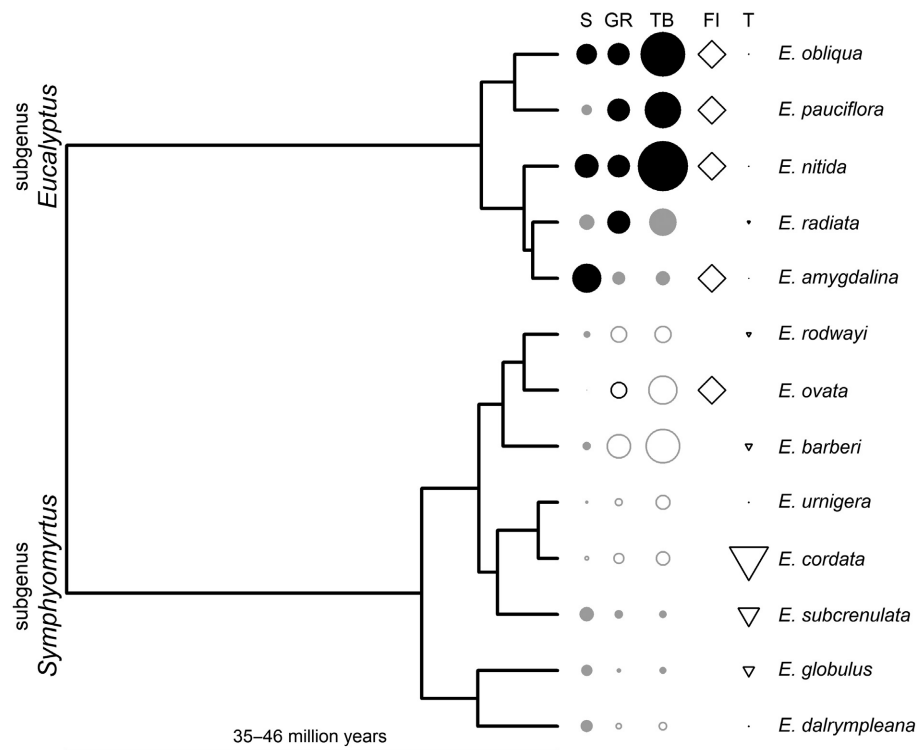
Fig. 1. Phylogenetic lineages differ in responses to soils conditioned by species of increasing phylogenetic distance, the presence of Fistulinaceae in their conditioned soils, and concentrations of terpenes within fine roots. Phylogeny of the eucalypt species (subgenus *Eucalyptus* and *Symphyomyrtus*) used in the present study with mapped slopes of linear relationships between the survival (S), growth rate (GR), and total biomass (TB) feedback ratios of each eucalypt species and phylogenetic distance between focal and conditioning species. The presence of Fistulinaceae (FI) within conditioned soils (diamonds) and concentration of terpenes (T) within the fine roots of soil conditioning species (triangles) are also mapped, where the increasing size of triangles represents increasing concentrations of terpenes. For the feedback regression slopes, full circles represent positive relationships between the relative performance of focal species in heterospecific conditioned soils and increasing phylogenetic distance to soil conditioning species, while open circles represent negative relationships. The size of circles represents the strength of the relationships, with larger circles representing stronger relationships, and black and gray colored circles represent significant and non-significant responses, respectively. This phylogenetic tree is based on a previously published tree (Senior et al. 2016), where the root of the phylogeny was assigned an arbitrary value of one. This was because divergence time of the subgenera is not well known, but estimated to be between 35 and 46 million years (Crisp et al. 2004, Thornhill et al. 2015).

fourth unidentified family(s) from order Microascales was present in most subgenus *Eucalyptus* soils, but completely absent in the soils of subgenus *Symphyomyrtus*. However, we only considered family Fistulinaceae and the unidentified family(s) from order Microascales for further analyses since they were generally only present in the soils of subgenus *Eucalyptus* species (Fig. 1) and thus potentially capable of explaining the significant feedbacks observed in these species. Indeed, regressions between the average growth rate and total biomass feedback ratios (i.e., performance in heterospecific/conspecific soils) of each species and the average abundance of Fistulinaceae in soils conditioned by each species detected a marginally significant ($F_{1,11} = 4.6$, $P = 0.054$, $R^2 = 0.23$) and significant relationship ($F_{1,11} = 12.2$, $P = 0.005$, $R^2 = 0.48$), respectively. Both relationships were positive, indicating that species that contained family Fistulinaceae
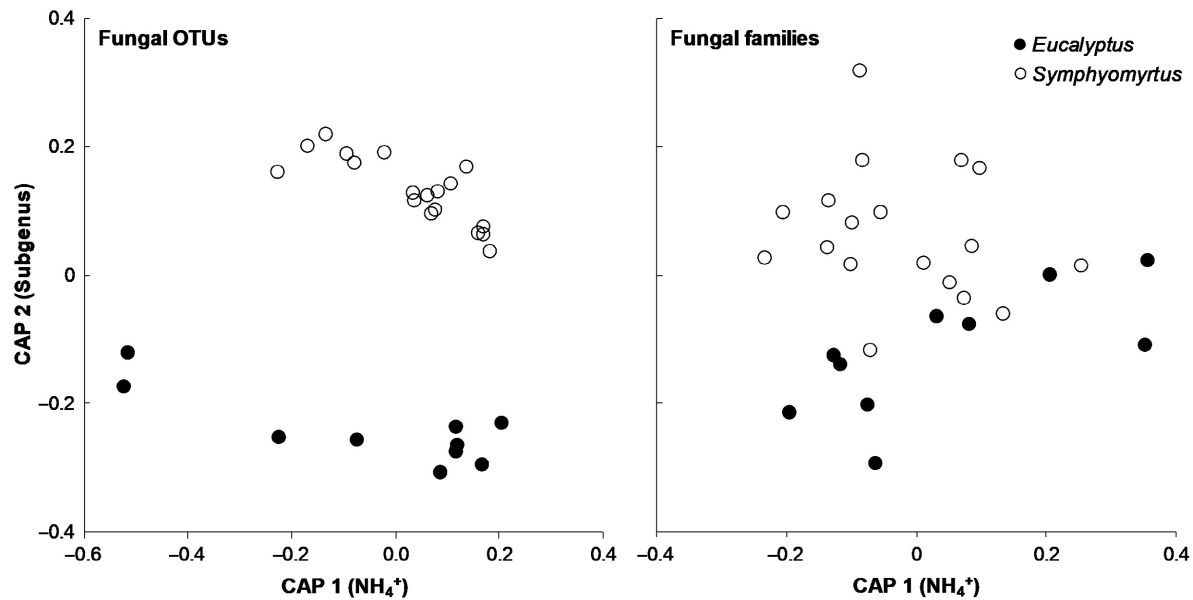
Fig. 2. Eucalypt subgenera condition distinct fungal communities. Constrained canonical analysis of principal coordinates (CAP) plot illustrating the effects of subgenus and NH_4^+ on fungal operational taxonomical units and families. The analysis is based on pot-level soil community data, where CAP1 is aligned with variation in soil NH_4^+ concentration (maximum linear correlation coefficient of 0.99), whereas CAP2 is aligned with subgenus (maximum linear correlation coefficient of 0.99).

Table 2. Fungal families contributing to differences in the soil communities of eucalypt subgenera.

Family	Contribution (%)	Presence in subgenus *Eucalyptus* soils (%)	Presence in subgenus *Symphyomyrtus* soils (%)	Significance χ_1^2	P
Fistulinaceae	1.2	70	11	7.3	**0.007**
Order Hysterangiales family unclassified	1.1	20	67	5.1	**0.023**
Order Microascales family unclassified	1.1	60	0	—*	—
Davidiellaceae	1.1	30	72	3.9	**0.047**
Kappamycetaceae	1.0	70	33	3.1	0.080
Bondarzewiaceae	1.0	60	22	2.7	0.102
Onygenaceae	1.0	40	72	2.4	0.123
Leotiomycetes family *Incertae sedis*	1.0	70	39	1.4	0.241
Marasmiaceae	1.0	60	33	1.5	0.224
Hymenochaetaceae	1.0	70	44	1.5	0.221

Notes: Results of an analysis of similarities (SIMPER) identifying fungal families that explain the greatest amount of dissimilarity between the subgenera and the results of generalized mixed models (Significance) analyzing for variation in the presence/absence of each fungal family in soils conditioned by each subgenus using pot-level data. The families explaining the first 10% of dissimilarity between subgenera are reported. Bold values indicate statistical significance at α = 0.05.

  * We detected no unclassified order Microascales family (families) in the soils of subgenus *Symphyomyrtus* species, and thus, these taxa could not be analyzed with a generalized mixed model.

within their conditioned soils performed better when inoculated with soils conditioned by heterospecifics.

In support of our final hypothesis, the abundance of family Fistulinaceae in conditioned soils was related to phylogenetic patterns in the root chemistry of soil conditioning species (Fig. 1). Non-parametric Kendall's rank correlations detected a significant effect of the average concentration of terpenes, but not total phenolics or condensed tannins ($P > 0.05$), within the fine roots of conditioning species on the average

abundance of Fistulinaceae in their conditioned soils ($\tau = -0.626$, $P = 0.016$). The relationship was negative, indicating that increasing concentrations of terpenes in fine roots were associated with a decrease in the abundance of this fungal family in conditioned soils.

DISCUSSION

A major goal in ecology is to understand the mechanisms that drive plant community structure. Recent studies suggest that phylogenetic signal in PSF may potentially explain plant community structure (Liu et al. 2012, Anacker et al. 2014), but the ubiquity and underlying mechanisms of such signals are largely unknown. We show that phylogenetic relatedness can shape PSF, where focal species respond to increasing phylogenetic distance to conditioning species depending on their phylogenetic lineage. Further, by sequencing microbial DNA extracted from experimentally conditioning soils and incorporating root chemical data from the same conditioning trees, we also provide a potential mechanistic explanation of why this phylogenetic signal might occur. By also including this microbial community and trait data, our study represents one of the most comprehensive explorations of the mechanisms behind phylogenetic signal in PSF to date and provides further support for phylogenetic-based PSF as a potential driver of plant community structure.

Our results suggest that phylogenetic relatedness can influence PSF in two ways. Firstly, we show that the degree of phylogenetic distance between focal and conditioning species can be important in determining PSF. We identified variable trends between seedling responses to conditioned soils and increasing phylogenetic distance between focal and conditioning species, ranging from positive (i.e., increased performance with increasing phylogenetic distance) to negative (i.e., decreased performance with increasing phylogenetic distance), although relationships were predominantly positive. Almost half of the focal species showed significant relationships between feedbacks to conditioned soils and phylogenetic distance to conditioning species. This is contrary to recent studies reporting little to no effect of phylogenetic distance on PSF (Mehrabi and Tuck 2015, Mehrabi et al. 2015,

Fitzpatrick et al. 2017). For example, a recent meta-regression of 329 experimental PSF effects demonstrated that the growth of 133 species in conditioned soils was poorly predicted by phylogenetic distance between focal and soil conditioning species (Mehrabi and Tuck 2015). Our results suggest this could arise with trends among species that vary from positive to negative. Furthermore, our results also suggest that when these same trends are pooled and examined across lineages, they bring about an overall neutral effect of phylogenetic relatedness on PSF. Finally, we show that species belonging to different phylogenetic lineages respond differently to soils conditioned by closely related species. We identified clear phylogenetic signals in the survival and growth responses of species to soils conditioned by species of increasing phylogenetic distance. These phylogenetic signals were driven by differences between subgenera, where subgenus *Eucalyptus* species performed better when inoculated with soils of more distantly related species, while subgenus *Symphyomyrtus* species typically did not differentially respond to conditioned soils. These feedbacks exhibited by subgenus *Eucalyptus* species were consistent with phylogenetic patterns in PSF observed by Liu et al. (2012), where the adverse effects of host-specific soil pathogens may diminish with decreasing phylogenetic relatedness between focal seedlings and conditioning species (i.e., a phylogenetic Janzen-Connell effect). However, we show that not all species are susceptible to this effect and this can be predicted to some extent by the phylogenetic lineage of focal species; in this case subgenus.

We found that the eucalypt subgenera conditioned different microbial communities, providing a clear candidate mechanism to explain the observed phylogenetic patterns in PSF. Our findings support recent studies showing that microbial communities are sensitive to phylogenetic distance among host plants (Bouffaud et al. 2014, Naylor et al. 2017) and plant species exhibit phylogenetic signal in the structure of soil microbial communities (Fitzpatrick et al. 2018), at least with regard to organisms inhabiting the root ecto- and endosphere. Here, we show that plant subgenera can assemble distinct soil fungal communities in the bulk soils. However, our results suggest that phylogenetic signal in PSF may only

be driven by a relatively few soil microbial taxa that are unique to plant phylogenetic lineages. While many observations of PSF have been generally associated with species-specific relationships with fungal pathogens or arbuscular mycorrhizal fungi (Bever 2002, Klironomos 2002, van der Putten et al. 2007), few studies have sought to identify more specific groups driving feedback responses. In one example, Packer and Clay (2000) identified *Pythium* spp. as the driver of negative PSF in black cherry. We identified a candidate fungal group which could have contributed to subgenus *Eucalyptus* species performing better in the soils of more distantly related species. Soils conditioned by subgenus *Eucalyptus* species generally contained *Fistulina* spp., a group known to exhibit pathogenic relationships with several subgenus *Eucalyptus* species (Keane et al. 2000), while soils conditioned by subgenus *Symphyomyrtus* species in most cases did not contain this pathogen.

Our results suggest that phylogenetic patterns in PSF can be related to underlying phylogenetic conservatism in plant traits. Phylogenetic signal in plant chemical traits has previously been implicated as an underlying driver of plant susceptibility to aboveground herbivores and pathogens (Johnson et al. 2014, Carrillo-Gavilán et al. 2015). Herein, we detected a significant negative relationship between the concentration of root terpenes, which exhibit significant phylogenetic signal in the eucalypt species studied (Senior et al. 2016) and have previously been shown to exhibit antimicrobial properties (Gilles et al. 2010), and the abundance of *Fistulina* spp. in conditioned soils, which were associated with negative feedbacks. Specifically, subgenus *Eucalyptus* species exhibited lower concentrations of terpenes and contained *Fistulina* spp. in their soils, while subgenus *Symphyomyrtus* species displayed higher concentrations and these taxa were generally absent in their soils. Such phylogenetic conservatism in plant traits associated with feedbacks might be used to explain variable PSF when evolutionary history alone may not (Fitzpatrick et al. 2017). In such cases, convergent selection could result in similar plant traits occurring among different phylogenetic lineages as a result of similar selective pressures (Crisp and Cook 2012) and thus erode phylogenetic signal in PSF.

Our findings further support the use of phylogenetic information in predicting species responses to the soils of co-occurring species and ultimately forest structure. Although our results are based on conditioning and feedbacks observed in a greenhouse environment, and many factors could affect their inference to natural systems (e.g., soil nutrient content and a full native soil community), these results raise the possibility that subgeneric differences in PSF may contribute to the structure of mixed eucalypt stands containing species belonging to each subgenus (Davidson and Reid 1980, Austin et al. 1983, Duff et al. 1983). Seedlings in this study reflect the early stages of eucalypt growth (seedling and sapling stages), where plants are particularly sensitive to soil conditions. Plant-soil feedbacks that develop during this crucial establishing phase could have important and lasting consequences for community structure. Specifically, subgenus *Eucalyptus* species may escape host-specific soil pathogens by growing in the soils of subgenus *Symphyomyrtus* species, allowing greater survival and growth. However, subgenus *Symphyomyrtus* species were generally unaffected by soil conditioning species, suggesting that soil biota may not limit the growth of these species to the same degree. In this case, other factors, such as insect herbivory (Stone et al. 1998), may limit the growth of subgenus *Symphyomyrtus* species and contribute to the coexistence of both subgenera.

CONCLUSIONS

In summary, we provide further evidence that phylogenetic relationships between focal and conditioning species can influence the direction and magnitude of PSF. We found the responses of eucalypt species to increasing phylogenetic distance to conditioning species were predicted by phylogenetic lineage and were associated with the conditioning of specific soil fungal organisms and variation in root chemistry. Although this study includes just 13 plant species representing two subgenera, our findings may provide important insight into why recent studies may have found little to no effect of phylogenetic relatedness. First, by pooling species responses across lineages with different responses to phylogenetic distance to conditioning

species might bring about an overall neutral effect. Second, we show that underlying phylogenetic conservatism in plant traits and differences in interactions with soil microorganisms is related to patterns of PSF. This suggests that if different lineages exhibit similar traits through convergent selection, they might also exhibit similar feedbacks. Therefore, future studies investigating which plant traits are associated with PSF and their phylogenetic distribution are vital to advancing the field as well as understanding how evolutionary history shapes ecological communities.

ACKNOWLEDGMENTS

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