

Experimental evidence of strong relationships between soil microbial communities and plant germination

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Abstract

1. Plant-associated microbes play essential roles in nutrient uptake and plant productivity, but their role in driving plant germination, a critical stage in the plant life cycle, is still poorly understood.
2. We used data from a large-scale, field-based soil seed bank study to examine the relationship among plants germinating from the seed bank and soil microbial community composition. We combined this with an experiment using 34 laboratory-based microcosms whereby sterile soil was inoculated with microbes from different field sites to examine how microbes affect the germination of nine plant species.
3. The community composition of plants in the soil seed bank was highly and significantly associated with bacterial and fungal community composition, with stronger correlations for soil beneath plant canopies. Microbes predicted a unique portion of the variation in the community composition of germinants after accounting for differences in environmental variables. The strongest correlations among microbes and plant functional traits included those related to perenniality, growth form, plant size, root type and seed shape. Our microcosm study showed that different plant species had their own associated germination microbiome, and most plant-microbe interactions were positive during germination.
4. *Synthesis.* Our study provides evidence for intimate relationships between plant and soil biodiversity during germination. Our work fills an important knowledge gap for plant-microbe interactions and reveals valuable insights into the shared natural history of plants and microbes in terrestrial ecosystems.

KEYWORDS

bacteria, fungi, germinants, microbes, plant germination, plant-microbial association, soil seed bank

1 | INTRODUCTION

Plants and soil microbes have co-evolved over millions of years (Field et al., 2015; Lutzoni et al., 2018). Plants synthesize a range of compounds and exudates that benefit specific microbial consortia

in the rhizosphere, resulting in complex interactions and feedbacks among microbes, soils and plants that regulate their growth, persistence and diversity (e.g. Bever et al., 1997; Bezemer et al., 2006; Herrera Paredes & Lebeis, 2016; Kulmatiski et al., 2008). Studies of plant-microbial feedbacks over the past few decades have

focussed on well-described interactions including pathogenesis (e.g. *Fusarium* sp.), symbiosis (e.g. mycorrhizal fungi) and decomposition (e.g. saprobes, Brundrett, 2002; van der Putten et al., 2013; Wang et al., 2010) or studies involving ecologically important species. Some of these studies involve seeds, and examples of seed-microbe associations include the suppression of germination of barley (*Hordeum* spp.) seeds by the bacterium *Azotobacter chroococcum* (Harper & Lynch, 1980) and greater germination of a range of native and invasive grasses, forbs and shrubs when seeds come into contact with *Methylobacterium* bacteria (Balshor et al., 2017). Inoculation of seeds with arbuscular mycorrhizal fungi (AMF) is known to enhance seed germination through mutualistic associations that improves the ability of these fungal-associated plants to access nutrients (Aprohmanian et al., 2016). Indeed, associations with AMF may have allowed plants to colonize land more than 400 million years ago (Schu ler & Walker, 2011) by enhancing their capacity to absorb water and nutrients (Wang et al., 2010). Given the many examples of plant-microbe associations, it is only natural that microbes have evolved as important drivers of plant productivity and diversity in terrestrial environments (Leff et al., 2018; van der Heijden et al., 2008).

Despite the acknowledgement that seeds provide a range of habitats for diverse microbial assemblages (Compant et al., 2010), we still know relatively little about seed-associated microorganisms and the critical early stages of plant life, compared with those from other plant compartments (Nelson, 2018; Nelson et al., 2018). Indeed, research to date has focussed mainly on the potential associations among soil microbes and established plants (Delgado-Baquerizo et al., 2018). Treatment of seeds with fungicides indicates that fungal associations with seeds are important (Nelson, 2018), but relatively little is known about the identity of those microbes that are implicated in these relationships. A greater understanding of how seeds and microbes interact is critical for understanding not only basic biology of seeds and plants, but also for advancing improvements in agricultural productivity (Nelson et al., 2018).

Microbiome studies evaluating potential associations among plants and microbes during the germination processes are largely absent (Nelson et al., 2018; van der Putten et al., 2013). The potential functional capabilities of soil microbes (e.g. potential pathogens, saprobes and mycorrhizal fungi) could regulate seed germination via signalling, pathogenesis, nutrient release and symbiotic relationships (Chee-Sanford et al., 2006). Similarly, microbes may be responsible for breaking seed dormancy by breaking down seed coats that prevent the seeds from imbibing water (Baskin & Basin, 1989). For example, bacteria have a close relationship with orchids, one that is critical to promote host-plant germination in orchids (Tsavkelova et al., 2007). Microbes have also been shown to be critical for reducing the reliance on agrichemicals in cropping systems (Rocha et al., 2019) and for enhancing the success of restoration programs (Koziol et al., 2018). Identifying the potential associations between soil microbes and plant germination in their natural ecosystems and across contrasting plant species is fundamental to a greater

understanding of the implications of changes in microbial communities under altered climates.

To advance our understanding of potential associations among soil microbial communities and plants from the germinable seed bank, we combined a field survey with a soil seed bank study using soils from 54 sites in eastern Australia and a laboratory-based microcosm experiment. The soil seed bank study identified a large number of seed bank species, some that were absent from the standing plant community (Val et al., 2020). We aimed to link seed bank information to detailed information on the microbial assemblage from the same soil (Eldridge et al., 2017) to test potential associations among different microbial assemblages and germinants from the seed bank. Given the correlative nature of such a study, we complemented this with a manipulative microcosm study. Specifically, we inoculated sterile soil with microbes (bacteria and fungi) from a subsample of the seed bank sites in order to demonstrate the range of potential positive and negative relationships among various microbial assemblages and our model plant species, thereby providing empirical support for the correlative study. Unravelling potential associations among taxonomic and functional attributes of soil microbes and plants will give us potential insights into the shared natural histories and ecological preferences of plants and microbes and the important role of microbes in plant germination.

2 | MATERIALS AND METHODS

2.1 | Field-based soil sampling and data collection

The field-based component of our study (seed bank-soil microbe relationships) was undertaken using soils from three woodland communities in semi-arid eastern Australia that were characterized by the dominant trees Blackbox *Eucalyptus largiflorens* F. Muell., River red gum *Eucalyptus camaldulensis* Dehnh. and White cypress pine *Callitris glaucophylla* Joy Thomps. & L.A.S. Johnson. Within each of the three communities, we sampled 18 sites ($n = 54$ sites). At each site, we established five large (5 m by 5 m) quadrats spaced every 50 m along a 200 m transect. Within each large quadrat we centrally located a small (0.5 m by 0.5 m) quadrat. At three points along the transect (0, 50 and 100 m), we located the nearest perennial grass, shrub, tree and bare microsite, and placed a 0.50-m² circular quadrat in these microsites within which the cover and abundance of all vascular plants were recorded. We collected 10 cores of soil from the top 5 cm, where most seeds occur (Traba et al., 2004), from each microsite, and pooled them at the site level ($n = 216$), to be used to extract germinable seeds and soil microbes.

Environmental data were collected from each site for use in our statistical analyses. For the 216 soil samples, we assessed total soil carbon (C: $3.09 \pm 0.03\%$, mean \pm SE) with high temperature combustion using a LECO CNS-2000 CNS Analyser (LECO Corporation) and pH (6.61 ± 0.02) in a 1:5 soil-water extract. We measured the concentrations of four enzymes (β -glucosidase, β -D-cellobiosidase, N-acetyl- β -glucosaminidase, phosphatase) that are proxies for C,

nitrogen (N) and phosphorus (P) degradation respectively. Enzyme activities were measured by fluorometry using 1.00 g of soil, as described in the study by Bell et al. (2013). Aridity was calculated as 1-Aridity Index (precipitation/potential evapotranspiration) using the FAO global aridity map (<https://data.apps.fao.org/>). Climatic data were obtained from the WorldClim database (<https://www.worldclim.org>).

At each site, we assessed recent grazing activity at all five points along the 200 m transect using dung and pellet counts (Marques et al., 2001). Dung of cattle, sheep/goat and kangaroos was counted in the large quadrats, and dung of rabbits, sheep/goat and kangaroos in the small quadrats. Dung counts were converted to mass per hectare based on relationships between abundance and mass developed for different herbivores (Eldridge et al., 2017). Recent grazing intensity, which represents grazing over the past few years, was defined as the total mass of herbivore dung.

2.2 | Seed bank emergence study

Soil samples were mixed and sifted to remove woody debris, and a 150-g subsample spread evenly (~5 mm deep) over sterilized sand in commercial germination trays (35 cm × 14 cm) and placed in an unheated greenhouse. The trays were watered regularly to maintain field capacity. The position of all trays was randomly allocated in order to account for a possible bias associated with tray position. Ten control trays, that is, trays containing only sterilized sand were evenly distributed in the poly house to control for glasshouse weeds and seeds within the sterilized sand. Emerging plants were counted and removed following identification, and different species repotted to grow on to confirm identification. The study ran for 242 days to allow for warm season and cool season germination cues.

2.3 | Laboratory-based microcosm experiment

To test for the presence of a causal relationship among soil microbes and plant germination, we undertook a germination microcosm experiment using nine plant species and 34 microcosms. These species came from a range of different Australian plant families that support key provisioning and cultural services. Because seeds of most of the species emerging from the seed bank study are either unavailable, could not be collected in the field or are too small with unknown and low germination rates, we used commercially available seed with known high germination rates from similar families as those in the seed bank study. These plant species were selected based on a range of different traits (seed size, nitrogen (N) fixation, seed ornamentation, plant type, e.g. forb vs. grass). Microcosms were established in small containers 26 cm by 33 cm by 7 cm deep, containing a single independent sterile soil (pH = 6.5, soil total carbon (C) = 5.5%, fine sandy loam) that had been autoclaved three times over a period of 8 days with an intervening period to allow

microbial species to germinate between subsequent autoclaving. Microcosms were then inoculated with a microbial slurry made up of 25 g of soil in 180 ml of sterile phosphate buffer adjusted to pH of 6.0. This slurry was then added to the autoclaved soil at a rate of 40 ml buffer/kg soil. Soil for the inoculations was obtained from 34 of the 54 sites used in the plant germination study, with 12 from the Blackbox community and 11 from each of the other two communities. Sampled locations were selected to cover the entire range of environmental conditions in our field survey. Our aim was not to isolate and reintroduce individual microbes, the majority of which cannot be cultivated, but rather, to inoculate whole communities, which are more likely to affect the germination process. Between 20 and 50 seeds of each of the nine plant species were sterilized with 10% sodium hypochlorite and sown. Inoculated microcosms were placed in a constant temperature room at 22°C and watered two to three times daily under Glo-Lux lights to promote germination. All seeds were treated equally prior to commencement of the germination study. None of the nine species is known to have allelopathic effects that might have limited the germination of other species in our study. The study was carried out for 61 days, and all germinants were removed, together, at the final date, and the number was recorded. Some germinants were grown out separately to confirm their identity.

2.4 | DNA extraction and bioinformatics for seed bank soils and microcosm soils

We characterized the microbial community (fungi and bacteria) for the 216 soils forming the seed bank study, and for the soils in the microcosm (at the completion of the microcosm study) as follows. Soil DNA was extracted from 0.5 g of defrosted soil samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories) according to the manufacturer's instructions. Amplicons targeting the bacterial 16S rRNA gene (341F-805R; Herlemann et al., 2011) and the fungal ITS region (ITS7-ITS4R; Ihrmark et al., 2012) were sequenced at the Western Sydney University NGS facility (Sydney, Australia) using Illumina MiSeq 2x 301 bp (bacteria) or 2x 280 bp (fungi) paired-end sequencing. Bioinformatic analyses were carried out, as explained in the study by Bell et al. (2013). Briefly, UPARSE (Edgar, 2013, 2016) was used for the OTU (Operational Taxonomic Unit) clustering at 97% sequence identity. The UPARSE approach provided similar community composition results to those using 100% ASV DADA2 (Mantel $r > 0.9$; $p < 0.001$). Representative sequences of each OTU were assigned against the Silva (Quast et al., 2013) and UNITE (Kõljalg et al., 2005) database for bacteria and fungi respectively. For the seed bank soil samples, OTU abundance tables were rarefied to 10,851 and 20,797 sequences for bacteria and fungi respectively (the minimum number of sequences for a soil sample). For the microcosm study, OTU tables were rarefied at 18,791 and 26,388 sequences for bacteria and fungi respectively. Alpha diversity metrics were then calculated using the *alpha_diversity.py* script within QIIME (Caporaso et al., 2010).

2.5 | Statistical analyses

2.5.1 | Seed bank study

For the seed bank study, we evaluated the correlation between the dissimilarity in the community composition of microbes and that of the community composition of plant germinants recorded in the seed bank (Val et al., 2020). First, we calculated the Bray–Curtis dissimilarity matrices for the community of bacteria and fungi, separately (at the phylotype or OTU level), and plant germinants (species level). We then correlated the matrix of fungal and bacterial community composition dissimilarity with that of the germinant composition dissimilarity using Mantel test correlations (Spearman).

We then conducted multivariate statistical modelling (variation partitioning; Legendre, 2008) to evaluate whether the community composition of microbial communities could predict a unique portion of the variation in the community composition of plant germinants while accounting for key environmental factors. These environmental variables were soil properties and processes (soil pH, total C, activity of enzymes associated with C, N and phosphorus (P) cycling), location (latitude and longitude), historical legacies of climate (data on mean annual temperature and aridity from each site) and grazing intensity (livestock, kangaroos and rabbits). Variation partitioning analyses were conducted with the R package VEGAN (Oksanen et al., 2015). Note that adjusted coefficients of determination (R^2) in multiple regression and canonical analysis can, on occasion, take negative values (Oksanen et al., 2015). In this case, negative values in explained variance for any group of predictors are interpreted as zeros and correspond to cases in which the explanatory variables explain less variation than that explained using random normal variables (Legendre, 2008).

Finally, we correlated the relative abundance of plant families (taxonomy) and traits (e.g. plant size, seed weight) with the relative abundance of bacterial and fungal phyla and fungal lifestyles (Spearman's ρ correlations) and present the data as a series of heat diagrams. To further advance our understanding of potential plant-microbial associations determining the ability of microbial communities to regulate germination success, we then examined the relationships among functional (e.g. saprobes, ectomycorrhizal fungi) and taxonomic (phyla) microbial attributes, and the relative abundance of germinants according to their functional (e.g. growth form, seed shape, dispersal mechanism) and taxonomic (family) attributes (via Spearman's correlations).

2.5.2 | Microcosm study

For the microcosm experiment, we generated a bivariate plant-microbial (bacteria and fungi) correlation network to identify potential associations among microbial and plant species during the germination process based on our 34 microcosms. Our network included information on the relative abundance (%) of 2,950 microbial phylotypes (1,111 fungal and 1,839 bacterial species) and

percentage germination of the nine plant species. Given the largely controlled nature of our microcosm experiment, we used a correlation cut-off of Spearman $p < 0.05$. The network was visualized with the interactive platform Gephi (<https://gephi.org>). See Delgado-Baquerizo et al. (2018) for a similar approach. We then estimated the total number of positive and negative plant-microbial connections (correlations) within our correlation network.

3 | RESULTS

In the seed bank study, Actinobacteria and Proteobacteria accounted for almost 50% of the total abundance of bacterial OTUs, and Ascomycetes accounted for 68% of fungal OTUs (Figure S1a,b). The germinable soil seed bank yielded 24,651 seedlings from 196 species and 45 families (Figure S1c,d). We found that the community composition of plants in the soil seed bank was highly and significantly associated with that of both bacterial (Mantel $\rho = 0.43$, $p < 0.01$) and fungal (Mantel $\rho = 0.40$, $p < 0.01$) communities, and these correlations were stronger for plant canopies (Mantel $\rho = 0.42$ – 0.51) than bare soils (Mantel $\rho = 0.30$ – 0.38). We also found a strong correlation between the richness of seed bank germinants and both bacterial (Figure 1a) and fungal (Figure 1b) richness. The strongest correlations among microbes and plant functional traits included those related to perenniality (with annuals mostly positive, and perennials mostly negative, Figure 2; Figure S2), growth form, plant size, root type and seed shape (particularly flat and spherical; Figure 3).

Our variation partitioning model provided further evidence that the community composition of soil microbes (fungi and bacteria) can predict a significant ($p < 0.001$) and unique portion of the variation in the community composition of germinants that is unaccounted for by soil, plants, grazing, climate or location (Figure 1c), which also explained significant portions of variations in all cases ($p < 0.001$). We detected a number of potential and important microbe-seed interactions such as strong positive correlations between the relative abundance of ectomycorrhizal fungi and that of the plants from the families Ranunculaceae, Juncaceae, Campanulaceae, Amaranthaceae and Myrtaceae, as well as some plant traits such as seed shape and N fixation (Figure 3; Figure S3).

All nine plant species germinated in at least six of the 32 microcosms. Germination rates varied markedly, from an average of 25% across all microcosms for the shrub *Einadia nutans* to 1% for the shrub *Grevillea havilandii*. We did not observe any obvious allelopathic effects among different seeds in the microcosms. The microcosm study, based on species from similar families to those found in the seed bank study, showed predominantly positive plant-microbe interactions during germination (Figures 4 and 5; Table S1; Figures S3 and S4) and included multiple ectomycorrhizal associations. Negative interactions included multiple fungal parasites, while saprobes and endophytic fungal taxa resulted in both positive and negative plant-microbial associations (Table S2; Figure S4). Of the total number of potential connections ($n = 41,292$ combinations), 2.3% of bacterial connections were positive and 1.2% negative. For fungi, 0.43% of

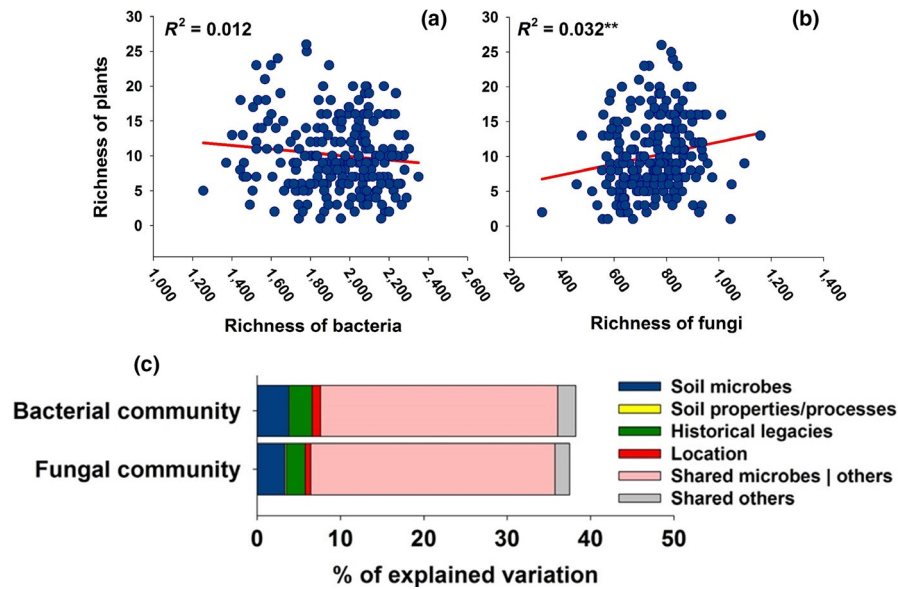


FIGURE 1 Relationship between the relative abundance of (a) bacterial and (b) fungal OTUs (Bray–Curtis) and the richness of seedlings germinated from the soil seed bank. The solid lines represent the fitted linear regressions and significant relationships were highlighted with **. (c) Variation partitioning model identifying the unique contribution of microbial communities in explaining the variation of the community composition of the germinable plants from the seed bank. ‘Shared microbe/other’ includes the shared explained variation in the distribution of emergent seeds between microbes, soil properties/processes, historical legacies and location. ‘Shared others’ includes the shared explained variation in the distribution of emergent seeds between soil properties/processes, historical legacies and location, not shared with microbes. Data based on the soil survey-seed bank study

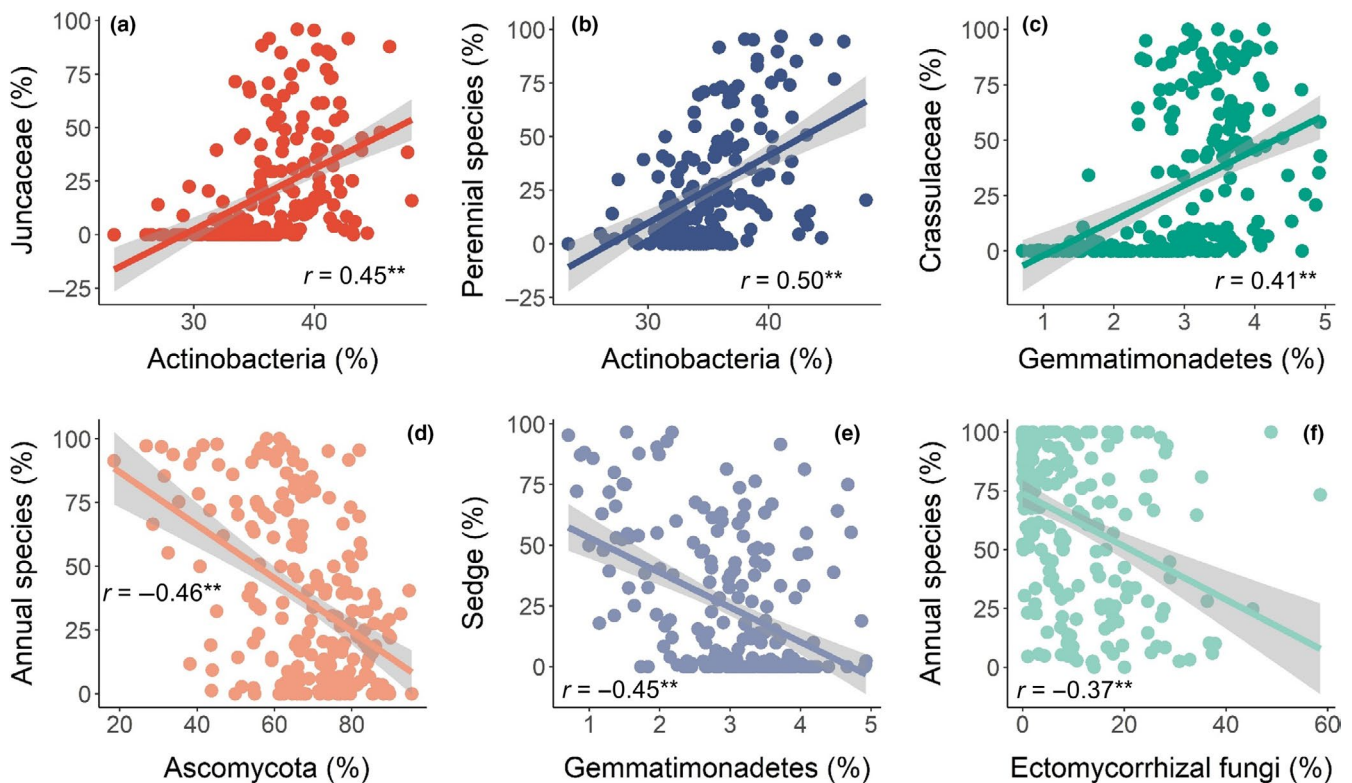


FIGURE 2 Relationships between (a–f) the relative abundance (%) of different microbial phyla and the relative abundance (%) of different plant groups or families (Pearson’s r). Significant relationships were highlighted with **

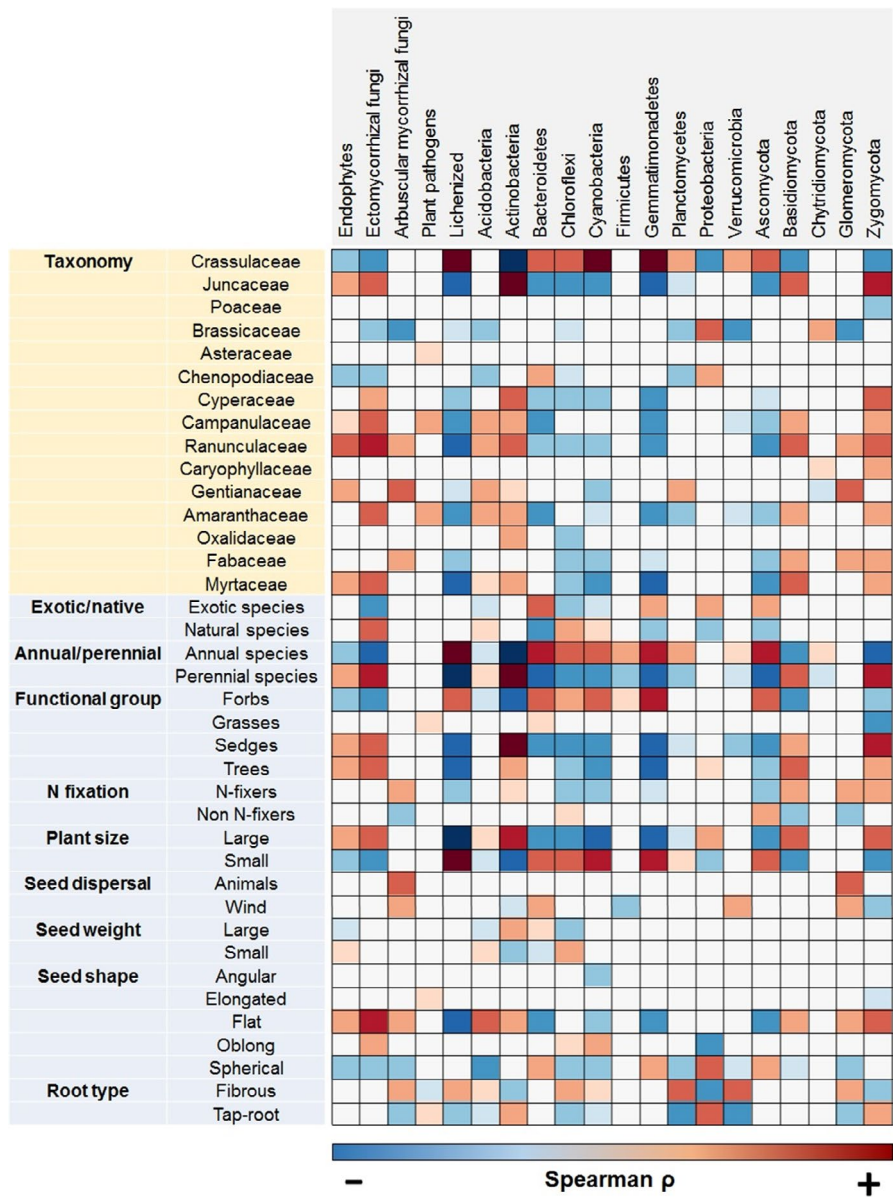


FIGURE 3 Significant correlations (Spearman's ρ ; $p \leq 0.05$) between the relative abundance (%) of different microbial phyla and fungal lifestyles and the relative abundance of germinants from 15 plant families, and of plant traits. Data based on the soil survey-seed bank study

connections were positive and 0.08% negative. In general, different plant species had their own associated germination microbiome (Figure 4b; Table S1), but we also found some microbial phylotypes that were positively associated with the germination of multiple plant species (Figure 4c,d; Table S1). These species included multiple bacterial taxa with a low number of reads (e.g. *Verrucomicrobia*, *Planctomycetes*) and some fungal saprobes (Table S1). Moreover, species within Alphaproteobacteria and Gammaproteobacteria were particularly important for promoting and suppressing the germination of multiple species (Figure 4c,d; Table S1).

4 | DISCUSSION

Our study demonstrates substantial correlations among seeds germinating in the seed bank and the soil microbial community and suggests that soil microbes might have a close relationship with

the community composition of plants emerging from the soil seed bank across large-scale environmental gradients. Despite the unique importance of microbial communities in predicting the variation in germinants, as expected, germination is not explained by microbes alone. Interestingly, a large part of the variation in germination is shared by microbial communities and the environment, suggesting that the potential interactions among microbes and their environment might also play a critical role in regulating plant germinants.

Multiple potential mechanisms might explain the linkages between soil microbial communities and germinants from the seed bank. The most likely explanations are either shared environmental preferences by plants and microbes, or potential interactions among plants and microbes during the germination of our focal plant species. Such potential interactions include the microbial breakdown of physical dormancy *via* physical and/or chemical changes to the seed coat that break physical dormancy (Chee-Sanford et al., 2006). Other potential mechanisms driving these results include the

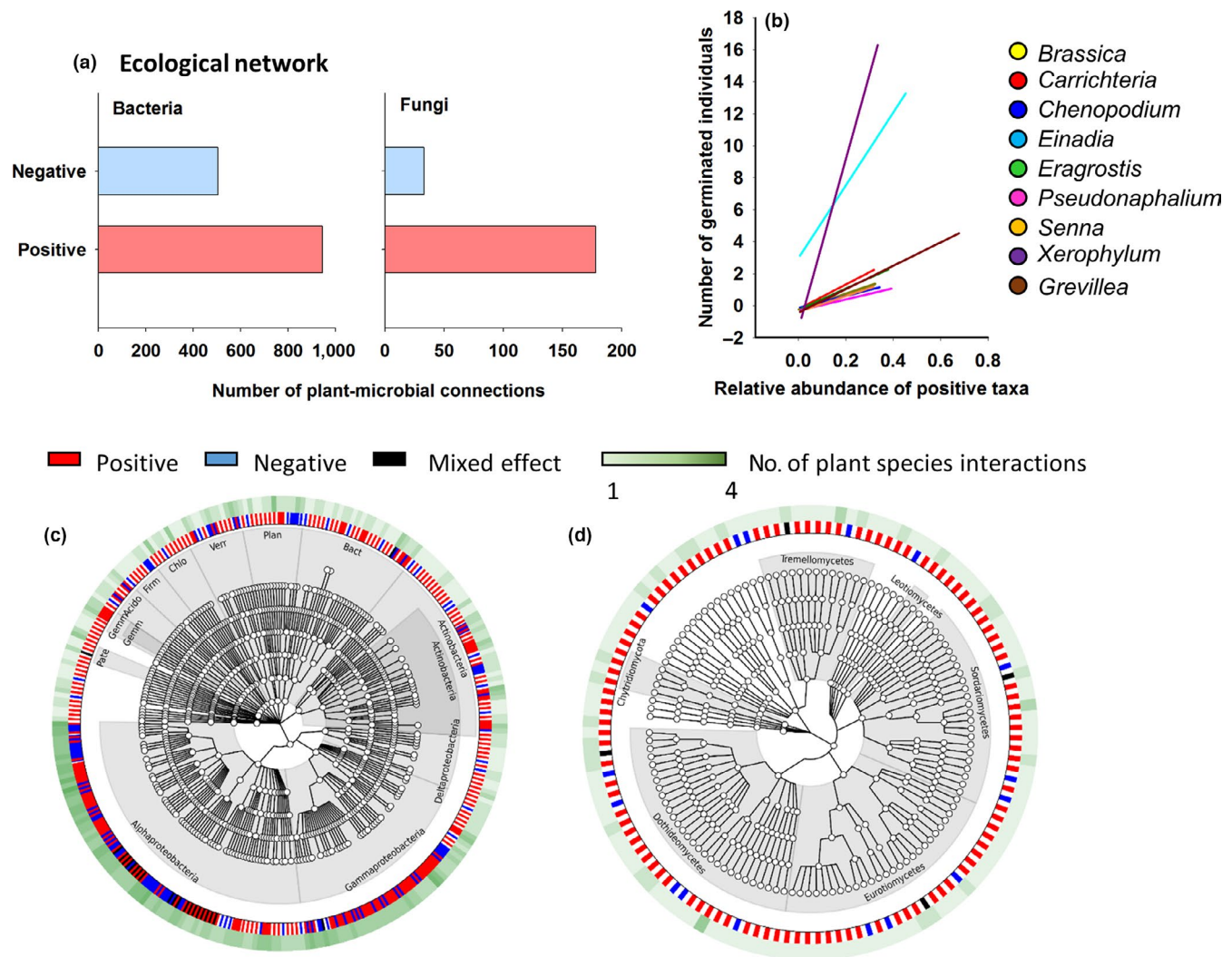


FIGURE 4 Microbiome associated with plant germination in a microcosm experiment. Panel (a) represents the number of negative and positive plant-microbial associations from a bivariate plant-microbe ecological network (Figure 5). Panel (b) includes the relationship between the relative abundance of positive taxa (Table S1) reported for each plant species and the number of individual germinants for each plant species. Panels (c) and (d) include circular tree diagrams including the taxonomic information on those bacterial and fungal taxa positively and negatively associated with multiple plant species in our microcosm experiment. Further details can be found in Table S1. Data based on the microcosm experiment

microbially driven release and competition for nutrient availability during germination (Nelson, 2018). Rapidly growing plants may be able to compete successfully with fast-growing microbial species for resources in soils.

We found that the strongest correlations among microbes and plant functional traits included those related to perenniality (with annuals mostly positive, and perennials mostly negative), growth form, plant size (Dinnage et al., 2019), root type and seed shape (particularly flat and spherical). Moreover, our results also provide evidence for multiple significant associations between taxonomic and functional groups of microbes (e.g. mycorrhizal fungi) and plant communities from the seed bank, which might reflect a preference for similar environmental conditions for plants and microbes, or their potential interactions. For example, we found that the relative abundance of small plants and annual plants was highly positively correlated with fast-growing microbial taxa such as Bacteroidetes

and Gemmatimonadetes (Fierer et al., 2007; Trivedi et al., 2013). Conversely, the relative abundance of large typically perennial plants such as *Juncus aridicola* or *Eleocharis acuta* (Juncaceae) was associated with microbial communities such as Actinobacteria (Arocha-Garza et al., 2017), whose relative abundance is typically high in environments such as deserts (Noy-Meir, 1979).

Our results also include some potential and important microbe-seed interactions. For example, there were strong positive correlations between the relative abundance of ectomycorrhizal fungi and that of the plants from the families Ranunculaceae, Juncaceae, Campanulaceae, Amaranthaceae and Myrtaceae (Bennett et al., 2017; Bougher, 1995; Newman & Reddell, 1987; Tawaray, 2003), as well as with the relative abundance of perennial and large plants, sedges and trees with flat seeds, which might benefit from symbiotic collaboration with mycorrhizal fungi (Tawaray, 2003). Another important association is the positive

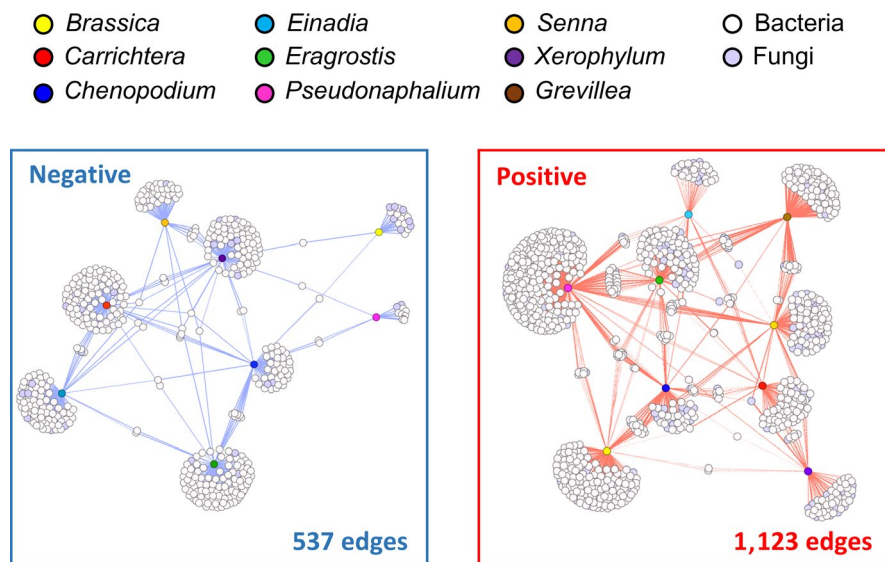


FIGURE 5 Bivariate network identifying the negative and positive links between microbial phylotypes and plant species in a microcosm experiment

relationship between the relative abundance of AMF and N-fixing plants. Nitrogen fixation requires high levels of P to support the high energy demand, and this P is provided by AMF fungi (Smith et al., 2011). Other examples include the positive correlations between lichenized fungi and plants such as members of the family Crassulaceae (*Crassula* spp.), tiny non-N-fixing annual plants growing at high densities on biocrusted dryland soils (Cunningham et al., 2002). The only significant, but weak, negative correlation among the abundance of potential fungal soil-borne plant pathogens and plant communities was associated with plants with fibrous root types. Our work also provides evidence for other less studied, yet potentially novel interactions among plants and microbes. For example, germinable plants with fibrous root types were positively associated with the relative abundance of Planctomycetes and Verrucomicrobia in soil, and negatively associated with the relative abundance of Proteobacteria, and the opposite was the case for germinants that develop tap roots. This differential effect of root type on microbes could be due to markedly greater surface area of fibrous than tap roots, and therefore potential greater microbial habitat. Further, fibrous roots are more likely to undergo continual breakdown. This root zone (detritosphere) is associated with a specific microbiome (Zhou et al., 2020), particularly with taxa associated with C and N utilization (Marschner et al., 2012). This filtering of both bacterial (Liu & Ludewig, 2019) and fungal (Yu et al., 2005) communities by root type provides further evidence of the specific physiological niches associated with plant roots that support different microbial taxa. This filtering of both bacterial (Liu & Ludewig, 2019) and fungal (Yu et al., 2005) communities by root type provides further evidence of the specific physiological niches associated with plant roots that support different microbial taxa.

The microcosm study indicated that plant-microbe interactions during germination were predominantly positive, particularly for six of the nine plant species. For example, *Grevillea* sp. included 100% of positive associations, with no negative

association detected (Figure S3; Table S1). This species, which is Gondwanan in origin and from the family Proteaceae, plays an important cultural role in aboriginal society and provides a critical ecosystem service as a food source for nectivorous birds. Positive associations included multiple ectomycorrhizal, while negative interactions include multiple fungal parasites. Saprobes and endophytic fungal taxa resulted in both positive and negative plant-microbial associations. In general, different plant species had their own associated germination microbiome, but we also found some microbial phylotypes that were positively associated with the germination of multiple plant species. These species included multiple bacterial taxa from rare phyla (e.g. *Verrucomicrobia*, *Planctomycetes*) and some fungal saprobes (Table S1). Moreover, species within Alphaproteobacteria and Gammaproteobacteria had important associations with the germination of multiple species.

Evidence suggests that the effects of microbes on germination could be due to specific seed-resident microbiota in the spermosphere, the environment immediately adjacent to the seed surface (Nelson, 2018). Different dispersal agents can influence those microbial communities acquired by seeds before they enter the soil seed bank. For example, bacteria can come into contact with seeds passed through the guts of birds (Hird et al., 2015) and mammals (Ley et al., 2008). In our study, we sterilized the seeds in order to control for seed-resident microbes prior to the microcosm study. Notwithstanding the strong results in our study, it is conceivable, however, that some seed bank microbes were transferred to the soil via the seeds, and therefore could be partly responsible for some of our results.

Our microcosm experiment provides experimental evidence to support the importance of soil biodiversity as a driver of the germination of plants in our study. Moreover, findings from this experiment provide support for research that strengthens work on microbially assisted plant cultivation (Figueira et al., 2019) or development towards greater resistant to unfavourable environmental

conditions. For example, inoculating barley plants with the root-colonizing fungus *Piriformospora indica* has been shown to increase its tolerance to both fungal disease and salt tolerance (Waller et al., 2005). Further, the use of rhizobacteria as agricultural bio-pesticides can substantially reduce the reliance on traditional, agricultural chemicals (Liu et al., 2017). These examples indicate the critical role of microbial-seed bank studies and their implications for improving the provision of essential ecosystem services such as food and forage production.

5 | CONCLUSIONS

Our study, combining an extensive field survey and laboratory microcosm experiment, provides strong evidence for the intimate and generally positive relationships among soil microbes and plant germination in a range of species from natural systems. Our field and laboratory results identified strong and significant associations among functional and taxonomic groups of microbes and the abundance of functional plant traits related to perenniality, plant growth form and size, root type and seed shape. These findings provide novel evidence for potential shared life strategies between plants and microbes at plant germination, the most critical stage in the life of plants, which is the major providers of food and habitat on Earth.

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AUTHORS' CONTRIBUTIONS

M.D.-B and D.J.E. designed the study; J.V., S.K.T. and D.J.E. collected the soil samples; J.V. undertook the seed bank study, and S.K.T. and J.D. managed the microcosm study; B.K.S. and J.-T.W. sequenced the microbes. The manuscript was written by D.J.E. and M.D.-B., and all the authors contributed to reviewing the manuscript.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.13660>.

DATA AVAILABILITY STATEMENT

Tables S1 and S2 data are archived with figshare <https://doi.org/10.6084/m9.figshare.11272298.v1> (Eldridge, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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