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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 seedbank study to examine the relationship among plants germinating from the seedbank 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 affected the germination of nine plant species.

3. The community composition of plants in the soil seedbank 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-microbial 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 seedbank

1 INTRODUCTION

Plants and soil microbes have co-evolved over millions of years (Field, Pressel, Duckett, Rimington, & Bidartondo, 2015; Lutzoni et al., 2018). Plants synthesise 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, Westover, & Antonovics, 1997; Bezemer et al., 2006; Herrera Paredes & Lebeis, 2016; Kulmatiski, Beard, Stevens, & Cobbold, 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 (Aprahamian et al., 2016). Indeed, associations with AMF may have allowed plants to colonize land more than 400 million years ago (Schußler & 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, Bardgett, & van Straalen, 2008).

Despite the acknowledgement that seeds provide a range of habitats for diverse microbial assemblages (Compant, Clément, & Sessitsch, 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, Simoneau, Barret, Mitter, & Company, 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 identify 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 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, Williams, Davis, & Sims, 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, Cherdyntseva, Botina, & Netrusov, 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 seedbank, we combined a field survey with a soil seedbank study using soils from 54 sites in eastern Australia, and a laboratory-based microcosm experiment. The soil seedbank study identified a large number of seedbank species, some that were absent from the standing plant community (Val, Travers, Oliver, & Eldridge, 2020). We aimed to link seedbank 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 seedbank. 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 seedbank 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 (seedbank–soil microbe relationships) was undertaken using soils from three woodland communities in semi-arid eastern Australia that were characterised 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, 100 m) we located the nearest perennial grass, shrub, tree and bare microsite, and placed a 0.50 m^2 circular quadrat in these microsites within which the cover and abundance of all vascular plants was recorded. We collected 10 cores of soil from the top 5 cm, where most seeds occur (Traba, Azcárate, & Peco, 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 ± 0.03%, mean ± SE) with high temperature combustion using a LECO CNS-2000 CNS Analyser (LECO Corporation, St Joseph, MI, USA) 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 1.00g of soil, as described in reference (Bell et al., 2013). Aridity was calculated as

1-Aridity Index (precipitation/potential evapotranspiration) using the FAO global aridity map (http://data.fao.org/en/map). 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., 2011). 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 a 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 Seedbank emergence study

Soil samples were mixed and sifted to remove woody debris and a 150 g sub-sample spread evenly (~ 5 mm deep) over sterilised sand in commercial germination trays (35 cm x 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 i.e., trays containing only sterilised sand were evenly distrusted in the poly house to control for glasshouse weeds and seeds within the sterilised sand. Emerging plants were counted and removed following identification and different species re-potted 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 seedbank 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 seedbank study. These plant species were selected based on a range of different traits

(seed size, 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 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 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^{-1} soil. Soil for the inoculations came 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 sterilised 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 recorded. Some germinants were grown out separately to confirm their identity.

2.4 DNA extraction and bioinformatics for seedbank soils and microcosm soils

We characterized the microbial community (fungi and bacteria) for the 216 soils forming the seedbank 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, Carlsbad, CA, USA) according to the manufacturer's instructions. Amplicons targeting the bacterial 16S rRNA gene (341F-805R; Herlemann et al., 2011) and the fungal ITS region (FITS7-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 Bell et al.

(2013). Briefly, UPARSE (Edgar, 2013; Edgar, 2016) were 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 seedbank 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

Seedbank study: For the seedbank 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 seedbank (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 to 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 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).

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 2950 microbial phylotypes (1111 fungal and 1839 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 seedbank study, Actinobacteria and Proteobacteria accounted for almost 50% of the total abundance of bacterial OTUs, and Ascomycetes 68% of fungal OTUs (Figs. S1a, S1b). The germinable soil seedbank yielded 24,651 seedlings from 196 species and 45 families (Figs. S1c, S1d). We found that the community composition of plants in the soil seedbank 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 $\rho = 0.42$ to 0.51) than bare soils (Mantel $\rho = 0.30$ to 0.38). We also found a strong correlation between the richness of seedbank germinants and both bacterial (Fig. 1a) and fungal (Fig. 1b) richness. The strongest correlations among microbes and plant functional traits included those related to perenniality (with annuals mostly positive, and perennials mostly negative, Fig. 2, Fig. S2), growth form, plant size, root type, and seed shape (particularly flat and spherical; Fig. 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 (Fig. 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 Ranunculacea, Juncaceae, Campanulaceae, Amaranthaceae and Myrtaceae, as well as some plant traits such as seed shape and N-fixation (Fig. 3; Fig. 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 sub-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 seedbank study, showed predominantly positive plant-microbe interactions during germination (Figs. 4 & 5, Table S1, Figs. S3 & 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, Fig. S4). Of the total number of potential connections ($n = 41292$ combinations), 2.3% of bacterial connections were positive and 1.2% negative. For fungi, 0.43% of connections were positive and 0.08% negative. In general, different plant species had their own associated germination microbiome (Fig. 4b, Table S1), but we also found some microbial phylotypes that were positively associated with the germination of

multiple plant species (Figs. 4c & 4d, 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 (Figs. 4c & 4d, Table S1).

4. DISCUSSION

Our study demonstrates substantial correlations among seeds germinating in the seedbank 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 seedbank 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 seedbank. 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 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., 2018), 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 seedbank, 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, Bradford, & Jackson, 2007; Trivedi, Anderson, & Singh, 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, Canales-Del Castillo, Eguiarte, Souza, & De la Torre-Zavala, 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 found strong positive correlations between the relative abundance of ectomycorrhizal fungi and that of the plants from the families Ranunculacea, Juncaceae, Campanulaceae, Amaranthaceae and Myrtaceae (Bennett et al., 2017; Bougher, 1995; Newman & Reddell, 1987; Tawaraya, 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 (Tawaraya, 2003). Another important association is the positive 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, Jakobsen, Gronlund, & Smith, 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, Mulham, Milthorpe, & Leigh, 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 (detritusphere) is associated with a specific microbiome (Zhou et al., 2020), particularly with taxa associated with C and N utilisation (Marschner, Marhan, & Kandeler, 2012). This filtering of both bacterial (Liu & Ludewig, 2019) and fungal (Yu, Lee, Kim, & Hwang, 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 (Fig. 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 seedbank. For example, bacteria can come into contact with seeds passed through the guts of birds (Hird, Sanchez, Carstens, & Brumfield, 2015) and mammals (Ley, Lozupone,

Hamady, Knight, & Gordon, 2008). In our study we sterilised 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 seedbank 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 plant germination, of our focal species. Moreover, findings from this experiment provide support for research that strengthens work on microbially-assisted plant cultivation (Figueira, Ferreira, Silva & Cunha, 2019) or development towards greater resistant to unfavourable environmental conditions. For example, inoculating barley plants with the root-colonising 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 biopesticides can substantially reduce the reliance on traditional, agricultural chemicals (Liu, Newman, McInroy, Hu, & Kloepper, 2017). These examples indicate the critical role of microbial-seedbank 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, 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 seedbank 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 authors contributed to reviewing the manuscript.

DATA AVAILABILITY

Tables S1 and S2 data are archived with Figshare https://figshare.com/articles/dataset/Microbe_seedbanks/11272298/1 (Eldridge, 2020).

REFERENCES

Aprahamian, A. M., Lulow, M. E., Major, M. R., Balazs, K. R., Treseder, K. K., & Maltz, M. R. (2016). Arbuscular mycorrhizal inoculation in coastal sage scrub restoration. *Botany*, 94, 493-499. https://doi.org/10.1139/cjb-2015-0226

Arocha-Garza, H. F., Canales-Del Castillo, R., Eguiarte, L. E., Souza,V., & De la Torre-Zavala, S. (2017). High diversity and suggested endemicity of culturable Actinobacteria in an extremely oligotrophic desert oasis. *PeerJ*, 5 e3247. doi: 10.7717/peerj.3247

Balshor, B. J., Garrambone, M. S., Austin, P., Balazs, K. R., Weihe, C., Martiny, J. B. H., Huxman, T. E., McCollum, J. R., & Kimball Sarah. (2017). The effect of soil inoculants on seed germination of native and invasive species. *Botany*, 95, 469-480. https://doi.org/10.1139/cjb-2016-0248

Baskin, J. M., & Baskin, C. C. (1989). in *Ecology of soil seed banks* (eds M. A. Leck, V. T. Parker & R. L. Simpson) 53-66 (Academic Press). https://doi.org/10.1016/b978-0-12-440405-2.50009-9

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments*, 15, p.e50961. https://doi.org/10.3791/50961

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M., & Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, 355, 181-184. https://doi.org/10.1126/science.aai8212

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology*, 85, 561-573. https://doi.org/10.2307/2960528

Bezemer, T. M., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R., & Van der Putten, W. H. (2006). Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology*, 94, 893-904. https://doi.org/10.1111/j.1365-2745.2006.01158.x

Bougher, N. L. (1995). Diversity of ectomycorrhizal fungi associated with eucalypts in Australia. In 'Mycorrhizas for plantation forestry in Asia'. (Eds M Brundrett, B Dell, N Malajczuk, G Mingqin) pp. 8–15. (ACIAR: Canberra).

Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytologist*, 154, 275-304. https://doi.org/10.1046/j.1469-8137.2002.00397.x

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., & Huttley, G. A. (2010). QIIME allows analysis of

high-throughput community sequencing data. *Nature Methods*, 7, 335-336. https://doi.org/10.1038/nmeth.f.303

Chee-Sanford J. C., Williams II, M. M., Davis, A. S., & Sims, G. K. (2006). Do microorganisms influence seed-bank dynamics? *Weed Science*, 54, 575-587. https://doi.org/10.1614/ws-05-055r.1

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, 42, 669-678. https://doi.org/10.1016/j.soilbio.2009.11.024

Cunningham, G. M., Mulham, W. E., Milthorpe, P. L., & Leigh, J. H. (2002). Plants of Western New South Wales. (CSIRO Publishing). https://doi.org/10.1071/9780643104273

Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., Benavent-González, A., Eldridge, D. J., Bardgett, R. D., Maestre, F. T., Singh, B. K., & Fierer, N. (2018). A global atlas of the dominant bacteria found in soil. *Science*, 359, 320-325. https://doi.org/10.1126/science.aap9516

Dinnage, R., Simonsen, A. K., Barrett, L. G., Cardillo, M., Raisbeck-Brown, N., Thrall, P. H., & Prober, S. M. (2019). Larger plants promote a greater diversity of symbiotic nitrogen-fixing soil bacteria associated with an Australian endemic legume. *Journal of Ecology*, 107, 977-991. https://doi.org/10.1111/1365-2745.13083

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, 10, 996–998. https://doi.org/10.1038/nmeth.2604

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*, 081257. http://dx.doi.org/10.1101/081257d

Eldridge, D. J., Delgado-Baquerizo, M., Travers, S. K., Val, J., Oliver, I., Hamonts, K., & Singh, B. K. (2017). Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores. *Ecology*, 98, 1922-1931. https://doi.org/10.1002/ecy.1879

Eldridge, D. J. (2020). Microbe seedbanks. figshare. Dataset. https://doi.org/10.6084/m9.figshare.11272298.v1

Field, K. J., Pressel, S., Duckett, J. G., Rimington, W. R., & Bidartondo, M. I. (2015). Symbiotic options for the conquest of land. *Trends in Ecology & Evolution*, 30, 477-486. https://doi.org/10.1016/j.tree.2015.05.007

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, 88, 1354-1364. https://doi.org/10.1890/05-1839

Figueira, C., Ferreira, M. J., Silva, H., & Cunha, A. (2019). Improved germination efficiency of *Salicornia ramosissima* seeds inoculated with *Bacillus aryabhattai* SP1016-20. *Annals of Applied Biology*, 174, 319-328. https://doi.org/10.1111/aab.12495

Harper, S. H. T., & Lynch, J. M. (1980). Microbial effects of microbes on the germination and seedling growth of barley. *New Phytologist*, 84, 473-481. https://doi.org/10.1111/j.1469-8137.1980.tb04554.x

Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME Journal*, 5, 1571-1579. https://doi.org/10.1038/ismej.2011.41

Herrera Paredes, S., & Lebeis, S. L. (2016). Giving back to the community: microbial mechanisms of plant–soil interactions. *Functional Ecology*, 30, 1043-1052. https://doi.org/10.1111/1365-2435.12684

Hird, S. M., Sánchez, C., Carstens, B. C., & Brumfield, R. T. (2015). Comparative gut microbiota of 59 neotropical bird species. *Frontiers in Microbiology*, 6, 1403. https://doi.org/10.3389/fmicb.2015.01403

Ihrmark, K., Bödeker, I., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, 82, 666-677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Kõljalg, U., Larsson, K. H., Abarenkov, K., Nilsson, R. H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., & Pennanen, T. (2005). UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist*, 166, 1063-1068. https://doi.org/10.1111/j.1469-8137.2005.01376.x

Koziol, L., Schultz, P. A., House, G. L. Bauer, J. T., Middleton, E. L., & Bever, J. D. (2018). The plant microbiome and native plant restoration: The example of native Mycorrhizal fungi. *BioScience*, 68, 996-1006. https://doi.org/10.1093/biosci/biy125

Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant–soil feedbacks: a meta-analytical review. *Ecology Letters*, 11, 980-992. https://doi.org/10.1111/j.1461-0248.2008.01209.x

Leff, J. W., Bardgett, R. D., Wilkinson, A., Jackson, B. G., Pritchard, W. J., Jonathan, R., Oakley, S., Mason, K. E., Ostle, N. J., Johnson, D., & Baggs, E. M. (2018). Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *The ISME journal*, 12, 1794-1805. https://doi.org/10.1038/s41396-018-0089-x

Legendre, P. (2008). Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *Journal of Plant Ecology*, 1, 3-8. https://doi.org/10.1093/jpe/rtm001

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., Schlegel, M. L., Tucker, T. A., Schrenzel, M. D., Knight, R., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*. 320, 1647-1651. https://doi.org/10.4161/gmic.21905

Liu, K., Newman, M., McInroy, J. A., Hu, C. H., & Kloepper, J. W. (2017). Selection and assessment of plant growth-promoting rhizobacteria for biological control of multiple plant diseases. *Phytopathology*, 107, 928-936. https://doi.org/10.1094/phyto-02-17-0051-r

Liu, Y., & Ludewig, U. (2019). Nitrogen-dependent bacterial community shifts in root, rhizome and rhizosphere of nutrient-efficient *Miscanthus x giganteus* from long-term field trials. *Global Change Biology Bioenergy*, 11, 1334-1347. https://doi.org/10.1111/gcbb.12634

Lutzoni, F., Nowak, M. D., Alfaro, M. E., Reeb, V., Miadlikowska, J., Krug, M., Arnold, A. E., Lewis, L. A., Swofford, D. L., Hibbett, D., & Hilu, K. (2018). Contemporaneous radiations of fungi and plants linked to symbiosis. *Nature Communications*, 9, 1-11. https://doi.org/10.1038/s41467-018-07849-9

Marques, F. F., Buckland, S. T., Goffin, D., Dixon, C. E., Borchers, D. L., Mayle, B. A., & Peace, A. J. (2001). Estimating deer abundance from line transect surveys of dung: sika deer in southern Scotland. *Journal of Applied Ecology*, 38, 349-363. https://doi.org/10.1046/j.1365-2664.2001.00584.x

Marschner, P., Marhan, S., & Kandeler, E. (2012). Microscale distribution and function of soil microorganisms in the interface between rhizosphere and detritusphere. *Soil Biology & Biochemistry*, 49, 174-183. https://doi.org/10.1016/j.soilbio.2012.01.033

Nelson, E. B., Simoneau, P., Barret, M., Mitter, B., & Company, S. (2018). Editorial special issue: the soil, the seed, the microbes and the plant. *Plant & Soil*, 422, 1-5. https://doi.org/10.1007/s11104-018-3576-y

Nelson, E. B. (2018). The seed microbiome: Origins, interactions, and impacts. *Plant & Soil*, 422, 7-34. https://doi.org/10.1007/s11104-017-3289-7

Newman, E. I., & Reddell, P. (1987). The distribution of mycorrhizas among families of vascular plants. *New Phytologist*, 106, 745-751. https://doi.org/10.1111/j.1469-8137.1987.tb00175.x

Noy-Meir, I. (1979). Structure and function of desert ecosystems. *Israel Journal of Botany*, 28, 1-19. https://doi.org/10.1080/0021213X.1979.10676851

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2015). vegan: Community Ecology Package. R package version 2.3-5.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41, D590-D596. https://doi.org/10.1093/nar/gks1219

Rocha, I. D., Ma, Y., Souza-alonso, P., Vosatka, M., Freitas, H., & Oliveira, R. S. (2019). Seed coating: a tool for delivering beneficial microbes to agricultural crops. *Frontiers in Plant Science*, 10, 1357. https://doi.org/10.3389/fpls.2019.01357

Schußler, A., & Walker, C. (2011). 7 Evolution of the 'Plant-Symbiotic' Fungal Phylum, Glomeromycota. In *Evolution of fungi and fungal-like organisms* (pp. 163-185). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-19974-5_7

Smith, S. E., Jakobsen, I., Gronlund, M., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiology*, 156, 1050-1057. https://doi.org/10.1104/pp.111.174581

Tawaraya, K. (2003). Arbuscular mycorrhizal dependency of different plant species and cultivars. *Soil Science and Plant Nutrition*, 49, 655-668. https://doi.org/10.1080/00380768.2003.10410323

Traba, J., Azcárate, F. M., & Peco, B. (2004). From what depth do seeds emerge? A soil seed bank experiment with Mediterranean grassland species. *Seed Science Research*, 14, 297-303. https://doi.org/10.1079/ssr2004179

Trivedi, P., Anderson, I. C., & Singh, B. K. (2013). Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends in Microbiology*, 21, 641-51. https://doi.org/10.1016/j.tim.2013.09.005

Tsavkelova, E. A., Cherdyntseva, T. A., Botina, S. G., & Netrusov, A. I. (2007). Bacteria associated with orchid roots and microbial production of auxin. *Microbiological Research*, 162, 69-76. https://doi.org/10.1016/j.micres.2006.07.014

Val, J., Travers, S. K., Oliver, I., & Eldridge, D. J. (2020). Perennial plant patches are sinks for seeds in semi-arid woodlands in varying condition. *Applied Vegetation Science*, 23, 377-385. https://doi.org/10.1111/avsc.12486

van der Heijden, M. G., Bardgett, R. D., & van Straalen, N. M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11, 296-310. https://doi.org/10.1111/j.1461-0248.2007.01139.x

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., & Suding, K. N. (2013). Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, 101, 265-276. https://doi.org/10.1111/1365-2745.12054

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., Von Wettstein, D., & Franken, P. (2005). The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proceedings of the National Academy of Sciences*, 102, 13386-13391. https://doi.org/10.1073/pnas.0504423102

Wang, B., Yeun, L. H., Xue, J. Y., Liu, Y., Ané, J. M., & Qiu, Y. L. (2010). Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist*, 186, 514-525. https://doi.org/10.1111/j.1469-8137.2009.03137.x

Yu, Y., Lee, C., Kim, J., & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering*, 89, 670-679. https://doi.org/10.1002/bit.20347

Zhou, Y., Coventry, D. R., Gupta, V. V., Fuentes, D., Merchant, A., Kaiser, B. N., Li, J., Wei, Y., Liu, H., Wang, Y., & Gan, S., (2020). The preceding root system drives the composition and function of the rhizosphere microbiome. *Genome Biology*, 21, 1-19. https://doi.org/10.1186/s13059-020-01999-0

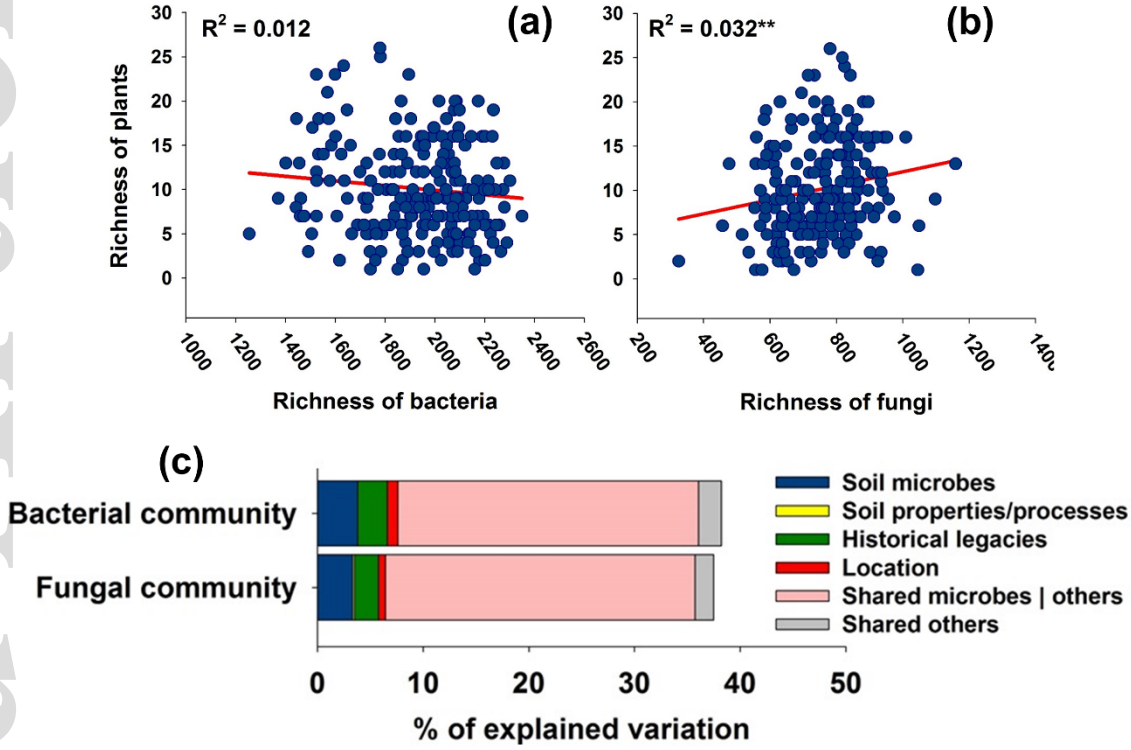

Figure 1 Relationship between the relative abundance of (a) bacterial and (b) fungal OTUs (Bray-Curtis) and the richness of seedlings germinated from the soil seedbank. The solid lines represent the fitted linear regressions. (c) Variation partitioning model identifying the unique contribution of microbial communities in explaining the variation of the community composition of the germinable plants from the seedbank. '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-seedbank study.

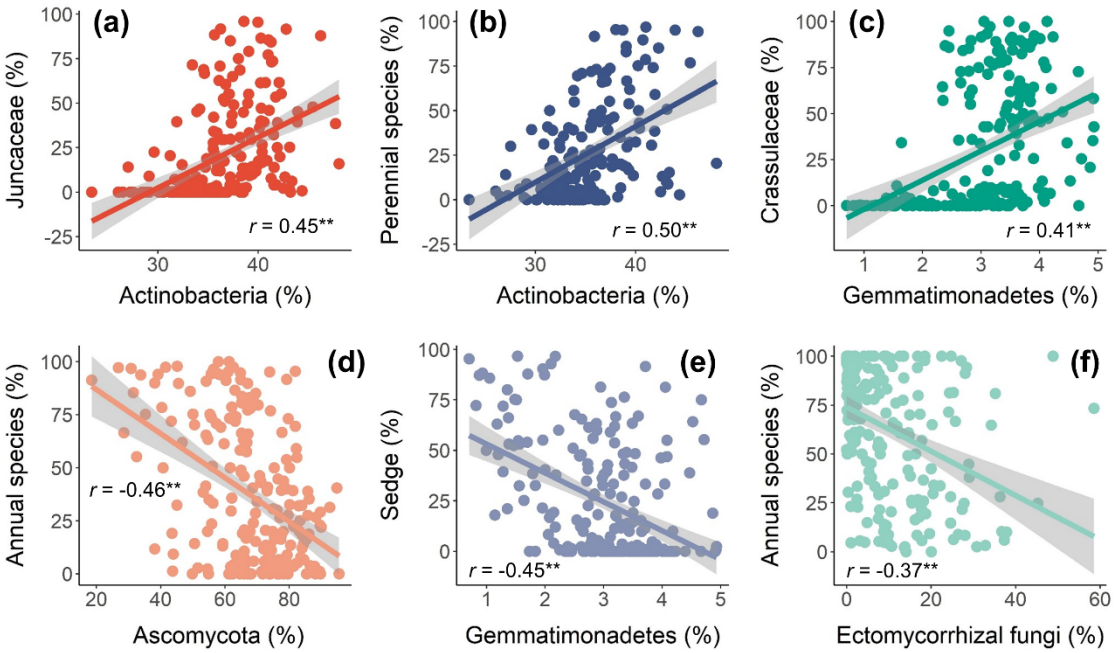

Figure 2. Relationships between the relative abundance (%) of different microbial phyla and the relative abundance (%) of different plant groups or families (Pearson's *r*).

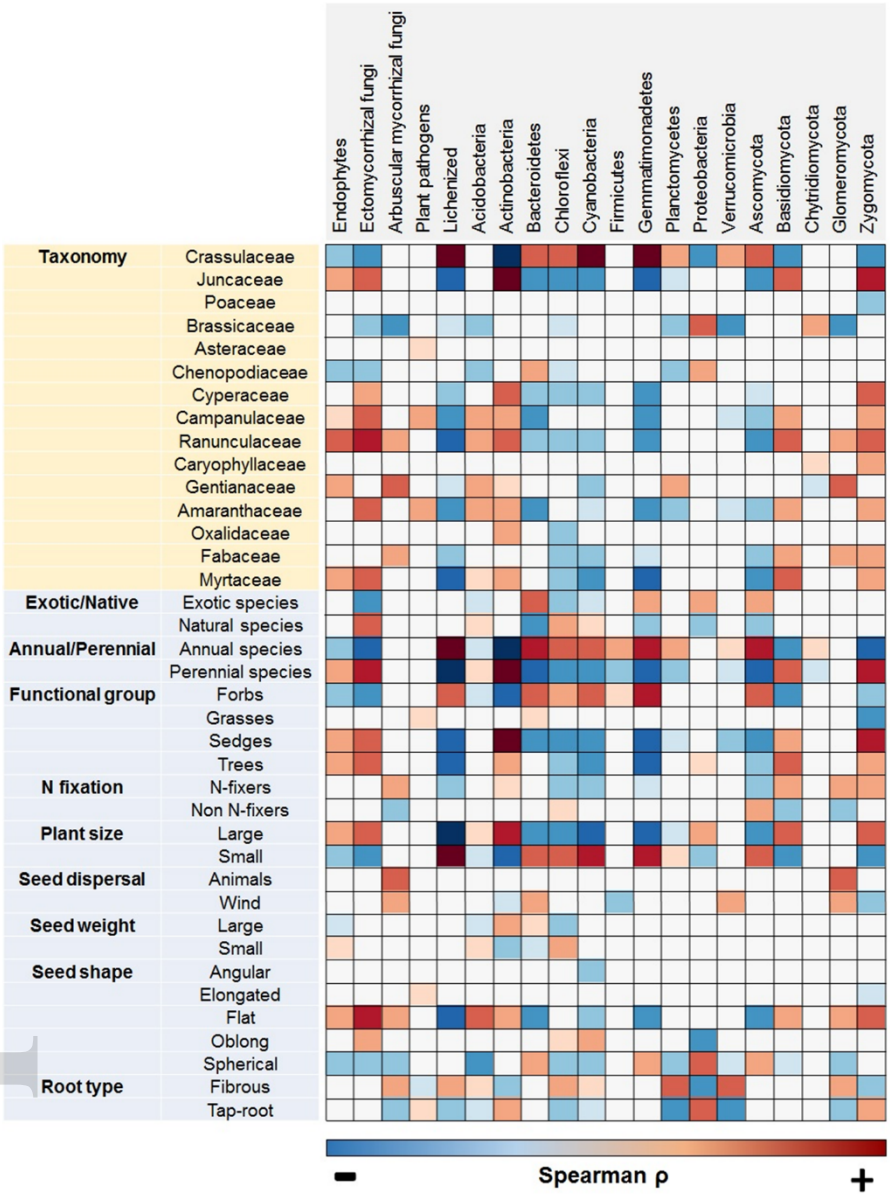

Figure 3. Significant correlations (Spearman's ρ; $P \leq 0.05$) between the relative abundance (%) of different microbial phyla and fungal lifestyles and the relative abundance of germinants from 15 plant families, and of plant traits. Data based on the soil survey-seedbank study.

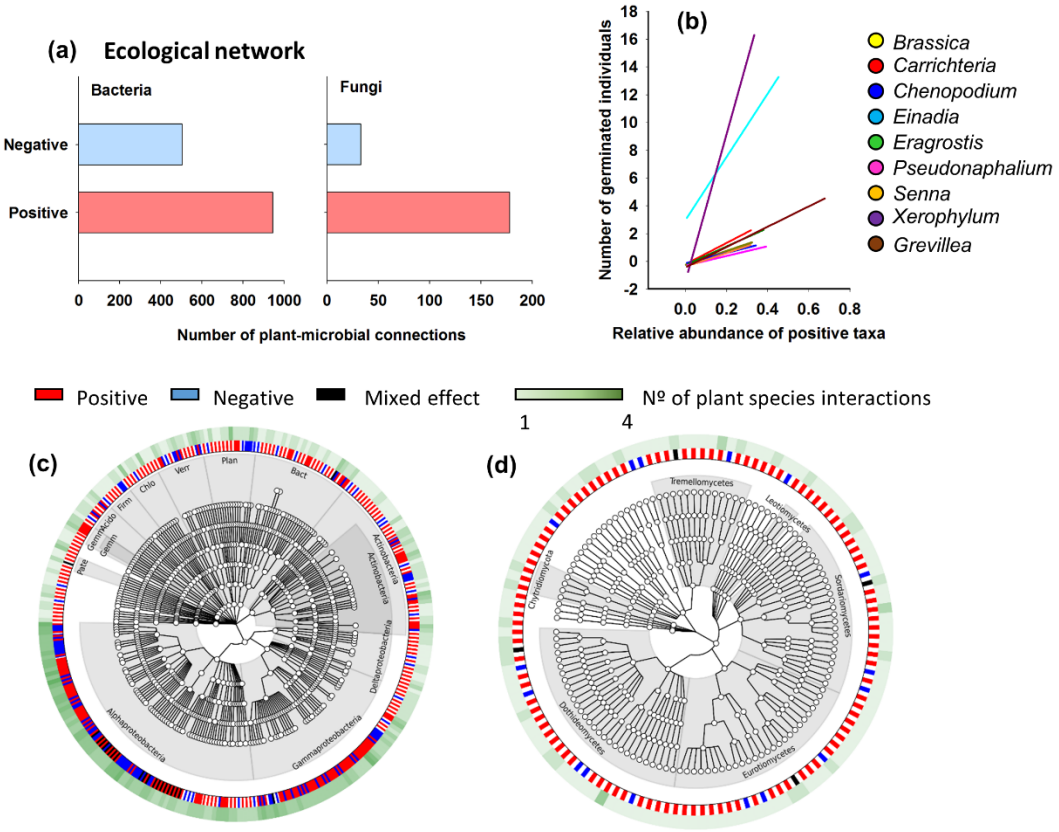

Figure 4. Microbiome associated with plant germination in a microcosm experiment. Panel (a) represents the number of negative and positive plant-microbial associations from a bivariate plant-microbe ecological network (Fig. 5). Panel (b) includes the relationship between the relative abundance of positive 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.

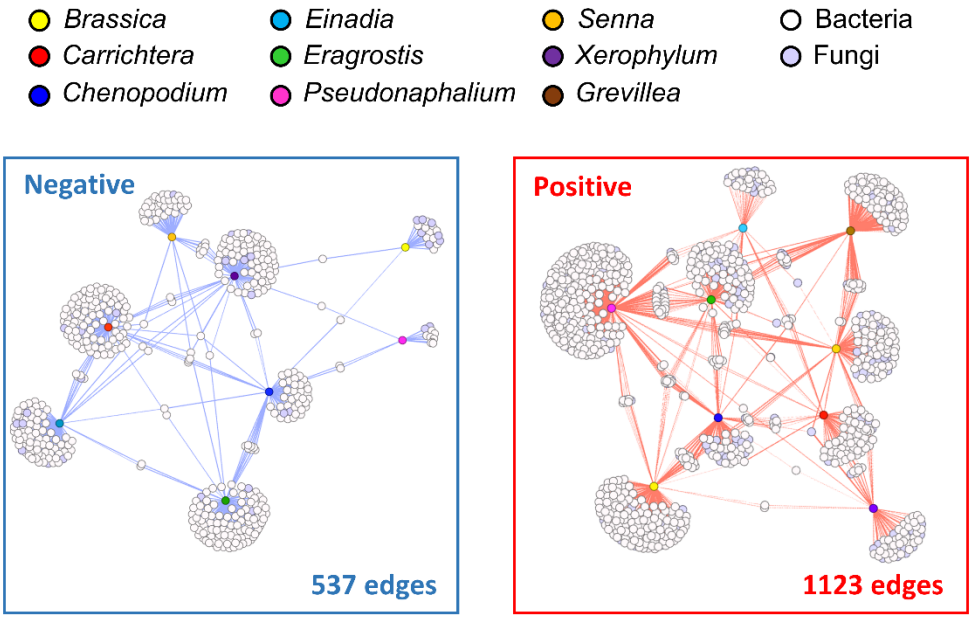

Figure 5. Bivariate network identifying the negative and positive links between microbial phylotypes and plant species in a microcosm experiment.