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ON THE NATURE OF THINGS

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Rewiring critical plant–soil microbial interactions to assist ecological restoration

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More than three quarters of terrestrial habitats have been transformed by human activities (Ellis et al., 2021). As a result, reversing land degradation is one of humanity's chief imperatives, with the United Nations declaring 2021–2030 the Decade of Ecosystem Restoration. Restoring degraded lands requires rebuilding and protecting ecological communities that support biodiversity and ecosystem functions. These efforts normally focus on macroscopic native organisms (usually plants) that through time have co-evolved and developed interaction networks with other species across a diverse range of niches. The rich interdependencies in such ecological interaction networks (EINs) enhance the integrity of ecosystems and improve resilience to stressors (e.g., Barnes et al., 2020). However, the restoration of these networks is often ineffective, with unpredictable outcomes, in part because restoration usually focuses on a few plant species or specific animal habitats, and therefore wider interaction networks are generally not considered. Some researchers have also suggested that networks (e.g., plant–pollinator) develop as "passengers" of restored plant communities (e.g., Menz et al., 2011), while others have argued that the restoration of key taxa has the potential to re-establish functional networks (Pocock et al., 2012). Restoration would thus benefit from approaches that facilitate the reassembly of EINs early in the process, e.g., through targeted rewiring of key biotic interactions. One promising approach is the direct manipulation of soil microbes to enhance restoration success (Koziol et al., 2022). Working with soil microbial communities to restore vegetation is not a new idea (Coban et al., 2022), but identifying and using specific soil microorganisms to shortcut the development of key plant–microbial interactions to enhance the colonization and persistence of diverse native plant communities is.

Soil microbes play a crucial role in the functioning of all ecosystems, e.g., influencing plant performance by maximizing nutrient uptake and alleviating the impacts of stressors (Trivedi et al., 2020). Microbes are heterogeneously distributed in soils, largely because of variations in abiotic properties such as pH and organic carbon (Fierer, 2017). Therefore, when ecosystems experience degradation that alters abiotic soil properties, their soil microbial communities (and associated functions) are also altered. It has therefore been suggested that restoring and maintaining healthy soil microbial communities would benefit ecological restoration (here referring to the re-establishment of native plant communities; Coban et al., 2022). However, a recent global meta-analysis has clearly shown that manipulations of soil microbial communities during ecological restoration have had varied success (Gerrits et al., 2023), suggesting that the wholesale manipulation of soil microbial communities is difficult, if not impossible, partly due to the altered abiotic soil properties of degraded lands.

A more feasible option than the wholesale manipulation of soil microbial communities to reassemble critical plant–microbial interactions during ecological restoration may be the manipulation of specific microbes. It can be argued that symbiotic mutualists (e.g., mycorrhiza, rhizobia) are the key microbes to target for the restoration of critical interactions. The benefit of manipulating such mutualists to improve plant performance has been well demonstrated in agricultural systems and, to some extent, in natural environments (e.g., Farrell et al., 2020). For example, large-scale revegetation trials in Australia showed that inoculation with generalist rhizobia

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enhances the establishment of *Acacia* seedlings in some, but not all, habitats (Thrall et al., 2005). Similarly, Koziol and Bever (2017) found that the benefits of inoculation with arbuscular mycorrhizal fungi (AMF) for grassland restoration in North America were dependent on the type of AMF used. These two examples illustrate that the outcomes of interventions to reassemble key plant–mutualist interactions during restoration are varied and context-dependent. Using the restoration of land previously invaded by alien species as an example, we provide ideas on how to identify the most-promising soil microbial mutualists to predictably re-establish key native species interactions during restoration.

IMPACTS OF INVASIVE PLANTS ON NATIVE SOIL MICROBIAL COMMUNITIES

We studied the impacts of African olive (*Olea europaea* subsp. *cuspidata*) invasion in the Critically Endangered Cumberland Plain Woodland (CPW) in southwestern Sydney, Australia. We grew seedlings of the native legume *Acacia implexa* (hickory wattle) in soils collected from sites that had been cleared of African olive and restored 25 years ago (restored soil), sites where dense African olive is still present (invaded soil), and reference sites where African olive has never invaded (uninvaded soil). Hickory wattle forms mutualisms with rhizobia. These bacteria stimulate the formation of specialized structures, called nodules, in the roots of legumes where they fix atmospheric nitrogen in exchange for plant-derived sugars. Levels of nodulation and nitrogen fixation, however, depend on the availability of compatible and effective rhizobia. For example, hickory wattle nodulates exclusively with *Bradyrhizobium* strains. We isolated DNA from the nodules of hickory wattle seedlings grown in the three soil types and used DNA barcoding to identify *Bradyrhizobium* strains that are characteristic of each soil type (i.e., indicator taxa; see Le Roux et al., 2018 for similar methodologies). We found the relative abundance of indicator *Bradyrhizobium* taxa of invaded and uninvaded soils to be negatively and positively correlated, respectively, with nodulation across all soil types, while the relative abundance of non-indicator taxa did not correlate with nodulation (Figure 1).

The example above clearly illustrates the impact of invasion on the availability of mutualists for native plants. In instances such as these, it is critical that we incorporate our knowledge of EINs (Moreno-Mateos et al., 2020) to determine the most effective mutualists at the community level to enhance restoration success; however, there are currently no guidelines on how to ascertain which will be most effective. Identifying suitable mutualists for use in restoration is further complicated by the fact that establishing ecological interactions requires the introduction of species with compatible traits, phenologies, and high encounter probability (Moreno-Mateos et al., 2020). We suggest that paying attention to the structural properties of EINs among plant and soil microbial mutualists can help ecologists identify the most-promising microbial mutualists for effective use in restoration.

A NETWORK PERSPECTIVE TO INFORM RESTORATION

All species interactions fall somewhere along a continuum of specialization with, at one end, specialist species interacting with only one or a few taxa, while at the other end, generalists can successfully interact with a wide range of taxa. Mutualistic EINs are typically nested, e.g., with specialist plant species interacting with generalist mutualists, while generalist plants interact with both generalist and specialist mutualists (e.g., Figure 2D). The mutualists interacting with generalist host plants are also often mutually substitutable in terms of the benefits they provide to their hosts, i.e., being functionally redundant. Conversely, the mutualists of specialist plants may not be substitutable. For example, network analyses between rhizobia and invasive and native legumes in South Africa showed that native legumes with highly specialized rhizobium interactions were unable to persist in invaded habitats, while generalist native legumes could, but only in association with novel rhizobia (Le Roux et al., 2016). The introduction, retention, or removal of specialists may thus strongly influence the functioning of EINs (Warwick et al., 2022). Introducing super-generalist mutualists that are compatible with both specialist and generalist plant species, identified via network analyses in reference sites (e.g., rhizobium species 1 in Figure 2D), will provide symbiotic benefits to the highest number of native plant species to increase plant functional diversity in degraded soils. For the hickory wattle example, we suggest that indicator *Bradyrhizobium* strains of uninvaded (i.e., reference) soil will be of high restoration value if network analysis identifies them as also having strong links with generalist native legume species.

Like super-generalist mutualists, the introduction of super-generalist host plants may be useful targets for ecological restoration, with possible knock-on benefits to other trophic networks (Pocock et al., 2012). These host plants could be introduced without mutualists, as they are likely to encounter compatible partners in degraded soils or, alternatively, they can be used to "trap" mutualists for inoculum development in reference sites. In networks with high connectivity and low modularity, super-generalist host species could also facilitate indirect interactions, such as mutualist spillover (Warwick et al., 2022).

Despite its promise, rewiring specific connections in EINs may have consequences for other networks. For instance, introducing certain plant species combinations to promote pollination may cause changes in parasitism or herbivory (e.g., Windsor et al., 2021). We suggest that future research explore the effectiveness of generalist endosymbionts that are also effective mutualists of specialist plants for use as inoculants in restoration (also see Pocock et al., 2012).

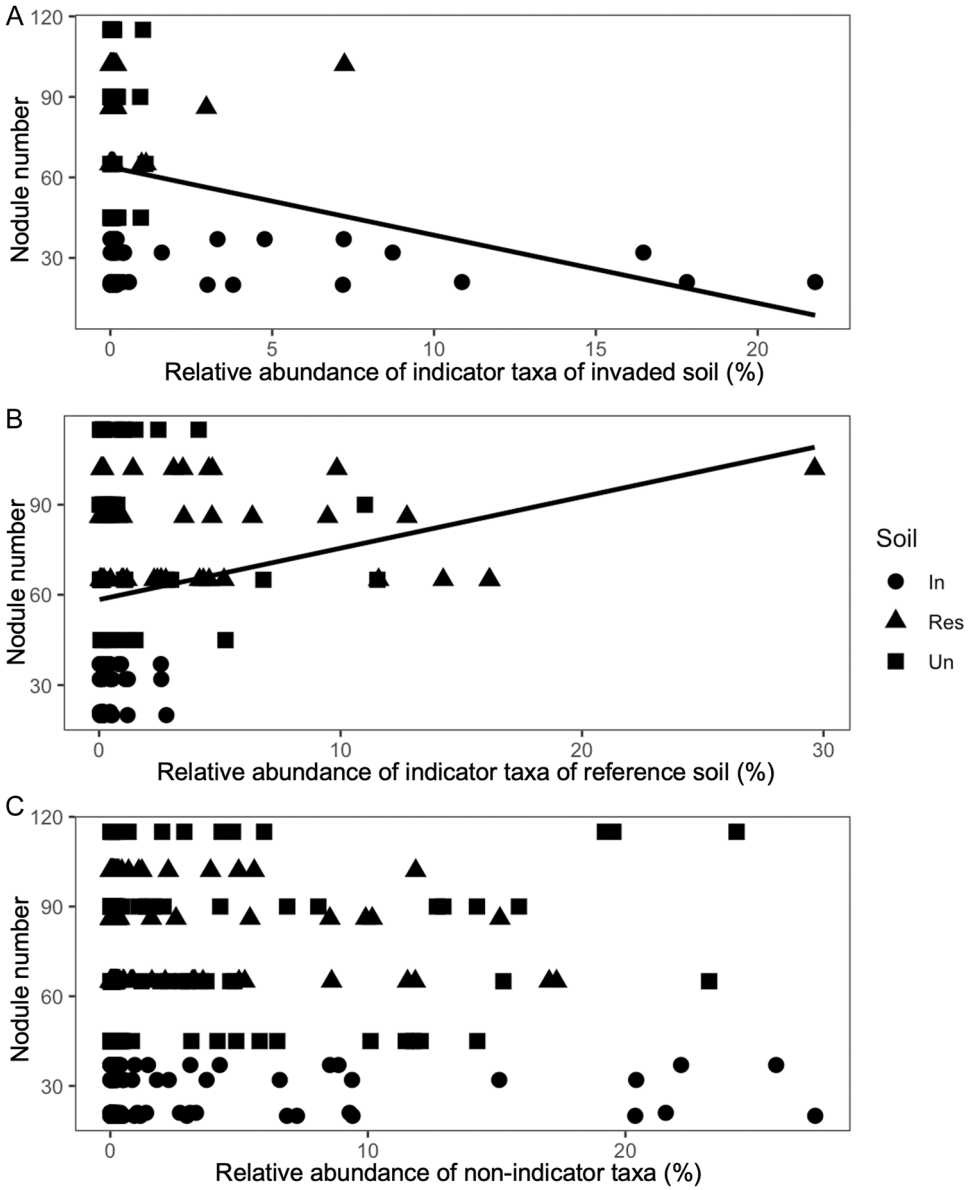


FIGURE 1 Correlations between nodulation (number of root nodules formed) by hickory wattle (*Acacia implexa*) and the relative abundance of (A) indicator *Bradyrhizobium* taxa from soil invaded by African olive (*Olea europaea* subsp. *cuspidata*) ($r = -0.23$, $P < 0.01$), (B) indicator taxa from reference (i.e., uninvaded) soils ($r = 0.23$, $P < 0.01$), and (C) non-indicator taxa, present in the root nodules of hickory wattle when grown in three soil types. Indicator taxa are defined as those that are characteristic of each soil type. Soil types: In, invaded; Un, uninvaded; Res, restored.

FUTURE PERSPECTIVES: CONSIDERING MICROBIOME ASSEMBLY PROCESSES DURING ECOLOGICAL RESTORATION

While plant endosymbiotic mutualists are promising targets for assisting ecological restoration, they only make up a small fraction of all the microbes that plants interact with, and plant-associated microbiomes are a vast untapped reservoir of plant-beneficial microbes. The assembly of these microbiomes is not a random process and is controlled by interactions among host plants, the abiotic environment, and microbial taxa. Firstly, we can consider that the microbial community of the bulk soil acts effectively as the regional pool, with both abiotic (e.g., soil nutrients) and biotic (e.g., plants) conditions creating a filter resulting in local microbial community composition. The trajectory of microbial community assembly is then influenced by a range of factors including local abiotic conditions, dispersal ability, priority effects, disturbance, propagule pressure, niche differentiation, and competition (Trivedi et al., 2020). Given this complexity, is it likely that we can design strategies to manipulate plant-associated microbiomes that will enhance restoration success? Network analyses again provide important insights by identifying microbes that

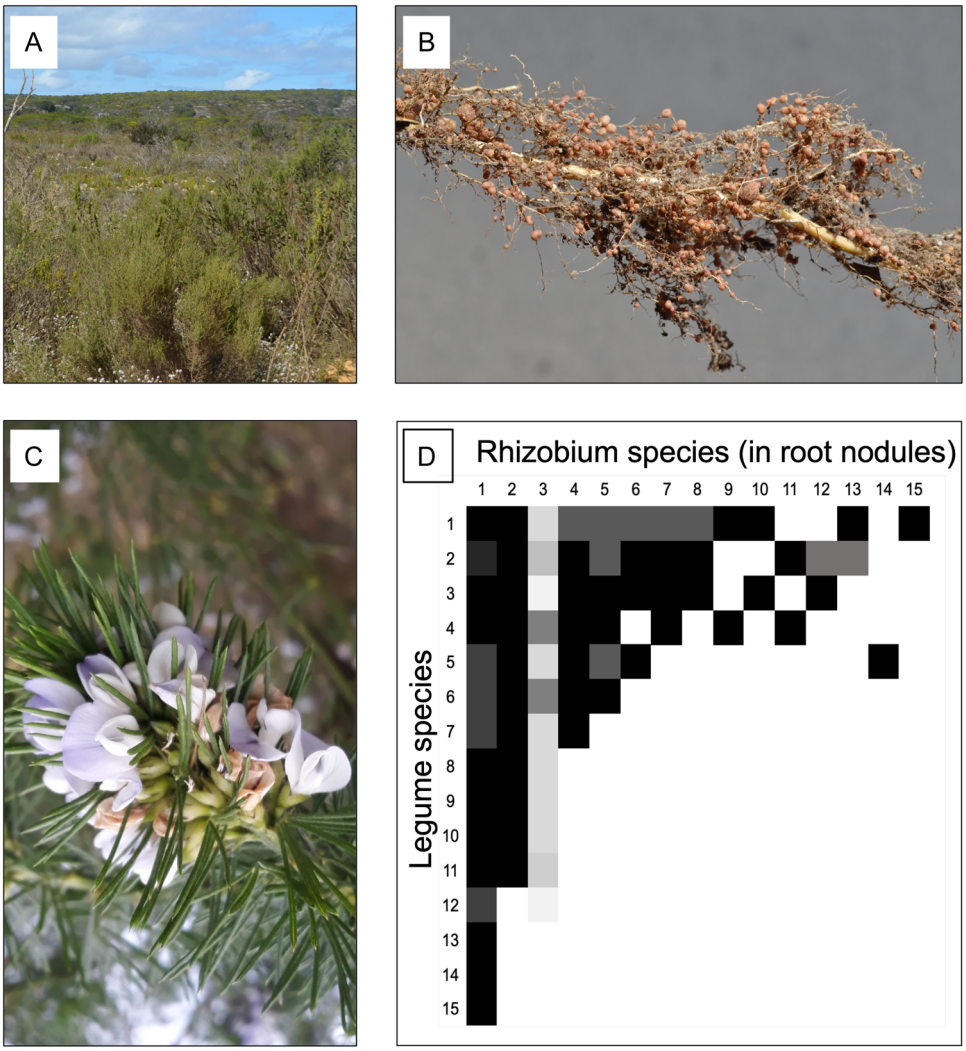


FIGURE 2 A network perspective for assisting ecological restoration. Observational data on plant–microbial mutualist interactions from reference sites (A), e.g., between rhizobia (B) and legumes (C) can be used to reconstruct bipartite ecological interaction networks (D) to infer network (e.g., modularity or nestedness) and node (e.g., specialization) properties to identify mutualists that are most likely to benefit restoration. Increasing intensity of grey represents the increasing frequency of species interactions in the interaction network (D). Photo credits: Johannes J. Le Roux (A and C) and Jan-Hendrik Keet (B).

frequently co-occur and play critical regulatory roles in microbiome assembly and functioning (so-called keystone taxa). For example, using a combination of network analysis and top-down experimental manipulation of soil microbial communities, Romdhane et al. (2022) showed that patterns of co-occurrence inferred from network analysis broadly matched the ecological interactions between microbial taxa under experimental conditions. Further, keystone microbial taxa influence the physiology, anatomy, behavior, and reproduction of higher organisms (Trivedi et al., 2020). For instance, interconnected phyllosphere keystone taxa are a strong predictor of health in *Arabidopsis thaliana* (Agler et al., 2016). A key challenge will be to enable the reassembly of plant-associated microbial networks around keystone taxa to enhance restoration outcomes in the long term, with agricultural research suggesting that we are still a long way from predictably assembling microbial communities (French et al., 2021). Our inability to isolate and cultivate most microorganisms also poses a significant challenge to implementing this strategy. These challenges represent important priorities of future research efforts.

AUTHOR CONTRIBUTIONS

J.L.R.: conceptualization (lead); writing original draft (lead); formal analysis (supporting); review and editing (equal); visualization (equal). M.L.: review and editing (equal). D.G.: review and editing (supporting); formal analysis (supporting); visualization (equal). A.M.: review and editing (equal); visualization (equal).

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