**Title: The interaction networks of plants and symbiotic fungi are not restored following reforestation of a degraded subtropical ecosystem**

Running Title: Restoration and mycorrhizal interactions

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**Abstract**

To ameliorate the negative impacts of deforestation, ecological restoration practices such as reforestation are often invoked. However, the success of these efforts is inconsistent and rarely leads to the reestablishment of target ecosystems or their services. One important factor that is often overlooked during restoration is the synergistic interactions of organisms with each other, for example plant interactions with microbial symbionts. This is a considerable oversight, as a multitude of studies have shown the importance of symbiotic microbes in plant community assembly and ecological succession.

We examined whether restoration practices focused aboveground, were successful in restoring belowground symbiotic fungal communities. We sampled remnant subtropical montane forests and restored forest patches, and characterized their root-associated symbiotic fungi to assess their ecological interactions.

Plant-fungal network analyses revealed that remnant forest networks harboured significantly more specialized, and less dense interactions than restored habitat patches. Analysis of fungal co-occurrence networks revealed that remnant forest keystone taxa were absent in restored sites. We also found that fungal community composition and potential function differed significantly. Combined, these findings have implications for the effectual re-establishment of the target ecosystem. We suggest that moving forward, restoration practices must take a multi-guild approach in order to increase their success.

**Introduction**

Through our actions humans have modified >50% of the Earth’s land surface (1). While concerted efforts have been taken to help minimize ecosystem losses through habitat preservation, there is a recognized need for effectual restoration of already degraded areas (2). However, recovery rates are often slow and restored ecosystems rarely achieve their targeted state (2, 3). A contributing factor could be that restoration methods over-simplify complex systems (4), and omit key ecosystem components or processes, thereby preventing full recovery. This is evident by restoration projects often being strongly biased towards single taxonomic groups (eg. plants, 5). Despite their well-recognized roles in local- and broad-scale ecosystem processes (6) microbes are often overlooked in the context of ecological restoration (5). Root symbiotic mycorrhizal fungi are a particularly salient guild of microorganisms to consider during restoration due to their ubiquity and significant impacts on plant success, biodiversity and ecosystem functioning (7, 8), as well as the trajectory of ecosystem succession (9). These fungi can potentially serve a valuable role during terrestrial ecological restoration, acting either as an ecosystem component to be manipulated (10, 11, 12), or as indicators of the state of recovery to a target ecosystem (13). In the present study, we set out to test the latter.

Ecosystem disturbance and secondary succession has been shown to affect plant symbiotic microbes such as mycorrhizal fungi by changing their abundance (14, 15), altering their community composition (16) and their functional traits (17). Due to the reliance of the majority of plants on mycorrhizal fungi to survive in the wild (18), the success of plant restoration projects and the sequential reestablishment of the food-webs for which plants form the foundation, may hinge upon whether the diversity and function of mycorrhizal interactions can also be restored. While previous studies have examined the potential role that mycorrhizal fungal pre-inoculation can have in steering successful ecological restoration (9, 19, 20, 21), information is lacking on the reestablishment of mycorrhizal interactions *in situ*, limiting our understanding of belowground ecosystem development and plant-soil feedbacks post-restoration. To assess this, we employed community ecology metrics and an ecological network approach within a landscape-scale reforestation project on Hawaii Island.

For symbiotic organisms, ecological network theory is an especially powerful tool to understand the complex interactions among hosts and their symbionts in nature (22). Rather than metrics of diversity, ecological networks measure the degree, extent and intensity of interactions among species within an ecosystem. Network patterns also provide valuable information on the stability of biotic interactions in the face of perturbations, and the degree of specificity among hosts and symbionts. Because they measure complex interactions, network approaches provide a framework for assessing the system-wide status of restored areas relative to a reference, such as primary forests. By assessing and comparing network properties we can target specific ecosystem components to manipulate that may accelerate or at least increase the success of future restoration efforts.

Previous work provides multiple insights about how ecological network properties are influenced by disturbance, and how networks vary during ecosystem recovery. For instance, examination of plant-pollinator networks has revealed that networks within intact ecosystems are more complex, relative to sites that have been disturbed (23). Investigations of networks between plants and soil biota have revealed similar patterns. In their examination of abandoned arable lands Morriën et al. (24) observed that connectance increased with successional age. Comparisons of mycorrhizal networks between young and old forests in Estonia have also shown that networks are more connected in old-growth forests, and increase in their number of specialists (25), indicating that niche partitioning is greater at later successional stages (26). Together these studies suggest that increasing network complexity and specialization are properties that should be targeted when restoring interaction networks, but these ideas remain to be explicitly tested.

Habitat reforestation, the practice of out-planting native woody plants into disturbed areas with the goal of spurring secondary succession, is a common practice in restoration ecology (2). We contend that focusing on individual species or taxonomic groups during restoration, while disregarding their ecological interactions, does not necessarily reassemble the target community. To assess this concept, we examined plant symbiotic mycorrhizal fungal communities and their interactions with their host plants within a large-scale reforestation project on Hawaii Island. We determined the network architecture of mycorrhizal communities between remnant forest patches that represent the benchmark for restoration, to those in adjacent areas that have been previously disturbed and have since undergone reforestation. We predicted that the mycorrhizal networks of remnant forests would be denser, more connected, and more specialized relative to restored forest networks. We expected that differing land-use histories between these habitats would result in disparate mycorrhizal fungal communities, coinciding with loss of keystone fungal taxa from restored forests. Additionally, we predicted that remnant forests would harbour a higher relative abundance fungal taxa with late successional life history strategies (*sensu* 27).

**Methods**

*Study Area*

Sampling for this study was conducted within the 13,240 ha Hakalau Forest National Wildlife Refuge (herein Hakalau) located on the eastern slope of Mauna Kea on the Island of Hawaiʽi (19°51’N; 155°18’W). During the 1800’s large swaths of land below Mauna Kea were converted to pastureland for livestock grazing, which included activities such as large-scale removal of native vegetation by means of logging, bulldozing and fires, and planting of exotic grasses for pasture (28). In 1987, one of the largest restorations efforts in Hawaii was initiated in Hakalau to re-establish habitat for rare and endangered native Hawaiian forest birds (28). Initially, over 390,000 seedlings of the native canopy tree *Acacia koa* (koa) were planted into open pastureland areas over a 2-3 year period (29). Additional out-plantings of other native woody species began approximately 10 years later.

*Sampling*

Sampling was undertaken in July and August 2017. At the time of sampling, a gradient of habitat types existed within Hakalau (SI Appendix, Fig. S1A) which included; 1) un-restored open-pasture consisting of exotic pasture grasses, 2) restoration corridors of out-planted *A. koa* and an understory of exotic grasses, 3) restoration areas of *A.* *koa* with additional out-planted native woody species and smaller remnant patches of grass, and 4) remnant forest patches that are co-dominated by the native canopy trees *A. koa*, and *Metrosiderios polymorpha*, and include subcanopy native trees such as *Cheirodendron trigynum* (ʻōlapa), *Myrsine lessertiana* (kōlea), and *Coprosma rhynchocarpa* (pilo); and shrubs such as *Rubus hawaiensis* (ʻākala), *Leptecophylla tameiameia* (pukiawe), and *Vaccinium calycinum* (ʻōhelo). These species were chosen for two reasons: 1) they are representative of the plant community composition within remnant forest patches, and 2) they provide important resources for native birds at risk for extinction (30). For the purposes of our study we focused on just two of the habitat types within Hakalau: restoration areas (herein restored forest) and remnant forest patches (herein remnant forest) as our reference sites.

We sampled the roots of the same host plant species in both remnant and restored forest plots. We chose restored plots that were in close proximity to remnant forest and had a plant community composition most similar to these neighboring forested areas. Plots were established along two parallel transects, both of which transitioned from pasture into each of the habitat types listed above (SI Appendix, Fig. S1A). Along each of the two transects, six plots were established, divided equally between remnant and restored forest habitat types, resulting in a grand total of 12 plots (SI Appendix, Fig. S1B). Within each plot we sampled roots from seven hosts to arbuscular mycorrhizal (AM) fungi, including six native host species; *M. polymorpha*, *A. koa*, *C. trigynum*, *M. lessertiana*, *C. rhynchocarpa*, and *R. hawaiensis*, and non-native grasses (most of which were *P. clandestinum* but were not identified to species). Plots were ~12 m in diameter and their perimeters were separated by ≥ 20 m (SI Appendix, Fig. S1B). Within each plot, we sampled roots from underneath up to eight individuals of each target species (SI Appendix, Appendix I). Generating a grand total of over 625 root samples (SI Appendix, Table S1). For each individual host, we sampled roots by tracing fine roots back to larger branching roots of the host. In the field, roots samples were bagged and stored on ice until they could be transferred to a 1ºC cold room where they were kept until they could be processed for DNA extraction.

*Molecular analysis*

Cleaned and dried root samples ≥ 0.5 g were cut into 1 cm fragments using sterilized scissors, and DNA was extracted from a 0.25 g subsample using the MP Bio FastDNA® spin kit for plant and animal tissue (MP Biomedicals, LLC, Santa Ana, California, USA), following the manufacturer’s instructions. Then, we carried out a two-step PCR reaction to first specifically amplify the small subunit (SSU) of arbuscular mycorrhizal fungi’s ribosomal RNA (rRNA) and then and second reaction adhered Illumina barcodes and adaptors to our amplicons (SI Appendix, Appendix II; 31).

*Soil chemical analysis*

For a subset of soil samples from within each plot (ranging 20-28), soil chemical analyses for organic matter (OM), estimated total nitrogen (N), readily available phosphorus (P), extractable cations (potassium (K), magnesium (Mg), calcium (Ca), sodium (Na)), hydrogen (H), Sulfate-S (S), pH, and cation exchange capacity (CEC). Analyses were performed by A & L Western Agricultural Laboratories, Inc. (Modesto, CA, USA).

*Bioinformatics*

Bioinformatic processing was conducted using the open-source platform quantitative insights into microbial ecology (QIIME version 2; https://qiime2.org). For details on demultiplexing, quality control methods, and taxonomic identification of sequences see SI Appendix, Appendix III.

*Data Analysis*

All analyses were conducted in R (version 3.5.2; 32), using R studio (33).

*Plant-AM fungal networks*

Bipartite plant-AM fungal networks were built using the bipartite package (34). For each plot, bipartite networks were assembled using an aggregated species-level matrix, where mean relative abundances of each AM fungal species were calculated for each plant species. Networks were assembled for each plot individually, and each network was considered an independent unit. Meaning, for each habitat type we had six independently assembled plant-AM fungal networks.

We examined multiple network-level characteristics including: connectance, nestedness, modularity, linkage density, and specialization of the entire network (H2’) (see SI Appendix, Table S2for descriptions of each characteristic**)**. For all network-level characteristics, we calculated mean values for both remnant and restored forest habitats by averaging the observed metrics across plots for each habitat type. We then used Welch unequal variance t-tests (35) to compare the metrics between the two habitat types.

Network characteristics of each host plant were examined by quantifying host symbiont range (an indicator of the capacity of a host to interact with different AM fungi) and host specialization on particular AM fungi (d’) (SI Appendix, Table S2). For both metrics, differences between habitat types and among plant hosts were evaluated using a generalized linear model (GLM) with a quasi-Poisson distribution and a log link function, using the *glm* function available in the R stats package (SI Appendix, Appendix IVa). Pairwise comparisons for every combination of host and habitat type were examined using the *emmeans* function in the emmeans package (36), followed by the *pairs* function in the R graphics package (v3.5.2).

To test whether observed network characteristics differed from random, we used a null model approach. We used two separate null model algorithms available in the bipartite package; *swap.web* and *r2dtable*. *Swap.web* was chosen because it provides a more constrained null analysis and is therefore more realistic. We concurrently used the *r2dtable* null model because similar to *swap.web,* *r2dtable* maintains marginal totals, but randomizes network connectance. Null bipartite networks generated using *r2dtable* were used to determine whether observed connectance differed from random, while bipartite generated using *swap.web* were used for the other network characteristics. For both null algorithms, observed matrices were randomized 1000 times using the *nullmodel* function, followed by network characteristics calculated on null matrices. For each network index, we compared observed network characteristics to the null calculations using Welch unequal variance t-tests (35).

*AM fungal co-occurrence networks*

AM fungal networks were assembled using the Sparse Inverse Covariance Estimation for Ecological Association Inference package in R (SPIEC-EASI; 37). We assembled AM fungal networks using the *spiec.easi* function with 9999 iterations (SI Appendix, Appendix IVb). Inferred networks were then converted to igraph objects using the *adj2igraph* function. and analyzed networks using the igraph package (38). Overall AM fungal network characteristics were examined by evaluating connectedness (degree) and centrality (betweenness) between habitat types (Table S2). Each metric was calculated on fungal-fungal networks that were assembled individually for each plot. Overall network topologies were compared between the two habitat types using Welch unequal variance t-tests (35).

To evaluate potentially important AM fungal taxa within the networks for each habitat type, we identified candidate keystone and indicator species (SI appendix, Appendix IVc). A keystone species has traditionally been described as a species that has a disproportionately large effect on its environment/community relative to its abundance (39). In our case, we branded keystone species within our networks as taxa that had a disproportionately large effect on the network relative to their prevalence (40). Candidate keystone species were identified on composite networks generated for each habitat type, and were denoted as nodes that were maximal in both centrality and degree, but with a low prevalence score (≤ 0.005, SI Appendix, Appendix IVb).

*AM fungal diversity*

To examine the influence of habitat type, host species, and their interaction on AM fungal richness, we used a GLM with a Poisson distribution and a log link function. Pairwise comparisons for every combination of host and habitat type were examined using the *emmeans* function, followed by the *pairs* function. To help interpret patterns of AM richness between habitats we compared the species abundance distributions (SADs) using the sads package (41). Observed SADs were assembled using the *octav* command, and then multiple species probability distribution models were fit to the observed distributions using the *fitsad* command. The best species distribution model was chosen using Akaike’s information criterion (AIC; 42) using the *AICtab* function in the bbmle package (43). The best species distribution model was chosen based on which model had the lowest AIC.

To examine patterns of AM fungal community nestedness within hosts and habitats, we compared observed nestedness “temperature” to 9999 permuted null model communities generated non-sequentially preserving sample (row) frequencies using the *oecosimu* function available in the vegan package (44), using the ‘r0’ algorithm and a seed of 96822.

Changes in AM fungal community composition among hosts and habitats were determined using the Bray-Curtis dissimilarity index (45). Variation in AM fungal community composition was visualized using nonmetric multidimensional scaling (NMDS) using the *metaMDS* function available in the vegan package (44). Soil chemical properties were fitted to the NMDS ordination using the *envfit* command in vegan. The effect of habitat type and host species on AM fungal community composition were determined by permutational multivariate analysis of variance using distance matrices (PERMANOVA; 46) using the *adonis* function and 9999 permutation in the vegan package (44). Pairwise community compositional differences among all combinations of habitat type by host species were then done using the *pairwise.perm.manova* function from the RVAideMemoire package in R (47).

To help further interpret diversity patterns, we examined changes in relative abundance of the major AM fungal families between the two habitat types. Differences in relative abundance of each family were determined by binning sequence reads by family and then comparing mean relative abundance for each family between the two habitat types using Welch unequal variance t-tests (35).

**Results**

*Plant-AM fungal networks*

As predicted, overall network specialization (H2’) was significantly higher in remnant than restored forests (Fig. 1A; Welch unequal variance t-test; t = -4.42, df = 6.64, p = 0.003). Null model analyses revealed that remnant forest networks were more specialized than expected by chance, while restored forest networks did not differ from null expectations (SI Appendix, Table S3). Similarly, host specialization on AM fungi (d’) was significantly higher in remnant than restored forests, and varied among species (Fig. 2A, SI Appendix, Table S4). Null model analyses revealed that within remnant forests three out of the seven hosts were more specialized than expected by chance, while in restored forest plots, only one of these seven hosts was significantly more specialized than randomly assembled networks (SI Appendix, Table S5). While no significant differences in host symbiont range were observed between habitat types (Fig. 2B), when we compared observed symbiont range to that of randomly assembled networks, we found that within restored forests *M. polymorpha* had a smaller symbiont range than expected by chance, while grasses had larger symbiont range than expected (SI, Appendix, Table S5).

Contrary to our expectations, overall density (the proportion of realized connections per species) of the plant-AM fungal networks was greater in restored versus remnant forests (Fig. 1B; Welch unequal variance t-test; t = 2.99, df = 5.76, p = 0.03) and our null models revealed that network density did not significantly differ from null expectations in restored forests and was lower than expected by chance in remnant forests (SI Appendix, Table S3). Related to network density, the mean number of AM fungal links per plant species was also significantly greater in restored versus remnant forests, indicating less host specificity (Fig. 1C; Welch unequal variance t-test; t = 3.03, df = 9.98, p = 0.01). Again, null model analyses revealed that plant species within remnant forests had fewer links than expected by chance, with no significant deviations from null expectations with restored plots (SI Appendix, Table S3). For both habitat types network connectance was lower -, while both nestedness and modularity were higher than expected by chance indicating interactions between plants and AM fungi are formed through non-random processes (SI Appendix, Table S3; 48).

*AM fungal networks*

Keystone species analysis revealed two keystone AM fungi within remnant forest networks; *Glomus* *VTX00290* (Degree = 8, Betweenness Centrality = 1578.5, Prevalence = 1.05E-05) and *Acaulospora* *VTX00227* (Degree = 7, Betweenness Centrality = 1822, Prevalence = 0.0045) (Fig. 3A). For restored forest AM fungal networks, we identified *Archaeospora trapeii*, which was not detected in within the remnant forest as a candidate keystone species (Fig. 3B; Degree = 50, Betweenness Centrality = 9467, Prevalence = 1.04E-09). Between the two habitats, *Gl.* *VTX00290* was detected in a similar percentage of samples (remnant forest = 14%, restored forest = 15%) and at a similar relative abundance (remnant forest = 7.31E-05 relative read abundance, restored forest = 7.89 E-05), but was not classified as a keystone species as it was not a hub within the network (Degree = 4, Betweenness Centrality = 27.5). The remnant forest keystone species *Ac.* *VTX00227* was not detected in restored forest plots.

Indicator species analysis detected 17 AM fungal taxa significantly associated with remnant forests, and 10 with restored forests (SI Appendix, Table S6). We also found that Claroideoglomeraceae (p = 8.37E-04), in addition to Ambisporaceae (p = 2.13E-02), Archaeosporaceae (p = 1.13E-03), and Paraglomeraceae (p = 3.75E-07) all had significantly higher relative abundances in restored forest plots relative to remnant forest (SI Appendix, Fig S4). Conversely, we observed Diversisporaceae (p = 1.72E-10) and Gigasporaceae (p = 5.74E-03) to both have higher abundances in remnant forests (SI Appendix, Fig. S4; Table S12).

*AM fungal diversity*

AM fungal richness was similar between habitat types, but differed significantly among hosts (SI Appendix, Fig. S5; Table S8). In total, 183 AM fungal taxa were detected in restored forest samples (sample mean and SD: 19.1 ± 5.5), and 184 taxa were detected in remnant forest samples (18.4 ± 6.1). Overall, we detected 212 AM fungal taxa from 9 families and 13 genera. Ninety-two of our taxa were matched VT in the MaarjAM or NCBI databases, and the remaining 120 were undetermined beyond the subphyla level of Glomeromycotina. Post-hoc comparisons revealed that *M. lessertiana* within remnant forests harboured the most AM fungal taxa, while *M. polymorpha* within remnant forests harboured the fewest (SI Appendix, Table S9). AM fungi from both remnant (Nested temperature = 15.00; P = 1e-04) and restored (Nested temperature = 7.58, P = 0.001) forest habitat types had significant nestedness patterns among plant hosts when compared to null assembled communities (Fig. 4). Meaning that plants from the same habitat type hosted subsets of the species pool of AM fungi, rather than entirely new AM fungal communities per host. Within remnant forests, AM fungal communities for all plant hosts were nested within *M. lessertiana* fungal communities (Fig. 4A), while in restored forests they were nested within grass fungal communities (Fig. 4B).

Observed SADs indicated that AM fungal communities from remnant forests had more rare species and a more symmetric SAD than restored forest communities (SI Appendix, Fig. S6). The observed SAD for restored forests was bimodal. Maximum likelihood estimation revealed that both remnant (Fig. 5A; P = 2.2e-16) and restored (Fig. 5B; P = 2.2e-16) AM fungal communities fitted to a Poisson log-normal type of SAD.

*AM fungal community membership*

AM fungal community composition was significantly influenced by habitat type, host, and their interaction (SI Appendix, Table S10). Pairwise comparisons revealed that AM fungal communities differed by habitat type (Pairwise Permutation MANOVA; p = 0.0001, permutations = 9999, Fig. 5), and soil pH (r2 = 0.42, p = 0.001) was the best abiotic predictor of community composition (Table S11). All soil chemical variables, except Ca, Na, and P were significantly correlated with the ordination obtained (Fig. 5; SI Appendix, Table S11). Additionally, all soil chemical variables, except Ca, Mg, and P differed between habitat types (SI Appendix, Table S12). Within both habitats, AM fungal communities differed significantly among hosts (SI Appendix, Table S13). Congruent with greater host specificity, NMDS analysis revealed that AM fungal communities from the same hosts within remnant versus restored forests clustered closer together (SI Appendix, Fig. S7).

**Discussion­­­**

We investigated whether the common restoration practice of out-planting native plant species into previously disturbed areas is successful in re-establishing critical belowground symbiotic networks. By comparing measures of diversity and biotic interactions, we found that even after 20 years post reforestation these areas do not mimic more intact remnant forests and have not recovered to their pre-disturbance state (Fig. 5).

In the current study, the best indicators for remnant forests were high overall network and individual host species specialization (Figs 1A & 2A). The degrees of specialization within remnant forests were significantly higher than expected by chance, while being essentially indistinguishable from random within restored sites (SI Appendix, Table S3). We propose three possible non-exclusive mechanisms that could be contributing to these patterns. First, due to the age differences of the forests, their environmental conditions are distinct. For instance, higher root densities and lower micronutrient pools in the older remnant forests, could lead to greater competition for soil resources than in the younger restored forest (SI Appendix, Table S12). This in turn, may lead to more niche partitioning belowground and hosts partnering with the specific fungi that provide them with the most benefits for the lowest costs (49). Second, fungal taxa that found in the remnant forests are not establishing within restored sites, which could be contributing to the lack of native plant recruitment (30, 50). This idea is supported by our findings that: restored forests were missing keystone AM fungal taxa present in the remnant forests, each habitat type had distinct indicator taxa, and overall fungal community composition differed significantly between the two habitats. Furthermore, analyses of the species abundance distributions (SADs) of AM fungi in the two habitat types indicated a drop-off of rare taxa between remnant and restored sites (SI Appendix, Fig. S6), and ordination analysis revealed greater intermixing of AM fungi among hosts in restored sites (SI Appendix, Fig. S7), both of which are indicative of a more uniform AM fungal community.

A third potential source leading to decreased specialization within restored sites is their historical occupation by invasive plant hosts, namely grasses. Previous work has shown that specialist AM fungal taxa reluctantly colonize novel plant hosts (51), meaning that generalist fungi will favourably associate with invasive hosts novel to an ecosystem. Subsequently, positive feedbacks between invasive hosts and generalist fungi would increase the abundance of these fungi creating a soil legacy affect even after the removal of these hosts (52, such as in our restored sites). The species interaction and nestedness patterns we observed support this concept. Within restored sites we observed grasses to associate with a broader range of AM fungal taxa than expected by chance, and all other out-planted native hosts’ AM fungal communities nested within grasses (Fig. 4). This suggests that within restored forests, grass AM fungi are the “donor” pool from which all other hosts accumulate their mycorrhizal symbionts, and these fungi may be less beneficial to native hosts thereby hindering their re-establishment. For example, within restored sites *M. polymorpha*,a dominant species in native Hawaiian forests (53),harboured symbionts that were distinct from its specialized partners in remnant forests (Fig. 4, SI Appendix, Table S5). In the remnant forests, *M. lessertiana* harboured basically all of the AM fungal diversity found in all other hosts (Fig. 4). Therefore isolating and pre-inoculating native hosts with these locally sourced fungi prior to outplanting may aid in the overall reestablishment of the target ecosystem.

Other possible consequences of specialization loss within the restored forests is that native hosts are not re-establishing their specific mycorrhizal partnerships, but are instead associating with a greater diversity of low-quality symbionts. This has led an interaction network with more links and greater density making it less susceptible to perturbations than randomly assembled, or the remnant forest networks (Fig. 1). At first, the creation of a more robust ecological network may seem like an unintended positive effect of reforestation. However, we would argue that this is not necessarily the case for the following reasons: 1) reforestation efforts have not led to natural regeneration despite a lack of seed limitation indicating that specific belowground interactions may be needed for hosts to reproduce successfully and, 2) now that these robust networks are established, they will be harder to manipulate and may represent an alternative stable state to late-stage forest succession. However, both of these concepts need to be explicitly tested.

Two keystone AM fungal taxa from remnant forest networks were either lost, or did not perform as such within the networks of restored forests (Fig. 3). Specifically, we found that a previously unidentified *Acaulospora* species was absent within restored forest, while playing a major role within remnant forest networks (Fig. 3). This is a notable observation, as a loss of keystone taxa within interaction networks has been shown to lead to cascading effects (54). For example, the loss of keystone taxa can potentially proliferate throughout networks resulting in the loss, or decreasing abundance of other taxa with similar traits. For plant communities, a potential consequence of losing fundamental mycorrhizal symbionts is the alteration of community composition and/or ecosystem functioning. This pattern has been observed previously in other studies by Duhamel et al. and Banerjee et al. (55, 56). Notably, they found that the effects of disturbance altered mycorrhizal community composition, and especially influenced a subset of rare taxa or keystone taxa which in turn affected plant establishment and bioegeochemical cycling. In our system we suspect a similar dynamic exists, where the loss or alteration of rare and keystone taxa could be contributing to stalled native forests restablished 20 years post-restoration (30). However, further work examining the specific roles of these keystone taxa, especially in terms of their function and physiological effect on hosts will be vital to understanding their potential importance in re-establishing forests (56).

Changes in AM fungal community composition between remnant and restored forest sites coincided with a decrease in the relative abundance of taxa with late successional life history strategies (SI Appendix, Fig. S4; 17, 27). Specifically, we observed a decrease in Diversisporaceae and Gigasporaceae taxa between remnant and restored forests (SI Appendix, Fig. S4). The latter family was also an indicator of remnant forests. Two potential interacting mechanisms could be contributing to their observed decline. First, these families are characterized by high investment into extraradical hyphae in the soil (57, 58), which would enable hosts to access sparse soil nutrient pools where competition is high (59). By enabling hosts to access these pools, fungal taxa with such traits would theoretically receive higher rewards from their hosts, thus securing their place in the community (59). Second, families such as Gigasporaceae have slow recovery rates after soil disturbance (60). Therefore, such taxa would be less likely to re-establish post disturbance (such as our restored sites). Coincidently, we observed relative increase in the abundance of Archaeosporaceae in restored habitats. A recent meta-analysis by van der Heyde et al. (60) identified this family as highly tolerant to disturbance. A potential consequence of these shifts in AM fungal community composition that coincide with the alteration of specific functional traits is stalled of native plant recruitment and ecosystem succession like we have observed at Hakalau (30, 55). To help mitigate these losses, and to help increase restoration success, locally sourced AM fungal taxa from groups with late successional life history strategies should be targeted for future reforestation efforts (9, 20).

*Conclusions*

We proposed that by focusing on individual guilds of organisms such as plants, while disregarding their interactions with symbionts, does not lead to the inherent reassembly of the target ecosystem community. Our examination of the symbiotic fungal communities in an area that has undergone large-scale restoration, revealed that the ‘restored’ mycorrhizal communities were substantially different from remnant forest sites, and lacked many of their properties. Furthermore, we found that the interaction patterns between plants and mycorrhizal fungi were not restored, especially the specialized interactions of foundational native plant species. To help mitigate this issue, we suggest adopting a holistic approach to restoration, which focuses on restoring not only individuals and species, but also their ecological interactions. While this requires a thorough understanding of the ecology of the target ecosystem, the pay-off is substantial, as these connections are essential to long-term ecosystem functioning and resilience.

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**Competing Interests**

The authors have no competing interests.

**References**

1. R.L.B. Hooke, J.F. Martín-Duque, J. Pedraza, Land transformation by humans: a review. *GSA today*, **22**(12), 4-10 (2012). doi: 10.1130/GSAT151A.1.
2. Suding KN. Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Annu Rev Ecol Evol Syst. 2011 Nov 4;42(1):465–87.
3. H.P. Jones et al., Restoration and repair of Earth’s damaged ecosystems. *Proceedings of the Royal Society B*, **285**(1873), pii: 20172577 (2018). doi: 10.1098/rspb.2017.2577.
4. M.A. Palmer, R.F. Ambrose, N. LeRoy Poff, Ecological theory and community restoration ecology. *Restoration Ecology*, **5**, 291-300 (1997). doi: 10.1046/j.1526-100X.1997.00543.x
5. C.E. Wainwright et al., Links between community ecology theory and ecological restoration are on the rise. *Journal of Applied Ecology*, **55**, 570-581 (2018). doi: 10.1111/1365-2664.12975
6. M.G.A. van der Heijden, R.D. Bardgett, N.M. van Straalen, The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296-310 (2008). doi: 10.1111/j.1461-0248.2007.01139.x
7. J.D. Bever, et al., Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology and Evolution*, **25**, 468-478 (2010). doi: 10.1016/j.tree.2010.05.004
8. R.D. Bardgett, W.H. van der Putten, Belowground biodiversity and ecosystem functioning. *Nature*, **515**, 505-511 (2014). doi: 10.1038/nature13855
9. L. Koziol, J.D. Bever, Mycorrhizal feedbacks generate positive frequency dependence accelerating grassland succession. *Journal of Ecology*, **107**(2), 622-632 (2019). doi: 10.1111/1365-2745.13063
10. P.H. Thrall et al., Seed inoculation with effective root-nodule bacteria enhances revegetation success. *Journal of Applied Ecology*, **42**(4), 740-751 (2005). doi: 10.1111/j.1365-2664.2005.01058.x
11. P. Kardol, D.A. Wardle, How understanding aboveground-belowground linkages can assist restoration ecology. *Trends in Ecology and Evolution*, **25**(11), 670-679 (2010). doi: 10.1016/j.tree.2010.09.001
12. E.R.J. Wubs, W.H. van der Putten, M. Bosch, T.M. Bezemer, Soil inoculation steers restoration of terrestrial ecosystems. *Nature Plants*, **2**, 16107 (2016). doi: 10.1038/NPLANTS.2016.107
13. J. Harris, Soil microbial communities and restoration ecology: facilitators or followers? *Science*, **325**, 573-574 (2009). doi: 10.1126/science.1172975
14. M.M. Alguacil et al., The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. *Ecological Applications*, **18**(2), 527-536 (2008). doi: 10.1890/07-0521.1
15. S.R. Holden, K.K. Treseder, A meta-analysis of soil microbial biomass response to forest disturbances. *Frontiers in Microbiology*, **4**, 163 (2013). doi.org/10.3389/fmicb.2013.00163
16. T.K. Schnoor, Y. Lekberg, S. Rosendahl, P. Axel Olsson, Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in a semi-natural grassland. *Mycorrhiza*, 21,211-220 (2011). doi: 10.1007/s00572-010-0325-3
17. M.M. Hart, P.D. Zaitsoff, M. van der Heyde, J. Pither, Testing life history and trait-based predictions of AM fungal community assembly. *Pedobiologia*, **59**, 203-213 (2016). doi: 10.1016/j.pedobi.2016.06.001
18. S.E. Smith, D.J. Read, Mycorrhizal symbiosis, 3rd edn. (Academic Press 2008).
19. E.L. Middleton et al., Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere*, **6**(12), 276 (2015). doi: 10.1890/ES15-00152.1
20. L. Koziol et al., The plant microbiome and native plant restoration: the example of native mycorrhizal fungi. *BioScience*, **68**(12), 996-1006 (2018). doi: 10.1093/biosci/biy125
21. L. Neuenkamp, S.M. Prober, J.N. Price, M. Zobel, R.J. Standish, Benefits of mycorrhizal inoculation to ecological restoration depend on plant functional type, restoration context and time. *Fungal Ecology*, **40**, 140-149 (2018). doi: 10.1016/j.funeco.2018.05.004
22. J. Bascompte, Disentangling the web of life. *Science*, **325**, 416-419 (2009). doi: 10.1126/science.1170749
23. M.L. Forup, K.S.E. Henson, P.G. Craze, J. Memmott, The restoration of ecological interactions: plant-pollinator networks on ancient and restored heathlands. *Journal of Applied Ecology*, **45**, 742-752 (2008). doi: 10.1111/j.1365-2664.2007.01390.x
24. E. Morriën et al., Soil networks become more connected and take up more carbon as nature restoration progress. *Nature Communications*, **8**, 14349 (2017). doi: 10.1038/ncomms14349
25. A.E. Bennett et al., Arbuscular mycorrhizal fungal networks vary throughout the growing season and between successional stages. *PLoS ONE*, **8**(12), e83241 (2013). doi.org/10.1371/journal.pone.0083241
26. N. Blüthgen, F. Menzel, N. Blüthgen, Measuring specialization in species interaction networks. *BMC Ecology*, **6**, 9 (2006). doi.org/10.1186/1472-6785-6-9.
27. P.L. Chagnon, R.L. Bradley, H. Maherali, J.N. Klironomos, A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science*, **18**(9), 484-491 (2013). doi: 10.1016/j.tplants.2013.05.001
28. United States Fish and Wildlife Service, Hakalau forest national wildlife refuge comprehensive conservation plan (2010). <https://www.fws.gov/pacific/planning/main/docs/HI-PI/Hakalau/Hakalau%20Forest%20NWR%20Draft%20CCP-EA.pdf> (accessed September 2018)
29. J. Jeffrey, B. Horiuchi, Tree planting at Hakalau forest national wildlife refuge - the right tool for the right stock type. *Native Plants*, **4**(1), 30-31 (2003). doi: 10.3368/npj.4.1.30
30. S.G. Yelenik, Linking dominant Hawaiian tree species to understory development in recovering pastures via impacts on soil and litter. *Restoration Ecology*, **25**(1), 42-52 (2017). doi: 10.1111/rec.12377
31. CP Egan, D.W. Li, J.N. Klironomos, Detection of arbuscular mycorrhizal fungal spores in the air across different biomes and ecoregions. *Fungal Ecology*, **12**, 26-31 (2014). doi: 10.1016/j.funeco.2014.06.004
32. R Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2019). URL http://www.R-project.org/.
33. R Studio Team, RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL (2016). <http://www.rstudio.com/.>
34. C.F. Dormann, B. Gruber, J. Fruend, Introducing the bipartite package: analysing ecological networks. *R news*, **8**(2), 8-11 (2008).
35. B.L. Welch, The generalization of “student”s’ problem when several different population varlances are involved. *Biometrika*, **34**(12), 28–35 (1947). doi: 10.1093/biomet/34.1-2.28
36. R. Lenth, emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.3.3. (2019).
37. Z. Kurtz, C. Mueller, E. Miraldi, R. Bonneau, SpiecEasi: Sparse Inverse Covariance for Ecological Statistical Inference. R package version 1.0.5 (2019).
38. G. Csardi, T. Nepusz, The igraph software package for complex network research, *Inter Journal, Complex Systems* 1695 (2006). http://igraph.org
39. R.T. Paine, A note on trophic complexity and community stability. *The American Naturalist*, **103**(929), 91–93 (1969). doi:10.1086/282586.
40. D. Berry, S. Widder, Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Frontiers in Microbiology*, **5**, 219 (2014). doi: 10.3389/fmicb.2014.00219
41. P.I. Prado, M.D. Miranda, A. Chalom, sads: Maximum Likelihood Models for Species Abundance Distributions. R package version 0.4.2. (2018). https://CRAN.R-project.org/package=sads
42. H. Akaike, Information theory and an extension of maximum likelihood principle. In: Petrov BN, Csaki F, Proceedings of the Second International Symposium of Information Theory. (Akademiai Kiado 1973), pp 267-281.
43. B. Bolker, bbmle: Tools for General Maximum Likelihood Estimation. R package version 1.0.20 (2017). https://CRAN.R-project.org/package=bbmle
44. J. Oksanen et al., vegan: Community Ecology Package. R package version 2.5.4 (2019). https://CRAN.R-project.org/package=vegan
45. J.R. Bray, J.T. Curtis, An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, **27**(4), 325-349 (1957). doi: 10.2307/1942268
46. M.J. Anderson, A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**, 32-46 (2001). doi: 10.1111/j.1442-9993.2001.01070.pp.x
47. M. Hervé, RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R package version 0.9.72. (2019). https://CRAN.R-project.org/package=RVAideMemoire
48. S.K. Sepp et al., Non-random association patterns in a plant-mycorrhizal fungal network reveal host-symbiont specificity. *Molecular Ecology*, **28**(2), 365-378 (2019). doi: 10.1111/mec.14924
49. E.T. Kiers et al., Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, **333**(6044), 880-882 (2011). doi: 10.1126/science.1208473
50. L. Koziol, J.D. Bever, Mycorrhizal response trades off with plant growth rate and increases with plant successional status. *Ecology*, **96**(7), 1768-1774 (2015). doi: doi.org/10.1890/14-2208.1
51. M. Moora et al., Alien plants associate with widespread generalist arbuscular mycorrhizal fungal taxa: evidence from a continental-scale study using 454 sequencing. *Journal of Biogeography*, **38**, 1305-1317 (2011). doi:10.1111/j.1365-2699.2011.02478.x
52. Q. Zhang et al., Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. *PLoS ONE*, **5**(8), e12380 (2010). doi: 10.1371/journal.pone.0012380
53. D. Mueller-Dombois, Forest dynamics in Hawaii. *Trends in Ecology & Evolution*, **2**(7), 216-220 (1987). doi: 10.1016/0169-5347(87)90024-3
54. P.R. Guimarães Jr, P, Jordano, J.N. Thompson, Evolution and coevolution in mutualistic networks. *Ecology Letters*, **14**(9), 877–885 (2011). doi: 10.1111/j.1461-0248.2011.01649.x
55. M. Duhamel et al., Plant selection initiates alternative successional trajectories in the soil microbial community after disturbance. *Ecological Monographs*, In Press, e01367 (2019). doi: 10.1002/ecm.1367
56. S. Banerjee, K. Schlaeppi, M.G.A. van der Heijden, Keystone taxa as drivers of microbiome structure and functioning. *Nature Reviews Microbiology*, **16**, 567-576 (2018). doi: 10.1038/s41579-018-0024-1
57. M.M. Hart, R.J. Reader, Host plant benefit from association with arbuscular mycorrhizal fungi: variation due to size of mycelium. *Biology and Fertility of Soils*, **36**(5), 357-366 (2002). doi: 10.1007/s00374-002-0539-4
58. H. Maherali, J.N. Klironomos, Phylogenetic trait-based assembly of arbuscular mycorrhizal fungal communities. *PLoS ONE*, **7**(5), e36695 (2012). doi: 10.1371/journal.pone.0036695
59. P.L. Chagnon, R.L. Bradley, J.N. Klironomos, Trait-based partner selection drives mycorrhizal network assembly. *Oikos*, **124**, 1609-1616 (2015). doi: 10.1111/oik.01987
60. M. van der Heyde, B. Ohsowski, L.K. Abbot, M.M. Hart, Arbuscular mycorrhizal fungus responses to disturbance are context-dependent. *Mycorrhiza*, **27**(5), 431-440 (2017). doi: 10.1007/s00572-016-0759-3

**Figure Legends**

**Fig. 1. Dot plots displaying bipartite arbuscular mycorrhizal fungal and plant host network metrics between remnant (green) and restored (blue) habitats within the Hakalau Forest National Wildlife Refuge.** Bipartite metrics that differed significantly between habitat include: overall network specialization (H2’; A), linkage density (B), and mean number of links per species in the network (C). Dots represent group means and whiskers represent group standard deviation around the mean. Differences in bipartite metric means between habitat types were determined using a Welch unequal variance t-test. Asterisks indicate statistical differences between habitat types where; ns = p > 0.05, \* = p ≤ 0.05, \*\* = p ≤ 0.01, \*\*\* = p ≤ 0.001, \*\*\*\* = p ≤0.0001.

**Fig. 2. Boxplots showing host specialization (A) and hosts’ symbiont range (B) on arbuscular mycorrhizal (AM) fungi in restored forest (left-side) and remnant forest (right-side) habitat types within the Hakalau Forest National Wildlife Refuge.** The bottom and the top of the boxes represent the first and third quartiles, the dark band inside boxes represents the median, the whiskers contain the upper and lower 1.5 interquartile range, and the dots represent outliers. Boxplots are coloured by host species. Symbiont range was significantly influenced by host species (p = 1.67 E-10, with a marginal effect of habitat type (p = 0.099). Host specialization was significantly influenced by both habitat type (p = 7.67 E-10) and host species (p = 0.016). For both metrics, pairwise comparisons were determined by estimated marginal means, where statistical differences are signified by boxes without shared letters.

**Fig. 3.**  **Scatterplots to identify potential keystone species. Betweenness centrality plotted against node degree for all arbuscular mycorrhizal (AM) fungal species within the networks from remnant (A) and restored forests (B) habitat types within the Hakalau Forest National Wildlife Refuge.** AM fungal species with high betweenness centrality represent potentially key connector species within networks, while AM fungal species with high degree represent hubs in the network. Points representing AM fungal species are sized by prevalence. Candidate keystone AM fungal species that had high betweenness centrality and degree values, while also exhibiting a prevalence score ≥ 0.005, are highlighted in both plots.

**Fig. 4. Matrices depicting the nested temperature of arbuscular mycorrhizal (AM) fungal communities in remnant (A) and restored (B) forest habitat types within Hakalau.** Host plant species are rows and AM fungal taxa are columns. Presences of an AM fungal taxon within hosts are indicated by colored boxes.

**Fig. 5. Non-metric dimensional scaling (NMDS) ordination plot of the community composition of arbuscular mycorrhizal (AM) fungal communities collected from Remnant (green circles) and Restored forest (blue diamonds) habitat types within Hakalau.** Compositional differences are based on Bray-Curtis dissimilarity among samples. Communities are colored by habitat type. Ellipses represent the 95% confidence region based on the centroid for each host. Environmental variables with significant correlation with the ordination are shown. Arrowhead size is proportional to the strength of correlation (SI Appendix, Table S1