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ORIGINAL ARTICLE

Soil Fertility and the Impact of Exotic Invasion on Microbial Communities in Hawaiian Forests

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Abstract Exotic plant invasions into Hawaiian montane forests have altered many important nutrient cycling processes and pools. Across different ecosystems, researchers are uncovering the mechanisms involved in how invasive plants impact the soil microbial community—the primary mediator of soil nutrient cycling. We examined whether the invasive plant, *Hedychium gardnerianum*, altered microbial community composition in forests dominated by a native tree, *Metrosideros polymorpha*, under varying soil nutrient limitations and soil fertility properties within forest plots of the Hawaii long-term substrate age gradient (LSAG). Microbial community lipid analysis revealed that when nutrient limitation (as determined by aboveground net primary production [ANPP]) and soil fertility were taken into account, plant species differentially altered soil microbial community composition. Microbial community characteristics differed under invasive and native plants primarily when N or P was added to the older, highly weathered, P-limited soils. Long-term fertilization with N or P at the P-limited site led to a significant increase in the relative abundance of the saprophytic fungal indicator (18:2ω6c,9c) under the invasive plant. In the younger, N-limited soils, plant species played a minor role in influencing soil microbial community composition. We found that the general rhizosphere microbial community structure was determined more by soil fertility than by plant species. This study indicates that although the aggressive invasion of a nutrient-demanding, rapidly decomposable, and invasive plant into Hawaiian forests had large impacts on soil microbial decomposers, relatively little impact occurred on the overall soil microbial community structure. Instead, soil nutrient conditions were more important determinants of the overall microbial community structure within Hawaii's montane forests.

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Introduction

Invasive plant species threaten the unique flora of the Hawaiian Islands by outcompeting native species for resources and space, and by potentially changing the soil chemical and biotic environment for native plant communities [30, 33]. Some invasive plants can alter the indigenous soil environment by changing soil nutrient pools, for example increasing soil nitrogen (N) availability to plants through N fixation [29, 46]. Other invasive species may deplete nutrient pools through the rapid uptake of soil N [3]. Finally, many invasive plants in Hawaii are associated with increased litter decomposition rates, which in turn, can lead to more rapid nutrient cycling in invaded soils [1].

Likewise, in other ecosystems and regions outside Hawaii, invasive plants can alter nutrient pools and processes. In a review of 79 research papers on plant invasions, Ehrenfeld [16] found that increases in N availability and litter decomposition rates are common with plant invasions. In addition, 11 of 16 studies showed increases in N mineralization and nitrification rates under invaded soils. The changes in N transformation rates may be attributed to concurrent changes in plant biomass or litter N concentration with invasion. Plant invasions alter important components of the C, N, and P cycles, but the

mechanisms that lead to the changes are still unclear across different ecosystems.

Much of the alteration to the soil environment may be the result of the invading plant's influence on the resident soil microbial community. Plant species can differentially impact the composition and activity of the soil microbial community, which may lead to important changes in nutrient cycling and fluxes [9, 14, 19, 26, 28]. For example, if an invasive species alters the quality or quantity of the resources available to soil microorganisms, community composition will likely shift. A shift in the composition or abundance of particular members of the microbial community can alter nutrient pools and fluxes [5, 7]. For instance, invasion by exotic grasses into California grassland led to a shift in the composition and abundance of the soil microbial community to favor ammonia-oxidizing bacteria (AOB) [23]. However, stepwise multiple regression revealed that the changes in AOB composition did not directly alter N cycling. Instead, AOB abundance explained the increase in gross N mineralization rates in invaded plots.

Shifts in the composition of other major microbial groups can also indicate changes in N or C cycling. Gram-negative bacteria tend to be more abundant in rhizosphere soils compared to bulk soil [40], and shifts in the proportion of Gram-negative and Gram-positive bacteria could indicate changes in microbial substrate use of C and cycling of nutrients [6]. For example, Fraterrigo *et al.* [21] found that the proportion of Gram-negative bacterial lipid indicators in forest soils were positively associated with N mineralization rates. The rhizosphere of several plant species tends to have a higher proportion of Gram-negative bacteria [42], and Gram-negative bacteria presence increases with closer proximity to plant roots [31]. Gram-negative bacterial levels also increase in rhizosphere soils as succession proceeds in grassland systems [44]. These results suggest that specific mechanisms controlling N cycling can be cryptic.

It is possible that ecosystem traits other than plant species composition may have greater control over the composition and activity of soil microorganisms, consequently enhancing or negating the impacts of plant invasions on the soil microbial community. For example, site differences (e.g., in dominant vegetation or soil fertility) can influence the impact of an invasive species on soil nutrient processes. Researchers found that the level of extractable NH_4^+ released in *Phragmites*-dominated systems differed in brackish versus freshwater wetlands [32]. Soil nutrient levels may also influence the magnitude of an invasive plant's impact on soil microorganisms. Kourtev *et al.* [28] found that the activity and composition of the soil microbial community under the invasive species differed from the native plant. Additional information on soil characteristics reveals that soil extractable NO_3^- levels were greater under the exotics compared to the native species [17]. The invaded soils also showed increased N mineralization and nitrification rates in relation to the soils under the native species. The higher soil N levels may have led to larger shifts in the soil microbial community, but a greenhouse experiment using the same species confirmed that within a 3-month period, soil microbial community composition and activity, in addition to nitrification rates, were altered despite starting with the same pool of soil [27]. However, long-term changes in soil N pools, resulting from invasion, may feedback to promote greater shifts in microbial community composition. This further complicates our ability to separate the effects of ecosystem traits (soil fertility) versus plant invasion (plant species) in shaping microbial communities.

In this study, we were able to separate the effects of soil fertility from plant invasion by using well-established nutrient addition plots located within the Hawaiian long-term substrate age gradient (LSAG) research site. Our objective was to assess the relative importance of soil environmental conditions (soil fertility) versus plant species (native and exotic) in altering soil microbial community composition. We examined the impact of an invasive plant *Hedychium gardnerianum* on soil microbial community structure in comparison to the native dominant tree *Metrosideros polymorpha* in N-limited versus P-limited plots receiving N, P, or no nutrient addition. Concurrently, we assessed the roles of N or P addition and site characteristics (N limitation versus P limitation) in altering soil microbial community composition. We hypothesized that the invasion of a nutrient-demanding species (with highly decomposable litter) into native forests dominated by *M. polymorpha* would alter the structure of the soil microbial community with a notable increase in the relative abundance of Gram-negative bacteria within the rhizosphere. In addition, increased root production should favor saprophytic fungi. This type of transition would complement faster nutrient turnover and cycling rates in invaded soils. We also hypothesized that alleviating soil nutrient limitations by adding P to P-limited soils or adding N to N-limited soils would enhance the differences in soil microbial community composition under the two plant species, as a result of *H. gardnerianum*'s potentially greater ability to compete for soil resources. In contrast to the slower-growing *M. polymorpha*, *H. gardnerianum* can take advantage of increased nutrient availability, possibly resulting in higher levels of activity in its rhizosphere.

Materials and Methods

Site Description and Experimental Design

We sampled soil at two sites along the Hawaiian LSAG, as described by Crews *et al.* [15]. The two sites differ by soil

nutrient availability and age sequence. Soils at Na Pali Kona Forest Reserve (22°08′ W, 159°37′ N) on the island of Kauai are characterized as P-limited for net primary productivity and are dated at 4.1 million years old, whereas soils at Hawaii Volcanoes National Park (19°25′ W, 155°15′ N) on the island of Hawaii are characterized as N-limited and are dated at 300 years old (Table 1). Nutrient limitation to aboveground net primary productivity was determined by Herbert and Fownes [25] and Vitousek and Farrington [45] through fertilization experiments. The soils at both sites are derived from volcanic ash or tephra and are classified as Plinthic Acrothox [15]. The soil surface horizon is highly organic, comprised of up to 20% carbon (Table 1). Both sites are located at 1,200 m elevation, receive approximately 2,500 mm of annual rainfall, and have a mean annual temperature of 16°C.

The myrtaceous tree *M. polymorpha* is native to the Hawaiian islands and is the dominant overstory vegetation at each site [15]. An invasive understory herb *H. gardnerianum* (kahili ginger) is also present at both sites, and forms dense rhizomatous stands that are 1–2 m tall. The presence of *H. gardnerianum* was detected in the 1950s and has spread across the Hawaiian islands from its origins in the Himalayan region of India [47].

The sites have received full factorial fertility treatments beginning in 1985 at the N-limited site and in 1991 at the P-limited site [25, 33]. In the study reported in this article, we include only plots fertilized with N or P and non-fertilized (control) plots. At each site, 15×15 m plots received semiannual applications of 100 kg ha^{-1} $year^{-1}$ of N (1:1 urea and ammonium nitrate) or 100 kg ha^{-1} $year^{-1}$ P (triple superphosphate). We had four replicate plots per fertility treatment at the N-limited site, whereas we included only three replicate plots at the P-limited site as a result of storm damage to a replicate block. Within each plot, we collected three soil samples from the rhizosphere of either *M. polymorpha* or *H. gardnerianum* and pooled the three subsamples for microbial lipid analysis. Collection of rhizosphere soil was within the top 15 cm of the soil surface directly under the different plant species roots. Soil adhering to the plant roots was separated using spatulas and was termed rhizosphere soil for microbial analysis. The final analysis included one composite soil sample for each species and replicate plot of the N, P, and control treatments (n=3 or 4 per treatment per plant species).

Microbial Community Analysis

We used a hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) to analyze microbial community composition [23]. The procedure is based on the extraction of 'signature' lipid biomarkers from the cell membrane and wall of microorganisms [48]. Samples are homogenized, frozen, and then freeze-dried before analysis. All glassware is baked at 550°C for 3 h. Membrane lipids are then extracted, purified, and identified using steps from a modified Bligh and Dyer [11] technique for lipid extraction, combined with FAME as described by Microbial ID (Hayward, CA, USA). We adjusted the proportion of lyophilized soil to the phosphate–methanol–chloroform mixture by a 1:6 ratio because the soil collected in this study has a high organic C content. Lipids were extracted from 0.5 g of freeze-dried soil using a chloroform–methanol extraction with a phosphate buffer (potassium phosphate [2.7 ml], methanol [6 ml], and $CHCl_3$ [3 ml]) in 25-mL glass tubes, shaken for 1 h, and centrifuged. Supernatant was then decanted to 30-mL tubes and potassium phosphate buffer and $CHCl_3$ were readded and the tubes were vortexed for 30 s. The phases were allowed to separate overnight at room temperature. The top layer was aspirated off (saving the chloroform phase), and volume was reduced in a RapidVap. We then followed the procedure for FAME as given by Microbial ID: sodium hydroxide is added for saponification; the solution is heated in a water bath for 30 min, followed by methanolysis; the FAMEs are rinsed with a dilute base wash; and the solution is reduced in a RavidVap, and reconstituted in a mixture of hexane and methyl *tert*-butyl ether for chromatographic analysis.

A 2-μl injection of the methyl ester derivatives of the extracted lipid was analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a flame ionization detector and split/splitless inlet and a 25 m×0.2 mm inside diameter×0.33 μm film thickness Ultra 2 (5% phenyl, 95% methyl) capillary column (Agilent) using hydrogen as the carrier gas, N as the make up gas, and air to support the flame. Gas chromatograph conditions are set by the MIDI Sherlock program (MIDI, Newark, DE, USA). Peaks were identified with bacterial fatty acid standards and Sherlock peak identification software (MIDI, Newark, DE, USA). Fatty acids were quantified by comparisons of peak areas

Table 1 Soil characteristics of the N-limited and P-limited sites

Site	Site age (years)	pH	Water content ($g \cdot g^{-1}$)	Ammonium ($\mu g \cdot g^{-1}$)	Nitrate ($\mu g \cdot g^{-1}$)	Total C (%)
N-limited	300	4.35±0.05	1.24±0.16	2.27±0.34	0.67±0.57	8.8±0.7
P-limited	4,100,000	3.18±0.06	0.57±0.12	2.81±0.39	7.36±4.3	18.0±2.5

Means are ±1 SE.

from the sample compared to peak areas of two internal standards, 9:0 (nonanoic methyl ester) and 19:0 (nonadeconoic methyl ester), of known concentration. Internal standard lipids were subtracted from the final lipid profiles, and in all subsequent analyses, we used only fatty acids that were identifiable and present at >0.5 mol%.

Lipids cannot confidently be used to represent specific microbial 'species' but are more commonly assigned functional guilds [43]. Terminology to describe fatty acids is described by 'A:BωC' where 'A' indicates the total number of carbon atoms, 'B' the number of double bonds (unsaturations), and 'ω' indicates the position of the double bond from the methyl end of the molecule. The prefixes 'i' and 'a' refer to iso and ante-iso methyl branching. Hydroxy groups are indicated by 'OH'. Cyclopropyl groups are denoted by 'cy' [2, 4, 41]. The monounsaturated guild represents lipids that are common in Gram-negative bacterial membranes, whereas the branched chain guild indicates lipids generally from Gram-positive bacteria.

Statistical Analysis

We performed principal components analysis (PCA) on the arcsine-transformed molar fractions of individual lipids using the JMP software, version 5.0 (SAS Institute, Cary, NC, USA). We analyzed microbial lipid absolute abundance using three-way ANOVA. The fixed factors include nutrient limitation (site), plant species, and fertility treatment (N, P, or control); significant differences were identified by Fisher's LSD. We tested each variable used in our analysis for normality and used arcsine-transformations of data to control for heterogeneity.

Results

General Rhizosphere Microbial Community Structure

Principal components analysis (PCA) of the full set of lipid variables and treatments indicated that the soil microbial community was distinct by site along PC1 (Fig. 1a). PC1 and PC2 explained 54% of the variability in the data. The general rhizosphere community in the N-limited (300-year-old) site was far more variable than that at the P-limited (4.1 million-year-old) site. Further evaluation of the component scores using ANOVA revealed that microbial community structure in the rhizosphere of the N-limited and P-limited soils was significantly different (F=135.4, P<0.0001). The lipid indicators that contribute to PC1 included several branched chain and monounsaturated fatty acids (typically indicative of Gram-positive and Gram-negative bacteria, respectively).

When PCA was performed for each site individually, the soil microbial community differed by nutrient addition treatment along PC1 for the P-limited site and along PC2 for the N-limited site (Fig. 1b and c). The first two components explained 49% of the variability in the P-limited dataset. At the P-limited site, the rhizosphere communities of the invasive and native plants species were distinct in the control treatments (no nutrients added, Fig. 1b), remained distinct when N was added, and appeared to converge when the limiting element, P, was added. However, for both plant species, the rhizosphere community shifted when either N or P was added to the soil. Adding P to the P-limited site altered microbial

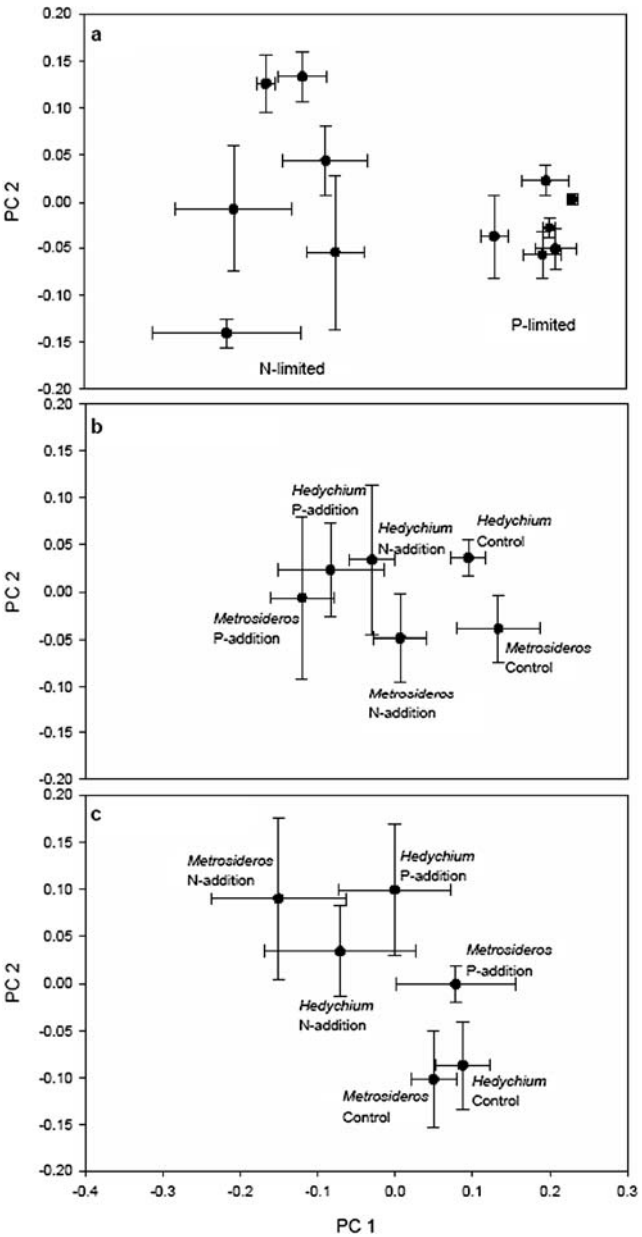

Figure 1 Principal components analysis of microbial lipid molar fractions (means±1 SE) from **a** N-limited and P-limited sites, **b** P-limited site, and **c** N-limited site. Data points indicate the average PCA scores for the replicate plots (P-limited site: n=3, N-limited site: n=4)

community structure (F=12.03, P=0.001) more for the native plant species than for the *Hedychium*. The branched chain, hydroxy, and monounsaturated fatty acids contributed greatly to the loadings along PC1 at the P-limited site.

At the N-limited site, PC1 and PC2 explained 54% of the variability with branched chain and monounsaturated fatty acids contributing to the loadings (Fig. 1c). The N-limited site also indicated differences in soil microbial community structure depending on fertility treatment and plant species (Fig. 1c). The N-limited site control treatment rhizosphere communities were similar under both plant species, which was in contrast to controls soils in the P-limited site that showed distinct communities under the two species. Adding N to N-limited soils grown under *H. gardnerianum* resulted in a change in microbial community structure, although smaller than the change in the native rhizosphere (PC2: F=6.02, P=0.01).

Although it appeared that adding the limiting element had the largest effect on microbial community structure under both plant species (with the magnitude of effect dependent on plant species), adding the nonlimiting element to soil also affected microbial community structure in the rhizosphere. In addition, the nonlimiting element appeared to have a disproportionate impact on the microbial community associated with the invasive plant (Fig. 1b and c). In other words, P added to the N-limited soil or N added to the P-limited soil resulted in a large shift in microbial community composition in the *Hedychium* rhizosphere and a smaller shift in the *Metrosideros* rhizosphere.

Relative Abundance of Select Microbial Lipid Indicators

In addition to general community patterns, some lipids indicate microbial groups, such as saprotrophic fungi, mycorrhizal fungi, or Gram-negative bacteria. In this study, the saprotrophic fungal indicator 18:2ω6,9c was the only major microbial lipid indicator that showed significant differences in relative abundance between *M. polymorpha* and *H. gardnerianum* (Fig. 2c, Table 2). Nutrient addition to P-limited soils led to an increase in the abundance of 18:2ω6,9c under *H. gardnerianum* relative to *M. polymorpha* (F=7.1, P<0.05), whereas the control soils showed no significant differences. In contrast, differences between the N-limited versus P-limited sites in general appeared to be related to differences in the abundance of many of the lipid guilds (Fig. 2).

Plant species did not affect the relative abundance of other microbial ecological guilds, but some interesting trends in soil fertility and nutrient limitation were found. The relative abundance of monounsaturated lipids (thought to be Gram-negative bacterial indicators) was greater in N-limited soils in comparison to P-limited soils

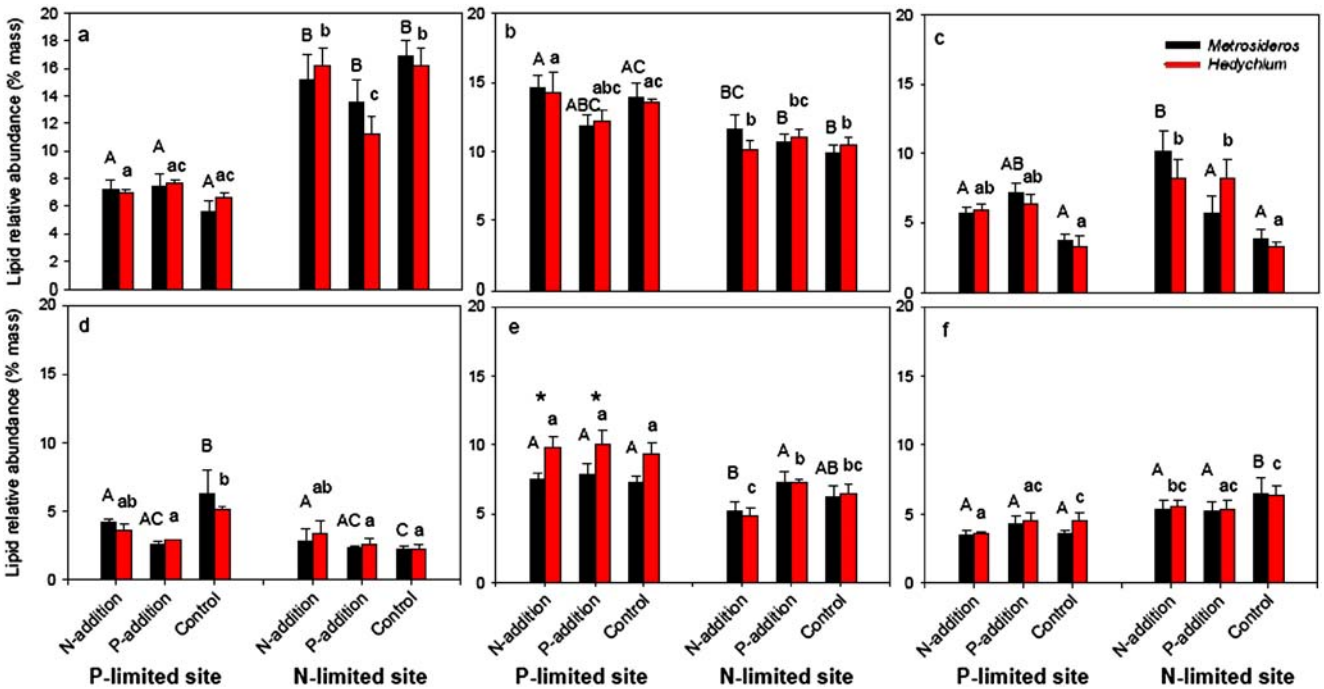

Figure 2 Microbial lipid relative abundance (mass percentage) of indicator guilds (means±1 SE). Microbial lipid relative abundance from soils collected under the native *Metrosideros* are represented by *black bars* and those collected under the invasive *Hedychium* are represented by *gray bars*. *Bars with capital case letters* indicate means comparisons among *Metrosideros* samples (bars with the same capital letters are not significantly different), whereas *bars with lowercase letters* indicate means comparisons among *Hedychium* samples (bars with the same lowercase letters are not significantly different; Fisher's LSD, P<0.05). An *asterisk* above two bars indicates a significant difference between the two plant species

Table 2 List of fatty acids within the major microbial lipid guilds

Major microbial lipid guilds	
Monounsaturated	12:1ω5c
	14:1ω6c
	15:1ω8c
	16:1ω7c
	17:1ω7c
Branched chain	*a*13:0
	*i*14:0
	*a*15:0
	*i*15:0
	*i*16:0
	*i*17:0
Hydroxy	*i*11:0 3OH
	12:0 3OH
	16:0 2OH
	18:0 2OH
Cyclopropyl	*cy*19:0
Saprotrophic fungi	18:2ω6c,9c
AM fungi	16:1ω5c

(F=103.9, P<0.0001; right-hand side of Fig. 2a), except when P was added to the N-limited plots. In contrast, the relative abundance of branched chain lipids (Gram-positive bacterial indicators) was generally greater in P-limited soils than in N-limited soils (F=24.4, P<0.0001) (Fig. 2b). For cyclopropyl lipids (often anaerobic bacterial indicators), the control plot at the P-limited site had greater relative abundance than at the N-limited site (F=16.0, P<0.001); however, the addition of N or P appeared to reduce differences in relative abundance of cyclopropyls between the two sites (Fig. 2d).

Discussion

General Rhizosphere Microbial Community Patterns

Our objective with this study was to assess whether soil fertility influences or moderates the belowground impacts of plant invasions into Hawaii's montane forests. We focused on the rhizosphere rather than bulk soil and sampled from an invasive and native plant species. We found that long-term nutrient addition and plant species influenced microbial community composition in the rhizosphere. As expected, addition of the limiting element had the largest effect on general rhizosphere community structure relative to control plots (Fig. 1b, c). Thus, plant species' response to nutrient limitation appears to result in a shift in rhizosphere microbial community lipid profile. Relieving P limitation (adding P to soil plots) resulted in the convergence of microbial community profiles from native and invasive rhizospheres relative to profiles in the control plots (Fig. 1b). In contrast, the rhizosphere microbial communities under native and invasive at the young (N-limited) site appear to have started out similarly (community profiles in control plots are similar) and diverged under long-term nutrient treatments (Fig. 1c). This observation is further supported by the relative abundance of indicator lipids in the P-limited versus N-limited sites (Fig. 2, right-hand versus left-hand sides of each). In both cases, the addition of the limiting nutrient had a greater impact on the microbial community in the native rhizosphere than in that of *Hedychium*.

The difference in rhizosphere microbial community shift (divergence versus convergence from control upon addition of the limiting nutrient) is intriguing. It is important to note that N and P limitation at the Hawaii study sites are determined by vegetation responses, not by microbial responses. Fertilization experiments conducted at the N-limited and P-limited sites showed that the addition of the limiting nutrient led to increased vegetation growth, which indicated the limitations to plant growth at the sites [45]. The microbial shift we found may indicate a unique plant response to N versus P limitation that is translated into an impact on microbial community profile in the plant rhizosphere.

We found a number of additional trends in N and P limitation that affected microbial community structure. Among these was an increase in the relative abundance of monounsaturated fatty acids (indicative of Gram-negative bacteria) at the N-limited site compared to the P-limited site (Fig. 2). The branched chain fatty acids (typical of Gram-positive bacteria) showed the reverse trend where the relative abundance increased in the P-limited site rather than the N-limited site. In other studies, we have found that Gram-negative bacterial lipid indicators are often associated with rapid (or tight) nitrogen cycling, whereas branched lipids tend to be relatively more abundant where there is lower pH or higher physical stresses in the soil [21, 38, 43]. The higher relative abundance of Gram-negative bacteria in the N-limited soil could indicate tighter N-cycling at the N-limited site, but we did not measure nitrification or N mineralization at each plot. An experiment conducted by Funk [22] at the N-limited site showed that N cycling did not increase with invasion of *Hedychium* in unfertilized soils, but our experiments were conducted at different years.

The effect of altering soil nutrient availability could change microbial community composition in unpredictable ways. Some researchers have found that increased soil fertility shifts microbial community structure with a noticeable decrease in fungi and increase in bacteria [12, 35]. Other researchers have shown that the long-term addition of N led to a decrease in microbial biomass and

activity in forest soils [20, 39]. However, in the aged and highly weathered soils in our study, competition for nutrients may be more intense, and thus the rhizosphere community profiles start out distinct (in control plots) and converge when the addition of the limiting element relieves nutrient competition. These ideas remain to be tested.

Specific Impacts of Invasive Species and Soil Properties

The invasion of *H. gardnerianum* into Hawaiian forests appears to have had limited impact on soil microbial community structure relative to the differences related to site and treatment history. General rhizosphere microbial community profiles varied more by site and nutrient status than by plant species. The P-limited site did show an effect of invasion on the overall structure of the soil microbial community in control soils (Fig. 1b), but the N-limited site showed no similar trend (Fig. 1c). Our study also indicates that when microbial guilds (groups of microorganisms with similar fatty acid structure) are taken into account, saprotrophic fungal relative abundance may be particularly sensitive to plant type and nutrient input in the highly weathered (older) soils. Plots in this study with highly weathered soils that have received long-term inputs of N or P showed a greater abundance of saprotrophic fungal lipid indicators under *H. gardnerianum* than under the native *M. polymorpha* (Fig. 2). The increase in abundance of saprotrophic fungi under the invasive plant is consistent with findings that show faster decomposition rates of *H. gardnerianum* litter when compared with the litter of several native species [1]. These researchers also found that the difference in litter decomposition rates between the invasive plant and four of the native species was greater when N and P were added to the plots. However, our findings indicate that simply adding fertilizers will not always result in species-specific impacts on the soil microbial community. Instead, soil properties may play an important role in shaping the magnitude of an invasive plant's impact on belowground properties and processes. We found that the increase in abundance of saprotrophic fungal indicators may have been more dependent on site characteristics than plant species. The P-limited soils from the island of Kauai showed differences in the relative mass of specific decomposer indicators only when N or P were added, whereas there were no differences in response to nutrient addition at the N-limited site on the big island of Hawaii (Fig. 2). Other researchers have also shown that soil fertility played an important role in shaping soil microbial community structure in forest sites along a fertility gradient [8, 35].

The results from this study (indicating large impacts of *Hedychium* invasion on soil decomposers and limited impact on overall microbial community composition) suggest that high decomposition rates and nutrient release from invasive plant litter does not necessarily lead to large overall changes in soil microbial composition or function. Instead, the effects of the plant invasion may be highly dependent on site characteristics and soil fertility, whereas changes in plant composition alone does not lead to a predictable effect on the soil microbial community. Many researchers predict that plant invasions associated with increased plant primary production, leaf nutrient concentrations, or litter decomposition rates will alter soil nutrient cycling processes [3, 18]. However, some studies indicate no changes in N mineralization and nitrification rates in invaded areas, despite significant changes to plant community traits [34]. In the Hawaiian forests, Funk [22] also found that *H. gardnerianum* invasion had no significant impact on N mineralization or soil N availability, despite the high N demand of the species. The results from our study indicate that invasion by *H. gardnerianum* significantly impacts the relative abundance of only a single major ecological group of soil microorganisms—the saprotrophic fungi. This relative lack of impact may explain the weak impacts of invasion on nitrogen cycling found by others. It may be that Gram-negative bacteria are a key to N cycling, and they are relatively robust to disturbance and invasion [21, 38, 43].

There are several possible reasons for why *H. gardnerianum* invasion did not lead to large subsequent changes in the composition of the soil microbial community at the N-limited site. Nutrients released from *H. gardnerianum* litter may be rapidly acquired by the plant, making the nutrients unavailable to soil microorganisms. Asner and Vitousek [3] found that the invasive plant was able to outcompete neighboring plants for soil nutrient resources. Using remote sensing, they found that *H. gardnerianum* reduces overstory foliar N concentrations in *M. polymorpha* forests, suggesting that the invasive plant is more effective in acquiring available soil N. This faster uptake may imply a lower reliance on microbial 'intermediaries' (e.g., to rapidly decompose litter and/or increase uptake surface area) or a modification of community 'intermediaries'.

Another explanation for the minor changes to the overall composition of the soil microbial community at the N-limited site may be the lack of a substantial self-fertilizing effect in invasive plant stands. Other researchers found that positive plant–soil feedback, such as promoting rhizosphere mutualists or increasing the availability of a limiting nutrient to the plant, are important mechanisms for invasive plant success [10, 13, 36]. We found that adding soil N or P to the forest plots was necessary to alter the general composition of the soil microbial community, possibly indicating that the invasive plant does not, on its own, sufficiently augment soil nutrient concentrations to a level that would impact

the structure and abundance of soil microorganisms. In addition, the relative mass of saprotrophic fungi increased under *H. gardnerianum* only when the oldest forest plots were fertilized with additional soil N and P. Other researchers have demonstrated that high nutrient-demanding invasive species can create their own nutrient-rich sites, thus possibly promoting their own invasion [17, 46].

A third possibility is that site differences could be more important in influencing soil microbial community composition than nutrient availability or plant community composition. Although both sampling sites share similar elevation, soil composition, vegetation, rainfall patterns, and climate, the overall structure of the soil microbial community was distinctly different by site. The major difference between the sites is the age of the parent material (300 years versus 4.1 million years); thus, simply the amount of time the soil has developed may control the composition of the microbial community. For instance, over time, the weathering of P from soils often leads to increased P limitation or higher C/P ratios, as seen in the Hawaiian LSAG [24], the Franz Josef Glacier chronosequence [37], and the Waitutu forest chronosequence [49]. The decrease in P availability in older soils affects plant growth, which could, in turn, affect microbial community growth and activity, but we know very little about other factors that could influence microbial community development over time. Evidence for substantial 'legacy effects' of land management and past plant communities indicates possible long-term patterns in microbial community change [21].

The results of the PCA for both sites (Fig. 1a) indeed showed that the microbial community was distinctly different by site, but the results also indicate that the variability in the soil microbial community was greater at the younger (300 year) site, and therefore, potentially less stable than at the older site. Similarly, Smithwick *et al.* [43] found that microbial community composition differed along stand age of lodgepole pine forests with the oldest sites having the most unique microbial community. They also determined that gross NH_4^+ mineralization rates were highest at the oldest sites and that the composition of the soil microbial community best explained the mineralization rates.

In this study, high nutrient uptake and litter decomposition rates were not adequate predictors of an invasive plant's impact on soil microbial community composition. In contrast to what was expected, we found that invasion of a nutrient-demanding, rapidly decomposable species into native Hawaiian forests significantly influenced the abundance of soil microbial decomposers, but had little impact on overall soil microbial community structure relative to the effects of site/soil age and long-term nutrient additions. The composition and relative mass of microbial lipids for major ecological guilds were similar under both the native and invasive species, except when N or P was added. Because the more dominant factors shaping microbial communities appear to be soil properties, such as nutrient limitation or fertility, we found that the presence of an invasive plant alone does not correspond to a large alteration in the composition of the soil microbial community. We suggest that future studies examining the effects of plant invasions on soil microbial community composition or activity take into account factors such as site history and soil fertility in influencing different belowground invasion scenarios.

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