

Elevated enzyme activities in soils under the invasive nitrogen-fixing tree *Falcataria moluccana*

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Abstract

Like other N-fixing invasive species in Hawaii, *Falcataria moluccana* dramatically alters forest structure, litterfall quality and quantity, and nutrient dynamics. We hypothesized that these biogeochemical changes would also affect the soil microbial community and the extracellular enzymes responsible for carbon and nutrient mineralization. Across three sites differing in substrate texture and age (50–300 years old), we measured soil enzyme activities and microbial community parameters in native-dominated and *Falcataria*-invaded plots. *Falcataria* invasion increased acid phosphatase (AP) activities to $>90 \mu\text{mol g}^{-1} \text{soil h}^{-1}$ compared to $30\text{--}60 \mu\text{mol g}^{-1} \text{soil h}^{-1}$ in native-dominated stands. Extracellular enzymes that mineralize carbon and nitrogen also increased significantly under *Falcataria* on the younger substrates. By contrast, total microbial biomass and mycorrhizal abundance changed little with invasion or substrate. However, fungal:bacterial ratios declined dramatically with invasion, from 2.69 and 1.35 to <0.89 on the 50- and 200-year-old substrates, respectively. These results suggest that *Falcataria* invasion alters the composition and function of belowground soil communities in addition to forest structure and biogeochemistry. The increased activities of AP and other enzymes that we observed are consistent with a shift toward phosphorus limitation and rapid microbial processing of litterfall C and N following *Falcataria* invasion.

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1. Introduction

Nitrogen-fixing plants dramatically increase nitrogen (N) inputs and cycling rates in many ecosystems (Vitousek et al., 1987; Vitousek and Walker, 1989; Bowman et al., 1996; Maron and Connors, 1996). Because photosynthesis and productivity are often limited by N availability, N-fixers may increase ecosystem carbon (C) inputs as well (Binkley et al., 1992; Welsh, 2000). In sites where exotic N-fixers invade native ecosystems, they can alter vegetation structure and the quality of litter inputs (Vitousek and Walker, 1989).

Recently, Hughes and Denslow (2005) demonstrated that invasion by the exotic N-fixing tree *Falcataria moluccana*

dramatically alters forest structure and litter inputs on young lava flows at low elevations on the island of Hawaii. Typically, these flows have minimal soil development and support native, open canopy forests dominated by *Metrosideros polymorpha*. On flows as young as 50 years, invading *Falcataria* can establish a closed canopy and thick organic root mat on top of the underlying substrate. Native vegetation declines under the dense canopy, and *Falcataria* facilitates invasion by other exotic species such as *Psidium cattleianum*. The biogeochemical effects of *Falcataria* invasion are dramatic: litterfall biomass inputs increase 1.3- to 8.6-fold, corresponding to four- to 55-fold increases in N inputs. *Falcataria* invasion also dramatically increases decomposition rates and soil nutrient availability by factors exceeding 10- and 100-fold, respectively, on the youngest lava flows (Hughes and Denslow, 2005; Hughes and Uowolo, in press). Compared to the native *Metrosideros*,

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N concentrations in *Falcataria* litter are four times greater, although lignin concentrations are also higher and cellulose concentrations are lower in *Falcataria* litter (Hughes and Uowolo, in press).

Aside from increasing rates of C and N cycling, N-fixers also affect phosphorus (P) cycling (Zou et al., 1995; Kaye et al., 2000). In plantations on the island of Hawai'i, *Falcataria* decreases available P pools more than adjacent, nonfixing plantation species with similar productivities (Binkley and Ryan, 1998; Binkley et al., 2000). Although Hughes and Denslow (2005) found elevated P availability with *Falcataria* invasion, the increase was not as strong as for N, suggesting that P supply may also be relatively limited in this system. Low P supply is consistent with the young age of these lava flows, where large available and organic P pools have not yet accumulated due to weathering processes (Raich et al., 1996). Under P limitation, plants and microbes may allocate resources toward P acquisition, particularly the production of phosphatase enzymes (Chróst, 1991; Olander and Vitousek, 2000; Treseder and Vitousek, 2001). Such changes in soil phosphatase activity can be driven by mycorrhizal fungi, and both *Falcataria* (Alexander et al., 1992) and the dominant native tree *M. polymorpha* are known to form associations with mycorrhizae.

Because of its abundant, N-rich litter inputs and effects on nutrient cycling, invasion by *Falcataria* is likely to affect soil microbiological and biochemical processes (Sinsabaugh et al., 1991; Ratledge, 1994; Sinsabaugh, 1994). Specifically, we hypothesized that elevated nutrient availabilities under *Falcataria* would cause a shift in the microbial community from fungal to bacterial dominance, since some fungi compete poorly under high N conditions (Fog, 1988). Since the relative availability of P may decline under *Falcataria*, we predicted that mycorrhizal abundance and acid phosphatase (AP) activity would increase. We also hypothesized that *Falcataria* litter inputs would provide resources for microbes to increase production of enzymes that degrade organic C compounds, while the synthesis of N-degrading enzymes would decrease due to higher N availability (Chróst, 1991; Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005). Finally, we predicted that the microbial and enzymatic effects of invasion would diminish with flow age because differences in stand structure and litter inputs were smallest on the oldest lava flow (Hughes and Denslow, 2005).

2. Methods

2.1. Site description

We used study sites established by Hughes and Denslow (2005) in the Malama Ki (19°26'53"N, 154°51'40"W) and Keauohana (19°25'11"N, 154°57'14"W) State Forest Reserves on the Island of Hawaii. Mean annual precipitation at the sites is ~2500 mm (Giambelluca et al., 1986), and mean annual temperature is ~23 °C. The climate is

aseasonal, with no less than 100 mm of precipitation falling in any given month, and monthly mean temperatures varying by <3 °C (NOAA, 2002). The Malama Ki site is located on a 213 year-old (y.o.) pahoehoe lava flow, while the Keauohana sites are located on 48 and 200–400 y.o. a'a flows. For simplicity, we refer to the sites as 50 y.o. a'a, 200 y.o. pahoehoe, and 300 y.o. a'a. The native vegetation at these sites is dominated by the ubiquitous Hawaiian tree *M. polymorpha*, with the diversity of understory shrubs and trees increasing with flow age. Forests on the two younger flows have open canopies, with lichens (50 y.o. a'a) or grasses (200 y.o. pahoehoe) covering the volcanic substrate between *Metrosideros* individuals. At the 50 y.o. site, soils are sparse and classified as a'a lava flows (Sato et al., 2005). Soils at the 200 y.o. site are classified as Opihikao extremely rocky mucks, with pahoehoe lava outcrops occupying 30–50% of the land area, while soils at the 300 y.o. site are classified as Malama extremely stony mucks. Both of these soil types have thin (~5 cm) organic horizons that are strongly acidic (Sato et al., 2005).

At each site, we set up 10 circular 0.01 ha plots in the native forest ('control') and 10 plots in adjacent *Falcataria*-invaded ('invaded') areas. To ensure that plot locations were comparable in vegetation cover, substrate type, and disturbance history prior to *Falcataria* invasion, we examined aerial photos of the invaded plots prior to invasion and compared size-class distributions of dead *Metrosideros* trees in the invaded plots with those of live trees in adjacent control plots (Hughes and Denslow, 2005).

2.2. Soil collection

In January 2003 and January 2004, we randomly chose five of 10 control and five of 10 invaded plots at each site for soil sampling. We used a trowel to remove five 10 × 10 cm samples to a depth of 5 cm near the center of each plot. Within each plot, we took samples at random locations 1–2 m apart, except in the 50 y.o. site where we only found soil accumulating in low spots between a'a blocks. Samples were composited, kept cool, and shipped to Stanford University within 2–3 days for further processing. We passed the samples through a 4 mm sieve to remove coarse roots, organic debris, and lava pieces, and homogenized the soil by hand for use in microbial and enzyme analyses. Soils were subsampled to determine water and nutrient contents (oven drying, 65 °C) and refrigerated (4 °C) for a maximum of 10 days before analyses.

Samples for phospholipid fatty acid (PLFA) analyses were collected similarly in July 2003, except that three cores were composited from within each of three control and three invaded plots. The composited samples were sieved to 2 mm and freeze-dried prior to analysis.

2.3. Soil nutrient concentrations

We determined total soil N and P concentrations using Kjeldahl digestion followed by colorimetric analysis on an

Alpkem autoanalyzer (OI Analytical, Wilsonville, OR). Soil samples from 2004 were run with apple leaf (NIST 1515) and Montana soil (NIST 2710) standards, and recoveries were >96% for both N and P.

2.4. Enzyme assays

We measured the activities of extracellular enzymes (Table 1) with assay techniques modified from Sinsabaugh et al. (1992, 1993). The P-releasing enzyme acid phosphatase (AP), the N-releasing enzymes urease (UR), glycine aminopeptidase (GAP), and *N*-acetyl-glucosaminidase (NAG), and the C-degrading enzymes β -glucosidase (BG) and polyphenol oxidase (PPO) were included in this study. Approximately 1–2 g fresh soil (~0.5 g dry weight equivalent) was combined with 60 ml 50 mM sodium acetate buffer, pH 5, and homogenized in a blender for 1 min. In a 2 ml centrifuge tube, 0.75 ml homogenate was combined with 0.75 ml substrate (Table 1) in 50 mM acetate buffer and shaken vigorously for 1–5 h at room temperature. Homogenates were diluted 10-fold before assaying PPO activity to reduce interference from native soil polyphenols.

After shaking, we centrifuged the tubes and measured the absorbance of reaction products in the supernatant on a Hitachi U-2000 spectrophotometer (Hitachi Instruments, Inc.). The *p*-nitrophenol (pNP) reaction product from the AP, NAG, and BG assays was measured at 410 nm after addition of sodium hydroxide. *p*-Nitroaniline (pNA) generated from the GAP reaction was also measured at 410 nm directly in the supernatant. We measured NH_4^+ concentrations from the UR reaction at 690 nm according to a procedure modified from Kandeler and Gerber (1988). PPO reaction products were measured directly in the supernatant at 460 nm. In each case, we included appropriate controls to account for the background absorbance of the substrate and homogenate. Enzyme activities are presented in units of $\mu\text{mol product g}^{-1} \text{ dry soil h}^{-1}$, except PPO activity which is presented as $\mu\text{mol substrate g}^{-1} \text{ dry soil h}^{-1}$. We converted absorbances to concentrations by measuring the absorbance of solutions with known concentrations of pNP (for pNP- and pNA-based assays) or NH_4^+ (for UR). We standardized PPO activities by completely oxidizing a known amount of pyrogallol with

commercially prepared PPO (Sigma-Aldrich, Inc.) and measuring the absorbance of the reaction products.

2.5. Microbial biomass

We determined microbial biomass C using the chloroform fumigation-direct extraction technique (Brookes et al., 1982; Brookes et al., 1985). We extracted soluble C from ~5 g (dry weight equivalent) soil in 50 ml 0.5 M K_2SO_4 before and after fumigation. Carbon concentrations in extracts were determined using a Shimadzu TOC-5000A analyzer (Shimadzu Corp.). Microbial biomass C was calculated based on the amount of additional C released by fumigation, assuming an extraction efficiency of 0.45.

2.6. PLFA analyses

We extracted, purified, and identified PLFAs from microbial cell membranes in lyophilized whole soil using a modified Bligh and Dyer (1959) technique. We extracted 5 g subsamples in a one-phase extraction mixture containing chloroform:methanol:phosphate buffer (1:2:0.8 v/v/v), and separated the phospholipids from the neutral and glycolipids on solid phase extraction columns containing 0.5 g silica gel (Agilent Technologies). Polar lipids were transesterified by mild alkaline methanolysis. We added 18:0 (octadecanoic methyl ester) and 10:0 (decanoic methyl ester) as internal standards, and analyzed samples using a Hewlett-Packard 6890 Gas Chromatograph with a 25 m \times 0.2 mm \times 0.33 μm Ultra 2 (5%-phenyl)-methylpolysiloxane column. We used the concentration of the PLFA 18:2 ω 6,9c as an indicator of total fungal biomass (Klamer and Bååth, 2004), 16:1 ω 5c as an indicator of the biomass of arbuscular mycorrhizal fungi, and the average of i17:0, cy17:0, a17:0, i16:0, i15:0, and a15:0 as an indicator of total bacterial biomass (Joergensen and Potthoff, 2005).

2.7. Statistics

We used a three-way analysis of variance (ANOVA) to test for the effects of site, invasion, and sampling date on soil microbiological and biochemical properties using SAS PROC GLM (SAS Institute, 2001). We did not use a repeated-measures design because the plots sampled in

Table 1
Extracellular enzymes and their functions and substrates

Enzyme	Function	Substrate
Acid phosphatase (AP)	Releases inorganic phosphate from organic matter	5 mM pNP-phosphate ^a
Urease (UR)	Degrades urea to ammonium	40 mM urea
Glycine aminopeptidase (GAP)	Protein degradation	2 mM glycine <i>p</i> -nitroanilide (pre-dissolved in a small volume of acetone)
<i>N</i> -acetyl-glucosaminidase (NAG)	Chitin degradation	2 mM pNP-acetyl- β -D-glucosaminide
β -glucosidase (BG)	Releases glucose from cellulose	5 mM pNP- β -D-glucopyranoside
Polyphenol oxidase (PPO)	Oxidizes lignin and humic compounds	50 mM pyrogallol 50 mM EDTA

^apNP = *p*-nitrophenyl.

2003 were not necessarily re-sampled in 2004. If sampling date or its interaction was significant in the three-way ANOVA, we presented and analyzed the data from 2003 and 2004 separately using a two-way ANOVA with site and invasion as main effects. If there was no significant effect of sampling date, we ran a two-way ANOVA on pooled data from 2003 and 2004. Tukey's HSD test was used for post hoc mean separations. AP activities, BG activities and fungi:bacteria ratios were log-transformed to improve the normality of the data prior to analysis.

3. Results

3.1. Soil nutrient concentrations

Compared to the native-dominated stands, soil N concentrations under *Falcataria* more than doubled to 31 g kg^{-1} at the 50 y.o. site, increased significantly at the 200 y.o. site, and did not respond in the 300 y.o. site (Table 2). *Falcataria* invasion increased soil P concentrations from 0.69 to 0.99 g kg^{-1} at the 50 y.o. site, but had no effect at the other two sites. Soil N:P ratios increased significantly across all sites under *Falcataria*, with increases $>50\%$ in the 50 and 200 y.o. sites.

3.2. Enzyme activities

All extracellular enzyme activities increased or showed no change under *Falcataria*, with AP increasing most dramatically. AP activity more than doubled under *Falcataria* across all sites and dates, reaching $>140 \mu\text{mol pNP g}^{-1} \text{ h}^{-1}$ in 2003 (Fig. 1). While overall AP activities declined slightly from 2003 to 2004, the pattern of significantly greater activity under *Falcataria* was consistent across both years.

The responses of C- and N-degrading enzymes varied by site and sampling year. All enzyme activities roughly doubled under *Falcataria* at the 200 y.o. site, except NAG and BG activities which did not respond in 2003 (Figs. 2 and 3). On the 50 y.o. lava flow, GAP activity increased significantly to $1.7 \mu\text{mol pNA g}^{-1} \text{ h}^{-1}$ (Fig. 2) and BG

Table 2
Soil nutrient concentrations

Site		Soil N	Soil P	Soil N:P
		(g kg^{-1})	(g kg^{-1})	Mean (SE)
50 y.o. a'a	Control	14.5 (0.5)	0.69 (0.03)	21.0 (0.7)
	Invaded	31.3 (0.7)***	0.99 (0.06)*	32.2 (2.1)***
200 y.o. pahoehoe	Control	13.8 (0.4)	0.84 (0.03)	16.6 (0.5)
	Invaded	25.9 (0.5)***	0.98 (0.02)	26.4 (0.9)***
300 y.o. a'a	Control	21.0 (0.9)	1.00 (0.06)	21.2 (0.5)
	Invaded	23.9 (2.5)	0.93 (0.12)	26.1 (2.2)*

Significance of *t*-test for invaded vs. control within a site:
* $P < 0.05$; *** $P < 0.001$.

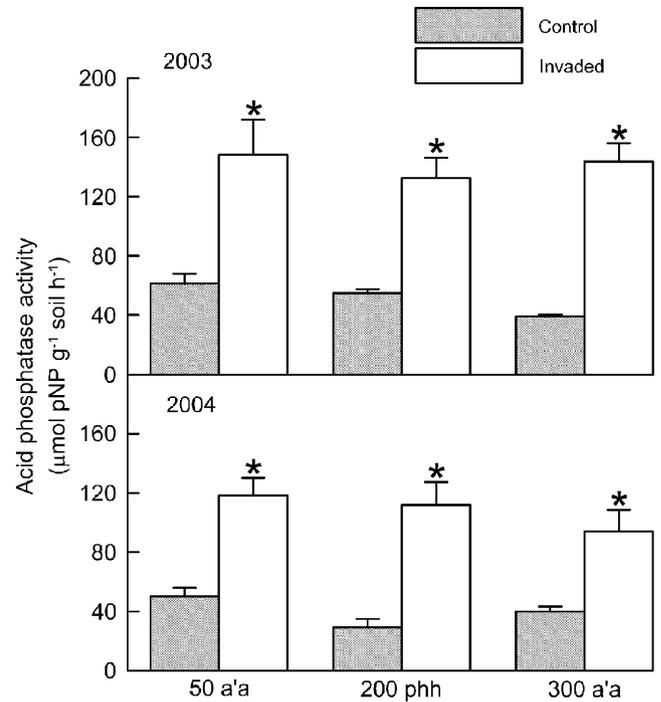


Fig. 1. Mean (\pm SE) acid phosphatase activity in control and *Falcataria*-invaded plots on different aged lava flows in 2003 and 2004. 50 a'a = 50 y.o. a'a flow, 200 phh = 200 y.o. pahoehoe flow, 300 a'a = 300 y.o. a'a flow. Significant ($P < 0.05$) differences between control and invaded plots are denoted with (*).

activity doubled (Fig. 3) under *Falcataria*, but no other enzyme activities differed significantly. No C- or N-degrading enzyme activities changed in response to *Falcataria* invasion on the 300 y.o. substrate, suggesting an attenuation of enzyme responses with substrate age.

3.3. Microbial biomass

Total microbial biomass C decreased slightly but significantly in response to *Falcataria* invasion overall. Although no pairwise comparisons were statistically significant, there was a trend toward lower microbial biomass C in 2004 at the 300 y.o. site (Table 3). On average, soils from the 300 y.o. site contained $\sim 12\%$ less microbial biomass than the other two sites ($P < 0.05$). The trend toward lower microbial biomass in the invaded soils indicates that enzyme activity (and probably production) per unit microbial biomass increased under *Falcataria*.

3.4. Microbial community composition

Ratios of fungal:bacterial biomass were significantly higher in native relative to *Falcataria*-dominated plots at the 50 and 200 y.o. sites, but not at the 300 y.o. site (site \times invasion effect $P < 0.05$; Table 3). This pattern was driven by a significant decrease in the fungal PLFA 18:2 ω 6,9c as well as a significant increase in bacterial indicator PLFAs under *Falcataria* at these sites (Table 3).

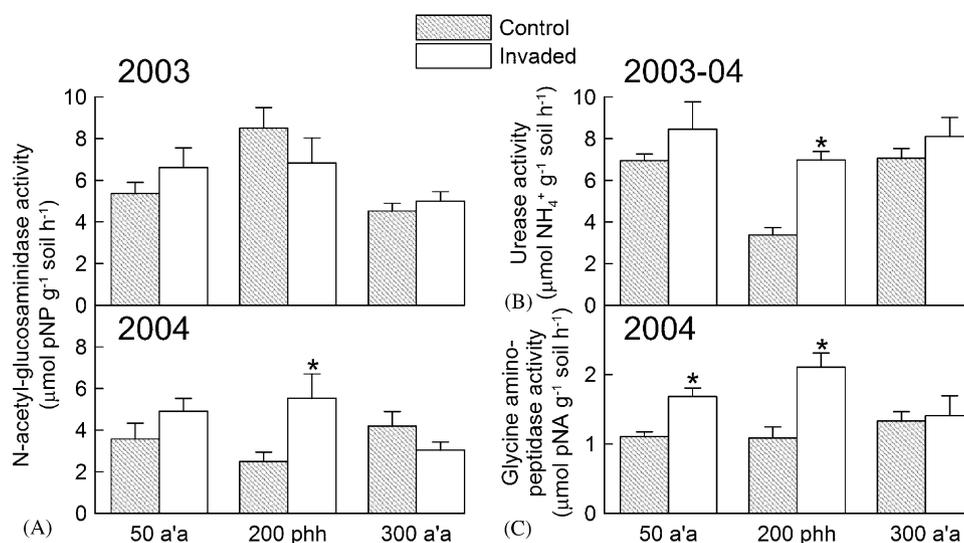


Fig. 2. Mean (\pm SE) N-releasing enzyme activities in control and *Falcataria*-invaded plots on different aged lava flows in 2003 and 2004. (A) N-acetylglucosaminidase; (B) urease; (C) glycine aminopeptidase. Symbols and significance as in Fig. 1.

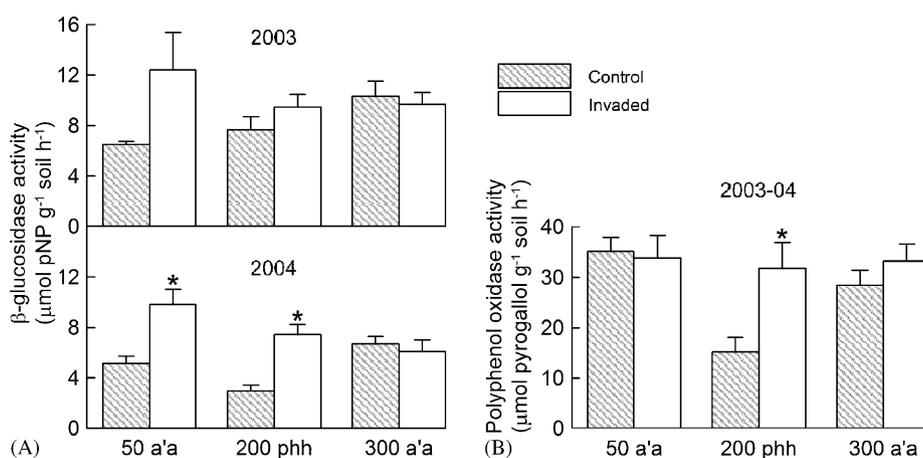


Fig. 3. Mean (\pm SE) C-degrading enzyme activities in control and *Falcataria*-invaded plots on different aged lava flows in 2003 and 2004. (A) β -glucosidase; (B) polyphenol oxidase. Symbols and significance as in Fig. 1.

Table 3
Microbial biomass and community composition

Site		Microbial biomass C ^a		Fungal indicator PLFA ^b	Bacterial indicator PLFAs ^c	Fungi:Bacteria	AMF indicator PLFA ^d
		January 2003 Mean (SE)	January 2004 Mean (SE)				
50 y.o. a'a	Control	4.50 (0.24)	4.08 (0.39)	28.8 (5.6)	10.6 (0.6)	2.69 (0.39)	18.0 (0.3)
	Invaded	3.86 (0.33)	3.91 (0.39)	14.4 (2.7)**	16.8 (1.2)*	0.89 (0.23)**	22.5 (5.6)
200 y.o. pahoehoe	Control	4.98 (0.41)	3.62 (0.39)	22.0 (1.6)	16.2 (0.3)	1.35 (0.07)	12.4 (1.0)
	Invaded	4.50 (0.33)	3.43 (0.11)	11.0 (1.6)*	23.0 (1.7)**	0.49 (0.10)**	19.9 (3.3)
300 y.o. a'a	Control	3.94 (0.33)	3.83 (0.18)	11.1 (2.2)	18.6 (2.1)	0.64 (0.19)	20.8 (2.3)
	Invaded	4.01 (0.12)	2.73 (0.21)	13.1 (3.2)	19.7 (2.0)	0.69 (0.22)	18.2 (3.6)

Significance of *t*-test for invaded vs. control within a site: * $P < 0.05$; ** $P < 0.01$.

^amg g⁻¹ soil.

^bnmol g⁻¹ soil of the phospholipid fatty acid 18:2 ω 6,9c.

^cAverage nmol g⁻¹ soil of the phospholipid fatty acids i17:0, cy17:0, a17:0, i16:0, i15:0, a15:0.

^dArbuscular mycorrhizal fungi; nmol g⁻¹ soil of the phospholipid fatty acid 16:1 ω 5c.

Concentrations of the fungal PLFA were lower in the 300 y.o. site compared to the 50 y.o. site ($P < 0.05$), while bacterial PLFAs were lowest in the 50 y.o. site ($P < 0.01$). Abundance of the arbuscular mycorrhizal biomarker 16:1 ω 5c did not differ significantly by site or in response to *Falcataria* invasion (Table 3). Because total fungal biomass declined under *Falcataria* but arbuscular mycorrhizal biomass did not, arbuscular mycorrhizae comprised a larger fraction of the fungal biomass in the 50 and 200 y.o. invaded plots.

4. Discussion

Falcataria-driven increases in litter production are accompanied by comparable increases in decomposition and resin-available nutrients, indicating an acceleration of biogeochemical cycling rates in *Falcataria* stands (Hughes and Denslow, 2005; Hughes and Uowolo, in press). In these invaded stands, dramatic changes in litter quality and quantity correspond to substantial changes in soil enzyme production and microbial community composition. In turn, these changes may feed back to increase nutrient availability and rates of decomposition, especially in the 50 and 200 y.o. sites.

One major effect of *Falcataria* invasion is to increase litterfall biomass (1.3–8.6 fold) and litter N inputs (4–55 fold) (Hughes and Denslow, 2005). These litter inputs provide C and N substrates that can support increased microbial growth and enzyme production. In particular, the abundant N in *Falcataria* litter is an essential resource for microbes that synthesize enzymes required for the degradation of cellulose, lignin, and protein present in *Falcataria* litter (Allison and Vitousek, 2005; Hughes and Uowolo, in press). Elevated activities of C- and N-degrading enzymes under *Falcataria* can then accelerate litter decomposition and nutrient mineralization rates in the invaded stands (Sinsabaugh et al., 1993; Sinsabaugh and Moorhead, 1994). Therefore, like many invasive species, *Falcataria* facilitates a positive feedback to nutrient cycling through its litter production (Vitousek and Walker, 1989; Allison and Vitousek, 2004; Hughes and Uowolo, in press).

As we predicted, *Falcataria* invasion increased the activity of soil AP. By increasing the supply of C and N, *Falcataria* litter inputs stimulate P demand and provide raw materials for the production of extracellular phosphatases. These enzymes cleave phosphate groups from the exterior of complex organic molecules and provide a mechanism for scavenging P from organic material (McGill and Cole, 1981). Phosphatase production often increases in response to P limitation (Chróst, 1991; Treseder and Vitousek, 2001), especially when C and N sources are readily available to aid in enzyme synthesis (Allison and Vitousek, 2005). Although forests on young lava flows in Hawaii are usually N-limited, sustained N inputs have been shown to induce secondary P limitation (Harrington et al., 2001; Vitousek, 2004), and we suspect that *Falcataria*

invasion has had a similar effect. Although litter P inputs increase by 2–28 fold and resin available P increases by 2–24 fold with invasion, N inputs and availability increase more than twice as much (Hughes and Denslow, 2005). Support for P limitation is also evident in the soil N:P ratios, which increased 23–60% under *Falcataria* (Table 2).

Despite likely P limitation, there is significant evidence that *Falcataria* efficiently acquires P from soil (Binkley et al., 2000). The dramatic increase in AP activity that we and others (Zou et al., 1995) have observed under *Falcataria* should allow roots and soil microbes to access organic forms of P more efficiently, thereby providing one mechanism for enhancing P supply. At the 50 y.o. site in particular, we hypothesize that *Falcataria* also meets its increased P demand by mobilizing P from rock. Total tree basal area at the 50 y.o. site increased by a factor of 13–33.1 m² ha⁻¹ in the invaded plots, indicating that biomass P pools increased dramatically, especially since biomass P concentrations also increased (Hughes and Denslow, 2005). Although not measured directly, soil organic P pools under *Falcataria* probably also equal or exceed those in native stands. The ratio of litter inputs to litter decomposition provides an estimate of forest floor biomass (Olson, 1963), and this value stayed constant at 4 Mg ha⁻¹ at the 50 y.o. site and increased from 4 to 8 Mg ha⁻¹ with invasion at the 200 y.o. site (Hughes and Denslow, 2005; Hughes and Uowolo, in press). Since soil P concentrations remained similar, it is unlikely that soil P pools declined in *Falcataria* stands, meaning that total ecosystem P (soil organic + biomass P) increases with invasion. Thus, we conclude that *Falcataria* meets at least part of its P demand by mining additional P from unweathered rock at the 50 and 200 y.o. sites.

Given the high N availability and the likelihood of P limitation under *Falcataria*, we expected that microbes might allocate fewer resources to N-releasing enzymes (Chróst, 1991; Sinsabaugh and Moorhead, 1994). However, the large quantity of *Falcataria* litter entering the system is probably more important than mineral N availability in determining enzyme production. Foliage and litter from N-fixing trees are rich in protein (McKey, 1994), and high N inputs make urea production more likely in soils under N-fixers. These inputs of complex organic N may stimulate soil microbes to produce enzymes such as proteases (i.e. GAP) and UR (Bolton et al., 1985; Asmar et al., 1994).

Our results revealed large shifts in microbial community composition under *Falcataria*, although arbuscular mycorrhizal abundance and total microbial biomass did not change. Fungal:bacterial ratios declined dramatically in the younger sites under *Falcataria*, which may correspond to dominance by bacteria adapted to nutrient-rich soil conditions. Some fungi may compete poorly under N-rich conditions (Fog, 1988; Carreiro et al., 2000), which could explain their decline in the N-rich soils under *Falcataria*. Despite the tendency for *Falcataria* to form associations with arbuscular mycorrhizal fungi (Alexander et al., 1992),

we found no significant difference in the mycorrhizal marker 16:1 ω 5c between control and invaded plots. While it is possible that the mycorrhizal community produces more AP under *Falcataria* than in control soils, we did not test this mechanism. Overall, *Falcataria* invasion results in a bacterially dominated microbial community that is highly effective at litter degradation and enzyme production. Although invasion did not increase microbial biomass per gram of soil, rapid biogeochemical cycling under *Falcataria* suggests that microbial turnover may have increased.

As we predicted, the effect of *Falcataria* invasion on soil microbes and enzymes diminished with substrate age. At the 300 y.o. a'a site, we observed few differences between control and invaded plots, consistent with smaller differences in forest structure and litter inputs (Hughes and Denslow, 2005). We expected large invasion effects on the 50 and 200 y.o. substrates, where native forest stands had not developed a closed canopy or accumulated substantial amounts of soil organic matter. Ecosystem development at the 200 y.o. site was also inhibited by a pahoehoe substrate, which weathers more slowly than comparably aged a'a (Aplet et al., 1998). Thus, *Falcataria* invasion caused relatively stronger microbial and biogeochemical changes in these younger sites.

The responses of soil microbes and enzyme activities show that the biogeochemical effects of *Falcataria* are dramatic and pervasive, especially on younger substrates. N-fixers clearly influence ecosystem development and increase ecosystem inputs of C and N (Vitousek and Walker, 1989; Walker and Vitousek, 1991), but our results indicate that invasive N-fixers also have the potential to alter microbial community composition and the production of extracellular enzymes, particularly phosphatase. These changes stem from large increases in litter quantity and N concentration, and probably facilitate positive feedbacks to nutrient cycling in *Falcataria*-invaded stands. This mechanism can allow invading N-fixers to increase nutrient availability and facilitate further invasion even in sites where P availability is expected to limit invader productivity.

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