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Fire modifies plant–soil feedbacks

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Abstract

Although plant–soil feedbacks (interactions between plants and soils, often mediated by soil microbes, abbreviated as PSFs) are widely known to influence patterns of plant diversity at local and landscape scales, these interactions are rarely examined in the context of important environmental factors. Resolving the roles of environmental factors is important because the environmental context may alter PSF patterns by modifying the strength or even direction of PSFs for certain species. One important environmental factor that is increasing in scale and frequency with climate change is fire, though the influence of fire on PSFs remains essentially unexamined. By changing microbial community composition, fire may alter the microbes available to colonize the roots of plants and thus seedling growth post-fire. This has potential to change the strength and/or direction of PSFs, depending on how such changes in microbial community composition occur and the plant species with which the microbes interact. We examined how a recent fire altered PSFs of two leguminous, nitrogen-fixing tree species in Hawai'i. For both species, growing in conspecific soil resulted in higher plant performance (as measured by biomass production) than growing in heterospecific soil. This pattern was mediated by nodule formation, an important process for growth for legume species. Fire weakened PSFs for these species and therefore pairwise PSFs, which were significant in unburned soils, but were nonsignificant in burned soils. Theory suggests that positive PSFs such as those found in unburned sites would reinforce the dominance of species where they are locally dominant. The change in pairwise PSFs with burn status shows PSF-mediated dominance might diminish after fire. Our results demonstrate that fire can modify PSFs by weakening the legume-rhizobia symbiosis, which may alter local competitive dynamics between two canopy dominant tree species. These findings illustrate the importance of considering environmental context when

evaluating the role of PSFs for plants.

Keywords

plant–soil feedbacks, PSF, fire, wildfire, restoration, rhizobia, legumes, Hawaiʻi, koa, māmane, *Acacia koa*, *Sophora chrysophylla*

Introduction

Plants, microbes, and soil interact in diverse ways, termed "plant soil feedbacks" (PSFs), influencing local biodiversity patterns across the world (Klironomos 2002, Kardol et al. 2006, Bever et al. 2015). These interactions can shape plant success and survival in both positive (e.g., mutualism) and negative (e.g., pathogens) ways, and this can lead to long-term consequences for biodiversity and coexistence (Chung and Rudgers 2016). Although it is increasingly evident that the nature of PSFs depends on the environmental context, it remains unclear exactly how and when environmental factors alter the strength or direction of PSFs (Yelenik and Levine 2011, Crawford et al. 2019).We evaluate how PSFs are modified by a key environmental factor: fire.

Due to the difficulty of assessing effects of environmental factors on PSFs, much attention has been paid to how a plant species' PSF influences its survival or performance when living in the space of a conspecific (Kulmatiski et al. 2008, Van der Putten et al. 2013, Crawford et al. 2019). The net outcome of such "individual PSFs" (sensu Kulmatiski et al. 2008) is the sum of all the positive, negative, and neutral interactions between a plant and its microbial communities (Bever et al. 1997). For example, diversity within tropical forests may relate to negative feedbacks between plants and their specialist antagonists, including microbial pathogens, which limit the abundance of any one species (Janzen 1970, Connell 1971). Such

patterns of plant performance being lower when nearer to a mature conspecific (negative density dependence) have been broadly documented across the world's biomes and in different plant functional groups (Kulmatiski et al. 2008, Crawford et al. 2019). However, PSFs may also increase plant performance, such that proximity to a mature conspecific may be beneficial (Smith and Reynolds 2012, Crawford and Knight 2017). Specialist mutualistic microbes, such as mycorrhizae or bacteria, may be more available nearer a mature conspecific, and such mutualistic interactions may balance those of the potential antagonists, resulting in a net positive effect of proximity to a conspecific on young plant performance (positive density dependence).

In addition to the PSFs affecting conspecifics, effects on neighboring heterospecifics can lead to pairwise feedbacks that also change patterns of plant performance and where/how plants occur in ecosystems (Bever et al. 1997, Kulmatiski and Kardol 2008). For example, if one species (X) increases soil-based pathogen loads but these pathogens are more negative for a given heterospecific (Y) than they are for conspecific individuals of species X, then there is a net positive pairwise feedback for species X (Yelenik and Levine 2011). All else being equal, such a "net pairwise PSF" (sensu Bever et al. 1997) would lead to competitive exclusion of species Y by species X. Additionally, species X and Y may both have positive to neutral PSFs when being grown in either conspecific or heterospecific soil (including heterospecific soil that is from neither X nor Y). In that case, the competitive outcome between species X and species Y may depend more on non-PSF-related factors, such as initial abundance of the two species of interest or their relative growth rates. Lastly, an additional possibility is that of a negative net pairwise PSF, where both species are hindered when grown in their own soil, relative to when grown in a heterospecific soil (Crawford et al. 2019). This sort of negative pairwise PSF can lead to the two species coexisting in the field, all else being equal, resulting in a pattern of negative density

dependence as in Janzen-Connell dynamics (Janzen 1970, Chung and Rudgers 2016). All of these pairwise PSFs may also be influenced by environmental context, including fire.

Fire is an important disturbance in terrestrial ecosystems worldwide and is forecasted to increase in frequency and extent with climate change (Flannigan et al. 2000, Moritz et al. 2012). In ecosystems where it was historically infrequent, fire can have devastating ecological consequences and can shift habitats into species-poor stable states where most native diversity is lost (Smith and Tunison 1992, Ellsworth et al. 2014, Mahood and Balch 2019). For example, in invasive-grass invaded forests and shrublands, fire can lead to a shift to grass-dominated landscapes with self-reinforcing short fire return intervals (D'Antonio and Vitousek 1992, Ellsworth et al. 2014). Typically, fire effects on vegetation are considered through the lens of direct relationships between fire and plants and how fire modifies competitive relationships between plants. Yet, fire may also affect plant communities by altering microbial communities.

Fire is known to alter microbial communities in ways that may then alter plant performance (Pourreza et al. 2014, Hedo et al. 2015, Prendergast-Miller et al. 2017). However, such results have yet to be placed in a PSF framework to more clearly understand community-level effects. For example, fire can lead to the death and modified community composition of ectomycorrhizal fungi (Glassman et al. 2016, Taudière et al. 2017), which are well known to be positive mutualists for plants. Such a result could lead to a potential shift towards a net negative PSF if pathogens are then more relatively abundant. However, fire could also eliminate pathogens, which would then favor more positive net PSFs. Although fire may have strong impacts on PSFs, how fire affects PSFs is poorly understood. Fire may change the direction of PSFs leading from coexistence to competitive exclusion of some species, or it may cause PSFs to vanish altogether (Van der Putten et al. 2013, Senior et al. 2018). Disentangling the complexities

of fire on PSFs requires detailed studies that explicitly compare PSFs both with and without fire, and among different species.

We studied how fire affects PSFs of two tree species in the Fabaceae. Like other legumes, these species engage in a mutualism with rhizobial bacteria in the soil (Andrews and Andrews 2017). Rhizobia fix nitrogen (N), which is shared with the plant, while the plant shares carbon and constructs nodules in which the bacteria live. Members of the legume family are ideal for studying PSFs and environmental context because they present a rich set of interactors, both positive (mutualism with rhizobia) and negative (such as with fungal pathogens) (Jack et al. 2019, Grman et al. 2020). In addition, leguminous trees have shown a range in specificity with different rhizobia (Sprent and Parsons 2000). How fire might modify the net outcome of these positive and negative interactions remains unknown.

We expected that fire would influence the PSFs of two leguminous tree species found in the Hawaiian Islands. To address this expectation, we asked three questions (1) How is plant performance affected by conspecific vs heterospecific soils and is this pattern affected by fire? We hypothesized that each N-fixer would do better in conspecific soil rather than heterospecific soils or soils of a non-N-fixing woody species, due to the rhizobial mutualism and the relatively low root-associated fungal diversity in Hawaiʻi (Hayward and Hynson 2014). (2) Does rhizobial nodule formation mediate the effect of soils on plant performance? Given the importance of the rhizobial mutualism to plant performance of legumes, we expected patterns of nodule formation to mediate this relationship. (3) Are net pairwise PSFs between these two species affected by fire? We hypothesized that fire would reduce the strength of net pairwise PSFs, as fire may cause death of the microbial members of PSFs, potentially leading to more neutral interactions.

Methods

Study Site and Species

We conducted our work within Hawaiʻi Volcanoes National Park on Hawaiʻi Island, Hawaiʻi. In August of 2018, a fire burned approximately 1500 hectares within the national park, in an area with mostly native upland forest (Appendix S1: Figure S1). Although fire has not been a significant ecological force in Hawaiʻi historically, introductions of invasive grasses, shifting temperatures, and increased ignitions have shortened fire return intervals dramatically (Smith and Tunison 1992, Trauernicht 2019). We examined the PSFs of two tree species endemic to the Hawaiian Islands: koa (*Acacia koa* A. Gray) and māmane (*Sophora chrysophylla* (Salisb.) Seem.). Both of these species are in the family Fabaceae and fix N through a mutualism with rhizobial bacteria. Additionally, both of these species are able to regenerate after fire, either from seed or from roots (Loh et al. 2007, McDaniel et al. 2008), though māmane is, anecdotally, somewhat less fire-tolerant than koa, which readily regenerates after fire (Hamilton et al. 2021).

Soil Sourcing, Experimental Design, and Data Collection

To examine the effect of fire on PSFs, we performed a factorial experiment using field-collected soils from within and outside the burned area (Appendix S1: Figure S2). We gathered soils from multiple focal trees within and outside the burned area to capture variation present in the soil microbial community following the fire   .

In June of 2019, we gathered soils from under both living koa and māmane, as well as from under living ʻaʻaliʻi (*Dodonaea viscosa* (L.) Jacq.), a Hawaiian-native, non-N-fixing member of the Sapindaceae that is common in these sites. We collected soils from under ʻaʻaliʻi so that we would have a consistent "far from conspecific" soil to examine (that likely lacked the rhizobia of our two focal species). We collected soils from under living trees so that effects that we observed were attributable to the burn, rather than to tree death. We termed the species from

which we collected soil the "source plant." We then factorially crossed these three source plants with the burn, by collecting soils from inside the burn and outside the burn (n = 3 source plants × 2 [burn vs not] = 6 possible types of soil inoculum). Within each source plant × burn combination, we collected soils from under 5 replicate trees (Appendix S1: Figure S2). For the coordinates and elevations of each of the locations from which we collected soil, please see Appendix S1: Table S1. We collected soils to a depth of up to ~12 cm, though we generally tried to collect soil from shallow depths as much as possible. The effects of fire on soil biota have been documented to penetrate into the soil profile for up to ~10 cm (Ahlgren 1974, Pattinson et al. 1999, Mataix-Solera et al. 2009), but are most often documented within the first 3 cm (Ahlgren 1974, Bradstock and Auld 1995, Mataix-Solera et al. 2009). The depth of the soils that we collected indicates that the results from this work may be conservative, in relation to effects of the fire on soil microbes.

We used an inoculation approach to minimize any effects of soil nutrients or other non-microbial factors, whereby we used an inoculum of field soil to pots containing sterilized potting medium (Crawford et al. 2019). We sterilized our potting medium (Professional Growing Mix SS#1, Sungro Horticulture, Agawam, Massachusetts, USA) by autoclaving it for 60 minutes at 121°C. We used a ratio of 85% sterile potting medium to 15% field soil per pot, by volume, following typical inoculation practices (Crawford et al. 2019). Pots were standard 4-inch pots and were surface-sterilized prior to use by submersion in a 0.6% concentration bleach solution for 12–14 hours. Each pot contained a total of 475 mL of soil (400 mL sterile medium and 75 mL field soil inoculum). From each of the 5 replicate trees for each plant species × burn combination, we inoculated a total of 15 pots filled with sterile potting media (n = 15 pots × 5 replicate trees × 3 source plants × 2 [burn vs not] = 450 pots per each of koa and māmane at

experiment outset; see Appendix S1: Figure S2). We grew a seedling of each species in an additional 15 pots of sterile potting media as a reference for the absence of soil biota for later visual comparisons, though due to the nested nature of our experimental design, we were unable to include these sterile soil seedlings in our statistical analyses. Because some seeds did not germinate, our final sample sizes for each species were 324 inoculated pots and 15 sterile pots for koa and 408 inoculated pots and 13 sterile pots for māmane. Seedlings were grown in a shadehouse located at Kīlauea Field Station, within Hawai'i Volcanoes National Park, and we randomized placement of the different sorts of inoculum across five benches.

Prior to planting, seeds were scarified and surface-sterilized, to enable rapid germination and to eliminate any confounding microbes potentially on the seed coats. The māmane seeds were scarified and sterilized in a solution of 100% sulfuric acid for 1 hour, and then rinsed to remove the acid solution (Sierra McDaniel & Makani Gregg, National Park Service, oral communication, 2019). The koa seeds were scarified in nearly boiling water (~90°C) for 60 seconds, rapidly cooled in a cold water bath, and then soaked in cool water overnight (Elevitch and Wilkinson 2003), then surface-sterilized in a sodium hypochlorite (bleach) solution (0.6%) for 10 minutes, followed by a deionized water rinse to remove the bleach solution. We planted three seeds of either māmane or koa into the inoculated or sterile pots (n=465 originally, per focal species), and thinned to one seedling in each pot at the time of the emergence of the first true leaf, after which seedlings were grown 10 weeks (Appendix S1: Figure S3). Some koa seedlings experienced insect herbivory, which affects biomass production (Barton 2016), and at harvest we recorded whether a plant experienced herbivory at any time during the 10 weeks. Māmane seedlings experienced essentially no herbivory. Following the 10-week growth period, we harvested all individuals, recorded the number of nodules present on the roots of each

seedling (Appendix S1: Figure S3) and then dried them at 60°C to a constant biomass (for a minimum of 24 hours), after which we measured total dry weight (g). We divided this dry weight by the number of days that an individual had grown from the emergence of the first true leaf to harvest, to standardize study duration across individuals.

Analysis

All analyses were conducted in R, version 3.6.3 (R Core Team 2020), through the interface of RStudio, version 1.2.5033 (RStudio 2019). Significance testing was done following null hypothesis significance testing with $\alpha = 0.05$.

How is plant performance affected by soil source and is this pattern affected by fire?

We ran separate linear mixed models for koa and māmane, to examine the effects of the burn. The response variable was total dry biomass, standardized to the number of growing days. The predictor variables were the burn, the source plant, the number of nodules present at the time of harvest, and the interaction between the burn and the source plant on biomass. We used two random effects: one for the replicate tree (to account for the 5 replicate trees in each field soil, see Appendix S1: Figure S2) and the second for the bench in the greenhouse, to account for any effects of where the plants were growing in the greenhouse. We treated the response as following a Gaussian distribution, as the biomass data were normal. Within this model, but only for koa, we included a term for herbivory. We additionally ran separate generalized linear mixed models (GLMMs) for koa and māmane, in which the number of nodules was the response, with the predictors of the source plant, the burn, and the interaction of those terms, and treating the response as following a negative binomial distribution (because the number of nodules is overdispersed count data). The random effects of these models followed the same structure as the models with biomass as the response. All four of these models were run through the lme4

package, version 1.1-23 (Bates et al. 2015). We derived the effect size of percent difference, from estimated marginal means from the model, using emmeans, version 1.4.8 (Lenth 2020), and using the percent difference formula

of: $|\mu_1 - \mu_2| / ((\mu_1 + \mu_2) / 2)$, where μ_i are the estimated marginal means.

Does nodule formation by rhizobia mediate the effect of soils on plant performance?

Based on the results of our models, we investigated the direct effect of source plant on biomass and its indirect effect (via nodulation) on biomass, using a structural equation model (SEM) for each of koa and māmane (realizing that the lack of latent variables in the model means this is technically a path model). We constructed SEMs using the piecewiseSEM package, version 2.1.1 (Lefcheck 2016). We constructed SEMs using the models that we used for our linear mixed models, with the modifications that, given that our soil source predictors consisted of three non-ordinal categories, we split soil source into three columns of binary responses (koa or not, 'a'ali'i or not, māmane or not), and removed the focal species binary variable from its own model, to avoid rank deficiency of the model. To calculate standardized effects, we multiplied the beta coefficient of the model by the standard deviation of the independent variables divided by the standard deviation of the dependent variable. We report these standardized betas in the Results using the letter β.

Are net pairwise PSFs between our two focal species affected by fire?

We calculated net pairwise PSF (noted using the interaction coefficient, I_s), following Bever et al. (1997). In this framework, $I_s = G(A)_\alpha - G(A)_\beta - G(B)_\alpha + G(B)_\beta$, where G(A) and G(B) represent growth (biomass, in our case) of species A and B, respectively, and α and β refer to soil sourced from under species A and B, respectively (Bever et al. 1997). Because this feedback is calculated between pairs of species, we only examined the pairwise feedbacks

between koa and māmane, as we did not grow any 'a'ali'i seedlings. First, we determined whether a pairwise PSF between these two species significantly differed inside vs. outside of the burn. To do this, we ran a linear mixed model in which the response was biomass and the predictor variables were the species (two focal species), the source plant, the burn, and all interactions. Here, a significant three-way interaction between focal species, source plant, and burn would indicate that PSFs differ between burn treatments for these two species. We then calculated I_s within and outside the burn using two linear mixed models, one inside the burn and one outside it. To do this, we ran a linear mixed model in which the response was biomass and the predictor variables were the focal species, the source plant, and their interaction. Here, a significant interaction would indicate a significant PSF between the two species. From this model, we extracted I_s, the coefficient associated with the interaction between the two species, and determined its significance using a Type III ANOVA with Satterthwaite's approximation for degrees of freedom from the lmerTest package, version 3.1-2 (Kuznetsova et al. 2017).

Results

How is plant performance affected by source plant and is this pattern affected by fire?

Koa biomass was not affected by soils from different source plants (Figures 1 and Appendix S1: Figure S4; Appendix S1: Table S2). However, more nodules resulted in higher koa biomass and nodulation was affected by source plant soil, with more nodules in conspecific soil (Appendix S1: Figure S5; Appendix S1: Table S3). There were 5.2% more nodules on koa seedlings grown in koa soil than in māmane soil and 10.5% more nodules on koa seedlings grown in koa soil than in 'a'ali'i soil (calculated as percent difference).

For māmane, biomass was affected by source plant soil (Figures 1 and Appendix S1: Figure S6; Appendix S1: Table S2), with higher biomass in conspecific soil. Māmane biomass

was 33.0% higher when grown in māmane soil than in koa soil, and 13.9% higher when grown in māmane soil than in ʻaʻaliʻi soil (percent difference). However, māmane nodulation patterns were unaffected by soil source (Appendix S1: Figure S7; Appendix S1: Table S3).

The recent fire did not affect biomass or nodulation for koa (Appendix S1: Tables S2 and S3), nor for biomass of māmane (Appendix S1: Table S2) (see Figure 1 for biomass data). Fire had an interactive effect on the nodulation of māmane, where māmane seedlings grown in māmane soil had more nodules outside the burn than māmane seedlings in any other treatment (Appendix S1: Table S3; range of 5.1–37.8% more nodules than other source plant × burn combinations [percent difference]).

Does nodule formation by rhizobia mediate the effect of soils on plant performance?

The koa SEM fit the data well (Fisher's C = 0.035; p = 0.98; Figure 2). Based on our SEM results, koa tended to obtain greater biomass in its own soils than in heterospecific soils (ʻaʻaliʻi and māmane), leading to a positive single species PSF. Biomass differences were indirectly mediated through number of nodules. Biomass was positively correlated with the number of nodules (β = 0.38, p < 0.0001). Koa produced fewer nodules when grown in ʻaʻaliʻi compared to its own soil (β = -0.0053, p < 0.0001), with the indirect effect of growing in ʻaʻaliʻi soil being β = -0.0020. Koa also produced fewer nodules when grown in māmane compared to its own soil (β = -0.0027, p=0.022), with the standardized indirect effect of growing in māmane soil being β = -0.0010. Biomass was negatively affected by herbivory (β=-0.19, p = 0.0001).

The māmane SEM fit the data well (Fisher's C = 0; p = 1; Figure 2). Māmane, similar to koa, attained greater biomass in its own soils than in heterospecific soils (ʻaʻaliʻi and koa) leading to a positive single species PSF. Unlike koa, however, this was only indirectly mediated through nodule biomass with one of the away soils: ʻaʻaliʻi. Māmane biomass was lower when

grown in ʻaʻaliʻi soil than when grown in its own soil (β = -0.15, p = 0.019), with the indirect

effect of growing in ʻaʻaliʻi soil being β = -0.0083. Māmane biomass was also lower when grown

in koa soil than when grown in its own soil (β = -0.35, p < 0.0001), with the indirect effect of

growing in koa soil being β = -0.0016. Biomass was positively affected by the number of

nodules (β = 0.39, p < 0.0001). The number of nodules was negatively affected by being grown

in ʻaʻaliʻi soil (β = -0.021, p = 0.0002).

Are net pairwise PSFs between our two focal species affected by fire?

Burning trended towards reducing the strength of otherwise positive PSFs between koa

and māmane ($F_{1,457}$ = 2.96; p = 0.086). Outside the burn, PSFs were positive (I_s = 0.0016) and

significant (t=3.70 , p = 0.00027). However, within the burn, I_s was 0.00061, and this was not

significant (t=1.43, p = 0.16) (Figure 3).

Discussion

In one of the first experimental studies to explicitly test the effects of fire on PSFs, we

found that fire can alter net pairwise PSFs with important implications for post-fire succession.

In our system, in unburned areas, net pairwise PSFs likely help to reinforce local species

dominance (Bever et al. 1997). These positive PSFs, however, become insignificant in burned

soils, suggesting that after fires species are more likely to coexist, which also translates into the

easier spread of species across current realized ranges (Bever et al. 1997, Yelenik and Levine

2011). Mechanistically, this may have been due to a breakdown in the positive effects of

conspecific soils, suggesting that positive benefits from rhizobia were weakened with the fire.

Specifically, in māmane, nodulation was lower in soils from burned sites. Our results illustrate

the importance of considering how environmental context, and specifically fire, alters PSFs.

Nodule formation positively affected seedling performance, and this was likely a major reason for the positive relationship that we see with our seedlings when they are grown in conspecific soil (Figures 1 and 2; Crawford and Knight 2017, Siefert et al. 2018). The patterns that we observed with nodulation may indicate that the rhizobial bacteria associated with our species are at least somewhat host-specific, as has been shown with rhizobial bacteria in other systems (Fauvart and Michiels 2008, Andrews and Andrews 2017). In our system, nodulation of koa was higher when grown in conspecific soil and lower in heterospecific soil (Figures 2 and 4), yet the nodulation rates were similar in heterospecific soil whether the other species was another legume or a non-N-fixer, which further demonstrates that koa rhizobia may be somewhat host-adapted. For māmane, although not significant, nodulation trended toward being higher in conspecific soil than heterospecific soil and higher in N-fixer soil than non-N-fixer soil (Figure 4). These patterns indicating potential host-specificity of rhizobia may be partly why PSFs are positive in conspecific soil in our system and suggest a mechanism for positive PSFs elsewhere.

For one of our study species (māmane) the pattern of nodulation was affected by fire (Appendix S1: Table S3), indicating that fire can change positive PSF interactions that would otherwise reinforce local māmane dominance (Carvalho et al. 2010, Jesus et al. 2020). Nodulation was higher in conspecific, non-burned soil than any other combination of burn and soil source (Appendix S1: Table S3), indicating that fire may negatively impact the ability of this species to form nodules. How fire affects PSFs and patterns of nodulation in legumes is poorly understood (De Long et al. 2019). PSFs broadly have only rarely been studied in the context of environmental factors. However, fire has been shown to lead to large-scale changes in bacterial and fungal communities, independent of plants (Dooley and Treseder 2012, Whitman et al. 2019). In a global meta-analysis, Dooley and Treseder (2012) found that fire reduced microbial

abundance by an average of 33% across studies. Within the legume-rhizobial symbiosis, it is increasingly recognized that nodulation can be modified by fire, especially with shifts in the bacterial community involved in this interaction (Jesus et al. 2020), but this has not been studied outside just a few systems. For example, Jesus et al. (2020) showed that the rhizobial community associated with roots of invasive *Acacia longifolia* (Andrews) Willd. in Portugal shifted with fire. In their study, however, nodulation increased after fire, which they attributed to different life stages (seedlings versus adults) in burned versus unburned sites. Nonetheless, few papers make the link between shifts in microbial communities due to fire and subsequent effects on plant growth. Our work shows that fire can modify nodulation patterns and seedling growth, and as a result reduce the strength of positive PSF with potential consequences for post-fire succession and restoration activities.

While our results do demonstrate the importance of incorporating the effects of fire when considering PSFs, our findings are also nuanced. It is possible that some of this nuance in our results is potentially due to fire severity. Fire severity is known to affect the soil microbial community, with more severe fires having correspondingly larger impacts on soil microbes (Dooley and Treseder 2012, Reazin et al. 2016), and this may then also reflect back on the microbes that participate in PSFs. We sampled beneath trees that survived the fire (to not confound our results with death of an individual in the field), and it is possible that by selecting these trees we selected for microsites of lower fire severity, which potentially muted our findings relative to that which may occur in microsites with higher fire severity. In addition, there could be a temporal effect, where fire's effects on PSFs decline over time. We collected soils ~10 months following the burn. Although some effects of fire on soil microbes have been shown to last for ~20 years (Pérez-Valera et al. 2018) and PSFs can carry on after the death of an

individual (Kardol et al. 2007, Mueller et al. 2019), these effects shift in composition and quantity over time (Pérez-Valera et al. 2018), and effects on PSFs are likely most pronounced immediately following the fire itself.

Although PSFs have been increasingly studied since being coined by Bever et al. (1997), such studies rarely incorporate environmental context and almost never incorporate the relationship with fire (Kulmatiski and Kardol 2008, Van der Putten et al. 2013, Senior et al. 2018, Crawford et al. 2019, Beals et al. 2020). There was a significant pairwise feedback between two species in non-burned areas, and while not significantly different from the lack of feedback within the burned area, the trend is towards stronger feedbacks before fire (Figure 3). This means that, likely, prior to fires, neighbor identity matters for koa and māmane in ways that reinforce local dominance, while after fire, this PSF pattern disappears. Similar patterns have been seen in the only other study of this sort of which we are aware (Senior et al. 2018) in Australia, where positive individual feedbacks between *Eucalyptus globulus* Labill. and soils disappeared once soils were burned. Such weakening of PSFs with fire follows from a priori assumptions where the abundance and richness of soil microbes decreases after fire (Dooley and Treseder 2012, Reazin et al. 2016). Given this assumption, our results, and those of Senior et al. (2018), it is possible that such patterns may be occurring in fire-impacted systems more generally.

Changes to PSFs may influence patterns of plant diversity, and understanding of the pairwise PSFs of our two focal species can help us to better understand the interactions between these two plant species in the field. Forests in the Hawaiian Islands support relatively few tree species (Little and Skolmen 1989), and our two focal species make up the vast majority of trees within our study site (C. Warneke, S. Yelenik, personal observation, 2019). Positive net pairwise

PSFs would reinforce the local dominance of each of these species, and interestingly, koa-dominated and māmane-dominated forests are spatially separated across an elevation gradient, possibly due to drier, cooler conditions upslope. However, in areas near our field site, koa abundance can rapidly increase (Hamilton et al. 2021), including in previously māmane-dominated habitats, after successive fires (Rick Warshauer, Hawai'i Cooperative Studies Unit, University of Hawai'i-Hilo oral communication 2021). A change from positive net-pairwise feedbacks to neutral feedbacks would facilitate the two N-fixers shifting their local dominance patterns towards increased coexistence or a change in local dominance towards koa. We also note that koa growth rates are higher than those of māmane (Yelenik et al. 2017, Barton and Shiels 2020), which would reinforce koa spreading into māmane-dominated forests. Comparing the strength of pre-fire PSFs (a density-dependent process) to differences simply due to differential growth rates (non-density-dependent), or stochastic community assembly processes such as seed dispersal or impacts of burn severity (Mahood and Balch 2019), would help tease out how important such PSFs are for plant community patterns and succession in this ecosystem (MacDougall et al. 2009, Yelenik and Levine 2011).

Our results suggest that managers may need to consider changes in microbial communities after fire when considering management actions (Dooley and Treseder 2012, Pérez-Valera et al. 2018, Grman et al. 2020). Due to the increase in fire frequency in the Hawaiian Islands in recent years and going forward (Trauernicht et al. 2015), land managers often plant seeds and seedlings of our study species into recently burned habitats to reestablish the native plant community (McDaniel et al. 2008). By adding conspecific soil or rhizobial inoculum to growing media, restoration practitioners may help grow bigger, more successful koa and māmane in the greenhouse, which, in turn may allow for better success of seedlings when

outplanted into field conditions (Elevitch and Wilkinson 2003). Decreases in nodule formation may alter growth rates of these species in post-fire reseeding and planting efforts, and countering that through inoculation may benefit restoration efforts. This is likely to more strongly benefit māmane, which has a slower growth rate than koa (Yelenik et al. 2017, Barton and Shiels 2020), making restoring populations of this species a challenge in degraded habitat conditions. In addition, koa generally reestablishes well after fires, regardless, making this step unnecessary in areas that were dominated by koa before the fire (Loh et al. 2007, Hamilton et al. 2021).

Overall, our results demonstrate that fire has the potential to modify PSFs and the legume-rhizobial symbiosis. The relationship between fire and PSFs has been unclear; yet, advancing knowledge around this topic is critical at a time when fire is increasing worldwide. Our work demonstrates the importance of considering environmental context for PSFs.

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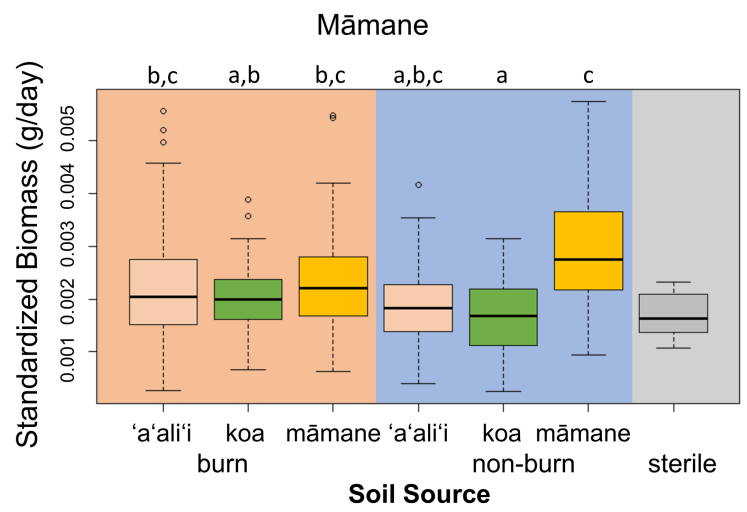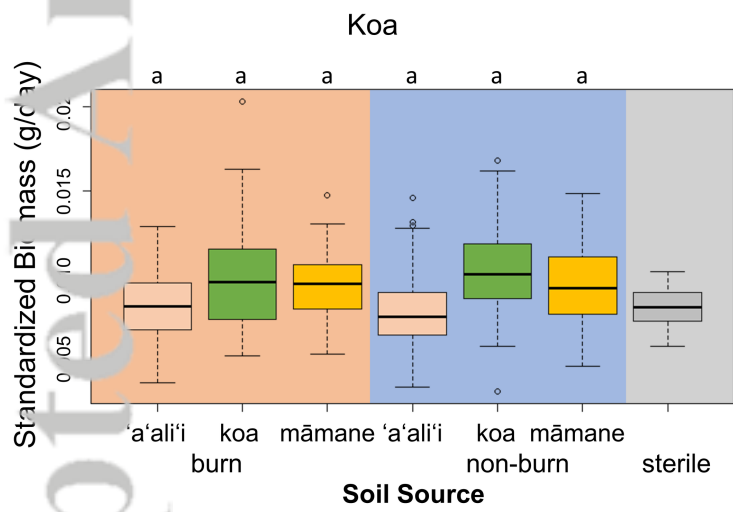
Figure Legends

Figure 1. Standardized biomass (g/day) for koa and māmane, by source plant crossed with burn. Background color indicates burn (light orange; left) or nonburn (light blue; middle), and bar color is color coded by source plant species. Letters above the plot indicate groups (within each species plot), determined by post-hoc comparisons. Interquartile ranges cover the middle 50% of the data and the whiskers are up to 1.5 times the interquartile range. Biomass tends to be higher in soil from conspecifics and N-fixing species. Sterile soil (gray bar with a gray background; right) is included for a visual comparison and was not included in statistical analyses.

Figure 2. SEM diagrams for each species. Solid arrows indicate positive relationships, while dashed arrows indicate negative relationships. Black arrows indicate significant relationships ($p<0.05$), while gray arrows indicate nonsignificant relationships ($p>0.05$). Line size is scaled to standardized effect size. Relationships for source soils are relative to conspecific soils (i.e., for māmane, the line from "'a'ali'i" to "biomass" indicates that māmane obtained less biomass in koa soil than its own soil).

Figure 3. Strength of net pairwise PSF (I_s) between koa and māmane inside and outside of the burn. The difference between the two is marginal ($p = 0.086$, marked with §). Outside the burn, PSFs were significant ($p = 0.00027$, marked with *), while within the burn they were not ($p = 0.16$).
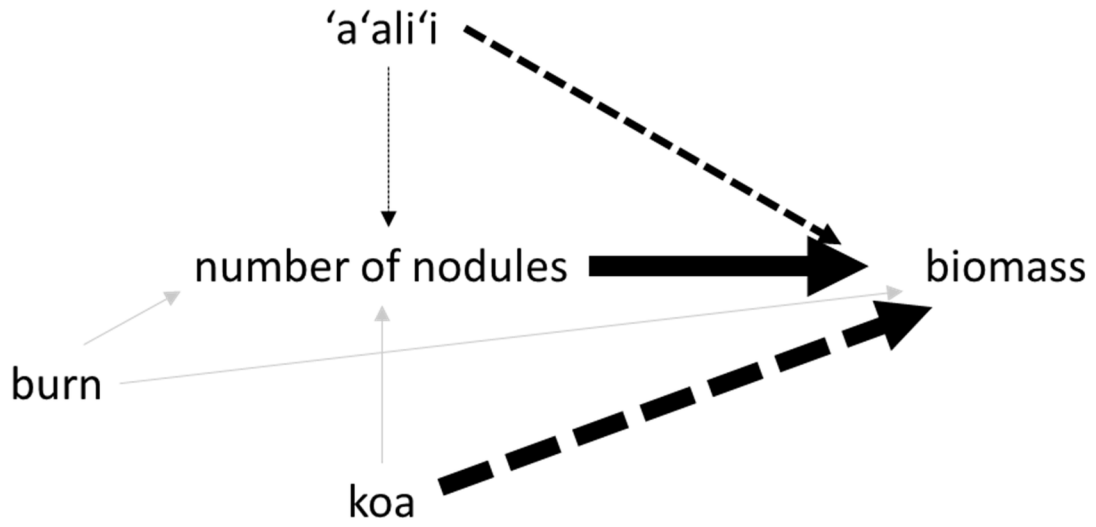
Figure 4. Number of nodules for koa and māmane, by source plant crossed with burn. Background color indicates burn (light orange; left) or nonburn (light blue; middle), and bar

color is color coded by source plant species. Letters above the plot indicate groups (within each species plot), determined by post-hoc comparisons. Interquartile ranges cover the middle 50% of the data and the whiskers are up to 1.5 times the interquartile range.
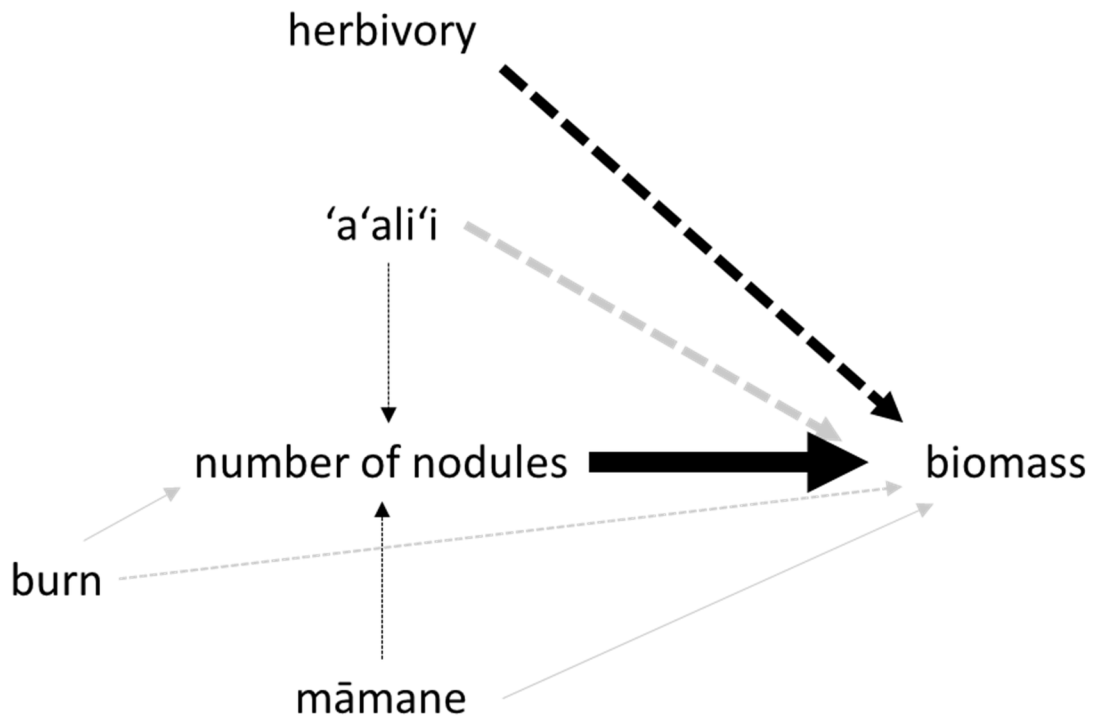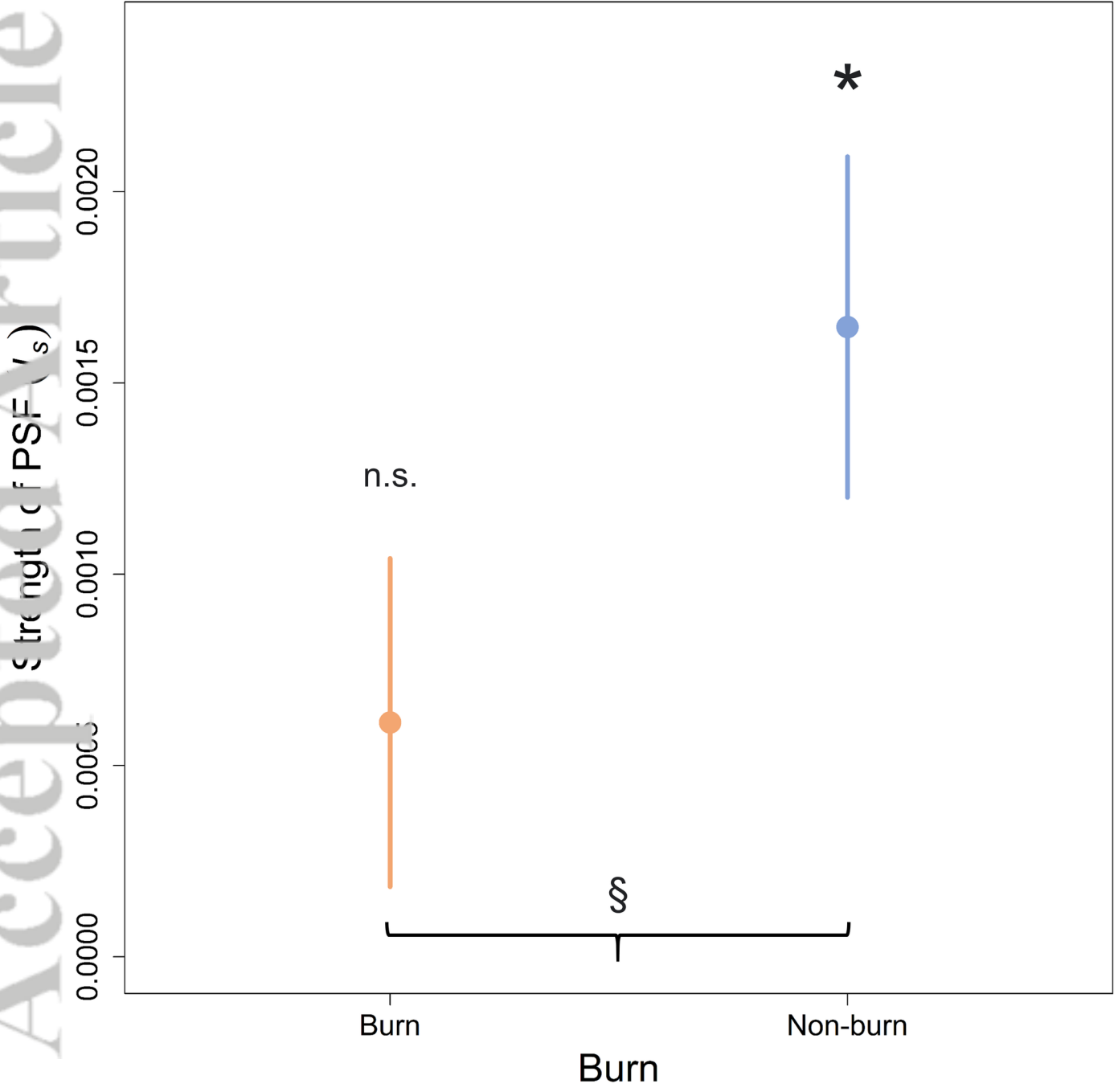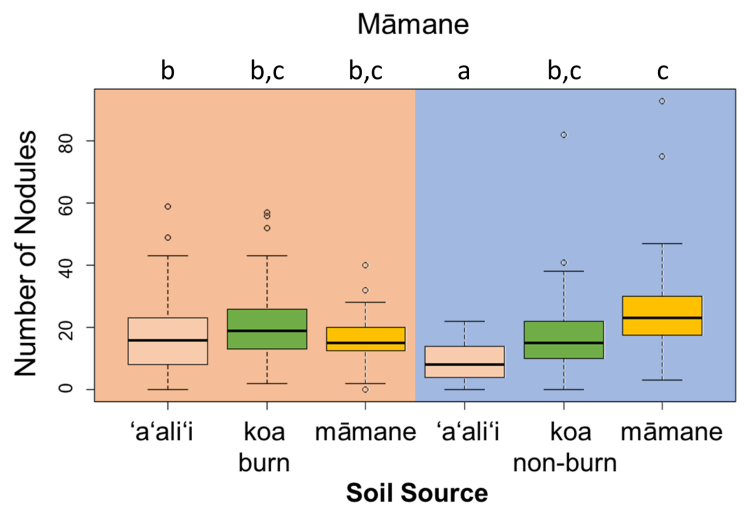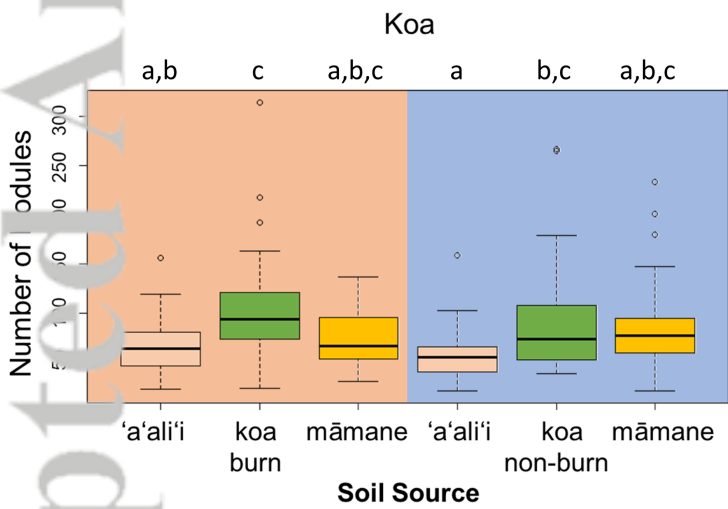
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MĀMANE

'a'ali'i

number of nodules → biomass

burn

koa

KOA

herbivory

'a'ali'i

number of nodules → biomass

burn

māmane

ECY_3994_FIG 2.tif

ECY_3994_Warneke_et_al_Fig_3.tif

Koa

a,b c a,b,c a b,c a,b,c

Number of Nodules

'a'ali'i koa burn māmane 'a'ali'i koa non-burn māmane

Soil Source

Māmane

b b,c b,c a b,c c

Number of Nodules

'a'ali'i koa burn māmane 'a'ali'i koa non-burn māmane

Soil Source

ECY_3994_FIG 4.tif