MECHANISMS AND MITIGATION OF SOIL LEGACIES OF INVASIVE GRASSES

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DEDICATION

For my dear late friend Arielle Davis, who saw in me more than I saw in myself.

May others be so lucky as to experience such a once-in-a-lifetime friendship.

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ABSTRACT

The grass-fire cycle can be accurately re-described as the human-grass-fire cycle, with the majority of invasive grass introductions being deliberate, and the majority of ignitions being anthropogenic in nature. With increased fire risk, and altered fire regimes favoring invasives, inhibiting natives, and depressing the resilience of native ecosystems, the stakes for restoring grass-invaded areas are higher than ever. Invasive grasses often grow in monocultures, outcompeting and suppressing other species, but even when removed, an abundance of research has demonstrated evidence of soil legacies that can hinder restoration efforts. Thus, there is a need to identify specific mechanism of soil legacies associated with invasive grasses that limit or inhibit restoration success, in order to translate that knowledge into mitigation strategies that better target these mechanisms.

To begin, I gathered studies examining grasses for allelopathic abilities, to see if phylogenetically conserved allelochemicals, including benzoxazinoids, which are mostly *Poaceae* specific, points to allelopathy as a key soil legacy mechanism that contributes to disproportionate invasive success in the grass family. By narrowing the frame of the landmark allelopathy analysis by Zhang et al. (2020), I found support for the novel weapons hypothesis in invasive grasses; specifically, the allelopathic impact on native recipients, was more negative when the allelopathic species was a non-native grass, compared to when the allelopathic species was a native grass. Additionally, I found support for the phylogenetic distance hypothesis, supporting other research suggesting that allelochemical impacts depend on the phylogenetic distance of the target plant. I did not find support for the biotic resistance hypothesis, specifically that the allelopathic impact when the allelopathic species was a native grass was more negative on a non-native recipient, than a native recipient. Through this analysis, I showed evidence suggesting that land managers ought to consider testing for allelopathy, or considering allelopathy-informed restoration practices, when trying to restore grass-invaded areas.

Next, in a field study at Camp Pālehua (Kapolei, O'ahu, Hawai'i) I compared soil characteristics and the soil microbial community between a *Megathyrsus maximus* invaded area, and an area that was formerly invaded by *Megathyrsus maximus* but had been restored by community partner Malama Learning Center 18 months prior to the comparison. The two sites shared a long history of grazing, climate and soil characteristics, and slope. While I could not

rule out inherent differences between the sites that existed prior to the restoration, I sought to identify differences in soil characteristics and the soil microbial community that could be attributed, at least in part, to the restoration. While the restoration practice at the Malama Learning Center section was relatively successful, the site continued to require frequent hand-weeding, so I was particularly interested in evidence of a soil legacy in the soil microbial community, specifically any "hold-over" or "hold-out" taxa, that may be contributing to on-going re-emergence of grass species in the native ecosystem. I found a genus of fungi (*Glomus*) and bacteria (*Candidatus Udaeobacter)* were abundant at both sites and these genera have been identified in the literature as being associated with pastures, suggesting that, in the restoration site, these genera were hold-overs. This suggests that specific members of the soil microbial community could be contributing to on-going, long-lasting soil legacy effects, such that modifying the soil microbial community could mitigate some of these effects.

In the third chapter, I conducted a greenhouse plant-soil feedback study, using a whole soil inoculum design, comparing germination, survival, and above/belowground growth in inoculum added soil to control soil. By using inoculum in less the 5% volume (w/w), I was able to isolate for the impact of the soil microbial community while holding soil characteristics and nutrients constant, to test for a soil microbial community mechanism for a soil legacy of an invasive grass *(Megathyrsus maximus)*. In addition, I implemented a moderate drought treatment at 60% pot capacity to test for the impact of drought on any plant-soil feedback, since drought is expected to impact ecosystems in Hawai'i in the future. The native species used to test for feedback from the invasive grass microbial community was the endemic dry forest shrub *Chenopodium oahuense.* I found evidence that there was positive con-specific feedback (the grass benefited from its own soil microbial community) and negative hetero-specific feedback (negative impacts of the grass soil microbial community on the native), impacting primarily the belowground growth of both species, suggesting that the soil microbial community mediates belowground competition for space and nutrients. In addition, I found an interaction between the presence of the grass soil microbial community and drought that was associated with delayed germination of *Chenopodium oahuense*, suggesting that a soil legacy effect may contribute to phenological mismatch for native species as climate change progresses.

In the fourth chapter, I conducted an experimental restoration at a separate *Megathyrsus maximus* invaded section of the Camp Pālehua property in Kapolei, O'ahu, Hawai'i using a soil amendment of activated carbon, alone and in combination with a locally sourced biowaste-based biochar fertilizer, to determine whether these amendments might mitigate the soil legacy mechanisms I had previously investigated. Two native species were used: *Plumbago zeylanica* and *Dodonaea viscosa.* I found that soil raking alone prior to planting improved the width of *Plumbago zeylanica* by 1 cm after 1 year. Raking had two effects that could have benefited plant growth: disruption of soil compaction, and suppression of the no-raking indicator fungi *Bionectriaceae*. In addition, I found that the height of *Dodonaea viscosa* increased 4 cm with 0.5 kg/m^2 of activated carbon added. Two potential explanations are the neutralization of any present allelochemicals, and the suppression of specific fungi which were indicators of the treatments lacking activated carbon, including *Coniophora,* which has been found in other studies examining the soil microbiome of restoration projects on former pastures. The hypothesis that after the initial suppression, the activated carbon treatment would create opportunity for the outplants to recruit new members to the soil microbial community, resulting in indicator species for the activated carbon level, was not supported.

Overall, I was able to contribute evidence that allelopathic ability and the soil microbial community contribute to the soil legacies of invasive grasses, and in *Megathyrsus maximus* in Hawai'i, activated carbon can be used to suppress fungi and/or allelopathy to improve outcomes for some native plants, while raking alone can improve outcomes, potentially by disrupt soil compaction suppressing certain fungi. Activated carbon could be used in small scale-projects to create sustaining native ecosystems that can later be used for soil transplants or to provide soil inoculum in larger areas. Additionally, some of the identified fungi suppressed by activated carbon could be tested in isolation, or in different combinations for their impact on native plants.

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CHAPTER 1

LITERATURE REVIEW ON BELOWGROUND MECHANISMS OF INVASIVE GRASS SOIL LEGACIES AND THEIR INFLUENCE ON RESTORATION

Global Change and Plant Invasions

Global change is an umbrella term for the cumulative impacts of anthropogenic change, including climate change. Plant invasions generally fall within the umbrella of global change, as most invasive plants have been and continue to be spread intentionally and unintentionally by humans. Invasive plants that tend to cause regime shifts, or lasting change in an ecosystem between two distinct conditions (Scheffer et al., 2001), often have ecosystem engineering abilities related to plant-soil feedback (PSF) and microbial-mediated nutrient cycling (Klironomos, 2002; Garbeva, van Veen & van Elsas, 2004; Gaertner et al., 2014; Aldorfová, Knobová & Münzbergová, 2020), with evidence pointing to plant-fungi associations (pathogenic or mutualist) in particular as being important to invasive plant success and impacts (Dickie et al., 2017; Chung, Collins & Rudgers, 2019).

Soil Microbial Community

The soil microbial community can mediate plant competitive interactions (Gan, Emmett & Drinkwater, 2021), influence ecosystem functions (Bardgett & van der Putten, 2014), drive the diversity-productivity relationship (Schnitzer et al., 2011; Chen et al., 2021), and contribute to the resilience and survival of the plant community (Chen et al., 2021). The microbial community is typically structured by a combination of niche and neutral factors (Dumbrell et al., 2010), meaning that both current and historical states of a site matter, making comparisons and generalizations challenging. Descriptions of soil microbial communities typically cover both the individual organisms composing the community and the community as a whole (Harris, 2003; Hall et al., 2018). Due to potential interactions among species within a community, the additive impact of individual organisms is not necessarily equivalent to the realized cumulative impact of the entire community (Hilbig & Allen, 2015). To consider each individual organism within a community would require data that can be hard to get (Escalas et al., 2019). On the other hand, one can consider the whole community operating as a "supra-organism" (Konopka, 2009) by assessing or quantifying specific genes of interest in the community (Escalas et al., 2019), but this approach is coarse and highly context dependent (Perreault et al., 2020).

Plant-Soil Feedback

Plant-soil feedback (PSF) is characterized by reciprocal relationships between plants, the abiotic soil and the biotic community in the soil, which includes the soil microbial community (Kardol et al., 2007; Chen et al., 2019). One meta-analysis found that on average, native plants tend to have positive conspecific feedback and/or positive feedback with soil conditioned by other natives, while invasive species generally had more variable PSF (Suding et al., 2013). At disturbed sites, soil can be significantly altered in both abiotic and biotic characteristics since the most recent presence of a native ecosystem, so if positive feedback with native-conditioned soil is key to the establishment and/or survival of native plants, loss of positive feedback may reduce the ability or natives to successfully establish at disturbed sites and benefit the establishment of invasive plants. Modeling suggests that rather than being a driving force in the further spread of invasive plants (Levine et al., 2006), PSF plays a more significant role in the long-term impact of invasive plants (Levine et al., 2006) because of the time required to build up significant effects, which is why feedbacks are important to consider in restoration practices. For example, nitrogen-fixing invaders increase nitrogen availability, alter the microbial community, and are associated with community-level effects like decreased representation among natives in the soil seedbank (Nsikani, van Wilgen & Gaertner, 2018).

The PSF and microbial communities associated with invasive plants can have a suppressive effect on native species growth, contributing to the difficulty of restoring invaded areas (Batten, Scow & Espeland, 2008; Perkins & Nowak, 2013; Suding et al., 2013; Skurski, Rew & Maxwell, 2014). Furthermore, PSF can contribute to soil legacies, which can be driven by PSF effects. As an example, an invasive plant can alter soil composition, not only during their growth through release of phenolics, but also after death, by contributing to bulk organic carbon composition. Both soil alterations can be slow to turnover, resulting in a soil legacy after invasive plants have been removed (Suseela et al., 2016). Plant community composition changes have been associated with significant differences in microbial communities up to thirty years after removal (Wubs, Melchers & Bezemer, 2018; López Zieher, Vivanco & Yahdjian, 2020). Mitigating PSF mechanisms, through soil transplants (Solans, Pelliza & Tadey, 2021), "re-culturing" soil with a new species (Brinkman et al., 2017), use of commercially produced inoculants (Perkins & Hatfield, 2016) and other methods, has had success as a restoration strategy.

Allelopathy: A Mechanism of Plant-soil Feedback

One key mechanism of plant-soil feedback is allelopathy, or secondary chemicals (Bennett & Klironomos, 2019). Allelopathy is intertwined with the influence of the soil microbial community, as soil microbes are typically responsible for the degradation of allelochemicals present in the soil (Cheng & Cheng, 2015). Allelopathy has been important to study in the context of invasion (Inderjit et al., 2008). One meta-analysis found that the literature contains evidence that just over 50% of invasive plants have at least one study providing evidence of allelopathic abilities (Kalisz, Kivlin & Bialic-Murphy, 2021). In addition to contributing to the negative impacts of invasive plants, in both an immediate and soil legacy sense (Suseela et al., 2016), allelopathy can also influence plant-pollinator interactions by influencing pollen and nectar production or impacting fruit attractiveness (Hale & Kalisz, 2012), and thus contribute to much wider impacts on aboveground communities. Allelopathy has both direct and indirect mechanisms (Figure 1.1), and allelopathic impacts can be highly dependent on soil characteristics (Scavo, Abbate & Mauromicale, 2019).

As more research is published, we have a better chance of finding commonalities, patterns and generalizations, which have been hard to find (Bezemer et al., 2006), but can be important for common issues like soil legacies (Dickie et al., 2017). PSF continues to be a popular research topic with many remaining research needs, four of which have been incorporated into the scope of this project, while also controlling for other factors known to influence PSF (Table 1.1).

Global Change: Soil Properties

Land-use, another important aspect of anthropogenic change, may alter N uptake in part through impacting soil organic matter (SOM) (Gan, Emmett & Drinkwater, 2021), shift the importance of drivers of microbial community structure (Jiao et al., 2020), impact variation in microbial diversity (Lauber et al., 2013), and influence functional group abundance (Morales, Cosart & Holben, 2010), all of which may contribute to losses and/or modification of ecosystem function and processes. Changes precipitated by temporary disturbances, such as prescribed fires can be supported and sustained by novel disturbance regimes associated with land-use, such as cattle grazing (Hernández et al., 2021). Grazing itself is associated with abiotic and biotic impacts to soil, including changes in soil carbon, soil nutrients (Zhu et al., 2021a), microbial

community composition (Wang et al., 2006; Ding, Travers & Eldridge, 2022) and microbial biomass (Wang et al., 2006).

Changing wildfire regimes can be categorized as an aspect of anthropogenic change, as they often have anthropogenic causes (Menezes et al. 2022), but wildfires are also discussed in the context of climate change, as they are within the group of extreme events whose increased frequency and intensity is influenced by climate change (Westerling et al., 2011). Additionally, wildfires are significant contributors to another major aspect of anthropogenic change, N deposition (Koplitz et al., 2021). The negative impact of N deposition on fungal abundance and activity can, over time, reduce nitrogen mineralization rates and enzyme activity (Hood-Nowotny et al., 2021). There is evidence, however, that N deposition can increase carbon sequestration in soil (Hood-Nowotny et al., 2021; Zhang & Suseela, 2021; Li et al., 2021), which is an important ecosystem function in terms of mitigating worsening climate change.

Global Change: Drought

Droughts themselves can have legacies that impact PSF (Hassan & Carrillo, 2021). In one study, root exudates from plants that experienced drought were more effective at inducing soil respiration that could compensate for reduced respiration through other mechanisms, thus potentially increasing microbial community activity, which can influence plant growth via the "nutrient highway" (de Vries et al., 2019). This finding suggests that drought may amplify allelopathic interactions with indirect mechanisms that interact with water competition. Drought and disturbances can interact in their impact on invasive plant establishment, and this interaction is dependent on plant species and life stage (Orbán et al., 2021). In some cases, the benefit disturbance provides to invasive plants can outweigh the negative impact of drought on the same species (Orbán et al., 2021). There is a need to better understand the functional traits that may contribute to the ability of invasive plants to adapt to drought as well as other environmental conditions (Drenovsky et al., 2012).

Competition for water constrains microbial activity (Li et al., 2021), so changes in precipitation regime can have significant impacts on microbial communities and their associated functions (Castro et al., 2010). Studies have found an interaction between the impact of microbial communities and drought treatment on plant biomass, suggesting that microbial communities may mediate drought survival (Delavaux, Smith-Ramesh & Kuebbing, 2017; Xi,

Chu & Bloor, 2018). Short-term effects of drought may impact stoichiometry and function measures significantly, such as microbial N, P, C:N, C:P. Evidence of the impact of drought on microbial biomass carbon is mixed (Cheng et al., 2021; Canarini et al., 2021). Long-term effects of drought may impact microbial composition measures significantly, such as decreased diversity in bacteria and fungi communities (Zhou, Wang & Luo, 2020; Yang et al., 2021). A meta-analysis of six studies examining the impact of drought on soil microbial communities found that drought was correlated with a decrease in abundance of unidentified/uncultured genera in the bacteria families Xanthobacteraceae, Pirullaceae, Coleofasciculaceae, Sphingobacteriaceae, Chitinophagaceae and Illumatobacteraceae, and decrease in abundance of the genera *Blastocatella* and *Bryobacter* (Isobe et al., 2020). In contrast, the same study found that drought was correlated with an increase in abundance of unidentified/uncultured genera in the bacteria families Cytophagaceae and Gemmatimonadaceae (Isobe et al., 2020). Evidence that indicates specific microbes play a role in drought tolerance can be utilized to improve restoration outcomes. For example, the addition of plant-growth promoting rhizobacterium *Bacillus subtilius* inoculant mediated drought tolerance by increasing soil water retention and lowering soil hydraulic conductivity, in some part through the release of extracellular polymeric substances by *Bacillus subtilius* into the soil environment (Jin et al., 2020). Evidence that drought can shift the bacteria:fungi ratio (de Vries et al., 2018; Wang et al., 2020), and generally has more discernable impacts on the bacterial community compared to the fungal community (de Vries et al., 2018), is important given the evidence that plant-soil legacies are often mediated by fungi (Heinen et al., 2020).

In a study focusing on grassland soil microbial communities, the bacteria genera *DA101, Candidatus xiphinematobacter,* and *Rhodoplanes* and genera in the fungus family Mortirellaceae were indicators of drought tolerance (de Vries et al., 2018). Furthermore, there is evidence that the bacteria community in grass soils may be more resistant to change in the face of frequent drying-rewetting compared to proximal non-grass soils of the same classification (Fierer, Schimel & Holden, 2003).

Invasive Grasses

Drought tolerance, an ecological performance (Violle et al., 2007) commonly attributed to many invasive grasses, may be associated with functional traits like specific nutrient uptake

strategies, which can contribute to a soil legacy (Craine et al., 2013). The presence of invasive grasses tends to increase the impact of drought on native plant growth, although this impact may differ based on resource allocation strategy of the native plants present (LaForgia, Harrison & Latimer, 2020). The ability of grasses to adapt to a wide range of environmental conditions (Linder et al., 2018), may help explain why the grass family (Poaceae) is among the most successful plant families in the world (Linder et al., 2018). Negative heterospecific PSF towards native plants is thought to contribute to the success of some invasive grasses (Perkins, Hatfield & Espeland, 2016). Previous investigations into invasive grass PSF found that the extreme climate shifts associated with climate change will favor non-native grasses by mitigating negative feedbacks on invasive grasses that make ecosystems more resilient (Duell et al., 2019). Invasive grasses are also problematic for many other reasons, one of which is that they can have a significantly higher fuel load than proximal native communities, greatly increasing the frequency and intensity of fire and disrupting the environment (Tomat-Kelly, Dillon & Flory, 2021; Zhu et al., 2021b).

Megathyrsus maximus: A Dominant Grass Invader in Hawaii

In the main Hawaiian islands, around one quarter of the land area is dominated by non-native grasses (Trauernicht et al., 2015). The initial establishment of many non-native grasses, such as *Megathyrsus maximus*, was intentional, to support grazing pastures (Cuddihy and Stone 1990), although this grass has spread widely as a naturalized invader since the initial pasture plantings. Importantly, the spread of *Megathyrsus maximus* in the past century may have contributed to lower carbon sequestration in some Hawaii's ecosystems, as *M. maximus* stands typically have less than 10 MgC/ha, while mesic native forests have over 30 MgC/ha (Hughes et al., 2017). Conversion of tropical forests to pasture can contribute to significant changes to the function and composition of soil microbial communities, depending on initial soil characteristics and forest type (Díaz-Vallejo et al., 2021). While ecological restoration research continues, restoration success seems to be dependent on the on-going use of herbicide or manual weeding (Ammondt et al., 2013), solutions which do not translate to self-sustaining, low-intervention, native ecosystems. In this dissertation, I aimed to improve our understanding of the key mechanisms contributing to invasive success and restoration failure at sites invaded by *Megathyrsus maximus.* I also aimed to explore restoration practices that can lead to the self-

sustaining native ecosystems necessary to make real progress towards reducing fire risk in Hawaii.

Hypotheses to be Addressed by this Dissertation Research

1) Allelopathy contributes to the disproportionate success in range expansion of grasses, which has resulted in many grasses being deemed invasive worldwide (Chapter 2)

   This hypothesis is proposed because research on grasses has recently identified mostly-family specific allelochemicals, benzoxazinoids (Niculaes et al., 2018; Hu et al., 2018), and there is evidence that the existence of benzaxazinoids outside of Poaceae may be attributable to convergent evolution (Frey et al., 2009). The traditional approach to study allelopathy posits that allelopathy contributes to invasion (Inderjit et al., 2008), however no studies to date have specifically reviewed evidence for allelopathic potential or strength of allelopathy impacts among invasive grasses. I conducted a search of the literature to gather studies where the species being examined for allelopathic potential is a member of the *Poaceae* family. After identifying those studies, I extracted data necessary for the calculation of effect size, as well as information about the study methodology. Finally, I conducted a Bayesian meta-analysis to test if non-native grasses had a more negative allelopathic effect on native recipient species, compared to native grasses.

2) In Hawaii, the grass *Megathyrsus maximus* is associated with a soil legacy that complicates restoration success in invaded areas (Chapter 3 & 4)

   This hypothesis is proposed because in at least one instance, native seed germination in *Megathyrsus maximus* invaded areas was low, even when the invader has been removed (Ammondt et al., 2013). There is evidence of plant-soil feedback in other invasive grasses in Hawaii (Yelenik & D'Antonio, 2013). In Chapter 3, I conducted a comparison of a restored and un-restored site in Kapolei, HI, focusing on abiotic and biotic soil properties. First, I recorded observations about physical soil characteristics. Then, I collected soil samples to utilize for next generation sequencing and compare

the diversity and composition of the bacteria and fungi communities. In addition, I analyzed the relative abundance of nitrogenase enzyme complex as a proxy for nitrogen fixing bacteria. I also conducted a plant-soil feedback experiment testing the impact of the *Megathyrsus maximus* soil microbial community on the germination of an endemic shrub. In Chapter 4, I conducted a plant-soil feedback experiment testing the impact of the soil microbial community associated with *Megathyrsus maximus* on the germination and growth of *Chenopodium oahuense*, an endemic shrub that is often utilized in restoration projects. I tested the impact of the soil microbial community on *Megathyrsus maximus* germling survival and growth. *Megathyrsus maximus* germlings were grown in commercial soil for 5 weeks to create soil inoculums, which were then be added to un-conditioned commercial soil at less than 5% v/v to add the effect of the soil microbial community, without modifying soil characteristics or nutrient profile. Growth species were grown for seven weeks, after which I harvested and weighed above and belowground biomass. Finally, I incorporated a moderate drought treatment to see if the associated soil microbial community has an influence on drought tolerance for either species.

3) The soil microbial community associated with *M. maximus* is associated with a plant-soil feedback effect that reduces germination and growth of endemic Hawaiian species *Chenopodium oahuense* (Chapter 4)

Evidence of *Megathyrsus maximus* having negative plant-soil feedback towards native plants has been found in other parts of the invasive range, most recently in Texas, with invaded soil being associated with reduced plant abundance and diversity compared to uninvaded soil with the same seed bank (Bowman, Plowes & Gilbert, 2023). The same study also found positive con-specific feedback, which would reinforce invasion, but the study utilized soil collected from the field, which leaves the mechanism of feedback in question. Specific microbial species, such as *Trichoderma* and *Azospirillum*, have been shown to improve root development and dry matter of *Megathyrsus maximus* (Argumedo-Delira, Gómez-Martínez & Mora-Delgado, 2022), suggesting that the soil microbial community should be considered as a potential mechanism. In Hawaii, some restoration projects implementing native

seed rain treatments in *Megathyrsus maximus* invaded areas have had little to no success (Ammondt et al., 2013). I conducted a plant-soil feedback experiment testing the impact of the soil microbial community associated with *Megathyrsus maximus* on the germination and growth of *Chenopodium oahuense*, an endemic shrub that is often utilized in restoration projects. *Megathyrsus maximus* germlings were grown in commercial soil for 5 weeks to create soil inoculums, which were then be added to un-conditioned commercial soil at less than 5% to add the effect of the soil microbial community, without modifying soil characteristics or nutrient profile. Growth species were grown for seven weeks, after which I will harvest and weigh above and belowground biomass.

4) Drought simulation worsens negative effects of the *M. maximus* soil microbial community on native *Chenopodium oahuense* (Chapter 4)

   Megathyrsus maximus appears to be drought adapted (Ammondt et al., 2013) and is generally considered to be stress tolerant (Rhodes et al., 2021; Oliveira et al., 2022). There is evidence that the soil microbial community of an invader can improve stress recovery for the invader, while decreasing the recovery for native species (Zhang, Oduor & Liu, 2022). In addition to being an endemic species, and a common choice for restoration, *Chenopodium oahuense* was selected due to it being native to drier areas (Leopold & Hess, 2019; Cole et al., 2021), making it a likely choice for restoration projects in areas that are expecting to experience drought in the near- or long-term future. In the previously discussed plant-soil feedback study, I incorporated a moderate drought treatment (watering at 60% pot capacity, compared to 90% pot capacity) to see if the associated soil microbial community has an influence on drought tolerance for either species.

5) Activated carbon, when used as a soil amendment, can significantly modify soil biotic and abiotic characteristics, leading to a dampening of any soil legacy effects driven by the previous invader, *Megathyrsus maximus* (Chapter 5)

   Activated carbon can neutralize allelochemicals (Madsen et al., 2014; Del Fabbro & Prati, 2015), decrease mycorrhization (Wurst, Vender & Rillig, 2010), decrease

bacterial abundance (Kulmatiski, 2011), and modify bacterial community composition (Shan et al., 2015; Nolan et al., 2015). I conducted an experimental restoration project in Kapolei, HI, at a site previously invaded by *Megathyrsus maximus.* First, I collected pre-practice soil samples from the site to utilize for future comparisons. In addition to the land manager's typical restoration practice of outplanting, and infrequent drip irrigation and weeding (weekly for the first four months, monthly for the following eight months) as a control, I incorporated four additional treatments. The first treatment was the activated carbon treatment, spread at 0.5 kg/m^2 and raked into the soil. The second treatment was the combination treatment, where activated carbon was spread at 0.25 kg/m^2, then biochar was spread at 0.25 kg/m^2, and then the surface level amendments will be raked into the soil. The combination treatment is based on research showing that mixing activated carbon with a fertilizer can mitigate nitrate increases (Lau et al., 2008). The third treatment was the biochar treatment, spread at 0.5 kg/m^2 and raked into the soil. Finally, the fourth treatment was the raking treatment, where no soil amendment is added, but the soil is raked, so that I could distinguish between effects associated with the physical disturbance to the soil and the addition of the amendment itself. After applying any assigned treatments, I conducted outplanting evenly across each plot, using *Plumbago zeylanica* and *Dodonaea viscosa.* At the six-month mark, I surveyed the site for outplant mortality and at the one-year mark, I measured the height and width of each outplant. At the six month and one year mark, I collected soil samples to utilize with next generation sequencing, to analyze impacts to the diversity and composition of the bacteria and fungi community, and compare samples between treatments, and to pre-practice samples. At the six month and one year mark, I collected soil samples to utilize for total carbon and nitrogen analysis. At the six month and one year mark, I deployed ion-resin bags for four weeks, after which I collected and performed an extraction on each bag and analyze the extract for inorganic nutrients (N/P). At the six month and one year mark, I conducted field infiltration tests at each plot and recorded the time to full penetration for the second pour at each plot.

For the success of conservation and restoration priorities in Hawai'i, there is a clear need for research that specifically examines potential PSF mechanisms that contribute to the long-term

impacts of invasive grasses. As climate change progresses, it also evident that climate events and environmental stressors must be considered in research being conducted in controlled environments, if the goal of research is to produce practical implications for contemporary practitioners. If these research aims prove fruitful in improving restoration outcomes in Hawai'i, they could result in healthy native communities, that can then be used as sources for soil transplants, to scale up restoration efforts. Additionally, the success of invasive grasses across the mainland of the United States and across the globe show that research advances that facilitate restoration following grass invasion could have far-reaching benefits for many local communities, including reduced wildfire frequency or intensity and associated improvements in air quality.

References

Aldorfová A, Knobová P, Münzbergová Z. 2020. Plant-soil feedback contributes to predicting invasivness of 68 alien plant species differing in invasive status. *Oikos* 129:1257–1270. DOI: 10.1093/annonc/mdy039/4835470.

Ammondt SA, Litton CM, Ellsworth LM, Leary JK. 2013. Restoration of native plant communities in a Hawaiian dry lowland ecosystem dominated by the invasive grass Megathyrsus maximus. *Applied Vegetation Science* 16:29–39. DOI: 10.1111/j.1654-109X.2012.01208.x.

Argumedo-Delira R, Gómez-Martínez MJ, Mora-Delgado J. 2022. Plant Growth Promoting Filamentous Fungi and Their Application in the Fertilization of Pastures for Animal Consumption. *Agronomy* 12:3033. DOI: 10.3390/agronomy12123033.

Balser TC, McMahon KD, Bart D, Bronson D, Coyle DR, Craig N, Flores-Mangual ML, Forshay K, Jones SE, Kent AE, Shade AL. 2006. Bridging the gap between micro - and macro-scale perspectives on the role of microbial communities in global change ecology. *Plant and Soil* 289:59–70. DOI: 10.1007/s11104-006-9104-5.

Bardgett RD, van der Putten WH. 2014. Belowground biodiversity and ecosystem functioning. *Nature* 515:505–511. DOI: 10.1038/nature13855.

Batten KM, Scow KM, Espeland EK. 2008. Soil microbial community associated with an invasive grass differentially impacts native plant performance. *Microbial Ecology* 55:220–228. DOI: 10.1007/s00248-007-9269-3.

Beals KK, Moore JAM, Kivlin SN, Bayliss SLJ, Lumibao CY, Moorhead LC, Patel M, Summers JL, Ware IM, Bailey JK, Schweitzer JA. 2020. Predicting Plant-Soil Feedback in the Field: Meta-Analysis Reveals That Competition and Environmental Stress Differentially Influence PSF. *Frontiers in Ecology and Evolution* 8:191. DOI: 10.3389/fevo.2020.00191.

Bennett JA, Klironomos J. 2019. Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist* 222:91–96. DOI: 10.1111/nph.15603.

Bezemer TM, Lawson CS, Hedlund K, Edwards AR, Brook AJ, Igual JM, Mortimer SR, Van Der Putten WH. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology* 94:893–904. DOI: 10.1111/j.1365-2745.2006.01158.x.

Bowman EA, Plowes RM, Gilbert LE. 2023. Evidence of plant-soil feedback in South Texas grasslands associated with invasive Guinea grass. *NeoBiota* 81:33–51. DOI: 10.3897/NEOBIOTA.81.86672.

Brinkman EP, Raaijmakers CE, de Boer W, van der Putten WH. 2017. Changing soil legacies to direct restoration of plant communities. *AoB PLANTS* 9. DOI: 10.1093/aobpla/plx038.

Canarini A, Schmidt H, Fuchslueger L, Martin V, Herbold CW, Zezula D, Gündler P, Hasibeder R, Jecmenica M, Bahn M, Richter A. 2021. Ecological memory of recurrent drought modifies soil processes via changes in soil microbial community. *Nature Communications* 12:5308. DOI: 10.1038/s41467-021-25675-4.

Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW. 2010. Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Applied and Environmental Microbiology* 76:999–1007. DOI: 10.1128/AEM.02874-09.

Chen W, Wang J, Meng Z, Xu R, Chen J, Zhang Y, Hu T. 2019. Fertility-related interplay between fungal guilds underlies plant richness–productivity relationships in natural grasslands. *New Phytologist* 226:1129–1143. DOI: 10.1111/nph.16390.

Chen D, Xi N, Cadotte M, Wu H, Chu C. 2021. Soil legacies and drought regulate plant diversity-productivity relationships. *Authorea Preprints*. DOI: 10.22541/AU.161407743.30028199/V1.

Cheng F, Cheng Z. 2015. Research Progress on the use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. *Frontiers in Plant Science* 6:1020. DOI: 10.3389/fpls.2015.01020.

Cheng H, Wang S, Wei M, Yu Y, Wang C. 2021. Alien invasive plant Amaranthus spinosus mainly altered the community structure instead of the α diversity of soil N-fixing bacteria under drought. *Acta Oecologica* 113:103788. DOI: 10.1016/j.actao.2021.103788.

Chung YA, Collins SL, Rudgers JA. 2019. Connecting plant–soil feedbacks to long-term stability in a desert grassland. *Ecology* 100:e02756. DOI: 10.1002/ecy.2756.

Coats VC, Rumpho ME. 2014. The rhizosphere microbiota of plant invaders: an overview of recent advances in the microbiomics of invasive plants. *Frontiers in Microbiology* 5:368. DOI: 10.3389/fmicb.2014.00368.

Cole RJ, Soper FM, Litton CM, Knauf AE, Sparks K, Gerow KG, Giardina CP, Sparks JP. 2021. Restoration benefits of soil nutrient manipulation and weeding in invaded dry and wet tropical ecosystems in Hawai‘i. DOI: 10.1111/rec.13390.

Craine JM, Ocheltree TW, Nippert JB, Towne EG, Skibbe AM, Kembel SW, Fargione JE. 2013. Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change* 3:63–67. DOI: 10.1038/nclimate1634.

Cuddihy LW, Stone CP. 1990. Alteration of native Hawaiian vegetation. *University of Hawaii Cooperative National Park Study Unit*.

Delavaux CS, Smith-Ramesh LM, Kuebbing SE. 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* 98:2111–2119. DOI: 10.1002/ecy.1892.

Díaz-Vallejo EJ, Seeley M, Smith AP, Marín-Spiotta E. 2021. A meta-analysis of tropical land-use change effects on the soil microbiome: Emerging patterns and knowledge gaps. *Biotropica* 53:738–752. DOI: 10.1111/btp.12931.

Dickie IA, Bufford JL, Cobb RC, Desprez-Loustau ML, Grelet G, Hulme PE, Klironomos J, Makiola A, Nuñez MA, Pringle A, Thrall PH, Tourtellot SG, Waller L, Williams NM. 2017. The emerging science of linked plant–fungal invasions. *New Phytologist* 215:1314–1332. DOI: 10.1111/nph.14657.

Ding J, Travers SK, Eldridge DJ. 2022. Microbial communities are associated with indicators of soil surface condition across a continental gradient. *Geoderma* 405:115439. DOI: 10.1016/j.geoderma.2021.115439.

Dostál P. 2021. The temporal development of plant-soil feedback is contingent on competition and nutrient availability contexts. *Oecologia* 1:3. DOI: 10.1007/s00442-021-04919-6.

Drenovsky RE, Grewell BJ, D’Antonio CM, Funk JL, James JJ, Molinari N, Parker IM, Richards CL. 2012. A functional trait perspective on plant invasion. *Annals of Botany* 110:141–153. DOI: 10.1093/aob/mcs100.

Dudenhöffer JH, Ebeling A, Klein AM, Wagg C. 2018. Beyond biomass: Soil feedbacks are transient over plant life stages and alter fitness. *Journal of Ecology* 106:230–241. DOI: 10.1111/1365-2745.12870.

Duell EB, Zaiger K, Bever JD, Wilson GWT. 2019. Climate Affects Plant-Soil Feedback of Native and Invasive Grasses: Negative Feedbacks in Stable but Not in Variable Environments. *Frontiers in Ecology and Evolution* 7:419. DOI: 10.3389/fevo.2019.00419.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME Journal* 4:337–345. DOI: 10.1038/ismej.2009.122.

Escalas A, Hale L, Voordeckers JW, Yang Y, Firestone MK, Alvarez-Cohen L, Zhou J. 2019. Microbial functional diversity: From concepts to applications. *Ecology and Evolution* 9:12000–12016. DOI: 10.1002/ece3.5670.

Del Fabbro C, Prati D. 2015. The relative importance of immediate allelopathy and allelopathic legacy in invasive plant species. *Basic and Applied Ecology* 16:28–35. DOI: 10.1016/j.baae.2014.10.007.

Fierer N, Schimel JP, Holden PA. 2003. Influence of Drying-Rewetting Frequency on Soil Bacterial Community Structure. *Microbial Ecology* 45:63–71. DOI: 10.1007/s00248-002-1007-2.

Forero LE, Grenzer J, Heinze J, Schittko C, Kulmatiski A. 2019. Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated. *Frontiers in Environmental Science* 7. DOI: 10.3389/fenvs.2019.00184.

Frey M, Schullehner K, Dick R, Fiesselmann A, Gierl A. 2009. Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* 70:1645–1651. DOI: 10.1016/j.phytochem.2009.05.012.

Gaertner M, Biggs R, Te Beest M, Hui C, Molofsky J, Richardson DM. 2014. Invasive plants as drivers of regime shifts: Identifying high-priority invaders that alter feedback relationships. *Diversity and Distributions* 20:733–744. DOI: 10.1111/ddi.12182.

Gan H, Emmett BD, Drinkwater LE. 2021. Soil management legacy alters weed-crop competition through biotic and abiotic pathways. *Plant and Soil*:1–18. DOI: 10.1007/s11104-021-04891-3.

Garbeva P, van Veen JA, van Elsas JD. 2004. Microbial Diversity in Soil: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. *Annual Review of Phytopathology* 42:243–270. DOI: 10.1146/annurev.phyto.42.012604.135455.

Grenzer J, Kulmatiski A, Forero L, Ebeling A, Eisenhauer N, Norton J. 2021. Placing plant-soil feedbacks in the context of plant community growth. *Authorea Preprints*. DOI: 10.22541/AU.161871517.76220563/V1.

Hale AN, Kalisz S. 2012. Perspectives on allelopathic disruption of plant mutualisms: a framework for individual- and population-level fitness consequences. *Plant Ecology* 213:1991–2006. DOI: 10.1007/s11258-012-0128-z.

Hall EK, Bernhardt ES, Bier RL, Bradford MA, Boot CM, Cotner JB, del Giorgio PA, Evans SE, Graham EB, Jones SE, Lennon JT, Locey KJ, Nemergut D, Osborne BB, Rocca JD, Schimel JP, Waldrop MP, Wallenstein MD. 2018. Understanding how microbiomes influence the systems they inhabit. *Nature Microbiology* 3:977–982. DOI: 10.1038/s41564-018-0201-z.

Harris JA. 2003. Measurements of the soil microbial community for estimating the success of restoration. *European Journal of Soil Science* 54:801–808. DOI: 10.1046/j.1351-0754.2003.0559.x.

Hassan K, Carrillo Y. 2021. The Effect of Prolonged Drought Legacies on Plant-Soil Feedbacks. DOI: 10.21203/rs.3.rs-282547/v1.

Heinen R, Hannula SE, De Long JR, Huberty M, Jongen R, Kielak A, Steinauer K, Zhu F, Bezemer TM. 2020. Plant community composition steers grassland vegetation via soil legacy effects. *Ecology Letters* 23:973–982. DOI: 10.1111/ele.13497.

Hernández E, Shaw EA, Aoyama L, Brambila A, Niederer C, Weiss SB, Hallett LM. 2021. Fire versus grazing as tools to restore serpentine grasslands under global change. *Restoration Ecology*:e13353. DOI: 10.1111/rec.13353.

Hilbig BE, Allen EB. 2015. Plant-soil feedbacks and competitive interactions between invasive Bromus diandrus and native forb species. *Plant and Soil* 392:191–203. DOI: 10.1007/s11104-015-2451-3.

Hood-Nowotny R, Schmittner K, Ziss E, Leitner S, Watzinger A, Gorfer M, Djukic I, Dirnböck T. 2021. Functional response of an Austrian forest soil to N addition. *Environ. Res. Commun* 3:25001. DOI: 10.1088/2515-7620/abe8c7.

Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, Van Der Heijden MGA, Schlaeppi K, Erb M. 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* 9:1–13. DOI: 10.1038/s41467-018-05122-7.

Hughes RF, Asner GP, Litton CM, Selmants PC, Hawbaker TJ, Jacobi JD, Giardina CP, Sleeter BM. 2017. Influence of invasive species on carbon storage in Hawai‘i’s ecosystems. *Baseline and projected future carbon storage and carbon fluxes in ecosystems of Hawai’i. U.S. Geological Survey Professional Paper 1834. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey: 43-55. Chapter 4* 1834:43–55.

Inderjit, Seastedt TR, Callaway RM, Pollock JL, Kaur J. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. *Biological Invasions* 10:875–890. DOI: 10.1007/s10530-008-9239-9.

Isobe K, Bouskill NJ, Brodie EL, Sudderth EA, Martiny JBH. 2020. Phylogenetic conservation of soil bacterial responses to simulated global changes. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 375:20190242. DOI: 10.1098/rstb.2019.0242.

Jiao S, Yang Y, Xu Y, Zhang J, Lu Y. 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME Journal* 14:202–216. DOI: 10.1038/s41396-019-0522-9.

Jin Y, Zeng S, Kaniz F, Zheng W, LaManna J, Bais H, Jin Y, Zeng S, Kaniz F, Zheng W, LaManna J, Bais H. 2020. Rhizobacteria Mediated Changes in Soil Physical and Hydrological Properties. In: *EGU General Assembly*. Online, 11651. DOI: 10.5194/egusphere-egu2020-11651.

Kalisz S, Kivlin SN, Bialic-Murphy L. 2021. Allelopathy is pervasive in invasive plants. *Biological Invasions* 23:367–371. DOI: 10.1007/s10530-020-02383-6.

Kardol P, Cornips NJ, Van Kempen MML, Bakx-Schotman JMT, Van Der Putten WH. 2007. Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs* 77:147–162. DOI: 10.1890/06-0502.

Klironomos JN. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417:67–70. DOI: 10.1038/417067a.

Konopka A. 2009. What is microbial community ecology. *ISME Journal* 3:1223–1230. DOI: 10.1038/ismej.2009.88.

Koplitz SN, Nolte CG, Sabo RD, Clark CM, Horn KJ, Thomas RQ, Newcomer-Johnson TA. 2021. The contribution of wildland fire emissions to deposition in the U S: implications for tree growth and survival in the Northwest. *Environmental Research Letters* 16:024028. DOI: 10.1088/1748-9326/ABD26E.

Kulmatiski A. 2011. Changing Soils to Manage Plant Communities: Activated Carbon as a Restoration Tool in Ex-arable Fields. *Restoration Ecology* 19:102–110. DOI: 10.1111/j.1526-100X.2009.00632.x.

LaForgia ML, Harrison SP, Latimer AM. 2020. Invasive species interact with climatic variability to reduce success of natives. *Ecology* 101. DOI: 10.1002/ecy.3022.

Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzländer M, Strauss SY, Hufbauer RA. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist* 178:412–423. DOI: 10.1111/j.1469-8137.2007.02360.x.

Lauber CL, Ramirez KS, Aanderud Z, Lennon J, Fierer N. 2013. Temporal variability in soil microbial communities across land-use types. *ISME Journal* 7:1641–1650. DOI: 10.1038/ismej.2013.50.

Lekberg Y, Bever JD, Bunn RA, Callaway RM, Hart MM, Kivlin SN, Klironomos J, Larkin BG, Maron JL, Reinhart KO, Remke M, van der Putten WH. 2018. Relative importance of competition and plant-soil feedback, their synergy, context dependency and implications for coexistence. *Ecology Letters* 21:1268–1281. DOI: 10.1111/ele.13093.

Leopold CR, Hess SC. 2019. Facilitating adaptation to climate change while restoring a montane plant community. *PLOS ONE* 14:e0218516. DOI: 10.1371/JOURNAL.PONE.0218516.

Levine JM, Pachepsky E, Kendall BE, Yelenik SG, Lambers JHR. 2006. Plant-soil feedbacks and invasive spread. *Ecology Letters* 9:1005–1014. DOI: 10.1111/j.1461-0248.2006.00949.x.

Li Z, Wang F, Su F, Wang P, Li S, Bai T, Wei Y, Liu M, Chen D, Zhu W, Eviner V, Wang Y, Hu S. 2021. Climate change drivers alter root controls over litter decomposition in a semi-arid grassland. *Soil Biology and Biochemistry* 158:108278. DOI: 10.1016/j.soilbio.2021.108278.

Linder HP, Lehmann CER, Archibald S, Osborne CP, Richardson DM. 2018. Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. *Biological Reviews* 93:1125–1144. DOI: 10.1111/brv.12388.

López Zieher XM, Vivanco L, Yahdjian L. 2020. Soil bacterial communities remain altered after 30 years of agriculture abandonment in Pampa grasslands. *Oecologia* 193:959–968. DOI: 10.1007/s00442-020-04736-3.

Madsen MD, Davies KW, Mummey DL, Svejcar TJ. 2014. Improving restoration of exotic annual grass-invaded rangelands through activated carbon seed enhancement technologies. *Rangeland Ecology and Management* 67:61–67. DOI: 10.2111/REM-D-13-00050.1.

Menezes LS, de Oliveira AM, Santos FLM, Russo A, de Souza RAF, Roque FO, Libonati R. 2022. Lightning patterns in the Pantanal: Untangling natural and anthropogenic-induced wildfires. *Science of The Total Environment* 820:153021. DOI: 10.1016/J.SCITOTENV.2022.153021.

Miller EC, Perron GG, Collins CD. 2019. Plant-driven changes in soil microbial communities influence seed germination through negative feedbacks. *Ecology and Evolution* 9:9298–9311. DOI: 10.1002/ece3.5476.

Morales SE, Cosart T, Holben WE. 2010. Bacterial gene abundances as indicators of greenhouse gas emission in soils. *The ISME Journal* 4:799–808. DOI: 10.1038/ismej.2010.8.

Münzbergová Z, Šurinová M. 2015. The importance of species phylogenetic relationships and species traits for the intensity of plant-soil feedback. *Ecosphere* 6:art234. DOI: 10.1890/ES15-00206.1.

Niculaes C, Abramov A, Hannemann L, Frey M. 2018. Plant Protection by Benzoxazinoids—Recent Insights into Biosynthesis and Function. *Agronomy* 8:143. DOI: 10.3390/agronomy8080143.

Nolan NE, Kulmatiski A, Beard KH, Norton JM. 2015. Activated carbon decreases invasive plant growth by mediating plant - Microbe interactions. *AoB PLANTS* 7. DOI: 10.1093/aobpla/plu072.

Nsikani MM, van Wilgen BW, Gaertner M. 2018. Barriers to ecosystem restoration presented by soil legacy effects of invasive alien N 2 -fixing woody species: implications for ecological restoration. *Restoration Ecology* 26:235–244. DOI: 10.1111/rec.12669.

Oliveira EM de, Martuscello JA, Jank L, Cunha D de NFV da, Santos MF. 2022. Evaluation of Megathyrsus maximus genotypes under water stress conditions. *Acta Scientiarum. Animal Sciences* 44:e54975. DOI: 10.4025/actascianimsci.v44i1.54975.

Orbán I, Szitár K, Kalapos T, Körel-Dulay G. 2021. The role of disturbance in invasive plant establishment in a changing climate: insights from a drought experiment. *Biological Invasions*:1–14. DOI: 10.1007/s10530-021-02478-8.

Perkins LB, Hatfield G. 2016. Can commercial soil microbial treatments remediate plant-soil feedbacks to improve restoration seedling performance? *Restoration Ecology* 24:194–201. DOI: 10.1111/rec.12302.

Perkins LB, Hatfield G, Espeland EK. 2016. Invasive grasses consistently create similar plant-soil feedback types in soils collected from geographically distant locations. *Journal of Plant Ecology* 9:180–186. DOI: 10.1093/jpe/rtv040.

Perkins LB, Nowak RS. 2013. Native and non-native grasses generate common types of plant-soil feedbacks by altering soil nutrients and microbial communities. *Oikos* 122:199–208. DOI: 10.1111/j.1600-0706.2012.20592.x.

Perreault L, Forrester JA, Wurzburger N, Mladenoff DJ. 2020. Emergent properties of downed woody debris in canopy gaps: A response of the soil ecosystem to manipulation of forest structure. *Soil Biology and Biochemistry* 151:108053. DOI: 10.1016/j.soilbio.2020.108053.

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, van de Voorde TFJ, Wardle DA. 2013. Plant-soil feedbacks: the past, the present and future challenges. *Journal of Ecology* 101:265–276. DOI: 10.1111/1365-2745.12054.

van der Putten WH, Bradford MA, Pernilla Brinkman E, van de Voorde TFJ, Veen GF. 2016. Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology* 30:1109–1121. DOI: 10.1111/1365-2435.12657.

Rhodes AC, Plowes RM, Goolsby JA, Gaskin JF, Musyoka B, Calatayud PA, Cristofaro M, Grahmann ED, Martins DJ, Gilbert LE. 2021. The dilemma of Guinea grass (Megathyrsus maximus): a valued pasture grass and a highly invasive species. *Biological Invasions* 23:3653–3669. DOI: 10.1007/S10530-021-02607-3/FIGURES/3.

Scavo A, Abbate C, Mauromicale G. 2019. Plant allelochemicals: agronomic, nutritional and ecological relevance in the soil system. *Plant and Soil 2019 442:1* 442:23–48. DOI: 10.1007/S11104-019-04190-Y.

Scheffer M, Carpenter S, Foley JA, Folke C, Walker B. 2001. Catastrophic shifts in ecosystems. *Nature 2001 413:6856* 413:591–596. DOI: 10.1038/35098000.

Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkel LL, Reich PB, Xiao K, Rillig MC, Sikes BA, Callaway RM, Mangan SA, Van Nes EH, Scheffer M. 2011. Soil microbes drive the classic plant diversity-productivity pattern. *Ecology* 92:296–303. DOI: 10.1890/10-0773.1.

Shan J, Ji R, Yu Y, Xie Z, Yan X. 2015. Biochar, activated carbon, and carbon nanotubes have different effects on fate of 14 C-catechol and microbial community in soil. *Scientific Reports* 5:16000. DOI: 10.1038/srep16000.

Singh M, Meyer WM. 2020. Plant-Soil Feedback Effects on Germination and Growth of Native and Non-Native Species Common across Southern California. *Diversity* 12:217. DOI: 10.3390/d12060217.

Skurski TC, Rew LJ, Maxwell BD. 2014. Mechanisms Underlying Nonindigenous Plant Impacts: A Review of Recent Experimental Research. *Invasive Plant Science and Management* 7:432–444. DOI: 10.1614/ipsm-d-13-00099.1.

Solans M, Pelliza YI, Tadey M. 2021. Inoculation with Native Actinobacteria May Improve Desert Plant Growth and Survival with Potential Use for Restoration Practices. *Microbial Ecology*:1–13. DOI: 10.1007/s00248-021-01753-4.

Suding KN, Stanley Harpole W, Fukami T, Kulmatiski A, Macdougall AS, Stein C, van der Putten WH. 2013. Consequences of plant-soil feedbacks in invasion. *Journal of Ecology* 101:298–308. DOI: 10.1111/1365-2745.12057.

Suseela V, Alpert P, Nakatsu CH, Armstrong A, Tharayil N. 2016. Plant–soil interactions regulate the identity of soil carbon in invaded ecosystems: implication for legacy effects. *Functional Ecology* 30:1227–1238. DOI: 10.1111/1365-2435.12591.

Tomat-Kelly G, Dillon WW, Flory SL. 2021. Invasive grass fuel loads suppress native species by increasing fire intensity and soil heating. *Journal of Applied Ecology*:1365-2664.13881. DOI: 10.1111/1365-2664.13881.

Trauernicht C, Pickett E, Giardina CP, Litton CM, Cordell S, Beavers A. 2015. The Contemporary Scale and Context of Wildfire in Hawai‘i. *Pacific Science* 69:427–444. DOI: 10.2984/69.4.1.

Trognitz F, Hackl E, Widhalm S, Sessitsch A. 2016. The role of plant–microbiome interactions in weed establishment and control. *FEMS Microbiology Ecology* 92:fiw138. DOI: 10.1093/femsec/fiw138.

Violle C, Navas M-L, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E. 2007. Let the concept of trait be functional! *Oikos* 116:882–892. DOI: 10.1111/j.0030-1299.2007.15559.x.

de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Hallin S, Kaisermann A, Keith AM, Kretzschmar M, Lemanceau P, Lumini E, Mason KE, Oliver A, Ostle N, Prosser JI, Thion C, Thomson B, Bardgett RD. 2018. Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications* 9:1–12. DOI: 10.1038/s41467-018-05516-7.

de Vries FT, Williams A, Stringer F, Willcocks R, McEwing R, Langridge H, Straathof AL. 2019. Changes in root-exudate-induced respiration reveal a novel mechanism through

which drought affects ecosystem carbon cycling. *New Phytologist* 224:132–145. DOI: 10.1111/nph.16001.

Wang Z, Li T, Li Y, Zhao D, Han J, Liu Y, Liao Y. 2020. Relationship between the microbial community and catabolic diversity in response to conservation tillage. *Soil and Tillage Research* 196:104431. DOI: 10.1016/j.still.2019.104431.

Wang KH, McSorley R, Bohlen P, Gathumbi SM. 2006. Cattle grazing increases microbial biomass and alters soil nematode communities in subtropical pastures. *Soil Biology and Biochemistry* 38:1956–1965. DOI: 10.1016/j.soilbio.2005.12.019.

Westerling AL, Bryant BP, Preisler HK, Holmes TP, Hidalgo HG, Das T, Shrestha SR. 2011. Climate change and growth scenarios for California wildfire. *Climatic Change 2011 109:1* 109:445–463. DOI: 10.1007/S10584-011-0329-9.

Wubs ERJ, Melchers PD, Bezemer TM. 2018. Potential for synergy in soil inoculation for nature restoration by mixing inocula from different successional stages. *Plant and Soil* 433:147–156. DOI: 10.1007/s11104-018-3825-0.

Wurst S, Vender V, Rillig MC. 2010. Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecology* 211:19–26. DOI: 10.1007/s11258-010-9767-0.

Xi N, Chu C, Bloor JMG. 2018. Plant drought resistance is mediated by soil microbial community structure and soil-plant feedbacks in a savanna tree species. *Environmental and Experimental Botany* 155:695–701. DOI: 10.1016/j.envexpbot.2018.08.013.

Yang Y, Li T, Wang Y, Cheng H, Chang SX, Liang C, An S. 2021. Negative effects of multiple global change factors on soil microbial diversity. *Soil Biology and Biochemistry* 156:108229. DOI: 10.1016/J.SOILBIO.2021.108229.

Yelenik SG, D'Antonio CM. 2013. Self-reinforcing impacts of plant invasions change over time. *Nature* 503:517–520. DOI: 10.1038/nature12798.

Yelenik SG, Levine JM. 2010. Native shrub reestablishment in exotic annual grasslands: Do ecosystem processes recover? *Ecological Applications* 20:716–727. DOI: 10.1890/08-2365.1.

Yelenik SG, Levine JM. 2011. The role of plant-soil feedbacks in driving native-species recovery. *Ecology* 92:66–74. DOI: 10.1890/10-0465.1.

Zhang X, Oduor AMO, Liu Y. 2022. Invasive plants have greater growth than co-occurring natives in live soil subjected to a drought-rewetting treatment. *Functional Ecology*. DOI: 10.1111/1365-2435.14244.

Zhang Z, Suseela V. 2021. Nitrogen availability modulates the impacts of plant invasion on the chemical composition of soil organic matter. *Soil Biology and Biochemistry* 156:108195. DOI: 10.1016/j.soilbio.2021.108195.

Zhou Z, Wang C, Luo Y. 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. *Nature Communications 2020 11:1* 11:1–10. DOI: 10.1038/S41467-020-16881-7.

Zhu Y, Delgado-Baquerizo M, Shan D, Yang X, Eldridge DJ. 2021a. Grazing impacts on ecosystem functions exceed those from mowing. *Plant and Soil*:1–13. DOI: 10.1007/s11104-021-04970-5.

Zhu TR, Litton CM, Giardina CP, Trauernicht C. 2021b. Moisture availability and ecological restoration limit fine fuels and modeled wildfire intensity following nonnative ungulate removal in Hawaii. *Journal of Applied Ecology*:1365-2664.13952. DOI: 10.1111/1365-2664.13952.

Table 1.1. Factors influencing PSF that we controlled for or examined, based on research need.

Controlled for	• **Plant lifestage:** direction (Dudenhöffer et al., 2018), intensity (Münzbergová & Šurinová, 2015) can vary. Examining influence on germination and/or seedling/germling establishment only (Miller, Perron & Collins, 2019). • **Competition:** can correlate with dominance (Grenzer et al., 2021), can outweigh influence of PSF (Yelenik & Levine, 2011), differs between monoculuture and interspecies mixture (Hilbig & Allen, 2015). Direct competition removed/minimized. • **Nutrient availability:** can influence direction (Lekberg et al., 2018), fertilizer can reduce negative intraspecific PSF (Dostál, 2021). Did not use sterilization method for greenhouse study which can increase nutrient availability (Singh & Meyer, 2020).
Research needs examined	• **Mechanisms (allelopathy, Ch. 2; soil microbial community, Ch. 3; both, Ch. 5):** need to examine and quantify specific mechanisms (Bennett & Klironomos, 2019), connect mechanisms to how invasive plants may respond to climate change (van der Putten et al., 2013). • **Variation in precipitation (Ch. 3):** can vary based on precipitation regime (Yelenik & Levine, 2011). • **Field study (Ch. 5):** discrepancy between greenhouse and field experiments (Forero et al. 2019, Beals et al. 2020, among others), need for field research utilizing 'natural' conditions (Yelenik & Levine, 2010; van der Putten et al., 2016). • **Applications (Ch. 5):** need to translate analysis of the soil microbial community into improved management and restoration practices against invasive plants (Balser et al., 2006; Coats & Rumpho, 2014; Trognitz et al., 2016).

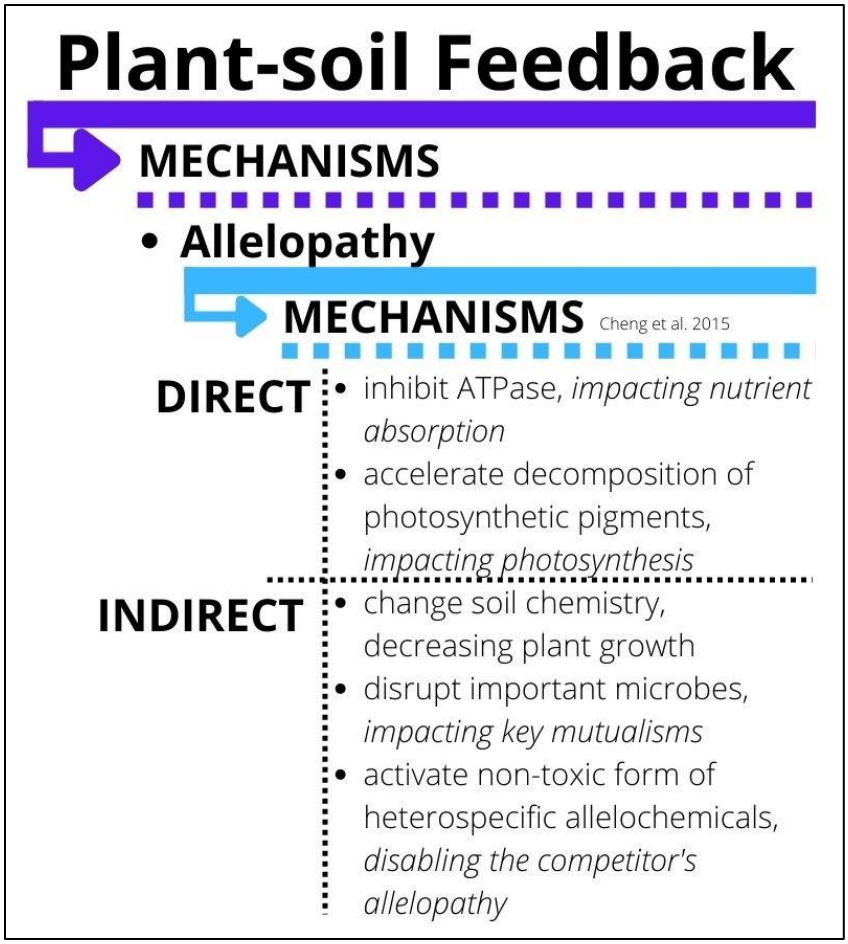

Figure 1.1. Examples of direct and indirect mechanisms of allelopathy, which is a mechanism of plant-soil feedback. Based on Cheng et al. 2015.

CHAPTER 2

META-ANALYTIC EVIDENCE THAT ALLELOPATHY MAY INCREASE THE SUCCESS AND IMPACT OF INVASIVE GRASSES

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Abstract

Background. In the grass family, a disproportionate number of species have been designated as being invasive. Various growth traits have been proposed to explain the invasiveness of grasses, however the possibility that allelopathy gives invasive grasses a competitive advantage has attracted relatively little attention. Recent research has isolated plant allelochemicals that are mostly specific to the grass family that can breakdown into relatively stable, toxic byproducts.
Methods. We conducted a meta-analysis of studies on grass allelopathy to test three prominent hypotheses from invasion biology and competition theory: 1) on native recipients, non-native grasses will have a significantly more negative effect compared to native grasses (novel weapons hypothesis); 2) among native grasses, their effect on non-native recipients will be significantly more negative compared to their effect on native recipients (biotic resistance hypothesis), and 3) allelopathic impacts will increase with phylogenetic distance (phylogenetic distance hypothesis). From 23 studies, we gathered a dataset of 524 observed effect sizes (delta log response ratios) measuring the allelopathic impact of grasses on growth and germination of recipient species, and we used non-linear mixed-effects Bayesian modeling to test the hypotheses.
Results. We found support for the novel weapons hypothesis: on native recipients, non-native grasses were twice as suppressive as native grasses (22% versus 11%, respectively). The phylogenetic distance hypothesis was supported by our finding of a significant correlation between phylogenetic distance and allelopathic impact. The biotic resistance hypothesis was not supported. Overall, this meta-analysis adds to the evidence that allelochemicals may commonly contribute to successful or high impact invasions in the grass family. Increased awareness of the role of allelopathy in soil legacy effects associated with grass invasions may improve restoration outcomes through implementation of allelopathy-informed restoration practices. Examples of allelopathy-informed practices, and the knowledge needed to utilize them effectively, are discussed, including the use of activated carbon to neutralize allelochemicals and modify the soil microbial community.

Introduction

As a prime example of anthropogenic change, grasses have been deliberately moved by human civilizations, often to feed livestock (D'Antonio & Vitousek, 1992; Fusco et al., 2021), and their invasive spread has devastated many ecosystems (Marshall et al., 2011; Wied et al., 2020; Kerns et al., 2020; Rhodes et al., 2021; Rayment, French & Bedward, 2022). The spread of non-native grasses can diminish native biodiversity by forming monocultures and modifying soil characteristics and nutrient cycling (Perkins, Johnson & Nowak, 2011; Gibbons et al., 2017; Wied et al., 2020; Musso et al., 2021; Nagy et al., 2021; Soti & Thomas, 2021). Non-native grasses may benefit from aspects of global change, including wildfire (Davies et al., 2022), drought (Leal et al., 2021; Sommers, Davis & Chesson, 2022), and nitrogen deposition (Cione, Padgett & Allen, 2002; Sigüenza, Corkidi & Allen, 2006). Non-native grass establishment can lead to increased wildfire frequency and/or intensity (D'Antonio & Vitousek, 1992; Fusco et al., 2019; Tomat-Kelly, Dillon & Flory, 2021; Walker & Morgan, 2022), and shortened fire cycles can push an ecosystem past the threshold of passive recovery (D'Antonio, Hughes & Tunison, 2011), which substantially increases costs of restoration and adds urgency to restoration planning in areas recently invaded by grasses.

Native (Hierro & Callaway, 2021), invasive (Kalisz, Kivlin & Bialic-Murphy, 2021) and domesticated/crop grasses (Niculaes et al., 2018) are reported to have allelopathic abilities. Across plant groups, allelochemicals differ in chemical structure and impart impacts through different mechanisms (Cheng & Cheng, 2015), but researchers have identified benzoxazinoids as allelochemicals that have been phylogenetically conserved within the Poaceae family (Frey et al., 2009; Dutartre, Hilliou & Feyereisen, 2012; Niculaes et al., 2018), with evidence supporting independent or convergent evolution of benzoxazinoids in some dicots (Schullehner et al., 2008; Dick et al., 2012). When considered together, the evidence of shared allelochemicals, disproportionate invasion success and impacts (Linder et al., 2018) and the large number of grass species, the grass allelopathy literature provides a unique opportunity to test important hypotheses in invasion biology and draw conclusions that can inform real world practices used to reduce the impacts of invasive grasses.

The aim of this meta-analysis was to test whether three key invasion biology theories are supported by studies investigating potential allelopathic abilities in grasses. First, we tested if the Novel Weapons Hypothesis (Callaway, 2000; Hierro & Callaway, 2003; Callaway &

Ridenour, 2004) was supported (on native recipient species, effect size of non-native grass < native grass). Second, we tested if the Biotic Resistance Hypothesis (D'Antonio & Thomsen, 2004; Cummings, Parker & Gilbert, 2012) was supported (for native grasses, effect size associated with non-native recipients < native recipients, assuming the native grass is an important contributor to native community resistance). Finally, we tested the hypothesis that increased phylogenetic distance is associated with increased allelopathic impact due to expected greater similarities in secondary chemicals among closer relatives and presumed resistance to self-produced allelochemicals (Phylogenetic Distance Hypothesis, co-efficient of smoothed phylogenetic distance < 0) (Wink, 2003; Zhang et al., 2020b).

Materials & Methods

In our comprehensive search, three terms were used in database searches to identify studies to be included in the invasive grass allelopathy metanalysis: "invas*", "allelo*" and "grass", where "*" indicated a wildcard character. Thus, agriculture-focused research was considered only if it was presented in the context of invasion. In May 2021, multiple search engines were used to identify relevant studies for use in the meta-analysis: Web of Science, SpringerLink, EBSCO, PubMed, Google Scholar and JSTOR (Figure 2.1). Specific journals were also searched: *Journal of Chemical Ecology* and *Plant and Soil* to allow searching a longer timeframe in these journals which have been historically popular for allelopathy research. Additionally, studies used in the Zhang et al. (2020b) meta-analysis (which included all volumes of *Allelopathy Journal*) that used grasses as the allelopathy species (species being tested for allelopathic potential) were included, but data from these studies was procured independently from each article to ensure that the methodology of extracting data remained consistent across all studies. Manya Singh performed the search strategy, and any disagreements were discussed between Manya Singh and Curt Daehler until a consensus was reached. This initial screening resulted in 477 studies, and after filtering for studies that included methodology that met criteria for inferring allelopathy (as described by Zhang et al. 2020b), grass species as the source of potential allelopathic abilities (referred to here as the 'allelopathy species'), ecological context of invasion, and separate reporting of control and test condition data with standard deviations or standard errors, 23 studies were left (Rasmussen & Rice, 1971; Rice, 1972; Orr, Rudgers & Clay, 2005; Blank & Sforza, 2007; Barbosa, Pivello &

Meirelles, 2008; Navarro-Cano, 2008; Rudgers & Orr, 2009; Hussain, Ahmad & Ilahi, 2010; Meksawat & Pornprom, 2010; Harnden, Macdougall & Sikes, 2011; Bennett, Thomsen & Strauss, 2011; Corbett & Morrison, 2012; Ghebrehiwot, Aremu & van Staden, 2014; Greer et al., 2014; Abu-Romman & Ammari, 2015; Ismail, Tan & Chuah, 2015; Perkins, Hatfield & Espeland, 2016; Oliveira et al., 2016; Jose et al., 2016; Uddin et al., 2017; Chen et al., 2018; Możdżeń et al., 2020; Guido et al., 2020).

From each study, we collected the following information: author, year published, table/figure where data are located, name of the allelopathy species (potentially allelopathic species), name of recipient species (species impacted by the allelopathy species), mean, standard error/deviation and sample size for both control and test conditions, lifespan of each species (annual or perennial), origin of each species, experimental method (as categorized by Zhang et al. 2020b), trait measured (germination or growth, for growth, aboveground preferred, then belowground, then total), duration in days, experimental environment (controlled or otherwise), condition of plant material allelochemicals were sourced from (fresh or dry), plant part used to source allelochemicals (aboveground, belowground or mixed source), dose, dose unit type, solvent and solvent polarity. Our use of 'recipient species' instead of 'test species,' which is used in other articles (including Zhang et al. 2020b) to refer to the species exposed to potential allelopathy, is a change made to improve clarity around the species pairs, as across ecology, 'test species' is often used to refer to the species that is of main importance (i.e. not the recipient species, but the species being tested for having or being involved in some key phenomena). Additional details about data collection and the *a priori* power calculator used prior to running the analyses are in the extended methods section (Supplemental File 1).

To account for small sample bias, the delta log response-ratio (delta LRR) formula was used to calculate one "observed" effect size from each pair of control and treatment means (and standard error, sample size) (Lajeunesse, 2015). Two observed effect sizes were dropped because both the control and treatment mean failed the Geary check (Lajuennese 2015, standard formula), indicating that these points violated the assumption of normality. After dropping those points, we were left with a total of 524 observed effect sizes. Of the whole dataset, 23% of pairs lacked a reported dose (or information that could be used to calculate a dose), so the "mice" package was used to impute missing values based on delta LRR, standard error, and all remaining predictors in the model (van Buuren & Groothuis-Oudshoorn, 2011).

From the “mice” function, 25 imputations were run, and for each observation missing a dose value, the median of the 25 imputed dose values was extracted for use in the modeling. Imputation via “mice” was done in place of the “missing values” feature included in the brms package, because dose had to be rounded and converted to a categorical variable to be used as a random effect, which is not supported by that feature. Plant species names were standardized using NCBI (Schoch et al., 2020). The article text and/or external sources were used to determine if each species was native (considered locally indigenous) or non-native. Other predictors collected from each study are listed in Appendix A- Table S1.

In R (R Development Core Team, 2022), analyses utilized the ‘brms’ package for non-linear, mixed-effect, multi-variate Bayesian modeling (Bürkner, 2017, 2018), using the Student’s t-distribution for the error components due to the presence of outliers. Predictors were chosen based on past evidence of significance (Zhang et al., 2020b) and the hypotheses to be tested. The “tree-linked” random variables refer to effects of species constrained by the phylogenetic covariance matrix, as a nested model (‘phyr’ package in R)(Li et al., 2020). The “phytools” package was used to generate the phylogenetic tree used in models (Revell, 2012), and the “aptg” package was used to generate a distance matrix for the full set of plant species (Benjamin, 2017), and the values from the distance matrix were included as a measure of phylogenetic distance in models. Phylogenetic distance was a log-scaled, smoothed term to allow for the model to inherently account for a non-linear relationship with effect size.

The non-linear model separated predictors into a “study” spline, with random effects associated with study design (study ID, nested sub-study, nested trait measured; method category, nested study duration; dose used), and a “species” spline, with random effects that capture species effects (grass and recipient species, and grass and recipient species linked to phylogenetic tree) and fixed effects for our hypotheses (origin status of grass, origin status of recipient species, phylogenetic distance). Past reviews and meta-analyses were referenced to determine which predictors were known to have correlations with allelopathic effect sizes, which we then included as random effects to account for variance (Zhang et al., 2020b).

To deal with the lack of independence among delta LRRs that came from the same study, the “study” spline consisted of random effects study ID (and nested variables sub study, and measured trait), dose (as a categorical variable) and experimental method (based on Zhang et al. (2020b) classification) (and nested variable study duration, as a categorical variable). The

"species" spline consisted of random effects grass species (allelopathy species), recipient species and both species tree-linked. The fixed effects on the "species" spline were origin status of grass (hereafter, grass origin), origin status of recipient species (hereafter, recipient origin), and smoothed, log-scaled phylogenetic distance between the grass species being tested for allelopathy and the recipient species.

Prior to running the full model, an intercept model was run, which did not include any fixed effects. After generating both models, the "loo_compare" function was used to compare the fit of both models, based on both leave-one-out cross validation (LOO) and widely applicable information criteria (WAIC) values (Vehtari, Gelman & Gabry, 2017). The "hypothesis" function was used to test hypotheses at the 95% confidence level. Explained variance was calculated from the posterior sigma estimate (regression noise scale) and standard deviation estimates of each random effect in the intercept model. To check for publication bias, we ran a modified intercept model with log-scaled year published as a smoothed fixed effect in the "study" spline, and an Egger's regression model based on the meta-analytic residuals from the original intercept model.

Results

Power analysis determined that there was sufficient power to find a difference in average allelopathic effect size, based on the number of studies and using Zhang et al (2020b) as the baseline for the difference (86%, Appendix B- Figure S1) (Steidl, Hayes & Schauber, 1997). In the intercept model, the study spline intercept was not significant (0.02, 95% CI [-0.21, 0.25]), but the species spline intercept was significantly negative, with grasses suppressing the growth or germination of the recipient species by approximately 24% (-0.28, 95% CI [-0.52, -0.04]). Around 35% of the variance was explained by study ID and nested variables sub-study and trait measured (15%, 8% and 11%, respectively). One-quarter of the variance was explained by method and nested variable duration (10% and 15% respectively). Another quarter of the variance was explained by grass species and recipient species (9% and 15% respectively). Dose explained 9% of the variance, meaning that only 7% of the variance in the dataset was unexplained at the observation (individual effect size) level. Phylogenetic signal from the tree-linked random effects for either the allelopathy species or the recipient species explained < 1% of the variance. The Egger's test and associated contoured funnel plot of the meta-analytic residuals did not indicate significant publication bias at the p=0.05 level (Appendix B- Figure

S2, y-intercept 95% CI [-0.03, 0.04]). Allelopathic impacts were not significantly related to publication year (Appendix B- Figure S3, y-intercept 95% CI [-0.32, 0.14], slope 95% CI [-0.29, 0.28]). The full model was better than the intercept model by LOO and WAIC criteria (Appendix A- Table S1).

Novel Weapons Hypothesis was supported by the full model (Table 1). For native recipients, non-native grasses on average were almost twice as suppressive (24%) as native grasses (13%). The predicted average effect size of native grasses on native recipients, was weakly significantly different from zero (-0.14, 95% CI [-0.29, 0.02], 90% CI [-0.26, -0.01], Figure 2.2). The predicted average effect size of non-native grasses on native recipients was significantly negative (-0.27, 95% CI [-0.44, -0.09], Figure 2.2).

The Biotic Resistance Hypothesis was rejected by the full model, with weakly significant support for the alternative hypothesis, that native grasses have more negative effects on native recipients compared to non-native recipients, instead of vice versa (0.09, 90% CI [0.02, 0.16], Table 1). On average, native grasses suppressed native recipients 9% more compared to non-native recipients, opposite to expectations for the biotic resistance hypothesis (positive model coefficient, Table 1). The predicted average effect size of native grasses on non-native recipients was not significantly different from zero (-0.05, 95% CI [-0.22, 0.14], with the model finding a 66% predicted probability that the average would be negative (Figure 2.3).

The Phylogenetic Distance Hypothesis was supported by the full model (Table 1). There was a significant negative correlation between smoothed, log-scaled phylogenetic distance and effect size. The co-efficient of a smoothed variable cannot be interpreted directly as magnitude of change between intervals, but from model posteriors, the average allelopathic effect size for conspecific species pairs is closer to zero, compared to other species pairs with increasing phylogenetic distance (Figure 2.4).

Discussion

Support for the novel weapons hypothesis and phylogenetic distance hypothesis

The novel weapons hypothesis (Callaway et al., 2008)(NWH) suggests that a lack of shared evolutionary history between non-native plants and native plants can result in allelochemical production by non-natives that has unusually large impacts on natives. We found that on a native recipient, non-native grasses are twice as suppressive as native grasses,

which supports NWH. Although non-native grasses may directly release allelochemicals that have large impacts on native plants, support for NWH can also be explained by novel microbial communities associated with non-native plants, which may produce novel allelochemicals that the existing soil microbial community (recruited by native plants), has not evolved the ability to degrade (Inderjit et al., 2011; Cipollini, Rigsby & Barto, 2012). The establishment of invasive plants is generally associated with modifications to the soil bacterial community (Torres et al., 2021), which plays a key role in degrading allelochemicals. The identity of the microbe degrading allelochemicals may be significant if different microbes result in different by-products, and stable by-products of allelochemical degradation can be toxic (Macías et al., 2006, 2007; Jilani et al., 2008; Hickman et al., 2021).

In some allelopathy studies, species are studied in a reciprocal design, where each species is examined as both a potential allelopathic and recipient species. The native grasses being studied may have been chosen based on their suspected susceptibility to the soil legacy of non-native grasses, thus resulting in an over-estimation of the impact of non-native grasses. In a reciprocal design, native grasses are tested as both an allelopathic and a recipient species. Only three studies used a native grass as both an allelopathy species and the recipient species of a non-native grass (*Andropogon gerardi* in Greer et al. 2014 and Harnden et al. 2011; *Nassella pulchra* in Chen et al. 2018), and these points comprise just over 7% of the dataset. In a post-hoc analysis, we examined the predicted average allelopathic effect of native grasses *Andropogon gerardi* and *Nasella pulchra* on a native recipient species and found that the average for these grasses was more negative than the overall average (Figure 2.2), suggesting that these grasses do not bias the NWH result by being less allelopathic than other grasses. Alternatively, native grasses used in studies of allelopathy may have been selected as closely related analogs of invasive species (congeneric approach, Inderjit et al. 2008). This type of species selection may bias allelopathic impacts downward. Less than 1% of the data consisted of a species pair where two species were of the same genus (*Eragrostis,* Appendix B- Figure S4), but at the family level, 39% of the data consisted of *Poaceae* pairs. Like other analyses of the allelopathy literature (Zhang et al., 2020b), we found support for an increasing magnitude of allelopathic impact with increasing phylogenetic distance, but the predicted average effect size on the grass recipient species ranged from positive (ex. *Agropyron cristatum)* to negative (ex. *Eragrostis bahiensis)* (Appendix B- Figure S4), indicating a high degree of variation in the

overall statistical pattern of increasing allelopathic impacts with increasing phylogenetic distance. Finally, it is possible that native grasses used in allelopathic studies were chosen based on evidence of their own allelopathic abilities, against native or non-native species, seen in the field, which could result in under-estimation of the difference in impact compared to non-native grasses. Without knowing the intention of each author, it is not possible to determine how common this explanation may be, which highlights how unstated aspects of experimental design can influence our meta-analytic interpretation and understanding of important phenomena.

Biotic resistance hypothesis

The biotic resistance hypothesis (D'Antonio & Thomsen, 2004) suggests that native plants may have stronger impacts on growth and establishment of non-native plants than they do on other native plants. Although biotic resistance is generally discussed in the context of an entire native community, in native plant communities that are locally characterized by one or just a few dominant species (as is often observed in modern native grasslands), a single plant species may be the most important contributors of biotic resistance (Prober & Lunt, 2009; Bennett et al., 2014). The weapons of a native grass would be naïve to a non-native recipient species, so the lack of support for the Biotic Resistance Hypothesis suggests that a difference in mechanism or magnitude of impact of weapons may be a separating feature between grasses that have seen significant range expansion (invasive grasses), and native grasses that have been studied for allelopathy in their native range. Observations of biotic resistance associated with some native grasses may result from other aspects of competition, such as being more resilient to stressors like drought (Conti et al., 2018). Additionally, it is possible that biotic resistance is reliant on soil characteristics, or the degree to which the native soil microbial community has avoided disturbance (disturbance hypothesis, Enders et al., 2020), which may be challenging to replicate in controlled experiments, and, potentially helping to explain the lack of evidence for the biotic resistance hypothesis in our study. Finally, the greatest chance of finding evidence for the biotic resistance hypothesis would be if the native species are dominants in their native communities. In general, we were not able to assess this, and therefore our study provides only a weak test of the biotic resistance hypothesis.

Variance explained by experimental design

The experimental design variables that were included as random effects in the intercept model (study/sub-study/trait, method/duration, dose, species, and tree-linked species, Appendix B-Figure S4 – S9) accounted for over 90% of variance in delta LRR. We included more variables as random effects compared to other meta-analyses of the allelopathy literature (Zhang et al., 2020b). The high level of explained variance may also be attributable to the choice of a Student's t-distribution over a Gaussian distribution for error terms, or to use of non-linear over linear formulation. One source of potential bias for the intercept model could be the imputed values for dose, as dose explained 10% of the variance in delta LRR. The magnitude of explained variance highlights the strength of Bayesian meta-analyses for mixed-effect modeling of complex, non-linear ecological phenomenon that are highly context dependent.

Allelopathy-informed restoration practices

Based on our finding of support for NWH, in non-native grass-invaded areas, practices that account for the impact of allelochemicals may contribute to improved restoration success. Because the impact of allelopathy is dose-dependent, and the concentration of an allelochemical is influenced by soil characteristics and processes (Kobayashi, 2004), amendments and practices that alter these processes may result in an indirect effect on the overall allelopathic effect. For many years, activated carbon was used as a way of neutralizing or ameliorating allelochemical impacts in the field (Callaway, 2000), but recent research suggests that in addition to a direct impact on allelochemicals, activated carbon has a broader impact on plant-soil feedback via modifying soil characteristics (Lau et al., 2008) and shifting the microbial community (Shan et al., 2015; Nolan et al., 2015). This suggests that activated carbon amendments may be useful in disrupting any dis-advantage to native plants created by soil legacy effects caused by allelopathy and altered soil feedback more generally; however it should be noted that carbon amendments do not universally benefit native plants (Zhang et al., 2020a), and that benefit from carbon amendments is better predicted by plant functional traits than native/invasive status (Knauf et al., 2021; Cole et al., 2021). Other options for field amendments to disrupt allelochemicals include re-conditioning the soil by growing another plant less susceptible to the allelochemicals (Li et al., 2017; Schütz et al., 2019); conducting a soil transplant from an area with a healthy native ecosystem or trying to reduce the concentration of allelochemicals with the addition of specific microbes via an inoculum

approach (Gong et al., 2018; He et al., 2020; Kheirabadi et al., 2020). Four allelopathy-informed restoration practices are summarized in Figure 2.5.

Some restoration projects in grass invaded areas have included native grasses based on their potential for resisting invasion through their functional traits (Funk et al., 2008) and/or limiting similarity (Hess et al., 2020), but we did not find support for the biotic resistance hypothesis in our analysis. Support for the phylogenetic distance hypothesis does contribute to evidence supporting the limiting similarity hypothesis, assuming that more closely related species will also share traits that have been evolutionarily conserved. There are, however, concerns about the utility and practicality of basing restoration efforts on the hypothesis that limiting similarity may lead to biotic resistance, due to the challenge of determining the necessary degree of similarity, and due to the specific conditions or amount of time needed for effects of limiting similarity to act (Hess et al., 2020).

Research Needs for Improved Allelopathy-informed Restoration Practices

For some of the allelopathy-informed restoration practices, background knowledge is needed for the practice to be implemented successfully (Figure 2.5). These "knowledge needs" point to areas where there is an urgent need for additional research. Research on the ability of specific microbes to degrade allelochemicals can contribute to the use of microbial inoculum in restoration practices. There are commercial soil amendments that include specific microbes for improving plant growth, so research into these microbes may contribute to similar commercial products that can be specifically targeted towards grass-invaded areas. Research testing the ability of different plant species to "re-culture" grass-invaded soil is also needed, and researchers may want to prioritize testing common resilient native plants or domesticated crop species, as these species may be more accessible for use in the field. Finally, the continued use of activated carbon in a variety of contexts can contribute to an improved understanding of what contexts are appropriate for activated carbon amendments. The consideration and simulation of climate change on the efficacy of allelopathy-informed restoration practices is critical, as there is evidence that some climate events like drought can increase the potency of allelochemicals (Borbély & Dávid, 2008). In addition, innovative communication strategies are needed for research to have meaningful impact on restoration practices outside of academia. Platforms like the Restor Foundation's RESTOR (restor.eco) have been developed during the UN's Decade of Restoration (United Nations, 2020) with the aim of collecting relevant data,

but practitioners may still need to invest substantial time and effort to determine the most appropriate, financially feasible practice for their context.

Conclusions

The rise and fall of allelopathy as a trending research topic has left research gaps, but our findings supporting allelopathy as a potential mechanism that can help explain strong dominance and impact (including legacy effects) by invasive grasses. By highlighting evidence that invasive grasses may often produce allelochemicals, we hope to stimulate further research and promote consideration of allelochemical amelioration strategies after invasive grass removal, as a strategy for producing tangible improvements in conservation and restoration outcomes. It's clear that in the UN Decade of Restoration, the stakes for restoration success are high, and when it comes to the broad impacts of invasive grasses worldwide, allelopathy research presents an important opportunity to make major headway.

References

Abu-Romman S, Ammari T. 2015. Allelopathic effect of *Arundo donax*, a mediterranean invasive grass. *Plant OMICS* 8:287–291.

Barbosa EG, Pivello VR, Meirelles ST. 2008. Allelopathic evidence in *Brachiaria decumbens* and its potential to invade the Brazilian Cerrados. *Brazilian Archives of Biology and Technology* 51:625–631. DOI: 10.1590/S1516-89132008000400021.

Benjamin C. 2017. Package "aptg": Automatic Phylogenetic Tree Generator.

Bennett JA, Stotz GC, Cahill Jr JF, Henrik Bruun Bennett H, corresponding author J, Jr C. 2014. Patterns of phylogenetic diversity are linked to invasion impacts, not invasion resistance, in a native grassland. *Journal of Vegetation Science* 25:1315–1326. DOI: 10.1111/JVS.12199.

Bennett AE, Thomsen M, Strauss SY. 2011. Multiple mechanisms enable invasive species to suppress native species. *American Journal of Botany* 98:1086–1094. DOI: 10.3732/ajb.1000177.

Blank RR, Sforza R. 2007. Plant-soil relationships of the invasive annual grass *Taeniatherum caput-medusae*: A reciprocal transplant experiment. *Plant and Soil* 298:7–19. DOI: 10.1007/s11104-007-9308-3.

Borbély M, Dávid I. 2008. Changeability of allelopathy depending on several factors. *Cereal Research Communications* 36:1383–1386.

Bürkner P-C. 2017. brms : An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software* 80:1–28. DOI: 10.18637/jss.v080.i01.

Bürkner P-C. 2018. Advanced Bayesian multilevel modeling with the R package brms. *The R Journal* 10:395. DOI: 10.32614/RJ-2018-017.

van Buuren S, Groothuis-Oudshoorn K. 2011. mice: Multivariate Imputation by Chained Equations in R. *Journal of Statistical Software* 45:1–67. DOI: 10.18637/JSS.V045.I03.

Callaway RM. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science* 290:521–523. DOI: 10.1126/science.290.5491.521.

Callaway RM, Cipollini D, Barto K, Thelen GC, Hallett SG, Prati D, Stinson K, Klironomos J. 2008. Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native europe. *Ecology* 89:1043–1055. DOI: 10.1890/07-0370.1.

Callaway RM, Ridenour WM. 2004. Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436. DOI: 10.2307/3868432.

Chen BM, D'Antonio CM, Molinari N, Peng SL. 2018. Mechanisms of influence of invasive grass litter on germination and growth of coexisting species in California. *Biological Invasions* 20:1881–1897. DOI: 10.1007/s10530-018-1668-5.

Cheng F, Cheng Z. 2015. Research Progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science* 6:1020. DOI: 10.3389/fpls.2015.01020.

Cione NK, Padgett PE, Allen EB. 2002. Restoration of a native shrubland impacted by exotic grasses, frequent fire, and nitrogen deposition in Southern California. *Restoration Ecology* 10:376–384. DOI: 10.1046/j.1526-100X.2002.02038.x.

Cipollini D, Rigsby CM, Barto EK. 2012. Microbes as targets and mediators of allelopathy in plants. *Journal of Chemical Ecology* 38:714–727. DOI: 10.1007/S10886-012-0133-7/TABLES/1.

Cole RJ, Soper FM, Litton CM, Knauf AE, Sparks K, Gerow KG, Giardina CP, Sparks JP. 2021. Restoration benefits of soil nutrient manipulation and weeding in invaded dry and wet tropical ecosystems in Hawai'i. *Restoration Ecology* 29:e13390. DOI: 10.1111/rec.13390.

Conti L, Block S, Parepa M, Münkemüller T, Wilfried Thuiller |, Acosta ATR, Mark Van Kleunen |, Dullinger S, Essl F, Dullinger I, Moser D, Klonner G, Bossdorf | Oliver, Carboni M. 2018. Functional trait differences and trait plasticity mediate biotic resistance to potential plant invaders. *Journal of Ecology* 106. DOI: 10.1111/1365-2745.12928.

Corbett BF, Morrison JA. 2012. The Allelopathic Potentials of the Non-Native Invasive Plant Microstegium vimineum and the Native Ageratina altissima : Two Dominant Species of the Eastern Forest Herb Layer. *Northeastern Naturalist* 19:297–312. DOI: 10.1656/045.019.0211.

Cummings JA, Parker IM, Gilbert GS. 2012. Allelopathy: A tool for weed management in forest restoration. *Plant Ecology* 213:1975–1989. DOI: 10.1007/s11258-012-0154-x.

D'Antonio CM, Hughes RF, Tunison JT. 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. *Ecological Applications* 21:1617–1628. DOI: 10.1890/10-0638.1.

D'Antonio CM, Thomsen M. 2004. Ecological resistance in theory and practice. *Weed Technology* 18:1572–1577. DOI: 10.1614/0890-037X(2004)018[1572:ERITAP]2.0.CO;2.

D'Antonio CM, Vitousek PM. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual review of ecology and systematics* 23:63–87.

Davies KW, Boyd CS, Bates JD, Hallett LM, Case MF, Svejcar L. 2022. What is driving the proliferation of exotic annual grasses in sagebrush communities? Comparing fire with off-season grazing. *Rangeland Ecology & Management* 82:76–85. DOI: 10.1016/j.rama.2022.02.009.

Dick R, Rattei T, Haslbeck M, Schwab W, Gierl A, Frey M. 2012. Comparative analysis of benzoxazinoid biosynthesis in monocots and dicots: Independent recruitment of stabilization and activation functions. *The Plant Cell* 24:915–928. DOI: 10.1105/tpc.112.096461.

Dutartre L, Hilliou F, Feyereisen R. 2012. Phylogenomics of the benzoxazinoid biosynthetic pathway of Poaceae: gene duplications and origin of the Bx cluster. *BMC evolutionary biology* 12:64. DOI: 10.1186/1471-2148-12-64.

Enders M, Havemann F, Ruland F, Bernard-Verdier M, Catford JA, Gómez-Aparicio L, Haider S, Heger T, Kueffer C, Kühn I, Meyerson LA, Musseau C, Novoa A, Ricciardi A, Sagouis A, Schittko C, Strayer DL, Vilà M, Essl F, Hulme PE, Kleunen M, Kumschick S,

Lockwood JL, Mabey AL, McGeoch MA, Palma E, Pyšek P, Saul W, Yannelli FA, Jeschke JM. 2020. A conceptual map of invasion biology: Integrating hypotheses into a consensus network. *Global Ecology and Biogeography* 29:978–991. DOI: 10.1111/geb.13082.

Frey M, Schullehner K, Dick R, Fiesselmann A, Gierl A. 2009. Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* 70:1645–1651. DOI: 10.1016/j.phytochem.2009.05.012.

Funk JL, Cleland EE, Suding KN, Zavaleta ES. 2008. Restoration through reassembly: plant traits and invasion resistance. *Trends in ecology & evolution* 23:695–703. DOI: 10.1016/j.tree.2008.07.013.

Fusco EJ, Balch JK, Mahood AL, Nagy RC, Syphard AD, Bradley BA. 2021. The human–grass–fire cycle: how people and invasives co-occur to drive fire regimes. *Frontiers in Ecology and the Environment*. DOI: 10.1002/fee.2432.

Fusco EJ, Finn JT, Balch JK, Nagy RC, Bradley BA. 2019. Invasive grasses increase fire occurrence and frequency across US ecoregions. *Proceedings of the National Academy of Sciences* 116:23594–23599. DOI: 10.1073/pnas.1908253116.

Ghebrehiwot HM, Aremu AO, van Staden J. 2014. Evaluation of the allelopathic potential of five South African mesic grassland species. *Plant Growth Regulation* 72:155–162. DOI: 10.1007/s10725-013-9847-y.

Gibbons SM, Lekberg Y, Mummey DL, Sangwan N, Ramsey PW, Gilbert JA. 2017. Invasive plants rapidly reshape soil properties in a grassland ecosystem. *mSystems* 2:e00178-e216. DOI: 10.1128/mSystems.00178-16.

Gong X, Huang D, Liu Y, Peng Z, Zeng G, Xu P, Cheng M, Wang R, Wan J. 2018. Remediation of contaminated soils by biotechnology with nanomaterials: bio-behavior, applications, and perspectives. *Critical Reviews in Biotechnology* 38:455–468. DOI: 10.1080/07388551.2017.1368446.

Greer MJ, Wilson GWT, Hickman KR, Wilson SM. 2014. Experimental evidence that invasive grasses use allelopathic biochemicals as a potential mechanism for invasion: chemical warfare in nature. *Plant and Soil* 385:165–179. DOI: 10.1007/s11104-014-2209-3.

Guido A, Quiñones A, Pereira AL, da Silva ER. 2020. ¿Las gramíneas invasoras Cynodon dactylon y Eragrostis plana son más fitotóxicas que una nativa coexistente? *Ecología Austral* 30:295–303. DOI: 10.25260/EA.20.30.2.0.1090.

Harnden J, Macdougall AS, Sikes BA. 2011. Field-based effects of allelopathy in invaded tallgrass prairie. *Botany* 89:227–234. DOI: 10.1139/b11-009.

He W, Megharaj M, Wu C-Y, Subashchandrabose SR, Dai C-C. 2020. Endophyte-assisted phytoremediation: mechanisms and current application strategies for soil mixed pollutants. *Critical Reviews in Biotechnology* 40:31–45. DOI: 10.1080/07388551.2019.1675582.

Hess MCM, Buisson E, Jaunatre R, Mesléard F. 2020. Using limiting similarity to enhance invasion resistance: Theoretical and practical concerns. *Journal of Applied Ecology* 57:559–565. DOI: 10.1111/1365-2664.13552.

Hickman DT, Rasmussen A, Ritz K, Birkett MA, Neve P. 2021. Review: Allelochemicals as multi-kingdom plant defence compounds: towards an integrated approach. *Pest Management Science* 77:1121–1131. DOI: 10.1002/ps.6076.

Hierro JL, Callaway RM. 2003. Allelopathy and exotic plant invasion. *Plant and Soil* 256:29–39. DOI: 10.1023/A:1026208327014.

Hierro JL, Callaway RM. 2021. The ecological importance of allelopathy. *Annual Review of Ecology, Evolution, and Systematics* 52. DOI: 10.1146/annurev-ecolsys-051120-030619.

Hussain F, Ahmad B, Ilahi I. 2010. Allelopathic effects of *Cenchrus ciliaris* L. and *Bothriochloa pertusa* (L.) A. camus. *Pakistan Journal of Botany* 42:3587–3604.

Inderjit, Seastedt TR, Callaway RM, Pollock JL, Kaur J. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. *Biological Invasions* 10:875–890. DOI: 10.1007/s10530-008-9239-9.

Inderjit, Wardle DA, Karban R, Callaway RM. 2011. The ecosystem and evolutionary contexts of allelopathy. *Trends in ecology & evolution* 26:655–62. DOI: 10.1016/j.tree.2011.08.003.

Ismail BS, Tan PW, Chuah TS. 2015. Assessment of the potential allelopathic effects of Pennisetum purpureum Schumach. On the germination and growth of *Eleusine indica* (L.) Gaertn. *Sains Malaysiana* 44:269–274. DOI: 10.17576/jsm-2015-4402-15.

Jilani G, Mahmood S, Chaudhry AN, Hassan I, Akram M. 2008. Allelochemicals: sources, toxicity and microbial transformation in soil —a review. *Annals of Microbiology* 58:351–357. DOI: 10.1007/BF03175528.

Jose CM, Brandão Torres LM, Torres MAMG, Shirasuna RT, Farias DA, dos Santos NA, Grombone-Guaratini MT. 2016. Phytotoxic effects of phenolic acids from Merostachys

riedeliana, a native and overabundant Brazilian bamboo. *Chemoecology* 26:235–246. DOI: 10.1007/s00049-016-0224-y.

Kalisz S, Kivlin SN, Bialic-Murphy L. 2021. Allelopathy is pervasive in invasive plants. *Biological Invasions* 23:367–371. DOI: 10.1007/s10530-020-02383-6.

Kerns BK, Tortorelli C, Day MA, Nietupski T, Barros AMG, Kim JB, Krawchuk MA. 2020. Invasive grasses: A new perfect storm for forested ecosystems? *Forest Ecology and Management* 463. DOI: 10.1016/J.FORECO.2020.117985.

Kheirabadi M, Azizi M, Taghizadeh SF, Fujii Y. 2020. Recent advances in saffron soil remediation: Activated carbon and zeolites effects on allelopathic potential. *Plants* 9:1714. DOI: 10.3390/plants9121714.

Knauf AE, Litton CM, Cole RJ, Sparks JP, Giardina CP, Gerow KG, Quiñones-Santiago M. 2021. Nutrient-use strategy and not competition determines native and invasive species response to changes in soil nutrient availability. *Restoration Ecology*:e13374. DOI: 10.1111/rec.13374.

Kobayashi K. 2004. Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management* 4:1–7. DOI: 10.1111/j.1445-6664.2003.00112.x.

Lajeunesse MJ. 2015. Bias and correction for the log response ratio in ecological meta-analysis. *Ecology* 96:2056–63. DOI: 10.1890/14-2402.1.

Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzländer M, Strauss SY, Hufbauer RA. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist* 178:412–423. DOI: 10.1111/j.1469-8137.2007.02360.x.

Leal RP, Silveira MJ, Petsch DK, Mormul RP, Thomaz SM. 2021. The success of an invasive Poaceae explained by drought resilience but not by higher competitive ability. *Environmental and Experimental Botany*:104717. DOI: 10.1016/j.envexpbot.2021.104717.

Li D, Dinnage R, Nell L, Helmus MR, Ives A. 2020. phyr: An R package for phylogenetic species-distribution modelling in ecological communities. *bioRxiv*. DOI: 10.1101/2020.02.17.952317.

Li Y-P, Feng Y-L, Kang Z-L, Zheng Y-L, Zhang J-L, Chen Y-J. 2017. Changes in soil microbial communities due to biological invasions can reduce allelopathic effects. *Journal of Applied Ecology* 54:1281–1290. DOI: 10.1111/1365-2664.12878.

Linder HP, Lehmann CER, Archibald S, Osborne CP, Richardson DM. 2018. Global grass (Poaceae) success underpinned by traits facilitating colonization, persistence and habitat transformation. *Biological Reviews* 93:1125–1144. DOI: 10.1111/brv.12388.

Macías FA, Marín D, Oliveros-Bastidas A, Castellano D, Simonet AM, Molinillo JMG. 2006. Structure-activity relationship (SAR) studies of benzoxazinones, their degradation products, and analogues. Phytotoxicity on problematic weeds *Avena fatua* L. and *Lolium rigidum* Gaud. *Journal of agricultural and food chemistry* 54:1040–8. DOI: 10.1021/jf050903h.

Macías FA, Oliveros-Bastidas A, Marín D, Carrera C, Chinchilla N, Molinillo JMG. 2007. Plant biocommunicators: their phytotoxicity, degradation studies and potential use as herbicide models. *Phytochemistry Reviews* 7:179–194. DOI: 10.1007/s11101-007-9062-4.

Marshall NA, Friedel M, van Klinken RD, Grice AC. 2011. Considering the social dimension of invasive species: the case of buffel grass. *Environmental Science & Policy* 14:327–338. DOI: 10.1016/j.envsci.2010.10.005.

Meksawat S, Pornprom T. 2010. Allelopathic effect of itchgrass (*Rottboellia cochinchinensis*) on seed germination and plant growth. *Weed Biology and Management* 10:16–24.

Możdżeń K, Barabasz-Krasny B, Stachurska-Swakoń A, Zandi P, Puła J, Wang Y, Turisova I. 2020. Allelopathic interaction between two common meadow plants: Dactylis glomerata L. and Trifolium pratense L. *Biologia* 75:653–663. DOI: 10.2478/s11756-020-00438-6.

Musso C, Fontenele HGV, Pinto G, Oliveira R, Correia C, Moutinho-Pereira JM, Soares AMVM, Loureiro S. 2021. Effects of water and nutrient availability on morphological, physiological, and biochemical traits of one invasive and one native grass of a Neotropical savanna. *Environmental and Experimental Botany* 182:104305. DOI: 10.1016/j.envexpbot.2020.104305.

Nagy RC, Fusco EJ, Balch JK, Finn JT, Mahood A, Allen JM, Bradley BA. 2021. A synthesis of the effects of cheatgrass invasion on US Great Basin carbon storage. *Journal of Applied Ecology* 58:327–337. DOI: 10.1111/1365-2664.13770.

Navarro-Cano JA. 2008. Effect of grass litter on seedling recruitment of the critically endangered *Cistus heterophyllus* in Spain. *Flora - Morphology, Distribution, Functional Ecology of Plants* 203:663–668. DOI: 10.1016/j.flora.2007.10.008.

Niculaes C, Abramov A, Hannemann L, Frey M. 2018. Plant protection by benzoxazinoids—Recent insights into biosynthesis and function. *Agronomy* 8:143. DOI: 10.3390/agronomy8080143.

Nolan NE, Kulmatiski A, Beard KH, Norton JM. 2015. Activated carbon decreases invasive plant growth by mediating plant - Microbe interactions. *AoB PLANTS* 7. DOI: 10.1093/aobpla/plu072.

Oliveira APP, Pereira SR, Cândido ACS, Laura VA, Peres MTLP. 2016. Gramíneas alelopáticas podem limitar a germinação de sementes e o crescimento de plântulas de Mutambo? Um teste com duas espécies de Braquiárias. *Planta Daninha* 34:639–648. DOI: 10.1590/S0100-83582016340400003.

Orr SP, Rudgers JA, Clay K. 2005. Invasive plants can inhibit native tree seedlings: Testing potential allelopathic mechanisms. *Plant Ecology* 181:153–165. DOI: 10.1007/s11258-005-5698-6.

Perkins LB, Hatfield G, Espeland EK. 2016. Invasive grasses consistently create similar plant-soil feedback types in soils collected from geographically distant locations. *Journal of Plant Ecology* 9:180–186. DOI: 10.1093/jpe/rtv040.

Perkins LB, Johnson DW, Nowak RS. 2011. Plant-induced changes in soil nutrient dynamics by native and invasive grass species. *Plant and Soil* 345:365–374. DOI: 10.1007/s11104-011-0788-9.

Prober SM, Lunt ID. 2009. Restoration of *Themeda australis* swards suppresses soil nitrate and enhances ecological resistance to invasion by exotic annuals. *Biological Invasions* 11:171–181. DOI: 10.1007/S10530-008-9222-5/TABLES/1.

R Development Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rasmussen JA, Rice EL. 1971. Allelopathic effects of *Sporobolus pyramidatus* on vegetational patterning. *American Midland Naturalist* 86:309. DOI: 10.2307/2423626.

Rayment J, French K, Bedward M. 2022. Understanding patterns and pathways of exotic perennial grass invasion in South-eastern Australian grassy communities. *Diversity and Distributions*. DOI: 10.1111/ddi.13520.

Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3:217–223. DOI: 10.1111/j.2041-210X.2011.00169.x.

Rhodes AC, Plowes RM, Goolsby JA, Gaskin JF, Musyoka B, Calatayud PA, Cristofaro M, Grahmann ED, Martins DJ, Gilbert LE. 2021. The dilemma of Guinea grass (Megathyrsus maximus): a valued pasture grass and a highly invasive species. *Biological Invasions* 23:3653–3669. DOI: 10.1007/S10530-021-02607-3/FIGURES/3.

Rice EL. 1972. Allelopathic effects of *Andropogon virginicus* and its persistence in old fields. *American Journal of Botany* 59:752–755.

Rudgers JA, Orr S. 2009. Non-native grass alters growth of native tree species via leaf and soil microbes. *Journal of Ecology* 97:247–255. DOI: 10.1111/j.1365-2745.2008.01478.x.

Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D, McVeigh R, O'Neill K, Robbertse B, Sharma S, Soussov V, Sullivan JP, Sun L, Turner S, Karsch-Mizrachi I. 2020. NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database : the journal of biological databases and curation* 2020. DOI: 10.1093/DATABASE/BAAA062.

Schullehner K, Dick R, Vitzthum F, Schwab W, Brandt W, Frey M, Gierl A. 2008. Benzoxazinoid biosynthesis in dicot plants. *Phytochemistry* 69:2668–2677. DOI: 10.1016/j.phytochem.2008.08.023.

Schütz V, Bigler L, Girel S, Laschke L, Sicker D, Schulz M. 2019. Conversions of benzoxazinoids and downstream metabolites by soil microorganisms. *Frontiers in Ecology and Evolution* 7:238. DOI: 10.3389/FEVO.2019.00238/BIBTEX.

Shan J, Ji R, Yu Y, Xie Z, Yan X. 2015. Biochar, activated carbon, and carbon nanotubes have different effects on fate of 14 C-catechol and microbial community in soil. *Scientific Reports* 5:16000. DOI: 10.1038/srep16000.

Sigüenza C, Corkidi L, Allen EB. 2006. Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. *Plant and Soil* 286:153–165. DOI: 10.1007/s11104-006-9034-2.

Sommers P, Davis A, Chesson P. 2022. Invasive buffel grass (*Cenchrus ciliaris*) increases water stress and reduces success of native perennial seedlings in southeastern Arizona. *Biological Invasions*:1–18. DOI: 10.1007/s10530-022-02750-5.

Soti P, Thomas V. 2021. Review of the invasive forage grass, guinea grass (*Megathyrsus maximus*): Ecology and potential impacts in arid and semi-arid regions. *Weed Research*. DOI: 10.1111/wre.12512.

Steidl RJ, Hayes JP, Schauber E. 1997. Statistical power analysis in wildlife research. *The Journal of Wildlife Management* 61:270. DOI: 10.2307/3802582.

Tomat-Kelly G, Dillon WW, Flory SL. 2021. Invasive grass fuel loads suppress native species by increasing fire intensity and soil heating. *Journal of Applied Ecology*:1365-2664.13881. DOI: 10.1111/1365-2664.13881.

Torres N, Herrera I, Fajardo L, Bustamante RO. 2021. Meta-analysis of the impact of plant invasions on soil microbial communities. *BMC Ecology and Evolution* 21:172. DOI: 10.1186/s12862-021-01899-2.

Uddin MN, Robinson RW, Buultjens A, al Harun MAY, Shampa SH. 2017. Role of allelopathy of *Phragmites australis* in its invasion processes. *Journal of Experimental Marine Biology and Ecology* 486:237–244. DOI: 10.1016/j.jembe.2016.10.016.

United Nations. 2020. UN decade on restoration. *www.decadeonrestoration.org*.

Vehtari A, Gelman A, Gabry J. 2017. Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Statistics and Computing* 27:1413–1432. DOI: 10.1007/s11222-016-9696-4.

Walker ZC, Morgan JW. 2022. Perennial pasture grass invasion changes fire behaviour and recruitment potential of a native forb in a temperate Australian grassland. *Biological Invasions*:1–11. DOI: 10.1007/s10530-022-02743-4.

Wied JP, Perotto-Baldivieso HL, Conkey A, Brennan LA, Mata JM. 2020. Invasive grasses in South Texas rangelands: historical perspectives and future directions. *Invasive Plant Sci. Manag* 13:41–58. DOI: 10.1017/inp.2020.11.

Wink M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry* 64:3–19. DOI: 10.1016/S0031-9422(03)00300-5.

Zhang Z, Liu Y, Brunel C, van Kleunen M. 2020a. Soil-microorganism-mediated invasional meltdown in plants. *Nature Ecology & Evolution* 4:1612–1621. DOI: 10.1038/s41559-020-01311-0.

Zhang Z, Liu Y, Yuan L, Weber E, van Kleunen M. 2020b. Effect of allelopathy on plant performance: a meta-analysis. *Ecology Letters*:ele.13627. DOI: 10.1111/ele.13627.

Table 2.1. Estimated difference and 95% CI for each hypothesis. Negative differences were predicted *a priori* for each hypothesis test.

	Estimate	95 LCI	95 UCI
Novel Weapons Hypothesis	-0.14	-0.25	-0.03 *
Biotic Resistance Hypothesis	0.09	-0.01	0.19
Phylogenetic Distance Hypothesis	-0.22	-0.36	-0.07 *

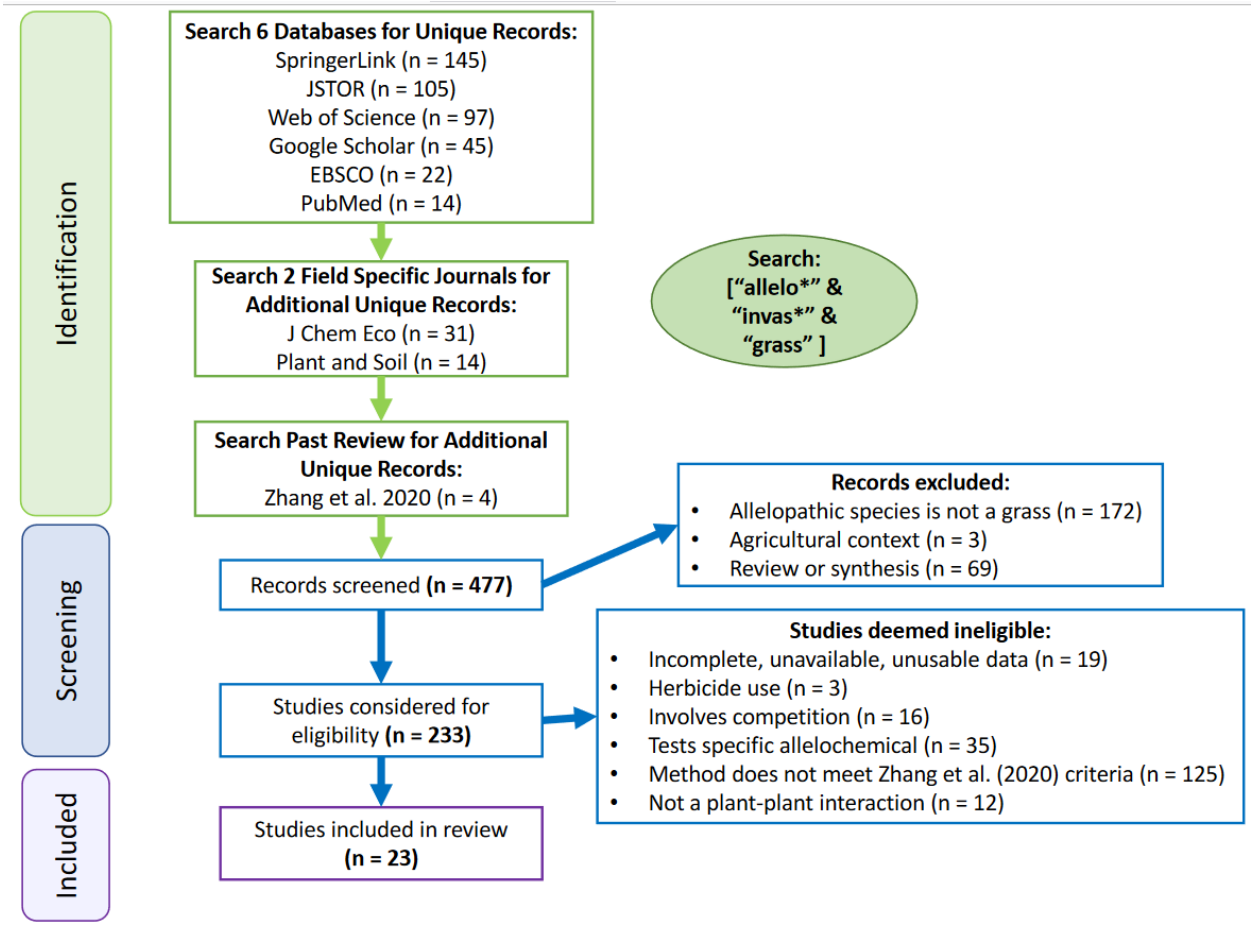

Figure 2.1. PRISMA flowchart.

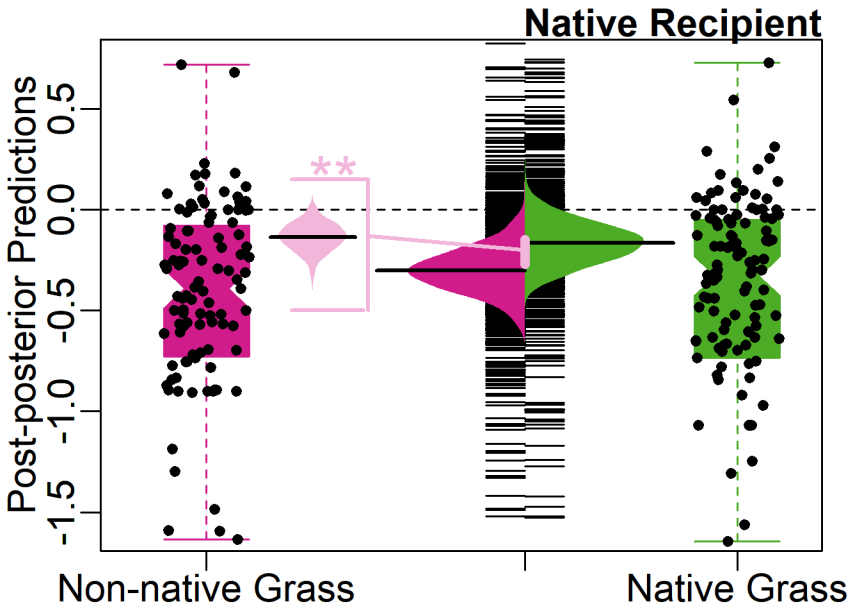

Figure 2.2. Test of the novel weapons hypothesis: Center, bean plot of distribution of predicted mean effect size with long line showing the average prediction, overlayed on strip-chart of distribution of predicted population. To each side, notched boxplot, overlayed with jittered points, showing distribution of observed effect sizes. Colors represent effect of native (green, right) and non-native (magenta, left) grasses on native recipients. Center-left, bean plot of predicted difference (light pink) between average effect of native grasses and average effect of non-native grasses on native recipients, with long line showing average predicted difference. Annotation "**" denotes significance at 95% CI level.

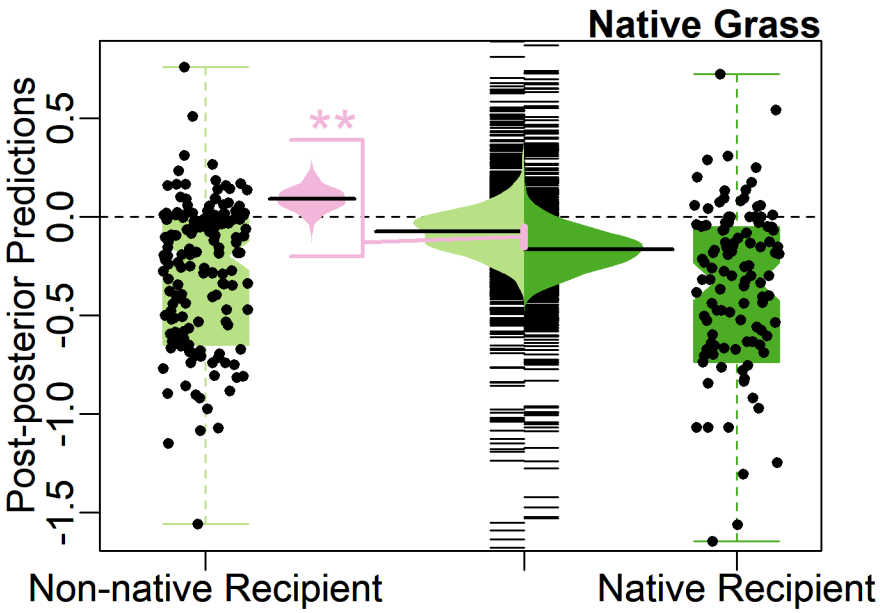

Figure 2.3. Test of the biotic resistance hypothesis: Center, bean plot of distribution of predicted mean effect size with long line showing the average prediction, overlayed on strip-chart of distribution of predicted population. To each side, notched boxplot, overlayed with jittered points, showing distribution of observed effect sizes. Colors represent effect of native grasses on native (green, right) and non-native (light green, left) recipients. Center-left, bean plot of predicted difference (light pink) in average effect size of native grasses on native recipients compared to non-native recipients, with long line showing average predicted difference. Contrary to the hypotheses, natives had stronger impacts on natives than on non-natives. Annotation "**" denotes significance at 95% CI level.

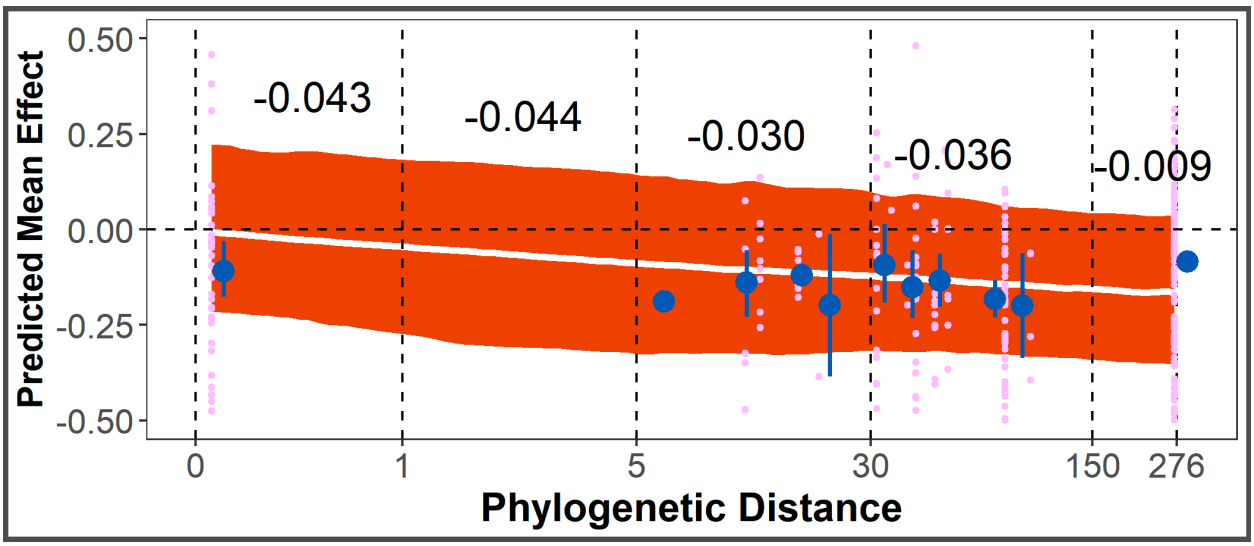

Figure 2.4. Post-posterior predicted mean effect size (+ 95% CI) across phylogenetic distance (unitless, from distance matrix calculated using aptg package), overlayed with points representing observed effect sizes (pink) and point-ranges (in blue) representing mean + SE of observed effect sizes within y-axis bounds. Black numbers are average predicted change in effect size for that interval of phylogenetic distance.

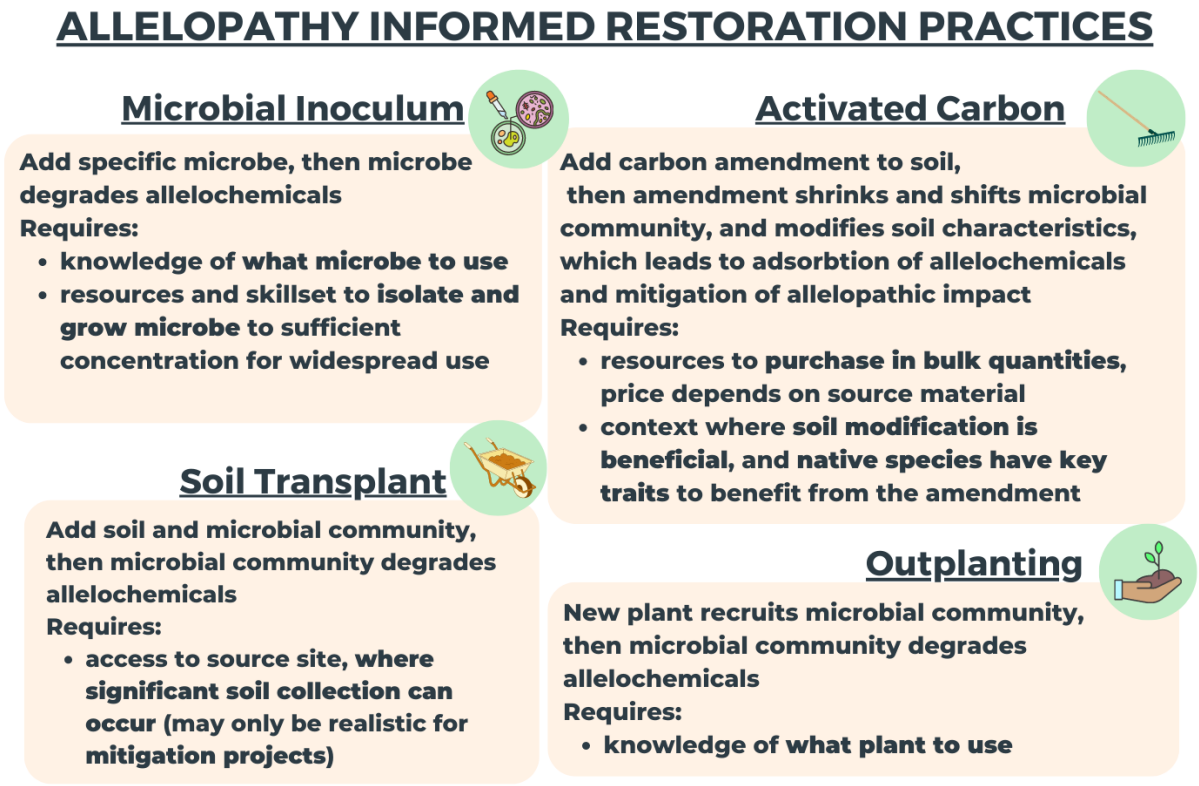

Figure 2.5. Four allelopathy informed restoration practices (out planting, microbial inoculum, soil transplant and activated carbon), with a summary of their underlying mechanisms and what is required to utilize the practice effectively.

Appendix A- Supplementary Tables

Table S1. LOO and WAIC comparison of full and intercept models.

Model	**LOO**	**WAIC**
m_full	0.000	0.000
m_intercept	-4.665	-4.616

Appendix B- Supplementary Figures

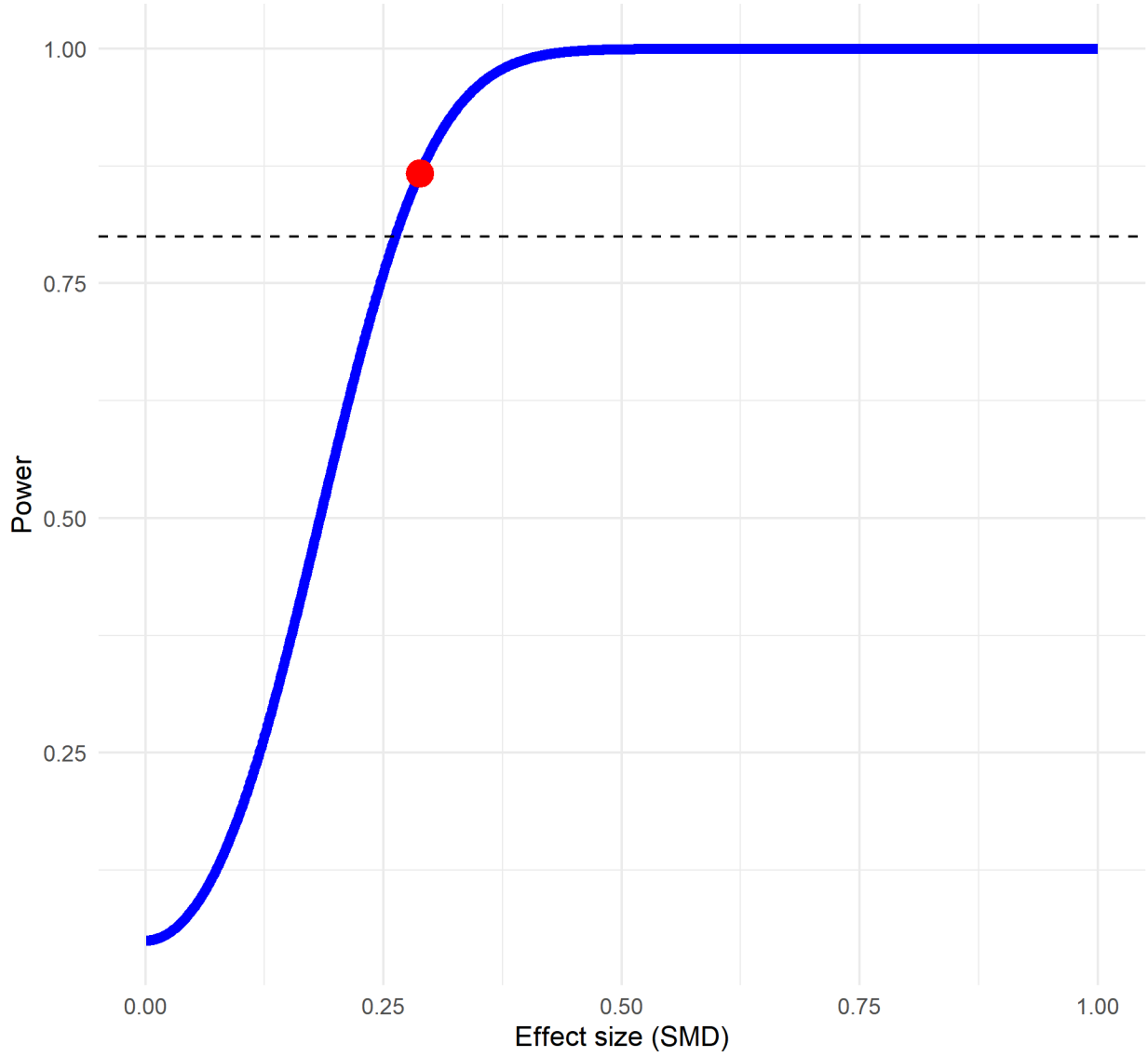

Figure S1. Power analysis graph based on number of studies used (23, n1 and n2=20). Heterogeneity was set to "high" and expected mean difference was set to 0.288 based on Zhang et al. (2020). Power was 86%.

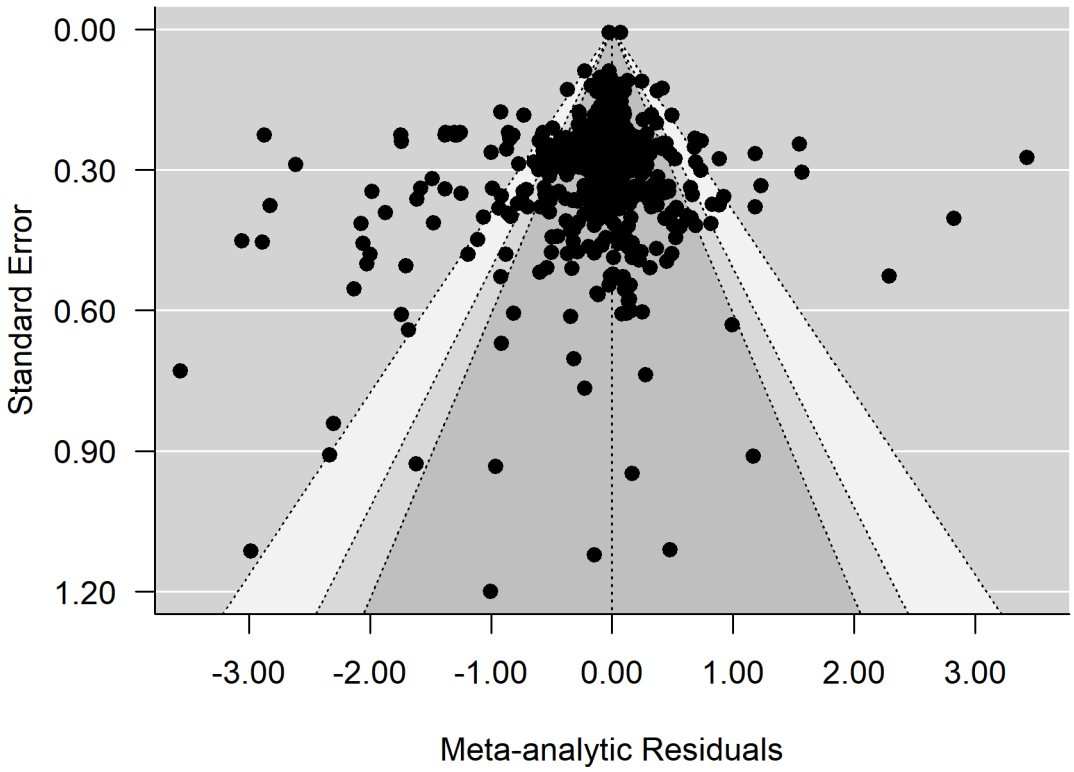

Figure S2. Funnel plot of standard error and meta-analytic residuals from the intercept model with contour zones. The spread of the points suggests publication bias, although Egger's test does not provide strong statistical support for publication bias (the y-intercept 95% confidence interval includes zero). Points outlie the funnel on both the negative and positive side. Some extreme outliers were excluded for clarity of visual.

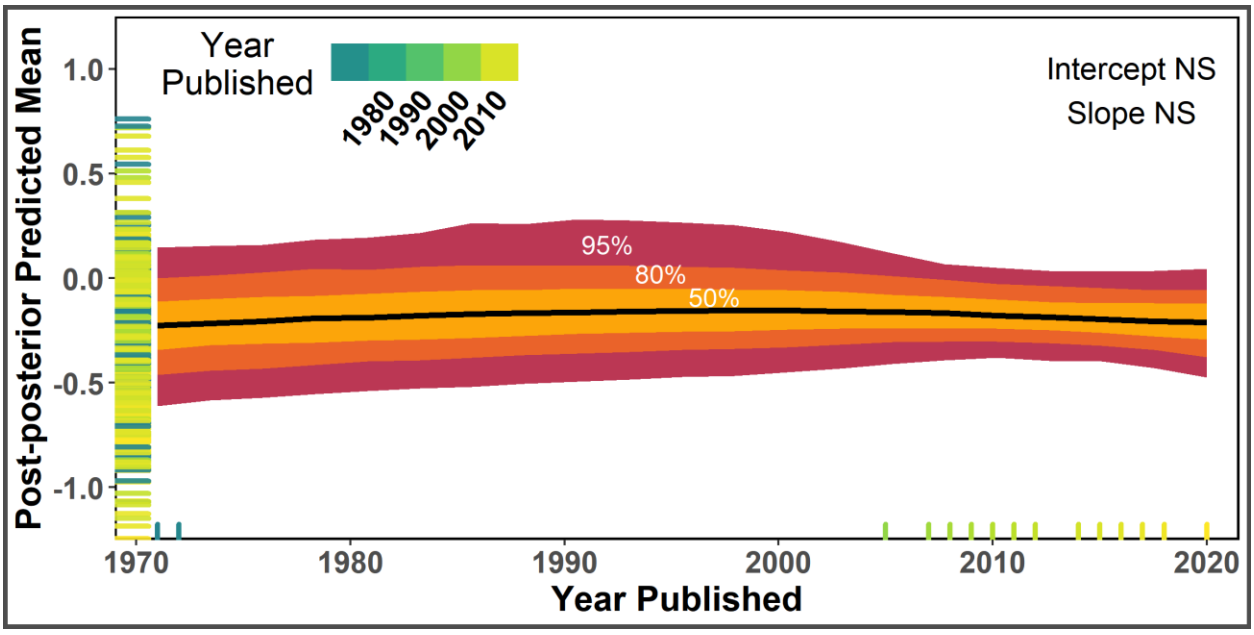

Figure S3. Post-posterior predicted smoothed relationship between year published and allelopathic effect size with confidence intervals. Rug plot on x and y axis represent distribution of observed effect sizes.

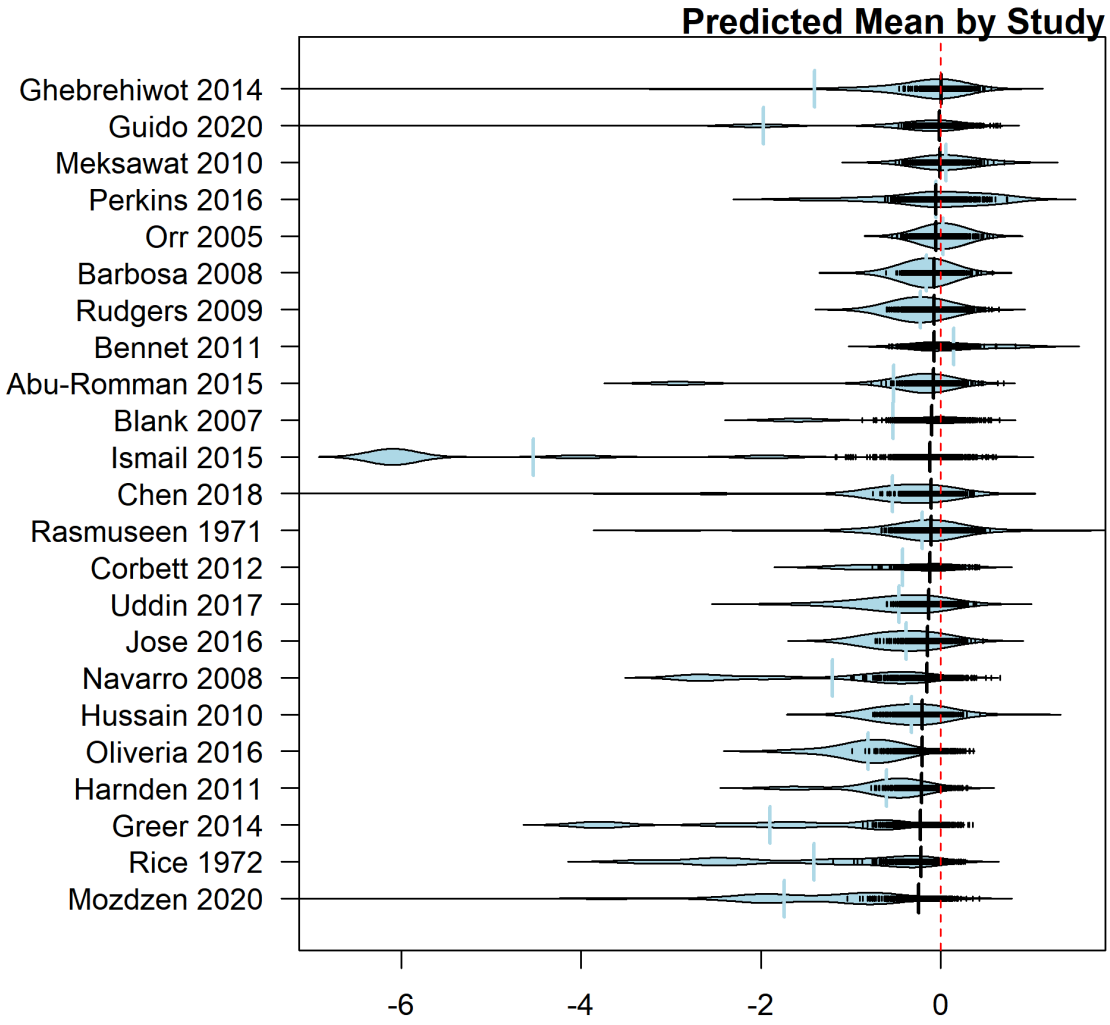

Figure S4. Bean plot of distribution of observed effect sizes (light blue) and strip chart (black) of post-posterior predicted mean by study (consolidated across any substudies and trait measured).

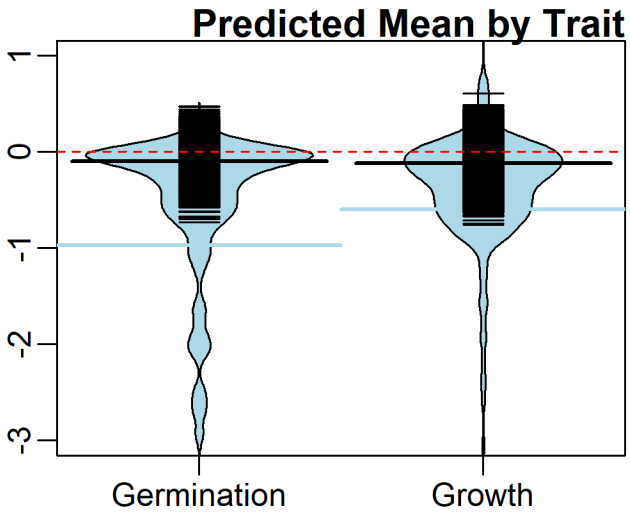

Figure S5. Bean plot of distribution of observed effect sizes (light blue) and strip chart (black) of post-posterior predicted mean by trait (consolidated across studies).

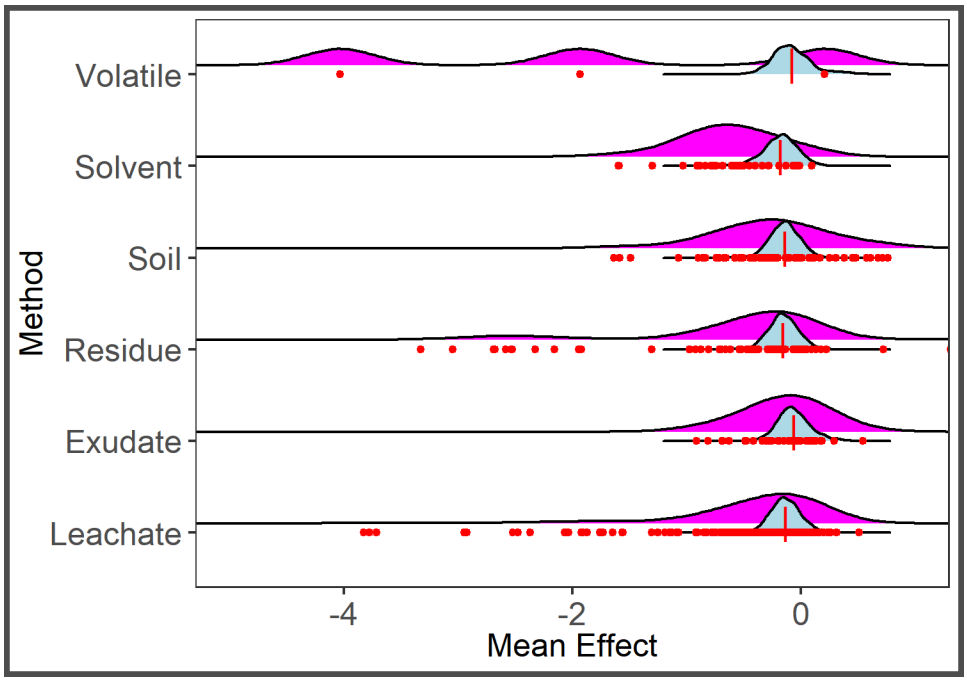

Figure S6. Ridges plot of distribution of observed effect sizes (magenta) and predicted average effect size (blue) by method. Points represent observed effect sizes (red) and long line represents mean of observed effect sizes (red).

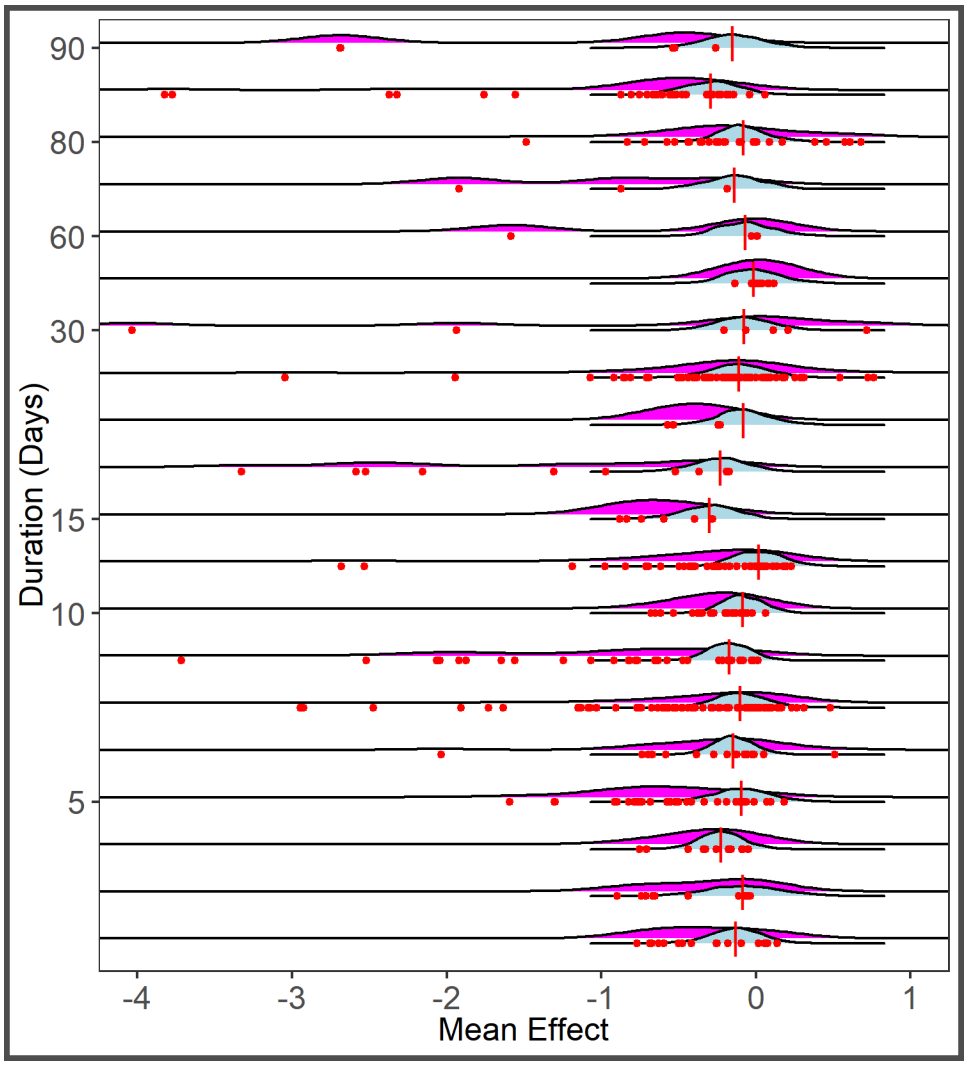

Figure S7. Ridges plot of distribution of observed effect sizes (magenta) and predicted average effect size (blue) by study duration (consolidated across methods). Points represent observed effect sizes (red) and long line represents mean of observed effect sizes (red).

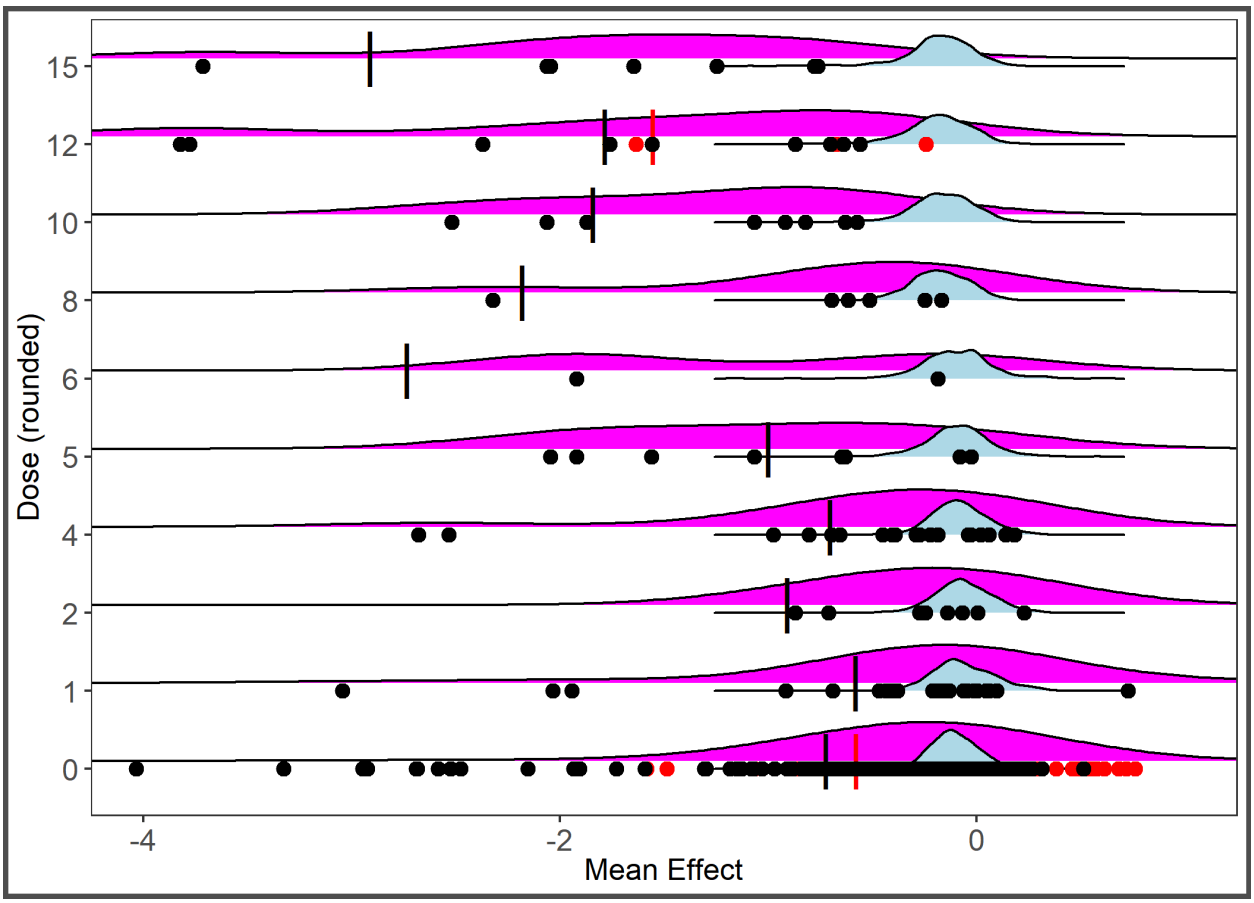

Figure S8. Ridges plot of distribution of observed effect sizes (magenta) and predicted average effect size (blue) by dose (or imputed dose). Points represent observed effect sizes by reported dose (black) and imputed dose (red). Long line represents mean with imputed dose values (red) and with only reported dose values (black).

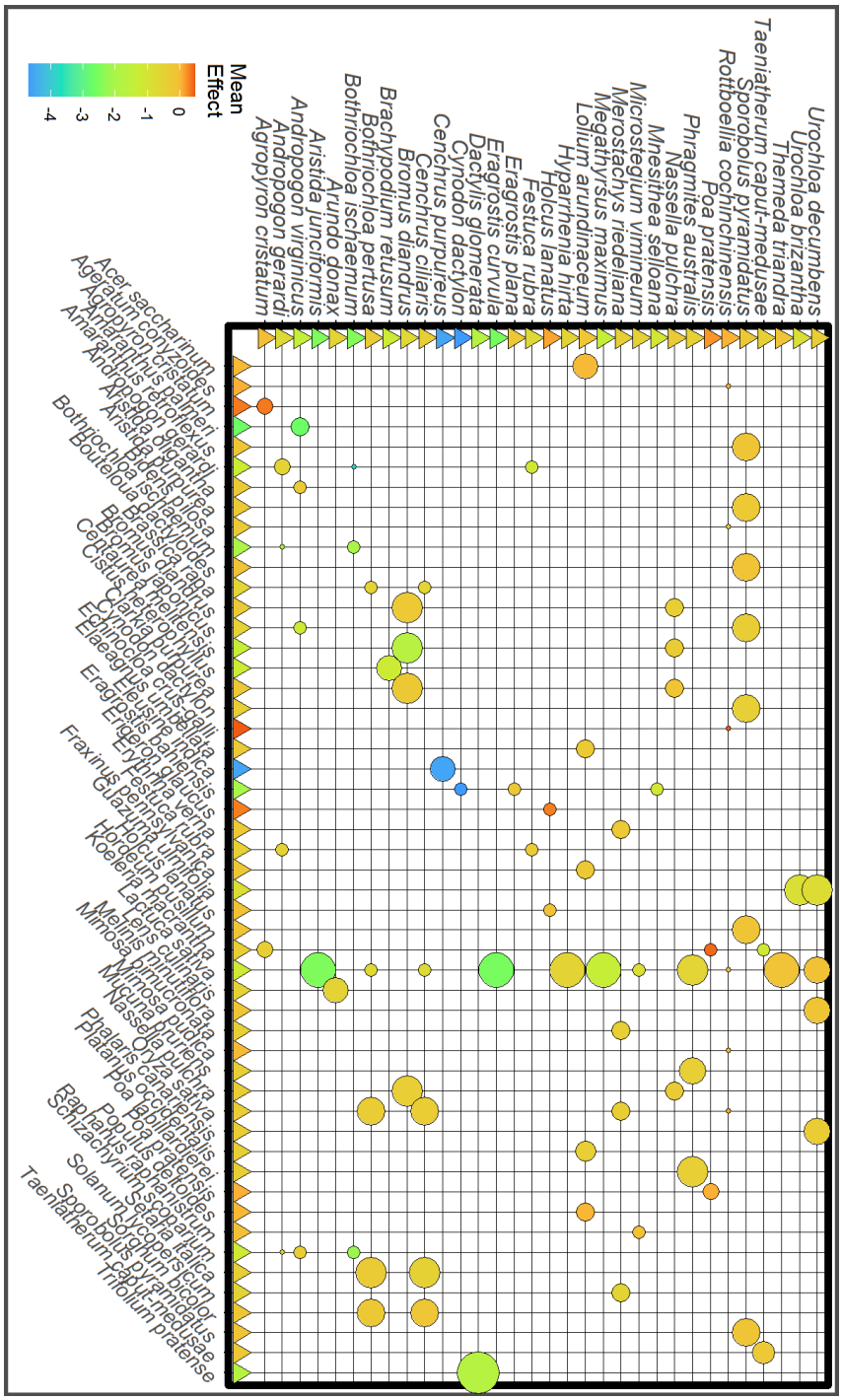

Figure S9. Balloon plot showing distribution of observed effect sizes by recipient species (x-axis) and grass species (y-axis). Balloon size represents frequency of each species pair. Triangle points represent the model predicted average effect size for each grass and recipient species. Color of balloons and triangles represents mean effect size.

CHAPTER 3

UTILIZING THE SOIL MICROBIOME AND SOIL CHARACTERISTICS TO INFORM RESTORATION PRACTICES AND SUCCESS: A CASE STUDY OF *MEGATHYRSUS* GRASS INVASION IN HAWAI'I

Abstract

Restoration projects are challenged when invasive grasses return after being controlled initially. We compared soil properties and the soil microbiome at a restored and grass-invaded site in Hawai'i and found differences in physical soil properties and microbiome communities; some key bacteria and fungi seem to be "hold outs" from pre-grass removal, even after native replanting. Soil legacy effects can affect restoration, and we suggest that increased attention to soil microbiomes can help inform and improve restoration strategies.

Introduction

Restoration of areas invaded by dense populations of non-native plants is one of the most pressing environmental issues of modern times (United Nations, 2020) . Non-native invasive plants can leave a soil legacy that negatively impacts the growth of restored native plants (Grman & Suding, 2010), and further examination of these resulting soil legacies is critical to consider in restoration, and where appropriate should be built into restoration goals, practices, and measures of success. Here, we demonstrate how incorporating belowground measures of the soil microbiome and soil characteristics can illuminate meaningful insights about the mechanisms and processes affecting restoration success.

Megathyrsus maximus (Guinea grass) is a perennial African grass that has been deliberately introduced throughout the tropics as a pasture forage species (Soti & Thomas, 2021), and is considered invasive throughout Hawai‘i and the Pacific Island Region (US Forest Service, 2021), so the development of successful restoration practices following invasions by this grass has broad relevance to conservation. In Hawai‘i, compared to native forest ecosystems, the rate of fire spread in invaded grasslands, such as those dominated by *M. maximus,* is up to 5 times faster (Ellsworth et al., 2014), making restoration particularly urgent. In abandoned or non-grazed areas of *M. maximus* on O‘ahu, restoration with grass removal alone or in combination with native seed supplementation have not been effective in restoring substantial native cover (Ammondt et al., 2013), which could be explained by many factors, including a potential soil legacy effect.

Beyond Hawai‘i, *M. maximus* pastures are associated with modification of soil characteristics, including increases in total N (Müller et al., 2004; Holland, Thomas & Soti, 2022), which can contribute to or drive soil legacies. *M. maximus* is known to benefit from

nitrogen-fixers, with evidence supporting a positive correlation between inoculation with nitrogen fixing bacteria *Azospirillum* and dry mass yield (Leite et al., 2019). In Hawai‘i, nitrogen-fixing bacteria and associated native and non-native plants have been identified as potentially contributing to plant-soil interactions that reduce native plant recruitment (Walker & Vitousek, 1991; Yelenik, D'Antonio & August-Schmidt, 2017) and improve conditions for non-native invaders (Allison & Vitousek, 2004).

Case Study: Camp Palehua, O‘ahu

To explore how the soil microbiome and soil characteristics can be integrated into restoration experiments, so as to help illuminate the mechanisms and processes affecting restoration success, we examine a case study at Camp Palehua located in south-west O‘ahu. The site has an annual precipitation of 925 mm (Giambelluca et al., 2013) and Grass Reference Surface potential evapotranspiration of 1437 mm (AI = .64)(Giambelluca et al., 2014). The soil is a well-drained Oxisol from volcanic ash (Mahana silty clay loam) (Soil Survey Staff, 2014), and acidity and nutrient deficiencies have been identified as key issues that may impact plant communities (Andie Gill, personal communication).

Two sites were compared for the purposes of examining differences in soil microbiome and soil characteristics that could provide insights into mechanisms that may promote or hinder restoration success. A restoration site at Camp Palehua was established in early 2020 through a collaboration with a non-profit community organization (Malama Learning Center) (Figure 3.1, "Restored Site"). Soil conditions at the restored site eighteen months post-restoration were compared to conditions at an actively grazed *M. maximus* area roughly 200m south-east of the restored Site (Figure 3.1, "Megathyrsus Site"). The two sites share the same soil classification, slope, elevation, climate conditions, and a multi-decade history of consistent cattle grazing use. Grazing can have impacts on the soil microbial community (Wang et al., 2006; Devi, Yadava & Garkoti, 2014) and soil characteristics (Braunack & Walker, 1985; Berhe, 2019), which can persist even 100 years after cessation (i.e. soil legacy) (Fichtner et al., 2014).

We hypothesized that the restored site would have different abiotic soil characteristics and a significantly different soil microbial community, compared to the Megathyrsus site. We hypothesized that microbial community diversity would be greater at the restored site compared to the Megathyrsus site (as indicated by greater PCA dispersion of within-site

community samples, as well as by greater mean Shannon Diversity Index and Simpson's Diversity Index) because soil microbial diversity is expected to be correlated with plant community diversity (Masumoto et al., 2021), which was higher at the restored site. Finally, we hypothesized that the average abundance of nitrogenase enzyme complex would be higher at the Megathyrsus site due to a previously reported association between Megathyrsus and nitrogen fixing microbes (Leite et al., 2019).

Methods

Prior to restoration, grass culms were cut off close to the base, and then the base and root were removed using a pick (Mashuri Waite, personal communication). Native plants such as *Acacia koa, Plumbago zeylanica, Sida fallax* and *Waltheria indica* were planted in clusters across an area of roughly 0.10 hectares in January of 2020. A clustering strategy was utilized due to the significant regrowth of *Megathyrsus maximus* in a previous project where outplants were deliberately spaced. In a nearest-neighbor survey (Cottam & Curtis, 1956) of 15 randomly generated points conducted on October 29th 2021, the nearest-neighbor estimate (Byth & Ripley, 1980; Krebs, 1999) was 5 plants per square meter and the average distance was 0.11 m. Each random point was within one of the plant clusters, and clusters were spaced around 3-5 m apart (Manya Singh, personal observation). The native outplants appeared to be growing at a decent pace given the age of the project, with most native tree/shrub species being at least 1 m tall, and most shrub/ground cover species being wider than 0.5 m at the widest point (personal observation, Manya Singh). Despite the relative success in maintaining a native community at the restored site 18 months post planting, the site required intensive weeding of *Megathyrsus*, meaning this practice cannot feasibly be scaled to larger areas without enormous labor inputs. Even with on-going weeding, Occasional *Megathyrsus maximus* individuals were present at the restoration site on the date of the nearest-neighbor survey (personal observation, Manya Singh). In contrast, at the *M. maximus* site, the *M. maximus* cover was 70%, with the remaining cover being bare ground (based on a survey of 50 randomly generated points). No unaltered or un-managed native ecosystem was proximally available to compare with the restored and Megathyrsus sites.

Soil descriptions and soil sample collection at the restored site and Megathyrsus site occurred on May 24th, 2021. Sampling points were randomly generated via ArcGIS. At the

restored site, the nearest plant species to each soil sampling point was noted. Samples were taken using a 2.5 cm diameter and 10 cm deep soil core. The probe was cleaned with a bleach wipe between samples. Basic soil descriptions were conducted in accordance with NRCS standards (Schoeneberger, Wysocki & Benham, 2012).

Soil samples were homogenized but not pooled, and for each sample DNA was extracted from four, 0.25 g replicates using the DNeasy PowerLyzer PowerSoil Kit as per manufacturer's directions (Qiagen Inc, Valencia, CA). The presence of DNA was verified via DNA quantification, then prepared for Illumina Inc. (Hayward, CA) NextGen sequencing. Primers were generated by Illumina Inc for the V3-V4 region of the 16S rRNA gene (Yadav et al., 2021) and ITS region (Raja et al., 2017) were amplified by PCR to generate raw sequences for our analyses.

The 16S sequences in fastq format were run through the general DADA2 workflow (Callahan et al., 2016). Then, IDTAXA from the "decipher" package (Wright, 2016) was utilized to assign taxonomy based on the SILVA database from 2019 (Quast et al., 2012; Yilmaz et al., 2014). The ITS1 sequences were also run through the DADA2 workflow. Then, IDTAXA from the "decipher" package was utilized to assign taxonomy based on the UNITE database from 2020 (Nilsson et al., 2018; Kõljalg et al., 2020). The "vegan" package (Oksanen et al., 2013) in R was used to generate diversity statistics and generate a Bray-Curtis dissimilarity matrix for principal coordinates analysis. Differences in centroid dispersion were tested using an ANOVA with the function betadisper, and each term was tested individually. Differences in centroid centers were tested using a PERMANOVA and the function adonis2. The "pheatmap" package (Kolde, 2019) was used to generate heatmaps to visualize community composition and determine clustering based on correlation between genera abundance.

Additionally, qPCR was performed to quantify the nitrogenase enzyme complex in 12 soil samples to compare the presence of nitrogen fixing bacteria in the restored site and Megathyrsus site. $C_{t.}$ values were assessed for samples in which detectable amounts of amplicon product were generated during the early exponential phase of the reaction. Rhizobium was collected from the root nodules of sunn hemp (*Crotalaria juncea*, harvested from the garden at Leeward Community College, Pearl City, HI) to serve as a positive control. DNA was extracted from the sunn hemp plant nodules and the *nifH* gene (Gaby &

Buckley, 2012) of the nitrogenase enzyme complex was amplified via qPCR. PolF and PolR primers (Integrated DNA Technologies, Coralville, IA) were chosen based on efficiency and two duplicates were established in a dilution series from 139 to 0.08 ng of template DNA. An ANOVA followed by a Bonferroni paired t-test was used to test for significant differences in average nitrogenase enzyme complex abundance between sites.

Results

Soil Abiotic Characteristics

The two sites differed in soil structure and color, but not in root or pore characteristics (Table 3.1). Soils from the two sites were similar in manner of failure (i.e,. response to increasing pressure on an aggregate), other than brittleness, where the Megathyrsus site was deformable (gradual response to pressure, also referred to as poor friability), while the restored site was brittle (sudden rupture, also referred to as "friable") (Table 3.1). Brittle failure can indicate that the soil was generally drier, and therefore stronger (Baver, Gardner & Gardner, 1972), and in restoration, the associated increased penetration strength has been linked to poor root growth (Davies, Younger & Chapman, 1992). Deformable soil, on the other hand, has been linked to plants with roots that spread out through the surface layers of the soil (Gao et al., 2016). Additionally, soil structural characteristics, such as pedality, the structure of the soil into peds that can take on various forms including blocky or granular, can correlate to abiotic features like shrink-swell capacity (Carter, 2004). High shrink-swell capacity is an issue seen in some Hawaiian soils that can complicate the ability to successfully build structure and/or cultivate plants (Deenik & McClellan, 2007)) and may become more problematic as climate change contributes to increased variability in precipitation regimes (Frazier & Giambelluca, 2017; Pendergrass et al., 2017).

Soil Fungal Community

Fungal community composition differed significantly by site (Figure 3.2), nearest plant species ($F_{(3, 36)} = 23.606$, $P = 0.001$, $R^2 = 0.274$), and sampling point ($F_{(7, 36)} = 12.942$, $P = 0.001$, $R^2 = 0.350$). Site explained 24% of the variation in fungi composition, while nearest plant species explained 27%, and sampling point explained 35%. Fungal community across-site diversity (i.e. dispersion of community samples) differed significantly by site (Figure 3.2), and sampling point ($F_{(11, 36)} = 3.911$, $P = 0.0009$), but not by nearest plant species ($F_{(4, 43)}$

= 2.471 $p > 0.05$). Fungal diversity averaged higher in the restored site compared to the Megathyrsus site, based on both Shannon and Simpson's Diversity (Figure 3.2).

Abundant fungi genera appearing in more than one sample at the Megathyrsus site were *Glomus, Leohumicola* and *Preussia,* while abundant fungi genera at the restored site were *Glomus, Coprinellus,* and *Fusarium* (Figure 3.3). The remaining genera only appeared at one sampling point, but some appeared at very high relative abundance (Figure 3.3).

Soil Bacterial Community

Bacteria community composition differed significantly by site (Figure 3.4), nearest plant species ($F_{(3, 46)} = 10.817$, $P = 0.001$, $R^2 = 0.126$), and sampling point ($F_{(7, 46)} = 18.706$, $P = 0.001$, $R^2 = 0.509$). Site explained 23% of the variation in bacteria composition, while nearest species explained 13%, and sampling point explained 51%. Bacteria community within-site diversity differed significantly by nearest plant species ($F_{(4, 42)} = 7.791$, $p < 0.01$), but not by site (Figure 3.4) or sampling point ($F_{(11, 35)} = 0.597$, $p > 0.05$). Bacterial diversity was higher in the restored site, compared to the Megathyrsus site, based on both Shannon and Simpson's Diversity (Figure 3.5).

Some of the most abundant bacteria genera (*Xanthobacteraceae, 67-14, Candidatus Udaebacter*) were present at similar levels at both sites (Figure 3.5). Some genera were only present at one site, such as *Bacillus* at the restored site, and *Conexibacter* at the Megathyrsus site. The average abundance of nitrogenase enzyme complex was significantly higher at the Megathyrsus site compared to the restored site (Figure 3.6). There were also significant differences based on nearest species ($F_{(3, 75)} = 21.885$, $p < 0.01$) and sampling point ($F_{(7, 75)} = 5.767$, $p < 0.01$).

Discussion

Differences in soil color suggest that the two sites differ in chemical composition (Torrent et al., 1983; Schwertmann, 1993; Shengli et al., 2001), which can contribute to the physical, structural characteristics of soil. Soil structure can influence water infiltration, with depth of infiltration tending to be increased in more structured soil (Flury et al., 1945), like that at the restored site. In silty clay loam, the growth of plants associated with arbuscular mycorrhizal fungi, such as *Sida fallax* and *Waltheria indica* (Koko, Swift & Hynson, 2021),

can improve soil aggregation (i.e., tendency to form larger peds like blocky peds, rather than smaller peds like granular peds) (Thomas et al., 1986), so the choice of outplanting species may explain or contribute to differences in soil structure. Poor friability (i.e., tendency to deform rather than crumble) at the Megathyrsus site is associated with high traffic areas and may be an indicator that the water-holding capacity of the soil is outside of the optimal range of water content (i.e., too low or too high) in a restoration context, meaning that an organic matter amendment may be useful (Munkholm, 2011).

Support for our hypothesis of differences in abiotic soil characteristics suggests that the restoration practice at the restored site has led to critical improvements in the soil. Although it is possible that there were inherent differences between the two sites prior to restoration, the two sites have many similarities (soil Order, slope, history of grazing, and proximity), with the restoration treatment being the largest obvious difference between the two sites that could account for observed soil differences. The native aboveground growth can contribute to shifts in soil abiotic characteristics through differences in litter quality (Wiegner & Tubal, 2010) or degradability (Allison & Vitousek, 2004) and by influencing light availability which can impact the soil microbial community (Koorem et al., 2017; Neuenkamp et al., 2020). Other former pasture restoration projects have found significant changes in soil microbial community measures in short time frames, such as a significant increase in microbial enzyme activity after two years (D'Angioli et al., 2022). Pasture use in formerly dry forest regions is associated with significant losses in critical soil functions, but there are instances of restoration practices resulting in significant improvements in soil characteristics, which can then influence the soil microbial community (Ayala-Orozco et al., 2018; Pedrinho et al., 2019).

The history of grazing may have impacted soil characteristics such as soil compaction (Hamza & Anderson, 2005), so another potential explanation for differences in soil characteristics would be the elimination of active grazing and/or the physical manipulation and disturbance of the soil that occurred at the restoration site during the planting stage. Soil compaction can influence root growth (Unger & Kaspar, 1994), so the disruption of compaction may provide some release to that effect, and root growth can influence physical soil structure (Angers & Caron, 1998). Root growth would also influence the availability and

impact of root exudates, which can influence nutrient cycling directly and indirectly via the soil microbial community (Coskun et al., 2017; Zhao et al., 2021; Wen et al., 2022).

Our hypothesis that the across-site diversity statistics in both bacterial and fungal communities would be higher at the restored site, compared to the Megathyrsus site, was supported, which is in line with research finding a positive correlation between microbial community diversity and plant community diversity (Hamonts et al., 2017). If we apply typical microbial community restoration goals retroactively to the original restoration practice, the results of our analysis would be evidence of restoration success. Another restoration goal could be to shift the composition of a specific functional group within the microbial community, such as nitrogen fixers. *Bacillus,* a nitrogen fixing bacteria, was detected solely at the restored site, while *Conexibacter,* another nitrogen fixing bacteria, was detected solely at the Megathyrsus site. The lower incidence of the nitrogenase enzyme complex in restored sites together with the sequencing data seem to suggest that the restoration modified the nitrogen-fixing functional group, with reduced nitrogenase activity, potentially supporting the success of native plants (Yelenik, D'Antonio & August-Schmidt, 2017).

We were interested in whether the high abundance genera in the bacteria or fungi communities remain the same across the unrestored and restored sites, or whether differing key genera are present in relatively similar abundance across the two sites. In our study, high abundance genera shared across the two sites include bacterium *Candidatus Udaeobacter* and fungi *Glomus*, both of which have been associated with grazed grasslands (Fox et al., 2022). If these genera are holdovers contributing to soil legacy effects (e.g., Jordan et al., 2012; Pickett et al., 2019; Hannula et al., 2021), it is possible that they can contribute to or support the re-establishment of *Megathyrsus,* or prevent or limit the ability of native plants to recruit key microbes necessary to their own establishment and/or growth, which could lead, at least in part, to the continued need for weeding at the restored site.

While the restoration practice that occurred at the restored site did not originally include any belowground restoration goals, this analysis demonstrates the importance and benefits of incorporating belowground restoration goals into restoration practices. Not only can belowground measures and goals improve our ability to assess the holistic nature of ecosystem restoration, but the measures used to assess belowground goals can illuminate

mechanisms that may contribute to invader re-emergence, allowing practitioners to improve upon restoration practices. With some commercial labs offering bacteria and fungi analyses at <US$200 per sample, microbial community analyses are becoming more accessible to practitioners. If such analyses are not within budget, insightful soil descriptions can still be made at little to no cost. Baseline belowground analyses, such as those presented here, can be usefully applied to help evaluate and facilitate restoration of large swaths of *Megathrysus* or other grass-invaded areas across the tropics (Perkins, Nimmo & Medeiros, 2012), and more generally, they may contribute insights any time a soil legacy effect of invasion is suspected.

References

Allison SD, Vitousek PM. 2004. Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. *Oecologia* 141:612–619. DOI: 10.1007/S00442-004-1679-Z/FIGURES/3.

Ammondt SA, Litton CM, Ellsworth LM, Leary JK. 2013. Restoration of native plant communities in a Hawaiian dry lowland ecosystem dominated by the invasive grass *Megathyrsus maximus*. *Applied Vegetation Science* 16:29–39. DOI: 10.1111/j.1654-109X.2012.01208.x.

Angers DA, Caron J. 1998. Plant-induced changes in soil structure: Processes and feedbacks. *Biogeochemistry* 42:55–72. DOI: 10.1023/A:1005944025343.

Ayala-Orozco B, Gavito ME, Mora F, Siddique I, Balvanera P, Jaramillo VJ, Cotler H, Romero-Duque LP, Martínez-Meyer E. 2018. Resilience of Soil Properties to Land-Use Change in a Tropical Dry Forest Ecosystem. *Land Degradation & Development* 29:315–325. DOI: 10.1002/ldr.2686.

Baver LD, Gardner WH, Gardner WR. 1972. *Soil physics*. Wiley New York.

Berhe AA. 2019. Drivers of soil change. In: Busse M, Giardina CP, Morris DM, Page-Dumroese DS eds. *Developments in Soil Science*. Elsevier, 27–42. DOI: 10.1016/b978-0-444-63998-1.00003-3.

Braunack M v., Walker J. 1985. Recovery of some surface soil properties of ecological interest after sheep grazing in a semi-arid woodland. *Austral Ecology* 10:451–460. DOI: 10.1111/j.1442-9993.1985.tb00906.x.

Byth K, Ripley BD. 1980. On sampling spatial patterns by distance methods. *Biometrics*:279–284.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581–583. DOI: 10.1038/nmeth.3869.

Carter MR. 2004. Researching structural complexity in agricultural soils. *Soil and Tillage Research* 79:1–6. DOI: 10.1016/J.STILL.2004.04.001.

Coskun D, Britto DT, Shi W, Kronzucker HJ. 2017. How plant root exudates shape the nitrogen cycle. *Trends in Plant Science* 22:661–673. DOI: 10.1016/j.tplants.2017.05.004.

Cottam G, Curtis JT. 1956. The use of distance measures in phytosociological sampling. *Ecology* 37:451–460.

D'Angioli AM, Giles AL, Costa PB, Wolfsdorf G, Pecoral LLF, Verona L, Piccolo F, Sampaio AB, Schmidt IB, Rowland L, Lambers H, Kandeler E, Oliveira RS, Abrahão A. 2022. Abandoned pastures and restored savannas have distinct patterns of plant–soil feedback and nutrient cycling compared with native Brazilian savannas. *Journal of Applied Ecology* 59:1863–1873. DOI: 10.1111/1365-2664.14193.

Davies R, Younger A, Chapman R. 1992. Water availability in a restored soil. *Soil Use and Management* 8:67–73. DOI: 10.1111/j.1475-2743.1992.tb00897.x.

Deenik J, McClellan AT. 2007. Soils of Hawai'i. *Soil and Crop Management* SCM-20:1–12.

Devi TI, Yadava PS, Garkoti SC. 2014. Cattle grazing influences soil microbial biomass in sub-tropical grassland ecosystems at Nambol, Manipur, northeast India. *Tropical Ecology* 55:195–206.

Ellsworth LM, Litton CM, Dale AP, Miura T. 2014. Invasive grasses change landscape structure and fire behaviour in Hawaii. *Applied Vegetation Science* 17:680–689. DOI: 10.1111/AVSC.12110.

Fichtner A, von Oheimb G, Härdtle W, Wilken C, Gutknecht JLM. 2014. Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more

than 100 years. *Soil Biology and Biochemistry* 70:79–87. DOI: 10.1016/j.soilbio.2013.12.015.

Flury M, Flfihler H, Jury WA, Leuenberger J. 1945. Susceptibility of soils to preferential flow of water: A field study. *Water Resources Research* 30. DOI: 10.1029/94WR00871.

Fox A, Widmer F, Barreiro A, Jongen M, Musyoki M, Vieira, Zimmermann J, Cruz C, Dimitrova-Mårtensson LM, Rasche F, Silva L, Lüscher A. 2022. Small-scale agricultural grassland management can affect soil fungal community structure as much as continental scale geographic patterns. *FEMS Microbiology Ecology* 97:148. DOI: 10.1093/FEMSEC/FIAB148.

Frazier AG, Giambelluca TW. 2017. Spatial trend analysis of Hawaiian rainfall from 1920 to 2012. *International Journal of Climatology* 37:2522–2531. DOI: 10.1002/JOC.4862.

Gaby JC, Buckley DH. 2012. A comprehensive evaluation of PCR primers to amplify the nifH gene of nitrogenase. *PLOS ONE* 7:e42149. DOI: 10.1371/JOURNAL.PONE.0042149.

Gao W, Hodgkinson L, Jin K, Watts CW, Ashton RW, Shen J, Ren T, Dodd IC, Binley A, Phillips AL, Hedden P, Hawkesford MJ, Whalley WR. 2016. Deep roots and soil structure. *Plant, Cell & Environment* 39:1662–1668. DOI: 10.1111/PCE.12684.

Giambelluca TW, Chen Q, Frazier AG, Price JP, Chen YL, Chu PS, Eischeid JK, Delparte DM. 2013. Online rainfall atlas of Hawai'i. *Bulletin of the American Meteorological Society* 94:313–316. DOI: 10.1175/BAMS-D-11-00228.1.

Giambelluca TW, Shuai X, Barnes ML, Alliss RJ, Longman RJ, Miura T, Chen Q, Frazier AG, Mudd RG, Cuo L, Businger AD. 2014. Evapotranspiration of Hawai'i. *Final report submitted to the US Army Corps of Engineers—Honolulu District, and the Commission on Water Resource Management, State of Hawai 'i*.

Grman E, Suding KN. 2010. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. *Restoration Ecology* 18:664–670. DOI: 10.1111/j.1526-100X.2008.00497.x.

Hamonts K, Bissett A, Macdonald BCT, Barton PS, Manning AD, Young A. 2017. Effects of ecological restoration on soil microbial diversity in a temperate grassy woodland. *Applied Soil Ecology* 117–118:117–128. DOI: 10.1016/J.APSOIL.2017.04.005.

Hamza MA, Anderson WK. 2005. Soil compaction in cropping systems: A review of the nature, causes and possible solutions. *Soil and Tillage Research* 82:121–145. DOI: 10.1016/J.STILL.2004.08.009.

Hannula SE, Heinen R, Huberty M, Steinauer K, de Long JR, Jongen R, Bezemer TM. 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nature Communications* 12:5686. DOI: 10.1038/s41467-021-25971-z.

Holland EP, Thomas V, Soti P. 2022. Low soil nitrogen and moisture limit the expansion of the invasive grass, *Megathyrsus maximus* (Guinea grass) in semi-arid soils. *Journal of Arid Environments* 204:104788. DOI: 10.1016/j.jaridenv.2022.104788.

Jordan NR, Aldrich-Wolfe L, Huerd SC, Larson DL, Muehlbauer G. 2012. Soil–occupancy effects of invasive and native grassland plant species on composition and diversity of mycorrhizal associations. *Invasive Plant Science and Management* 5:494–505. DOI: 10.1614/IPSM-D-12-00014.1.

Koko JH, Swift SOI, Hynson NA. 2021. Hawaiian Island endemic and indigenous plant species have higher mycorrhizal incidence than the global average. *American Journal of Botany* 108:1635–1645. DOI: 10.1002/AJB2.1731.

Kolde R. 2019. Pheatmap: pretty heatmaps. *R package version 1.0.12*.

Kõljalg U, Nilsson HR, Schigel D, Tedersoo L, Larsson K-H, May TW, Taylor AFS, Jeppesen TS, Frøslev TG, Lindahl BD, Põldmaa K, Saar I, Suija A, Savchenko A, Yatsiuk I, Adojaan K, Ivanov F, Piirmann T, Pöhönen R, Zirk A, Abarenkov K. 2020. The Taxon Hypothesis Paradigm—On the unambiguous detection and communication of taxa. *Microorganisms* 8:1910. DOI: 10.3390/microorganisms8121910.

Koorem K, Tulva I, Davison J, Jairus T, Öpik M, Vasar M, Zobel M, Moora M. 2017. Arbuscular mycorrhizal fungal communities in forest plant roots are simultaneously

shaped by host characteristics and canopy-mediated light availability. *Plant and Soil* 410:259–271. DOI: 10.1007/S11104-016-3004-0/TABLES/1.

Krebs CJ. 1999. *Ecological methodology*.

Leite R da C, Santos AC dos, Santos JGD dos, Leite R da C, Oliveira LBT de, Hungria M. 2019. Mitigation of Mombasa Grass (*Megathyrsus maximus*) dependence on nitrogen fertilization as a function of inoculation with *Azospirillum brasilense*. *Revista Brasileira de Ciência do Solo* 43. DOI: 10.1590/18069657rbcs20180234.

Masumoto S, Kitagawa R, Nishizawa K, Kaneko R, Osono T, Hasegawa M, Matsuoka S, Uchida M, Mori AS. 2021. Functionally explicit partitioning of plant β - diversity reveal soil fungal assembly in the subarctic tundra. *FEMS Microbiology Ecology*:1–11. DOI: 10.1093/femsec/fiab129.

Müller MML, Guimarães MF, Desjardins T, Mitja D. 2004. The relationship between pasture degradation and soil properties in the Brazilian amazon: a case study. *Agriculture, Ecosystems & Environment* 103:279–288. DOI: 10.1016/J.AGEE.2003.12.003.

Munkholm LJ. 2011. Soil friability: A review of the concept, assessment and effects of soil properties and management. *Geoderma* 167–168:236–246. DOI: 10.1016/J.GEODERMA.2011.08.005.

Neuenkamp L, Zobel M, Koorem K, Jairus T, Davison J, Opik M€, Vasar M, Moora M. 2020. Light availability and light demand of plants shape the arbuscular mycorrhizal fungal communities in their roots. DOI: 10.1111/ele.13656.

Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Gï Ockner 10 FO, Tedersoo L, Saar I, Abarenkov K. 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* 47:259–264. DOI: 10.1093/nar/gky1022.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. Package 'vegan.' *Community ecology package, version* 2:1–295.

Pedrinho A, Mendes LW, Merloti LF, de Cassia Da Fonseca M, de Souza Cannavan F, Tsai SM. 2019. Forest-to-pasture conversion and recovery based on assessment of microbial communities in Eastern Amazon rainforest. *FEMS Microbiology Ecology* 95:236. DOI: 10.1093/FEMSEC/FIY236.

Pendergrass AG, Knutti R, Lehner F, Deser C, Sanderson BM. 2017. Precipitation variability increases in a warmer climate. *Scientific Reports 2017 7:1* 7:1–9. DOI: 10.1038/S41598-017-17966-Y.

Perkins KS, Nimmo JR, Medeiros AC. 2012. Effects of native forest restoration on soil hydraulic properties, Auwahi, Maui, Hawaiian Islands. *Geophysical Research Letters* 39:n/a-n/a. DOI: 10.1029/2012GL051120.

Pickett B, Irvine IC, Bullock E, Arogyaswamy K, Aronson E. 2019. Legacy effects of invasive grass impact soil microbes and native shrub growth. *Invasive Plant Science and Management* 12:22–35. DOI: 10.1017/inp.2018.32.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41:D590–D596. DOI: 10.1093/nar/gks1219.

Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017. Fungal identification using molecular tools: A primer for the natural products research community. DOI: 10.1021/acs.jnatprod.6b01085.

Schoeneberger PJ, Wysocki DA, Benham EC. 2012. *Field book for describing and sampling soils*. Government Printing Office.

Schwertmann U. 1993. Relations between iron oxides, soil color, and soil formation. *Soil color. Proc. symposium, San Antonio, 1990*:51–69. DOI: 10.2136/SSSASPECPUB31.C4.

Shengli Y, Li J, Zhisheng AN, Shiyue C, Fukusawa H. 2001. Transformation functions of soil color and climate. *Science in China, Series D* 44.

Soil Survey Staff. 2014. Web Soil Survey. *Natural Resources Conservation Service, United States Department of Agriculture*:1–2.

Soti P, Thomas V. 2021. Review of the invasive forage Grass, Guinea grass (*Megathyrsus maximus*): Ecology and potential impacts in arid and semi-arid regions. *Weed Research*. DOI: 10.1111/wre.12512.

Thomas RS, Dakessian S, Ames RN, Brown MS, Bethlenfalvay GJ. 1986. Aggregation of a silty clay loam soil by mycorrhizal onion roots. *Soil Science Society of America Journal* 50:1494–1499. DOI: 10.2136/SSSAJ1986.03615995005000060023X.

Torrent J, Schwertmann U, Fechter H, Alferez F. 1983. Quantitative relationships between soil color and hematite content. *Soil Science* 136.

Unger PW, Kaspar TC. 1994. Soil compaction and root growth: A review. *Agronomy Journal* 86:759–766. DOI: 10.2134/agronj1994.00021962008600050004x.

United Nations. 2020. UN decade on restoration. *www.decadeonrestoration.org*.

US Forest Service. 2021. Pacific Island Ecosystems at Risk. *www.hear.org/pier*.

Walker LR, Vitousek PM. 1991. An invader alters germination and growth of native dominant tree in Hawai'i. *Ecology* 72:1449–1455. DOI: 10.2307/1941117.

Wang KH, McSorley R, Bohlen P, Gathumbi SM. 2006. Cattle grazing increases microbial biomass and alters soil nematode communities in subtropical pastures. *Soil Biology and Biochemistry* 38:1956–1965. DOI: 10.1016/j.soilbio.2005.12.019.

Wen T, Yu G-H, Hong W-D, Yuan J, Niu G-Q, Xie P-H, Sun F-S, Guo L-D, Kuzyakov Y, Shen Q-R. 2022. Root exudate chemistry affects soil carbon mobilization via microbial community reassembly. *Fundamental Research* 2:697–707. DOI: 10.1016/j.fmre.2021.12.016.

Wiegner TN, Tubal RL. 2010. Comparison of dissolved organic carbon bioavailability from native and invasive vegetation along a Hawaiian river. *Pacific Science* 64:545–555. DOI: 10.2984/64.4.545.

Wright ES. 2016. Using DECIPHER v2.0 to analyze big biological sequence data in R. *The R Journal* 8:352–359.

Yadav S, Caliboso KD, Nanquil JE, Zhang J, Kae H, Neupane K, Mishra B, Jha R. 2021. Cecal microbiome profile of Hawaiian feral chickens and pasture-raised broiler (commercial) chickens determined using 16S rRNA amplicon sequencing. *Poultry Science* 100:101181. DOI: 10.1016/J.PSJ.2021.101181.

Yelenik SG, D'Antonio CM, August-Schmidt E. 2017. The influence of soil resources and plant traits on invasion and restoration in a subtropical woodland. *Plant Ecology* 218:1149–1161. DOI: 10.1007/s11258-017-0757-3.

Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research* 42:D643–D648. DOI: 10.1093/nar/gkt1209.

Zhao M, Zhao J, Yuan J, Hale L, Wen T, Huang Q, Vivanco JM, Zhou J, Kowalchuk GA, Shen Q. 2021. Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth. *Plant, Cell & Environment* 44:613–628. DOI: 10.1111/pce.13928.

Table 3.1. Basic soil description of soil structure, color, roots, pores, brittleness, fluidity, and smeariness based on NRCS Field Book.

	Megathyrsus Site	**Restored Site**
Soil structure	Fine, granular	Medium, blocky, angular
Dry color	5 YR 3/ 6	7.5 YR 3/ 6
Wet color	5 YR 2/ 4	7.5 YR 3/ 4
Roots	Many fine roots throughout	
Pores	Many, fine dendritic tubular	
Brittleness	Deformable	Brittle
Fluidity	Non-fluid	
Smeariness	Non-smeary	

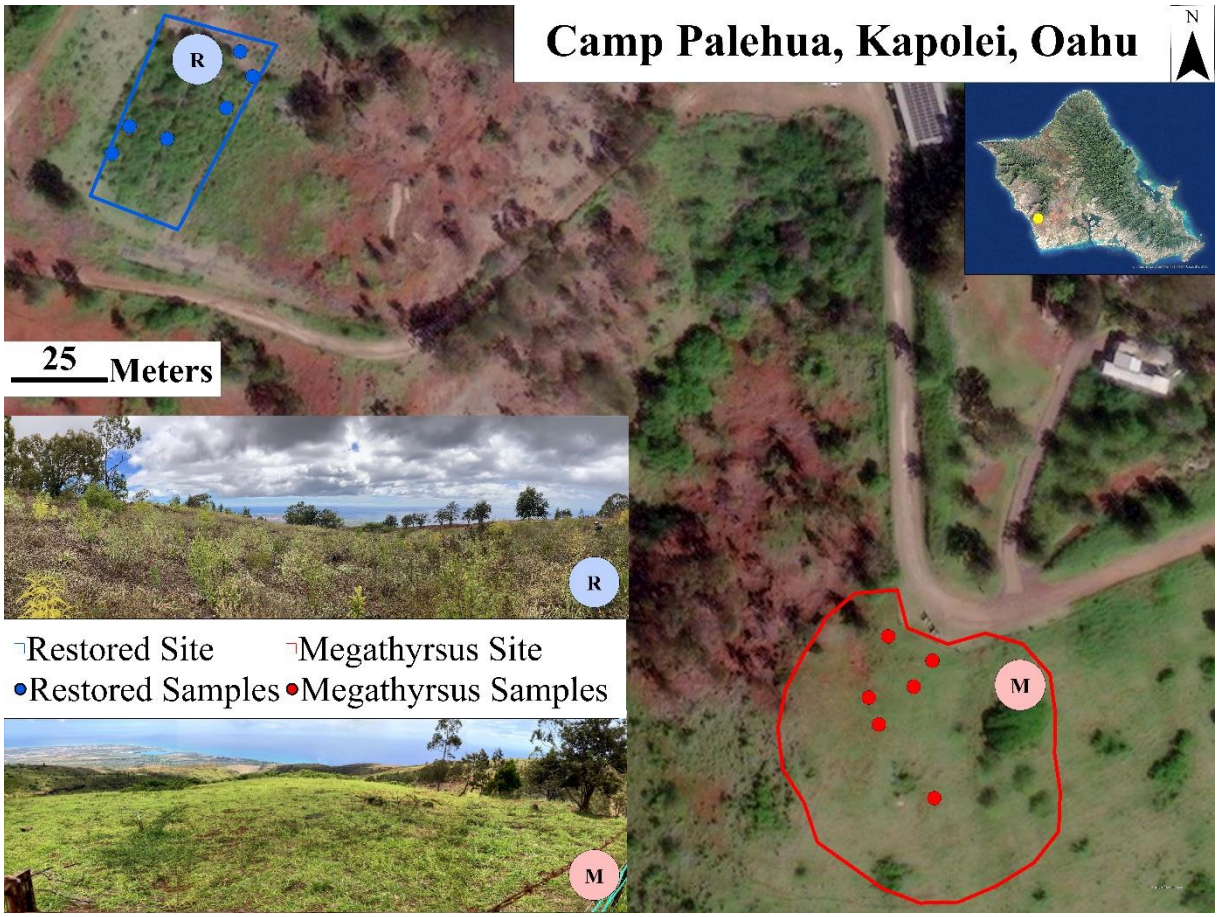

Figure 3.1. Map showing location of Restored Site (R) and Megathyrsus Site (M). Circular points (red and blue) indicate location of soil sampling for microbial community analysis. Upper right corner, the yellow point indicates the location of Camp Palehua on the island of O‘ahu. Lower left corner, overview images of each site, labeled R for Restored Site and M for Megathyrsus site.

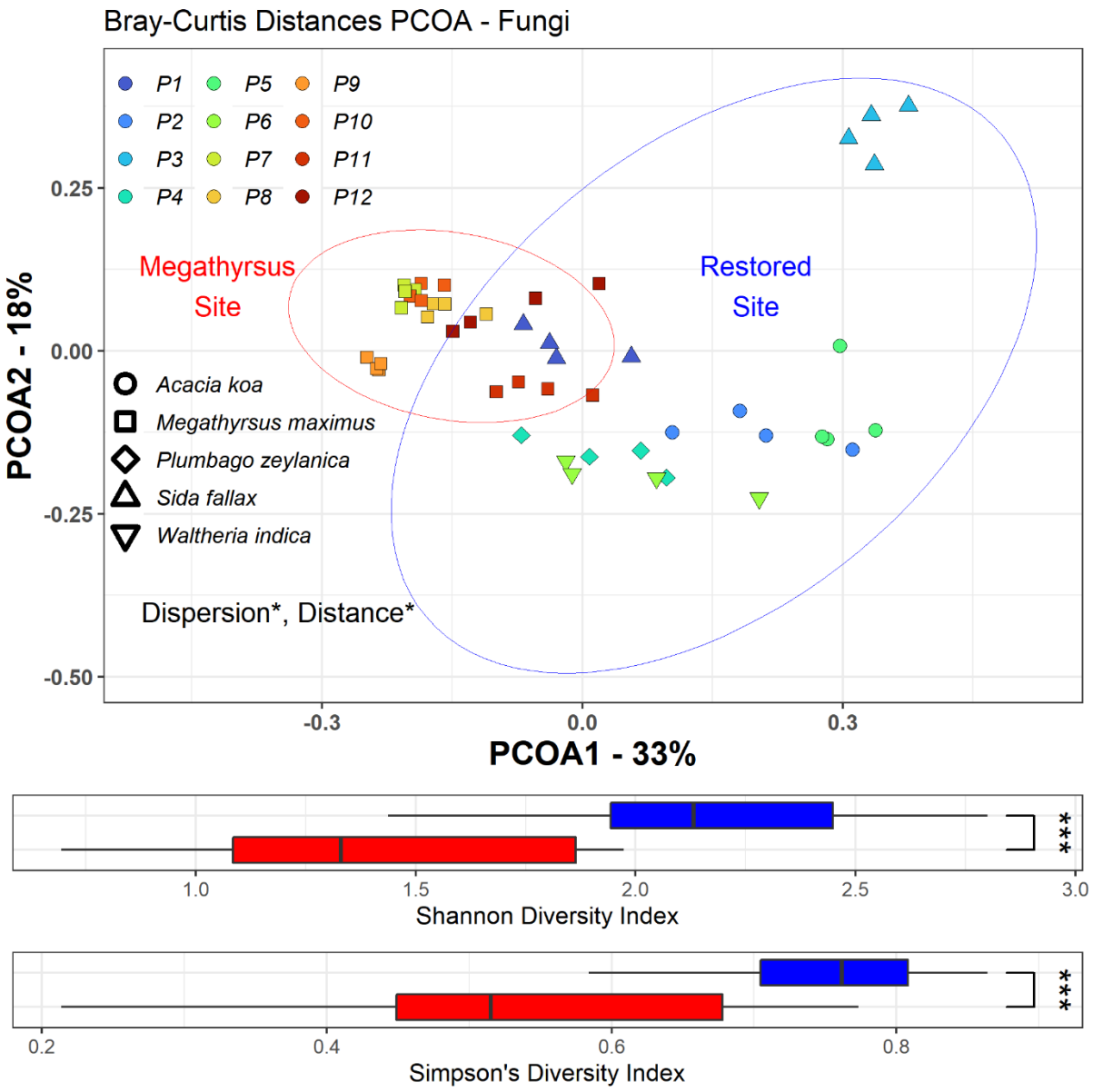

Figure 3.2. Top, Bray-Curtis PCoA of fungal community by site. Point color denotes sampling point of each sub-sample, and point shape denotes the closest plant species at each sampling point. P1-P6 are samples from the restored site and P7-P12 are samples from the Megathyrsus site. Dispersion of samples (ANOVA, $F_{(1, 46)} = 23.865$, $P = 0.0001$) and centroid location (PERMANOVA, $F_{(1, 36)} = 61.343$, $P = 0.001$, $R^2 = 0.237$) differed between sites. Bottom, Shannon Diversity Index ($F_{(1, 36)} = 222.640$, $P < 0.001$) and Simpson's Diversity Index ($F_{(1, 36)} = 238.838$, $P = 0.00$) by site. *** denotes significance at $p < .01$.

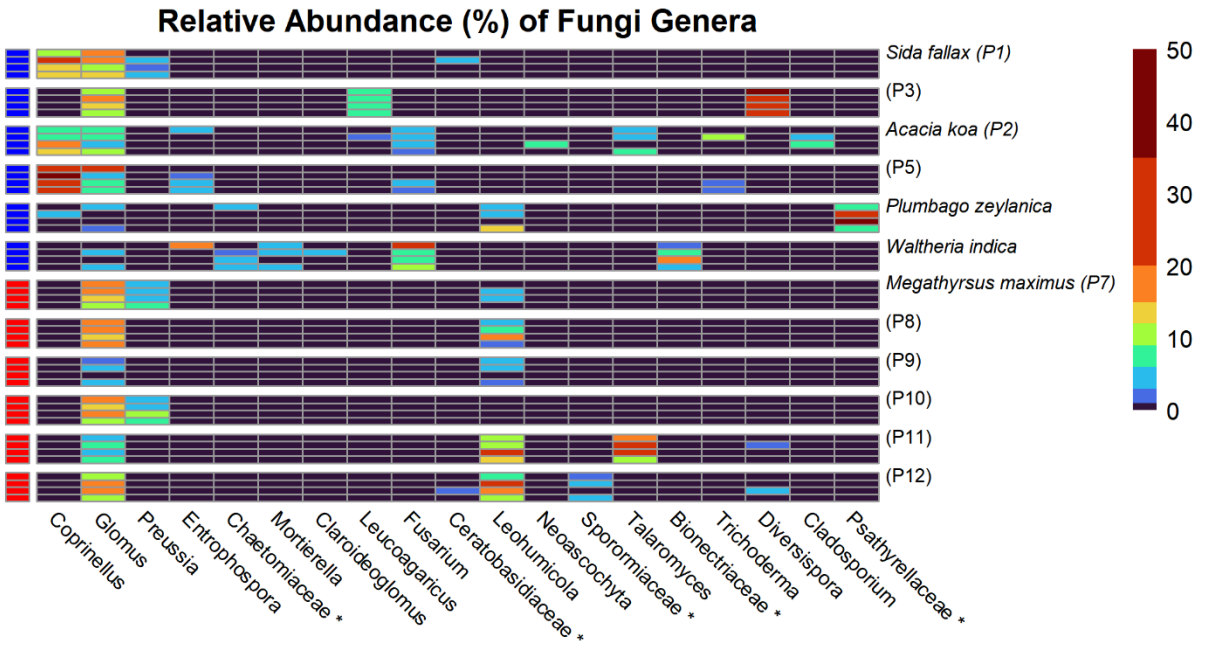

Figure 3.3. Heatmap of percent relative abundance of fungi genera. Each row represents one subsample, and grouped rows represent one sampling point. Left sidebar denotes site, with red indicating the sample was from the Megathyrsus site, and blue indicating the sample was from the Restored site. Right sidebar indicates nearest plant species and point number. * indicates unclassified genera within a family.

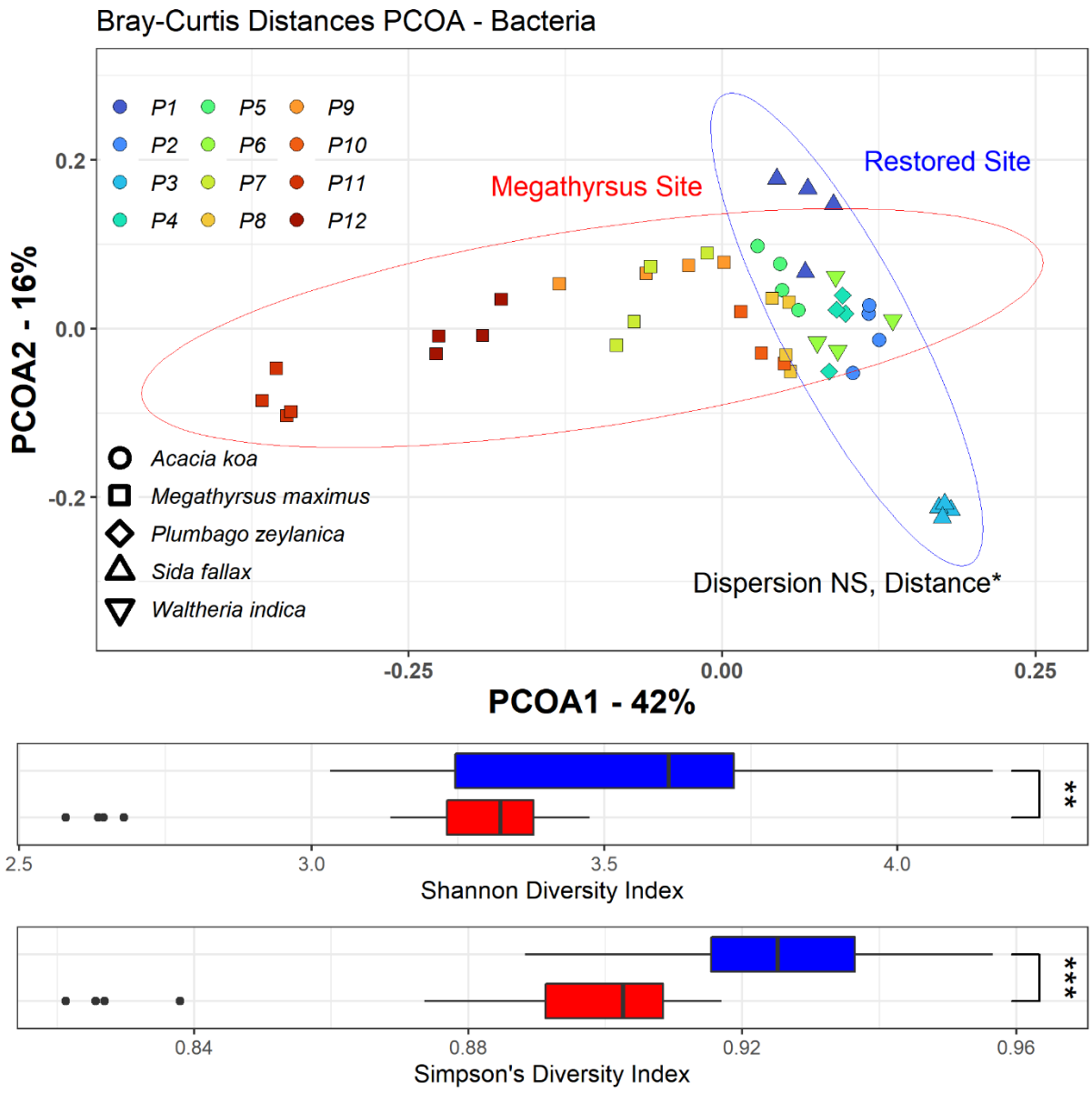

Figure 3.4. Top, Bray-Curtis PCoA of bacterial community by site. Point color denotes sampling point of each sub-sample, and point shape denotes the closest plant species at each sampling point. P1-P6 are samples from the restored site and P7-P12 are samples from the Megathyrsus site. Centroid location, but not sample dispersion, significantly differed between sites (PERMANOVA, $F_{(1, 45)} = 9.9789$, $P = 0.001$, $R^2 = 0.1815$). Dispersion did differ, however, by closest plant species (ANOVA, $F_{(4, 42)} = 7.927$, $p < 0.001$), as did centroid location (PERMANOVA, $F_{(4, 42)} = 4.7421$, $P = 0.001$, $R^2 = 0.311$). Bottom, Shannon Diversity Index and Simpson's Diversity Index by site. *** denotes significance at $p < 0.01$.

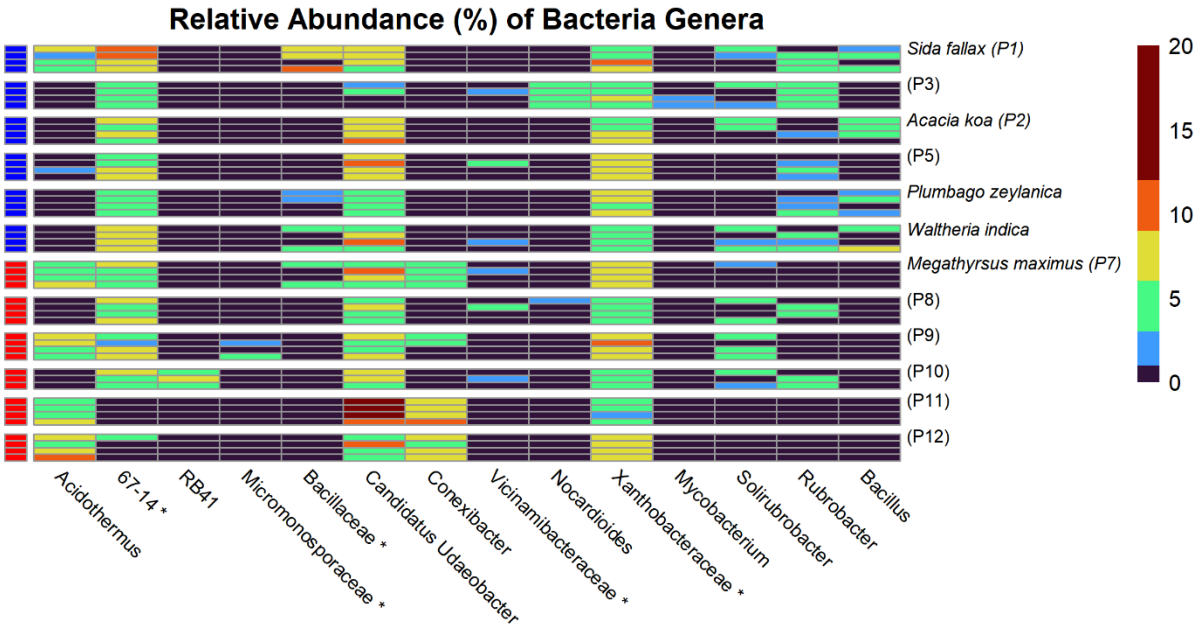

Figure 3.5. Heatmap of percent relative abundance of bacteria genera. Each row represents one subsample, and grouped rows represent one sampling point. Left sidebar denotes site, with red indicating the sample was from the Megathyrsus site, and blue indicating the sample was from the Restored site. Right sidebar indicates nearest plant species and point number. * indicates unclassified genera within a family.

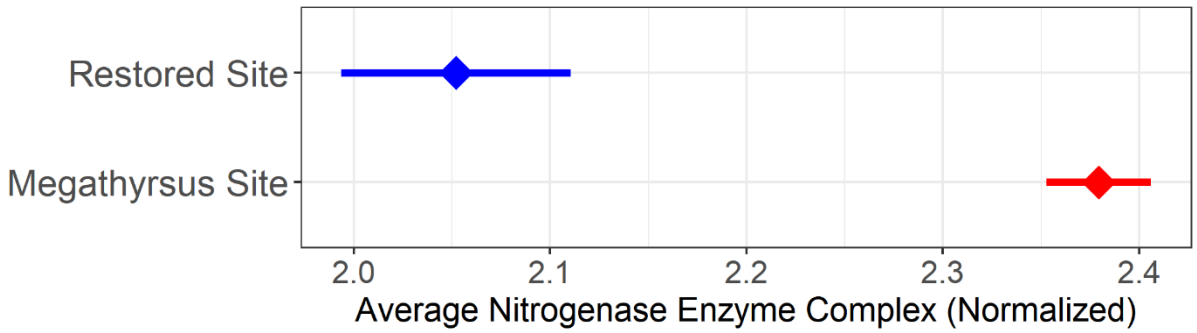

Figure 3.6. Point-range (average and standard error) of normalized average abundance of nitrogenase enzyme complex by site. The difference between sites was significant (ANOVA, $F_{(1, 75)} = 60.146$, $p < 0.001$; Bonferroni paired t-test, $p < 0.001$).

CHAPTER 4

MEGATHYRSUS MAXIMUS SOIL MICROBIOME DRIVES BELOWGROUND COMPETITION WITH AN ENDEMIC HAWAIIAN SHRUB *CHENOPODIUM OAHUENSE*

Abstract

The invasive grass *Megathyrsus maximus* has a wide range of impacts across much of the tropics, including the Hawaiian Islands. Restoration of grass-invaded areas has faced challenges due to low native seed germination, suggesting, among other explanations, that there is potentially a soil legacy effect at play. We conducted a greenhouse plant-soil feedback (PSF) study, using a whole soil inoculum approach to determine the effect of the *Megathyrsus* soil microbial community on *Megathyrsus* as well as on the Hawaiian endemic dry forest shrub *Chenopodium oahuense,* a common choice for restoration projects in the Hawaiian Islands. We also employed a moderate drought treatment to test if PSF was impacted by water availability. We hypothesized that there would be positive con-specific and negative hetero-specific PSF, with PSF effects being enhanced by the presence of drought. We found evidence of positive con-specific PSF (i.e., *Megathyrsus* inoculum on *Megathyrsus* growth) that increased belowground growth, suggesting the *Megathyrsus maximus* soil microbiome mediates belowground competition between *Megathyrsus* and native plants. In addition, we found that moderate drought interacted with the presence of the *Megathyrsus* soil microbiome to delay *Chenopodium oahuense* germination, suggesting that drought enhances negative PSF effects on the native, which can worsen the disadvantage that native plants face due to competition with the fast-growing invasive grass. Delayed germination and reduced resource availability from increased *Megathyrsus maximus* root growth, imparted by the soil microbiome of *Megathyrsus maximus,* could have a negative impact on restoration projects that include *Chenopodium oahuense*; other native plants might be similarly disadvantaged by the *Megathyrsus* soil microbiome.

Introduction

Reciprocal interactions between the abiotic soil environment, soil microbial community and plant community, known as plant-soil feedbacks (PSF), can have positive, negative, or neutral impacts on measures of plant fitness, such as growth and germination (Bardgett & Wardle, 2010; Miller, Perron & Collins, 2019). Here, PSF refers to direct or individual PSFs (Kulmatiski et al., 2008), meaning that the soil was conditioned by a single plant species, and PSF is measured as the effect of that conditioned soil on the growth of the conditioning plant species or a competitor. There is evidence that PSF can contribute to the long-term impacts of

invasive plants, making restoration more challenging (Yelenik & D'Antonio, 2013); therefore, distinguishing the mechanism(s) responsible for negative PSF effects can improve interventions intended to mitigate their impact (Kama, Sun & Afzal, 2020). Some of the most prolific invasive plants are invasive grasses that have been deliberately introduced as pasture grasses (Fusco et al., 2021; Rhodes et al., 2021; Rayment, French & Bedward, 2022). The impacts of these grasses include increasing wildfire intensity (Fusco et al., 2019, 2021; Kerns et al., 2020; Walker & Morgan, 2022), and threats to native ecosystems, home to at-risk endemic species, in places like Hawai'i (Goergen & Daehler, 2002; Cabin et al., 2002; Daehler & Goergen, 2005; D'Antonio, Hughes & Tunison, 2011; Ammondt et al., 2013; Kerns et al., 2020). Multi-dimensional PSF research has emerged as a pressing research need (Baxendale et al., 2014; Gundale & Kardol, 2021), and in Hawai`i, incorporating the context of climate change in PSF research is important for projecting future impacts of PSF. Climate change is expected to increase the frequency or intensity of drought (Foster, 2001; Chu, Chen & Schroeder, 2010); however, below-ground ecosystem responses to drought have been understudied, with one review estimating that around ¾ of the drought literature examines aboveground responses only (Vilonen, Ross & Smith, 2022).

Megathyrsus maximus, commonly known as Guinea grass, and previously known as *Panicum maximum*, is native to Africa, but has been widely introduced elsewhere as a pasture grass. It is known to be especially successful as an invader in tropical and warm temperate areas and is drought and fire resistant. In *Megathyrsus maximus* populations in Texas, there is evidence of positive con-specific feedback that reinforces invasion, although the mechanism requires further investigation (Bowman, Plowes & Gilbert, 2023). In Hawai`i, restoration efforts in areas invaded by *Megathyrsus maximus* have found that grass removal alone was not an effective restoration strategy (Ammondt et al., 2013), suggesting that there could be a soil legacy effect. Native seed additions in grass removal areas were largely unsuccessful (Ammondt et al., 2013), which could likewise suggest that a soil legacy was limiting germination of native species. On the other hand, past studies have shown that biomass of *M. maximus* increased in the presence of common native Hawaiian plants used in restoration (Ammondt & Litton, 2012), which implies that native plants could have a facilitating effect on grass growth.

An invader's competitive advantage due to direct competition may be less relevant to the early stages of a restoration project, where herbicide and mechanical removal of the grass

invader minimize competition from the invader. It is during such early life stages that soil legacy effects may be the most important for determining plant community trajectories. In this study, we focus on the potential for soil legacy effects by an invasive grass on a native plant, in the absence of direct competition with the invader. Soil legacy effects can influence early stages of a restoration project, which are critical to the long-term restoration success (Grman & Suding, 2010; Middleton & Bever, 2012; Wolfsdorf et al., 2021). Our objective was to determine if the *Megathyrsus maximus* soil microbiome may have a negative effect on native plant germination and/or native plant growth, leading to a negative, heterospecific PSF. We also investigated if the *Megathyrsus maximus* soil microbiome had a positive effect on *M. maximus* seedling survival and/or growth, which would lead to a positive, conspecific PSF.

The native plant used in this experiment was *Chenopodium oahuense*, an endemic shrub that is a common choice for restoration projects in Hawai`i (Daehler & Goergen, 2005; Ammondt et al., 2013; Medeiros, Allmen & Chimera, 2014; Ellsworth, Litton & Leary, 2015; Koko, Swift & Hynson, 2021). The growth of *C. oahuense* is known to be sensitive to nutrient availability (Cole et al., 2021), but previous studies have not characterized its potential response to soil microbiota, We hypothesized that *Chenopodium oahuense* would experience negative heterospecific PSF in response to *Megathyrsus* soil microbiota. Conversely, we hypothesized that *Megathyrsus maximus* would experience positive con-specific PSF when exposed to its own microbiota. We also hypothesized that drought would make negative heterospecific feedback more negative, and positive conspecific feedback more positive (Veresoglou et al., 2022). We tested for potential PSF on *Megathyrsus maximus* seedling survival, *Chenopodium oahuense* germination, days to germination, and seedling survival, as well as: aboveground biomass, belowground biomass, total biomass and root-to-shoot ratio of both species.

Methods

This PSF study used the ‘conditioned’ vs ‘control’ design, a modification of the ‘self’ vs ‘other’ design (Brinkman et al., 2010), with the soil inoculum method. The inoculum method is ideal for isolating the impact of soil biota when the inoculum volume is less than 5% (Smith-Ramesh & Reynolds, 2017).

For simulating drought in our pot studies, we employed slow drying and re-wetting cycles (Turner, 2019), rather than abrupt irrigation discontinuation, which does not resemble

drying rates in typical ecosystems (Poorter et al., 2012). More gradual drought stress exposure allows species to display osmotic adjustment (Bartlett et al. 2014), rehydration capacity, and adjustments to photosynthetic yield (Harb et al., 2010; Trueba et al., 2019), all of which are critical to drought tolerance and competition dynamics in drought conditions.

On October 2nd, 48 conetainers (3.8 cm diameter, 30 cm height) were filled with 90 g of Niu Brand Professional Blend Potting Soil (Niu Nursery, Honolulu, HI). The conetainers were thoroughly drenched daily for the next three days to break up soil surface tension. On October 9th 2022, three of the drenched conetainers were each wetted with 100 mL of water, and allowed to drain for 24 hours before the volume of the drainage was measured. The average water absorption (100 ml – volume of drainage) was 17 ml, which we considered to be 100% pot capacity (PC). For the soil conditioning phase and the regular water condition of the growth phase, each pot received 15 ml (90% PC) every three to four days (i.e. infrequent deficit irrigation). In the drought water condition of the growth phase, each pot received 10 ml (60% PC) every three to four days. In the greenhouse, a 70% aluminet shade cloth was set-up to protect conetainers from direct sunlight and reflectance from south side of the greenhouse (Figure 1). Remaining sides were also partially shaded due to presence of a neighboring building and a storage area within the greenhouse.

Megathyrsus maximus seedlings were collected from around the Campus Services Building at the University of Hawaii at Manoa, with care taken to extract seedlings with the roots intact. From all the seedlings collected, seedlings with no more than 2 leaves, and with a height less than 7.5 cm were selected. The root of each seedling was rinsed in water until no more soil particles were visible prior to planting each seedling in its own pot. *Chenopodium oahuense* was grown from seeds gifted by the Malama Learning Center nursery staff, sourced from plants on their property in Kapolei, Oahu. For each pot, approximately 10-15 *Chenopodium oahuense* seeds were added. Whether adding seeds or seedling, a blunt probe was used to create a depression in the center of the soil surface, a few mm deep, and the seeds or seedling were added into that depression, and then the probe was used to gently push soil back to lightly cover seeds or cover the root of the seedling. The probe was wiped with an unscented bleach wipe between use for each pot.

The three conetainers used to determine pot water holding capacity were set aside, and not used further in the conditioning phase. *Megathyrsus maximus* seedlings were added to the remaining 45 conetainers. Twenty-four *Megathyrsus maximus* individuals survived the five-week conditioning phase (October 10th-November 13th, 2022). At the end of the conditioning phase, the aboveground biomass was removed, and after emptying the contents of the conetainer into a paper bag, the belowground biomass was removed as well, leaving only the soil. On the same day, the growth phase conetainers were filled with either 86 g (inoculum conetainers) or 90 g (control conetainers) of Niu Brand Professional Blend Potting Soil and thoroughly drenched. A randomizer (RANDOM.org) was used to randomly assign inoculum from each of the surviving conditioning trial pot numbers (hereafter, inoculum IDs) to one growth phase pot for each species, (i.e., one *Chenopodium* pot, and one *Megathrysus* pot). From each inoculum pot, 4g of soil were transferred from the bag containing soil from the assigned inoculum ID and added on top of the commercial 86 g of soil.

For the growth phase, we started with 12 trials per soil condition (inoculated or uninoculated soil) per growth species (*Megathyrsus maximus, Chenopodium oahuense)* per water condition (regular water/drought). On November 14th, growth species were added to each conetainer, the conetainers were thoroughly drenched, and then the conetainers were immediately transitioned to their assigned watering regime. The earliest germination in *Chenopodium oahuense* trials was recorded on December 12th. If more than one seed germinated in a pot, the second and any subsequent seedlings were thinned such that the oldest seedling remained. Due to a heat wave, all *Megathyrsus maximus* seedlings added on November 14th died, and were replaced on November 27th, and all but four conetainers (ID: 21, 40, 42, 59) were replaced for a final time on December 12th. Only one of those four individuals survived the full growth period (ID: 42), and this individual was excluded from biomass-based analyses. In January 2023, any *Chenopodium oahuense* seedlings that had to be thinned from conetainers with existing seedlings were lightly rinsed in water and placed into conetainers without germinants to increase replicates in the drought condition. Transferred seedlings were excluded in statistical analysis of germination rates and time to germination. Ultimately, the drought condition did not have sufficient surviving replicates for biomass analysis, so transferred seedlings did not impact any biomass-based analyses. After 7 weeks, each pot was assessed for presence of absence of germination and seedlings were harvested, with the last pot harvested

occurring on March 6th 2023. Seedling biomass was then dried for 24 hrs at 50 C. Aboveground and belowground dry mass measurements were recorded for each trial.

A logistic regression was used to test the predictive power of soil condition and water condition on *Megathyrsus maximus* survival rate and *Chenopodium oahuense* germination rate and survival rate, using the "binomial" family in the glm function (R Development Core Team, 2022). A linear regression was also used to examine effects of soil and water condition on days to germinate for *Chenopodium oahuense*, using "glm.nb" from the *MASS* package (Ripley et al., 2013). For all non-biomass models, we tested soil condition, water condition, and the interaction of the two, as predictors. The anova function using the "Chisq" test and the "drop1" function was utilized to test the significance of each predictor, and the *emmeans* package (Lenth et al., 2019) was utilized to determine confidence intervals and treatment pair comparison ratios or log-odd ratios based on the models.

To calculate PSF effects on biomass, we paired inoculated and uninoculated conetainers within the same growth species and water condition, pairing each uninoculated conetainer to the inoculated conetainer directly adjacent or directly diagonal on the greenhouse bench (Figure S1). We used the pairwise comparison formula from Kulmatiski et al. 2016 (Formula 1), rather than condition averages, which can lead to inflated type 1 errors (Brinkman et al., 2010).

Formula 1:

PSF = (Trial_inoculated-Trial_uninoculated)/[max(Trial_inoculated,Trial_uninoculated)]

In Formula 1**,** PSF is assessed from paired trials of species grown in the inoculated condition and same species grown in uninoculated condition, in the same water condition. Negative values represent depressed biomass production in the presence of inoculum, while positive values represent increased biomass production in the presence of inoculum.

PSF was calculated for aboveground biomass, belowground biomass, total biomass and root-to-shoot ratio. Finally, for each PSF measure, we used a mixed effect, generalized Gaussian linear model, with fixed effects growth species and water condition, and random effect inoculum ID, to account for non-independence in conetainers that shared an inoculum source. If the sample size for drought trials was insufficient ($n < 5$), we ran the mixed-effects linear model on the regular water trials only and removed water condition as a fixed effect. We utilized "glht" with

the "holm" adjustment from the *multcomp* package (Hothorn, Bretz & Westfall, 2008) to test the hypothesis that for aboveground, belowground and total growth, PSF was greater, or more positive in *Megathyrsus,* compared to *Chenopodium*. We also used "glht" to test the hypothesis that, for aboveground, belowground, and total growth feedback, *Megathyrsus maxmius* would have positive feedback (i.e., greater than 0) and *Chenopodium oahuense* would have negative feedback (i.e., less than 0). For the root-to-shoot ratio, the alternative hypothesis remained two-sided for all tests. The *DHARMa* package was used for model diagnostics (Hartig, 2020).

Results

Plant-soil feedback effects on germination and survival

Neither soil condition, nor water condition were significant predictors of *Megathyrsus* survival (P > 0.1, Figure 2D). There were also no significant differences between any of the groups (uninoculated soil-regular water, uninoculated soil-drought, inoculated soil-regular water, inoculated soil-drought). In contrast, water condition was a significant predictor of *Chenopodium* germination (ANOVA, P = 0.02, "drop1" P = 0.03). The difference in germination between drought and regular water conetainers in the uninoculated soil condition was significant (P = 0.045, log-odds ratio 1.95, 95% CI [0.04, 3.85]). The average germination rate in regular uninoculated conetainers was 83% (95% CI [52%. 96%], Figure 2A) while the average germination rate in drought uninoculated conetainers was 42% (95% CI [19%, 69%], Figure 2A). The interaction between soil and water condition was not significant (P > 0.05).

For the time to germination, the interaction between soil condition and water condition was significant (ANOVA, P = 0.01, "drop1", P = 0.01). In uninoculated soil, there was no difference between regular water and drought conditions in time to Chenopodium germination (mean = 41 days versus 38 days, respectively, P = 0.45 Figure 2C). However, in inoculated soil with drought conditions, Chenopodium time to germination was delayed, relative inoculated soil with regular water conditions (52 days versus 41 days respectively, P = 0.01, Figure 2C). Thus, the significant interaction term indicated that, while drought alone or inoculum alone did not delay germination, the drought and inoculum combined delayed germination significantly, compared to all other groups (uninoculated soil-regular, uninoculated soil-drought, inoculated soil-regular), with the delay ranging from 11 to 14 days. The interaction term had to be dropped from the *Chenopodium* survival model due to model fitting issues (standard error over 2000 for

some predictors), but *Chenopodium* survival did not differ among inoculation and water treatments ($P > 0.05$, Figure 2B). Survival in the regular water condition was 84% and 92% in the uninoculated and inoculated conditions, respectively, and survival in the drought water condition was 55% and 72%, respectively.

Plant-soil feedback effects on growth

Due to low sample size in the drought condition (n = 3 *Chenopodium* pairs, n= 3 *Megathyrsus* pairs), we did not test for any effect of drought condition on biomass PSF. The average PSF valude for *Chenopodium* growth was –0.05, and the probability that the population average feedback value *Chenopodium oahuense* growth would be negative was 71% for belowground growth and 75% for total growth. The difference in average feedback value between *Chenopodium oahuense* and *Megathyrsus maxmius* was significantly greater than zero for belowground and total biomass ($p = 0.047$, $p = 0.03$, respectively, Figure 3). The presence of the *Megathyrsus maximus* microbiome improved belowground/total growth 20% more in *Megathyrsus maxmius,* compared to *Chenopodium oahuense* (belowground PSF = 0.20, one-sided hypothesis, 95% lower bound 0.003; total biomass PSF = 0.20, one-sided hypothesis, 95% lower bound 0.03). The average feedback value for belowground *Megathyrsus maximus* growth was significantly greater than 0 (0.16, one-sided hypothesis, 95% lower bound 0.01). Additionally, the average feedback value for total *Megathyrsus maximus* growth was significantly greater than 0 (0.15, one-sided hypothesis 95% lower bound 0.03).

Discussion

Phenological Mismatch: Delayed Germination of the Native with Drought and *Megathyrsus* Soil Inoculum

For the endemic shrub *Chenopodium oahuense,* we found no evidence of a difference in germination or survival between uninoculated and inoculated condition treatments, indicating lack of a PSF on germination or survival. However, as expected, regular water improved germination compared to drought, and this effect was consistent across soil conditions. Time to germination in drought uninoculated, well-watered uninoculated, and well-watered inoculated treatments was 27%, 22% and 21% faster, respectively, compared to germination in the drought and inoculated treatment (Figure 2C). This suggests that the negative impact of the *Megathyrsus*

maximus soil microbial community on *Chenopodium oahuense* germination becomes important under drought stress, resulting in delayed germination.

Delayed germination by *C. oahuense* could have implications for establishment and persistence of *C. oahuense*. There is evidence that species invading outside of their native range often exhibit less delayed germination compared to native species in their native range under drought or reduced water availability (Young et al., 2015; Funk, Hoffacker & Matzek, 2015). Germination timing is a key factor affecting the outcome of interspecific competition (Gioria, Pyšek & Osborne, 2018), especially in the case of *Megathyrsus maximus,* which quickly builds up a large seed bank (Soti & Thomas, 2021) and is known to outcompete native Hawaiian species for light very quickly (Ammondt & Litton, 2012). There is evidence that shifting lifecycle timelines and inter-species competition for light can influence resource allocation strategies as time goes on (Heberling et al., 2019). Ultimately, delayed germination may further contribute to the already significant disadvantages that *Chenopodium oahuense* faces in *Megathyrsus maximus* invaded areas.

<u>Belowground Competition and the Soil Microbiome: Conspecific PSF is significantly more positive than Heterospecific PSF</u>

We found that inoculation of soil with the *Megathyrsus* soil microbiome resulted in significantly more positive feedback for conspecific individuals compared to heterospecific individuals (15% vs -5%, respectively). The average feedback of *Megathyrsus* inoculum for belowground and total growth of *Megathyrsus maximus* was 15% (i.e., 15% greater growth in the presence of inoculum compared to without inoculum), and the model predicted that the probability that the population average feedback value for total growth of *Chenopodium oahuense* would be negative was over 70%, indicating that PSF on *C. oahuense* is most likely negative or near neutral. Our finding of positive con-specific feedback agrees with evidence of positive con-specific feedback in other parts of *Megathyrsus maximus'* non-native range (Bowman, Plowes & Gilbert, 2023). Our finding of positive feedback specifically for belowground biomass could be related to specific bacteria commonly found in pastures which have been found to improve root development and biomass in *M. maximus* (Argumedo-Delira, Gómez-Martínez & Mora-Delgado, 2022).

Here, the evidence suggests that the *M. maximus* soil microbiome may have a key role in mediating belowground competition for space and resources by increasing con-specific growth, while potentially suppressing heterospecific growth. Root biomass contributes to an individual's soil resource acquisition zone (Casper & Jackson, 1997; Craine & Dybzinski, 2013), meaning that increased root growth in *Megathyrsus maxmius* in the presence of its own microbiome limits the space in which any other individuals, native or non-native, can develop a resource acquisition zone sufficient to support nutrient requirements. Many *Megathyrsus maxmius* invaded areas already have degraded soils with limited nutrient availability related to grazing, soil erosion or other past land use, like sugarcane. If the *M. maximus* microbiome contributes to further limiting the resource acquisition zone of other species, this could be a significant obstacle in successful restoration, or even conversion to any other plant community more generally.

Our findings of PSF should be followed up by field experiments, since results of greenhouse experiments aren't always consistent with field studies (Hemrová, Knappová & Münzbergová, 2016; van der Putten et al., 2016; Schittko et al., 2016; Forero et al., 2019), potentially because soil conditions, such as nutrient availability can impact strength and direction of plant-soil feedback (Revillini, Gehring & Johnson, 2016; in 't Zandt et al., 2019). Field studies will be substantially more challenging, due to difficulties estimating root biomass and manipulating soil in the field. One experimental approach that could be attempted in a field setting is to neutralize or modify the *M. maximus* soil microbiome by adding activated carbon to field soils (Wurst, Vender & Rillig, 2010). Additionally, the experiment could be expanded within the greenhouse by incorporating competition between native plants and *Megathyrsus maximus* at various densities and life stages.

Implications for Restoration in Hawai`i

To mitigate the positive impact of the *Megathyrsus maximus* microbiome on *M. maximus* growth, further research is needed to establish more specific mechanisms of impact, and to determine and test methods of suppressing soil microbiomes. Activated carbon, which was formerly used to test for allelopathic effects, has a suppressive impact on the soil microbial community (Wurst, Vender & Rillig, 2010; Kulmatiski, 2011; Shan et al., 2015), but can be costly to use (Kulmatiski & Beard, 2006). Another option could include finding other plant species that are effective at "re-conditioning" the soil (Brinkman et al., 2017) to recruit a soil

microbiome that is more favorable to native Hawaiian plants and less favorable to Megathyrsus. Finally, use of soil transplants (Benetková et al., 2020), or a soil inoculum (Wolfsdorf et al., 2021), either from existing native plant communities, or artificially developed in controlled environments, may be a less expensive option in terms of material costs, but would require an investment of time and resources into the necessary research.

Conclusion

The soil microbiome of invasive grasses, like *Megathyrsus maximus*, may influence competitive outcomes by shifting belowground competition in favor of the grass, by imparting positive con-specific feedback and negative hetero-specific feedback towards native plants. In addition, we found that the soil microbiome can interact with moderate drought to delay a native plant's germination, which can worsen some of the disadvantages that native plants already face against fast-growing, high seed producing, non-native grasses. Restoration is constrained by the limits of time and resources, and failed restoration projects can reduce public support for allocating more resources towards future restoration. Improving restoration practices in grass-invaded areas by investigating potential mechanisms of PSF can have a benefical impact on the trajectory of restoration science and outcomes for the next decade.

References

Ammondt SA, Litton CM. 2012. Competition between Native Hawaiian Plants and the Invasive Grass *Megathyrsus maximus*: Implications of Functional Diversity for Ecological Restoration. *Restoration Ecology* 20:638–646. DOI: 10.1111/j.1526-100X.2011.00806.x.

Ammondt SA, Litton CM, Ellsworth LM, Leary JK. 2013. Restoration of native plant communities in a Hawaiian dry lowland ecosystem dominated by the invasive grass *Megathyrsus maximus*. *Applied Vegetation Science* 16:29–39. DOI: 10.1111/j.1654-109X.2012.01208.x.

Argumedo-Delira R, Gómez-Martínez MJ, Mora-Delgado J. 2022. Plant Growth Promoting Filamentous Fungi and Their Application in the Fertilization of Pastures for Animal Consumption. *Agronomy* 12:1–19. DOI: 10.3390/agronomy12123033.

Bardgett RD, Wardle DA. 2010. *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change*. Oxford University Press.

Bartlett MK, Zhang Y, Kreidler N, Sun S, Ardy R, Cao K, Sack L. 2014. Global analysis of plasticity in turgor loss point, a key drought tolerance trait. *Ecology Letters* 17:1580–1590. DOI: 10.1111/ele.12374.

Baxendale C, Orwin KH, Poly F, Pommier T, Bardgett RD. 2014. Are plant-soil feedback responses explained by plant traits? *New Phytologist* 204:408–423. DOI: 10.1111/nph.12915.

Benetková P, Tichý L, Háněl L, Kukla J, Vicentini F, Frouz J. 2020. The effect of soil and plant material transplants on vegetation and soil biota during forest restoration in a limestone quarry: A case study. *Ecological Engineering* 158:1–7. DOI: 10.1016/J.ECOLENG.2020.106039.

Bowman EA, Plowes RM, Gilbert LE. 2023. Evidence of plant-soil feedback in South Texas grasslands associated with invasive Guinea grass. *NeoBiota* 81:33–51. DOI: 10.3897/NEOBIOTA.81.86672.

Brinkman EP, Van der Putten WH, Bakker E-JJ, Verhoeven KJF. 2010. Plant-soil feedback: Experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* 98:1063–1073. DOI: 10.1111/j.1365-2745.2010.01695.x.

Brinkman EP, Raaijmakers CE, de Boer W, van der Putten WH. 2017. Changing soil legacies to direct restoration of plant communities. *AoB PLANTS* 9. DOI: 10.1093/aobpla/plx038.

Cabin RJ, Weller SG, Lorence DH, Cordell S, Hadway LJ, Montgomery R, Goo D, Urakami A. 2002. Effects of Light, Alien Grass, and Native Species Additions on Hawaiian Dry Forest Restoration. *Ecological Applications* 12:1595–1610. DOI: 10.2307/3099925.

Casper BB, Jackson RB. 1997. Plant Competition Underground. *Annual Review of Ecology and Systematics* 28:545–570. DOI: 10.1146/annurev.ecolsys.28.1.545.

Chu PS, Chen YR, Schroeder TA. 2010. Changes in Precipitation Extremes in the Hawaiian Islands in a Warming Climate. *Journal of Climate* 23:4881–4900. DOI: 10.1175/2010JCLI3484.1.

Cole RJ, Soper FM, Litton CM, Knauf AE, Sparks K, Gerow KG, Giardina CP, Sparks JP. 2021. Restoration benefits of soil nutrient manipulation and weeding in invaded dry and wet tropical ecosystems in Hawai‘i. *Restoration Ecology* 29:e13390. DOI: 10.1111/rec.13390.

Craine JM, Dybzinski R. 2013. Mechanisms of plant competition for nutrients, water and light. *Functional Ecology* 27:833–840. DOI: 10.1111/1365-2435.12081.

Daehler CC, Goergen EM. 2005. Experimental restoration of an indigenous Hawaiian grassland after invasion by Buffel grass (*Cenchrus ciliaris*). *Restoration Ecology* 13:380–389. DOI: 10.1111/j.1526-100X.2005.00047.x.

D’Antonio CM, Hughes RF, Tunison JT. 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. *Ecological Applications* 21:1617–1628. DOI: 10.1890/10-0638.1.

Ellsworth LM, Litton CM, Leary JJK. 2015. Restoration impacts on fuels and fire potential in a dryland tropical ecosystem dominated by the invasive grass Megathyrsus maximus. *Restoration Ecology* 23:955–963. DOI: 10.1111/rec.12263.

Forero LE, Grenzer J, Heinze J, Schittko C, Kulmatiski A. 2019. Greenhouse- and Field-Measured Plant-Soil Feedbacks Are Not Correlated. *Frontiers in Environmental Science* 7. DOI: 10.3389/fenvs.2019.00184.

Foster P. 2001. The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Science Reviews* 55:73–106. DOI: 10.1016/S0012-8252(01)00056-3.

Funk JL, Hoffacker MK, Matzek V. 2015. Summer irrigation, grazing and seed addition differentially influence community composition in an invaded serpentine grassland. *Restoration Ecology* 23:122–130. DOI: 10.1111/rec.12162.

Fusco EJ, Balch JK, Mahood AL, Nagy RC, Syphard AD, Bradley BA. 2021. The human–grass–fire cycle: how people and invasives co-occur to drive fire regimes. *Frontiers in Ecology and the Environment* 20:117–126. DOI: 10.1002/fee.2432.

Fusco EJ, Finn JT, Balch JK, Nagy RC, Bradley BA. 2019. Invasive grasses increase fire occurrence and frequency across US ecoregions. *Proceedings of the National Academy of Sciences* 116:23594–23599. DOI: 10.1073/pnas.1908253116.

Gioria M, Pyšek P, Osborne BA. 2018. Timing is everything: does early and late germination favor invasions by herbaceous alien plants? *Journal of Plant Ecology* 11:4–16. DOI: 10.1093/JPE/RTW105.

Goergen E, Daehler CC. 2002. Factors affecting seedling recruitment in an invasive grass (*Pennisetum setaceum*) and a native grass (*Heteropogon contortus*) in the Hawaiian Islands. *Plant Ecology* 161:147–156. DOI: 10.1023/A:1020368719136.

Grman E, Suding KN. 2010. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. *Restoration Ecology* 18:664–670. DOI: 10.1111/j.1526-100X.2008.00497.x.

Gundale MJ, Kardol P. 2021. Multi-dimensionality as a path forward in plant-soil feedback research. *Journal of Ecology*:1365-2745.13679. DOI: 10.1111/1365-2745.13679.

Harb A, Krishnan A, Ambavaram MMR, Pereira A. 2010. Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Plant Physiology* 154:1254–1271. DOI: 10.1104/pp.110.161752.

Hartig F. 2020. DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *R package version 0.3* 3.

Heberling JM, McDonough MacKenzie C, Fridley JD, Kalisz S, Primack RB. 2019. Phenological mismatch with trees reduces wildflower carbon budgets. *Ecology Letters* 22:616–623. DOI: 10.1111/ELE.13224.

Hemrová L, Knappová J, Münzbergová Z. 2016. Assessment of Habitat Suitability is affected by plant-soil feedback: Comparison of field and garden experiment. *PLoS ONE* 11:e0157800. DOI: 10.1371/journal.pone.0157800.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous Inference in General Parametric Models. *Biometrical Journal* 50:346–363. DOI: https://doi.org/10.1002/bimj.200810425.

in ’t Zandt D, van den Brink A, de Kroon H, Visser EJW. 2019. Plant-soil feedback is shut down when nutrients come to town. *Plant and Soil* 439:541–551. DOI: 10.1007/s11104-019-04050-9.

Kama R, Sun J, Afzal MU. 2020. Soil microorganism impact on the native plant growth under single and co-invasion with invasive plants: Responses through plant-soil feedback. *Biomedical Letters* 6:1–10.

Kerns BK, Tortorelli C, Day MA, Nietupski T, Barros AMG, Kim JB, Krawchuk MA. 2020. Invasive grasses: A new perfect storm for forested ecosystems? *Forest Ecology and Management* 463:1–15. DOI: 10.1016/J.FORECO.2020.117985.

Koko JH, Swift SOI, Hynson NA. 2021. Hawaiian Island endemic and indigenous plant species have higher mycorrhizal incidence than the global average. *American Journal of Botany* 108:1635–1645. DOI: 10.1002/ajb2.1731.

Kulmatiski A. 2011. Changing Soils to Manage Plant Communities: Activated Carbon as a Restoration Tool in Ex-arable Fields. *Restoration Ecology* 19:102–110. DOI: 10.1111/j.1526-100X.2009.00632.x.

Kulmatiski A, Beard KH. 2006. Activated Carbon as a Restoration Tool: Potential for Control of Invasive Plants in Abandoned Agricultural Fields. *Restoration Ecology* 14:251–257. DOI: 10.1111/j.1526-100X.2006.00127.x.

Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. 2008. Plant-soil feedbacks: a meta-analytical review. *Ecology Letters* 11:980–992. DOI: 10.1111/j.1461-0248.2008.01209.x.

Lenth R, Singmann H, Love J, Buerkner P, Herve M. 2019. Package ‘emmeans.’

Medeiros AC, Allmen EI von, Chimera CG. 2014. Dry Forest Restoration and Unassisted Native Tree Seedling Recruitment at Auwahi, Maui. *Pacific Science* 68:33–45. DOI: 10.2984/68.1.3.

Middleton EL, Bever JD. 2012. Inoculation with a Native Soil Community Advances Succession in a Grassland Restoration. *Restoration Ecology* 20:218–226. DOI: 10.1111/j.1526-100X.2010.00752.x.

Miller EC, Perron GG, Collins CD. 2019. Plant-driven changes in soil microbial communities influence seed germination through negative feedbacks. *Ecology and Evolution* 9:9298–9311. DOI: 10.1002/ece3.5476.

Poorter H, Fiorani F, Stitt M, Schurr U, Finck A, Gibon Y, Usadel B, Munns R, Atkin OK, Tardieu F, Pons TL. 2012. The art of growing plants for experimental purposes: A practical guide for the plant biologist. *Functional Plant Biology* 39:821–838. DOI: 10.1071/FP12028.

van der Putten WH, Bradford MA, Pernilla Brinkman E, van de Voorde TFJ, Veen GF. 2016. Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology* 30:1109–1121. DOI: 10.1111/1365-2435.12657.

R Development Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rayment J, French K, Bedward M. 2022. Understanding patterns and pathways of exotic perennial grass invasion in South-eastern Australian grassy communities. *Diversity and Distributions* 28:1136–1150. DOI: 10.1111/ddi.13520.

Revillini D, Gehring CA, Johnson NC. 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems. *Functional Ecology* 30:1086–1098. DOI: 10.1111/1365-2435.12668.

Rhodes AC, Plowes RM, Goolsby JA, Gaskin JF, Musyoka B, Calatayud PA, Cristofaro M, Grahmann ED, Martins DJ, Gilbert LE. 2021. The dilemma of Guinea grass (*Megathyrsus maximus*): a valued pasture grass and a highly invasive species. *Biological Invasions* 23:3653–3669. DOI: 10.1007/S10530-021-02607-3/FIGURES/3.

Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D, Ripley MB. 2013. Package 'mass.' *Cran r* 538:113–120.

Schittko C, Runge C, Strupp M, Wolff S, Wurst S. 2016. No evidence that plant–soil feedback effects of native and invasive plant species under glasshouse conditions are reflected in the field. *Journal of Ecology* 104:1243–1249. DOI: 10.1111/1365-2745.12603.

Shan J, Ji R, Yu Y, Xie Z, Yan X. 2015. Biochar, activated carbon, and carbon nanotubes have different effects on fate of 14 C-catechol and microbial community in soil. *Scientific Reports* 5:16000. DOI: 10.1038/srep16000.

Smith-Ramesh LM, Reynolds HL. 2017. The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. *Journal of Vegetation Science* 28:484–494. DOI: 10.1111/jvs.12519.

Soti P, Thomas V. 2021. Review of the invasive forage Grass, Guinea grass (*Megathyrsus maximus*): Ecology and potential impacts in arid and semi-arid regions. *Weed Research*. DOI: 10.1111/wre.12512.

Trueba S, Pan R, Scoffoni C, John GP, Davis SD, Sack L. 2019. Thresholds for leaf damage due to dehydration: declines of hydraulic function, stomatal conductance and cellular integrity precede those for photochemistry. *New Phytologist* 223:134–149. DOI: 10.1111/nph.15779.

Turner NC. 2019. Imposing and maintaining soil water deficits in drought studies in pots. *Plant and Soil* 439:45–55. DOI: 10.1007/S11104-018-3893-1/FIGURES/4.

Veresoglou SD, Li GC, Chen J, Johnson D. 2022. Direction of plant–soil feedback determines plant responses to drought. *Global Change Biology* 28:3995–3997. DOI: 10.1111/GCB.16204.

Vilonen L, Ross M, Smith MD. 2022. What happens after drought ends: synthesizing terms and definitions. *New Phytologist* 235:420–431. DOI: 10.1111/NPH.18137.

Walker ZC, Morgan JW. 2022. Perennial pasture grass invasion changes fire behaviour and recruitment potential of a native forb in a temperate Australian grassland. *Biological Invasions* 24:1755–1765. DOI: 10.1007/s10530-022-02743-4.

Wolfsdorf G, Abrahão A, D'Angioli AM, de Sá Dechoum M, Meirelles ST, F. L. Pecoral L, Rowland L, da Silveira Verona L, B. Schmidt I, B. Sampaio A, S. Oliveira R. 2021. Inoculum origin and soil legacy can shape plant–soil feedback outcomes for tropical grassland restoration. *Restoration Ecology* 29:1–11. DOI: 10.1111/rec.13455.

Wurst S, Vender V, Rillig MC. 2010. Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecology* 211:19–26. DOI: 10.1007/s11258-010-9767-0.

Yelenik SG, D'Antonio CM. 2013. Self-reinforcing impacts of plant invasions change over time. *Nature* 503:517–520. DOI: 10.1038/nature12798.

Young TP, Zefferman EP, Vaughn KJ, Fick S. 2015. Initial success of native grasses is contingent on multiple interactions among exotic grass competition, temporal priority, rainfall and site effects. *AoB PLANTS* 7:1–9. DOI: 10.1093/AOBPLA/PLU081.

Table 1. Mean (+/- se) mass (g) by growth species and soil condition.

Mean (+/- se) Mass (g) by Growth Species and Condition

	Aboveground		Belowground		Total		Root-to-shoot	
Growth Species	Control	Inoculum	Control	Inoculum	Control	Inoculum	Control	Inoculum
Chenopodium oahuense	0.034 +/- 0.007	0.024 +/- 0.008	0.089 +/- 0.024	0.054 +/- 0.016	0.122 +/- 0.03	0.079 +/- 0.023	2.368 +/- 0.36	2.623 +/- 0.496
Megathyrsus maximus	0.127 +/- 0.023	0.137 +/- 0.024	0.187 +/- 0.035	0.309 +/- 0.089	0.313 +/- 0.044	0.446 +/- 0.089	1.782 +/- 0.435	2.806 +/- 0.965

Only data paired to generate feedback values reported. All means for regular water condition.

Figure 1. Photo taken at the start of the experiment, showing how the aluminet cloth shaded the conetainers (aluminet also covered the top of the table, not shown in photo).

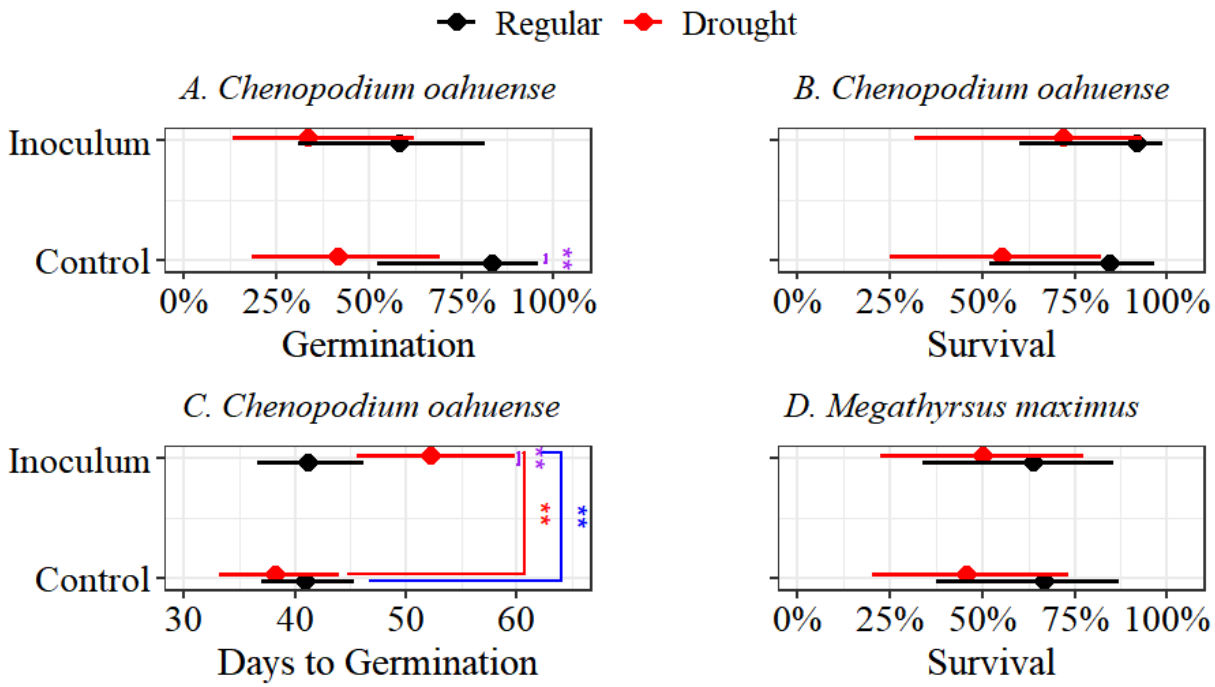

Figure 2. Average and 95% CI for non-biomass measures, by soil condition (y-axis) and water condition (point range color). Red point ranges represent drought (60% PC), while black point ranges represent regular (90% PC). "**" indicates significance at the $p < 0.05$ level.

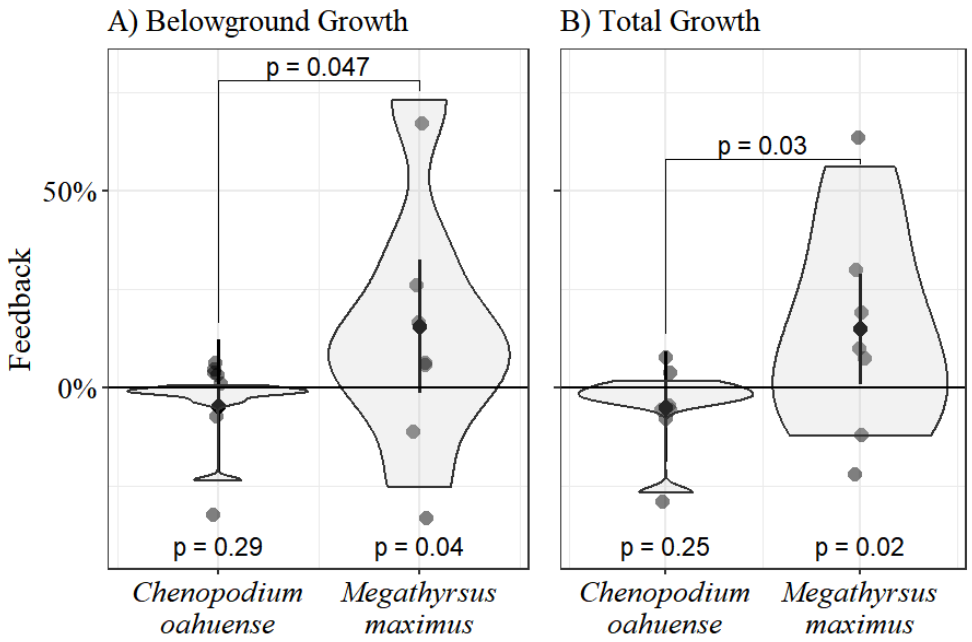

Figure 3. Distribution of observed belowground (left) and total (right) biomass feedback values as violin plot (gray) by growth species (x-axis), with point-range indicating mean and 95% CI. P value above bracket represents coefficient significance from hypothesis testing for difference between growth species. P value just above x-axis represents significant difference of predicted average of each growth species from 0.

Appendix C- Supplementary Figures

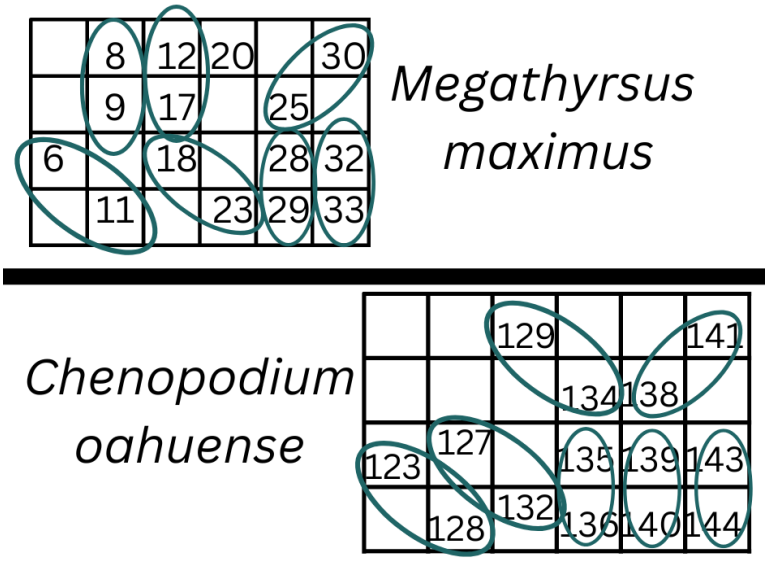

Figure S1. Image depicting how individuals were paired to generate feedback values for the biomass-based analyses.

CHAPTER 5

ACTIVATED CARBON AND RAKING DISRUPT BELOWGROUND SOIL LEGACY OF *MEGATHYRSUS MAXIMUS* TO IMPROVE GROWTH OF NATIVE HAWAIIAN OUTPLANTINGS

Abstract

Megathyrsus maximus is a pasture grass that has had wide-spread invasive success, including in Hawai'i. Here, we conducted an experimental restoration, incorporating activated carbon (AC) and sewage-sludge based biochar (BC) into an active outplanting restoration site. In addition to monitoring outplant survival and growth, we monitored total C and N, plant-available inorganic nutrients, infiltration rate, and the soil microbial community, taking measurements and soil samples six months and one year after initiating the restoration. We found that the height of *Dodonaea viscosa* increased by 8% with the application of AC at 0.5 kg/m². In addition, we found that, for the soil fungi community, AC was associated with reduced community dispersion based on Bray-Curtis Dissimilarity, reduced abundance of three fungi genera, and lower Shannon Diversity Index compared to that in control plots, based on a community-level physiological profile of carbon preferences of the soil bacteria community. Observed changes in the soil microbial community, specifically in terms of carbon preferences and degradation, may explain the improvement in *Dodonaea viscosa* growth. Activated carbon in the fine granular form applied here is expensive, and we discuss options for more cost-effective approaches to utilizing AC in restoration. In addition to the benefit of AC, we found that raking was associated with an improvement in the width of *Plumbago zeylanica*, by 24%. Future research could focus on suppression of the soil microbial community and disruption of soil compaction, to mitigate the effects of the *Megathyrsus maximus* soil legacy on native Hawaiian plants, which may contribute to increases in restoration success.

Introduction

In the last decade, there has been an effort to connect aboveground-belowground biotic interactions, including plant-soil feedback, to restoration theory and practices (Kardol & Wardle, 2010; Farrell et al., 2020). Plants can play a significant role in influencing C, N, P (Rumpel & Chabbi, 2019) and Si (Yang & Zhang, 2018; Schaller et al., 2021) cycling. The majority of plant dry biomass is made up of C, N and P biomass (Rumpel & Chabbi, 2019) and there is evidence that Si addition can improve plant productivity (Xu 2020). Exotic invasive plants may be associated with a soil legacy that negatively impacts the growth of native plants, which is why priority effects, and resulting soil legacies are critical to consider in restoration (Grman & Suding, 2010). Soil legacies can also contribute to instances of secondary invasion, which can

then disrupt restoration efforts and increase the spread of additional invasive species (Pearson et al., 2016). Invasive plants that have been investigated for microbial community impact were often found to modify soil characteristics and microbial community structure (30-40% of cases studied) (Dawkins et al., 2022), which together may be driving negative feedback on native plants. Recent research has focused on the goal of reducing the competitive advantage of invasive plants, including potential advantages associated with the soil microbial community, including mycorrhizae (Shahrtash & Brown, 2021), and decades of research has established the importance of mycorrhizae in restoration more generally (Smith, Charvat & Jacobson, 1998; Allen, 2009; Liu et al., 2019). Further research is required to establish the potential roles of soil microbial communities in failed and successful plant restoration (Farrell et al., 2020).

Isolating the role of soil microbial communities in the field is challenging, but one emerging method involves adding activated carbon (AC) to the soil and tracking the impacts on both the soil microbes and plants growing in the treated area. Activated carbon can be made from a variety of sources and is designed to be used to decontaminate gasses or liquids by increasing surface area available for chemical reactions (Hagemann et al., 2018). Activation is achieved through chemical reactions involving $ZnCl_2$, KOH, and H_3PO_4 or physical processes like exposure to steam (Hagemann et al., 2018). Activated carbon has commonly been used to suppress potential allelopathic activity, but studies have shown that it may also impact microbial communities directly (Nolan et al., 2015). Activated carbon can significantly reduce microbial biomass (Shan et al., 2015) and microbial abundance (Kulmatiski, 2011; Que et al., 2019). More specifically, AC addition was associated with decreases in bacterial community diversity through suppression of existing soil bacteria (Liu et al., 2018a; Que et al., 2019), and in some instances, recruitment of new dominant soil bacteria (Liu et al., 2018a), although these effects may be dose-specific (Que et al., 2019). In addition, AC addition has been associated with decreased abundance of mycorrhizae (Weißhuhn & Prati, 2009).

In addition to direct impacts on the microbial community, AC can affect general soil chemistry, which further impacts microbial communities (Lau et al., 2008; Weißhuhn & Prati, 2009; Wurst, Vender & Rillig, 2010). Activated carbon can increase soil pH, nitrate, K, and Fe, and conversely can decrease ammonium, Ca, Mg, Zn, and Mn (Lau et al., 2008). In some instances, AC addition was associated with increases in plant-available P (Weißhuhn & Prati, 2009). Use of carbon amendments (AC or BC) to modify resource availability is especially

relevant in PSF research, as nutrient availability can impact the magnitude and direction of feedback in invasion contexts (Klinerová & Dostál, 2020; Wang et al., 2020). Activated carbon added to soils has facilitated successful plant restoration, leading to increases in native cover in a variety of contexts (Kulmatiski, 2011; Nolan et al., 2015).

Activated carbon falls under the umbrella of pyrogenic carbonaceous material, along with the term "biochar." Biochar is produced from renewable/sustainable sources, and when used in non-agricultural contexts, can be used for the goal of carbon sequestration. In contrast, AC can be produced from fossils, waste, or renewable/sustainable sources, and is specific to chemical or physical processes of activation to maximize surface area, with physical activation being distinguished from general pyrolysis by the involvement of oxidative gases (Hagemann et al., 2018). If used in a project where carbon sequestration is a goal, a coconut shell-based AC amendment would also be considered an activated biochar, while a sewage-sludge based commercial fertilizer would be considered biochar but not activated carbon because it was heat-dried, and not chemically or physically activated. Biochar has been used to mitigate the allelopathic impact of two common tropical invaders, *Psidium cattleianum* and *Cymbopogon flexuosa* (Sujeeun & Thomas, 2017, 2022). Priming effects, which examine the impact of the addition of an organic amendment that modifies available carbon via microbial activity, have been observed with BC additions to soil and can be associated with modifications to functional diversity within a microbial community (Blagodatskaya & Kuzyakov, 2008).

Megathyrsus maximus grass invasions and impacts

Past restoration efforts in areas invaded by *Megathyrsus maximus* have found that grass removal alone was not an effective restoration strategy (Ammondt et al., 2013), suggesting that there could be a soil legacy effect left by *M. maximus*. Soil volumetric measurements showed that areas invaded by *M. maximus* were significantly drier than restoration areas (Ammondt et al., 2013). Analysis of photosynthetic rates suggests that *M. maximus* goes through dormancy in Hawai'i as on average, temperatures increase and precipitation decreases starting in August (Ammondt et al., 2013). Native seed additions following *M. maximus* removal were largely unsuccessful, which could suggest that the microbial community may be limiting germination of native species (Ammondt et al., 2013). Here, we hypothesize that AC will improve measures of restoration success, such as survival and growth of native outplantings. We hypothesize that, in accordance with previous studies, AC will have a suppressive impact on the soil microbial

community diversity and composition. Further, we hypothesize that the suppressive impact will allow native outplantings to establish and recruit new members to the soil microbial community, which we will test for using representative native species that have been commonly used in restoration. Finally, we hypothesize that AC will significantly improve field infiltration rate and soil organic carbon, while increasing plant-available nutrients. We applied AC (12x40 Coconut Shell Activated Carbon, Carbon Bulk Sales, Irvine, CA) independently as a soil amendment, and in combination with a locally sourced, sewage sludge based BC (Granulite® by Synargo®, Sand Island Wastewater Treatment Plant, Honolulu, HI) to test if similar results can be achieved at lower cost, by using half the amount of AC in combination with a BC fertilizer (i.e., fertilizer that meets criteria to be considered BC).

Methods

Restoration Site Description

The restoration site at Camp Palehua, Oahu qualifies as a dryland (Aridity Index, or AI < 0.65), with an annual precipitation of 925 mm (Giambelluca et al., 2013) and Grass Reference Surface potential evapotranspiration of 1437 mm (Giambelluca et al., 2014). The soil is classified as Mahana silty clay loam, and past analyses have identified acidity and nutrient deficiencies (C, Ca, K, organic matter) as key issues that may impact plant communities (Andie Gill, personal communication). Most of the property consists of abandoned or active pastures of *Megathyrsus maximus,* which was likely deliberately planted to support cattle (Texas Longhorns), which are active in areas surrounding the fenced restoration area. The Restoration Site is up slope from the main Camp Palehua property, is generally steep (6-12%), and is bordered on the north-west end by a section of the property that has a significant presence of Eucalyptus trees. While the restoration site is fenced-in, the belowground impacts of prior cattle grazing, including decreasing water infiltration rate (Franzluebbers & Stuedemann, 2008), total and macroporosity, and air permeability (Drewry & Paton, 2005), increasing soil erosion (Berhe, 2019), compaction, surface hardness (Braunack & Walker, 1985), bulk density (Drewry & Paton, 2005), and microbial biomass C, N and P (Clegg, 2006; Wang et al., 2006; Devi, Yadava & Garkoti, 2014) and shifting microbial community composition and abundance (Clegg, 2006; Wang et al., 2006; Fichtner et al., 2014), can be significant even 100 years after the cessation of the practice (i.e. soil legacy) (Fichtner et al., 2014). The site has been fenced-in for many years,

but cattle were able to frequently ingress, until the fences were improved in the last five to ten years (Mashuri Waite, personal communication). In 2019, a native tree restoration project focused on establishing over 100 native trees was conducted in the general vicinity of the site, however outplantings experienced very high mortality (Mashuri Waite, personal communication). The general goals of the land manager at the site were to restore native vegetation, to improve soil quality, to improve water infiltration to reduce run-off and erosion, and to improve carbon sequestration.

Experimental design and treatments

After partnering restoration practitioners (Mashuri Waite, Gill Ewa Lands) conducted grass removal and herbicide treatments, thirty 4 m^2 plots were established at the Restoration Site, with six plots randomly assigned to each of five treatments (control, raking only, AC, BC, combination AC and BC) (Figure 1). The control condition was the standard restoration practice that the land manager has been using at the site, including in the previous unsuccessful restoration planting, as follows: outplanting, followed by periodic irrigation, weeding, and thatching from dead *Megathyrsus maximus* biomass in between rows of outplantings. Irrigation, hand weeding, and re-application of thatching was done weekly for the first four months, then monthly for the following eight months. Hand weeding during restoration has been recommended when weeds are easy to remove (Humphries & Florentine, 2021) and when no or light competition between native and invasive plants is the target condition (O'Dwyer & Attiwill, 2000). Hand weeding has been used successfully to establish native plant communities in areas formerly invaded by perennial grasses (Bourdôt & Saville, 2019). Plots were arranged in blocks of three rows at roughly the same elevation, hereafter referred to as rows. Three rows (blocks) were arranged on each side of a natural gully-like depression resulting from erosion, with the side closer to the road referred to as the "road side" and the side further from the road referred to as the "mountain side." The rows were referred to as row 1,2, and 3, with the numbering starting at the row with the lowest elevation (Figure 1).

Unlike AC, the BC is a slow-release organic matter fertilizer made from local sewage that the land manager was interested in testing to see if the nutrient-poor quality of the soil could be remedied. The combination treatment used half strength of AC and BC (0.25 kg/m^2 each) compared to AC or BC alone plots (0.5 kg/m^2). On February 6th, 2022, soil amendments were

added to the designated plots and lightly raked into soil surface. The raking only condition allowed us to distinguish between the impact of any soil amendment (which included raking) and the impact of the disturbance associated with raking used to blend soil amendments with soil.

Dodonaea viscosa grown from seeds and *Plumbago zeylanica* grown from cuttings were purchased from Malama Learning Center (Kapolei, Oahu) for outplanting. These plants were started in pots at the same time, using the same potting mix base, such that within each species, outplants were roughly of the same height, with minimal width at the widest point. For each species, outplants were started in containers of the same size, such that the volume of potting mix and space for root growth were consistent. Due to the slope of the site, outplants were planted in a cup-like depression created by compressing backfilled soil around the stem to a depth ~ 1 cm below the surrounding soil, to improve water availability for each plant. At the time of outplanting, all outplants were less than 5 cm tall as measured from the soil surface (Figure 1C). In each plot, we marked five rows in 0.5 m intervals starting from the bottom boundary and ending with the top boundary, and four columns in 0.5 m intervals starting from the right boundary. Not counting the corner positions on the right boundary, that created 18 planting points, each separated from any other outplant by 0.5 m in any direction. Plots were immediately next to each other, so the six plots on the right most edge had 21 planting spots. In row 2, there was a separation between Plot 15 and Plot 14 due to a tree stump, so Plot 16 also had 21 planting spots since it was the right most edge of that section of row 2.

Two to four weeks after applying appropriate amendments, outplanting took place over 6 planting days (February 19th, 25th, March 1st, 5th, 18th and 22nd). We planted up to 4 *Dodonea viscosa* plants and up to 17 *Plumbago zeylanica* plants in a specific pattern (Figure 1A). There was occasional incidental variation in number of outplants and positioning due to physical limitations such as plants or rocks that needed to be avoided, as well as some human error in placing plants in the correct location. This variation was accounted for in analysis by using models that account for the number of trials (i.e., number of plants). The land manager also spread native seeds (*Sida fallax,* 10 per plot) and planted single individuals of other native plants (*Santalum haleakalae, Sesbania tomentosa, Erythrina sandwicensis,* 1 per plot) across all plots (personal communication, Mashuri Waite), but the seed addition and additional single plant outplantings failed to establish across all plots, so no analyses could be done regarding this

aspect of the restoration practice. Plots were monitored for one year (February 2022 - February 2023). Since outplanting occurred within a time span of less than a month, we did not distinguish between the outplanting date blocks in analyses where months post restoration was a predictor.

Plant Surveys

Six and twelve months after outplanting, surveys were done to assess survival of each outplanting. Percent survival of each species was analyzed through a logistic regression and likelihood ratio test. For the final analysis twelve months after outplanting, plant growth was assessed by measuring the height and width at the widest point of each native outplanting. Plants were measured in centimeters, to the nearest millimeter. Plant measurements were taken across three days (February 14th, February 21st and March 4th). All measurements were taken by Manya Singh.

Soil Properties

All soil samples were taken using a 2.5 cm diameter corer (LaMotte™ Soil Sampling Tube, Chestertown, MD), which was malleted into the soil to 10 cm deep. Any organic material was removed from the soil surface prior to placing the corer down. The corer was then gently removed from the soil, and the extracted soil core was removed. The corer was sterilized with alcohol wipes in between samples. After being air-dried and homogenized, the soil from the core was split into two containers, one was stored at 4˚C for microbial community analysis, and the other was dried and stored at room temperature for C/N analysis. Total carbon and total nitrogen analysis was performed by the University of Hawai'i at Manoa Agricultural Diagnostic Services Center using a combustion analyzer.

Baseline soil sample collection occurred on February 2nd 2022, after the herbicide treatment and marking of the plots. Samples were collected from the un-treated, unaltered areas of *Megathyrsus maximus* directly adjacent to the plots (hereafter referred to as 'pre-practice samples'). Funding limitations only allowed analyses of six baseline samples, so we collected one sample from each of the six row/side blocks and took the sample from the *Megathyrsus maximus* area on the low-elevation end of the center-most plot in each block. Since the pre-practice samples were not taken directly from any of the plots, they were not included in repeated-measures models analyzes mid-point and final soil later sampled within the plots. Mid-

point and final soil samples were taken from each plot between the bottom-left most *Dodonaea viscosa* and the nearest *Plumbago zeylanica* directly to its right when facing up slope.

Plant-available nutrients

Soil nutrients were tracked since they can be altered by plant growth. Ion-exchange resin bags were used to measure available soil N, P and Si pools (Sitters, Edwards & Olde Venterink, 2013), and were used in combination with soil total C and N to provide a wholistic picture of the nutrient cycling. Ion-exchange resin bags, made from 100 sq cm of nylon mesh (60 micrometer Sefar AG) containing 2 g of mixed-bed ion-exchange resin (Amberlite IRN 150, H+- and OH— forms, Sigma Aldrich), were used to monitor soil N, P and Si release rates. Conditioning consisted of 2 h of shaking in a 1M KCl solution. Resin bags that were buried 5 cm below the soil surface (with no bag exposed at the soil surface) in September 2022 were unretrievable due to loss, presumably due to excavation by birds or rats. The land manager observed some activity by birds and noted that a higher level of predation seemed to be impacting outplant mortality in the mountain side, row 1 block (Mashuri Waite, personal communication). We modified the resin bag shape, from rectangular to circular and used zipties rather than thread to seal them, in accordance with NutNet Resin Bag Protocol (Harpole, 2009). A second set of midpoint resin bags was installed in October 2022, and a final set of resin bags was installed in January 2023. Due to high rates of plant mortality on the mountain side, presumably due to predation, row 1 block was excluded from the experiment. Bags were installed in the remaining 25 plots, and not in the five plots in mountain side, row 1 block. In each plot, when facing up slope, one resin bag was installed between the top right *Dodonea viscosa* and the *Plumbago zeylanica* directly to the left for both midpoint and final analyses and left buried in the soil for four weeks. At the mid-point sampling, one bag (plot 23) was not retrievable. After bags were removed, they were rinsed in distilled H_2O, and then extracted via 1 h of shaking in 30 ml of 2 M KCl solution. The thrice filtered extract was analyzed colorimetrically for $NO_3^- + NO_2^-$, PO_4^{3-}, NH_4^+, and H_4SiO_4 using a Seal AA3 AutoAnalyzer (SEAL Analytical Inc., Mequon, WI) at the University of Hawai'i at Manoa School of Ocean and Earth Science and Technology Laboratory for Analytical Biogeochemistry.

Soil Water-Holding Capacity

To measure soil water holding capacity, field infiltration tests were conducted at the midpoint of the field experiment using a 16.5 cm diameter bucket with the bottom removed. The bucket became damaged after mid-point measurements, so the end-of-experiment field infiltration tests were conducted using a 19.1 cm diameter cardboard tubes meant for forming concrete columns. Multiple tubes of the same brand and size (QUIKRETE® 8-in Tube Form, Quikrete, Columbus, OH) were used for different plots, as the tubes started to deteriorate after two uses. The procedure consisted of two 1 L pours, with time to full infiltration recorded for the second pour, which was used to calculate infiltration rate. The difference in diameter and resulting surface area of the two different sized tools was accounted for in calculating the field infiltration rates.

Field infiltration tests were conducted at the Restoration site in October and November of 2022 for midpoint analysis, and January and February of 2023 for final analysis, with one infiltration test done per plot at each time point. Infiltration tests were done in roughly the same area as the soil sampling, but occurred after soil sampling was completed. Additionally, local weather data was sourced from the National Centers for Environmental Information, pulling from hourly and daily reports by the Ewa Kalaeloa Airport weather station, which is around 10 km from the site. For each sampling day and the two days prior, we recorded the dry bulb temperature and relative humidity at noon (11:53:00 AM) and the daily rainfall. Temperature and humidity were averaged across the three days, and the rainfall was summed up across the three days. Noon was chosen over maximum or minimum because measurements occurred either in the morning or afternoon, overlapping with noon either way. All measures were mean centered (i.e., the mean of the observed values was subtracted from each observed value to create a dummy variable centered around 0) for analyses to avoid multicollinearity issues.

Microbial Community Description and Analysis

A randomizer was used to determine which baseline soil samples and plot soil samples for each soil treatment would be sent for next-generation sequencing analysis, with 4 samples selected for baseline, 5 samples selected for control and raking only, and 4 samples selected for AC, and 2 samples selected for combination AC and BC. Plots in the mountain side, row 1 block were excluded from the randomization due to previously discussed concerns. Next-generation sequencing was conducted by the University of Hawai'i Cancer Center Genomics and

Bioinformatics Shared Resource, and DNA extraction was performed in accordance with manufacturer instructions using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA). Sequencing was done on an Illumina iSeq100 (Illumina, Inc., San Diego, CA), and raw fastq files were exported for analyses. The 16s fastqs were run through the general DADA2 workflow (Callahan et al., 2016) and then the function 'assign.taxonomy' was used, based on the SILVA database (Quast et al., 2013; Yilmaz et al., 2014) . The ITS1 fastqs were run through the DADA2 workflow, and then the function 'assign.taxonomy' was used, based on the UNITE database (Nilsson et al., 2018). Because the fastqs were short reads, they did not overlap during the merge step of DADA2, so forward and reverse reads were processed seperately, and the taxonomic matrices were joined as the final step.

The *vegan* package (Oksanen et al., 2013) in R was used to generate diversity statistics, and generate a Bray-Curtis dissimilarity matrix for principal coordinates analysis. A repeated-measures ANOVA was run on diversity metrics, using "lmer" from the *lme4* package (Bates et al., 2015) and "glht" from the *multcomp* package (Hothorn, Bretz & Westfall, 2008). Differences in dispersion among community samples from different treatments were tested using an ANOVA, with the function "betadisper", and each term (outplanting, raking, AC, BC, side, row, plot ID) was tested individually. Differences in community centroids of different treatments were tested using a PERMANOVA, and the function "adonis2". Indicator species analysis, using the *indicspecies* package (Cáceres & Legendre, 2009), was performed on the 50 most abundant species, testing for indicators of each level of each predictor.

One year (end of experiment) soil samples were assayed using Biolog EcoPlates to estimate functional diversity and average metabolic response to various forms of carbon. From the mid-point samples, sufficient soil was only available for 22 of the 30 plots, so mid-point soil samples were not assayed. Inoculation of the Biolog Plates consisted of 150 microliters from the 1:10,000 serial dilution of 1g of soil using 0.15 M NaCl (Sugiyama et al., 2013). Plates were incubated at 20 C and read at 595 nm every 24 hrs for 96 hrs (Garland & Mills, 1991; Sugiyama et al., 2013; Deng et al., 2019). Substrate specific average well color development was calculated from the 72 hrs point (Sugiyama et al., 2013). Overall average well color development was calculated per replicate for each incubation time point using the formula from Deng (2019). The 72 hr time point was used for calculating Shannon and Simpson indices (Deng et al., 2019), and

richness and evenness. Samples were analyzed at Leeward Community College in Pearl City, Hawai'i on a plate reader (Model 680, Bio-rad Laboratories, Hercules, CA).

Additional Statistical Analyses

To distinguish between the effects of different components of the restoration treatments: outplanting (yes or no); raking (yes or no); and any soil amendment, we coded these predictors separately, rather than treating the assigned treatment as one predictor. For outplanting, all pre-practice (pre-outplanting) samples were coded as 0, and all data from midpoint and final point (post-outplanting) were coded as 1. For raking, we coded baseline and the control treatment as 0 (no raking), and raking, and all amendment conditions (which included raking) as 1. Each soil amendment had its own predictor. For AC, the AC treatment was coded as 1, the combination treatment was coded as 0.5, to account for utilizing half the concentration, and all other treatments were coded as zero. For BC, the BC treatment was coded as 1, the combination treatment was coded as 0.5, to account for utilizing half the concentration, and all other treatments were coded as zero.

All analyses used a base formula (Formula 1), with some variations. Outplanting was included as a predictor where pre-outplanting samples were available: total carbon and nitrogen, plant-available nutrients, and next-generation sequencing. Time was included in repeated-measure analyses for total carbon and nitrogen and plant-available nutrients. Climate variables (average temperature at noon, summed rainfall, and average humidity) were included in the infiltration rate analysis. Row and Side were added as random variables to account for possible spatial variation, with Row nested within Side. Plot ID or plot number was included as a random variable in repeated-measures analyses: total carbon and nitrogen, plant-available nutrients, next-generation sequencing, and plant growth.

Dependent Variable ~	[Outplanting +] **Raking + AC + BC+ AC:BC** [+Average Temperature at Noon + Summed Rainfall +Average Humidity] [+ Time + AC:Time + BC:Time + AC:BC:Time] **+ (1|Side/Row)**

[+ (1|Plot)]

Formula 1. Formula used in statistical analyses. Bolded terms indicate the main formula, while predictors in brackets represent predictors that only appear in some analyses. Random variables are indicated with parentheses. Interactions are indicated with colons; "|" indicates by group; "/" indicates nesting.

Models were chosen based on assumptions necessary for each dependent variable. For outplant survival, we used the "quasibinomial" family in glm, with the logit link. For outplant growth, plant-available nutrients and infiltration rate, we used the "gaussian" family in glmer with the log link. For total carbon and nitrogen, we used the "beta" family in glmmTMB (Brooks et al., 2017) for both the repeated measures and non-repeated measures models. Model visualizations were generated using the "plot_model" function in the "sjPlot" package (Lüdecke, 2018). Hypotheses about differences in the impact of different predictors were tested using the "confint," "pairs," and "lsmeans" functions from the "lsmeans" package (Lenth, 2016; Lenth & Lenth, 2018). All code used to generate analyses and figures are available at doi.org/10.6084/m9.figshare.22177823.

All statistical analyses that included pre-practice (pre-outplanting) measures but did not include repeated measures used the formula: ~ Outplanting + Raking + AC + BC + AC:BC + (1|Side/Row). All statistical analyses that excluded baseline measures used the formula: ~ Raking + AC + BC + AC:BC + (1|Side/Row). All analyses that were repeated measures, excluding microbial community data, used a mixed effects design, with Plot ID included as a random effect. For repeated measures microbial community data, we included plot ID as a fixed effect in the model, for the purposes of using adonis2, which does not allow for random effects. The inclusion of plot ID as a fixed effect meant that the model dropped the interaction term (AC:BC) from the output. For the infiltration model, we first tested the full formula (Formula 1, bolded, plus climate variables average temperature, average humidity, sum rainfall) and then we tested the full model against a second model where we dropped any non-significant climate variables. If the full model was stronger ($p < 0.05$, ANOVA, type III), we used that model, but if the full model was not stronger ($p > 0.05$, ANOVA, type III), we used the reduced model, to avoid overfitting with unnecessary variables. Due to significant differences in weather between

the mid-point (temperature average: 29.14 C, humidity average: 55%, rain average: 0.01 cm) and final point (temperature average: 26.51 C, humidity average: 60%, rain average: 0.59 cm), for infiltration rate, we separated data for the midpoint and final point, and analyzed each dataset as a separate model without repeated measures.

Results

Outplant Survival and Growth

There were no discernable effects of treatments on *Dodonaea viscosa* survival. For mid-point survival of *Dodonaea viscosa*, out of 30 plots, five plots had one dead plant, so five plots had a survival rate of 75%, while the remaining plots had a survival rate of 100%. Only two plots had additional mortality between the mid-point and final point, and seven plots had a survival rate of 75% at the end point. Two treatments had no *D. viscosa* mortality (AC, BC), while the remaining three treatments had two or three dead plants total. Some treatments having no mortality at all would make it challenging for the model to find significant differences, since a confidence interval cannot be generated. Due to this limitation, outplant survival was not statistically analyzed for *Dodonaea viscosa.* All of the plots in the row 1, mountain block had 100% survival of *Dodonaea viscosa*, so despite earlier concerns about mortality, this block of plots was not excluded as outliers from the *Dodonaea viscosa* analyses.

For both the mid-point survival data and final survival data, none of the predictors had significant coefficients for *Plumbago zeylanica* survival (Table S1). Mean *Plumbago zeylanica* survival for each treatment ranging from 86% to 89% (SE 4 – 5%). Mean survival for mid-point was very similar, with treatment means ranging from 86% to 90% (SE 3 – 5%). Between the mid-point and final surveys, eight plots had one additional plant death, and one plot had two additional plant deaths. Plot 10 had the lowest *P. zeylanica* survival, with seven mortalities at the six-month mark, and eight mortalities total at the final survey, but it had 100% survival of *Dodonaea viscosa*, so it was not removed.

Activated carbon tended to benefit *Dodonaea viscosa* growth, while BC had negative effects. There were three significant predictors for *Dodonaea viscosa* height: AC, BC and the interaction between AC and BC ($p < 0.001$, Table S2). Use of AC at 0.5 kg m^-2 was associated with a height increase of around 4 cm (+ 8%), while use of BC at 0.5 kg m^-2 was associated

with a height decrease of around 4 cm (- 12%, Figure 2). The combination of AC and BC seemed to mediate the negative effect of BC, with a decrease in height of 1-2 cm compared to the control (- 4%, Figure 2). In testing additional hypotheses (Table S3), the effect of AC on *Dodonaea viscosa* height was significantly more positive compared to raking (+ 6%), AC:BC (+ 15%), and BC (+ 22%). Furthermore, the coefficient of raking was significantly more positive compared to AC:BC (+ 8%) and BC (+ 14 %). Finally, the effect of AC:BC was significantly more positive compared to BC (+ 6%). There were no significant predictors for *Dodonaea viscosa* width ($p > 0.05$, Table S2), and no significant support for additional hypotheses (AC & BC & AC:BC > Raking, AC & AC:BC > BC, $p > 0.05$).

Biochar tended to reduce *Plumbago zeylanica* height and width, while other treatments had no discernable effect. Use of BC was a significant negative predictor for *Plumbago zeylanica* height and width ($p < 0.001$, Table S2), and raking was a significant positive predictor for *Plumbago zeylanica* width ($p = 0.02$, Table S2). Use of BC at 0.5 kg m^-2 was associated with a decrease of roughly 5 cm in both height (- 27%) and width (- 38%) of *Plumbago zeylanica*, while use of raking was associated with an increase of roughly 2 cm in width of *Plumbago zeylanica* (+ 24%, Figure 3). In additional hypothesis testing (Table S3), we found that the effect of BC on *Plumbago zeylanica* height was significantly more negative compared to raking (- 37%) and AC (- 28%) and the effect of BC on *Plumbago zeylanica* width was significantly more negative compared to raking (- 50%). Additionally, the effect of AC:BC on *Plumbago zeylanica* width was significantly more negative compared to raking (- 38%).

Soil Properties

In the AC and BC treatments, total carbon and nitrogen seemed to decrease over time. Additionally, the raking treatment seemed to increase total nitrogen. For the repeated measures model for total carbon and nitrogen, the AC/time interaction term was significant (TC: $p < 0.001$, TN: $p = 0.01$, Table S4). For total carbon, AC and time were also significant individually ($p = 0.02$, $p = 0.01$, respectively). The model predicted that, with no AC there was a positive linear relationship between months post-restoration and total carbon and total nitrogen, but with increasing amounts of AC, the slope became negative (Figure S1). Additionally, raking was a significant predictor of total nitrogen ($p = 0.02$, Table S4). Raking was associated with an increase in total nitrogen of roughly 0.15% (Figure S2).

In the model comparing the pre-practice samples to final samples, both AC and BC were significant predictors of total carbon ($p < 0.001$) and total nitrogen ($p < 0.001$, $p = 0.01$, respectively, Table S5). Use of AC had a negative linear relationship with total carbon for the final samples (Figure 4), but AC use was not a significant predictor of total carbon when comparing *M. maximus* samples to mid-point samples (Table S5). BC application had a negative linear relationship with total carbon (Figure 4) and total nitrogen (Figure S3). For total nitrogen, raking was also a significant predictor for the final samples ($p = 0.01$, Figure S3), but not for mid-point samples (Table S5). Raking was associated with an increase of roughly 0.25% total nitrogen (Figure S3). Additional hypotheses comparing the effects of predictors were not significant for any total carbon or total nitrogen models (TC & TN: AC & BC & AC:BC & Raking > Outplanting, AC & BC & AC:BC > Raking, AC & AC:BC > BC, AC > AC:BC, $p > 0.05$) .

Plant-available nutrients

Over time, P cycling seemed to decrease, and both the raking and BC treatments were associated with decreases in Si cycling. For P cycling, time was the only significant predictor ($p < 0.001$, Table S7), with P cycling having a negative linear relationship with months post restoration (Figure S5). For Si cycling, raking and BC use were significant predictors ($p < 0.001$, $p = 0.03$, respectively, Table S7). Use of raking was associated with a decrease in Si cycling of roughly 0.015 ug g resin^-1 day^-1, while use of BC at 0.5 kg m^-2 was associated with a decrease in Si cycling of roughly 0.05 ug g resin ^-1 day^-1 (Figure 5). For the repeated-measures models, there were no significant predictors of inorganic N cycling ($p > 0.05$). Additional hypotheses comparing the effects of predictors were not significant for all inorganic nutrient cycling models (N, P, Si).

Soil Water Holding Capacity

There was a positive linear relationship between average temperature at noon and infiltration rate (Figure S4). For the mid-point infiltration rates model, climate variables were dropped after the model fitting process, and raking was the only significant predictor ($p = 0.03$, Table S6). Raking was associated with a decrease in infiltration rate of roughly 0.25 cm/min (Figure S4). The final infiltration rate model had only one climate variable after the model fitting process: average temperature during the infiltration test (Table S6), which was significant ($p =$

0.01, Table S6). Additional hypotheses comparing the effects of predictors were not significant for any infiltration rate models (AC & BC & AC:BC > Raking, AC & AC:BC > BC, AC > AC:BC, $p > 0.05$).

Microbial Community Analysis

For the fungi community, two predictors significantly shifted composition: raking and BC ($p = 0.03$, $p = 0.04$, respectively, Table 1). Additionally, AC was associated with differences in community dispersion ($p = 0.01$, Table 1), which is an estimate of alpha diversity, with AC having a homogenizing effect. Shannon Diversity Index, an estimate of community diversity, differed with BC addition ($p = 0.05$, Table 1), with use of BC associated with decreases in diversity. Simpson's Diversity Index, another estimate of community diversity, differed by the interaction of AC and BC ($p < 0.001$, Table 1), with the use of both amendments associated with decreases in diversity.

In the fungi indicator species analysis, there was evidence suggesting that AC addition suppressed specific fungi genera and families. For the fungi indicator species analysis, there were indicators found for the outplanting, raking, AC and BC predictors (Table 1). Outplanting was associated with increased abundance of indicators *Arachnomyces* ($p = 0.05$, specificity 0.83, sensitivity 0.94) and *Talaromyces* ($p = 0.01$, specificity 0.87, sensitivity 1). Outplanting was associated with the suppression of indicators *Alfaria* ($p = 0.03$, specificity 0.83, sensitivity 1), *unclassified Pezizaceae* ($p = 0.03$, specificity 0.93, sensitivity 1), and *Trichodemra* ($p = 0.02$, specificity 0.83, sensitivity 1). Within the raking predictor, *unclassified Bionectriaceae* was an indicator of no raking ($p = 0.01$, specificity 0.21, sensitivity .81). For the AC predictor, indicators of the no AC level were *Coniophora* ($p = 0.03$, specificity 0.99, sensitivity 0.79), *Saitozyma* ($p = 0.02$, specificity 0.83, sensitivity 0.82) and *Talaromyces* ($p = 0.01$, specificity 0.64, sensitivity 1). For the BC predictor, *Absidia* was a predictor of the no BC level ($p < 0.001$, specificity 0.68, sensitivity 1); *Aspergillus* ($p = 0.01$, specificity 0.66, sensitivity 1) and *Distoseptispora* ($p = 0.05$, specificity 0.85, sensitivity 1) were predictors of the BC level; *Naganishia* was a predictor of the combination level ($p = 0.04$, specificity 0.77, sensitivity 1).

For the bacteria community, no predictor was associated with a significant shift in composition (PERMANOVA of centroid center, $p > 0.05$). Outplanting and BC were associated with differences in dispersion (ANOVA of community samples, $p = 0.03$, Table 2), a measure of

beta diversity, with outplanting having a diversifying effect, while BC had a homogenizing effect. The interaction between AC and BC was associated with a decrease in Shannon Diversity Index ($p = 0.05$, Table 2). For the bacteria indicator species analysis, the predictors outplanting, AC and BC were associated with indicator taxa. Within the outplanting factor, the control (i.e., pre-practice) level had the indicators *FCPS473* ($p = 0.01$, specificity 0.91, sensitivity 1) and *unclassified Ktedonobacteriaceae* ($p = 0.04$, specificity 0.81, sensitivity 1) while outplanting indicators were *Soilrubrobacter* ($p = 0.02$, specificity 0.66, sensitivity 1) and *unclassified Thermkmonosporaceae* ($p = 0.01$, specificity 0.85, sensitivity 0.97). Within the AC predictor, use of the combination of AC and BC was associated with increased abundance of *unclassified Soilrubrobacteraceae* ($p = 0.03$, specificity 0.56, sensitivity 1). Within the BC predictor, BC use was associated with suppression of *Candidatus Soilbacter* ($p = 0.03$, specificity 0.52, sensitivity 1, for no BC level) and increased abundance of *unclassified Beijerinckiaceae* ($p = 0.04$, specificity 0.62, sensitivity 1).

Generally, AC and BC addition seemed to homogenize the carbon preferences of the soil bacteria community, increasing the usage of polymer sources (Table 3). By contrast, the combination treatment seemed to shift preferences away from polymers towards amino acids (Table 3). For the community level physiological profile focused on carbon preference within the bacteria community, none of the predictors were significantly associated with differences in composition or dispersion ($p > 0.05$, PERMANOVA, ANOVA). Activated carbon had significantly lower Shannon Diversity Index, compared to the control condition ($p = 0.04$, Table 3), while the combination of AC and BC had increased Shannon Diversity Index compared to the control ($p = 0.05$, Table 3). None of the predictors were associated with differences in evenness, richness, or Simpson's Diversity Index ($p > 0.05$). In the model testing if treatment influenced carbon preferences by category, there were significant interactions between soil amendments and two carbon preference categories (Table 3). For the polymer category, AC ($p < 0.001$) or BC ($p = 0.01$) soil amendment alone was associated with an increase in average well development color, while AC and BC in combination was associated with a decrease in average well color development, a measure of preference or usage ($p <0.001$). For the amino acid category, use of AC and BC in combination was associated with an increase in average well color development ($p = 0.01$). Additional hypotheses comparing the effects of predictors were not significant for any

microbial community models (AC & BC & AC:BC > Raking, AC & AC:BC > BC, AC > AC:BC, $p > 0.05$).

Discussion

Overall, both AC and BC altered the soil microbial community, and were associated with impacts on plant growth. The impacts on plant growth differed by outplant species and differed in direction. While AC improved some measures of plant growth, BC decreased other measures of plant growth. Other impacts of AC and BC were aligned, such as the shift in carbon source preferences towards polymer sources. Out of the factors we examined, the unique finding for the AC treatment that may explain or contribute to the improved plant growth was the suppression of specific fungi genera and families, based on the indicator species analysis.

Impact of Outplanting and Raking

We found that outplanting was associated with an increase in an estimate of beta diversity in the bacteria community, but no changes to the fungi community, or total C or N Within the bacteria community, outplanting seemed to suppress *FCPS473* (Family Ktedonobacteraceae, specificity 0.91), while recruiting or supporting the abundance of *Thermomonosporaceae* (specificity 0.85). *FCPS473* has been found to be a pre-dominant genus in soil samples from *Lolium* and *Pennisetum sp.* dominated grasslands in China (Deng et al., 2021). Additionally, there is evidence that reclamation of post-coal mining soils in South Africa was associated with reduced abundance of the genus *FCPS473* (Ezeokoli et al., 2020). The family *Thermomonosporaceae* is associated with increased soil temperature (Cardenas Gomez et al., 2022), so increased abundance may be attributable to 2022 being the 14th warmest year on record for Hawai'i (NOAA National Centers for Environmental Information).

For fungi, outplanting seemed to suppress the abundance of *Pezizaceae* (specificity 0.93), while recruiting and/or supporting the abundance of *Talaromyces* (specificity 0.87). The family *Pezizaceae* is associated with grasslands in China (Hu et al., 2008; Zhang et al., 2018), Iraq (Abdulla Salih et al., 2019), Minnesota (Dickie & Reich, 2005) and with non-fertilized *Megathyrsus maximus* pastures in Wailua, on the neighboring island of Kaua'i (Heisey et al., 2022). Multiple *Talaromyces sp.* have been investigated for plant-growth promotion, biocontrol, and bio-remedation potential, with positive findings (Naraghi et al., 2012; Vinale et al., 2017;

Goh et al., 2020; Liu et al., 2022). *Talaromyces*, however, was also an indicator of the no AC condition, but at a lower specificity (0.64). Activated carbon treatments were not associated with decreased outplanting growth, so this genus likely is not likely to be a driver of differences in outplanting growth observed in our experiment.

Raking was associated with a decrease in Si cycling, total nitrogen, and infiltration rate in the short-term (6 months), but was also associated with a significant shift in fungi community composition, and increase in *Plumbago zeylancia* height of roughly 2 cm. One potential explanation is that long-term grazing can increase soil compaction (Daniel et al., 2002; Sharrow, 2007; Drewry et al., 2008), and soil compaction can limit root and rhizome spread (Kirby & Bengough, 2002; Hamza & Anderson, 2005), so the release of upper soil layer compaction through raking may have allowed roots to spread more quickly through soil to better access resources, which in turn could allow increased resource allocation towards aboveground growth. Furthermore, there is evidence that the soil microbial community of *Megathyrsus maximus* mediates belowground competition through positive feedback towards its own belowground growth (Chapter 3). In the indicator species analysis, no-raking was associated with one low-specificity fungi indicator (*Bionectriaceae*, specificity = 0.21, Table S7). In Washington, *Bionectriaceae* was an indicator of the deepest strata of soil in a no-tillage wheat system (Schlatter et al., 2018), supporting a negative association between this family and mechanical soil disturbance. It is possible that suppressing this family of fungi disrupted belowground positive feedback on *Megathyrsus maximus*, contributing to its increased growth, in combination with the direct effects of releasing soil compaction.

Impact of AC

Activated carbon was associated with an increase in *Dodonaea viscosa* height of 8% (Figure 4). Based on hypothesis testing, AC had a more positive coefficient compared to BC (height of both species), raking (*Dodonaea viscosa* height) and the combination treatment (*Dodonaea viscosa* height) in plant growth outcomes. In addition to impacting outplant growth, AC was associated with impacts to total C and N and the soil microbial community. We found that AC was associated with a decrease in total C and N at the one-year mark, but not at the six-month mark, suggesting that there may be a delay or lag in this decreasing effect. The effect of AC in the literature on C and N is mixed, but our results are in line with studies that have shown

an overall decrease (Kulmatiski & Beard, 2006; Weißhuhn & Prati, 2009). AC was not associated with any changes in nutrient cycling, so increased nutrient availability from microbial decomposition is not a likely explanation for the benefit to the growth of *Dodonaea viscosa.*

We found that AC was associated with reduced beta diversity in the fungi and bacteria community (Table 1, Table 2), and decreased alpha diversity in carbon source preference in the bacteria community (Table 3). In the analysis of carbon preferences, we found that AC addition was associated with an increase in preference for polymers (Tween 40, Tween 80, Alpha-cyclodextrin, Glycogen), suggesting an increase in capacity for polymer digestion. Overall, the homogenizing effects on the fungi and bacteria community, and the carbon-source preferences of the bacteria community suggest an overall suppressive effect on the soil microbial community, which is supported by the literature (Kulmatiski, 2011; Shan et al., 2015; Liu et al., 2018b; Que et al., 2019). These impacts are unlikely to have driven the increased growth, as suppressed soil microbial community diversity and functional diversity and increased polymer preference was also found to be associated with BC, which lacked the increase in growth.

AC was associated with the suppression of fungi *Coniophora* (specificity 0.99 for no AC level), so it is possible that *Coniophora* plays some role in belowground feedback, such that suppression may contribute to improved growth. In abandoned cattle pastures turned native tree plantations in Costa Rica, *Coniophora* has been identified as capable of degrading complex C (Eaton et al., 2019). Our hypothesis that after initial suppression, over the course of the year, the native outplants would have opportunity to recruit new soil microbiome members, such that there would be indicators for the AC treatment that grew in abundance, was not supported. In a three-year study, Nolan et al. (2015) found that AC addition was associated with an increase in Actinomycelates and unidentified bacterium, so it may be that it takes more time to see AC addition have promote or support the abundance of specific microbes. Activated carbon is known to have a neutralizing effect on allelochemicals, and there is evidence that allelopathic abilities contribute to the success of non-native grasses (Singh & Daehler, 2023), and this impact can be dose-dependent, so this may explain increased growth in the AC but not combination treatment if the negative impacts of BC could not be compensated for by the positive impacts of AC, which is supported by the difference in magnitude between AC:BC and BC impacts.

BC and AC-BC Combination

While raking and AC were associated with improved plant growth compared to the control, BC, and to a lesser extent, the combination treatment, were associated with decreased plant growth. *Plumbago zeylanica* had decreased plant height and width with BC addition, and *Dodonaea viscosa* had decreased height in both the BC and combination treatments, with AC appearing to mediate the depressive impact of BC on height. Based on additional hypothesis testing, BC suppressed growth compared to raking alone (both *Plumbago zeylanica* measures + *Dodonaea viscosa* height), AC (height of both species) and the combination treatment (*Dodonaea viscosa* height). As previously mentioned, BC was associated with an increase in preference for carbon polymer sources in the community-level physiological profile of carbon preferences in the bacteria community. BC addition was associated with decreased fungi alpha diversity, and significantly shifted fungi community composition (centroid test, PERMANOVA). Additionally, BC was associated with decreases in Si cycling and total carbon and nitrogen. Based on visual inspection at the end of the experiment, BC was still present in plots, suggesting that its direct effects may be persistent for at least one-year (Manya Singh, personal observation). It is possible that as a slow-release fertilizer, it takes more than one year to see benefits, or that climate conditions at the site, such as the record-setting warmth, and shifting precipitation regime towards heavy but infrequent rain, contributed to delays in seeing benefits to plant growth, or that unrecognized negative impacts of BC may outweigh potential fertilization benefits. Ultimately, these limitations suggest that land managers and conservation practitioners in Hawai'i should consider the potential downsides, or lack of benefits, when determining whether to incorporate it into restoration practice.

The rationale for the combination treatment was that the combination of AC and BC would mitigate some of the downsides of AC established by the literature, specifically the high cost (Kulmatiski & Beard, 2006) and decreased nutrient availability (Lau et al., 2008). Had this been the case, then in interaction graphs, the combination line would be higher, or have a more positive slope, compared to the AC only line. We found no impact to nutrient availability associated with AC or AC:BC, contributing to the mixed evidence on this effect (Wurst, Vender & Rillig, 2010). In every instance where AC:BC was a significant predictor in a model (Figure 2, Figure 4, Figure S3), the AC only treatment was superior to the combination treatment, suggesting that instead of BC mitigating negative impacts of AC, AC mitigated negative impacts of BC. In additional hypothesis testing, we found that the impact of AC:BC was significantly

more positive than BC on *Dodonaea viscosa* height, but AC still outperformed AC:BC on the same measure. One interesting finding unique to the combination treatment is the reversal of preference for carbon polymers, instead favoring amino acid carbon sources. Thus, the mitigating effect of AC on BC impacts could be due to additive effects, or could be due to unique impacts of the combination treatment, such as the shift in carbon preferences in the bacteria community. The magnitude of the negative impact of the combination treatment on *Dodonaea viscosa* height is less than the negative impact of BC, but the lack of improvement in plant outcomes when BC was included as an amendment suggests that land managers would be better off with the control practice (raking only) compared to the combination treatment, while AC appeared to be the best performing treatment overall. If the cost of AC is prohibitive, our findings suggest that the raking alone treatment is superior to use of BC, due to decreased *Plumbago zeylanica* width one year after outplanting with BC addition. However, our study is limited in that we are unable to assess longer term patterns of growth.

Recommendations and Outlook

Overall, if money was unlimited, based on our one-year study, the restoration practice of outplanting, plus raking, plus AC would have the best outcomes for growth of native outplantings. Due to the cost of shipping AC from continental USA to Hawai'i, our AC amendments cost $32 per plot, or $16/m^2, for the AC only treatment. Without the cost of shipping, the cost would come down to $14 per plot, or $7/m^2. We used an AC powder, but the cost for the same treatment using AC in pellet form (Penn-Plax Activated Aquarium Filter Carbon, Penn-Plax, Hauppauge, NY) could be accomplished for $7 per plot, or $3.50/m^2. Activated carbon may become increasingly affordable as it becomes more commonly used in water filtration systems, both commercial and in home aquariums. Ultimately, AC may never be financially feasible for large scale projects, but at lower concentrations and less-expensive forms and in smaller areas, AC may be a good way to disrupt a soil legacy driven by bacteria and/or fungi, and create a small, sustaining native plant community, from which soil samples could be taken in the future for soil transplants, or soil inoculum treatments to be used in combination with fungicide if inhibitory fungi are present. Additionally, AC may be beneficial in directly neutralizing allelochemicals (Del Fabbro & Prati, 2015) and/or commercial herbicides (Madsen et al., 2014). Activated carbon may be used more cost effectively in protection pods around

native seeds (Madsen et al., 2014), and future research could try to identify an equivalent technique that would work with outplanting, such as coating outplant roots in fine AC powder prior to installation in the ground. For cost-limited projects, the raking treatment was an improvement upon the BC and raking treatment in three of the four plant measures.

Conclusion

This project was limited in scope to one site, with a specific land use history, climate, and soil characteristics. Nevertheless, our experimental design allowed us to identify statistical trends in plant outcomes across different restoration practices, and illuminated mechanisms through which *Megathyrsus maximus* soil legacies may hamper restoration outcomes. We found that AC, in combination with raking, had the best outcomes, and if the cost of AC is prohibitive, raking alone has better outcomes than BC and the AC:BC combination treatment. While our restoration practice itself may not be a prescription that is sure to work in every context, we have shown evidence that soil compaction, allelochemicals and/or specific fungi that are present after multiple decades of grass invasion may hamper restoration, and considering the broad invasive range of *Megathyrsus maximus* across the tropics, we recommend exploring whether soil legacies may hamper restoration at other *Megathyrsus maximus* sites, and to consider implementation of AC soil amendments or other mitigating treatments to support increased growth of outplantings.

References

Abdulla Salih S, Mohan Aday Al-Zubaidy A, Ali Nadir H, Hameed AlKhafaji M. 2019. New interesting records of three species from Pezizaceae and Pyronemataceae families (Pezizalea, Ascomycota) to Kurdistan region-Iraq. *Plant Archives* 19:55–61.

Allen MF. 2009. Arid Land Research and Management Mycorrhizae and rehabilitation of disturbed arid soils: Processes and practices Mycorrhizae and Rehabilitation of Disturbed Arid Soils: Processes and Practices. *Arid Soil Research* 3:229–241. DOI: 10.1080/15324988909381201.

Ammondt SA, Litton CM, Ellsworth LM, Leary JK. 2013. Restoration of native plant communities in a Hawaiian dry lowland ecosystem dominated by the invasive grass *Megathyrsus maximus*. *Applied Vegetation Science* 16:29–39. DOI: 10.1111/j.1654-109X.2012.01208.x.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67.

Berhe AA. 2019. Drivers of soil change. In: Busse M, Giardina CP, Morris DM, Page-Dumroese DS eds. *Developments in Soil Science*. Elsevier, 27–42. DOI: 10.1016/b978-0-444-63998-1.00003-3.

Blagodatskaya E, Kuzyakov Y. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. *Biology and Fertility of Soils* 45:115–131. DOI: 10.1007/s00374-008-0334-y.

Bourdôt GW, Saville DJ. 2019. *Nassella trichotoma* – plant growth rates and effects of timing of grubbing on populations in North Canterbury grassland. *New Zealand Journal of Agricultural Research* 62:224–245. DOI: 10.1080/00288233.2018.1483954.

Braunack M v., Walker J. 1985. Recovery of some surface soil properties of ecological interest after sheep grazing in a semi-arid woodland. *Austral Ecology* 10:451–460. DOI: 10.1111/j.1442-9993.1985.tb00906.x.

Brooks ME, Kristensen K, Van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Machler M, Bolker BM. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal* 9:378–400.

Cáceres M De, Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* 90:3566–3574. DOI: https://doi.org/10.1890/08-1823.1.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods 2016 13:7* 13:581–583. DOI: 10.1038/nmeth.3869.

Cardenas Gomez K, Rose A, Gibb KS, Christian KA. 2022. Microbial communities associated with mounds of the Orange-footed scrubfowl *Megapodius reinwardt*. *PeerJ* 10. DOI: 10.7717/PEERJ.13600/SUPP-5.

Clegg CD. 2006. Impact of cattle grazing and inorganic fertiliser additions to managed grasslands on the microbial community composition of soils. *Applied Soil Ecology* 31:73–82. DOI: 10.1016/j.apsoil.2005.04.003.

Daniel JA, Potter K, Altom W, Aljoe H, Stevens R. 2002. Long–term grazing density impacts on soil compaction. *Transactions of the ASAE* 45:1911-. DOI: 10.13031/2013.11442.

Dawkins K, Mendonca J, Sutherland O, Esiobu N. 2022. A systematic review of terrestrial Plant invasion mechanisms mediated by microbes and restoration implications. *American Journal of Plant Sciences* 13:205–222. DOI: 10.4236/ajps.2022.132013.

Deng Q, Zhang T, Xie D, Yang Y. 2021. Rhizosphere microbial communities are significantly affected by optimized phosphorus management in a slope farming system. *Frontiers in Microbiology* 12. DOI: 10.3389/fmicb.2021.739844.

Deng J, Zhou Y, Bai X, Luo J, Yin Y, Zhu W. 2019. Soil microbial functional diversity responses to different revegetation types in baishilazi nature reserve. *Polish Journal of Environmental Studies* 28:3675–3686. DOI: 10.15244/pjoes/99100.

Devi TI, Yadava PS, Garkoti SC. 2014. Cattle grazing influences soil microbial biomass in sub-tropical grassland ecosystems at Nambol, Manipur, northeast India. *Tropical Ecology* 55:195–206.

Dickie IA, Reich PB. 2005. Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology* 93:244–255. DOI: 10.1111/j.1365-2745.2005.00977.x.

Drewry JJ, Cameron KC, Buchan GD, Drewry JJ, Cameron KC, Buchan GD. 2008. Pasture yield and soil physical property responses to soil compaction from treading and grazing — a review. *Soil Research* 46:237–256. DOI: 10.1071/SR07125.

Drewry JJ, Paton RJ. 2005. Soil physical quality under cattle grazing of a winter-fed brassica crop. *Australian Journal of Soil Research* 43:525–531. DOI: 10.1071/SR04122.

Eaton WD, Mcgee KM, Donnelly R, Lemenze A, Karas O, Hajibabaei M. 2019. Differences in the soil microbial community and carbon-use efficiency following development of *Vochysia guatemalensis* tree plantations in unproductive pastures in Costa Rica. *Restoration Ecology* 27:1263–1273. DOI: 10.1111/rec.12978.

Ezeokoli OT, Bezuidenhout CC, Maboeta MS, Khasa DP, Adeleke RA. 2020. Structural and functional differentiation of bacterial communities in post-coal mining reclamation soils of South Africa: bioindicators of soil ecosystem restoration. DOI: 10.1038/s41598-020-58576-5.

Del Fabbro C, Prati D. 2015. The relative importance of immediate allelopathy and allelopathic legacy in invasive plant species. *Basic and Applied Ecology* 16:28–35. DOI: 10.1016/j.baae.2014.10.007.

Farrell HL, Léger A, Breed MF, Gornish ES. 2020. Restoration, soil organisms, and soil processes: emerging approaches. *Restoration Ecology* 28:S307–S310. DOI: 10.1111/rec.13237.

Fichtner A, von Oheimb G, Härdtle W, Wilken C, Gutknecht JLM. 2014. Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more than 100 years. *Soil Biology and Biochemistry* 70:79–87. DOI: 10.1016/j.soilbio.2013.12.015.

Franzluebbers AJ, Stuedemann JA. 2008. Soil physical responses to cattle grazing cover crops under conventional and no tillage in the Southern Piedmont USA. *Soil and Tillage Research* 100:141–153. DOI: 10.1016/j.still.2008.05.011.

Garland JL, Mills AL. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology* 57:2351–2359. DOI: 10.1128/aem.57.8.2351-2359.1991.

Giambelluca TW, Chen Q, Frazier AG, Price JP, Chen YL, Chu PS, Eischeid JK, Delparte DM. 2013. Online rainfall atlas of Hawai'i. *Bulletin of the American Meteorological Society* 94:313–316. DOI: 10.1175/BAMS-D-11-00228.1.

Giambelluca TW, Shuai X, Barnes ML, Alliss RJ, Longman RJ, Miura T, Chen Q, Frazier AG, Mudd RG, Cuo L, Businger AD. 2014. Evapotranspiration of Hawai'i. *Final report submitted to the US Army Corps of Engineers—Honolulu District, and the Commission on Water Resource Management, State of Hawai 'i*.

Grman E, Suding KN. 2010. Within-year soil legacies contribute to strong priority effects of exotics on native California grassland communities. *Restoration Ecology* 18:664–670. DOI: 10.1111/j.1526-100X.2008.00497.x.

Hagemann N, Spokas K, Schmidt H-P, Kägi R, Böhler M, Bucheli T. 2018. Activated carbon, biochar and charcoal: Linkages and synergies across pyrogenic carbon's ABCs. *Water* 10:182. DOI: 10.3390/w10020182.

Hamza MA, Anderson WK. 2005. Soil compaction in cropping systems: A review of the nature, causes and possible solutions. *Soil and Tillage Research* 82:121–145. DOI: 10.1016/J.STILL.2004.08.009.

Harpole S. 2009. *NutNet Resin Bag Protocol*.

Heisey S, Ryals R, Maaz TM, Nguyen NH. 2022. A single application of compost can leave lasting impacts on soil microbial community structure and alter cross-domain interaction networks. *Frontiers in Soil Science* 2:1–16. DOI: 10.3389/FSOIL.2022.749212.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50:346–363. DOI: https://doi.org/10.1002/bimj.200810425.

Hu DM, Dai XH, Guo DY, Hyde KD, Zhang KQ. 2008. The diversity of coprophilous fungi from Dahuadian and Zhongdian grasslands, Yunnan, China. 29:355–364.

Humphries T, Florentine SK. 2021. A comparative review of six invasive *Nassella* species in Australia with implications for their management. *Plants* 10:1–17. DOI: 10.3390/plants10061036.

Kardol P, Wardle DA. 2010. How understanding aboveground–belowground linkages can assist restoration ecology. *Trends in Ecology & Evolution* 25:670–679. DOI: 10.1016/j.tree.2010.09.001.

Goh YK, Marzuki NF, Tuan Pa TNF, Goh T-K, Kee ZS, Goh YK, Yusof MT, Vujanovic V, Goh KJ. 2020. Biocontrol and plant-growth-promoting traits of *Talaromyces apiculatus* and *Clonostachys rosea* consortium against *Ganoderma* basal stem rot disease of oil palm. *Microorganisms* 8:1–20. DOI: 10.3390/microorganisms8081138.

Kirby JM, Bengough AG. 2002. Influence of soil strength on root growth: experiments and analysis using a critical-state model. *European Journal of Soil Science* 53:119–127. DOI: 10.1046/J.1365-2389.2002.00429.X.

Klinerová T, Dostál P. 2020. Nutrient-demanding species face less negative competition and plant–soil feedback effects in a nutrient-rich environment. *New Phytologist* 225:1343–1354. DOI: 10.1111/nph.16227.

Kulmatiski A. 2011. Changing soils to manage plant communities: Activated carbon as a restoration tool in ex-arable fields. *Restoration Ecology* 19:102–110. DOI: 10.1111/j.1526-100X.2009.00632.x.

Kulmatiski A, Beard KH. 2006. Activated carbon as a restoration tool: Potential for control of invasive plants in abandoned agricultural fields. *Restoration Ecology* 14:251–257. DOI: 10.1111/j.1526-100X.2006.00127.x.

Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzländer M, Strauss SY, Hufbauer RA. 2008. Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist* 178:412–423. DOI: 10.1111/j.1469-8137.2007.02360.x.

Lenth R V. 2016. Least-squares means: the R package lsmeans. *Journal of statistical software* 69:1–33.

Lenth R, Lenth MR. 2018. Package 'lsmeans.' *The American Statistician* 34:216–221.

Liu H, Liang C, Ai Z, Zhang J, Wu Y, Xu H, Xue S, Liu G. 2019. Plant-mycorrhizae association affects plant diversity, biomass, and soil nutrients along temporal gradients of natural restoration after farmland abandonment in the Loess Plateau, China. *Land Degradation and Development* 30:1677–1690. DOI: 10.1002/ldr.3372.

Liu S, Liu Y, Tan X, Zeng G, Zhou Y, Liu S, Yin Z, Jiang L, Li M, Wen J. 2018a. The effect of several activated biochars on Cd immobilization and microbial community composition during in-situ remediation of heavy metal contaminated sediment. *Chemosphere* 208:655–664. DOI: 10.1016/j.chemosphere.2018.06.023.

Liu Y, Liu M, Xu X, Tian Y, Zhang Z, van Kleunen M. 2018b. The effects of changes in water and nitrogen availability on alien plant invasion into a stand of a native grassland species. *Oecologia* 188:441–450. DOI: 10.1007/s00442-018-4216-1.

Liu Y, Zheng Y, Jahn L V., Burns JH. 2022. Invaders responded more positively to soil biota than native or noninvasive introduced species, consistent with enemy escape. *Biological Invasions*:1–14. DOI: 10.1007/S10530-022-02919-Y/FIGURES/2.

Lüdecke D. 2018. sjPlot: Data visualization for statistics in social science. *R package version* 2.

Madsen MD, Davies KW, Mummey DL, Svejcar TJ. 2014. Improving restoration of exotic annual grass-invaded rangelands through activated carbon seed enhancement technologies. *Rangeland Ecology and Management* 67:61–67. DOI: 10.2111/REM-D-13-00050.1.

Naraghi L, Heydari A, Rezaee S, Razavi M, Afshari-Azad H. 2012. Promotion of growth characteristics in greenhouse cucumber and tomato by *Talaromyces flavus*. *International Journal of Agricultural Science and Research* 2:129–141.

Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Gï Ockner 10 FO, Tedersoo L, Saar I, Abarenkov K. 2018. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* 47:259–264. DOI: 10.1093/nar/gky1022.

NOAA National Centers for Environmental Information.Monthly Global Climate Report for Annual 2022. *Available at https://www.ncei.noaa.gov/access/monitoring/monthly-report/global/202213* (accessed April 12, 2023).

Nolan NE, Kulmatiski A, Beard KH, Norton JM. 2015. Activated carbon decreases invasive plant growth by mediating plant - Microbe interactions. *AoB PLANTS* 7. DOI: 10.1093/aobpla/plu072.

O'Dwyer C, Attiwill PM. 2000. Restoration of a native grassland as habitat for the golden sun moth *Synemon plana* Walker (Lepidoptera; Castniidae) at Mount Piper, Australia. *Restoration Ecology* 8:170–174. DOI: 10.1046/j.1526-100x.2000.80024.x.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. Package 'vegan.' *Community ecology package, version* 2:1–295.

Pearson DE, Ortega YK, Runyon JB, Butler JL. 2016. Secondary invasion: The bane of weed management. *Biological Conservation* 197:8–17. DOI: 10.1016/j.biocon.2016.02.029.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* 41:D590-596. DOI: 10.1093/nar/gks1219.

Que W, Zhou Y hui, Liu Y guo, Wen J, Tan X fei, Liu S jia, Jiang L hua. 2019. Appraising the effect of in-situ remediation of heavy metal contaminated sediment by biochar and activated carbon on Cu immobilization and microbial community. *Ecological Engineering* 127:519–526. DOI: 10.1016/j.ecoleng.2018.10.005.

Rumpel C, Chabbi A. 2019. Plant–soil interactions control CNP coupling and decoupling processes in agroecosystems with perennial vegetation. In: *Agroecosystem Diversity*. Elsevier, 3–13. DOI: 10.1016/B978-0-12-811050-8.00001-7.

Schaller J, Puppe D, Kaczorek D, Ellerbrock R, Sommer M. 2021. Silicon cycling in soils revisited. *Plants* 10:1–33. DOI: 10.3390/plants10020295.

Schlatter DC, Kahl K, Carlson B, Huggins DR, Paulitz T. 2018. Fungal community composition and diversity vary with soil depth and landscape position in a no-till wheat-based cropping system. *FEMS Microbiology Ecology* 94. DOI: 10.1093/FEMSEC/FIY098.

Shahrtash M, Brown SP. 2021. A path forward: Promoting microbial-based methods in the control of invasive plant species. *Plants* 10:943. DOI: 10.3390/plants10050943.

Shan J, Ji R, Yu Y, Xie Z, Yan X. 2015. Biochar, activated carbon, and carbon nanotubes have different effects on fate of 14 C-catechol and microbial community in soil. *Scientific Reports* 5:16000. DOI: 10.1038/srep16000.

Sharrow SH. 2007. Soil compaction by grazing livestock in silvopastures as evidenced by changes in soil physical properties. *Agroforestry Systems* 71:215–223. DOI: 10.1007/S10457-007-9083-4/TABLES/3.

Singh M, Daehler CC. 2023. Meta-analytic evidence that allelopathy may increase the success and impact of invasive grasses. *PeerJ* 11:e14858. DOI: 10.7717/PEERJ.14858.

Sitters J, Edwards PJ, Olde Venterink H. 2013. Increases of soil C, N, and P pools along an *Acacia* tree density gradient and their effects on trees and grasses. *Ecosystems* 16:347–357. DOI: 10.1007/s10021-012-9621-4.

Smith MR, Charvat I, Jacobson RL. 1998. Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. *Canadian Journal of Botany* 76:1947–1954. DOI: 10.1139/b98-205.

Sugiyama A, Bakker MG, Badri D V., Manter DK, Vivanco JM. 2013. Relationships between Arabidopsis genotype-specific biomass accumulation and associated soil microbial communities. *Botany* 91:123–126. DOI: 10.1139/cjb-2012-0217.

Sujeeun L, Thomas SC. 2017. Potential of biochar to mitigate allelopathic effects in tropical island invasive plants: Evidence from seed germination trials. *Tropical Conservation Science* 10. DOI: 10.1177/1940082917697264/ASSET/IMAGES/LARGE/10.1177_1940082917697264-FIG2.JPEG.

Sujeeun L, Thomas SC. 2022. Biochar rescues native trees in the biodiversity hotspot of Mauritius. *Forests* 13:277. DOI: 10.3390/f13020277.

Vinale F, Nicoletti R, Lacatena F, Marra R, Sacco A, Lombardi N, d'Errico G, Digilio MC, Lorito M, Woo SL. 2017. Secondary metabolites from the endophytic fungus *Talaromyces pinophilus*. *Natural Product Research* 31:1778–1785. DOI: 10.1080/14786419.2017.1290624.

Wang KH, McSorley R, Bohlen P, Gathumbi SM. 2006. Cattle grazing increases microbial biomass and alters soil nematode communities in subtropical pastures. *Soil Biology and Biochemistry* 38:1956–1965. DOI: 10.1016/j.soilbio.2005.12.019.

Wang Y, Ni G, Hou Y, Wang Q, Huang Q. 2020. Plant–soil feedbacks under resource limitation may not contribute to the invasion by annual Asteraceae plants. *Oecologia* 194:165–176. DOI: 10.1007/s00442-020-04756-z.

Weißhuhn K, Prati D. 2009. Activated carbon may have undesired side effects for testing allelopathy in invasive plants. *Basic and Applied Ecology* 10:500–507. DOI: 10.1016/j.baae.2008.10.009.

Wurst S, Vender V, Rillig MC. 2010. Testing for allelopathic effects in plant competition: does activated carbon disrupt plant symbioses? *Plant Ecology* 211:19–26. DOI: 10.1007/s11258-010-9767-0.

Yang JL, Zhang GL. 2018. Silicon cycling by plant and its effects on soil Si translocation in a typical subtropical area. *Geoderma* 310:89–98. DOI: 10.1016/J.GEODERMA.2017.08.014.

Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO. 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research* 42:D643–D648. DOI: 10.1093/nar/gkt1209.

Zhang C, Liu G, Song Z, Wang J, Guo L. 2018. Interactions of soil bacteria and fungi with plants during long-term grazing exclusion in semiarid grasslands. *Soil Biology and Biochemistry* 124:47–58. DOI: 10.1016/J.SOILBIO.2018.05.026.

Table 1. Results for fungi community composition PERMANOVA, dispersion ANOVA (top-left) and diversity indices analyses (top-right). Dropped predictors were ns at $p < 0.05$. Bottom, results from fungi indicator species analysis, by predictor (second column) and level (fourth column) and reporting specificity ("A") and sensitivity ("B").

Fungi Composition PERMANOVA

Predictor	*df*	*Sum Sq*	*r²*	*F*	*Pr(>F)*
Raking	1	0.25	0.05	2.10	0.02
BC	1	0.24	0.04	2.01	0.04
Plot	15	2.32	0.43	1.32	0.04

Fungi Dispersion ANOVA

Predictor	*df*	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
AC	2	0.1	0.05	4.97	0.01

Fungi Diversity, Model Coefficients

	Shannon					Simpson's				
		95% CI					95% CI			
	Est.	*L*	*U*	*t*	*p*	*Est.*	*L*	*U*	*t*	*p*
Intercept	2.88	2.45	3.32	12.92	0	0.86	0.80	0.92	28.62	0
BC	-0.62	-1.19	-0.05	-2.15	0.05	-0.04	-0.11	0.03	-1.11	ns
AC:BC	-1.52	-3.77	0.72	-1.33	ns	-0.51	-0.79	-0.24	-3.65	0

Fungi Indicator Species Analysis

	Predictor	*p*	*level*	*A*	*B*
Alfaria	Outplanting	0.03	Control	0.83	1.00
Arachnomyces	Outplanting	0.05	Outplanting	0.83	0.94
Pezizaceae unclassified	Outplanting	0.03	Control	0.93	1.00
Talaromyces	Outplanting	0.01	Outplanting	0.87	1.00
Trichoderma	Outplanting	0.02	Control	0.83	1.00
Bionectriaceae unclassified	Raking	0.01	No Raking	0.21	0.81
Coniophora	AC	0.03	No AC	0.99	0.79
Saitozyma	AC	0.02	No AC	0.83	0.82
Talaromyces (AC)	AC	0.01	No AC	0.64	1.00
Absidia	BC	0.00	No BC	0.68	1.00
Aspergillus	BC	0.01	BC	0.66	1.00
Distoseptispora	BC	0.05	BC	0.85	1.00
Naganishia	BC	0.04	Combo	0.77	1.00

Table 2. Results for bacteria community dispersion ANOVA (top-left) and diversity indices analyses (top-right). Dropped predictors were ns at $p < 0.05$. All predictors were ns for composition PERMANOVA. Bottom, results from bacteria indicator species analysis, by predictor (second column) and level (fourth column) and reporting specificity ("A") and sensitivity ("B").

Bacteria Dispersion ANOVA

Predictor	*df*	*Sum Sq*	*Mean Sq*	*F value*	*Pr(>F)*
Outplanting	1	0.03	0.03	5.12	0.03
BC	2	0.04	0.02	3.74	0.03

Bacteria Diversity, Model Coefficients

	Shannon					Simpson's				
		95% CI					95% CI			
	Est.	*L*	*U*	*t*	*p*	*Est.*	*L*	*U*	*t*	*p*
Intercept	3.20	2.85	3.56	17.80	0	0.86	0.82	0.91	41.24	0
AC:BC	-1.72	-3.38	-0.07	-2.04	0.05	-0.16	-0.35	0.03	-1.62	ns

Bacteria Indicator Species Analysis

	Predictor	*p*	*level*	*A*	*B*
FCPS473	Outplanting	0.01	Control	0.91	1.00
Ktedonobacteraceae unclassified	Outplanting	0.04	Control	0.81	1.00
Solirubrobacter	Outplanting	0.02	Outplanting	0.66	1.00
Thermomonosporaceae unclassified	Outplanting	0.01	Outplanting	0.85	0.97
Solirubrobacteraceae unclassified	AC	0.03	Combo	0.56	1.00
Beijerinckiaceae unclassified	BC	0.04	BC	0.62	1.00
Candidatus Solibacter	BC	0.03	No BC	0.52	1.00

Table 3. Left, summary of diversity indexes analyses. All predictors were ns at $p < 0.05$ for Simpson's Diversity Index, richness, and evenness. Right, summary of carbon category model. Dropped predictors and carbon source categories were ns at $p < 0.05$.

Ecoplate Diversity, Model Coefficients

	Shannon				
		95% CI			
	Est.	*L*	*U*	*t*	*p*
Intercept	2.72	2.22	3.22	10.69	0
AC	-0.79	-1.49	-0.08	-2.19	0.04
AC:BC	2.62	0.17	5.06	2.10	0.05

Carbon Category, Model Coefficients

		95% CI				
Predictor	*Est.*	*L*	*U*	*df*	*t*	*p*
Polymer						
AC	1.49	0.86	2.11	4.65	874	0.00
BC	0.79	0.19	1.39	2.59	874	0.01
AC:BC	-6.85	-8.95	-4.74	-6.38	874	0.00
Amino Acid						
AC:BC	2.30	0.58	4.02	2.63	874	0.01

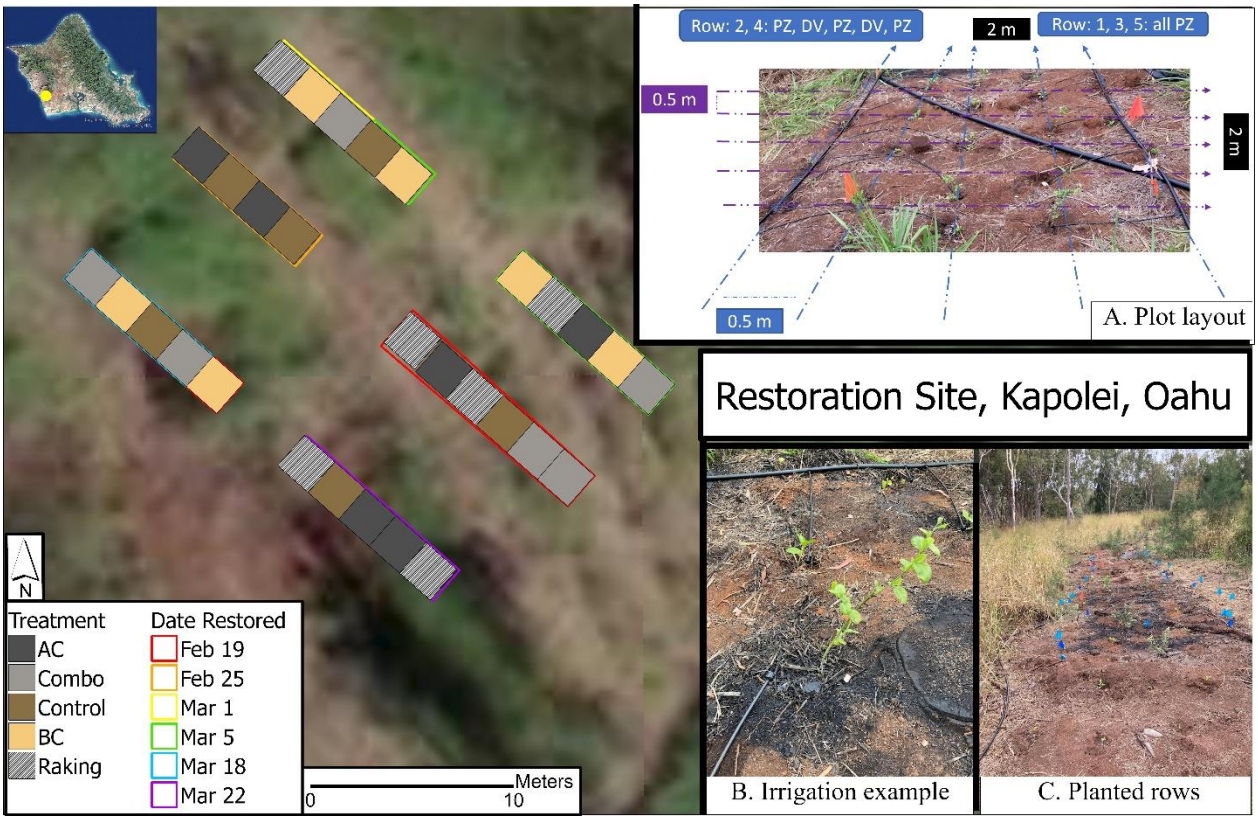

Figure 1. Map showing layout of plots at restoration site at Kapolei, Oahu. Plot fill represents treatment, and plot outline represents date restored. Upper-left callout, Oahu, with point representing location of Camp Palehua. Right: A) diagram demonstrating the layout of each plot; B) example of irrigation set-up in an AC plot; C) picture of planted row.

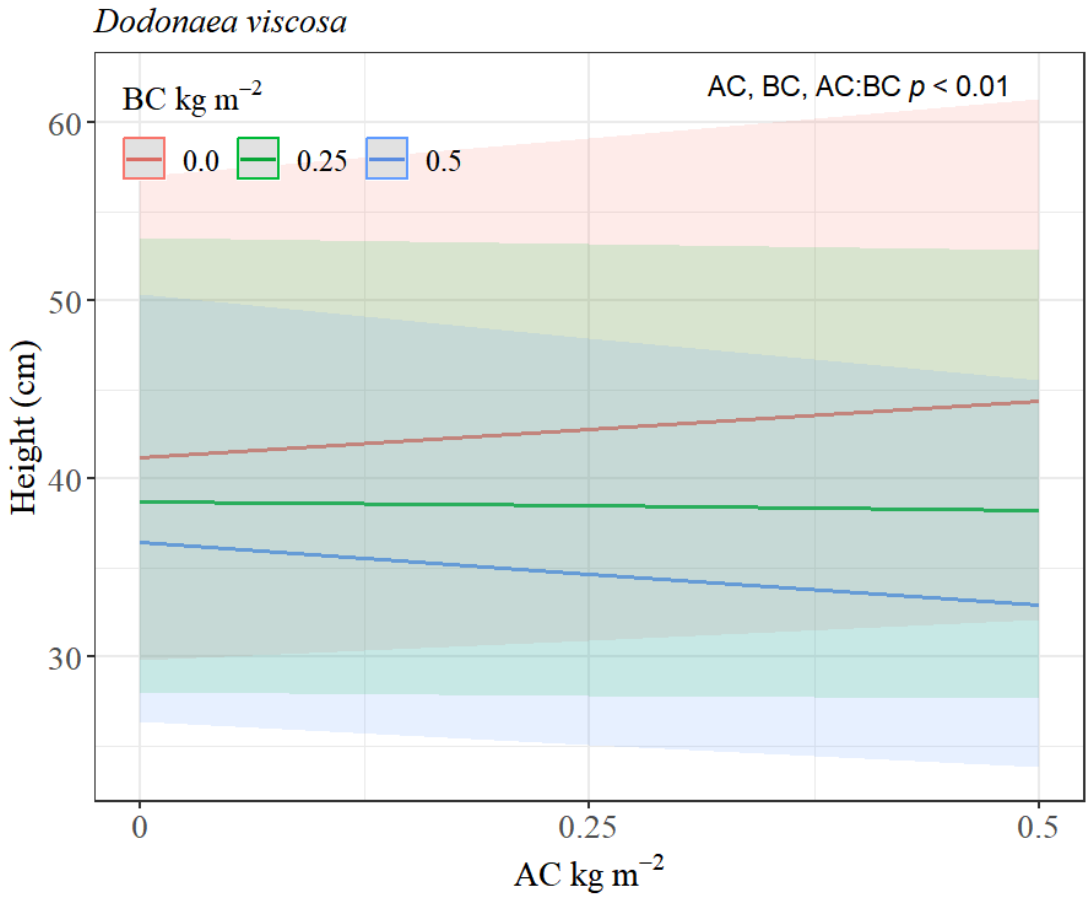

Figure 2. Model predictions for *Dodonaea viscosa* height by AC use (x-axis) and BC use (line and fill color).

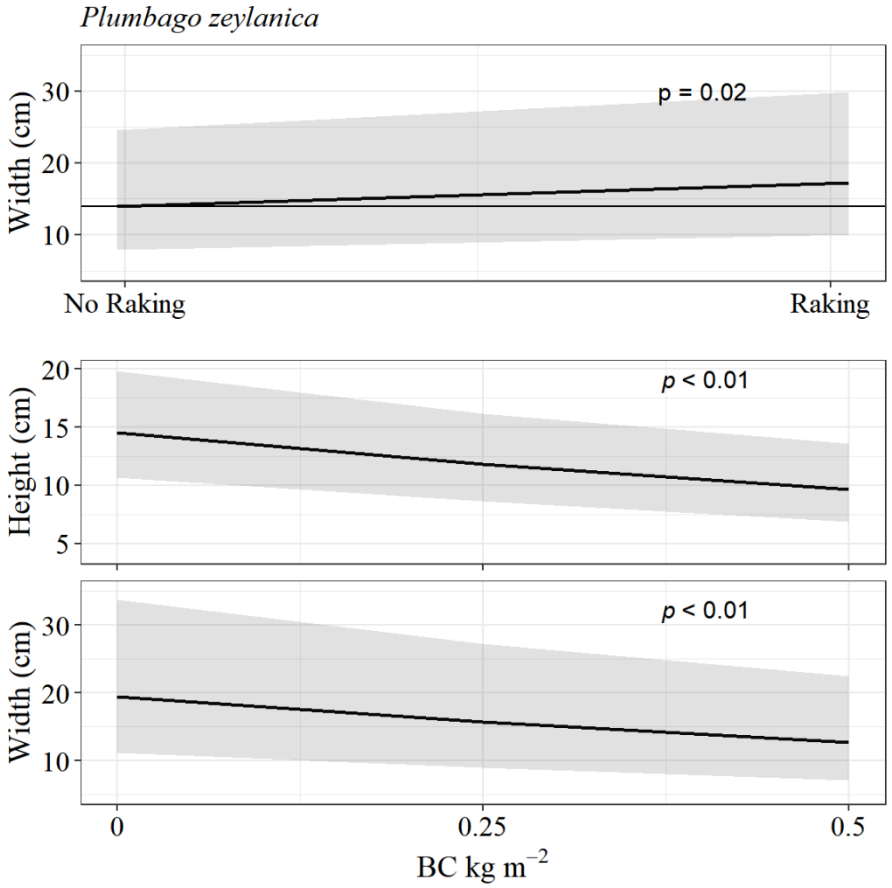

Figure 3. Model predictions for *Plumbago zeylanica* width (top, bottom) and height (center) by use of raking (top) and use of BC (center, bottom).

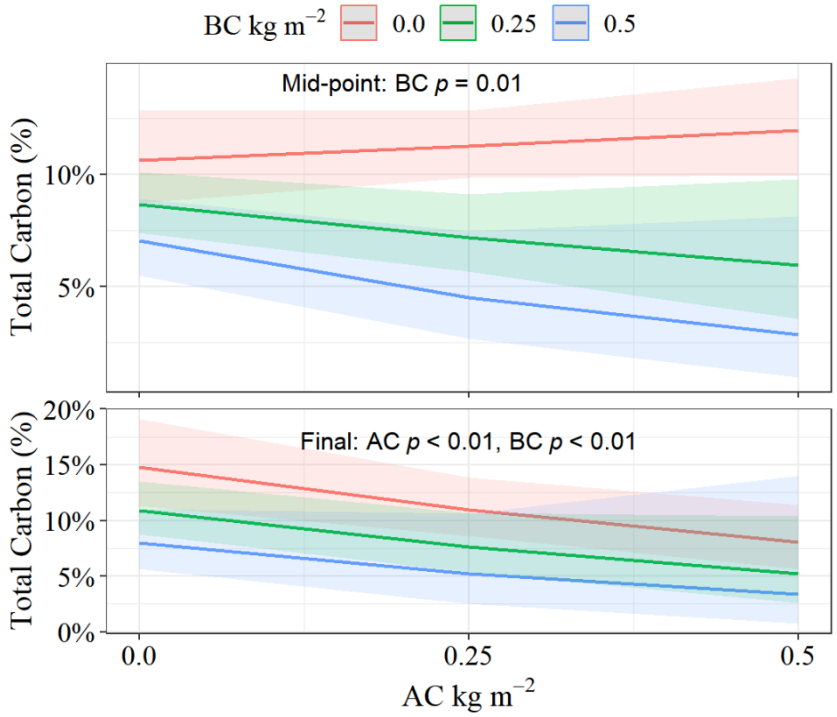

Figure 4. Model predictions for total carbon (%) by AC use (x-axis) and BC use (line and fill color) in the non-repeated models (top: mid-point model, bottom: final point model).

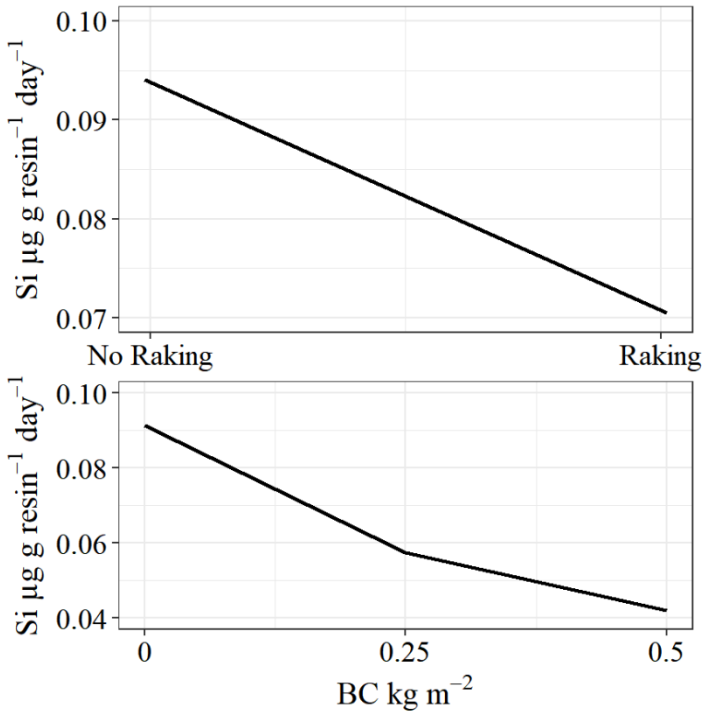

Figure 5. Model predictions for Si cycling across use of raking (above) and use of BC (below).

Appendix D- Supplementary Tables

Table S1. Model summary for *Plumbago zeylanica* outplant survival.

Survival Rate, Model Coefficients

Mid-point					Final				
		95% CI					95% CI		
Predictor	**Odds Ratio**	**L**	**U**	***p***	**Predictor**	**Odds Ratio**	**L**	**U**	***p***
Intercept	9.22	3.9	21.81	0	Intercept	8.39	3.46	20.32	0

Dropped for ns: Raking, AC, BC, AC:BC

Table S2. Model summary for *Plumbago zeylanica* (left) and *Dodonaea viscosa* (right) height and width. Predictors were ns if $p > 0.05$.

Growth Dimensions, Model Coefficients

	Plumabgo zeylanica								*Dodonaea viscosa*							
	HEIGHT				**WIDTH**				**HEIGHT**				**WIDTH**			
		95%	CI			95%	CI			95%	CI			95%	CI	
Predictor	**Odds Ratio**	**L**	**U**	***p***	**Odds Ratio**	**L**	**U**	***p***	**Odds Ratio**	**L**	**U**	***p***	**Odds Ratio**	**L**	**U**	***p***
Intercept	12.62	9.13	17.45	0	16.4	9.33	28.84	0	40.92	29.6	56.57	0	23.31	15.69	34.63	0
Raking	1.15	0.98	1.34	ns	1.24	1.03	1.49	0.02	1.01	0.99	1.02	ns	0.98	0.73	1.3	ns
AC	1.01	0.88	1.16	ns	0.86	0.73	1.01	ns	1.08	1.06	1.09	0	1.19	0.95	1.49	ns
BC	0.73	0.62	0.85	0	0.62	0.49	0.77	0	0.88	0.87	0.9	0	0.9	0.65	1.24	ns
AC:BC	0.73	0.41	1.31	ns	1.22	0.6	2.48	ns	0.84	0.83	0.85	0	1.68	0.68	4.15	ns

Table S3. Summary of hypothesis testing for plant growth.

Growth Dimensions Hypotheses

Contrast	Ratio	SE	df	95% CI L	95% CI U
Plumbago zeylanica					
Height					
Control / BC	1.38	0.11	Inf	1.03	1.83
Raking / BC	1.58	0.20	Inf	1.01	2.46
AC / BC	1.39	0.12	Inf	1.02	1.90
Width					
Control / BC	1.62	0.19	Inf	1.09	2.43
Raking / AC:BC	1.62	0.22	Inf	1.00	2.63
Raking / BC	2.01	0.32	Inf	1.15	3.52

Ratio > 1 indicates contrast: first > second
Ratio < 1 indicates contrast: first < second

Growth Dimensions Hypotheses

Contrast	Ratio	SE	df	95% CI L	95% CI U
Dodonaea viscosa					
Height					
Control / AC	0.93	0.01	Inf	0.91	0.95
Control / AC:BC	1.07	0.01	Inf	1.05	1.09
Control / BC	1.13	0.01	Inf	1.10	1.16
Raking / AC	0.94	0.01	Inf	0.90	0.97
Raking / AC:BC	1.08	0.01	Inf	1.04	1.11
Raking / BC	1.14	0.01	Inf	1.10	1.18
AC / AC:BC	1.15	0.01	Inf	1.13	1.17
AC / BC	1.22	0.01	Inf	1.18	1.26
AC:BC / BC	1.06	0.01	Inf	1.04	1.08

Ratio > 1 indicates contrast: first > second
Ratio < 1 indicates contrast: first < second

Table S4. Summary of ANOVA on repeated-measures models for TC% and TN%. Dropped predictors were ns at $p < 0.05$.

ANOVA, Type III Wald

	TC%			TN%		
Predictor	*χ2*	*df*	*p*	*χ2*	*df*	*p*
Intercept	122.9	1	0	566.9	1	0
Raking	2.2	1	ns	5.2	1	0.02
AC	5.8	1	0.02	1.9	1	ns
Time	7.1	1	0.01	0.9	1	ns
AC:Time	11.5	1	0	6.8	1	0.01

Dropped for ns: BC, AC:BC, BC:Time, AC:BC:Time

Table S5. Summary of ANOVA for models comparing mid-point samples (left) and final samples (right) to pre-practice samples for total carbon and total nitrogen. Dropped predictors were ns at $p < 0.05$.

ANOVA, Type III Wald

	Mid-point			Final		
Predictor	*χ2*	*df*	*p*	*χ2*	*df*	*p*
TC%						
Intercept	384.3	1	0	180.8	1	0
AC	0.8	1	ns	9.0	1	0
BC	7.0	1	0.01	8.7	1	0
TN%						
Intercept	1945.4	1	0	906.4	1	0
Raking	2.4	1	ns	6.1	1	0.01
AC	0.1	1	ns	12.0	1	0
BC	11.3	1	0	7.7	1	0.01

Note: Due to model fitting issues,TN-mid-point model block random effect was changed to Side:Row
Dropped predictors were ns

Table S6. Summary of infiltration rate analysis. Dropped predictors were ns at $p < 0.05$.

Infiltration Rate, Model Coefficients

		Mid-point 95% CI				Final 95% CI		
Predictor	**Odds Ratio**	**L**	**U**	***p***	**Odds Ratio**	**L**	**U**	***p***
Raking	0.4	0.18	0.89	0.03				ns
Climate					2.53	1.26	5.06	0.01

Dropped for ns: AC, BC, AC:BC
Climate: Avg. Temp. (°C) for Final

Table S7. Summary of ANOVA results for inorganic nutrient models. Dropped predictors were ns at $p < 0.05$. All predictors were ns for N.

ANOVA, Type III Wald

	P			Si		
Predictor	**χ2**	***df***	***p***	**χ2**	***df***	***p***
Raking	1.8	1	ns	14.9	1	0
BC	0.1	1	ns	5	1	0.03
Time	9.1	1	0	3.4	1	ns

Dropped ns: AC, AC:BC

All N predictors were ns

Appendix E- Supplementary Figures

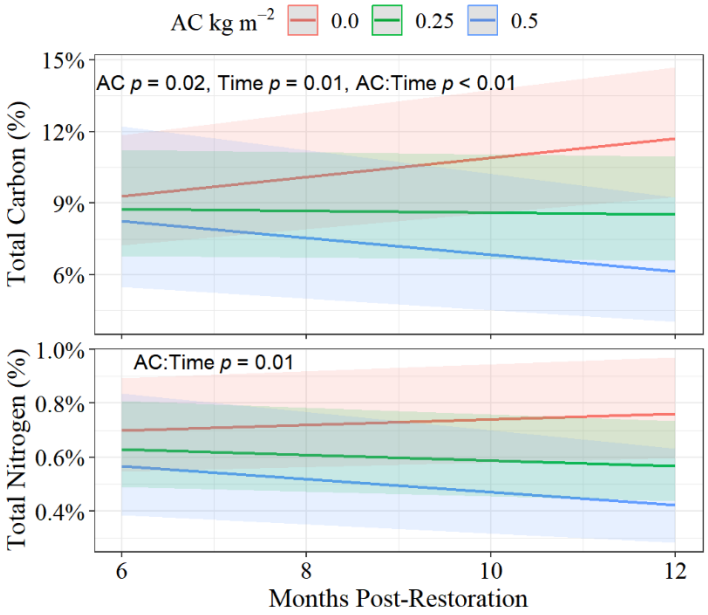

Figure S1. Total carbon (above) and total nitrogen (below) across months post-restoration (x-axis) and by AC use (line color and fill), based on repeated-measures model.

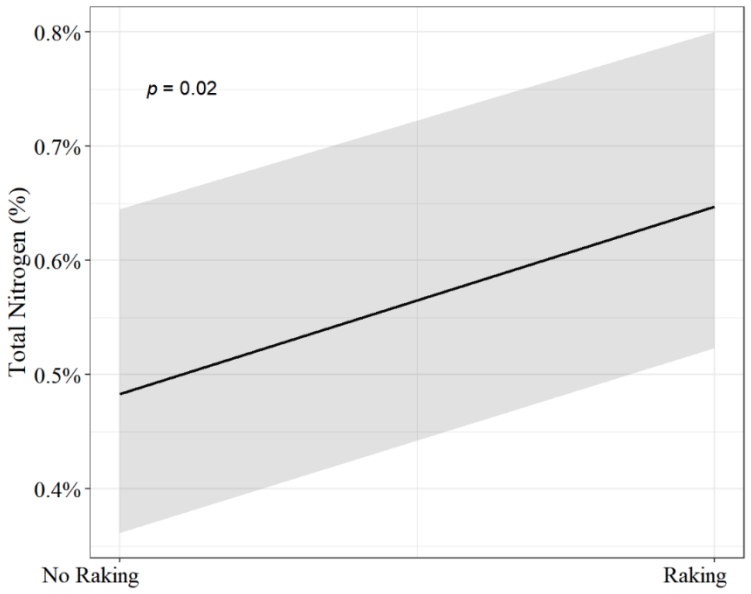

Figure S2. Total nitrogen (%) across use of raking, based on the repeated-measures model.

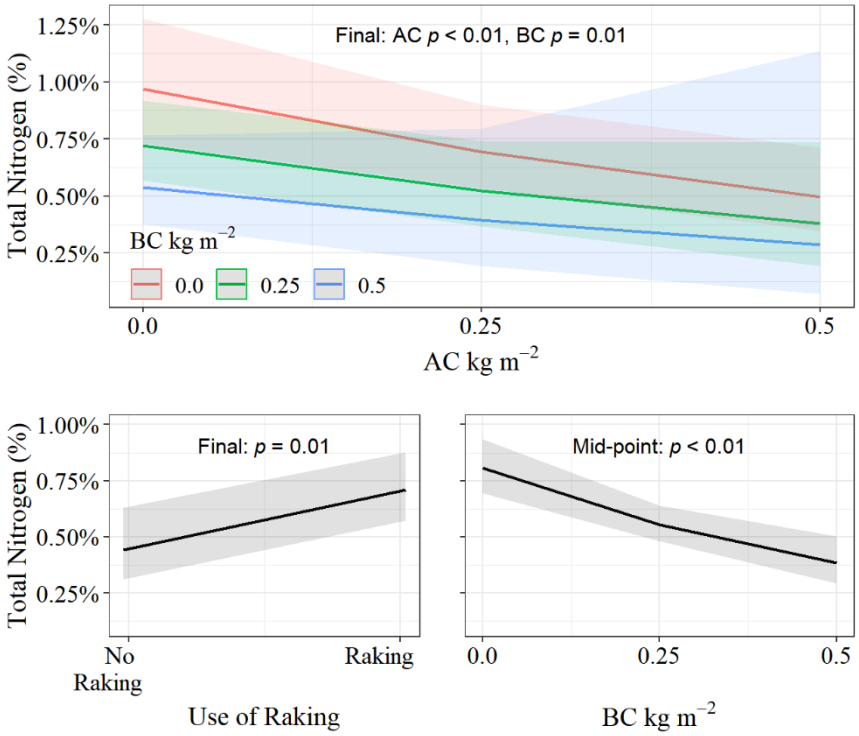

Figure S3. Above, total nitrogen by AC use (x-axis) and BC use (line color and fill), from the final point model. Bottom-left, total nitrogen across raking use from the final point model. Bottom-right, total nitrogen across BC use from the mid-point model.

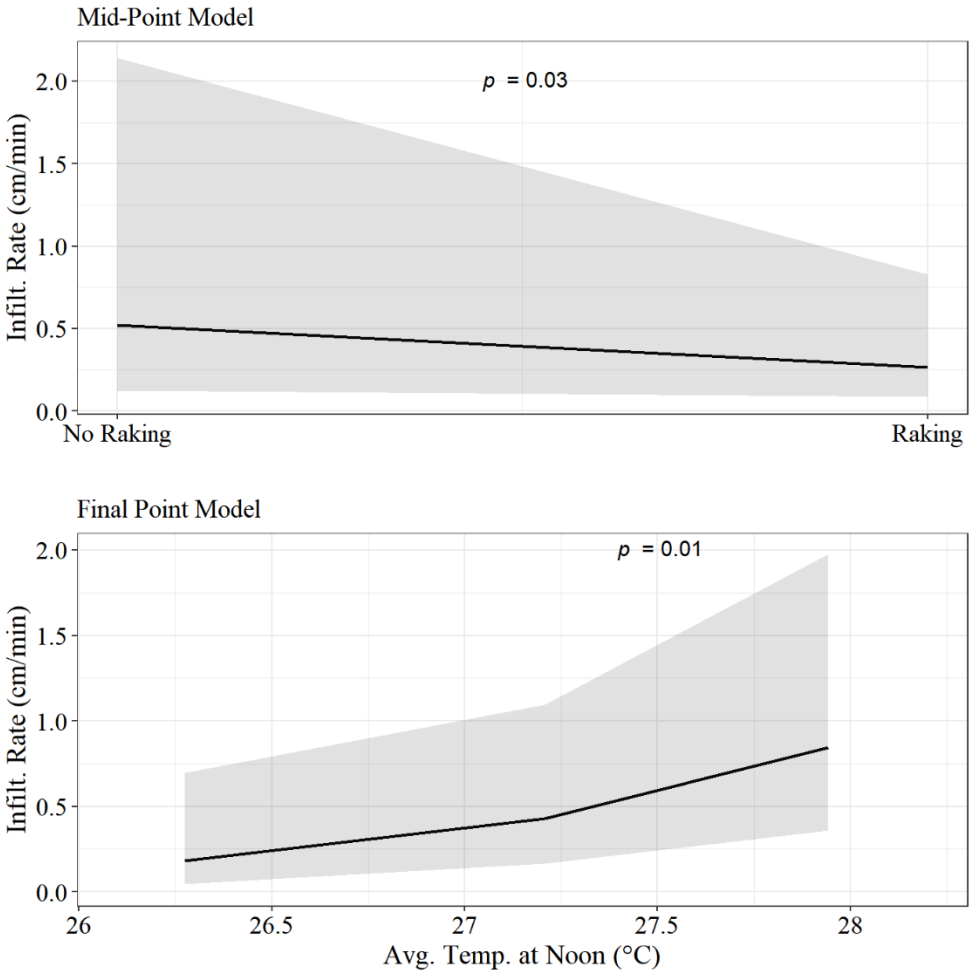

Figure S4. Above, infiltration rate across raking use based on the mid-point model. Below, infiltration rate across average temperature at noon based on the final point model.

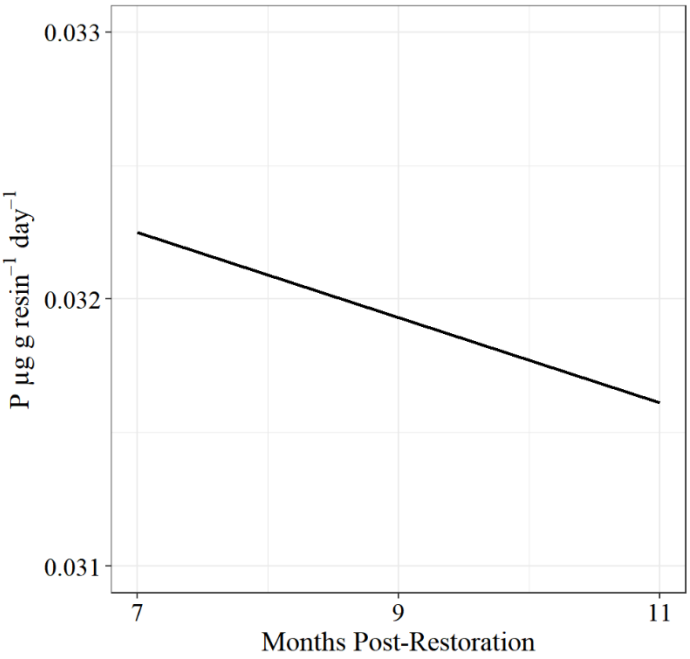

Figure S5. P cycling across months post-restoration.

CHAPTER 6

CONCLUSION

The aim of this work was to improve our understanding of specific mechanisms of invasive grass soil legacies, and to improve our ability to mitigate the mechanisms in restoration. I focused specifically on allelopathy and the soil microbial community, two mechanisms that are not mutually exclusive. Elucidating the details of mechanisms is challenging, given the role of context and the complex inter-locking nature of belowground processes. Improving soil legacy mitigation strategies, and improving restoration success more generally, is not just critical to conserving native plants and ecosystems. Restoration projects require resources, and allocation of resources from many sources requires the public to view restoration as worth the investment. While there is much to learn from failed restoration projects, failure has an undeniable cost in the world of public opinion. Restoration ecology, and many of its' key concepts, are ultimately based in pragmatism (Morse et al., 2014; Miller & Bestelmeyer, 2016; Backstrom et al., 2018). In this body of work, I hoped to contribute pragmatically to the field of restoration ecology, and by focusing on invasive grasses, I am hopeful that this project provides practical, actionable information for land managers in places far beyond Hawai'i.

In the second chapter, I found support for the role of allelopathy in the disproportionate invasive success among grasses. Allelochemicals in the *Poaceae* family are increasingly of interest, particularly benzoxazinoids (Frey et al., 2009; Niculaes et al., 2018; Hu et al., 2018). This finding suggests that incorporating allelopathy-informed restoration practices in management of grass-invaded areas may improve restoration success, and allelopathy-informed restoration practices that practitioners may want to consider are summarized in Figure 2.5. There is still a lot of room to explore and develop allelopathy-informed restoration practices further, as some, like soil amendment with activated carbon, are cost-prohibitive, and others, like soil transplants, will only be possible in very specific mitigation project contexts. The full list of identified mechanisms of allelopathy is expansive (Cheng & Cheng, 2015), and further efforts towards identifying specific mechanisms, and identifying commonalities in mechanisms within *Poaceae,* could assist in improving the efficacy of mitigation strategies as well. As previously mentioned, the mechanisms of allelopathy and feedbacks involving the soil microbial community are not mutually exclusive, and studies have found that benzoxazinoids have anti-fungal properties (Niculaes et al., 2018), suggesting that suppressing specific fungi may be a mechanism of allelopathic impact.

In the third chapter, I examined a previously restored site, and compared the soil properties and the soil microbiome to a currently invaded, actively grazed area of the invasive grass *Megathyrsus maximus.* Since we could not compare the restoration site before and after restoration, we selected a *M. maximus* site that shared soil classification, slope, aspect and land-use history with the restoration site with the objective of identifying soil characteristics that may be associated with grass invasion or restoration In this comparison, we found that there was diversification of the soil microbial community at the restored site, which is in-line with the general tend towards soil microbial community diversity having a positive correlation with plant community diversity. We also found that there were key pasture-associated fungi and bacteria genera that were highly abundant at both sites, suggesting that despite the restoration, it is possible that these genera at the restored site were "hold-overs" from prior grass invasion. As hold-overs, they may contribute to on-going issues at the restoration site, including the continuing grass re-emergence, requiring intensive weeding the site to maintain the native plant community. In considering how to effectively scale restoration to larger sections of land, this analysis suggests that, if hold-over microbes promote grass re-emergence or other negative impacts to the native plant community, adapting the practice to shift or suppress the soil microbial community may help to ameliorate these issues.

In the fourth chapter, I conducted a plant-soil feedback study with *Megathyrsus maximus* and a dry forest endemic shrub, *Chenopodium oahuense.* I found that the soil microbial community associated with *Megathyrsus maximus* seems to drive belowground competition by having positive feedback on *M. maximus* belowground and total growth. Specifically, the increased *Megathyrsus maximus* root biomass is expected to limit the soil resource acquisition zone that any other species can access. In addition, the *Megathyrsus* soil microbiome was associated with a significant germination delay in *Chenopodium oahuense,* under moderate drought conditions, which can increase the competitive growth advantage that *M. maximus* has by giving *M. maximus* a headstart as precipitation regimes shift toward dryer conditions.

Both findings are relevant to restoration projects in *M. maximus* invaded areas. While we did not find that the *M. maximus* microbiome had significantly negative feedback towards *Chenopodium oahuense* belowground growth, the feedback of the *Megathyrsus* soil microbiome on *Chenopodium* belowground growth had a ~70% probability of being negative, suggesting that

further studies may find that the *M. maximus* microbiome mediates belowground competition through positive con-specific feedback and negative hetero-specific feedback. Additionally, outplanting typically has a higher cost compared to dispersal of seeds, so as precipitation decreases, restoration may require more resources if establishment by seeds is essentially ineffective. Since in the previous chapter we found that even in a successful restoration project, high-abundance microbes can be hold-overs from the days of *M. maximus* invasion, it seems that directly incorporating some method of suppressing or shifting the soil microbial community could contribute to neutralizing or at least moderating the feedback effects.

Finally, in the fifth chapter, I tested the efficacy of activated carbon (AC) as a mitigator of soil microbiome mediated or driven *Megathyrsus maximus* soil legacy effects. We found that application of AC at 0.5 kg/m² was associated with an improvement in height of *Dodonaea viscosa* of 8%. For the soil fungi community, activated carbon was associated with reduced community sample dispersion based on Bray-Curtis Dissimilarity. In addition, the indicator species analysis found that AC addition was associated with the suppression of three genera, including *Coniophora,* which had a specificity of 0.99, meaning that that if *Coniophora* is present in a sample, the probability that the sample came from a plot without use of AC is 99%. *Coniophora* has previously been identified in former pastureland converted to native tree plantations as a complex C degrader (Eaton et al., 2019). Additionally, in the community-level physiological profile of carbon preferences of the soil bacteria community, AC addition was associated with significantly lower Shannon Diversity Index values compared to the control plot samples. Total C did decrease with AC addition, but only at the one-year mark, and not at the six-month mark, even though total C generally decreased with time. Thus, among the aspects of the restoration that we examined, a likely explanation for the improvement in *Dodonaea viscosa* growth is the impact to the soil microbial community, specifically in terms of carbon preferences and degradation, and this may be related to the lagging C decrease in AC added plots. The addition of AC was also associated with decreased total N and increased soil bacteria preference for polymer sources, but these findings were also found in the combination AC and biochar treatment, which did not have the same increase in plant growth.

While activated carbon is quite expensive, it may be a worthwhile investment for small areas to disrupt soil legacy effects, and potentially create a “source” native ecosystem for soil

transplants or soil inoculum. The cost of AC could also be moderated by changing to a different source material, or switching from powdered or granular AC to pellet-based AC. Even if activated carbon use is unreasonable for many contexts, our findings suggest that further research could focus on suppression of the soil microbial community and disruption of soil compaction, to mitigate the effects of the *Megathyrsus maximus* soil legacy on native Hawaiian plants. If a fungicide is used, it may be worthwhile to include AC in protection pods around native seeds, to protect them from the fungicide in the soil (Madsen 2014), which may be more cost-effective than direct application of AC to the soil. If outplanting is being used, perhaps dipping, or coating the roots or belowground growth of the outplant in AC prior to installation in the soil could achieve the same effect at a lower cost than using an amendment. One more cost-effective approach would be to integrate raking alone into restoration practices, as it seems to benefit the growth of *Plumbago zeylanica,* a native plant often used in restoration projects throughout Hawai'i. We found that raking was associated with an improvement in the width of *Plumbago zeylanica,* by 24%. The raking treatment shifted the fungi community, increased total N but not N cycling, and mechanically disturbed soil compaction, all of which alone or in combination could have mitigated soil legacy effects on *Plumbago zeylanica.*

While chapters three through six focus heavily on *Megathyrsus maximus* in Hawai'i, our findings have broader relevance because of the wide reach of this pasture grass, and of pasture grasses in general. Since increasing nutrient availability generally benefits invasive plants (Daehler, 2003), we must consider what alterations to restoration practices can more reliably be used to benefit natives over invasive grasses. Here, we show that practitioners ought to consider the suppression and/or alteration of the soil microbial community as an area worth additional consideration. Further research into soil microbial community focused restoration practices has the potential to change the trajectory of conservation of native ecosystems and native plants, not only in Hawai'i, but worldwide. Restoration is not only a matter of preserving important native species that are the fabric of critical and complicated ecosystems. Restoration contributes to the continuation of people and culture, and ultimately, I am grateful to have had the wonderful opportunity to contribute to restoration efforts in Hawai'i.

References

Backstrom AC, Garrard GE, Hobbs RJ, Bekessy SA. 2018. Grappling with the social dimensions of novel ecosystems. *Frontiers in Ecology and the Environment* 16:109–117. DOI: 10.1002/fee.1769.

Cheng F, Cheng Z. 2015. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science* 6:1–16. DOI: 10.3389/fpls.2015.01020.

Daehler CC. 2003. Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics* 34:183–211. DOI: 10.1146/annurev.ecolsys.34.011802.132403.

Eaton WD, Mcgee KM, Donnelly R, Lemenze A, Karas O, Hajibabaei M. 2019. Differences in the soil microbial community and carbon-use efficiency following development of *Vochysia guatemalensis* tree plantations in unproductive pastures in Costa Rica. *Restoration Ecology* 27:1263–1273. DOI: 10.1111/rec.12978.

Frey M, Schullehner K, Dick R, Fiesselmann A, Gierl A. 2009. Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* 70:1645–1651. DOI: 10.1016/j.phytochem.2009.05.012.

Hu L, Robert CAM, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, Van Der Heijden MGA, Schlaeppi K, Erb M. 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* 9:1–13. DOI: 10.1038/s41467-018-05122-7.

Miller JR, Bestelmeyer BT. 2016. What's wrong with novel ecosystems, really? *Restoration Ecology* 24:577–582. DOI: 10.1111/rec.12378.

Morse NB, Pellissier PA, Cianciola EN, Brereton RL, Sullivan MM, Shonka NK, Wheeler TB, McDowell WH. 2014. Novel ecosystems in the Anthropocene: A revision of the novel ecosystem concept for pragmatic applications. *Ecology and Society* 19:1–10. DOI: 10.5751/ES-06192-190212.

Niculaes C, Abramov A, Hannemann L, Frey M. 2018. Plant Protection by Benzoxazinoids—Recent Insights into Biosynthesis and Function. *Agronomy* 8:1–24. DOI: 10.3390/agronomy8080143.