RESPONSES OF SOIL INVERTEBRATE AND BACTERIAL COMMUNITIES TO THE

REMOVAL OF NONNATIVE FERAL PIGS (*SUS SCROFA*) FROM A HAWAIIAN

TROPICAL MONTANE WET FOREST

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ABSTRACT

Feral pigs (*Sus scrofa*) are perhaps the most abundant, widespread, and economically significant large, introduced vertebrate across the Pacific Island region. This species has played a role in the degradation of native ecosystems and the extinction of multiple species of plants and animals on Pacific islands and has negative effects on both the ecotourism and agricultural industries. Despite numerous published studies on feral pigs in the Pacific Island region, some fundamental aspects of feral pig ecology remain poorly characterized, particularly belowground. To address these knowledge gaps, this thesis analyzed relationships between soil macroinvertebrates and microbes and feral pigs using nine sites located inside and outside of feral pig removal units representing a ~25-year environmentally-constrained chronosequence of removal in tropical montane wet forests in Hawai'i. The results of these studies indicate that areas with active trampling by feral pigs correlate with lower abundance, biomass, and species richness of all soil macroinvertebrates. Comparatively, active rooting correlated with higher abundance and biomass of nonnative earthworms (*Lumbricidae* and *Megascolicidae*) and ground beetles (*Carabidae*). Further, my results indicate an overall increase in the net biodiversity of soil bacterial communities following feral pig removal, with biodiversity positively correlating to time since removal. Comparatively, environmental characteristics, including mean annual temperature and elevation, are better predictors of differences in functional and phylogenetic biodiversity among soil bacterial communities than feral pig removal. Collectively, these results indicate that the removal of feral pigs largely does not affect soil macroinvertebrates but does increase the diversity of bacterial communities, which could increase ecosystem stability.

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CHAPTER 1

Introduction

Feral pigs (*Sus scrofa*) are recognized globally as ecosystem engineers capable of dramatically altering the habitats they invade. Their ability to transform ecosystems has earned them renown as one of the most widespread and destructive invasive species on the planet (Barrios-Garcia and Ballari 2012; Campbell and Long 2009; Hess 2016). The broad diaspora of *S. scrofa* can primarily be attributed to their generalist diet, ability to live in variable climates (Campbell and Long 2009), and their historic utility to humans (Frantz et al. 2016). The effects of feral pigs have been studied worldwide and vary from damage to freshwater turtle habitats (Doupé et al. 2009) and sugarcane fields in Australia (Wurster et al. 2012) to alterations of soil properties in Tennessee (Singer et al. 1984) to the consumption of garbage in the Middle East (Hafeez et al. 2011).

In the Pacific Island region, feral pigs are arguably the most abundant, widespread, and economically significant large, introduced vertebrate. Unlike many other non-native, invasive species, feral pigs have both cultural and recreational importance in the region, complicating their management (Ikagawa 2013). Today, Pacific Island feral pigs are a mixture of several strains of domestic swine, Asiatic wild boar, and European wild boar (Linderholm et al. 2016). Due to their generalist diet and rooting behavior, feral pigs alter soils and watersheds and negatively affect native and non-native ecosystems (Atkinson and LaPointe 2009; Cole and Litton 2014; Long et al. 2017). As a result, feral pigs have played a role in the extinction of multiple species of plants and animals on Pacific islands and have had negative effects on both ecotourism and agricultural industries (Peh et al. 2015).

Despite numerous published studies on feral pigs in the Pacific Island region, of which the majority include systematic analyses of original empirical data, some fundamental aspects of feral pig ecology remain poorly characterized. These knowledge gaps can be attributed, at least in part, to the remote and inaccessible environments that feral pigs often inhabit. To address this knowledge gap, I used a previously constructed chronosequence on the Island of Hawaiʻi as a model system for describing the effects of feral pigs on tropical montane wet forests, with a focus on soil macroinvertebrates and bacterial communities. Hawaiʻi represents an ideal system for studying feral pig effects because of the existence of fenced, feral pig removal units of various ages across a landscape with similar climate, soils, and disturbance history. Specifically, the high level of control across exclusion sites of various ages allows for well-controlled studies with global applicability (Cole and Litton 2014; Cole et al. 2012; Loh and Tunison 1999; Long et al. 2017).

This thesis consists of five chapters with relevance throughout the Pacific Island Region and globally. Chapter 2 is a literature review summarizing the origins, history, ecology, environmental effects, and current management of feral pigs in the Pacific Island Region. This review integrates regional scientific findings with other insular and continental systems and identifies current knowledge gaps requiring further research to inform the ecology and management of this widespread invasive species. In Chapters 3 and 4, I present two empirical studies focused on the long-term effects of feral pig removal on soil macroinvertebrate and bacterial communities, addressing the lack of available data on these ecological relationships. These two studies rely on an established ~25 year-old chronosequence of feral pig removal management units on the Island of Hawaiʻi (Cole and Litton 2014). Chapter 5 summarizes and integrates the results of the prior chapters.

Thesis Overview

This thesis contains three primary chapters (2, 3 and 4) examining the current knowledge gaps in the ecology and management of feral pigs in the Pacific Island Region. Chapter 2 is a review of the present knowledge of feral pigs throughout the Pacific. Being a literature review, the goal of this chapter was to summarize information currently available on feral pigs to provide land managers and researchers with a concise collection of resources on feral pigs' origin, history, ecology, environmental effects, and management in the Pacific region. Further, this chapter identifies knowledge gaps helping to guide future research in the field.

Chapter 3 of my thesis addresses the effects of feral pigs on soil macroinvertebrate communities. Previously, alterations to the earthworm and arthropod communities by feral pigs have been studied in the context of seasonal changes in Australia (Taylor et al. 2011). My study, however, is the first to examine how the long-term removal of feral pigs effects soil macroinvertebrate communities. This information provides important insights into the ecological relationships between feral pigs and soil macroinvertebrates, including earthworms, that are collectively known to play important roles in the environment (Barrios-Garcia and Ballari 2012; Dechaine et al. 2005; Lavelle et al. 2006; Lincoln 2014; Taylor et al. 2011).

Chapter 4 of my thesis examines the effects of feral pig removal on the soil bacterial community. Overall, very little is presently known regarding the effects of feral pigs on soil microbial communities (Barrios-Garcia and Ballari 2012). As such, this study provides novel insight into an important knowledge gap due to the critical role of soil microbes in moderating ecosystem processes and overall ecosystem stability (Barrios 2007; Browning et al. 2008; Kaller and Kelso 2006; Naeem et al. 2009).

CHAPTER 2

Biology and Impacts of Pacific Island Invasive Species: *Sus scrofa***, the Feral Pig**

The second chapter of my thesis is a review paper for the regional scientific journal

Pacific Science. This chapter has been published, and the citation for this publication is below.

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Abstract

Feral pigs (*Sus scrofa*) are perhaps the most abundant, widespread, and economically

significant large, introduced vertebrate across the Pacific Island region. Unlike many other non-

native, invasive species, feral pigs have both cultural and recreational importance in the region,

complicating their management. Today, Pacific Island feral pigs are a mixture of several strains

of domestic swine, Asiatic wild boar, and European wild boar. Due to their generalist diet and

rooting behavior, feral pigs alter soils and watersheds and negatively affect native and non-native

flora and fauna. As a result, feral pigs have played a role in the extinction of several species of

plants and animals on Pacific islands and have negative effects on both ecotourism and

agricultural industries in the region. Despite numerous published studies on feral pigs in the

Pacific Island region, of which the majority include systematic analyses of original empirical

data, some fundamental aspects of feral pig ecology remain poorly characterized, at least partly

due to the remote and inaccessible environments that they often inhabit. To address these

knowledge gaps, effort should be made to integrate research conducted outside the Pacific Island

region into local management strategies. This review summarizes the origins, history, ecology, environmental effects, and current management of feral pigs in the Pacific Island region, integrates regional scientific findings with other insular and continental systems, and identifies current knowledge gaps requiring further research to inform the ecology and management of this invasive species.

Introduction

Anderson and Stone (1993) described feral pigs (*Sus scrofa*) as "… the most pervasive and disruptive alien influence on the unique native forests of the Hawaiian islands." This quote is representative of the wide-ranging effects feral pigs have globally, where they inhabit every continent except for Antarctica (Barrios-Garcia and Ballari 2012) and represent an invasive species in almost every part of their range. The widespread habitat degradation caused by feral pigs is unparalleled in the Pacific, and for more than thirty years management of feral pigs has been limited only by the availability of funding and policies reflecting public sentiment, including advocacy by both hunters and cultural practitioners. Management projects have primarily focused on fencing and removal in conservation areas throughout the region to minimize habitat degradation associated with feral pigs (Tomich 1986).

Feral pigs are unique because undomesticated populations of *S. scrofa* still existed in the wild when domesticated pigs first became feral (Diong 1982). Additionally, *S. scrofa* interbreeds with other members of the *Sus* genus, furthering the distinctive nature of this species. Overall, *S. scrofa*'s existence as wild, feral, domestic, and interbred forms has caused a great deal of confusion regarding their taxonomy (Diong 1982; Moutou and Pastoret 2010). Recently, the implementation of genetic tests to differentiate between wild, feral, and domestic lineages has

allowed researchers to track historical introductions of individual populations (Aravena et al. 2015; Linderholm et al. 2016), and further implementation of this strategy in the Pacific represents an opportunity to fill important knowledge gaps clarifying feral pig introductions and their taxonomic status.

Name

Sus scrofa (Linnaeus, 1758).

Common names: feral pig, hog, or swine; Old World swine; razorback; Eurasian or Russian wild boar, hog or pig.

It is important to clarify that the term "feral pig" is representative of those populations descended from historically domesticated populations. In Europe and Asia, undomesticated lineages of wild boar still exist. However, suides throughout the Pacific should generally be recognized as feral because nearly all pig populations in the Pacific are genetic hybrids of domestic, feral, and, on some islands, wild populations which may never have been domesticated (Diong 1982). This distinction often causes confusion due to the interchanging use of common names for feral pigs (Figure 2.1).

Figure 2.1. Lactating feral pig, *Sus scrofa*, on Hawaiʻi Island (Photo: U.S. Geological Survey).

Life History Traits

Feral pigs are considered old world swine, but they have been widely distributed in the new world due to domestication (Diong 1982; Graves 1984). As newborns, piglets stand minutes after birth and begin forming dominance relationships with their siblings within hours (Graves 1984). Worldwide, juvenile feral pigs are generally all black, but additional varieties include a wild type with reddish striping. As adults, feral pigs have a sparse but highly coarse coat, which is usually all black (Graves 1984; Long 2003). However, occasional spotting and/or white feet occurs in adults. These coloration patterns are indicative of remnant genetic traits from prior domestication (Tomich 1986). Adult feral pigs have upward curving canine teeth, which form tusks in males (Giffin 1978; Graves 1984). On average, feral pigs weigh between 45-70kg in the Pacific Island region, but cases have been reported of females reaching weights of 90kg and males reaching up to 250kg (Giffin 1978).

Historically, domestic pigs were bred to maximize growth rate and reach maturity quickly (Lega et al. 2016), and female pigs can begin reproducing in their first year (Taylor et al. 1998). Once they become reproductively active, a Texas study showed feral pigs generally became pregnant every twenty-one months, with roughly 40% of all females becoming pregnant each year (Taylor et al. 1998). However, it is possible for two litters to be born per year under ideal conditions, as is frequently the case in the Pacific where feral pigs breed year-round (Diong 1982). A study of feral pigs in Hawai'i showed 24% of 327 females sampled were pregnant with a median number of seven embryos per pregnant sow (Hess et al. 2006), while a Costa Rican study showed that 46% of all females sampled were either pregnant or suckling (Sierra 2001). Socially, the reproductive habits of feral pigs include moderate levels of polygyny but almost no polyandry (Hampton et al. 2004a). Among feral pigs, older, heavier females became pregnant

more often than their younger, smaller counterparts, with these older, larger females producing larger litters (Fonseca et al. 2011; Taylor et al. 1998). Typically, litter sizes consist of five or six piglets and show a slight bias towards being male (Diong 1982; Fonseca et al. 2011; Taylor et al. 1998).

Feral pigs live in matrilineal groups known as "sounders." Sounders are composed of several generations of related females and their dependent offspring (Gabor et al. 1999; Kaminski et al. 2005). These sounders, however, are generally confined to a single family unit and therefore not considered to be herds (Graves 1984). Many sounders have maximum sizes of 30-50 individuals, however, some rare cases of sounders as large as 400 pigs have been observed (Long 2003), and within larger sounders it is common for subgroups to form (Gabor et al. 1999). Generally, sounders are stable social units, with most female offspring remaining with the sounder after weaning (Boitani et al. 1994; Kaminski et al. 2005). Sounders can have territorially exclusive, non-overlapping core home ranges (Sparklin et al. 2009). However, feral pigs do not defend their home ranges from other sounders, and individuals within sounders often have non-exclusive, overlapping home ranges (Graves 1984; Sparklin et al. 2009). It is therefore likely that movement between sounders facilitates gene flow (Hampton et al. 2004b).

Little information is available regarding the size of feral pig home ranges and distance of juvenile dispersal, especially on Pacific islands. A single study conducted in Hawai‘i estimated feral pig home ranges using four individuals. The resultant GPS telemetry data produced home ranges between 0.11 and 0.86 km^2 (Salbosa 2009). In New Zealand, home ranges were shown to be somewhat larger covering 0.3 to 2.1 km^2 (McIlroy 1989). In contrast, a study conducted in Texas calculated average home range for females much larger than on Pacific islands, ranging between 4.6 to 6.1 km^2 with core ranges of 2.7 to 3.1 km^2, with the smaller ranges on Pacific

islands likely due to the high concentration of food resources available there (Hampton et al. 2004b). In terms of juvenile dispersal, a Polish study suggested that, while some movement between sounders occurs, dispersal ranges are generally similar to home ranges indicating juveniles remain within their birth sounders (Podgorski et al. 2014).

Population sizes are difficult to estimate, but several techniques have been used including damage transects (Hess et al. 2006; Leopold et al. 2016), predictive modelling (Holland et al. 2009), and feces counts (Ferretti et al. 2016). Random encounter models that do not require specific individual recognition have the potential to more accurately estimate feral pig populations in the Pacific (Rowcliffe et al. 2008). However, these models are not currently used on Pacific islands due to the rugged and heavily vegetated terrain present on many islands. Other techniques being employed include: DNA analyses, aerial surveys, mark/recapture, and hunter surveys (Engeman et al. 2013). Despite difficulties in estimating population densities, an Australian study estimated average densities of 12 to 43 pigs per km^2 in areas without hunting compared to densities of 3 to 8 pigs per km^2 in nearby heavily hunted sites (McIlroy 1989). In Hawai'i, Scheffler et al. (2012) estimated population densities between 0.6 to 16.3 feral pigs per km^2. Variation in reported population densities of feral pigs is likely dependent on food availability, with reproductive habits often mirroring mast seeding/fruiting events (Graves 1984; Salinas et al. 2015).

Feral pigs are well-known for two primary behaviors: wallowing and rooting. Feral pigs can create large wallows many meters wide, where the primary use of wallows is to help remove parasites from the hide, heal cuts, and create a protective lathering of mud against pests (Campbell and Long 2009; Graves 1984). Some research has also suggested that wallowing may serve a role in obtaining mates because older, stronger males are seen wallowing more frequently

during reproductive periods (Fernandez-Llario 2005). Wallows are destructive, creating bare patches throughout the landscape. Similarly, feral pig rooting behaviors lead to habitat degradation. Rooting is one of the primary foraging strategies utilized by feral pigs and involves digging or scraping into the top layer of soil to obtain food, particularly earthworms and plant roots (Lincoln 2014). On average, the pits resulting from feral pig rooting are approximately 10 cm deep (Hancock et al. 2015), and in tropical wet forests an individual rooting pig can disturb up to 200 m^2 of soil each day (Anderson and Stone 1993).

In terms of their diet, feral pigs are opportunistic omnivores relying primarily on their sense of smell to locate forage, and numerous studies have highlight their wide-ranging diets. For example, a seven year study in Texas showed that around 40% of all feral pigs sampled had vertebrates, mostly carrion, in their stomach contents, which the authors attributed to the scarcity of easily obtained protein in the summer and fall (Wilcox and Van Vuren 2009). By comparison, a New Zealand population was shown to obtain up to 27% of its diet from underground invertebrate species alone (Parkes et al. 2015). In Costa Rica, feral pig diets were primarily comprised of fruit regardless of the season (Sierra 2001), and Argentinian feral pigs had diets composed of 95% vegetation (Cuevas et al. 2010). A study in Pakistan showed *S. scrofa* obtained 58% of their diets from garbage (Hafeez et al. 2011), a true testament to their omnivorous habits. In Hawai'i, Diong (1982) showed the three most common food sources by volume for feral pigs were tree ferns (*Cibotium* spp.), strawberry guava fruit (*Psidium cattleianum*), and earthworms. Due to the opportunistic feeding habits of feral pigs, agricultural crops can also represent a large portion of their diets. Examples include feral pigs that obtained 25% of their diets from sugarcane (Wurster et al. 2012), and a separate study showing a third of all feral pigs near sugar beet fields relied on beets for >50% of their diet (Zeman et al. 2016) (Figure 2.2).

Figure 2.2. Top panel: a feral pig wallow on the island of Hawaii; bottom panel: the difference between feral pig exclusion (left) and feral pig presence (right).

History of Introductions

Globally, feral pigs inhabit every continent excluding Antarctica (Barrios-Garcia and Ballari 2012; Long 2003). Even within the native range of undomesticated wild boar, numerous releases of feral pigs have led to interbreeding between the two groups. The domestication of *S. scrofa* originally occurred in Europe to utilize wild boar for agricultural husbandry (Moutou and Pastoret 2010). As such, Europe became one of the first places to experience the effects of feral pigs after domesticated *S. scrofa* returned to the wild (Wilson 2004). With European trade expansion, domesticated pigs began to spread across the world and become a prominent food source for expanding human populations. It is important to note that, before the advent of contemporary methods using genetic techniques and mitochondrial DNA, the phylogeography of Suidae was based solely on comparative morphology of skeletal remains compared to modern specimens, cytogenetic determination, and utilization of other less informative characteristics such as the presence of facial warts. Conclusive phylogenetic work has only recently become available with the use of genetic tests to differentiate between wild, feral, and domestic lineages and track historical introductions of individual populations (Aravena et al. 2015; Linderholm et al. 2016).

The history of feral pigs on Pacific islands originates in Island Southeast Asia with the oceanic voyages of the Neolithic Lapita culture, ancestors of the Polynesians (Larson et al. 2005). All five *Sus* species endemic to Island Southeast Asia (*S. barbutus*, *S. celebensis*, *S. philippensis*, *S. scrofa*, and *S. verrucosus*) originated from the region including the Malay Peninsula, Sumatra, Java, Borneo, and the Philippines. Two separate human-mediated dispersals of domestic pigs from Island Southeast Asia into Oceania have been documented using mitochondrial DNA from ancient and modern pig specimens (Larson et al. 2007). A Pacific

clade of *S. scrofa* was dispersed from mainland Southeast Asia to Java, Sumatra, and the islands

of Wallacea and Oceania, with a separate dispersal occurring with the translocation of *S. scrofa*

from mainland East Asia to western Micronesia, Taiwan, and the Philippines. The first

appearance of *S. scrofa* in Wallacea is closely associated with the arrival of the Lapita culture

between 5000-1500 B.C.

Evidence suggests domesticated *S. scrofa* were not brought to the Hawaiian Islands by

Polynesian voyagers until between AD 1000 and 1200 (Dye and Pantaleo 2010; Kirch 2011).

This evidence arose from recent radiocarbon dating from an archeological site at Bellows, Oahu

(Dye and Pantaleo 2010), corroborated by data suggesting Pololu Valley on Hawaii Island was

first settled by Polynesians between 1200 and 1300 AD (Field and Graves 2008). Additionally,

Burney et al. (2001) documented pig bones from prehistoric habitation near a sinkhole and cave

system on Kaua'i dating from 1430-1665 A.D., well before the arrival of Europeans. Pigs did

not, however, reach Australia and New Zealand until the age of European exploration through

Polynesia in the late 1700s, which brought European *S. scrofa* to numerous islands throughout

the Pacific (Clarke and Dzieciolowski 1991; Gongora et al. 2004).

Both skeletal remains of pigs and early historic observers indicate *pua'a* (the Polynesian

lineage of *S. scrofa*) was smaller than contemporary feral pigs in Hawai'i, weighing only 27-45

kg (Ziegler 2002). It was historically related that, not long before European contact, well over a

thousand animals would be consumed at the consecration of important *heiau* (temples).

Following European contact, Captain James Cook's voyage was provisioned with ~600 pigs

during a four month stay in Hawai'i in 1778-1779. Later, Captain Cook noted that, when trading

for pigs, "We could seldom get any above fifty or sixty pounds weight" (Diong 1982). In 1823,

Missionary William Ellis observed that natives possessed, "a small species of hogs, with long

heads and small erect ears (Ellis 1827)," which further confirms a Polynesian origin of pigs in Hawai'i. This information regarding the historic ranges of feral pigs provides reason to believe *pua'a* brought by the original Hawaiian settlers differed substantially from the feral pigs found in Hawai'i today.

Originally, *pua'a* held close relationships with humans and are believed to have stayed close to the *kauhale* (family compound) where taro and sweet potato agriculture was well-developed (Maly et al. 1998). Ellis (1827) described the relationship of Hawaiians with *pua'a*: "In the pre-European contact era, Polynesian man-pig interaction was essentially a loose one… pigs were never contained by any method. They were never confined in sites, but range about in search of food." Cook observed that, "pigs were abundant, formed an important part of the natives' culture, and were sometimes found wild in the mountains" (Diong 1982). Ellis (1827) further noted, "The pigs, herded with dogs, acted as scavengers and were left unattended to roam freely and without restraint." This traditional and historic evidence indicates *pua'a* remained largely domesticated, living on the periphery of *kauhale* and extending no further than nearby lowland forests (Ziegler 2002). They relied largely on the food and shelter provided by the *kauhale* because, in pre-contact times, native Hawaiian forests were devoid of large non-native fruits such as mangos (*Mangifera indica*) and strawberry guava (*Psidium cattleianum*), as well as major sources of protein that would eventually support the large populations of feral pigs today (e.g., nonnative earthworms). Without such fodder, early roaming populations would have been chiefly dependent on people for food and survival. Therefore, it is highly likely that small populations of loosely controlled and free-roaming animals existed historically (Ziegler 2002).

Domesticated *pua'a* carried strong cultural value in traditional Polynesian cultures. Aside from being an important possession and food source, oral tradition describes the adventures of

Kamapuaʻa (the pig god) in Hawaiʻi, a powerful demi-god who ranged over the islands and into

the sea (Charlot 1987). Even the name of the traditional land management system in Hawaiʻi

(*ahupuaʻa*) refers directly to the *puaʻa*, highlighting their importance amongst the resources

collected and given as offerings during annual *makahiki* (tributes). However, *puaʻa* were not

hunted as game by ancient Polynesians. Instead, the Polynesians' interaction with *puaʻa* was one

of near-complete domestication (Ziegler 2002). Despite references to hunting rats with bow and

arrows, no historic or traditional knowledge sources describe ancient Hawaiians hunting pigs for

food or recreation. Even in the legend of *Kamapuaʻa*, where the demi-god is pursued by man, he

is sought so that he might be punished for his mischievous actions, not for sport or subsistence

(Charlot 1987).

Today, a cultural shift has taken place where recreational hunters strongly support the

presence of feral pigs in the Hawaiian Islands for sport and subsistence, while conservation

biologists consider their removal as a prerequisite step for protecting native vegetation. There are

segments of present-day society who argue pigs and other ungulates serve a historically and

culturally important role and, as such, should be allowed to roam freely (Ikagawa 2013), often

citing unverifiable information to support this viewpoint. My goal is not to debase the cultural

and recreational importance of feral pigs in the Pacific Island region, but rather to summarize

existing information regarding their effects on the environment. First, conservation and

restoration projects on Pacific islands promoting native vegetation are severely hampered by the

presence of ungulates (Cole and Litton 2014; Hess 2016; Reeser and Harry 2005). Second, the

pigs inhabiting the islands alongside original Polynesian settlers were smaller and more easily

controlled, serving as localized food sources that were not explicitly hunted (Ellis 1827). Finally,

the influx of invasive plants, as well as interbreeding with larger European pigs following the

arrival of Captain Cook, has led to an expansion of negative effects generated by feral pigs on Pacific islands.

Inevitably, debate over these viewpoints arises at least in part regarding the genetic purity of feral pigs as entirely European *S. scrofa*. Evidence suggests Pacific Island feral pigs are derived from multiple lineages, and the number of chromosomes in crossbred pigs ranges from 36-38, depending on the combination of Asiatic, European, domestic, and hybridized backgrounds of individuals (Diong 1982; Giffin 1978). It is therefore important to clarify that feral pigs on Pacific islands should be recognized as feral *S. scrofa*. Recently, Linderholm et al. (2016) showed that ~70% of the genetic makeup of feral pigs tested in Hawaii was composed from *S. scrofa* of Polynesian ancestry. The hunting community used this information to argue that feral pigs should not be removed from Hawaiian islands. However, this study drew inference from the investigation of only one allele among a relatively small sample of *S. scrofa* (fifty-seven pigs sampled across four islands). I therefore conclude that more comprehensive genetic work needs to be conducted in order to draw conclusions regarding feral pig genetics in the Pacific region.

Geographic Distributions

In the Pacific, feral pigs inhabit nearly every island throughout the region (Table 2.1). Only the Cocos Islands (Woodroffe and Berry 1994), Easter Island (Allen et al. 2001; Giovas 2006), Pitcairn Island (Giovas 2006), and Wake Island (Griffiths et al. 2014) were recorded as being devoid of feral pigs. However, it is important to note that these four islands likely had pigs introduced at some point, but due to a general lack of resources and distance from other land masses, feral pigs likely never established there (Allen et al. 2001). Further, I assume that feral

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pigs are not present on Cocos and Wake Islands because they were not listed among other mammals described in literature from those respective locations (Griffiths et al. 2014; Woodroffe and Berry 1994).

Globally, feral pigs are one of the most widespread invasive species on the planet. This diaspora is primarily due to their generalist diet and ability to live in variable climates (Campbell and Long 2009), as well as their historic utility to humans. Generally, feral pigs show an inability to withstand harsh winters experienced in polar climates or hot summers of desert regions. However, a poleward shift in continental populations is occurring, likely due to current climate change, and microclimate exceptions do exist (McClure et al. 2015).

Table 2.1. Presence of feral pigs on select Pacific Islands

Pacific Islands	Present	Absent	Notes
American Samoa	X[a]		
Australia	X[a,b]		
Bonin Islands	X[c]		
Cocos Islands		X[d]	
Easter Island		X[e]	
Fiji	X[a,b]		
French Polynesia	X[a,b]		
Galapagos Islands	X[a,b]		
Guam	X[a,b]		
Hawaiian Islands	X[a,b]		
Indonesia	X[a,b]		
Japan	X[a,b]		
Juan Fernandez	X[a,b]		
Kiribati	X[a]		
Marshall Islands	X[a]		
Micronesia	X[a]		
Nauru	X[a]		
New Caledonia	X[a,b]		
New Zealand	X[a,b]		
Niue	X[a]		
Norfolk Island	X[b]		
Northern Mariana Islands	X[a,b]		
Palau	X[a,b]		
Papua New Guinea	X[a,b]		
Philippines	X[f]		
Pitcairn Island		X[e]	
Solomon Islands	X[a,b]		
Taiwan	X[a]		
Tokelau Island	X[b]		loosely-domestic[b]
Tonga	X[a,b]		
Tuvalu	X[g]		loosely-domestic[g]
Vanuatu	X[b]		
Wake Island		X[h]	
Wallis and Futuna	X[a]		

[a](Barrios-Garcia and Ballari 2012), [b](Long 2003), [c](Okochi and Kawakami 2010), [d](New World Encyclopedia Contributors 2017; Woodroffe and Berry 1994), [e](Allen et al. 2001; Giovas 2006), [f](Oliver 2014), [g](McKinnon 2009), [h](Griffiths et al. 2014)

Degradation of Natural Environments

Feral pigs are well-known for their role as ecosystem engineers, and they generate alterations at multiple trophic levels across both localized and landscape spatial scales (Barrios-Garcia and Ballari 2012; Hess 2016). While few studies have accurately determined pig abundance beyond presence or absence, their environmental alterations have been demonstrated throughout the Pacific, largely via comparisons of habitats inside and outside of fenced feral pig removal units (Cole and Litton 2014; Cole et al. 2012).

Effects on Fauna

While it is possible to extrapolate effects from the degradation of natural environments and ecosystem-level effects, there are few studies in the Pacific examining the direct effects of feral pigs on other fauna. However, one noteworthy direct effect is egg predation and the destruction of important nesting sites used by Pacific Island ground-nesting avifauna (Challies 1975). Additionally, one indirect effect widely reported in Hawaiʻi is the spread of avian malaria with feral pig activity. Specifically, wallowing and rooting behaviors create pools of standing water that provide habitat for non-native mosquitoes carrying avian malaria, and a lack of resistance to avian malaria in native Hawaiian forest birds has resulted in significant population reductions and even extinctions of numerous endemic avifauna (Atkinson and LaPointe 2009). Climate change, particularly rising temperatures allowing mosquitoes to expand habitat into higher elevation, mosquito-free zones, poses an immediate extinction threat for remaining forest bird populations in Hawaiʻi, especially on lower-elevation islands (Paxton et al. 2016).

Beyond avifauna, feral pigs affect many other animals. For example, compaction of soils has been shown to negatively affect arthropod communities (Vtorov 1993), but feral pigs have

also been shown to aid in dispersing aquatic invertebrates (Vanschoenwinkel et al. 2008).

Additional negative effects include: the destruction of turtle habitats in Australia stemming from

trampling in mudflats surrounding marshes (Doupé et al. 2009); outcompeting of native

peccaries in Texas (Galetti et al. 2015) and Brazil (Keuroghlian et al. 2009); and outcompeting

of fox populations in California (Melstrom 2014). Other direct effects on fauna and indirect

effects on freshwater and marine communities due to soil erosion and sedimentation are likely,

but largely undescribed.

Effects on Flora

The remote location of many Pacific islands prevented most terrestrial mammals from

colonizing these habitats historically. Now, due to anthropogenic transport, these island

ecosystems face an inundation of non-native mammals such as feral pigs (Hess 2016), which are

typically highly destructive to native vegetation (Aplet et al. 1991). Prior to the introduction of

mammals to Hawai'i, for example, terrestrial herbivores were comprised of snails, insects, and

flightless birds, with only two species of bats representing native terrestrial mammals (Ziegler

2002). Defense against herbivory arose in at least ten native Hawaiian plant species (Givnish et

al. 1994; Olson and James 1982). However, due to the absence of terrestrial mammalian

herbivores, especially ungulates, many Hawaiian plant species lost evolved defenses to

mammalian herbivory, such as woody thorns, stinging nettles, and alkaloids (Bowen and Van

Vuren 1997; Carlquist 1970). As such, direct herbivory can cause significant damage to Pacific

Island plant communities. Feral pigs are known to consume the roots, buds, flowers, and fruits of

plants (Cole and Litton 2014; Diong 1982; Katahira et al. 1993; Pratt et al. 1999; Ralph and

Maxwell 1984; Tomich 1986), and up to 95% of feral pigs' diets seasonally can come directly

from vegetation (Cuevas et al. 2010; Diong 1982). Feral pigs are also well-established seed

predators, limiting the recruitment of some plant species, particularly in continental mast-seeding

environments (Lott et al. 1995; Sanguinetti and Kitzberger 2010; Sweitzer and Van Vuren 2008).

In turn, feral pigs readily disburse many non-native seeds, particularly those found in fleshy

fruits (Diong 1982).

Feral pigs damage plants via rooting, in addition to direct herbivory. Rooting behaviors

cause extensive damage to both seedlings and adult plants (Campbell and Long 2009; Cole and

Litton 2014; Cole et al. 2012; Diong 1982; Murphy et al. 2014). The effects of feral pigs on

seedlings is exemplified by an experiment analyzing the role of small-scale physical disturbance

in seedling mortality in a Hawaiian rain forest. After one year, the percentage of seedlings

damaged was significantly greater among terrestrial seedlings versus epiphytic seedlings in the

presence of feral pigs, and significantly more terrestrial seedlings were damaged with feral pigs

present (Drake and Pratt 2001). Direct damage to plants can also result from nest-building (Ickes

et al. 2005), trampling, wallowing, and rubbing on trees and other woody plants to remove skin

parasites (Murphy et al. 2014).

One of the most notable effects of feral pig damage to native plant communities is the

potential for native plants to be replaced by non-native, invasive plants. An examination of the

association between feral pig disturbance and the composition of non-native plant assemblages in

Hawai'i Volcanoes National Park suggested a strong relationship between feral pig activity and

non-native plant presence (Aplet et al. 1991). For example, many mesic and wet forests in

Hawai'i are being heavily invaded by the highly invasive strawberry guava (*Psidium

cattleianum*), which is thought to have expanded dramatically due to feral pig activity (Aplet et

al. 1991; Diong 1982). Feral pigs eat strawberry guava fruits and then release the seeds via their

feces, providing excellent conditions for further recruitment and dispersal (Diong 1982; Huenneke and Vitousek 1990). Other invasive plants known to be spread by feral pigs in the Pacific region include banana poka (*Passiflora tarminiana*) (Beavon and Kelly 2015), mesquite (*Prosopis pallida*) (Lynes and Campbell 2000), and freshwater pond apple (*Annona glabra*) (Setter et al. 2002), all of which show high levels of germination or recruitment following feral pig-mediated dispersal. The replacement of native plants with invasive plants due to feral pig activity results in a positive feedback loop generating progressively more opportunities for invasive plants to establish which may, in turn, alter wildfire regimes (Trauernicht et al. 2015).

Following the removal of feral pigs via exclusion fencing, plant species recover quickly where they still exist at the time of removal (Spear and Chown 2009). Using a chronosequence of feral pig removal, Cole and Litton (2014) found that common understory native vegetation recovered within 6.5 years of feral pig removal from Hawaiian tropical montane wet forests. Similarly, Weller et al. (2011) showed improvement among at least some native plants following ungulate removal from Hawaiian mesic forests, and Busby et al. (2010) showed improved native seedling survival inside ungulate exclosures in Hawaiian wet forests. Another study in wet forest habitat in Hawai'i Volcanoes National Park documented that feral pig removal increased native understory cover by 48% in seven years, with most recovery occurring in the first two years following feral pig removal (Loh and Tunison 1999). A reexamination of these plots sixteen years after feral pig removal documented extended recovery of commonly occurring native understory woody plants (Cole et al. 2012). Importantly, these studies documented that non-native plants also benefited from feral pig removal, with as much as a 190% increase in non-native understory vegetation cover following removal (Cole et al. 2012; Loh and Tunison 1999). The important takeaway from these studies is that once feral pigs are removed, all plants that are

present tend to benefit as a result of release from top-down control, regardless of whether the plants are native.

Effects on Soils

Broadly speaking, the effects of feral pigs on the physical, chemical, and biological properties of soils are much less well understood than their aboveground effects (Long et al. 2017; Spear and Chown 2009). Feral pigs primarily affect soils through trampling, wallowing, and rooting, as well as the deposition of feces and urine and aboveground effects on plant communities. The presence of feral pigs can alter soil structure via reduced aggregate stability and increased compaction (Beever et al. 2006), which can in turn affect arthropod communities (Vtorov 1993). Further, the degradation of understory plant communities can lead to long-term effects on soils given the close linkages between above- and belowground biota (Wardle et al. 2004). Wallowing, much like trampling, leads to increased soil bulk density (Bueno et al. 2013), and can also affect soil structure by creating depressions that allow water to pool.

Feral pigs have been shown to alter soils differently in various habitats. For example, in Tennessee's deciduous forests, soil bulk density was decreased by feral pigs (Singer et al. 1984), while soil bulk density increased with feral pig presence in Spanish alpine habitats (Bueno et al. 2013). Removal of feral pigs from Hawaiian wet forests resulted in decreased soil bulk density within 6.5 years (Long et al. 2017). Physical alteration of soil organic matter via fragmentation during feral pig foraging was also recorded in some (Hobbs 1996; Siemann et al. 2009; Wirthner et al. 2012), but not all cases (Bruinderink and Hazebroek 1996). Feral pigs variably affect soil nutrient cycling and availability as well, with several studies documenting increased soil nitrogen with feral pig activity (Bueno et al. 2013; Siemann et al. 2009; Wirthner et al. 2012), while

another observed increased cycling and availability of soil nitrogen with feral pig removal (Long et al. 2017). Variability in the effects of feral pigs on soil properties may result from methodological differences across disparate studies (Davidson and Hewitt 2014; Long et al. 2017).

Rooting is a common foraging activity used by feral pigs to support specific dietary needs. As a result, during foraging activities individual feral pigs can generate pits roughly 10 cm deep over up to 200 m^2 of soil daily (Anderson and Stone 1993; Hancock et al. 2015). Feral pigs prefer sandy soils for rooting over those composed of clay or rock because of the ease of access (Elledge et al. 2013). Further, it has been documented that feral pigs exhibit a strong propensity to root in places where the soil has been previously disturbed (Elledge et al. 2013; Hancock et al. 2015; Krull et al. 2016). The return of feral pigs to previously disturbed sites is important because it indicates a positive feedback loop leading to continued soil degradation. A potential facilitative relationship between feral pigs and earthworms may explain why feral pigs preferentially return to sites with disturbed soils. Supporting this concept, studies in Hawai'i and Australia correlated worm abundance and feral pig activity (Lincoln 2014; Taylor et al. 2011), and in Spain rooting disturbance was shown to directly increase the abundance and diversity of earthworm species (Bueno and Jiménez 2014). However, a Slovenian study suggested the increased presence of earthworms did not lead to increased damage by feral pigs due to their highly opportunistic foraging strategy (Laznik and Trdan 2014). It is important to further understand the ecological implications of potential interactions between feral pigs and earthworms, particularly in the Pacific region where earthworms are largely non-native.

Associated with soil disturbance, feral pigs are known to generate numerous negative effects on watersheds. These effects include increased soil erosion and runoff, thereby decreasing

water quality at multiple scales (Browning et al. 2008; Cuevas et al. 2010; Doupé et al. 2010; Strauch et al. 2016). For example, feral pig associated runoff results in elevated nitrogen levels (Cuevas et al. 2010; Strauch et al. 2016) and increased acidity in nearby water, which can occur over extended periods of time (Doupé et al. 2009). Additionally, increased downstream sedimentation rates have been traced to feral pig activity (Dunkell et al. 2011a), as have alterations of wetland plant communities (Arrington et al. 1999; Setter et al. 2002). Feral pig activity has also been associated with the presence of fecal matter in watersheds (Strauch et al. 2016), increased levels of enterococci (Bovino-Agostini et al. 2012; Browning et al. 2008; Dunkell et al. 2011b), and the presence of leptospira (Browning et al. 2008).

Economic Importance

Due to the longstanding importance of feral pigs as a cultural icon in the Pacific, putting a direct price on their value is difficult. Culturally, feral pigs serve an important role in the folklore and traditions of contemporary native peoples (Charlot 1987). Further, the species today is important to hunters throughout the Pacific who view it as a primary target for recreation and subsistence. Based on current harvest numbers for Hawaiʻi, however, access to feral pigs for cultural and recreational purposes could be accomplished with much smaller areas containing free-ranging feral pigs than currently exist (Hess and Jacobi 2014). When considering land designation, the needs of cultural and recreational communities requiring access to feral pig populations must be balanced with conservation efforts resulting from the negative effects feral pigs have on native ecosystems and the suite of goods and services that they provide.

Feral pigs can also cause significant declines in agricultural production, and transmit diseases to livestock and humans. The most straightforward aspect of direct effects on agriculture

is crop herbivory; feral pigs may consume excessive amounts of crops, thereby reducing farm production, as demonstrated in Australia (Gentle et al. 2015; Wurster et al. 2012). Feral pigs can also transmit diseases to livestock (Machackova et al. 2003), with diseases of primary concern including African swine fever, classical swine fever, Aujeszky's disease (Gortázar et al. 2007), and bovine tuberculosis (Essey et al. 1983; Machackova et al. 2003; Naranjo et al. 2007). Additionally, disease transmission to humans is another important risk to consider with feral pigs, especially for hunters. Previously, feral pigs have been associated with the increased risk of contracting enterococcus in Hawai'i (Dunkell et al. 2011b), Japanese encephalitis virus and brucellosis in Japan (Nidaira et al. 2007; Watarai et al. 2006), hepatitis E virus in Japan and Australia (Meng et al. 2009), trichinella in Papua New Guinea (Owen et al. 2005), and swine influenza virus (Meng et al. 2009).

A case study on the island of Montserrat in the Caribbean revealed it was five times more economical to manage than to ignore feral pigs because the high level of damage caused to the environment decreased profits in the ecotourism industry (Peh et al. 2015). This is important in the Pacific Island region because so many islands are economically dependent on ecotourism.

Management Strategies

The removal of feral pigs and other invasive ungulate species is a well-documented and important step toward effectively improving the habitat of ecosystems damaged by non-native ungulates (Courchamp et al. 2003). As such, a great deal of time, money, and effort is put into the management of feral pigs each year. Management efforts may use either lethal or non-lethal techniques depending on site-specific goals and resource availability. Overall, the most cost-effective means of managing ecosystems affected by feral pigs is to address problems directly

using an adaptive framework in which researchers test a variety of techniques to determine the most effective way to eliminate feral pigs (Firn et al. 2015; Keiter and Beasley 2017). Recently, technological advances have greatly improved the ability to remotely monitor feral pigs, thereby allowing for more studies of the effects of various management activities (Engeman et al. 2013).

When lethal removal of feral pigs is selected as the best management option, typically where complete eradication is the management goal, the most frequently used methods include hunting and toxicants (Campbell and Long 2009; Keiter and Beasley 2017). The types of hunting involve snares, sharpshooters, and, frequently in the Pacific, dogs. The challenge with hunting techniques is that, as the number of feral pigs dwindles, the return-on-investment for time spent hunting becomes progressively lower, particularly at population levels <1 pig/km^2 (Anderson and Stone 1993; Barron et al. 2011; Krull et al. 2016). Aerial culling using sharpshooters has shown some success in removing the last remaining pigs from an area (Parkes et al. 2010). However, this method has little utility in densely forested areas where feral pigs are often found on Pacific islands. Strategies involving both infrared spotting of animals and the use of Judas pigs (released pigs equipped with tracking collars used to lead managers to existing populations) have also been implemented successfully (McCann and Garcelon 2008; McIlroy and Gifford 1997; Melstrom 2014). Comparatively, toxicants used to target invasive mammals in Australia have shown initial population decreases of up to 86% in localized areas (Campbell et al. 2012; Cowled et al. 2006; Cowled et al. 2007). Furthermore, the use of toxicants has been demonstrated as more cost-effective than hunting for feral pig population reduction (Coblentz and Baber 1987; West et al. 2009). As such, it is likely that future feral pig management in the Pacific, where complete removal is the objective, will increasingly rely on toxicants due to the large land areas invaded and difficulty and expense in removing feral pigs via traditional methods. Recently, the United

States Environmental Protection Agency approved the use of a single warfarin-based toxicant, but the product has yet to be approved for application by any state government.

While many lethal management programs for the removal of feral pigs have the ultimate goal of complete eradication of the species, this is not true of all programs. Weeks and Packard (2009) showed some success with the management of feral pig populations on private land with hunting in a continental system. Success in controlling feral pig populations via hunting has also been seen in Argentina (Gürtler et al. 2017). However, it is unlikely that this strategy would be feasible throughout most of the Pacific Island region due to low numbers of hunters and remote, rugged terrain. As such, eradication is the goal for most programs and is especially common on islands where the removal of populations can succeed without the fear of natural reintroduction via expansion (Hone and Stone 1989; Parkes et al. 2010).

The one non-lethal management tool that has shown great success in controlling feral pig populations is fencing, which is by far the most common form of non-lethal feral pig management and, perhaps, the most common form and highest expense of land management throughout the Pacific Island region. Woven wire fences have been repeatedly shown to be effective in limiting feral pig movement (Giffin 1978; Lavelle et al. 2011). These fences require a minimum height of 1.2 m, as feral pigs can jump over shorter fences (Giffin 1978; Lavelle et al. 2011). Other types of fencing, such as electric fences and mist nets were shown to be ineffective because feral pigs simply pushed through the mist nets and electrical shocks (Lavelle et al. 2011; Reidy et al. 2008). Once fences are constructed, feral pigs must be completely removed from inside of the exclosures. This is almost exclusively completed using the lethal techniques described above, particularly hunting. Other non-lethal management techniques for feral pigs have been attempted. However, the use of non-lethal baiting to concentrate feral pigs

away from protected areas has proved ineffective (Campbell et al. 2012). Additionally, in spite of suggestions that feral pigs' olfactory sense is overlooked in management strategies (Nogueira et al. 2007), the use of odor-based repellants showed no benefit in preventing crop destruction by feral pigs (Schlageter and Haag-Wackernagel 2012).

While no single method has emerged as a panacea for feral pig control, combinations of both lethal and non-lethal techniques have resulted in cases of successful feral pig eradication. For example, by fencing off large areas into smaller units, Hawaiʻi Volcanoes National Park was able to combine the use of snares and hunters with dogs to eradicate pigs from management units on a block-by-block basis (Hone and Stone 1989; Katahira et al. 1993). Another attempt to eradicate pigs from sites on the Hawaiian island of Maui involved using hunters accompanied by dogs to take broad sweeps through fenced areas, which successfully reduced pigs to zero or near zero numbers in all control units (Barron et al. 2011). Eradication from Santa Cruz Island, California was successful using a multi-step process, which started by fencing the island into five zones, trapping for 1,660 trap-nights, shooting feral pigs from helicopters, hunting with dogs, and finally using radio-collared, sterile Judas pigs to track down the final few individuals (Parkes et al. 2010).

Prognosis

Feral pigs have repeatedly been shown to cause a broad range of systematic changes to Pacific ecosystems due to a myriad of cascading direct and indirect effects (Nogueira-Filho et al. 2009) ranging from damage to soils and watersheds (Cuevas et al. 2010; Doupé et al. 2010) to both direct and indirect effects on endemic flora (Campbell and Long 2009; Cole and Litton 2014; Cole et al. 2012) and fauna (Atkinson and LaPointe 2009). To combat ecosystem

degradation from feral pigs, their removal has been established as an effective means for restoring and conserving native ecosystems (Hess 2016).

Despite the clear negative effects of feral pigs on native ecosystems and the positive results seen following their removal, feral pig management in the Pacific Island region remains a topic of intense debate. Natural resource managers and government officials largely recognize the damage caused by feral pigs and concentrate resources on their eradication in areas deemed important for the protection of native ecosystems. Alternatively, native cultural practitioners and recreational hunters argue feral pig populations require management for sustainable harvest as a game species because of the cultural history that surrounds the hunting of feral pigs and their status as a ceremonial food source (Ikagawa 2013). Deepening this debate, an increasing ability to use genetic tests to accurately analyze the ancestry of feral pigs throughout the Pacific has resulted in the argument both for and against eradication based on historical lineages (Linderholm et al. 2016). As a result of these differences in opinion, feral pig eradication occurs almost exclusively on state and federal managed lands and other conservation areas so that native ecosystems can be protected without the complete removal of feral pigs from the whole island (Ikagawa 2013; Katahira et al. 1993). In turn, other areas are typically managed for the sustainable harvest of nonnative ungulate populations. Because areas with disparate management objectives related to feral pigs are often found interspersed throughout the landscape, a greater focus on the spatial arrangement of management units holds promise for reducing conflict and the resources required for implementing management activities. Moving forward, expanding the size and number of areas devoid of feral pigs around already existing feral pig-free areas would further protect native ecosystems and the services they provide from the negative effects of feral pigs. For cultural and recreational purposes, smaller populations in more readily accessible areas

may be sufficient to support the needs of these individuals while allowing protection of larger areas of native ecosystems throughout the Pacific Island region (Hess and Jacobi 2014).

The current body of literature focuses primarily on documenting changes involving alterations to plant communities in the presence of feral pigs. Comparatively, there is a paucity of information regarding the effects of feral pigs on soils and vertebrate and invertebrate communities. This lack of knowledge includes a need to analyze direct effects on Pacific avifauna, as well as traditionally overlooked communities of invertebrates. It is also worth noting that the effects of future climate change on feral pig ecology and distribution have not yet been fully examined (Firn et al. 2015). Feral pig ranges will likely expand northward in continental ecosystems as winters become progressively less harsh (McClure et al. 2015), however, implications are much less clear for island ecosystems that are predicted to be strongly affected by climate change.

CHAPTER 3

Relationships between Soil Macroinvertebrates and Nonnative Feral Pigs (*Sus scrofa*) in Hawaiian Tropical Montane Wet Forests

The third chapter of my thesis is an analysis of soil macroinvertebrate response to the removal of feral pigs. It is currently in review with the journal *Biological Invasions*.

Abstract

Nonnative feral pigs (*Sus scrofa*) are recognized throughout the New World as one of the most noteworthy introduced ecosystem engineers. Similarly, nonnative soil macroinvertebrates (e.g., earthworms, ground beetles, centipedes) invade and alter the structure and function of native habitats globally. However, ecological relationships between these organisms remain largely unstudied. This study analyzed relationships between soil macroinvertebrates and feral pigs using nine sites located inside and outside of feral pig removal units representing a ~25 year chronosequence of removal in tropical montane wet forests in Hawai'i. Soil macroinvertebrates were sampled from plots categorized as: actively trampled by feral pigs, actively rooted by feral pigs, feral pigs present with no signs of recent activity, or feral pigs removed. In total, I found 13 distinct families of soil macroinvertebrates representing 11 orders. Plots with active trampling correlated with lower total macroinvertebrate abundance, biomass, and family richness of all soil

macroinvertebrates. Comparatively, plots with active rooting correlated with higher abundance of nonnative earthworms (*Lumbricidae* and *Megascolicidae*) and ground beetles (*Carabidae*). Additionally, the abundance, biomass, and biodiversity of all macroinvertebrate species showed no changes following feral pig removal. Collectively, these results suggest that: 1) trampling by large ungulates (in this case, feral pigs) has negative influences on soil macroinvertebrates; 2) feral pigs either modify habitats while rooting thereby facilitating earthworm and ground beetle habitat use or selectively seek out target prey species of soil macroinvertebrates; 3) the removal of feral pigs largely does not affect soil macroinvertebrates.

Introduction

Nonnative feral pigs (*Sus scrofa*) alter ecosystem processes and biodiversity throughout temperate and tropical ecosystems worldwide (Anderson and Stone 1993; Campbell and Long 2009; Wehr et al. 2018), and their activity has been shown to affect soils (Bueno et al. 2013; Long et al. 2017; Singer et al. 1984), plant communities (Cole and Litton 2014; Drake and Pratt 2001; Setter et al. 2002), animal abundance and distribution (Atkinson and LaPointe 2009; Galetti et al. 2015; Keuroghlian et al. 2009), and watershed function (Browning et al. 2008; Cuevas et al. 2010; Dunkell et al. 2011a; Strauch et al. 2016) earning them distinction as ecosystem engineers. In 2000, the management of feral pigs cost ~$800 million in the United States alone (Pimentel et al. 2000), and has likely increased notably since. Due to their widespread environmental alterations, fencing and the subsequent removal of feral pigs is a common management strategy utilized to protect native ecosystems and species (Wehr et al. 2018).

In general, the effects of feral pigs on the physical, chemical, and biological properties of soils are poorly understood compared to their alterations to plant communities (Long et al. 2017; Spear and Chown 2009). Prior research has, however, shown that trampling, rooting, wallowing, and the deposition of feces and urine can also alter soil processes including indirect alterations to plant communities (Bueno et al. 2013; Wardle and Bardgett 2004; Wehr et al. 2018). The presence of feral pigs has been shown to alter soil structure via reduced aggregate stability and increased compaction (Beever et al. 2006; Long et al. 2017), which can in turn affect soil macroinvertebrate communities (Vtorov 1993). Specifically, feral pig rooting and wallowing has been associated with increased soil carbon, nitrogen, and phosphorous, increased pH, and increased volumetric water content and water-filled pore space at my study sites (Long et al. 2017). Further, the alteration of understory plant communities by feral pigs likely leads to long-term alterations on soil macroinvertebrates given the close linkages between above- and belowground biota (Wardle et al. 2004).

These macroinvertebrate species are frequently introduced and become invasive, including >4,500 introduced species of nonnative invertebrates in the United States alone, with more than half of these introductions occurring in Hawai'i (Pimentel et al. 2000). Further, soil macroinvertebrates compose more than 98% of all non-microbial soil fauna worldwide, with vertebrates representing the remaining 2% (Decaëns et al. 2006). Many of these are soil macroinvertebrates that inhabit the upper layers of soil and aid in the fragmentation and decomposition of leaf litter thereby altering the overall structure of forest habitats by increasing soil nutrient availability and decreasing understory recruitment (Bernier 1998; Buse et al. 2008; Lavelle et al. 2006; Meyer et al. 2011; Negrete-Yankelevich et al. 2008; Snyder and Hendrix 2008). Soil macroinvertebrate species are often sensitive to localized soil chemical and physical

properties, including pH (Huerta and van der Wal 2012; Kuperman 1996), nutrient content (Huerta and van der Wal 2012; Warren and Zou 2002), soil texture, soil organic matter content, and litter density (Ponsard et al. 2000), all of which can be altered by feral pigs (Bueno et al. 2013; Hobbs 1996; Long et al. 2017; Siemann et al. 2009; Singer et al. 1984; Wirthner et al. 2012).

Feral pigs target soil macroinvertebrates, including snails, beetles, and earthworms, as a food source while rooting, and the importance of soil macroinvertebrates as a protein source in the diets of feral pigs has been well-documented (Diong 1982; Massei and Genov 2004; Meads et al. 1984; Parkes et al. 2015). While rooting, feral pigs can disturb up to 200 m^2 of soil daily, generating pits averaging depths of ~10 cm (Anderson and Stone 1993; Hancock et al. 2015; Meads et al. 1984). Importantly, feral pig rooting has been shown to occur more frequently when prey species are readily available in the top layer of soils, such as following rain events, (Elledge et al. 2013; Krull et al. 2016; Laznik and Trdan 2014) and in places where the soil has been previously disturbed by feral pigs (Elledge et al. 2013; Krull et al. 2016). Further, rooting has been correlated to higher abundance and/or diversity of earthworms in a variety of environments (Bueno and Jiménez 2014; Lincoln 2014; Taylor et al. 2011). Given that feral pigs alter ecosystems and are nonnative throughout much of the world, understanding their relationship with soil macroinvertebrates as a target food source is of great importance particularly in light of the worldwide spread of nonnative macroinvertebrate species (Barrios-Garcia and Ballari 2012; Hendrix et al. 2008; Pimentel et al. 2000). However, mechanistic explanations of these patterns have not been determined.

To better understand the potential relationship between feral pigs and soil macroinvertebrates, I asked the following questions: 1) How does rooting and trampling by feral

pigs correlate to the abundance, biomass, and biodiversity of soil macroinvertebrates? 2) How does the abundance, biomass, and biodiversity of soil macroinvertebrates change over time following the removal of feral pigs? To address these questions, I examined soil macroinvertebrate communities from plots characterized by active rooting, active trampling, no recent activity despite feral pigs being present, and after 13, 15, 16, 23, and 25 years following feral pig removal. I hypothesized that: *i*) Soils actively trampled by feral pigs would have lower abundance, biomass, and biodiversity of soil macroinvertebrates (Vtorov 1993); *ii*) Soils with active feral pig rooting would have higher abundance, biomass, and biodiversity of nonnative soil macroinvertebrates (Elledge et al. 2013; Krull et al. 2016); and *iii*) The removal of feral pigs would increase soil macroinvertebrate abundance, biomass, and biodiversity over time (Decaëns et al. 2006; Taylor et al. 2011).

Methods

Study Site

This study utilized a previously established chronosequence of large feral pig removal management units on the Island of Hawai'i ranging from 13 to 25 years since removal and in size from 117-1,024 ha (Cole and Litton 2014). The experimental design consists of nine sites located on the eastern side of Mauna Loa Volcano in the Ola'a tract of Hawai'i Volcanoes National Park and the adjacent Pu'u Maka'ala Natural Area Reserve. All study sites are located in areas of canopy-intact native tropical montane wet forest classified as *Metrosideros polymorpha*/*Cibotium* spp. (Wagner et al. 1999). Sites range in elevation from 1,140-1,370 m above sea level, with mean annual temperatures between 14.4-15.9 °C and mean annual precipitation between 2,910-3,985 mm with no distinct seasonality. All study sites occur on

2,000-10,000 year-old tephra-derived andisols from two closely related soil series: Puaulu (medial over ashy, aniso, ferrihydritic over amorphic, isothermic Aquic Hapludands) and Eheuiki (medial, ferrihydritic, isothermic Typic Hydrudands). These soils are characterized by deep, moderately well-drained soils formed from basic volcanic ash deposited over basic lava with slopes of 2-5% (Cole and Litton 2014; Long et al. 2017; NRCS 2010). Forests surrounding the exclosures are managed for limited recreational hunting of feral pigs (Cole and Litton 2014), with densities estimated at 0.6-16.3 feral pigs/km^2 (Scheffler et al. 2012).

The nine total sites are arranged in pairs with five having feral pigs removed and four having feral pigs present, with one present site serving as the pair for two pig removal sites. In each of the four sites with feral pigs present, three sampling plots were utilized representing areas with soil actively rooted by feral pigs, soil actively trampled by feral pigs, and soil with no recent sign of feral pig activity despite the presence of feral pigs. In these sites, each of the plots was systematically selected to represent the three categories of feral pig activity (i.e. rooting, trampling, or no activity), with each site being preliminarily assessed for activity two and four months prior to sampling. Trampling results in increased soil compaction (Barrios-Garcia and Ballari 2012) and these plots represent areas with repeated compaction by feral pigs occurring in narrow corridors formed by feral pig movement. Rooted plots represent areas with significant repeated rooting occurring, which is typically associated with mixing of soil layers, decreased soil compaction, and increased nutrient cycling (Singer et al. 1984). On average, rooted areas surrounding the plot covered an area of 21 m^2. In the five sites representing the chronosequence of feral pig removal, plots were established in areas representing 13, 15, 16, 23, or 25 years since removal, again with three plots at each site (Cole and Litton 2014). Within each site, plots were located randomly and were placed 75-200 m apart. Across all plots, canopy cover was controlled

for by placing each plot within 1 m of the base of a randomly selected mature tree fern (*Cibotium* spp.).

Data Collection

Prior to assessing soil macroinvertebrate communities, localized feral pig sign was recorded along two 25 m transects extending north and south from the center of each plot. The amount of sign was determined by the presence/absence of recent feral pig sign at each meter along the transects. Additionally, percent litter cover was assessed over the surface of each pit. To then assess the soil macroinvertebrate community in each plot, pits were excavated to 30 cm depth, width, and length, resulting in 27,000 cm^3 total soil volume, including surface litter in the center of each plot (Chan and Munro 2001; Lincoln 2014). Once excavated, the soil was sieved through 6.4 mm mesh to overcome clumping. All macroinvertebrates were then extracted by hand from both the mesh and the sieved soils. To ensure that no earthworms escaped collection by burrowing below the excavated pits and to sample potential species residing at lower depths, a solution of 75 g of powdered mustard in 2 L of water was poured into the bottom of each pit (Chan and Munro 2001; Nuutinen et al. 2001; Pelosi et al. 2014). However, no additional earthworms were obtained using this method.

Macroinvertebrates sampled were identified to family. More accurate taxonomic classifications were not possible due to similarities among related taxa. Voucher specimens for each taxonomic group were collected in a solution of 70% isopropyl alcohol for identification in the laboratory and were then stored in the University of Hawaiʻi at Mānoa Insect Museum. Images are available on Scholarspace, the University of Hawaiʻi at Mānoa's open-access repository (Wehr 2018). For earthworms, those that had not yet developed distinct adult

markings (those typically <10 mm) were considered juveniles and their discrete family was not

identified. The status of each taxa was determined from prior literature as either native,

nonnative, or unknown. Length was recorded for the first 25 specimens collected for each family

from each plot. Once 25 individuals were collected, additional specimens were only counted,

with their length estimated from the mean of the first 25 specimens sampled. For earthworms, a

subsample was collected from each site, dried for 48 hours at 50 °C, weighed, processed in a

muffle furnace at 400 °C for 24 hours, and weighed again to estimate ash-free dry biomass to

convert length measurements into biomass estimates (Hale et al. 2004). For all other soil

macroinvertebrates, only length was recorded, and this measurement served as a proxy for

biomass and a standardized value across taxa.

Statistical Analyses

All statistical analyses were conducted using R-Studio statistical software version 3.4.3.

To compare the abundance, biomass, and biodiversity of soil macroinvertebrates across each of

the plot categories (i.e. rooting, trampling, no activity, and time since removal), Kruskal-Wallis

rank sum tests were used because of the non-parametric, categorical nature of the data. For those

comparisons found to be statistically or near-statistically significant, Dunn's test of multiple

comparisons using rank sums was used to explore differences in distributions of soil

macroinvertebrates between individual categories (package: <dunn.test>). Finally, linear

regression analyses were used to model the relationship between soil macroinvertebrates and

time since feral pig removal, as well as percent litter cover and the presence of soil

macroinvertebrates. Because of the small sample size, tests were considered to be significant at

or below $\alpha = 0.10$ for Kruskal-Wallis rank sum tests and linear regressions, and at or below $\alpha =$

0.05 for Dunn's tests (Long et al. 2017). Additionally, Bonferroni corrections were applied to systematic analyses in order to correct for family-wise error rates when making multiple comparisons ($m = 6$).

Results

Across all plots combined, I collected 2,352 total specimens from 13 unique families representing 11 orders (Table 3.1). Combined, earthworms (*Lumbricidae*, *Megascolicidae*, and juveniles) accounted for ~42% of total individuals sampled and ~66% of total soil macroinvertebrate biomass. *Julidae* was the second most common taxa accounting for ~31% of all individuals sampled and ~27% of total biomass. *Hemiptera* individuals were the third most abundant sampled (~14%), while *Lithobiidae* individuals represented the third largest amount of biomass (~7%). Trampling resulted in a decrease of 74% in mean abundance ($T = 2.09$, $p = 0.02$), a decrease of 79% in mean biomass ($T = 2.63$, $p = 0.004$), and a decrease of 41% in mean family richness ($T = 2.40$, $p = 0.008$) of all soil macroinvertebrates combined compared to plots with no activity (Fig. 3.1A, B, C). Comparatively, rooting did not result in significant differences in total macroinvertebrate abundance, biomass, or family richness when all specimens were combined in comparison to plots with no activity (Fig. 3.1A, B, C). Time since feral pig removal also did not correlate to any significant differences in the abundance, biomass, or family richness of soil macroinvertebrates (Fig. 3.1A, B, C).

Correlating to the patterns observed when examining all macroinvertebrates collectively, trampling showed marginally significant decreases in both the abundance and biomass of individual taxonomic groups respectively including *Hemiptera* ($T = 1.81$, $p = 0.04$; $T = 1.83$, $p = 0.03$), *Oxychilidae* ($T = 1.84$, $p = 0.03$; $T = 1.83$, $p = 0.03$), *Parasitidae* ($T = 1.69$, $p = 0.05$; $T =$

1.69, $p = 0.05$), and *Lepidoptera* ($T = 2.33$, $p = 0.01$; $T = 2.61$, $p = 0.005$), when compared to plots with no activity. Comparatively, rooting correlated to marginal increases in the abundance of both earthworms, including *Lumbricidae*, *Megascolicidae*, and juveniles combined ($T = -1.67$, $p = 0.05$; Fig. 3.1D), and *Carabidae* ($T = -2.38$, $p = 0.009$; Fig. 3.1E). There were no significant correlations to change over time among any individual taxa. However, *Lepidoptera* abundance did show a marginally non-significant negative trend over time following time since feral pig removal ($n = 6$, $F = 4.66$, $p = 0.10$, $r^2 = 0.42$).

Litter cover was negatively and linearly related to earthworm abundance ($n = 8$, $F = 6.5$, $p = 0.04$, $r^2 = 0.44$), but not biomass. Litter cover was not correlated to any other taxa's abundance or biomass. It is important to note that, litter cover was highly correlated to the presence of localized feral pig sign at each plot ($n = 26$, $F = 37.75$, $p < 0.001$, $r^2 = 0.59$). This included significant differences between rooted and trampled plots and nearly all other plot categories ($n = 8$, $\chi^2 = 23.02$, $p < 0.001$; Fig. 3.1F), with specific decreases in litter cover associated with rooting ($T = 3.51$, $p < 0.001$; Fig. 3.1F) and trampling ($T = 2.85$, $p = 0.002$; Fig. 3.1F).

Table 3.1. Soil macroinvertebrate taxa collected during this study, their current status in Hawai'i, the total number and relative percentage of specimens found, and their total and relative length (mm), serving as a proxy for biomass, combined across all sites. Please reference [a](Hayes 2015), [b](Chamberlin 1953), [c](Hoffman 1997), and [d](González et al. 2006).

Taxa	Status	Total N	% Total N	Total Length	% Total Length
Arionidae	Nonnative[a]	3	<1.0%	9	<1.0%
Carabidae	Unknown	22	<1.0%	60	<1.0%
Geophilidae	Native[b]	16	<1.0%	262	1.6%
Hemiptera	Unknown	334	14.2%	662	4.1%
Julidae	Nonnative[c]	731	31.1%	3,967	24.3%
Juvenile Earthworms	Nonnative[d]	797	33.9%	5,098	31.2%
Lepidoptera	Unknown	56	2.4%	348	2.1%
Lithobiidae	Unknown	145	6.2%	993	6.1%
Lumbricidae	Nonnative[d]	164	7.0%	3,112	19.1%
Megascolicidae	Nonnative[d]	31	1.3%	1,608	9.9%
Oniscidea	Unknown	13	<1.0%	70	<1.0%
Oxychilidae	Unknown	23	<1.0%	77	<1.0%
Parasitidae	Unknown	10	<1.0%	10	<1.0%
Talitridae	Unknown	7	<1.0%	43	<1.0%
All Macroinvertebrates	N/A	2,352	100.0%	16,319	100.0%
All Earthworms	Nonnative[d]	992	42.2%	9,818	60.2%
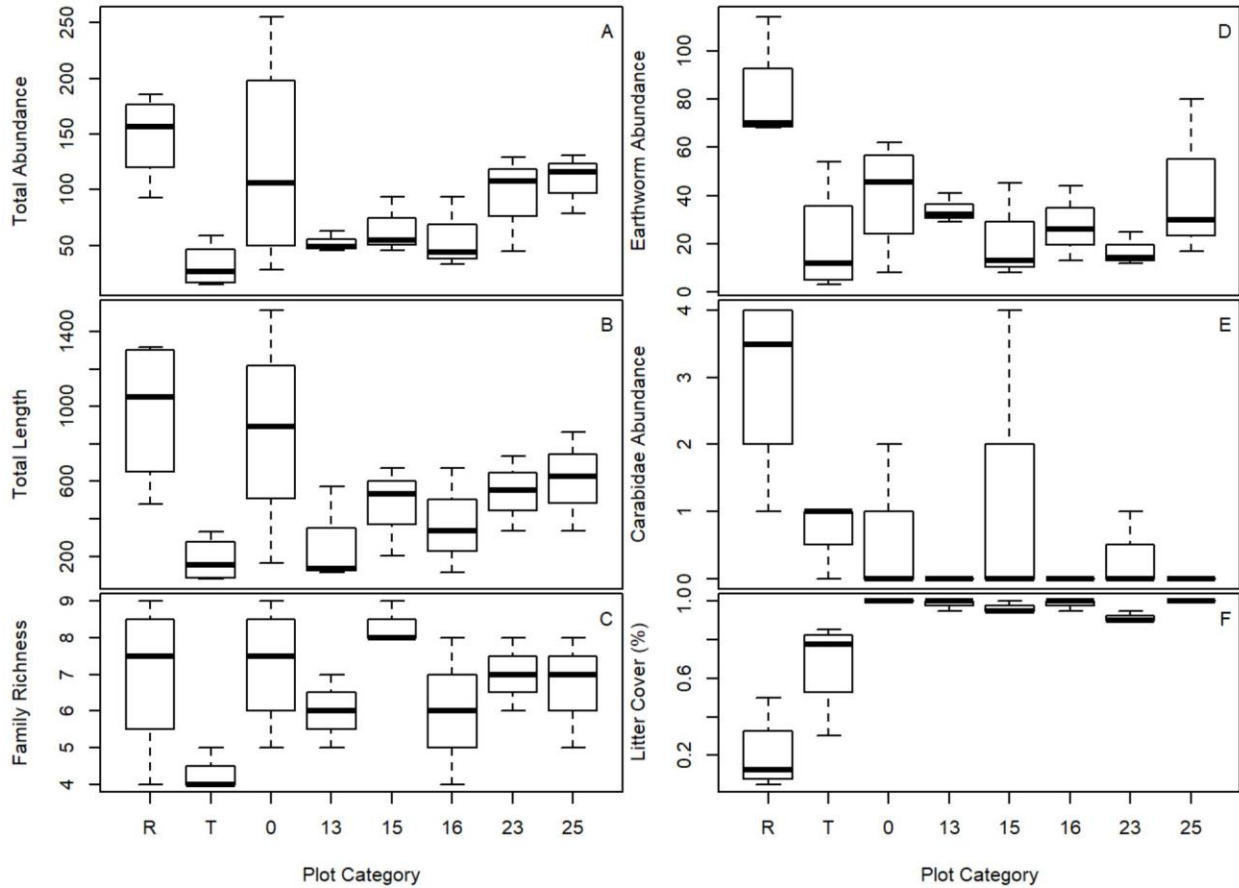
Fig. 3.1. Comparisons of six continuous variables labeled along the y-axes including: A) total abundance of all soil macroinvertebrates; B) total length (mm) (i.e. biomass) of all soil macroinvertebrates; C) the family richness of soil macroinvertebrates; D) the abundance of all earthworms combined including *Lumbricidae, Megascolicidae*, and juveniles; E) the abundance of *Carabidae*; F) the percent litter cover, at each plot type. These plot types are labeled along the x-axes: "R" representing plots with active rooting; "T" representing plots with active trampling; "0" representing plots with pigs present, but no recent activity; 13, 15, 16, 23, and 25 represent years since feral pig removal (for R, T, and 0, N = 4; for 13, 15, 16, 23, and 25, N = 3). Dunn's tests indicated that trampling resulted in lower abundance ($T = 2.09$, $p = 0.02$; A), biomass ($T = 2.63$, $p = 0.004$; B), and family richness ($T = 2.40$, $p = 0.008$; C) of all soil macroinvertebrates combined compared to plots with no activity. Rooting correlated to marginal increases in the

abundance of both earthworms, including *Lumbricidae*, *Megascolicidae*, and juveniles combined ($T = $ -1.67, $p = 0.05$; D), and *Carabidae* ($T = $ -2.38, $p = 0.009$; E). Litter cover was explained by the localized feral pig activity, with specific decreases in litter cover associated with rooting ($T = 3.51$, $p < 0.001$; F) and trampling ($T = 2.85$, $p = 0.002$; F).

Discussion

Throughout the study, plots trampled by feral pigs had lower levels of total macroinvertebrate abundance, biomass, and family richness. In examinations of specific taxa, *Hemiptera*, *Oxychilidae*, *Parasitidae*, and *Lepidoptera* were specifically identified as families with decreased abundance and biomass at trampled sites. Given that these four families made up <20% of the total population and <10% of the total biomass, these results indicate that, across the board, soil macroinvertebrate species are less prevalent in sites trampled by feral pigs although this may not be viewed as significant on a case-by-case basis. The decreased presence of soil macroinvertebrates at trampled sites is in-line with my original hypothesis based on work by Vtorov (1993) in similar forests, who documented soil macroinvertebrate species as negatively influenced by soil compaction, which has been associated with feral pigs in my study site (Long et al. 2017). This result is suggestive of a larger pattern where soil trampling by ungulates could decrease the survival of soil macroinvertebrates in trampled microhabitats worldwide.

Opposite to trampled sites, I originally hypothesized that I would observe an increase in soil macroinvertebrate abundance and biomass at sites rooted by feral pigs. However, this hypothesis largely was not supported. Instead, only earthworms (*Megascolicidae*, *Lumbricidae*, and juveniles combined) and ground beetles (*Carabidae*) were found to have higher abundances in the presence of feral pig rooting. This result is of particular interest because earthworms and ground beetles are important food sources for feral pigs (Diong 1982; Massei and Genov 2004; Parkes et al. 2015). Additionally, other studies have shown that feral pigs tend to return to sites they have previously rooted, despite removing target food sources during rooting (Elledge et al. 2013; Krull et al. 2016), and in my study, rooted plots were only examined after rooting had

occurred across a four month time period. It is therefore likely that the earthworms and ground beetles should have been recently and consistently predated upon by feral pigs prior to data collection. If the earthworm and ground beetle populations were not being predated, it is unlikely that feral pigs would repeatedly root at these sites because there would not be an energetic benefit to the activity. I therefore suggest two possible explanations for these results: 1) *Worm-Farming Hypothesis* – alterations to soils from feral pig rooting increases earthworm and ground beetle presence by rejuvenating populations; or 2) *Truffle-Worm Hypothesis* – feral pigs actively select sites that have more earthworms and ground beetles, and their consumption of target prey does not affect earthworm or ground beetle populations.

In support of my *Worm-Farming Hypothesis*, decreased soil compaction, increased nutrient deposition, and increased mixing of organic material are typical alterations to soils associated with rooting by feral pigs (Singer et al. 1984). These alterations to the soil could potentially facilitate the increased presence of earthworms I observed at rooted plots due to earthworms' general preference for soils with mixed compositions of organic and mineral material (Doube et al. 1997). Mechanistically, this would be possible because both families of earthworms present in my study system can begin reproducing 1-2 months after birth with short cocoon incubation times making it possible for earthworms as food resources to replenish over short time periods in the ideal conditions resultant from feral pig rooting (Butt 1993; Edwards et al. 1998; Karmegam and Daniel 2009; Venter and Reinecke 1988). In the case of ground beetles, while they would be unable to reproduce quickly enough to overcome this predation, they are capable of greater dispersion than earthworms and may recolonizing the area in order to predate on repopulating earthworms themselves (Lövei and Sunderland 1996). If future work supports *Worm-Farming Hypothesis* as true, it would be indicative of a facilitative relationship existing

between feral pigs, earthworms, and ground beetles synergistically accelerating the alterations of invasive species on Hawai'i's native ecosystems supporting the invasional meltdown hypothesis (Simberloff and Von Holle 1999). It is important to note, however, that this invasional meltdown likely would have occurred historically mirroring the introduction of feral pigs and earthworms and is likely limited to range expansion fronts presently due to climate change because feral pigs have largely maximized their use of available habitat in Hawai'i.

Truffle-Worm Hypothesis is equally likely to *Worm-Farming Hypothesis*. Mechanistically, *Truffle-Worm Hypothesis* suggests that feral pigs selectively choose to root in areas with a greater abundance of earthworms and ground beetles. Supporting this possibility, feral pigs have long been recognized for their adaptations suitable for the consumption of belowground food resources (Hatley and Kappelman 1980), but this is most commonly associated with truffles (*Tuber* spp.) that produce an aromatic compound similar to *S. scrofa* sex pheromones (Patel 2012). As such, it may be possible that feral pigs are capable of seeking out areas with greater availability of belowground food resources, but the mechanism cannot be confirmed by my study. If *Truffle-Worm Hypothesis* is true, repeated rooting in these areas could be explained by the increased availability of food, and the increased presence of ground beetles could then be explained again by the increased presence of earthworms as a target food source (Lövei and Sunderland 1996). Further, the increased presence of earthworms and ground beetles in these rooted areas could then be correlated more directly to soil alterations previously associated with feral pig rooting and wallowing in my study sites by Long et al. (2017) including increased soil carbon, nitrogen, and phosphorous, increased pH, and/or increased volumetric water content and water-filled pore space. Due to the limitations of this study, I am unable to clearly discern, which of these two explanations (i.e. *Worm-Farming Hypothesis* or *Truffle-*

Worm Hypothesis) could provide answers regarding the increased abundance of earthworms and ground beetles at rooted sites. However, based-off the prevailing literature and my own observations in the study system, I would favor *Worm-Farming Hypothesis* as the more likely explanation.

In consideration of sites with feral pigs removed, my initial hypothesis was that soil macroinvertebrate abundance, biomass, and biodiversity would increase over time following feral pig removal. However, my results did not support this hypothesis. Instead, none of the soil macroinvertebrate families showed any significant changes to their abundance or biomass over time following removal. As such, I conclude that the presence/removal of feral pigs largely does not affect soil macroinvertebrate communities over an extended period of time.

Although, I did not originally hypothesize that a relationship would exist, my results indicated a negative correlation exists between earthworms and litter cover. Three potential explanations for this are that: 1) feral pig activity alters both earthworms and litter cover independently; 2) litter cover influences earthworm abundance; or 3) earthworm abundance influences litter cover. Previous research has shown that earthworms decrease the volume of leaf litter (Bohlen et al. 1997) and that earthworm populations correlate to canopy cover, and thereby leaf litter (Lincoln 2014; Ponsard et al. 2000). This research would lend support to the third explanation. However, while I did observe a negative correlation between earthworm abundance and leaf litter, this did not mirror patterns of feral pig activity. Specifically, trampled plots had the lowest abundance of earthworms despite having a middling amount leaf litter cover. These plots therefore alter the pattern I am seeing in litter cover's influence on earthworms and indicate that the alterations to earthworm abundance are better explained by feral pig activity independently, which supports the first proposed explanation.

Conclusions

This study corroborates results previously reported by Vtorov (1993) suggesting that soil macroinvertebrates largely select against trampled habitats likely due to their inability to overcome soil compaction. This information will be useful in future examinations of soil macroinvertebrate species broadly, particularly in the presence of large, nonnative ungulates.

Additionally, the increased presence of earthworms and ground beetles under predation pressure from feral pigs is a novel result that suggests rooting, a common strategy used by feral pigs to forage for target prey species, either correlates with increases in the presence of earthworms and ground beetles (i.e. *Worm-Farming Hypothesis*) or with feral pigs' ability to intentionally seek out and find earthworms and ground beetles as a target food source belowground (i.e. *Truffle-Worm Hypothesis*). If rooting does alter soil conditions favorable for earthworms and ground beetles, this result would support the existence of a facilitative relationship between these taxa and feral pigs. Conceptually, this result would provide evidence for the invasional meltdown hypothesis (Simberloff and Von Holle 1999). Comparatively, if feral pigs instead are capable of seeking out soil macroinvertebrates as prey, then this would support prior work describing the evolution of *S. scrofa* to utilize belowground food sources (Hatley and Kappelman 1980). A mechanistic examination of this relationship is a necessary next step to understanding this relationship.

CHAPTER 4

Soil Bacterial Community Response to Removal of Nonnative Feral Pigs from Tropical

Montane Wet Forests

The fourth chapter of my thesis is an analysis of the soil microbiome response to the removal of feral pigs. It is currently targeted for submission to the journal *Scientific Reports*.

Abstract

Nonnative feral pigs (*Sus scrofa*) are ecosystem engineers that modify habitats and alter biogeochemical processes. Similarly, soil microbial communities play important roles in overall ecosystem structure and function, but the response of soil microbes to large scale management strategies, such as nonnative ungulate removal, remains understudied. With this research, I examined changes in the soil bacterial community following the removal of feral pigs from tropical montane wet forests on the Island of Hawaiʻi spanning a ~25 year chronosequence of removal. Bacterial eDNA was extracted from soil samples collected from multiple points within and outside of each exclosure and sequenced using the Illumina platform. Results demonstrate an overall increase in the biodiversity of soil bacterial communities following feral pig removal, with biodiversity positively correlated with time since removal. However, functional and phylogenetic diversity were not significantly altered by the removal of feral pigs. Rather, two environmental site characteristics (mean annual temperature and elevation) better explained differences in phylogenetic and functional diversity across sites. Collectively, feral pig removal, elevation, and mean annual temperature effectively described dissimilarities among soil bacterial communities. These results suggest that feral pig removal increases net soil bacterial diversity,

but does not lead to functional or phylogenetic changes, which were found to be more dependent on environmental characteristics.

Introduction

Due to recent technological advances, studies examining soil microbial communities, their function, and alterations to them are becoming increasingly common. As a result, ecosystem science has been able to elucidate the importance of numerous functional groups residing in soils, including a suite of species that regulate ecosystem processes and their services (Barrios 2007). Functionally, soil microbes play critical roles in water retention and detoxification, nutrient cycling, primary photosynthetic production, soil formation and processing, and carbon sequestration, with individual microbes often contributing highly specific roles (Lavelle et al. 2006). Given that individual microbial taxa often fill specific niches, numerous factors have been shown to play a role in determining dissimilarities among soil microbial communities. Such factors include soil properties such as pH, soil water content (Docherty et al. 2015; Selmants et al. 2016), cation exchange capacity (Docherty et al. 2015), and soil organic matter content (King 2003). These factors can then allow for explanations in the differences among communities.

Soil microbes are also known to share close relationships with the plant and animal communities where they reside (Kao-Kniffin and Balser 2008; Lavelle et al. 2006; Mohr et al. 2005; Selmants et al. 2016). For example, a study conducted on the Island of Hawai'i showed that soil microbial communities had higher biodiversity under native tree canopies than under canopies of non-native plants (Lynch et al. 2012). Microbial biomass and respiration were also shown to increase with increased plant species richness in closed-system trials in Minnesota (Zak

et al. 2003), and globally, beta-diversity of grassland plants was strongly correlated to the beta-diversity of soil microbial communities (Prober et al. 2015). Further, soil microbes have been shown to both inhibit and be inhibited by the growth of aboveground plants (Callaway et al. 2008; Callaway and Ridenour 2004).

The effects of animals, such as nonnative feral pigs (*Sus scrofa*), on the environment are often readily visible and can translate into effects on the belowground community of microorganisms. Prior research has indicated that, as ecosystem engineers, feral pigs affect nearly all aspects of their habitat even at broad scales (Barrios-Garcia and Ballari 2012; Campbell and Long 2009; Wehr et al. 2018). These effects alter plant communities (Cole and Litton 2014; Cole et al. 2012), fungi (Soteras et al. 2017), wildlife (Atkinson and LaPointe 2009; Galetti et al. 2015), watersheds (Browning et al. 2008; Dunkell et al. 2011a), and particularly soils (Doupé et al. 2010; Drake and Pratt 2001; Long et al. 2017). However, across a variety of habitats, feral pigs have been shown to alter soils variably, with primary effects resulting from their trampling, wallowing, and rooting behaviors. For example, in Tennessee's deciduous forests, soil bulk density was decreased by feral pigs (Singer et al. 1984), while soil bulk density increased with feral pig presence in Spanish alpine habitats (Bueno et al. 2013). Physical alteration of soil organic matter via foraging was also documented in some (Hobbs 1996; Siemann et al. 2009; Wirthner et al. 2012), but not all ecosystems where it has been examined (Bruinderink and Hazebroek 1996). Moreover, feral pigs have variable effects on soil nutrient cycling and availability, with several studies documenting increased soil nitrogen (N) availability with feral pig presence (Bueno et al. 2013; Siemann et al. 2009; Wirthner et al. 2012).

Likely as a result of these soil alterations, feral pigs have been shown to affect soil microbial communities directly and indirectly. Specifically, feral pigs have been shown to affect

the soil microbial community through defecation (Mohr et al. 2005), herbivory (Pastor et al. 1988), and soil turbation (Doan et al. 2013), and the resultant effects include alterations to the taxonomic structure of soil communities (Bovino-Agostini et al. 2012; Kaller and Kelso 2006). For example, feral pigs have been associated with an increased presence of fecal coliform in watersheds (Kaller and Kelso 2006; Strauch et al. 2016) and have been shown to increase the presence of specific bacteria, such as enterococci and leptospira, in soil runoff (Bovino-Agostini et al. 2012; Browning et al. 2008; Dunkell et al. 2011b; Yang et al. 2012). The presence of feral pigs in ecosystems also increases overall soil microbial activity; generally, this increase in activity is attributed to increased soil N availability due to the deposition of feces and urine (Mohr et al. 2005; Parkes et al. 2015). Interestingly, these changes to the soil microbial community have resulted in soils with an improved ability to store soil carbon (Wirthner et al. 2011). Further, the presence of feral pigs has been associated with faster microbial community recovery following rapid unexpected changes in the soil environment, such as the application of fumigants (Fujino et al. 2008).

In Hawaiʻi, feral pigs are a highly invasive species, and due to the many associated habitat alterations outlined above, the primary non-lethal management strategy for feral pigs in Hawaiʻi is exclosure fencing coupled with removal (Wehr et al. 2018). Prior research examining the results of this strategy have indicated that the removal of feral pigs from Hawaiian tropical montane wet forests resulted in increased plant species richness and density, increases in cycling and availability of soil N, and decreased soil bulk density, (Cole et al. 2012; Long et al. 2017). However, few published studies have examined the effects of feral pig removal in relation to soil microbial communities, particularly over an extended time period. As such, my goal was therefore to examine a previously untested aspect of feral pig removal by examining the soil

microbial community over a 25 year chronosequence of removal. Specifically, I addressed the following questions: 1) How does the removal of feral pigs affect the biodiversity of soil bacterial communities?; and, 2) Do these effects change with time since feral pig removal?

I hypothesized that feral pig removal would result in a less diverse soil bacterial community due to the system inputs associated with feral pigs including urine and feces deposition as well as changes to the physical structure of soils. This hypothesis reflects work conducted by Mohr et al. (2005) showing decreased soil microbial activity two years after the removal of wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) from a German ecosystem. I further hypothesized that bacterial diversity would not continue to change over time following the extended removal of feral pigs due to the ability of soil bacterial communities to rapidly adapt to environmental changes and ultimately reach equilibrium following feral pig removal (Allison and Martiny 2008; Buscardo et al. 2018; Cole et al. 2012).

Methods

Study Site

This study utilized a chronosequence of feral pig removal on the Island of Hawaiʻi initially characterized by Cole and Litton (2014). The experimental design consists of five pairs of sites arrayed across feral pig removal units located on the eastern side of Mauna Loa Volcano in the Olaʻa Unit of Hawaiʻi Volcanoes National Park and the adjacent Puʻu Makaʻala Natural Area Reserve. These pairs include five sites with feral pigs removed at intervals ranging from ~13 to ~25 years prior to my study. Each of these sites is paired to one of four control sites where feral pigs are currently present, with one such site serving as the pair for two of the removal sites (Cole and Litton 2014).

All study sites occur on 2,000-10,000 year-old tephra-derived andisols that are characterized by deep, moderately well-drained soils from basic volcanic ash deposited over basic lava. These soils stem from two closely related soil series: Puaulu (medial over ashy, aniso, ferrihydritic over amorphic, isothermic Aquic Hapludands) and Eheuiki (medial, ferrihydritic, isothermic Typic Hydrudands) with slopes of 2-5% (Cole and Litton 2014; Long et al. 2017; NRCS 2010). Sites range in elevation from 1,140-1,370 m above sea level, with mean annual temperatures (MAT) inversely correlating to elevation and covering a range of 14.4-15.9 °C. Mean annual precipitation (MAP) is between 2,910-3,985 mm at the sites with no distinct seasonality. Vegetation is characterized by large areas of canopy-intact native tropical montane wet forests classified as *Metrosideros polymorpha*/*Cibotium* spp. (Wagner et al. 1999). Excluding recreational hunting, forests surrounding the exclosures have never been managed for feral pigs (Cole and Litton 2014; Long et al. 2017), and feral pig densities have been estimated at 0.6-16.3 feral pigs/km^2 at these sites (Scheffler et al. 2012).

Soil Collection

During May of 2017, three soil samples were collected from unique subplots within each of the nine study sites described above for a total of 27 samples. Each sample was collected by gathering eight subsamples from 1 and 3 m intervals in the four cardinal directions from each subplot's center. Leaf litter was cleared to expose Oa- and A-horizon soils, and a clean spatula, latex gloves, and Falcon tube were used to collect each soil subsample (<12 mL in volume) from the top 1-2 cm of the soil from each of the eight subsample points. Once the subsamples were collected and sealed into a single Falcon tube representing the entire sample, the sample was

shaken by hand to homogenize the soil and placed in a cooler for ~3-7 hours until it could be transferred to a -20.0 °C freezer for longer-term storage until analysis.

eDNA Analysis

To assess the soil bacterial community in each subplot, eDNA was extracted from the soil samples using MP Biomedicals' FastDNA SPIN Kit for Soil (MP Biomedical, Santa Anna, California, USA). Manufacturer provided protocols were followed, including extended centrifugation at 14,000 rpm to remove excessive debris and an extra 5 minutes of incubation at 55 °C to increase eDNA yields. The Illumina© 16S Metagenomic Sequencing Library Preparation workflow was used to prepare samples for sequencing ~460 bp in the variable V3 and V4 regions of the 16s rRNA gene (Illumina 2013). To amplify the 16S rRNA region of interest, the following primers were applied to the eDNA sample extracts using qPCR: (Forward Primer = 5'

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; Reverse Primer = 5'

GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) (Klindworth et al. 2013). Here I provide a summary of qPCR and sequencing procedures used for my analysis, detailed protocol can be accessed at Illumina (2013). 16S rRNA primers were annealed to eDNA sample extracts and negative control samples at 55 °C for 30 seconds. Amplified products were washed twice using AMPure XP beads (Beckman Coulter, Brea, California, USA) to purify the final product. Subsequent qubit quantification was completed using Biotium's AccuBlue High Sensitivity dsDNA Solution Kit on a Qubit 3.0 fluorometer per manufacturer guidelines (Biotium, Fremont, California, USA). The final library was sequenced

at the University of Hawaiʻi at Mānoa Advanced Studies in Genomics, Proteomics, and Bioinformatics (ASGPB) genomics core facility on an Illumina© MiSeq platform. All samples were multiplexed using Illumina© NextEra XT index kit.

Molecular sequences were processed using the QIIME 2 bioinformatics platform version 2018.4 within a Virtual Box Core (Caporaso et al. 2010). Initial sequences were demultiplexed and truncated at the 10th bp from the left for both the forward and reverse reads. From the right, the sequences were truncated from the 290th bp on the forward read and from the 250th bp on the reverse read. The Divisive Amplicon Denoising Algorithm (DADA2) was then used to merge paired reads, create a sequence table, and remove chimeras. Sequences were then assigned to specific operational taxonomic units (OTUs) using the Greengenes 13_8 99% OTU database, and OTUs occurring <10 times across all 27 samples were removed. The resultant OTU CSV file was then exported for external data analysis and is available on Scholarspace, the University of Hawaiʻi at Mānoa's open-access repository (Wehr 2018). Secondarily, sequences were reassigned to functional groups using the Greengenes 13_5 99% OTU database in combination with the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) bioinformatics software. This step was completed with an open reference search within the QIIME 2 platform of the Greengenes 13_5 database. The resultant file listed the predicted functional groups based-off specific 16S rRNA OTUs (Langille et al. 2013). This CSV file was then exported for external analysis and is also available on Scholarspace (Wehr 2018). QIIME 2 was then used to obtain biodiversity scores using the <Phylogeny 2> plugin. Biodiversity scores utilized included Shannon-Weiner biodiversity index, Alpha diversity, rarified richness, and Faith's phylogenetic distance as well as Shannon-Weiner biodiversity index and rarefied richness describing functional diversity.

Statistical Analyses

Data from each of the 27 samples collected was averaged across the three samples from each site resulting in statistical analyses of the nine sites existing in the chronosequence ($n = 9$). Statistical analyses were then conducted using R-Studio Version 3.3.4. Biodiversity scores obtained in QIIME 2 were compared against available site characteristics obtained from Cole and Litton (2014) (time since feral pig removal, the presence/absence of feral pigs, removal unit size, MAP, MAT, elevation, and soil series). Due to the non-parametric nature of the data, Kruskal-Wallis rank sum tests were used to determine if any differences in biodiversity were associated with categorical site characteristics including presence/absence of feral pigs and soil series. Any significant categorical results were further analyzed using PERMANOVA within the QIIME 2 platform. Comparatively, continuous site characteristics (time since removal, removal unit size, MAP, MAT, and elevation) were compared in relation to biodiversity metrics using linear regression.

In addition to comparisons of biodiversity metrics, non-metric multidimensional scaling (NMDS) vector fitting was used to interpret the fit of site characteristics. These analyses were conducted using the <vegan> package available within R for complete coverage of all OTUs (Oksanen 2011). For each subset, basic ordination of community structure was established at each site using linear directional gradients <envfit>. These vectors were then plotted with surface fitting for improved clarity of nonlinear vector relationships <ordisurf>. Finally, constrained correspondence analysis (CCA) was used to determine site characteristics significantly affecting community composition dissimilarities to 1,000 iterations. Permutation tests were then conducted to select the best fitting model.

Results

Across all samples, eDNA extraction resulted in 53,140 to 81,823 reads per sample. These values were correlated to qubit quantification results, which ranged in value from 42.0-85.6 ng/uL for each subplot. Negative controls taken both before and after the separation of these aliquots resulted in values of -1.26 ng/uL. Initial delineation of OTUs organized the bacteria identified by QIIME 2 into 8,503 total OTUs. PICRUSt analysis further organized these OTUs into 328 functional groups.

Comparisons of biodiversity metrics with environmental variables led to three primary results. First, the removal of feral pigs was associated with higher Shannon diversity scores ($n = 9$, $\chi^2 = 4.86$, $p = 0.03$; Fig. 4.1), which was mirrored by results showing a positive linear correlation between Alpha diversity scores and time since feral pig removal ($n = 6$, $F = 14.81$, $p = 0.02$, $r^2 = 0.73$; Fig. 4.2). These results were also shown by PERMANOVA analyses conducted in QIIME 2 ($p = 0.001$). Second, rarified functional richness was positively and linearly correlated to MAT ($n = 9$, $F = 32.82$, $p < 0.001$, $r^2 = 0.80$; Fig. 4.3). Third, Faith's phylogenetic diversity scores were also positively and linearly correlated to MAT ($n = 9$, $F = 10.00$, $p = 0.02$, $r^2 = 0.53$; Fig. 4.3). It is important to note when considering these results that MAT exhibits a strong inverse correlation to elevation across these sites. As such, similar results were obtained explaining functional richness ($n = 9$, $F = 37.05$, $p < 0.001$, $r^2 = 0.81$) and phylogenetic diversity ($n = 9$, $F = 8.10$, $p = 0.02$, $r^2 = 0.47$) with a negative linear correlation to elevation. The results of which inversely mirror those results explained by MAT.

In the vector fitting phase of my analysis, the presence/absence of feral pigs ($p = 0.004$, $r^2 = 0.99$; Fig. 4.4), time since removal ($p = 0.003$, $r^2 = 0.88$), MAT ($p = 0.003$ $r^2 = 0.93$; Fig. 4.4),

and elevation ($p = 0.006$, $r^2 = 0.88$) were all considered good predictors of dissimilarity among bacterial communities (note: the r^2 values may be inflated due to my small sample size relative to the test statistic). None of the other available site characteristics were useful predictors of dissimilarity among bacterial communities. The vector axes representing elevation and MAT were highly correlated as were axes representing the presence/absence of feral pigs and time since removal.
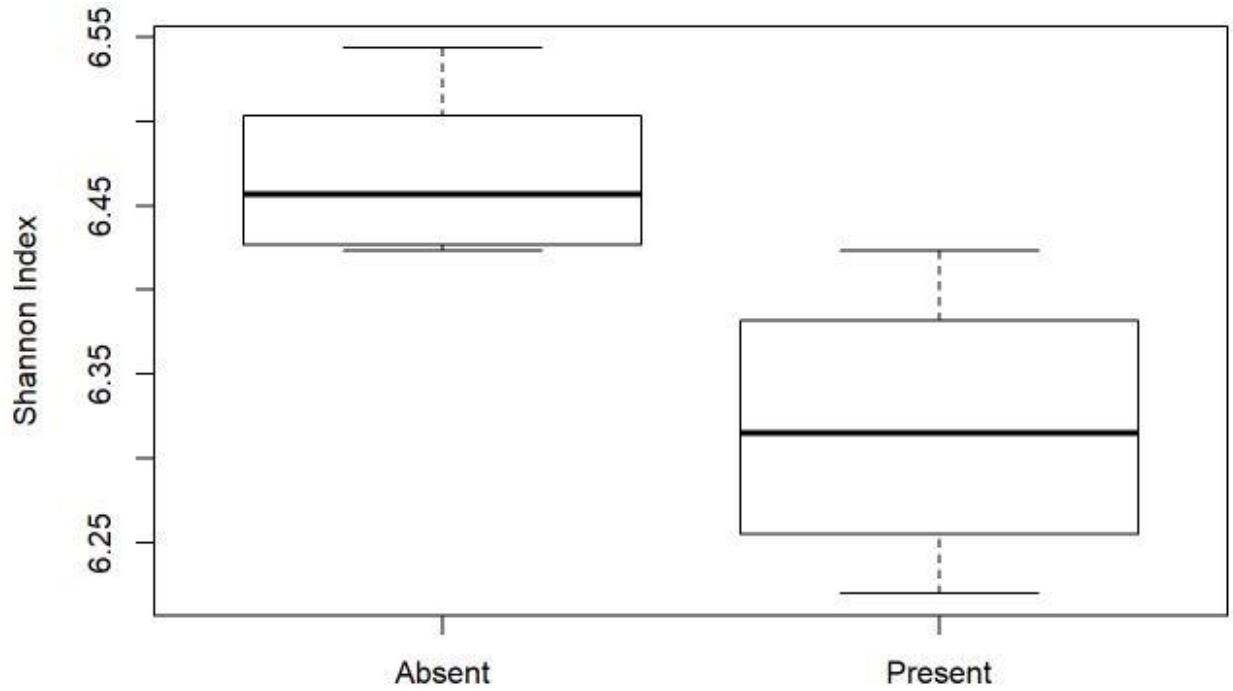
Fig. 4.1. Sites were categorized into those with feral pigs removed and sites with feral pigs present. The mean Shannon Diversity Index for these sites indicates that soil bacterial communities are more diverse following the removal of feral pigs ($n = 9$, $\chi^2 = 4.86$, $p = 0.03$).
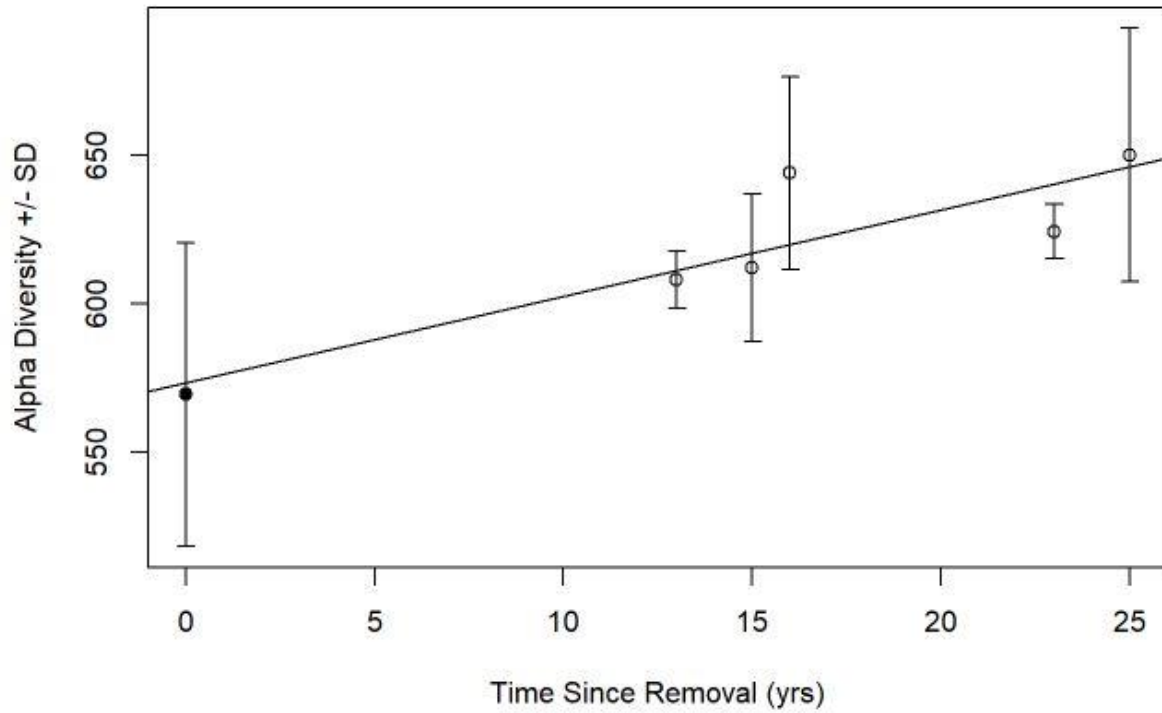
Fig. 4.2. The removal of feral pigs correlated positively to a linear increase in the Alpha diversity of soil microbial communities ($n = 6$, $F = 14.81$, $p = 0.02$, $r^2 = 0.73$). Along the left side of the figure at time $= 0$, the solid circle represents the mean of the four sites with feral pigs still present. The remaining five points symbolized with empty circles represent each of the five plots with feral pigs removed. Error bars represent standard deviation.
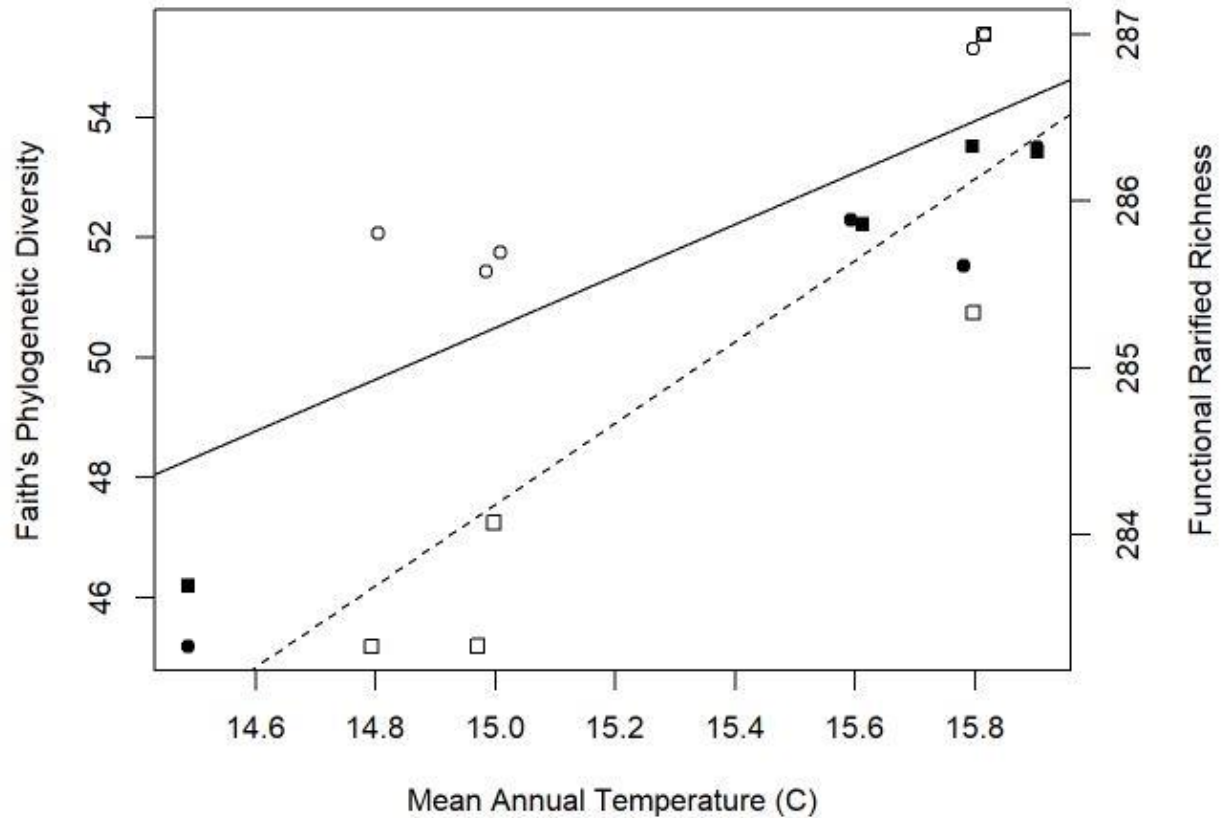
Fig. 4.3. Both Faith's phylogenetic diversity ($n = 9$, $F = 10.00$, $p = 0.02$, $r^2 = 0.53$) and functional richness ($n = 9$, $F = 32.82$, $p < 0.001$, $r^2 = 0.80$) were positively and linearly correlated with mean annual temperature. Represented by the left-side y-axis, Faith's phylogenetic diversity is labeled with circles and the solid line. Solid circles represent feral pigs being present and empty circles are sites where feral pigs have been removed. On the right-side y-axis, functional richness is indicated in the figure by the squares and dashed line, with solid squares having feral pigs present and empty squares having pigs removed.
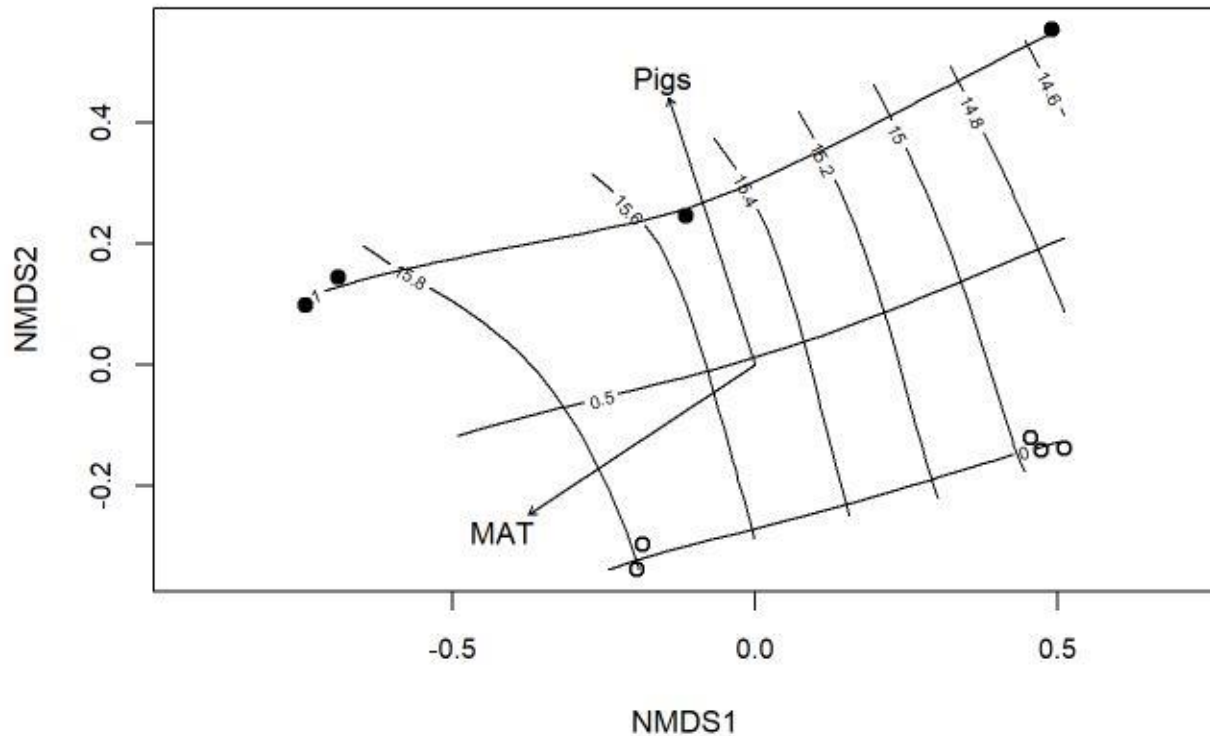
Fig. 4.4. Dissimilarities among bacterial communities were scored along two axes established by differences in the frequencies of individual OTUs at each site and placed into a dissimilarity index, displayed here. Each non-metric multidimensional scaling (NMDS) axis describes dissimilarity between communities along an arbitrary scale; these two scales are the x and y axes of this figure. Within the figure, the black circles represent the bacterial community at the individual sites in my study; solid circles have feral pigs present and empty circles represent sites with feral pigs removed. The sub-axis labeled "Pigs" represents the presence/absence of feral pigs at each site, and the horizontal lines within the figure show the scale of this axis where 1 represents sites with pigs present and 0 represents sites with pigs absent. This presence/absence is a good predictor of the dissimilarity among the bacterial communities ($p = 0.004$, $r^2 = 0.99$). The second sub-axis labeled "MAT" represents mean annual temperature at each site. The vertical lines within the figure scale the MAT from 14.6-15.8 °C. MAT was a significant

predictor of dissimilarity among the bacterial communities ($p = 0.003$ $r^2 = 0.93$). In addition to the presence/absence of feral pigs and MAT, time since the removal of feral pigs ($p = 0.003$, $r^2 = 0.88$) and elevation ($p = 0.006$, $r^2 = 0.88$) were both good predictors of dissimilarity. They are unlabeled in the figure because time since removal of feral pigs is represented by the same axis as presence/absence when inversed, and elevation is similarly represented by the same axis as MAT when inversed.

Discussion

I hypothesized that the biodiversity of soil bacterial communities would decrease following the removal of feral pigs. In addition, I hypothesized that the biodiversity of soil bacterial communities would not vary over time following the removal of feral pigs. My results, however, did not support either of these hypotheses. Instead, the biodiversity of soil bacterial communities was significantly higher in sites where feral pigs had been removed compared to sites where feral pigs remain present. Similarly, biodiversity was shown to positively and linearly increase with time since the removal of feral pigs. However, changes in the overall biodiversity of soil microbial communities were not evident in functional or phylogenetic metrics of diversity. Rather, functional diversity and phylogenetic diversity remained relatively constant in spite of feral pig removal and over time following removal.

Prior research has indicated that urine and feces deposition significantly increases the abundance of bacteria in soils, which would suggest that the removal of feral pigs would decrease bacterial abundance (Buscardo et al. 2018; Liu et al. 2015). Along the same lines, soil disturbance by feral pigs was shown to increase basal respiration in lowland New Zealand forests (Parkes et al. 2015), with similar results indicating decreased activity in soil microbial communities following the removal of wild boar disturbance in Germany (Mohr et al. 2005). It is possible that my results, conflicting with those above, can be explained by the pattern of extended aboveground recovery following the removal of feral pigs. Important changes previously recorded following the removal of feral pigs at these study sites include increased litter cover as well as increased abundance and richness of plant species, notably those in the understory (Cole and Litton 2014; Cole et al. 2012; Cushman et al. 2004). As these changes occur, the heterogeneity of the ecosystem would show corresponding increases. This increased

heterogeneity could account for the differences in overall biodiversity that I observed over time following the removal of feral pigs given that microbial communities can vary on small scales (Becker et al. 2006; Franklin and Mills 2003). The lack of change in the functional and phylogenetic diversity in these systems could then be explained because the newly present plant species in recovered plots likely rely on similar functions from soil bacteria as the already present species.

It is important to recognize that dissimilarities in soil bacterial communities are not only explained by the general increase in biodiversity following the removal of feral pigs. My results also indicated that elevation and MAT are good predictors of dissimilarity among bacterial communities. This result was reflected in the increased functional and phylogenetic diversity at warmer, lower elevation sites in this study. This result suggests that, while feral pig removal does result in changes to the soil bacterial community, overall community structure is sensitive to fine-scale differences in environmental variables (e.g., elevation and MAT). This result mirrors work indicating that soil microbial communities are highly sensitive to environmental factors at fine scales (Docherty et al. 2015; Lynch et al. 2012). Specifically, studies conducted worldwide have indicated that elevation plays a critical role in the diversity and composition of soil microbial communities (Corneo et al. 2013; Meng et al. 2013; Singh et al. 2014), as does temperature (Garcia-Pichel et al. 2013; Zogg et al. 1997). This result parallels recent work suggesting microbes follow patterns of tropical plant species richness decreasing in correlation to decreased temperature and increased elevation (Nottingham et al. 2018). However, it is important to note that my results contradict those previously reported by another field study conducted in Hawaiʻi suggesting that soil microbial communities are only minimally affected by changes in MAT (Selmants et al. 2016).

Conclusions

The results of this study provide important information for understanding changes over time in soil bacterial communities following the removal of nonnative ungulates, an increasingly common management approach globally. These results are the first documenting changes in soil microbial communities over an extended period of time following the removal of large nonnative ungulates from ecosystems dominated by native plants. In this study, I observed a linear increase in soil bacterial biodiversity over time following feral pig removal that did not correlate to any increases in functional or phylogenetic diversity. This result indicates increases in functional overlap, which for some communities can increase ecosystem stability (Naeem et al. 2009). My results are therefore compatible with a possible increase in microbial-driven ecosystem stability for at least the first 25 years following removal of feral pigs from Hawaiian tropical montane wet forests. It is important to recognize that, because the removal of feral pigs did not significantly alter the functional or phylogenetic diversity of soil bacterial communities, changes to broad-scale function of soil bacterial communities should not be expected by land managers following the removal of feral pigs. Instead, environmental factors including elevation and MAT may be more accurate predictors of the functional and phylogenetic structure of soil bacterial communities, regardless of the presence or absence of feral pigs.

CHAPTER 5

Conclusion

Feral pigs have been repeatedly shown to cause a broad range of systematic changes to ecosystems, primarily viewed as negative, from both direct and indirect effects (Nogueira-Filho et al. 2009). However, despite the clear negative effects of feral pigs on native ecosystems and the positive results typically observed following their removal, feral pig management in the Pacific Island region remains a topic of intense debate. This contention is particularly prevalent when management is ineffective and because there are no current ecosystems that have not been affected by feral pigs (Prior et al. 2018; Wehr et al. 2018). Natural resource managers and government officials largely recognize the damage caused by feral pigs, and concentrate resources on their eradication in areas deemed important for the protection of native biodiversity and ecosystems. Alternatively, both subsistence and recreational hunters contend that feral pig populations require management for sustainable harvest as a game species because of the cultural history that surrounds the hunting of feral pigs and their status as a ceremonial and subsistence food source (Ikagawa 2013). Presently, these conflicting goals are reconciled by removing feral pigs from conservation areas while allowing them to propagate elsewhere for use as a game animal.

The current body of literature focuses primarily on documenting changes involving alterations to plant communities in the presence of feral pigs or following their removal. Comparatively, there is a paucity of information regarding the effects of feral pigs, and their removal, on soils, including invertebrate, and microbial communities. In addition, the effects of future climate change on feral pig ecology and distribution have not yet been fully examined (Firn et al. 2015). Feral pig ranges will likely expand northward in continental ecosystems as

winters become progressively less harsh (McClure et al. 2015). However, implications are much less clear for island ecosystems that are predicted to be strongly affected by climate change in terms of precipitation.

In my thesis, I have begun to address the knowledge gaps that exist regarding the relationship between feral pigs and soil invertebrates and microbes. My first hypothesis-driven study examining the response of soil macroinvertebrates to the removal of feral pigs corroborates results previously reported by Vtorov (1993) suggesting that soil macroinvertebrates are negatively affected by feral pig activity (e.g., trampling). This information will be useful in future examinations of soil macroinvertebrate species in the presence of large, nonnative ungulates. Further, the elevated presence of earthworms and ground beetles under predation pressure from feral pigs is a novel result that suggests rooting, a common strategy used by feral pigs to forage for target prey species, may correlate with increases in the presence of earthworms and ground beetles. If true, this provides support that a facilitative relationship exists between these ecosystem engineers whereby feral pigs improve soil conditions for earthworms and ground beetles, and soil macroinvertebrates provide a food source for feral pigs. Alternatively, these results may suggest that feral pigs are capable of intentionally targeting areas with increased abundances of earthworm and ground beetle populations. This interaction is highly important given the global ranges of both *S. scrofa* and nonnative earthworms (Barrios-Garcia and Ballari 2012; Hendrix et al. 2008).

In the second hypothesis-driven chapter of my thesis, I examined the response of soil bacterial communities to the removal of feral pigs. The results of this study provide an important step forward in analyzing changes over time in soil bacterial communities. Specifically, these results are the first to analyze changes occurring over an extended period of time following the

removal of large nonnative ungulates from a primarily native ecosystem. The increase in biodiversity I observed following feral pig removal is important for land managers in considering the improvement to ecosystem stability that can occur following the removal of feral pigs. These results are important not only in Hawai'i, but throughout the nonnative range of *S. scrofa* where management via removal is common.

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