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Resolving Ecological, Evolutionary, and Global Change Effects on Biodiversity Dynamics: A Review and Empirical Study

By

Natalie R. Graham

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

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in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Rosemary G. Gillespie, Chair
Professor George K. Roderick
Professor Ian J. Wang
Professor Mike Boots

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Abstract

Resolving Ecological, Evolutionary, and Global Change Effects on Biodiversity Dynamics: A Review and Empirical Study

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Doctor of Philosophy in Environmental Science, Policy, and Management

University of California, Berkeley

Professor Rosemary G. Gillespie, Chair

Understanding why we find different types and numbers of species in different places has been a long-standing question for biologists. Ecological communities provide a conceptual and literal framework for unraveling generalizations about the composition, distribution, and dynamics of biodiversity. Communities assemble via ecological and evolutionary mechanisms that lead to the accumulation of species and the development of species interactions. Insights into the dynamics of community assembly have relied extensively on theoretical work or studies with limited spatiotemporal scale. Directly studying community structure through time using long-term time-series data offers an exciting opportunity to link evolutionary relatedness across multiple taxa and the ecological attributes of the communities in which they occur, within a dynamic framework. Oceanic archipelagos provide a chance to sample communities from discrete time points during community assembly. Present-day communities face threats, such as climate change and invasive species, demanding more holistic approaches that consider all species in an ecosystem and the interactions between them. Advancements in molecular techniques, bioinformatics, and data science have led to the possibility of a more comprehensive understanding of the structural properties of communities and of biodiversity overall.

The research presented here explores the interplay of ecological and evolutionary processes on biodiversity dynamics over deep time. I employ multiple research approaches including field collections, laboratory work with environmental DNA and museum specimens, macroecological theory, and network thinking to create a novel synthesis of information for understanding the evolution of ecological communities. First, I highlight the singular importance of insular systems for studying fundamental questions in ecology and evolutionary biology. By recording patterns of species accumulation and genetic distances in multiple arthropod lineages, I explore patterns of community assembly over time and how they differ between lineages. Additionally, I investigate systematic deviations in observed species abundance distributions from predictions of theory for understanding community steady state. Last, I demonstrate the implications of shifting network architecture metrics for understanding ecosystem change. My work makes critical advances in understanding biodiversity, community assembly, and the consequences of environmental perturbations on ecological communities.

This dissertation is dedicated to the baby boy I carry and the man he will become,
with love and the hope that he and his generation will know ecological communities rescued
from environmental catastrophe.

Introduction

The variety of life on earth is astounding. Arguments for the preservation of biodiversity vary from the inherent value of life (Wilson, 2016), to the ecosystem services provided (Mace et al., 2012), and the unknown cascade of consequences that may result from species removal (Eisenberg, 2013). There are multiple scales for the consideration of biodiversity, from what biologists term species and denote through hierarchical taxonomic strategies (Mayr, 1996), to the genetic underpinnings of these differences (Vellend & Geber, 2005), and the complex architecture of the interactions of species with the environment and one another (Pimm & Lawton, 1977). Evaluating factors that influence community composition and structure (e.g. genetic distances, species accumulation, abundance distributions, and species interaction networks) during community assembly would allow for a straightforward, comprehensive, multi-scale reconstruction of biodiversity dynamics.

One hurdle to overcome for accurate representation of indigenous biodiversity dynamics during community assembly is the influence of rapid Anthropogenic changes on current communities. Anthropogenic pressures are homogenizing previously discrete biological communities (Loreau et al., 2001; Martinez, 1996; Wilcove et al., 1998; Woodward et al., 2010) and ecological tipping points may fast be approaching (Barnosky et al., 2012). A second complicating factor to accurately assessing biodiversity dynamics is being able to untangle the processes that both create and maintain diversity (Heywood & Watson, 1995; Ricklefs & Schluter, 1993; Rosenzweig, 1995; Thompson et al., 2012). Many approaches to capturing biodiversity dynamics are limited to studying a few linages across deep time (evolutionary) or many lineages over spatial (and short temporal) scales (ecological) (McGill et al., 2019). A system that allows for the simultaneous study of whole communities across deep time would make it possible to unravel the consequences of both ecological and evolutionary mechanisms shaping biodiversity at multiple scales simultaneously.

To fully appreciate, catalogue, and protect biodiversity, we need a way of detecting biodiversity holistically, together with a simple system to study the inherent spatial and temporal dynamics of communities. Sensitive molecular techniques have been developed recently to comprehensively sample entire biological communities. Environmental DNA (eDNA) from a variety of sources (e.g. soil, water, plant tissues, faeces, gut content) contains the imprint of both macroorganisms and microbial species which can be identified at the species level by using reference taxonomic databases (e.g. GenBank, BOLD, SILVA) (Leray et al., 2019; Quast et al., 2012; Ratnasingham & Hebert, 2007). Sampling communities using eDNA across a time series would thus allow the spatial, temporal, ecological, and evolutionary factors that shape biodiversity during stages of community development to be disentangled (Bálint et al., 2018).

Here, I present the first empirical study to utilize high-throughput sequencing of entire biological communities using eDNA sampling across an evolutionary time-series. I collected arthropods associated with native plants across the natural geological age structure of the Hawaiian Islands. By holding all other abiotic and biotic variables constant, the chronosequence provides a natural experiment for examining changes in biodiversity over extended time (Funk & Wagner, 1995; Vitousek, 2002). In my research I have pioneered the integration of museum collections materials, ecological field study design, genomic sequencing of environmental DNA, and advanced statistical analyses, to contribute to a more multi-scaled, holistic field of biodiversity science. My dissertation focuses on the changes in genetic distance, species richness, abundance distributions, and ecological network structure as these community attributes reflect changes in community assembly over both ecological and evolutionary timescales.

Specific predictions I generated regarding the effects of community assembly processes on the empirically evaluated communities arise from a theoretical understanding of the development of communities. Community assembly theory predicts species will accumulate through the relative influence of ecological and evolutionary processes (Mittelbach & Schemske, 2015). The expectation for younger communities is that species will colonize a new habitat from a regional species pool (Cornell & Lawton, 1992). At the early stages of community development, when few species have colonized, and abundances are low, there is little competition (Wilson, 1969). As such, the ecologically ‘open’ space of young communities provides little opportunity for specialized mutualistic and antagonistic interactions. However, as the number and abundance of colonist species increases, intra- and inter-specific competition will also increase (Chesson, 2000; HilleRisLambers et al., 2012; Loreau & Mouquet, 1999; Wilson, 1969). The partitioning of resources and division of niche space (Chase & Leibold, 2009) decreases the overlap between closely related or functionally similar taxa. Therefore, as assembly continues, subsets of the population that specialize on different resources or experience different selective pressures, may speciate (Schluter, 2009). The resulting accumulation of additional taxa from speciation will result in a positive feedback for niche partitioning, partner specialization, and speciation (Ricklefs & Bermingham, 2002; Schluter, 2009; Wilson, 1969). As such, older communities will be composed of more specialist species and specific interaction partners.

In Chapter 1, I conduct a literature review to highlight the importance of island systems for understanding the influence of ecological and evolutionary processes, as well as global change phenomena, on biodiversity dynamics. I show how the power of islands to serve as ‘natural laboratories’ can be improved through functional classifications of both the biological properties of, and human impact on, insular systems. I review five categories of environmental perturbation (climate change, habitat modification, direct exploitation, invasion, and disease) and I discuss how insular properties have heightened the effects of these perturbations. Last, I show how taxonomists might meet the challenge of biodiversity assessment before the biodiversity disappears using an analysis of taxonomic checklists for the arthropod biotas of three well-studied island archipelagos. The aim of the review is to promote discussion on the tight correlations of the environmental health of insular systems to their continued importance as singular venues for discovery in ecology and evolutionary biology, as well as to their conservation significance as hotspots of endemism.

In Chapter 2, I introduce the empirical study methodology of eDNA sampling, and high-throughput sequencing of whole communities (specifically DNA metabarcoding), across the chronosequence of the Hawaiian Islands. First, I demonstrate how the genetic distances among the taxa that make up ecological communities change through time. Next, I investigate patterns of richness accumulation in multiple native arthropod lineages, comparing the results to predictions about how diversity might accumulate depending on the evolutionary history of the organisms. Last, I explore changes in beta diversity within each stage of community development. Together these results show how the relative influence of ecological and evolutionary mechanisms varies over time to shape biodiversity. Initially, processes are acting over the short-term to fill empty space in ecologically open communities. Over longer-time

scales, ecological processes give way to evolutionary, with diversification of lineages or the splitting of geographic space. I highlight a gap in understanding of long-term biodiversity dynamics that requires a view of the whole community simultaneously.

In Chapter 3, to test macroecological properties of communities across the Hawaiian chronosequence, I compare the observed shape of species abundance distributions (SADs), generated from the DNA metabarcoding data, to the predicted shape of SAD under the predictions from the maximum entropy theory of ecology (METE). This is the first test of METE over an evolutionary timescale. Systematic deviations from METE can help inform on where the community is changing. I separately test predictions for communities with and without non-native species to help assess the influence of anthropogenic change on community steady state. Results indicate that under the natural dynamics of communities prior to human arrival, new taxa are accumulating by evolutionary processes at the middle-aged sites, causing relatively rapid change in state variables compared to new colonists arriving by ecological processes at the youngest sites. However, the infiltration of very abundant non-native taxa appears to disrupt steady state in ecological communities at younger stages of development. Our results support the utility of using deviations from METE predicted macroecological patterns to understand ecosystem change and highlights the importance of separating non-native species to navigate the expectations for natural system versus modified system dynamics.

In Chapter 4, I evaluate changes in ecological network structure associated with the evolutionary assembly of communities by using the biotic associations of arthropods and plants, measured by DNA metabarcoding at each site over the island chronosequence. I calculate quantitative network metrics to evaluate the changing architecture for communities of different age. I find that the youngest communities are more nested and have link properties as well as consumer-prey asymmetries that reflect their lack of specialization. Older communities are more modular, with higher values for an index of specialization and interaction evenness, indicating that network specialization increases over time. I discuss the link of network architecture to community stability and the evidence that younger communities may be less resistant to environmental perturbations. This study provides the first empirical evidence of the shifting architecture of ecological communities during distinct stages of community assembly over deep time.

Together these chapters reveal a detailed view of biological communities and the factors influencing biodiversity dynamics. My research begins to tease apart the differences between natural and perturbed biological communities, and I make inferences about the consequences of ignoring rapidly changing ecosystems. It is my sincere hope that this work will help to expand our understanding of basic biological principles of communities as well as further an appreciation for the necessity of conservation action.

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Chapter 1

Island ecology and evolution: challenges in the Anthropocene

N. R. GRAHAM[1*], D. S. GRUNER[2], J. Y. LIM[1,3], R. G. GILLESPIE[1]

[1] Department of Environmental Sciences Policy and Management, University of California Berkeley, Mulford Hall, Berkeley, California, USA
[2] Department of Entomology, University of Maryland, College Park, Maryland 20742, USA
[3] Department of Integrative Biology, University of California, Berkeley, Valley Life Sciences Building, Berkeley, California, USA

*Corresponding author
Email: n.graham@berkeley.edu

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SUMMARY

Islands are widely considered to be model systems for studying fundamental questions in ecology and evolutionary biology. The fundamental state factors that vary among island systems - geologic history, size, isolation, age – form the basis of mature phenomenological and predictive theory. In this review, we first highlight classic lines of inquiry that exemplify the historical and continuing importance of islands. We then show how the conceptual power of islands as 'natural laboratories' can be improved through functional classifications of both the biological properties of, and human impact on, insular systems. We highlight how global environmental change has been accentuated on islands, expressly because of their unique insular properties. We review five categories of environmental perturbation: climate change, habitat modification, direct exploitation, invasion, and disease. Using an analysis of taxonomic checklists for the arthropod biotas of three well-studied island archipelagos, we show how taxonomists are meeting the challenge of biodiversity assessment before the biodiversity disappears. Our aim is to promote discussion on the tight correlations of the environmental health of insular systems to their continued importance as singular venues for discovery in ecology and evolutionary biology, as well as to their conservation significance, as hotspots of endemism.

VALUE OF ISLANDS FOR INSIGHTS IN ECOLOGY AND EVOLUTIONARY BIOLOGY

Islands are widely considered to be model systems for studying fundamental questions in ecology and evolutionary biology (Grant & Grant, 2011; MacArthur & Wilson, 1967; Vitousek, 2002). The study of islands has inspired a multitude of core theories in ecology and evolutionary biology (Warren et al., 2015). Arguably the most pivotal is that of Darwin who, through observation of thraupid finches on the Galapagos, famously theorized the role of natural selection and specialization to different diets to account for the observed diversity in beak morphology (Darwin, 1859).

Islands have also played a role in the birth of historical biogeography. Wallace (1880) through his extensive fieldwork in the Indo-Pacific discovered and characterized faunal affinities along distinct western (Asian origin) and eastern (Australian origin) lines, providing enduring insight into the role of historical and geological factors in driving species distributions (Holt et al., 2013). Likewise, islands have provided insights into the biogeographical imprint of extremely distant events in the Earth's history, connected with the plate-tectonic processes that have seen the break-up of super-continents, as well as the relative role of vicariance and dispersal in colonization history (Rosen, 1975).

Islands have also played an instrumental role in the development of several fundamental theories in ecology. They are a key element in one of the most robust generalizations in ecology – the species-area relationship (Arrhenius, 1921; Preston, 1960) – which has been used extensively to predict the magnitude of species extinction from habitat loss (e.g. Thomas et al., 2004). MacArthur and Wilson's Equilibrium Theory of Island Biogeography (MacArthur & Wilson, 1967) suggested species-area relationship manifested from a dynamic equilibrium between immigration and extinction, and that this dynamic was in turn influenced by the effect of isolation and island area on immigration and extinction rates. Diamond (1975) also used islands to argue that competitive interactions between species could explain non-random patterns of species co-occurrence in communities, arguing for the importance of biotic interactions in shaping local community structure. Associated rules of 'forbidden species combinations' and 'reduced niche overlap' have sparked more recent debate regarding the formation of appropriate null models (Chase & Myers, 2011; Gotelli & McCabe, 2002). Likewise, the concept of community nestedness in which the species composition of small assemblages is a subset of larger assemblages, drew upon data from islands (Darlington, 1957).

Islands have also been important in the development of insights at the interface between ecology and evolution. Thus, the idea of the 'taxon cycle' (Wilson, 1961), developed with studies of undisturbed island ant faunas in the Moluccas-Melanesian arc, argued that predictable ecological and evolutionary changes proceeded through iterative range expansion and colonization followed by evolutionary specialization within island populations. Ultimately, taxa either went extinct or progressed through a new phase of range expansion, thus renewing the cycle (Wilson, 1961). Thus, over the last century, ecological and evolutionary research focusing on multiple facets of islands – from the colonization of species and the development of uniquely evolved biotas, to the predictability of ecosystem development and community assembly over space and time – has shaped our fundamental vision of both pattern and process in ecology and evolutionary biology.

Considering the centrality of islands to fundamental development of these disciplines, we ask, what are the properties of insular systems that make them exceptional, and how are these hallmarks at risk in the Anthropocene? In the following sections our objectives are threefold: (1) to consider the biological properties of insular systems, in order to show how an island-based conceptual framework is usefully applied to situational derivatives of ‘true islands’ formed de novo (e.g. oceanic islands, caves, salt lakes) or by fragmentation (e.g. sky islands, kīpuka); (2) to review how predictions from theory and extensive empirical study can provide solutions to anthropogenic problems facing all island systems; and (3) to summarize a few important consequences of anthropogenic change on islands, but also highlight where scientific inquiry, at the most fundamental level of exploration of alpha-diversity, is providing a way to catalogue the changing world. Our review of human impacts is necessarily superficial, designed to categorize rather than provide a comprehensive overview. Moreover, specific examples are drawn primarily from literature on oceanic islands for which we are most familiar. Our intention is to stimulate broad discourse on the value of, and future challenges to, island environments that have generated so many seminal insights to the fields of ecology and evolution.

BIOLOGICAL PROPERTIES OF INSULAR SYSTEMS

Island Characteristics

From a biological perspective insular habitats can be defined as any discrete habitat that is isolated from other similar habitats by a surrounding inhospitable matrix (Gillespie & Clague, 2009). Both the habitat and the isolating matrix are relative to the organism in question. Water bodies present a stark barrier for terrestrial lineages on islands; in the same way intervening land around water bodies creates a barrier for aquatic organisms (Gillespie & Roderick, 2002). Patches of habitat (characterized for example by vegetation, soil, and/ or microclimate) separated by areas of unsuitable terrain, are also essentially isolated for species of narrow environmental tolerance. Islands may thus include sky islands, whale falls in the ocean, lakes within a land mass, and forest fragments in a matrix of secondary growth, pasture lands, or anthropogenic development. Because a greater understanding of the spectrum of island attributes will allow us to compare and contrast the processes playing out on them, we next review four primary attributes that dictate the biological properties of any given insular system: formation history, area, isolation, and time.

Formation history

It was the view of Darwin (1859) that biota on *de novo* islands were a product of colonization from continents followed by *in situ* evolutionary change. In contrast, Wallace (1880) studied islands in Indonesia, which were fragments of continents, and so his ideas revolved around the faunal affinities of islands relative to their source pools. Insular systems that are formed *de novo* can only gain species initially by colonization, and the biota grows through colonization or speciation (Warren et al., 2015). Thus, it is an island’s degree of isolation that dictates how much subsequent species accumulation will be a consequence of *in situ* diversification (neo-endemics) (M. Lomolino, 2000; MacArthur & Wilson, 1967; Rosindell & Phillimore, 2011). In contrast, insular fragments created by submergence or changes in climate of surrounding areas, are formed with a full biotic complement, and lose species during their formation through relaxation (Terborgh et al., 2001; Wilcox, 1978). Given a long period of time on fragment islands, distinct species may form (paleo-endemics). Thus, the history of island formation is key to the biological characteristics of a given insular community (Figure 1).

Time

In general, older islands have more species when the effects of formation history, area and latitude are removed (Wilcox, 1978). Time can make up for isolation by allowing for more time for immigration and/or speciation on *de novo* islands (P. A. V. Borges & Brown, 1999; Gruner, 2007; Willis, 1922). On fragment islands, the primary effect of time is greater paleo-endemism (Gillespie & Roderick, 2002).

Area

Species richness has long been recognized to scale as a log-linear function of area (Arrhenius, 1921; Rosenzweig, 1995). One of MacArthur and Wilson's (1967) key insights was that larger areas have the potential to support larger populations of species, thereby reducing extinction rates from demographic stochasticity and thus greater species richness. Larger populations also lead to greater standing genetic diversity, which may enhance rates of evolutionary change (Frankham, 1996). Area is also broadly correlated with other aspects of the landscape that promote speciation, including habitat diversity and heterogeneity and the potential for allopatric barriers (Losos & Parent, 2009).

Isolation

De novo islands close to a source of migrants are expected to reach an equilibrium species diversity with continuous turnover (MacArthur & Wilson, 1967). Increasing isolation leads to speciation playing a larger role in species accumulation, with anagenesis giving way to cladogenesis (Rosindell & Phillimore, 2011) and adaptive radiation on the most remote islands (Gillespie & Roderick, 2002). Fragment islands are generally less isolated than oceanic islands. However, those that are very isolated for extended periods, can also serve as a backcloth for adaptive radiation, presumably facilitated by fluctuations in land area and climate opening ecological opportunity for new colonists (Yoder et al., 2016).

Island Dynamics

The formation history, area, isolation, and time, are not static. Geologic and climatic processes are dynamic: islands are formed, and area and isolation may change over time through cycles of fusion, and fission of land masses (Price & Elliott-Fisk, 2004). The relative temporal scales of these processes will determine the interplay of area and isolation, which in turn will influence the biological properties of insular environments (Gillespie et al., 2017). For example, the relative abundance of different habitats on geologically ancient islands such as Madagascar has varied through time due to climatic oscillations, giving rise to a biota with both paleoendemic and neoendemic elements (Yoder et al., 2016).

Over shorter time scales, the geologic life cycle of oceanic 'hotspot' islands, from subaerial emergence to its eventual erosional demise, strongly affect the tempo of evolutionary radiation and decline on islands (Borregaard et al., 2017). The sequential formation of the Hawaiian archipelago has played host to multiple rapid species radiations that slowed with increasing age and declining island area (Lim & Marshall, 2017). In hotspot archipelagoes with a defined geological 'life cycle' (Lim & Marshall, 2017; Whittaker et al., 2008), there tends to be a progression of lineages colonizing from older to younger islands(Funk & Wagner, 1995; Shaw & Gillespie, 2016), and the composition of the biota shifts over geological time from primarily colonizing species on the youngest islands to those dominated by endemic species arising *in situ*

(Rominger et al., 2016). This sequential formation within an archipelago serves as a chronosequence; each island can be conceptualized as a trial in an experiment, with each new island a younger replicate of one of these experiments(Gillespie, 2016; Simon, 1987).

On yet shorter time scales, climate cycles play a role as 'species pumps', driving repeated changes in habitat isolation and thus promoting diversification. For example, changes in sea level have repeatedly isolated and reconnected the islands of the Galápagos, which may have enhanced species diversity (Grant & Grant, 2016). Alternating periods of warming and cooling have resulted in iterated episodes of isolation facilitating differentiation and endemism in Caribbean crickets (Papadopoulou & Knowles, 2015). Other drivers of fusion and fission cycles, such as lava flows that periodically isolate forest patches ('kīpuka') on a geological landscape mosaic, may act as 'crucibles' for evolution (Carson et al., 1990).

Ecological assembly

The Equilibrium Theory of Island Biogeography (ETIB) (MacArthur & Wilson, 1967) proposed that species richness on an island is a balance between immigration, which decreases with increasing distance from a mainland source, and extinction, which decreases with increasing island size. Subsequent work demonstrated how isolation affects extinction (in addition to immigration) through the 'rescue effect' (Brown & Kodric-Brown, 1977). The ETIB, though developed for *de novo* oceanic islands, has been applied to diverse insular systems, most notably to examine conservation implications of fragmentation on species diversity (Triantis & Bhagwat, 2011). In addition, the ETIB fortified concepts of metapopulations, in which colonization-extinction dynamics of a population of populations is modeled in ecological time (Levins, 1969) was later extended for communities (Leibold et al., 2004).

The main aspect of the ETIB used for conservation comes out of the SLOSS (Single Large Or Several Small) debate (Simberloff & Abele, 1976). Because larger and less isolated areas support more species, emphasis is placed on mitigating effects of habitat fragmentation to allow habitats to be as large and as contiguous as possible (Whittaker et al., 2005). While there is a tremendous body of research that supports these overall premises (Warren et al., 2015), there are two key elements in the ETIB that are still debated, namely equilibrium (Harmon et al., 2015) and turnover (Shaw & Gillespie, 2016). For example, it has been argued that communities are rarely at equilibrium, either because there has not been sufficient time to reach an equilibrial state or because the rate of temporal change to habitats or islands outpaces that of biological processes (Chambers et al., 2013). Moreover, the idea of continual species turnover contrasts strikingly with the phenomenon of priority effects, in which an early colonizing species in an area has an advantage over subsequent colonizers (Fukami, 2015) and can lead to patterns of endemism over evolutionary timescales (Shaw & Gillespie 2016). Understanding the transition between ecological and evolutionary processes is a promising new avenue for research using island chronosequences, which allow study of communities over ecological and evolutionary time (Rominger *et al.* 2016).

Both concepts of turnover and equilibrium have key relevance to conservation. For instance, given the time required for evolution, the interplay between colonization and speciation will necessarily be modified through disturbance, whether geological or ecological, natural or anthropogenic. Disturbances may effectively 'reset' a community to a more simple species composition, opening it to colonization by available propagules, the time required to reach an equilibrium depending on propagule availability. On isolated islands, native communities are largely the product of within-archipelago colonization and, associated with the paucity of colonizers, speciation. With the advent of humans and associated commensals, the availability of propagules has greatly increased. Thus, based on the arguments above, the impact of the increased extra-archipelago propagule pressure will depend on local disturbance, ecological turnover, and biotic resistance (Florencio et al., 2016).

Ecosystem function

Ecologists have long debated the relationship between diversity and the functioning of ecosystems (Hooper et al., 2005). At one level, species have been thought to make singular, unique contributions to the ecosystems. A famous analogy likened species to the rivets in a plane, their combined loss eventually causing the entire structure to fall apart (Ehrlich & Ehrlich, 1981). Alternatively, species have been viewed as redundant in a system with multiple species performing similar functional roles and so resilient to some loss of species (Walker, 1992). Others have said that the role of specific species in a community is context dependent and the effect following their removal is individualist or unpredictable (Lawton, 1994). Thus, effects of disturbance and species loss or gain may be more easily studied on islands and understanding of the factors leading to increased vulnerability across island systems is invaluable.

HUMAN IMPACT ON INSULAR SYSTEMS

The need for local understanding of how the biodiversity and the associated environment have been impacted by anthropogenic introductions (Kueffer et al., 2010), and what trajectories they will follow given current and future extinction and climate change (Kueffer et al., 2014), are particularly critical on islands. Islands often have limited biological and human resources, are isolated and exposed to storms and sea level rise, and therefore have limited resilience to new perturbations. Further, island economies that rely on the quality of their natural environment, notably through tourism, fishing and subsistence farming, are deeply affected by degradation of their environment (Connell, 2013). Some islands, including Britain and many Mediterranean islands, have had a long history of human influence, such that it is difficult to discern natural from anthropogenic influences. In contrast, the impacts of humans have generally been more recent on remote island systems. Here, paleontological studies have helped discern where natural processes or human impact, whether direct or indirect, have triggered prehistoric turnover in dominant vegetation types (Burney et al., 2001; Crowley et al., 2016). Reasons for high extinction rates include the vulnerability of narrowly endemic species associated with their smaller population sizes, combined with their evolutionary isolation. Body size appears to also influence vulnerability to extinction, with larger species being more extinction-prone (Terzopoulou et al., 2015). Introduction of new species and other forms of disturbance, including climate change, which weaken ecological and spatial barriers, may lead to hybridization as opposed to extinction, particularly among certain lineages of plant species, given that strong post-zygotic isolating barriers are poorly developed in island plants (Crawford & Archibald, 2017). These extinction factors, combined with global change phenomenon, have led to the loss of many island endemic lineages. For example, up to 2,000 species of birds, mostly flightless rails, were lost following human colonization in Melanesia, Micronesia and Polynesia (Steadman, 1995). Likewise, achatellinid tree snails in the Pacific islands, with estimated densities up to 500 per tree in 1903 in the Hawaiian lowlands, have largely disappeared in the last 100 years (Hadfield et al., 1993). However, the overall accumulation of extirpated species might be mediated by the taxonomic disharmony on isolated islands, where introduced species are more likely to play unique or underrepresented functional roles than on the mainland (Cushman, 1995).

Human impacts on island systems can be grouped into five categories, recognizing these categories are neither independent nor comprehensive: climate change, habitat modification, direct exploitation, invasion, and disease, the relative importance varying across islands and many island systems facing multiple anthropogenic pressures simultaneously and synergistically (Figure 2).

Climate change

The human inhabitants of oceanic islands are already dealing with the reality of climate change, in particular as reflected in rising sea levels (Shenk, 2011). Governments of smaller islands have already purchased land on larger islands as a last-ditch effort for human survival (Caramel, 2014). In terms of direct effects of climate, mean annual temperatures are expected to rise on oceanic islands despite buffering effects from surrounding oceans; also slightly more annual precipitation is predicted for the majority of islands in the future, with an increasing trend towards the end of the century, although large-scale precipitation projections might disregard the influences of island topographies on precipitation patterns on smaller scales (Harter et al., 2015). Likewise, the projected increase in intensity of extreme weather events (*e.g.* droughts, storm surges, hurricanes) has the potential for greater damage in delicate island habitats (Nurse et al., 2014; Seneviratne et al., 2012).

Habitat modification

The modification of habitats for human habitation, agriculture, livestock, and other resource extraction, is a major global conservation concern not limited to islands (Foley et al., 2005). However, the insular nature and smaller geographic size of islands can lead to two distinct consequences. First, the biota is more vulnerable to reduction in habitat for simple demographic reasons, as population sizes are usually small, coupled with the higher uniqueness, or endemism, of the biota. Second, islands that are independent nations often have a very limited economic base; thus, of the 48 countries on the UN's list of Least Developed Countries, nine are islands (five in the Pacific, four in the Indian Ocean). Poverty itself places tremendous demands on resources, leading to further exploitation and habitat modification.

Direct exploitation

Linked to habitat modification, direct exploitation of natural resources has had a major historical impact on island species, perhaps most notably flightless birds (Steadman, 1995). In the Pacific, ancestors of the Polynesians spread across most island groups in Oceania, clearing forests, cultivating crops, raising domesticated animals, and hunting megafauna to extinction (Steadman & Martin, 2003). Currently, direct exploitation is most apparent on economically poorer islands, such as Madagascar (Golden et al., 2014), where there are substantial pressures on the natural resources on which people depend for day-to-day survival (Connell, 2013).

Invasion

On oceanic islands, more extinctions have been attributed to introduced species than to habitat loss alone (Brooks et al., 2002). Humans have eroded biogeographical barriers by mediating dispersal of species into new regions where they can naturalize and cause ecological damage. A global database of 481 mainland and 362 island regions shows that in total 13,138 plant species (3.9% of extant global vascular flora) have become naturalized somewhere on the globe as a result of human activity (van Kleunen et al., 2015), with the Pacific Islands showing the fastest increase in species numbers with respect to land area. Although the relative vulnerability of continents and islands to biotic invasions has long been debated (Elton, 1958), it is generally held that the severity of invasive species impacts have been greater in isolated insular systems (D'Antonio & Dudley, 1995).

The impact of invasive species can become more profound due to their ability to modify the environment (so-called "ecosystem engineers") which can lead to facilitation with other non-natives (Borges et al., 2006). In plants, characteristics such as prolific seed production, dispersal ability, shade tolerance, nitrogen fixation, and production of allelopathic compounds can cause dramatic ecosystem-level effects. For example, the Macaronesian nitrogen-fixing tree *Morella faya* appears to facilitate subsequent invasions by enhancing nitrogen availability in nutrient poor soils (Vitousek et al., 1987). Such facilitation demonstrates the potential for 'invasional meltdown' (Simberloff & Von Holle, 1999), with synergistic impacts greater than with either species alone. Likewise, endangerment and extinction of native species may precipitate co-extinctions of mutualists (Cox & Elmqvist, 2000).

Disease

Introduction of non-native species (including humans) to islands frequently has been accompanied by diseases that find targets in naïve species and cause rapid extinction events. Among indigenous peoples, catastrophic declines in population sizes associated with the arrival of mainland human populations in the 1800s is well known in islands ranging from the offshore Scottish islets of St Kilda (Keay & Keay, 1994) to the remote islands of the Pacific (Kunitz, 1996), and is generally attributed to the effects of disease as a function of the susceptibility of the population. Native species have suffered similar impacts. Thus, in the Marquesas islands, the endemic genus of *Pomarea* flycatchers appear to have succumbed to malaria, while the more recently arriving Marquesan reed warblers may be resistant (Gillespie et al., 2008). In Hawai'i, avian malaria has been linked to the decline or extinction of 60 endemic forest bird species (Sodhi et al., 2009).

The impacts of disease are closely tied with other anthropogenic effects such as non-native species and climate change. Thus, avian malaria and avian pox, which have been important agents in the extinction of many endemic island birds and have also caused substantial population change and range contraction (Ralph & Fancy, 1994), rely on the introduced mosquito vector *Culex quinquefasciatus* for transmission. Likewise, global warming is expected to increase the occurrence, distribution, and intensity of avian malaria and threaten high-elevation refugia (LaPointe et al., 2012).

UNDERSTANDING AND ADDRESSING CONSERVATION CHALLENGES

It is clear that islands vary widely in biophysical properties. Therefore, as biologists, significant challenges are (1) to identify and catalogue biological diversity and characterize its structure across diverse landscapes and archipelagoes, and (2) to assess the nature of interactions with other taxa in order to predict the attributes of susceptible versus resilient communities in the face of abiotic and biotic change.

Discovery and taxonomic characterization of biodiversity

One of the first challenges is to recognize (and then close) the taxonomic impediment gap on islands. Islands have higher proportions of endemic species (Kier et al., 2009) but bear a disproportionate burden of global extinctions (Manne et al., 1999). In order to gauge more closely the relative impact of anthropogenic change on island systems we need an accurate picture of both richness and endemism on islands; yet a sizeable portion of island diversity probably remains undescribed, considering that archipelagos such as Hawaii are relatively well-studied with regard to eco-evolutionary theory (Wagner & Funk, 1995), and with two centuries of taxonomic work (Figure 3a). This shortfall varies between archipelagos, but can be substantial, and variation in taxonomic effort within and between archipelagoes has the potential to obscure biogeographic patterns in species richness (Gray & Cavers, 2014). For example, the Azores (Portugal) may have so few of its extant species described that reliable quantitative estimates of its true diversity are not possible (Lobo & Borges, 2010). This taxonomic impediment is especially worrying, given the multitude of threats that island biotas face; much diversity may have gone or will go extinct without ever being taxonomically described. These anthropogenic extinctions are already likely masking pre-human biogeographic patterns of diversity (Cardoso et al., 2010).

There are, however, some promising signs. Using comprehensive taxonomic checklists for the arthropod biotas of three archipelagos (Borges et al., 2005; Nishida, 1994), the rate of species description does not appear to be abating (Figure 3b). In particular, the rate of growth in the number of taxonomists working on Canary Island arthropods appears to be increasing (Figure 3b). This trend of increasing numbers of taxonomists appears to be true globally as well for a variety of taxonomic groups (Joppa et al., 2011). Further, the species-to-taxonomist ratio appears not to be declining (Figure 3b), suggesting that the declining pool of undescribed species (through cumulative taxonomic effort and/or species extinctions) has yet to limit the pace of current taxonomic efforts (cf. Costello et al., 2013; Joppa et al., 2011).

However, research on these archipelagoes might be a non-representative subset of global islands as a whole; Macaronesia and Hawaii are among the most well-studied oceanic islands in the world, and coordinated effort has led to the compilation of taxonomic checklists for their respective described biota (Borges et al., 2005; Nishida, 1994). Further, it is unclear for how long this trend of unabating taxonomic description will be sustained. Hawaii appears to have a decreasing trend in numbers of taxonomists despite greater taxonomic efficiency, perhaps because most Hawaiian taxonomic effort is driven by proportionally fewer people (Figure 3c).

Use of molecular methods may enhance the ability to identify cryptic species and stimulate subsequent taxonomic description. For example molecular evidence suggests that the notoriously low endemism of the Azorean biota may be due to cryptic diversity (Schaefer et al., 2011), and it is increasingly recognized that cryptic species may be a significant part of island biotas (Crawford & Stuessy, 2016). Advancements in rapid biodiversity assessment techniques (e.g. metabarcoding) now allow rapid and bulk recovery of molecular sequences from pooled community samples or environmental DNA, which may help elucidate community structure from samples that would otherwise be limited by traditional taxonomic approaches (Papadopoulou et al., 2015; Rees et al., 2014).

Community resilience in the face of change

Given that islands harbor high species diversity, local endemism, and particular niche affinities, a key challenge is to understand how communities of organisms will respond to human-mediated change in both biotic and abiotic variables. With regards to climate, recent approaches now incorporate the role of climatic cycles and associated changes in ocean currents, in conjunction with changes in area, isolation and elevation, all which may have shaped biodiversity on many islands in the past (Fernández-Palacios et al., 2016). For instance, there is clear evidence that communities are under pressure from changing climate, from the islands of the Mascarenes, Greece (Triantis & Mylonas, 2009), the Azorean Islands (Ferreira et al., 2016), and Bermuda (Glasspool & Sterrer, 2009). Small low-elevation topographically-homogeneous islands are least resilient to climate change pressures as a result of rising sea levels, and in many instances local habitat alteration interacts synergistically with novel abiotic perturbations causing even greater consequences for island communities (Harter et al., 2015).

Another challenge is to determine how communities will change from additions and deletions of species. Existing communities may be compiled of well-established non-native taxa currently playing similar functional roles to those species that have become extirpated (Davis et al., 2011). Network theory is providing an increasingly robust framework to understand species interactions and predict consequences of disturbance at a community level (Traveset, Tur, et al., 2016), showing both how alien species infiltrate receptive communities and how and to what extent they can impact and modify the structure of such communities (Romanuk et al., 2017). Combining plant–pollinator networks with islands as model systems serves to identify quantitative metrics that can describe changes in network patterns relevant to conservation (Traveset, Tur, et al., 2016). Some metrics may be suitable indicators of anthropogenic changes in pollinator communities that may allow assessment of structural and functional robustness and integrity of ecosystems (Kaiser-Bunbury & Blüthgen, 2015). In some cases native pollinators may be more resistant to exotic fauna than predicted by theory (Picanço et al., 2017).

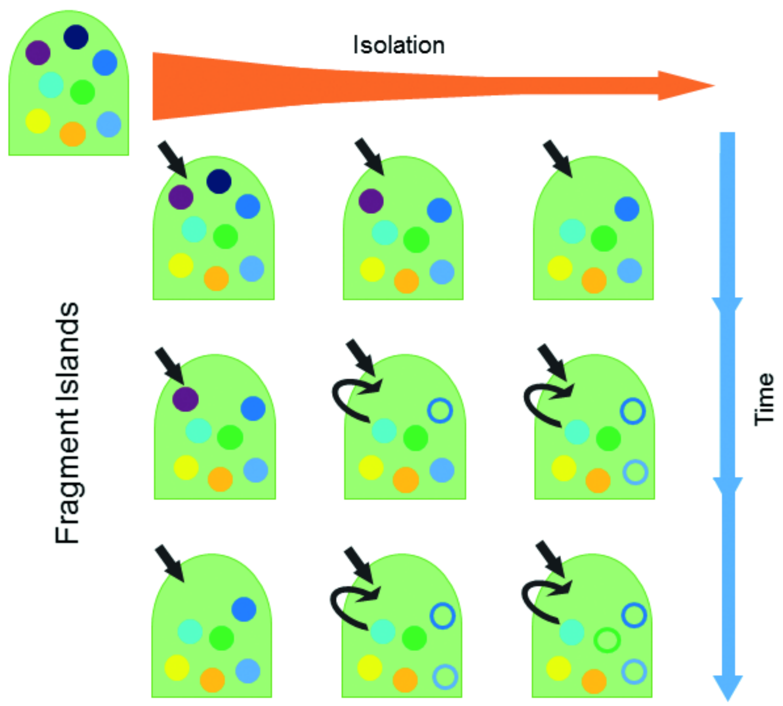
CONCLUDING REMARKS

Beyond the anthropogenic pressures impinging upon the ecological and evolutionary study of islands, human institutions may even at times impede fundamental research and environmental conservation. Research may be hindered on islands due to confusion in political jurisdiction, as islands often are managed on a regional basis by multiple institutions or countries. Additional institutional challenges include: mobilizing and accessibility of natural history collections and associated data, balancing public stakeholders with conservation objectives (e.g., hunting lobby vs. eradication), and navigating national and international funding agencies. Paradoxically, often the best economic health for islands is provided by programs such as tourism and agriculture that can, when managed poorly, may be dilapidating to the future of island ecosystems.

Current discourse analyzes how island systems negotiate with the challenges of balancing development with sustainability (Connell), incorporating indigenous and local knowledge to improve island environmental futures (Lauer), and harnessing environmental education on islands (Fumiyo). Strides towards uniting researchers across disciplines for the study of island biology and conservation are now evident. The fledgling Society for Island Biology (SIB), founded at the Summer 2016 Island Biology meeting in the Azores (Gabriel et al., 2016), is one such example. There have been cross-disciplinary conferences organized around current research for island biology (Kueffer et al., 2014) and special issues of journals focusing on the discourse of such symposia (Traveset, Fernández-Palacios, et al., 2016). A working group of researchers has put together a survey of the 50 fundamental questions in island biology (Patino et al., 2017). Such open dialogue will help to uncover the similarities in island systems and find suitable solutions that may be applied across islands and the mainland for anthropogenic disturbance. Moreover, the characteristics of islands that make them both exquisite study systems for fundamental ecology and evolution, and the archetypal endangered systems on the leading edge of global change, also position islands as the irreplaceable testing grounds for conservation solutions.

Figures

(a)

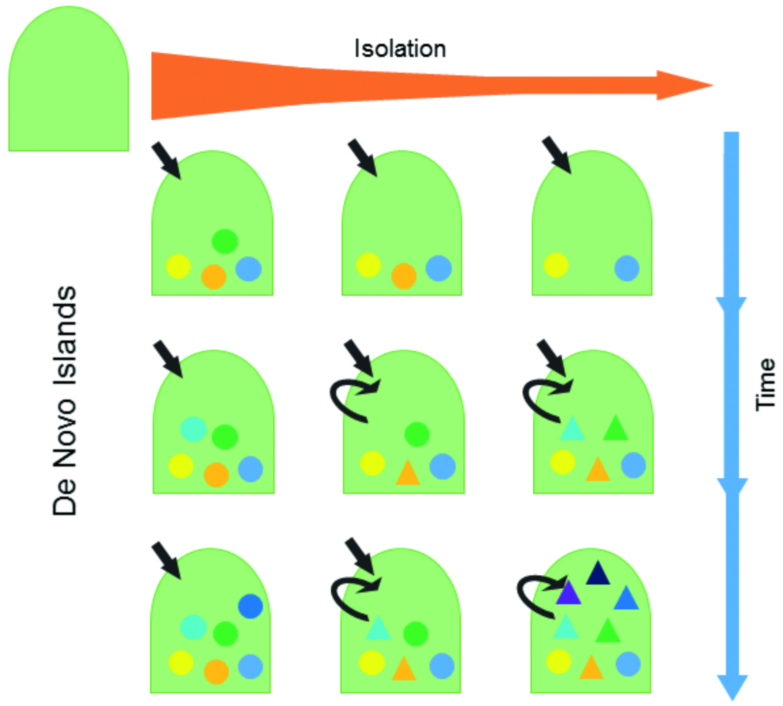


Low levels isolation: metapopulations dynamics; 'source sink' relationship

Intermediate isolation: opportunity for genetic differentiation between patches

High isolation: characterized by relictual lineages

(b)



Low levels isolation: colonization high; species exist as metapopulations similar to those of fragment islands

Intermediate isolation: species diverge genetically; colonization too frequent for adaptive radiation

High isolation: number colonizing species close to zero, rate of immigration slower than rate new species form through evolutionary process

Figure 1. Evolutionary assembly of de novo and fragment islands at varying levels of time and isolation. Colored shapes represent species as they accumulate, through colonization and through the formation of new species by both processes of anagenesis and cladogenesis. Black arrows indicate whether species are primarily accumulated by colonization (incoming arrow) or genetic divergence (arching arrow). (a) Fragment islands begin with biota similar to source. The number of species will decrease over ecological time as a result of relaxation and simply because of reduced area. Over evolutionary time, species diverge from original stock through anagenesis (unfilled circles), with the formation of paleo-endemics. (b) *De novo* islands are formed without life, thus the ecological space is open and available when they first appear. Over time species increase through both colonization and the formation of new species by cladogenesis (triangles). At high isolation, multiple neo-endemics may form through adaptive radiation.

Figure 2. Examples of major threats to island ecosystems, with illustrations from the Hawaiian Islands and Micronesia. (a) Urban development. Aerial photo of Honolulu, showing the entirely modified urban environment of Waikiki. The initial wetlands were first modified in the mid-15th century to cultivate wetland taro and for fishponds then drained in the 1920's with construction of the Ala Wai Canal to mitigate mosquito-borne human disease with further urbanization. Photo credit: George K. Roderick. (b) Crops. Pineapples, with sugar, were major plantation crops in Hawaii starting in the mid-1800s (Perroy et al. 2016). Acreage in pineapple and sugar halved between 1980 and 2015 and Maui Land and Pineapple company (shown here) closed in 2009. Currently, many plantations lie idle and weed-ridden, with an uncertain future. Photo credit: George K. Roderick. (c) Pasture. Cattle were introduced into the Hawaiian Islands in the 1790s, and ranching started in the early-to-mid 1800s and patches of native forest became increasingly restricted to inaccessible gulches, as shown in this photo of Hakalau Forest National Wildlife Refuge. Recent efforts are restoring areas of degraded pasture and forest, such as those at Auwahi on Maui (Cabin 2013). Photo credit: George K. Roderick. (d) Climate change. The many atolls that make up Micronesia now suffer frequent inundation as a result of sea level rise, as illustrated in this photo of Majuro in the Marshall Islands, which has become a vocal participant in global climate-change discussions (Labriola 2016). Many low-lying island nations of the Pacific are buying tracts of land on higher islands (Green 2016). Photo credit: George K. Roderick. (e) Invasions. One of the most insidious invaders of native ecosystems in the Hawaiian Islands is kahili ginger, Hedychium gardnerianum (The Nature Conservancy of Hawaii 2011), as shown: The left side shows a monotypic stand of ginger, the right a relatively pristine forest. The fence in front of the ginger, marking the boundary of the Waikamoi Preserve, in no way inhibits the spread, which is controlled to the extent possible by the diligence of The Nature Conservancy employees. Photo credit: George K. Roderick. (f) Disease. Studies, such as shown here at Hakalau Forest National Wildlife Refuge, show that the native honeycreepers such as the 'apapane, are vulnerable to pox and malaria (LaPointe 2008) and might be affected by upslope expansion of avian diseases (Camp et al. 2010). Photo credit: George K. Roderick. (g) Direct exploitation. In the Hawaiian Islands, the strongest evidence of direct exploitation is the use of

native bird feathers in capes. Perhaps most dramatic was the ‘O‘o, common in the 1800s, but extinct by the mid-1900s, which was exploited for a small tuft of yellow shoulder feathers (Lovette 2008). Photo credit: Honolulu Museum of Art.

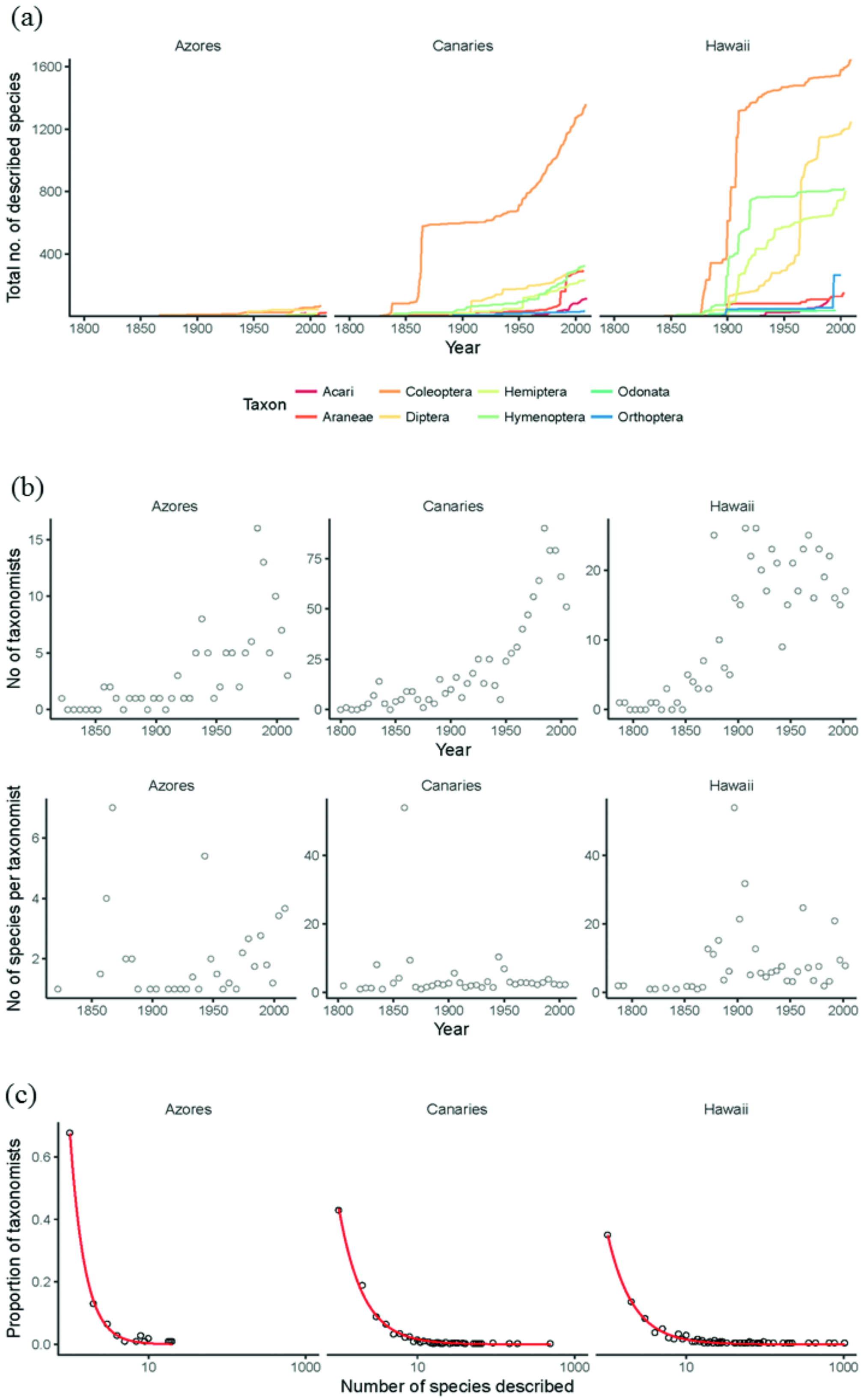

(a)
Azores
Canaries
Hawaii
Total no. of described species
1600
1200
800
400
1800
1850
1900
1950
2000
Year
Taxon
Acari
Araneae
Coleoptera
Diptera
Hemiptera
Hymenoptera
Odonata
Orthoptera
(b)
No of taxonomists
15
10
5
0
75
50
25
20
Year
No of species per taxonomist
6
4
2
40
(c)
Proportion of taxonomists
0.6
0.4
0.2
0.0
10
1000
Number of species described

Figure 3. Temporal trends in taxonomic description and taxonomic effort. (a) Cumulative number of described species over time, for various arthropod orders for the Azorean, Canarian, and Hawaiian archipelagoes. Data were obtained from recent arthropod checklists (Nishida 2002; Borges *et al.* 2005). (b) Trends over time (5 year bins) in the number of taxonomists and the number of species described per taxonomist. Black line represents a non-parametric smoothed best-fit line (loess regression). Black line represents a non-parametric smoothed best-fit line (loess regression). (c) Distribution of species descriptions among taxonomists. Most taxonomists describe few species, but there are fewer singletons (taxonomists that describe only one species) on Hawaii.

In the next Chapter, I introduce the empirical study that I performed. Analyses using the data from the empirical study are the subject of chapters 2-4. To understand how communities change over time we sampled arthropods (e.g. insects, arachnids, millipedes, amphipods) using vegetation beat sampling at 14 sites across the Hawaiian Islands, representing 14 different stages of community development. In Chapter 2, I first show how the genetic diversity of the community overall changes with time. I then discuss how the number of species (i.e. richness) changes for individual lineages of arthropods over time. Finally, I show how the diversity of whole communities changes as communities age. With this work I demonstrate how communities are developing in response to ecological processes (e.g. dispersal) and evolutionary processes (e.g. speciation) and how the response of individual lineages can vary depending on their evolutionary history.

Chapter 2

Ecological and evolutionary processes during community assembly evaluated using DNA metabarcoding of arthropods on islands

N. R. GRAHAM[1*], H. KREHENWINKEL[2], D. S. GRUNER[3], R. G. GILLESPIE[1]

[1] Department of Environmental Sciences Policy and Management, University of California Berkeley, Mulford Hall, Berkeley, California, USA
[2] Department of Biogeography, Trier University, Faculty of Regional and Environmental Sciences, Trier 54286, Germany
[3] Department of Entomology, University of Maryland, College Park, Maryland 20742, USA

*Corresponding author

Email: n.graham@berkeley.edu

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INTRODUCTION

The processes of community assembly unfold to shape the identity of species within ecological communities, such that biodiversity at any point in space and time is governed not only by local and regional processes, but also by large-scale biogeographic, historical and evolutionary processes (Loreau, 1994; Chase, 2003; Mouquet et al., 2003; Snyder & Chesson, 2004; Leibold et al., 2017). However, explanations for the assembly and maintenance of species diversity in ecological communities have traditionally been sought from two distinct perspectives. First, species diversity has been explained by local and short-term ecological processes (e.g. competition, predation, mutualism, and spatio-temporal variations in abiotic factors) following immigration from a regional species pool. Under this paradigm species assemblages are the result of environmental filtering (Kraft et al., 2015), competitive exclusion (Gause, 1934) and other ecological fitting mechanisms. A second approach focuses on larger scales of space and time, considering rates of speciation (Emerson & Gillespie, 2008), niche conservatism (Wiens & Graham, 2005), and diversity dependence (Rabosky et al., 2015). Clearly, both ecological and evolutionary processes play a role in species accumulation, and across scales of space and time, but their relative importance varies according to the situation (Ricklefs, 1987; Ricklefs & Schluter, 1993; Mittelbach & Schemske, 2015; Vanoverbeke et al., 2016). However, most studies of assembly processes have focused on either short-term ecological (and spatial) dynamics or long-term evolutionary (and temporal) dynamics, and few merge the two because of the difficulty of studying ecological dynamics over extended temporal scales. Thus, the explicit link between macroecological and macroevolutionary patterns and processes on the formation of biodiversity is mostly unexplored (McGill et al., 2019). To begin to integrate community assembly processes occurring over both ecological and evolutionary time scales, the mechanisms operating over extended spatial and temporal scales, and the interactions within and between scales, must be disentangled.

Clearly, there is a pressing need to bridge ecology and evolution across scales, since doing so is essential for understanding how the biodiversity that accumulated in the past is maintained in the present and might be potentially resilient to anthropogenic pressures in the future. Key questions are whether metrics of biodiversity change in a concerted manner across communities through time, and how we can associate concepts of specialization, turnover, species packing, and equilibrium or steady state over extended spatial and extended temporal scales, and hence infer how the overall dynamics of biological communities change through time. To tease apart the interplay of short-term and long-term processes we need a well-defined and simple system that allows measurement of biodiversity dynamics in the process of community assembly and how it plays out over extended time. Isolated islands provide comparatively small areas of land that are geographically discrete settings for measuring community ecology variables (Vitousek, 2002). Islands formed *de novo* can be used to provide insights into the assembly process (e.g. Florida Keys, Surtsey, Krakatau, etc). In particular, multiple islands of different age, as is found in many hotspot archipelagoes, can serve as ecological and evolutionary time series, providing the opportunity for studying assembly processes over extended evolutionary time (Gillespie, 2007; Whittaker & Fernández-Palacios, 2007; Shaw & Gillespie, 2016; Whittaker et al., 2017).

The Hawaiian archipelago, because of the island chronology, is an ideal system within which to evaluate the relative influence of macroecological and macroevolutionary processes affecting community assembly. As with other hotspot archipelagoes, the volcanic origin of the islands results in a stepping-stone like geographic arrangement where older islands in the northwest of the archipelago give way to successively younger islands at the southeast end of the archipelago (Simon, 1987; Gillespie, 2016). This geological history is mirrored in the pattern of evolution for many Hawaiian radiations (Funk & Wagner, 1995; Shaw & Gillespie, 2016). Thus, there is a tendency for many lineages to progress from older to younger islands (referred to as the 'progression rule'), meaning that multiple lineages are establishing and assembling, interacting, and adapting, over a similar timeframe that plays out over extended evolutionary time (Shaw & Gillespie, 2016). Thus, ecological and evolutionary metrics at different time slices of the community assembly process can be evaluate to measure how biodiversity properties (richness, abundance, trophic interactions) change over extended time (Loreau et al., 2002; Rominger et al., 2017) and how the interplay between immigration, adaptation, and speciation, in the assembly of communities through time, affects these properties (Whittaker, 1970; Urban et al., 2008; Urban & De Meester, 2009; Gillespie, 2016; Evans et al., 2017).

To clearly understand the processes shaping biodiversity we need to measure properties of entire communities across the chronosequence. Until recently, such an approach, which requires sampling of communities at a massive scale, has been prohibitive. However, the advent of DNA metabarcoding, an emerging methodology that allows rapid and efficient multi-species detection, has opened new avenues (Taberlet et al., 2012; Yu et al., 2012; Ji et al., 2013). A unique feature of metabarcoding data is its potential taxonomic breadth, allowing simultaneous investigation of many taxonomic groups of organisms (Deiner et al., 2017). Time series data can be compared for organisms of different trophic levels and functional groups, allowing a ready comparison of biodiversity dynamics that require long timescales and broad taxonomic coverage (Bálint et al., 2018).

As a focal taxonomic group, arthropods on the Hawaiian Islands are ideal for evaluating metrics for community assembly processes because they are locally abundant, often demonstrating high local diversity (α-diversity) and high rates of turnover (β-diversity) (Zimmerman, 1948a; Swezey, 1954; F. G. Howarth, 1990; F. Howarth & Mull, 1992; Roderick & Gillespie, 1998). Monitoring of entire arthropod communities using DNA metabarcoding over gradients of space and time has the potential to provide the needed insights into the combined influence immigration, extinction, adaptation, and speciation in the formation of communities (Bohmann et al., 2014; Watts et al., 2019). The shared temporal framework of the diversification history and community assembly of native and endemic taxa on the Hawaiian Islands provide a means to disentangle how biodiversity dynamics are changing over long spatial and temporal scales (Rominger et al., 2016). Here, we are leveraging the power of DNA metabarcoding to examine the role of extended time and space in dictating assembly patterns across entire communities. Specifically, we compare changes in community metrics of diversity, richness, and genetic relatedness of arthropods across the island chronosequence to develop insights into the interplay between ecological and evolutionary processes governing assembly patterns at different stages of community development.

We generated DNA metabarcoding data for arthropods at a set of plots at 14 sites across the archipelago. The general expectation is that diversity should increase over time as species accumulate through colonization and speciation. However, patterns of change should differ between lineages; moreover, the relative contribution of ecological (immigration) and evolutionary (adaptation, speciation) processes should vary through time. First considering changes in diversity across the chronosequence, we expect to find lowest diversity on the youngest sites. Diversity will increase through time, though species can increase at a given site through several mechanisms depending on the lineage: (i) immigration and colonization of an ecologically distinct taxon from an older site; (ii) *in situ* adaptation and divergence increase through time, though in ways that differ between lineages; and (iii) taxa tend to become increasingly specialized through time, which will be associated with increasingly finer scales of differentiation and divergence between members of a community.

(i) We expect the ecological processes of immigration to dominate the entire community at the youngest sites, giving way to the evolutionary processes, as shown by both (ii) and (iii), over time. Younger communities will therefore be composed up of colonists that arrive from older, and most likely closer, parts of the island or archipelago, to fill the open ecological space; as such, in the youngest communities we expect to see long branches between nearest neighbor taxa (Figure 1A). As species are added and the relative importance of competition increases, we expect ecological and evolutionary sorting to reduce the number of branches (Figure 1B). Once sufficient time has led to diversification, we expect to see more species and shorter branches among nearest neighbor taxa (Figure 1C). Thus, we expect to detect a signature of decreasing genetic distance among the taxa at the tips of a phylogenetic tree within each community over time.

(ii) In terms of differences between lineages, some lineages diverge through adaptation to different niches within a given geographic location such that multiple sister taxa co-occur at a site (Gillespie, 2004; Gillespie et al., 2018, 2020). Other lineages may diverge between different geographic locations (e.g. through divergent natural or sexual selection across the landscape), as has been shown for crickets (Shaw, 2002; M. Xu & Shaw, 2020). These two processes will lead to different expectations in terms of changes in diversity that we would detect with our data at a given site. For taxa that diverge between niches within a geographic location, species diversity will increase over time; for taxa that diverge between different geographic locations, we will detect little change in diversity at a given site (Figure 2).

(iii) In terms of specialization through time, the expectation is that all species in a community will become increasingly specialized through time. This will be reflected in the average community-wide differences between plots within a given site, with β-diversity (i.e. heterogeneity or turnover) expected to be greatest on the oldest island.

MATERIALS AND METHODS

Study sites

A replicated series of fourteen sampling sites spanning a gradient of substrate ages, from 48 years to 4.15 million years old was selected high-elevation wet forest in which *Metrosideros polymorpha* is the dominant canopy tree with patches of sub-dominant *Acacia koa* and numerous associated understory trees, shrubs, herbs, and ferns (Gagne & Cuddihy, 1990). Site selection was constrained to ranges of elevation (1000-1300 m) and precipitation (rainfall 2500-3000 mm) to hold abiotic and biotic factors affecting community formation as constant as possible and focus on the effect of age (Figure 3). Detailed information exists on the geological landscape of the Hawaiian Islands, with a matrix of volcanic substrates mapped to fine scales and with great precision in chronological age and geochemical composition (Lockwood et al., 1988; Wolfe & Morris, 1996). We used a combination of vegetation classification GIS (Hawaii Gap Analysis program, usgs.gov/core-science-systems/science-analytics-and-synthesis/gap), satellite and airborne imagery (NASA AVIRIS, aviris.jpl.nasa.gov; GAO-LiDAR, gao.asu.edu) and software analysis for land use and degradation (CLASlite, claslite.org), to select six site replicates for each candidate site. LiDAR spatial data was used to construct high-resolution vegetation density profiles using a physical model to derive surface (top-of-canopy) and ground digital elevation models (DEM) and calculate their differences. We combined these data layers to classify and prioritize the fourteen sites with differing geological histories within each volcanic zone on Hawaii Island, and for a single site on Maui, Molokai and Kauai. Sites are well documented from floristic and ecosystem-level perspectives, as described from the Mauna Loa environmental matrix (Vitousek et al., 1992, 1994) and the long substrate age gradient, which spans multiple islands (Crews et al., 1995; Vitousek et al., 1995; Chadwick et al., 1999). If a site replicate showed signs of invasive plant establishment during ground truthing that area was passed over in favor of one dominated by native vegetation.

Collection protocol

We collected arthropods using quantitative vegetation beating at six replicate 15 m radius plots per site (Figure 2D) during May 2015 through January 2016. The percent cover of each understory plant species was calculated and each plant species was sampled for some proportion of 420 seconds of total vegetation beating time in each plot with respect to its relative abundance in the plot, including ground covers, epiphytes and lianas, for a total of 35 plant genera across the islands. We placed custom constructed 1 m x 1 m white beating sheets under individual plants while gently agitating the foliage using a one meter length pvc pole for 3-5 second intervals and subsequently aspirating the arthropods which drop onto the beating sheet into a vial containing

95% ethanol. Each plant associated arthropod community sample was transferred to one or more 2 ml vials containing fresh 95% ethanol, labeled, and transported to the lab where it was stored at -20 °C. We dropped site replicates that were not sampled adequately from further analysis, based on if the sampling time for the replicate was within one standard deviation of the mean replicate sampling time, resulting in a total of 50 replicates and 11 sites (Figure S1).

Specimen sorting and DNA extraction

In the lab each plant beating sample was sorted under a stereoscope into four size categories (0-2 mm, 2-4 mm, 4-7 mm, 7 mm and up), individuals of each size category were counted and placed into fresh ethanol in a well of a 96-well plates (Krehenwinkel, Wolf, et al., 2017). An icepack was placed on the stereoscope stage beneath the sample to reduce the chance for DNA degradation. The Collembola, where abundances were orders of magnitude higher than the remaining arthropods in the 0-2 mm category, were separated into a 1.5 ml Eppendorf tube and processed for DNA extraction and sequencing parallel to the remaining arthropod community samples. The process of size sorting and Collembola separation should reduce or negate the amplification bias that is inherently caused by differential starting tissue amounts (Krehenwinkel, Wolf, et al., 2017). Specimens from public and private collections were used to generate a 912 sequence DNA barcode reference library (Accession numbers XXX-XXX).

Genomic DNA extraction of size sorted community samples was performed in 600 ul volumes using the Tissue protocol described in the Qiagen Puregene kit modified for automation. Briefly, ethanol was drawn off and evaporated from each well, Lysis buffer with proteinase K was added to each well and digested overnight, followed by the addition of RNase and further digestion. Protein Precipitation Solution was added, shaken to mix, and spun to pellet the insoluble debris. To purify the gDNA, a robot at the UC Berkeley DNA Sequencing Facility was used to transfer 140 ul of the cleared lysate to a plate containing 20 ul of Silica-coated magnetic beads. The DNA binds to the beads, is immobilized on the magnet, and is washed in 70% ethanol. After drying, DNA was eluted in 50 ul DNA Hydration Solution.

PCR amplification, library preparation and sequencing

Each sample was amplified with a primer pairs combination (ArF1/ Fol-degen-rev) (Gibson et al., 2014; Yu et al., 2012) that targets a 418 bp fragment in the barcode region of the Cytochrome Oxidase I (COI) gene. Compared to other markers, COI is distinguished by an exceptionally well-developed reference database (Leray et al., 2019; Ratnasingham & Hebert, 2007), which often allows species identification. We previously determined from mock communities that using this highly degenerate primer pair successfully recovers the majority of arthropod species in Hawaii (Krehenwinkel, Wolf, et al., 2017; Krehenwinkel, Kennedy, et al., 2018). Library preparation followed that described in Lange et al. (2014) with the following modifications. PCRs were run in 10 µl volumes using the Qiagen Multiplex PCR kit at an annealing temperature 46 °C, with 1 µl (15 ng) of DNA and 0.5 µl of each 10 µM primer. A first round of PCR consisted of 32 cycles using tailed primers, each primer additionally had a unique 6 bp inline barcode so that multiple plates of the same primer could be pooled prior together. PCR products were cleaned of residual primer using 1 X SPRI beads (Sera-Mag™) and pooled together based on based on band intensity on an agarose gel relative to a DNA ladder (NEB) and using the Gel Doc XR System with the Quantity One software (Bio-Rad). A second indexing PCR of 6 cycles was performed on the pooled amplicons to introduce dual indexes and Illumina® TruSeq sequencing adapters to 5′-tails of the locus-specific PCR primers. We used the same TruSeq primers and dual indexes as suggested in Lange et al. (2014). The indexed products were cleaned again with SPRI beads, quantified using a Qubit Fluorometer (Fisher Scientific) then pooled in equal amounts into a single tube. The final libraries were quantified using qPCR with a KAPA Library Quantification Kits for Illumina® platforms, then all samples were pooled in equimolar amounts and sequenced on an Illumina® MiSeq using V3 (600 cycles) chemistry and 2 × 300 bp reads according to the manufacturer's protocol (Illumina, San Diego, USA). We aimed for a total of 30,000 reads per sample for each primer pair.

Sequencing analysis

We generated 2276 metabarcode libraries with each library representing the total arthropods collected for each plant genus for each plot (a sampling event), sorted into one of four size categories (a sequencing pool). Sequences were demultiplexed on Illumina® BaseSpace by sample well based on the two 8-bp indexes. We merged paired reads using PEAR (Zhang et al., 2014) with a minimum overlap of 50 bp and a minimum quality of Q20. Merged reads were quality filtered (≥90% of bases ≥Q30) and transformed into fasta files using the FastX Toolkit (Gordon & Hannon, 2010). The resulting fasta files were demultiplexed by amplicon primer and 6 bp inline barcode combination, using the forward and reverse primer sequences as indices with the grep command in UNIX and primer sequences then trimmed using the UNIX stream editor.

Rarefaction

We rarefied each sample using a custom script that drew from the total reads of the metabarcoding analysis a number of reads that was equivalent to the numerical abundance of individual arthropods counted into each well of the 96-well plate, repeating the draw of sequences 100 X with replacement. The process of rarifying by repeating random draw based on the expected individual specimen abundance should correct the disproportionate abundance of sequences that accumulate for larger specimens compared to smaller specimens, due to the amplification bias that is inherently caused by differential starting tissue amounts.

Pseudogene removal

We generated zero-radius OTUs (ZOTUs), from the rarefied raw reads with the unoise3 command (Edgar, 2016) following the recommended protocols in the USEARCH v11 pipeline (Edgar, 2010). We removed non-Arthropod ZOTUs by filtering results of the top hit from a blastn search on a local nucleotide database downloaded May 2020 with BLAST+ (Camacho et al., 2009) and our custom DNA reference library for Hawaiian taxa. We aligned these 5046 ZOTUs using default settings in Clustal Omega (Sievers et al., 2011). To remove putative pseudogenes from the ZOTU dataset we ran metaMATE with default specifications and the example specifications file to detail how per-ZOTU read frequencies should be assessed (Andújar et al., 2021). Using the output of metaMATE we applied the least stringent Numt removal strategy so that we could retain as many putatively true ZOTUs as possible (Graham et al., 2021), this reduced the number of ZOTUS from 5046 to 4330.

Taxonomic matching and abundance estimates

About a quarter of the ZOTUS (901) were matched to the Blast or voucher DNA reference library with less than 85 percent similarity. To validate the taxonomic identification for each ZOTUs at higher taxonomic levels (e.g. order, family) we compared the top 10 blast and reference library hits with phylogenetic clustering from a ML tree. A ML tree with bootstop autoMRE bootstrap support was generated by running RAxML-HPC v.8 on XSEDE on the

Cipres science gateway (Miller et al., 2010) under the GTR evolutionary model with a gamma distribution plus invariant sites. For 28 ZOTUs Arthropod order could not be determined via a DNA reference voucher or phylogenetic clustering and these was marked as undetermined and removed from downstream analysis.

To create an table with ZOTUs abundances for community analyses we mapped a query set of raw reads to the filtered and taxonomically identified search database of ZOTUS in USEARCH v11 (Edgar, 2010) using the otutab command with the default 97% percent similarity mapping threshold. After mapping the number of unique sequences was reduced by 133 ZOTUs to 4197, suggesting that a small portion (3%) of the reads were due to experimental error. We dropped ZOTUs when they occurred with a sequence abundance of fewer than 5 reads, basing this cutoff from the negative control sequencing pool, given that storage of arthropods together likely carried DNA over between pools after they were sorted into size categories (Krehenwinkel, Fong, et al., 2018).

Community analysis

Data aggregation and statistical analyses were performed in R (Team, 2013). We grouped taxa into 14 'ordinal groups' by aggregating orders with few species or with spotty distributions into more inclusive categories for analysis (e.g. Class Myriapoda, Subclass Acari), hereafter referred to as order. To assess the completeness of sampling at each site we plotted species accumulation curves for each site from rarefaction analyses at both the sampling unit level and individual level, using the 'rarc' function in the R package *rich* (Rossi, 2011) with the number of randomizations set to 999.

Separation of native and invasive taxa

Given our interest in exploring the natural patterns of arthropod diversity and evolutionary assembly it was necessary to exclude the species introduced through human activities. Taxonomic assignment ambiguity, due to the lack of a complete barcode reference library, means it is not possible to assign introduction status based on species identification. Although some ZOTUs are straightforward to assign if they are identifiable to genus because they are a part of a large, well-studied, endemic Hawaiian radiation (e.g. *Tetragnatha* spiders), and assignment is straightforward in the case of some taxonomic groups which are wholly non-native at the family (e.g. Braconidae and Formicidae, Hymenoptera) and order (e.g. Blattodea) level, assignment ambiguity is troublesome for the large percentage of ZOTUs. Instead, we use a machine learning approach, to separate out native and non-native taxa for all ZOTUs regardless of taxonomic assignment certainty, based solely on sequence characteristics, based on the premise outlined in Anderson (2019). This machine learning strategy was implemented using random forests in sklearn and packaged with multiple utilities and a graphical user interface in niclassify (https://github.com/tokebe/niclassify). The sequence characteristics show a higher amount of neutral (or otherwise) sequence variation among endemic taxa, as they have evolved from a common ancestor on the islands, when compared to non-native taxa that evolved elsewhere and have no close relatives. By annotating the introduction status for sequences which are identifiable to species level (98% or above match to databases) niclassify can accurately assign status for the remaining sequences.

Role of ecological (immigration) versus evolutionary (adaptation) processes through time

We measured genetic distance as the mean nearest taxon distance for each community using 'nti' in the package *picante* (Kembel et al., 2010). We regressed genetic distance for each site (i.e. each community) by log-transformed substrate age data using a general linear model with gamma distribution. Gamma distribution was chosen because the values of genetic distance cannot be below zero. Because the size of the community will influence the genetic distances measured among taxa, a multiple regression model with number of species as a covariate was compared to a model with just substrate age. AIC scores were used to compare model fit.

Changes in diversity through time and differences between lineages

We compared ZOTU richness data (number of unique ZOTUs per order per site) with the mean age of the substrate for each community to examine patterns of diversity over time. The untransformed substrate age data departed significantly from normality, so comparisons were performed using linear regressions on log-transformed substrate age data, as is customary in the literature (Cowie, 1995; Peck et al., 1999; Gruner, 2007). Because the standard model of species diversity, $s = cA^z$ (MacArthur & Wilson, 1967) is equivalent to $\log s = \log c + z \log A$, there is a linear relationship in the log-transformed data. For completeness, we also grouped ZOTUs into putative species using a Poisson Tree Processes (PTP) model to infer putative species boundaries on a given phylogenetic input tree (Zhang et al., 2013) and regressed species richness (number of unique species per order per site) within orders over time as well, using the same log-transformed substrate age data.

Specialization through time and average community-wide differences between plots within a site

To determine β-diversity for each community we measured the within-site homogeneity with the Sørensen similarity index using 'vegdist' with binary set to true, in the package *vegan* for each of our sampling sites. The Sørensen similarity index uses presence/absence data to determine how much in common are the species in two measured sampling areas (Marion et al., 2017). Because the within site homogeneity may also be related to geographic distance between each site replicate, we calculated within site mean geographic distance using 'st_distance' in the package *sf*. We regressed the mean geographic distance among the site replicates by log-transformed substrate age. We regressed mean geographic distance among site replicates by the Sørensen index for each site. Finally, we did a multiple regression with both mean geographic distance among site replicates and log-transformed substrate age as predictors of homogeneity (Sørensen index) and included an interaction term.

RESULTS

Characterization of taxa with metabarcoding plus DNA reference library material

The number of raw reads and number of ZOTUs before filtering for each size class are presented (Figure S2). After all quality control filtering steps, the final number of ZOTUs was 3517, distributed across six classes: Collembola, Malacostraca, Insecta, Arachnida, Chilopoda, and Diplopoda. There were 2747 endemic ZOTUs and 770 classified as non-native. The barcode reference library increased taxonomic assignment to species or genus level for 401 ZOTUs. Taxonomic assignment was considered trustworthy if the percent similarity of the metabarcoding sequence to the NCBI GenBank or DNA reference voucher was: between 88-94% for family, between 94%-98% for genus and greater than 98% percent similarity for species, while matches below 88% similarity were made only to order. Confident assignment was accomplished for a percentage of ZOTUs at each taxonomic level: Order 99.9%, Family 67.3%, Genus 38.1% and Species 24.9% (Table S1). Data availability DOI (zenodo.org or dryad.org). Of the endemic ZOTUs, Hemiptera were the dominant order, (652 ZOTUs), followed by Araneae (467 ZOTUs), then Diptera (327 ZOTUs) and Coleoptera (266 ZOTUs).

Sampling completeness

The asymptote signature of both the individual based and sampling-unit based rarefaction curves demonstrate that most communities were amply sampled (Figure S3). Not all samples successfully passed through the data acquisition pipeline and the resultant under sampling is reflected in shorter and steeper climbing rarefaction curves at the Hippnet site, LSAG site, Kohala younger lava flow site. These sites were dropped when the site replicates were normalized by sample time. The final number of sample replicates was 50 replicates at eleven sites across four islands.

Role of ecological (immigration) versus evolutionary (adaptation) processes through time

Genetic diversity decreases significantly with log-transformed substrate age (Figure 4). While substrate age is a significant predictor of genetic diversity in the general linear model without number of species, the best model based on AIC score was a multivariate linear regression which included log-transformed substrate, number of species, and an interaction between covariates (AIC -76.555 univariate, AIC -91.685 multivariate). In the multivariate model number of species is the strongest predictor, with substrate age and the interaction of age and number of species significant to alpha <= 0.1.

Changes in diversity through time and differences between lineages

For lineages in which multiple species co-occur at a site reflecting niche differences (Araneae, Coleoptera, Diptera, Hemiptera, and Lepidoptera), we expect that diversity should increase steadily through time. For lineages which show mostly divergence between geographical locations (Orthoptera) we expect no change in diversity given that samples were from a single area. For certain orders (Collembola, Hymenoptera, Malacostraca, Myriapoda, Neuroptera, non-spider arachnids, and Psocoptera) there was insufficient information on levels of co-occurrence to generate predictions. The results matched our predictions in that there is a significant increase in richness over time for Araneae, Coleoptera, Diptera, Hemiptera, and Lepidoptera and no change for Orthoptera. The relationship of richness compared to substrate age is non-linear for Collembola, Hymenoptera, Malacostraca, non-spider Arachnida, and Psocoptera; and there was an increase in diversity through time for Myriapoda and Neuroptera. Linear equations of the correlation between ZOTU richness and log-transformed substrate age for each order are presented (Figure 5). The pattern is predominately the same for richness measured at the putative species level and regressed against log-transformed substrate age (Figure S4). However, when using putative species Neuroptera are not linearly associated and the non-spider Arachnida are significantly linearly associated.

Specialization through time and average community-wide differences between plots within a site

We find that there is a significant decrease in within-site homogeneity over time and thus a significant increase in β-diversity. There was a significant linear relationship of the Sørensen index and log-transformed substrate age ($R2 = 0.2941$, $p = 0.049$; Fig. 6). There was not a significant linear relationship between mean geographic distance among site replicates and log-transformed substrate age ($R^2 = 0.0586$, $p = 0.235$). There was not a significant linear relationship between geographic distance among site replicates and the Sørensen index for each site ($R^2 = -0.0762$, $p = 0.602$). A multiple regression of mean geographic distance among site replicates, log-transformed substrate age, and an interaction between the co-variates ($R^2 = 0.4423$, $p = 0.0721$) yielded no significant predictors of within-site homogeneity.

DISCUSSION

Here we show that metabarcoding whole communities of arthropods provides the opportunity to measure biodiversity across multiple scales (genetic, intraspecific, interspecific) of biological organization. Measuring multiple scales of biodiversity across a time series such as the Hawaiian Islands chronosequence allows an examination into the interplay between ecological and evolutionary processes among arthropods as diversity accumulates and communities assemble. Community structure – from a high-level whole community perspective – changes predictably with time, as the relatedness of communities and within-site β-diversity both significantly increase with substrate age. The patterns of species accumulation for Hawaiian endemic lineages over time are non-uniform, either increasing over time or having no significant relationship with time. Previous work shows how biodiversity accumulation may differ depending on the evolutionary history of a given clade (Gillespie & Baldwin, 2009; Lim & Marshall, 2017). Therefore, we must examine whether these differences in richness accumulation over time are biologically meaningful and how might they relate to the whole community perspective.

Role of ecological (immigration) versus evolutionary (adaptation) processes through time

A transition seems to occur in the relative strength of community assembly processes – from more ‘ecological’ to more ‘evolutionary’ – that we measure as the changing relatedness of biological communities over time. We find that the genetic distances among the nearest neighbor taxa are greatest in the youngest communities and decrease over time (Figure 4). This result is consistent with our prediction that younger communities are influenced by ecological processes; and environmental filtering, dispersal limitation, and species interactions, are all playing a role in successful establishment (Marteinsdóttir & Eriksson, 2014) while older communities have had more time for specialization, adaptation and *in situ* speciation (Gillespie, 2004).

In new environments, such as the bare lava, environmental filtering and changes in the landscape configuration prevent the establishment of species (Belinchón et al., 2019). The idea of an environmental filter, or abiotic factors that prevent the establishment or persistence of species in a particular location, can help quantify the role of the environment in shaping a community (Kraft et al., 2015) and how the role of that changes over time. In the youngest Hawaiian communities that establish on recent lava flow early colonists are adapted to persist under these conditions (Miller, 1960; Atkinson, 1970; Drake & Mueller-Dombois, 1993). The role of environmental filtering can also be context dependent, for example forest age affected the fungal communities associated with ancient forest species, but not the communities associated with early-successional tree species (Boeraeve et al., 2018). Nevertheless, as community age increases the relative influence of abiotic versus biotic factors seems to shift.

At the next stages of community assembly biotic interactions appear to play a larger role in determining species assemblages (Esselstyn et al., 2011; Resetarits et al., 2005; Ulrich et al., 2016). Experiments have shown that even if a community can support more species in the early phase of assembly while most species are rare, as population sizes increase and competition becomes stronger, species numbers drop and more highly coadapted sets of species tend to persist (Simberloff & Wilson, 1969; Wilson, 1969; Simberloff & Wilson, 1970). In many of the most specious orders, diversity is very high at intermediate age and drops off at the oldest aged island; while in less species rich groups the increase is more gradual, leveling off (Figure 5). This is consistent with observations from phylogenetic data of Hawaiian lineages (Emerson & Gillespie, 2008), and it was suggested that in the groups with a steady increase in richness, equilibrium has not yet been reached. The overshoot, with species packing into mid-aged communities, is more pronounced in some of the large high speciation rates lineages (Emerson & Gillespie, 2008).

For many of the Hawaiian radiations, there appears to be no absolute limit to the number of species that can coexist, a question that has long interested ecologists (Cornell & Lawton, 1992; Storch & Okie, 2019). It also seems clear that species recognition must play a role in species packing if sister species are to occupy the same landscape. With *Tetragnatha* spiders on Maui a large number of species and multiple members of the same ecomorph are found on the same volcano and we are just beginning to understand that species recognition in this group occurs through vibration cues and elaborate chemical recognition systems (Adams et al., 2021).

We provide evidence that communities, overall, are composed of more closely related biota as they age because of a shift in the relative importance of *ex situ* (e.g. dispersal) compared to *in situ* (e.g. adaptation) processes for the accumulation of taxa through time. Thus, at a high-level, across taxonomic groups, there is a change in relatedness among individuals at the tips of the tree over time. However, individual arthropod lineages respond uniquely to the ecological and evolutionary mechanisms at work in community evolution, so that the accumulation of biodiversity (e.g. gradual vs rapid) varies considerably among groups, as discussed below.

Changes in diversity through time and differences between lineages

The main groups that show increasing richness over time are Araneae, Coleoptera, Diptera, Hemiptera, and Lepidoptera (Figure 5). This matches our prediction that for lineages with co-occurring species tending to differentiate between niches within a geographic area, we would detect increasing diversity through time. Among spiders, all lineages show low diversity on the youngest island (Gillespie, 2016). Taxa in the spiny-leg (cursorial, no webs) clade of *Tetragnatha* exhibit discrete ecomorphs, with few species on the youngest island, and each of the older islands having a set of four co-occurring ecomorphs (Gillespie, 2004; Cotoras et al., 2018). The genus *Ariamnes* also displays discrete ecomorphs that frequently co-occur at a site. In both genera, ecomorphs show the highest diversity on islands of intermediate age (Maui and Molokai), declining on Kauai (Gillespie et al., 2018).

Intra-island radiation is often associated with shifts in ecological affinity, promoting co-occurrence of species. Patterns of diversification among Tribe Platynini (Family Carabidae) beetles are associated with both environmental shifts between different elevations (Liebherr & Zimmerman, 1998) and between ecologies, with sympatry common, and the component species occupying different ecological microhabitats (streamsides, arboreal epiphytes, moss mats, and under bark) (Cryan et al., 2001). Other radiations (e.g. Proterhinus, Plagithmysus) diverged so widely in food adaptations it can be expected to find species in every endemic woody plant as well as in most of the larger ferns in Hawaii (Zimmerman, 1970; Gressitt, 1975). Among beetles, diversity increases through time, with the highest diversity on the older islands (Gressitt, 1975; Liebherr & Zimmerman, 1998).

Specialization, by partitioning resources (e.g. food, substrate), sexual recognition, or both simultaneously can result in species co-occurrence. Among Diptera, the Hawaiian picture-wing *Drosophila* flies are known for having multiple species co-occurring within a site, separated by differences in sexual recognition (Kaneshiro & Boake, 1987) and ecological affinities (Montgomery, 1975), and with the high diversity on the islands of Maui and Molokai (Carson, 1983; Gillespie & Baldwin, 2009). In the Dipteran Family Pipunculidae, successive radiation combined with island hopping also has led to a peak of diversity on the mid-aged islands, Maui and Molokai (De Meyer, 1993, 1996).

Niche specialization is also linked to species co-occurrence in the oligophagous or monophagus sap-feeding Hemiptera (e.g. planthoppers and leafhoppers) (Swezey, 1954; Zimmerman, 1948b). *Nesodyne* (Family Delphacidae), shows a peak of diversity on Maui and Oahu, with multiple species on each volcano or island, but having differentiated by host plant (Asche, 1997). *Nesophrosyne* (Family Cicadellidae) diversity is again lowest on the young island of Hawaii, with evidence of high speciation rates associated with host plant transitions (Bennett & O'Grady, 2013) and a diversity-dependent decline in speciation on the older islands. Parallel diversification of clades restricted to different host plants suggests a major role of host switches in the diversification process (Bennett & O'Grady, 2013; Goodman et al., 2019) and the ability for congeners to co-occur.

In the Lepidoptera, the *Hyposmocoma* micromoths (Family Cosmopterigidae) are found ubiquitously, including barren areas where there is little other insect life (Zimmerman, 1970). Interestingly, much of the ecological diversity of *Hyposmocoma* appears to have evolved very early on (~15 mya) and more recent diversification is largely driven by the process of dispersal to, and subsequent isolation on, new islands, as opposed to purely being driven by niche partitioning (Haines et al., 2014). As such, some *Hyposmocoma* (e.g. cone-cased) have the greatest diversity on the oldest island of Kauai, with no species on the youngest island (Rubinoff, 2008). In the macrolepidoptera, male secondary sexual characteristics involved with pheromone dispersal in genus *Scotorythra* (Family Geometridae) appear to have evolved multiple times, suggesting an adaptive role for these structures; more species have accumulated on the mid-aged islands in this genus (Heddle, 2003).

One group that does not show increasing richness over time was, as predicted, Orthoptera (Fig. 5). Speciation in *Laupala* crickets (Family Gryllidae) seems to have occurred primarily in allopatry (Shaw, 2002). Closely related species have no ecologically distinguishable features: they are dietary generalists, without host-plant dependency (Shaw, 2002; M. Xu & Shaw, 2020). Most closely related species are not co-occurring (Gillespie et al., 2020); instead after independent colonization of Oahu, Maui, and Hawaii, representatives from each species group established sympatric communities together (Shaw, 2002). The *Banza* katydids (Family Tettigoniidae), are another example of a non-adaptive radiation within Orthoptera, with two minimally differentiated clades across the islands (Shapiro et al., 2006). Both *Laupala* crickets and *Banza* katydids are acoustic insects, with female preference for the male courtship song of their own species allowing some congener co-occurrence (Shapiro et al., 2006; M. Xu & Shaw, 2020).

There was insufficient information on levels of co-occurrence to generate predictions for several orders. High numbers of sampled taxa in Psocoptera, Collembola, and non-spider Arachnida (e.g. Acari, Pseudoscorpiones) provide strong evidence for a lack of accumulation of diversity over time. Within the Psocoptera, the genus *Pycta* (Family Psocidae) contains particularly high diversity on the youngest island of Hawaii, and Maui (Bess, 2011). Bark lice species are generally not very dispersive, and 85% of *Pycta* species are single-island endemics (Thornton, 1984). However, repeated movement between islands, relying on passive wind dispersal (Thornton, 1984) has resulted in back-colonization of younger to older islands (Bess, 2011), altering the pattern of concomitant species accumulation with increasing substrate age. Within Collembola (non-insect arthropods, the so-called "Springtails") the highest richness is again on the youngest island of Hawaii (Christiansen & Bellinger, 1992, 1994). With broad ecological tolerances, these arthropods do not exhibit conspicuous ecological specialization (except for some cave or littoral species). Only two small groups found in Hawaii (Sminthurides, Bourletiella) have any type of courtship (Christiansen & Bellinger, 1992, 1994). Interestingly, in comparison to insects, there is a much higher fraction of families of Collembola represented in Hawaii and a very low rate of speciation (Christiansen & Bellinger, 1994). Regarding the non-spider Arachnida (e.g. Acari, Pseudoscopiones), because of an unequal number of investigated localities on the islands, it is difficult to determine the correlation between island age and the number of species (Nishida, 1994; Niedbala, 1998).

These results are consistent with our predictions concerning richness accumulation. Among the lineages that do display a linear relationship of richness and time one commonality is niche specialization, with sister species co-occurring within a geographic site. As predicted, we detect this as a steady accumulation of diversity peaking on mid-aged and older islands (α-diversity). Among lineages that do not display a relationship of richness and time, lineages are non-adaptively radiating, resulting in non-overlapping distributions of sister species. As predicted, when species mostly differentiate between sites, we don't detect the signal of increasing diversity through time (β-diversity).

Specialization through time and average community-wide differences between plots within a site

Regarding the cumulative diversity of arthropod communities, our approach documents decreasing within-site homogeneity over time (Figure 6). Site homogeneity, measured using the Sørensen index, is a direct way of measuring the number of species that are shared, or turnover, within each site, without taking into account the abundance of taxonomic groups (Marion et al., 2017). Patterns of site-to-site variation in species composition, known as β-diversity, can provide fundamental insights into the processes that create and maintain biodiversity (Anderson et al., 2011; Kraft et al., 2011). Because β-diversity is known to scale consistently with geographic distance (Harrison et al., 1992) (Harte et al., 1999; Krishnamani et al. 2004) we confirmed that it was not a contributing factor in the decreasing within-site homogeneity over time.

For arthropods, fine scale habitat utilization (e.g. at the plant level) can lead to higher levels of β-diversity on the older islands as the result of allopatric speciation with little secondary contact or greater spatial dispersion among otherwise similar species. Using published data (Craven et al.,

2019) found a similar change in β-diversity for woody plant species over the Hawaiian archipelago; older islands (Kauai, Oahu) had a greater number of species and were more spatially clumped indicating they have higher within-island β-diversity than younger islands. Although the link between β-diversity and community evolution has not been well studied, the correlation of increasing β-diversity with increasing productivity is well established (Chase & Leibold, 2002; Harrison et al., 2006; Bai et al., 2007). Higher β-diversity at higher productivity sites may result from a stronger role for stochastic (e.g. ecological drift, dispersal limitation, and differential colonization/extinction) relative to deterministic (e.g. different niches to which different groups of species are favored) dynamics across localities (Chase, 2010); a similar mechanism may be at work in the link between increasing β-diversity and community evolution.

Utility of approach

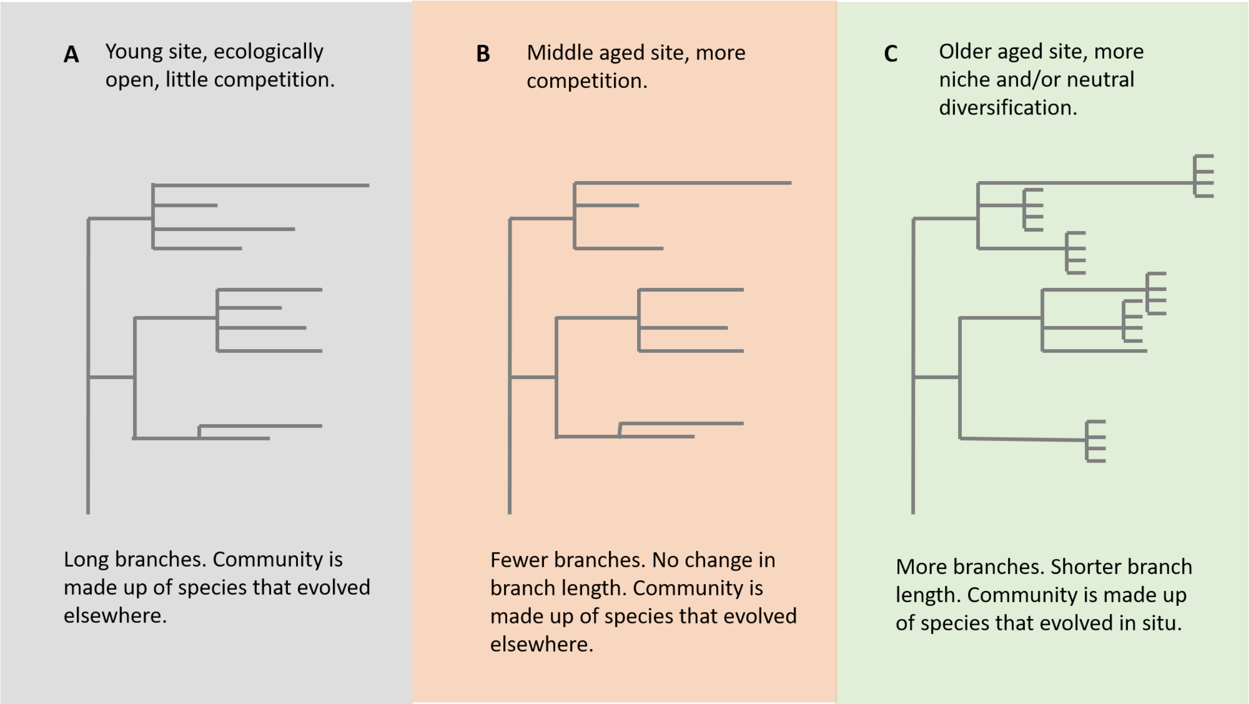
Here we show here the utility of metabarcoding data for community ecology. This powerful biodiversity assessment method captures phenotypically cryptic taxa, is inclusive of taxa from all life stages, and characterizes hyperdiverse biota encumbered by a lack of taxonomic descriptions. Coupled with time series data such comprehensively sampled community datasets provide an unparalleled opportunity to assess community assembly mechanisms over extended spatial and temporal scales. The power of a *de novo* island chain for investigating community assembly is the set of predictive constraints surrounding island formation, growth, senescence, and the corresponding soil nutrient availability, productively, habitat complexity, and biotic diversity (Cowie, 1995; Peck et al., 1999; Gruner, 2007; Lim & Marshall, 2017; Leopold et al., 2020). Metabarcoding data evaluates the consistency of these patterns over a nearly comprehensive sampling of arthropod lineages simultaneously, including taxonomically neglected groups (e.g. Acari), so patterns of diversity accumulation can be corroborated across large scales of space and time.

We recognize some noise may remain after bioinformatics processing that can inflate biodiversity estimates with metabarcoding. First, when working with bulk specimens collected in ethanol there will be some contamination of low coverage sequences among sequencing pools, especially carry over from soft bodied tissue specimens (Krehenwinkel, Fong, et al., 2018; Zenker et al., 2020), and best practices to remove these do not guarantee complete erasure. Second, there will be ZOTU inflation if nuclear mitochondrial like sequences (NuMts) remain running metaMATE (Andújar et al., 2021) to remove putative pseudogenes. Heteroplasmy, the presence of more than one type of mitochondrial genome, can also lead to differences in sequencing outcomes and ZOTU inflation. The methodology will be improved by practices which reliably recover quantitative data (Lamb et al., 2019), including specific primer choice (Andújar et al., 2018; Marquina et al., 2019), or alternatives such as PCR free methods or bait capture enrichment (Gauthier et al., 2020). Improvements to DNA voucher libraries (Leray et al., 2019) and a growing database of biotic interactions (Compson et al., 2018) and network analysis (Evans et al., 2016) will contribute to the utility of the approach for answering basic and applied questions about biodiversity dynamics rapidly and accurately. Rapid assessment of biodiversity dynamics is demanded by the speed of modern global change phenomenon (Loreau et al., 2001; Deiner et al., 2017; Graham et al., 2017).

Conclusions

Our results highlight the utility of examining community assembly processes for multiple lineages simultaneously, across large spatial and temporal scales. Past work that was lineage specific (e.g. Emerson & Gillespie, 2008) had limitations because it was only a sampling of radiations with phylogenetic data. By employing a short sequence of DNA, we can visualize changes across all coexisting lineages, regardless of how well studied they may be, across multiple levels of biological organization. We show that community assembly occurs through processes that change through time. Initially, processes are acting over the short-term to fill empty space in ecologically open communities. Over longer-time scales, ecological processes give way to evolutionary, with diversification of lineages or the splitting of geographic space. We highlight a gap in understanding of long-term biodiversity dynamics that requires a view of the whole community simultaneously.

Figures



A Young site, ecologically open, little competition.

B Middle aged site, more competition.

C Older aged site, more niche and/or neutral diversification.

Long branches. Community is made up of species that evolved elsewhere.

Fewer branches. No change in branch length. Community is made up of species that evolved elsewhere.

More branches. Shorter branch length. Community is made up of species that evolved in situ.

Figure 4. Hypothesis 1 infographic. Genetic relatedness of taxa at the tips of the phylogeny – i.e. the nearest neighbor taxa—should change predictably with age. A few long branches will represent the collection of taxa at youngest community. As the importance of interactions increases competition will remove some branches. After enough time there will be an accumulation of closely related species at the tips due to in situ speciation.

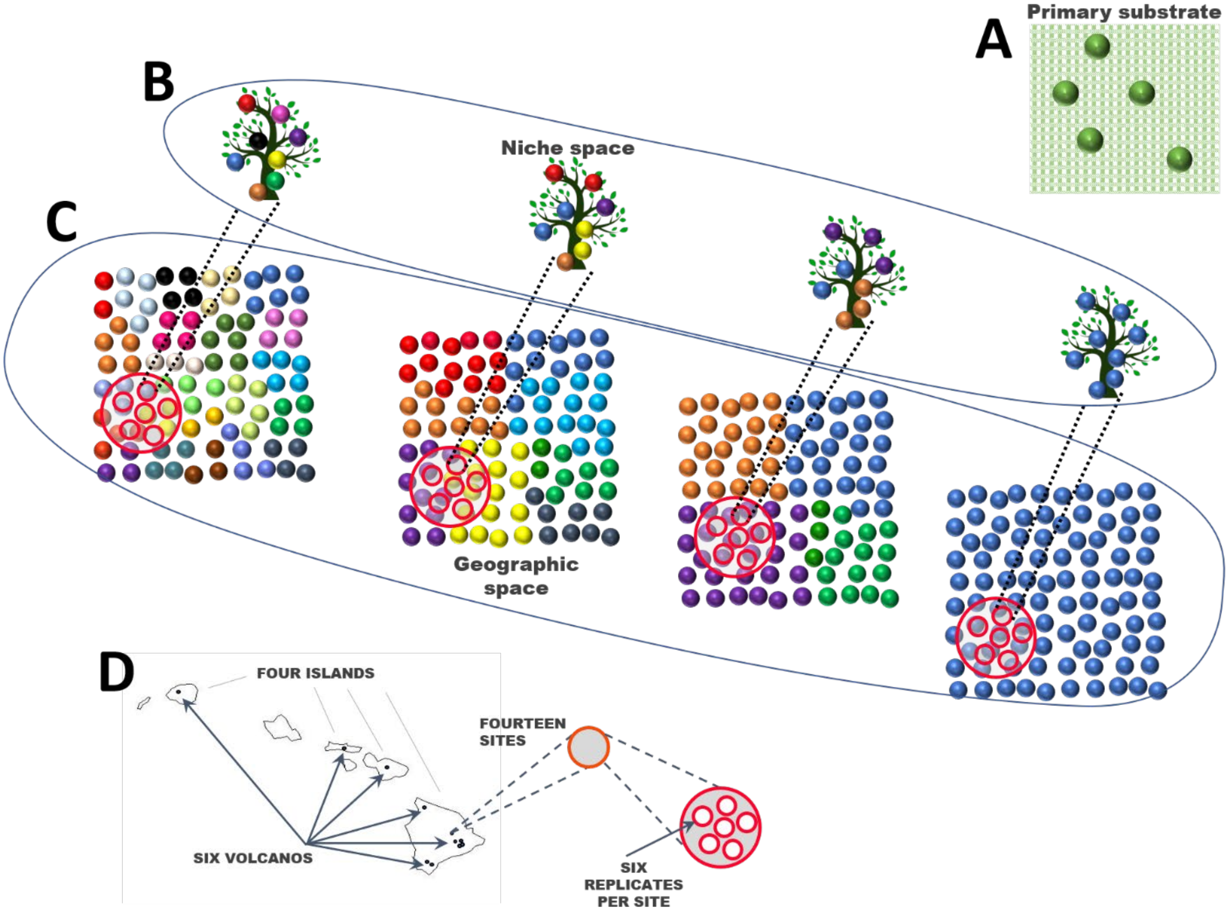


Figure 5. Hypothesis 2 infographic. A. A strong environmental filter limits the number of species that establish at the youngest site B. Change in α-diversity at a site: Diversity accumulates through diverging sister species at a site. Expected to increase quickly, with the increase detected within a site. May level off with time or keep increasing C. Change in β-diversity between sites: Diversity accumulates through specialization to a given environment. Expected to increase gradually, but no change detected within a site except at the oldest sites. May keep increasing through time. D. Overview of sampling scheme depicting the replication of collection events within each site across the archipelago.

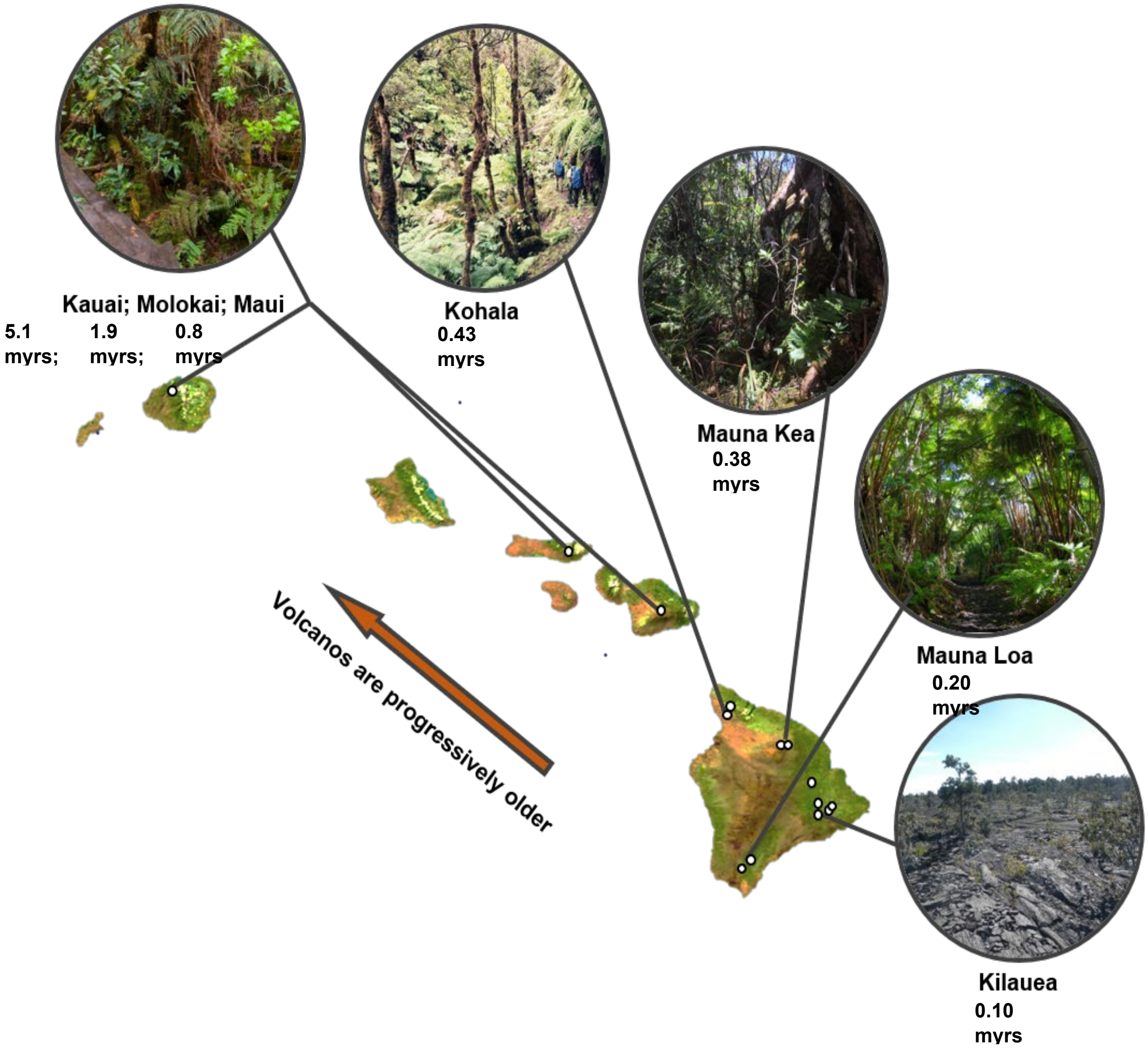


Figure 6. Sampling sites. Fourteen sites across a substrate age gradient on four islands across the Hawaiian archipelago. Sites were selected for continuity in elevation, temperature and rainfall patterns, and intact native Hawaiian rainforest.

Figure 7. Genetic diversity. Model of genetic distance and number of species regressed with log-transformed substrate age. Significance values: log-transformed substrate age $p = 0.0723$ and number of taxa $p = 0.0015$ and the interaction term $p = 0.069$. The genetic distance of nearest neighbor taxa (i.e. relatedness of taxa at the tips) decreases linearly with log substrate age.

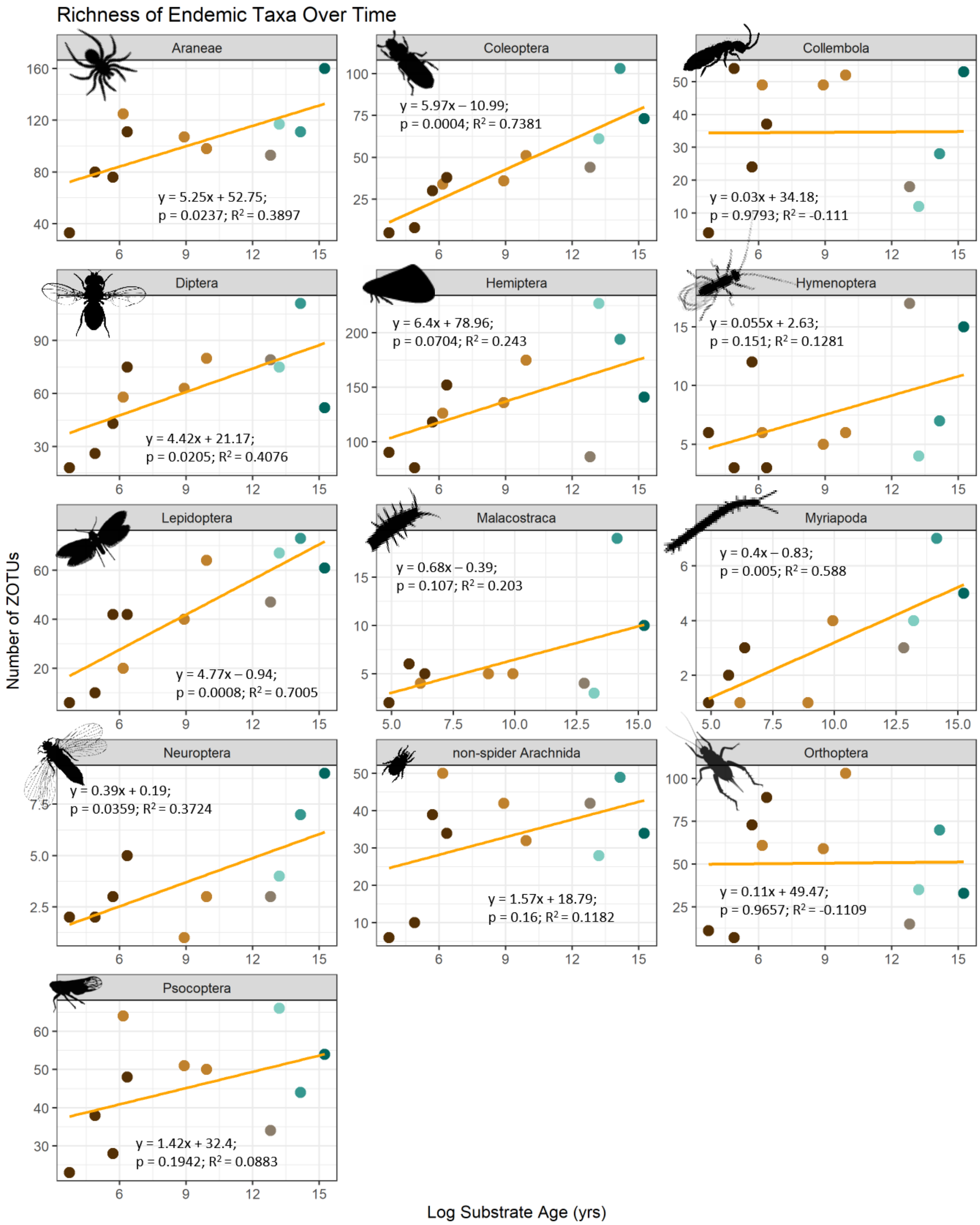


Figure 8. Richness over time. Correlation of taxon richness, measured as the number of unique ZOTUs from each order at each site, and the log-transformed substrate age of each community. Linear equations with significance values for regressions are presented along with adjusted R^2 values. Many arthropod orders (Araneae, Coleoptera, Diptera, Hemiptera, Lepidoptera, Myriapoda, Neuroptera) show a significant increase of richness with community age ($p<0.05$; Hemiptera $p=0.07$). Richness in other groups (Collembola, Hymenoptera, Malacostraca, non-spider Arachnida, Orthoptera, Psocoptera) does not accumulate linearly with time.

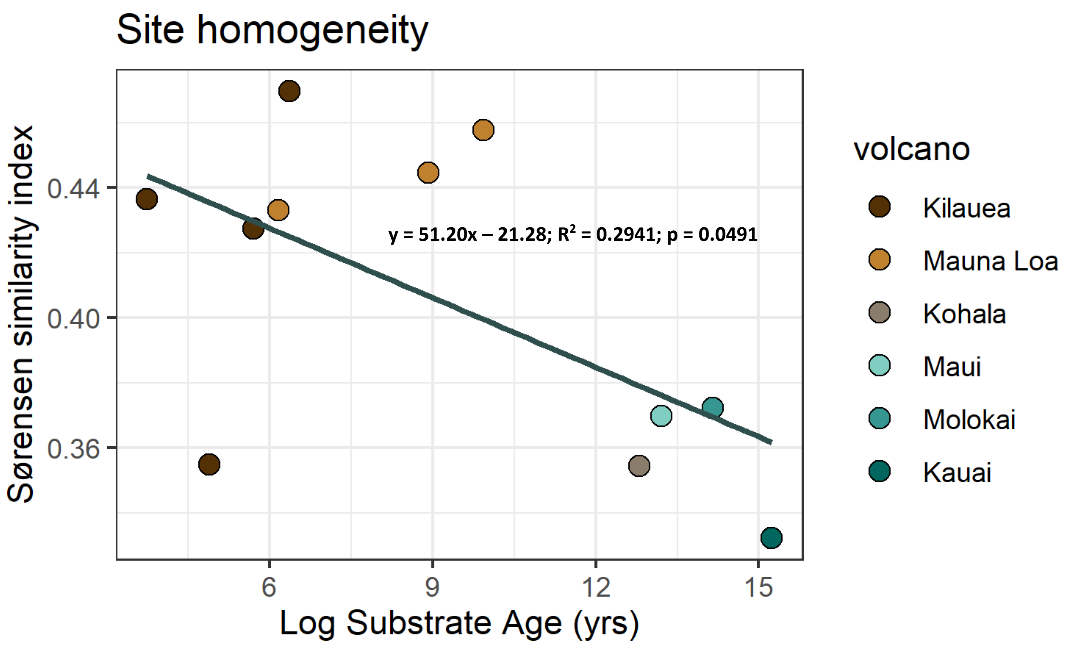


Figure 9. β-diversity over time. Correlation of within site homogeneity, measured using the Sørensen similarity index, and log-transformed substrate age at each site. There is a significant decrease in within site homogeneity over time, indicating that β-diversity at the local scale is increasing as communities age.

In the next chapter I discuss how species abundance distributions (SADs) are changing over time. I compare the observed species abundance distributions with the expected distributions under the theory of maximum entropy theory of ecology (METE). Deviations from METE can help predict where communities are most rapidly changing. I explore the deviations in SADs for communities under natural dynamics (without non-native species) and under disturbed dynamics due to anthropogenic effects. I show that community steady state differs depending on the presence of non-native taxa, and that METE is useful for understating community dynamics over long-term temporal scales of community assembly.

Chapter 3

An empirical test of steady state in ecological communities over evolutionary time

N. R. GRAHAM[1*], H. KREHENWINKEL[2], A.J. ROMINGER[3], J. HARTE, R. G. GILLESPIE[1]

[1] Department of Environmental Sciences Policy and Management, University of California Berkeley, Mulford Hall, Berkeley, California, USA
[2] Department of Biogeography, Trier University, Faculty of Regional and Environmental Sciences, Trier 54286, Germany
[3] School of Biology and Ecology, University of Maine, Murray Hall, Orono, Maine, USA
[4] Energy and Resources Group, University of California, Berkeley, Berkeley, CA, United States

*Corresponding author
Email: n.graham@berkeley.edu

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INTRODUCTION

The idea that species richness is dictated by equilibrial processes has been demonstrated for systems covering temporal scales (ecological to geological) and spatial scales (Chesson, 2000; Tilman, 2004; Rabosky, 2009; Alroy, 2010; Harte, 2011; Hubbell, 2001) and form the basis of many theories in biodiversity dynamics (Hubbell, 2001). Thus, large-scale diversity gradients in latitude (Pontarp et al., 2019) and elevation (M. V. Lomolino, 2001; McCain & Grytnes, 2010) are well known patterns with multiple causes related to climatic features (Currie, 1991; Hawkins et al., 2003), topographical heterogeneity (Davies et al., 2007) and time or stability (Jetz & Fine, 2012). In all of these, the concept of diversity limits plays a central role. This limit is not rigid, but is rather a stable equilibrium of the dynamics (Storch & Okie, 2019).

To be able to infer equilibrium dynamics, the structure of ecological communities needs to be quantitatively and systematically described. Conducting controlled experiments on ecosystems at large spatial scales and over long time-periods is generally not feasible. Hence our knowledge of large-scale phenomena, such as effects of global climate change on biodiversity, is often based on observed correlations over time or over spatial gradients. At relatively small spatial and temporal scales controlled manipulation experiments are often carried out on ecosystems, unfortunately the theory needed to scale the consequences up from plots to biomes, and from years to millennia, has been lacking, as has our understanding of emergent mechanisms that may only operate at these larger scales. There are three well known macroecological patterns to explain species diversity: the species abundance distribution (SAD), the species area relationship (SAR), and the metabolic relationship between energy and body size. These are not independent and both process-based neutral theories (Rosindell & Cornell, 2007; Hubbell, 2001) and statistical approaches that look at properties arising from constraints imposed by state variables (Brown et al., 2004; Harte, 2011) are utilized for predictions. Neutral theories can predict species-abundance distributions and species-area relationships by assuming equal demographic rates across individuals regardless of species identity. The predictive success and mechanistic simplicity of this approach has allowed exploration of a wide range of ecological patterns (Etienne & Olff, 2004; Wootton, 2005; Rominger et al., 2009; Rosindell & Phillimore, 2011), with deviations from the theory being used to understand additional mechanisms needed to make a more accurate, universal theory.

Other unified theories are, in contrast, largely statistical, with predictions for emergent macroecological patterns in which the influence of individual processes disappears within the context of large and complex systems. Thus, the attributes of equilibrium can be predicted by maximizing information entropy relative to the constraints of the state variables. These theories include the metabolic theory of ecology (MTE) (Brown et al., 2004) and the maximum entropy theory of ecology (METE). With the development of METE, Harte et al. (2008, 2009) showed that maximum information entropy inference procedure (Jaynes, 1957, 1982) can be used as a theoretical framework for predicting relatively accurately the macroecological metrics describing the abundance, spatial distribution, and energetics of species in an ecological community. Here, we focus specifically on the maximum entropy theory of ecology (METE) because of its broad utility in showing that patterns such as species-abundance distributions or species-area relationships, derive from the universal operation of statistical laws. METE allows the derivation of all the major metrics of macroecology from prior knowledge of state variables S (number of species), N (number of individuals), E (metabolic rate summed over individuals), and A (area of system), in loose analogy with thermodynamics, where the state variables pressure, volume, temperature and particle number characterize a system. These metrics include the relative abundance distribution of the species, the species-area and endemics-area relationships, the distribution of metabolic rates (or body sizes if a mass-metabolism relationship is assumed) across individuals and species, ecological network structures, and the spatial distributions of individuals within species. Tests of the theory using census data for plants, birds, and arthropods from a variety of habitats and over spatial scales ranging from square meters to thousands of square kilometers indicate that the theory generally predicts the major features of observed patterns (Harte et al., 2008, 2009; White et al., 2012; Harte & Kitzes, 2015; Xiao et al., 2015; Harte et al., 2017). While this theory's predictive success is well demonstrated for species abundance distributions and species area relationships at a single point in time (Harte et al., 2008; Harte & Kitzes, 2015; White et al., 2012), some systematic discrepancies are noted for communities in rapidly evolving communities (Supp et al., 2012; Rominger et al., 2016; Kunin et al., 2018; Franzman et al., 2021). In particular, when state variables are changing as a consequence of succession or anthropogenic disturbance, the values of the state variable at any moment in time no longer accurately predict the shapes of the macroecological metrics at that same moment in time; but see Harte et al. (2021) for an extension of METE that deals with dynamic systems. Deviations from the predictions of theory can be used as an indicator of disturbance or rapid change (Harte, 2011; Harte et al., 2021).

The behavior of METE over evolutionary time scales, i.e., when species arrive through speciation rather than immigration, is largely unknown. However, by understanding whether it behaves in the same way over extended evolutionary time, as it does over short-term ecological change, will allow insights into how invasive species infiltrate a given ecosystem. On such longer time scales METE is expected to work well in relatively static systems, failing in systems changing relatively rapidly. In order to examine this question, we focus on the Hawaiian Islands where the pre-human rate of arrival of colonists was extremely low (MacArthur & Wilson, 1967; Emerson & Gillespie, 2008) and many current lineages diversified on the islands since the formation of Kauai ~ 5 mya (Price & Clague, 2002). We also take advantage of the natural geological formation of the Hawaiian Islands, where the age of the substrate after deposition of lava can be used as a reliable proxy for community age (Vitousek, 2002). The evolutionary history of many lineages has mirrored the formation of the Hawaiian Islands (Funk & Wagner, 1995) and much of the diversity has been derived from speciation. Preliminary analysis of the behavior of METE across the Hawaiian chronosequence using data on arthropod richness and abundance showed that deviation was greatest at an intermediate aged site (Harte, 2011), although data from degree distributions of insect-plant associations find the greatest deviation from the METE expectations at the youngest and oldest sites rather than at intermediate aged site (Rominger et al., 2016). The current study uses the changing landscape of the Hawaiian Islands to provide detailed insights into the behavior of METE among communities where speciation plays a dominant role in the addition of taxa, to understand systematic departures of theory from observed species abundance distributions over long-term community evolution. We measured the richness and abundance of arthropods in communities in different stages of development from ~50 years old to ~4.15 million years. We focused on native forest sites with similar elevation and precipitation, that were dominated by native *Metrosideros polymorpha* forest, and differed only in age.

We characterized the diversity in each arthropod community by employing high-throughput sequencing, specifically DNA metabarcoding, which allows species delimitation and identification for whole communities simultaneously (Taberlet et al., 2012; Yu et al., 2012). High-throughput DNA sequencing provides a method to accurately sample the biodiversity of a system without bias. In particular, all individuals, regardless of size and life stage, can be detected and identified. Moreover, by accommodating for issues such as taxon size and primer bias, we can generate estimates of the relative abundance of molecular operation taxonomic units (OTUs) at a site (Krehenwinkel, Wolf, et al., 2017). Thus, we can compare biodiversity dynamics with time series data over ecological and evolutionary timescales, across organisms of different trophic levels and functional groups, and with broad taxonomic coverage (Bálint et al., 2018). Moreover, metabarcoding data allows us to separate our dataset according to whether a given OTU is native or non-native, due to the genetic distances associated with time periods spent within the archipelago (Andersen et al., 2019).

We used METE to test the macroecological properties of communities across the Hawaiian chronosequence. Given that deviation from METE should be greatest when a community is under the most rapid change, our expectations were as follows: (1) Under natural dynamics, prior to human arrival, we expect the greatest deviation from METE to occur at middle-aged sites in which there has been sufficient time for evolutionary change. Prior to human arrival, colonization rate from outside the archipelago would have been almost zero, the immigration of colonists to new habitat at the youngest sites will have been limited to a few arrivals of native taxa from other parts of the island, with much of the addition of taxa to a given community occurring through adaptation and speciation, coupled with extinction, which is most pronounced on the island of Maui (Gillespie & Baldwin, 2009; Gruner, 2007). (2) Under modified dynamics after human arrival, the immigration of non-native colonists is high, and these novel colonists settle most quickly in the youngest sites with the lowest diversity, resulting in a tighter fit to the METE predictions across all sites. Specific deviations in SAD shape inform on ecosystem dynamics (Harte, 2011; Harte et al., 2021). We expect any significant deviation from METE, based on the shape of the observed SAD compared to the predicted log-series shape SAD, to inform on where the community change is occurring. The METE predicted Fisher log-series shape for species abundance distributions is a straight line for intermediate and rare taxa, with an up-turn for the most common taxa. Thus, specifically we will inspect systematic deviations at the tails of the distribution, as well as at intermediate abundances where the shape of the observed SAD will be flatter where METE underpredicts (more species observed than predicted) and steeper where METE overpredicts (fewer species observed than predicted).

To get at these predictions, we used DNA metabarcoding data, separating OTUs according to native and non-native, and then used log(abundance) vs. species rank graphs to display SADs. The ability to use METE as a predictive tool to assess community ecosystem health has so far not depended on the separation of native and non-native taxa, however, since most tests of METE have been over ecological time, this has perhaps been less relevant. It will be instructive to see if separating the censused community by introduction status has a predictable effect on understanding deviations from steady state in natural vs perturbed systems.

METHODS

Site selection and collection protocol

Site selection and specimen collection was performed as described in Chapter 2. Briefly, a replicated series of fourteen sampling sites across the Hawaiian Islands was selected. Each site was within a narrow band of elevation (1000-1300 m) and hence temperature (14-17ºC), and precipitation (rainfall 2500-3000 mm), and all were within forests characterized by the dominant canopy tree in Hawaii, *Metrosideros polymorpha*. This selection of sites allowed us to hold abiotic and biotic factors affecting community formation as constant as possible and focus on the effect of age, with sites spanning a gradient of substrate ages from 48 years to 4.15 million years old (Chapter 2, Figure 3). We combined data layers on vegetation classification, satellite and airborne imagery including LiDAR, and land use and degradation, to classify and prioritize sites with differing geological histories. We excluded sites showing signs of invasive plant establishment. We collected arthropods under individual plants using quantitative vegetation beating at six replicate 15 m radius plots per site during May 2015 through January 2016. Each plant-associated arthropod community sample was transferred to a 2 ml vial containing 95% ethanol and stored at -20 °C. We dropped site replicates if the sampling time for the replicate was within one standard deviation of the mean replicate sampling time, resulting in a total of 50 replicates and 11 sites (Chapter 2, Figure S1).

DNA sequencing and analysis

The processes of DNA extraction, DNA sequencing, and DNA analysis were performed as described in Chapter 2. Briefly, each plant beating sample was sorted into four size categories (0-2 mm, 2-4 mm, 4-7 mm, 7 mm and up) in the lab under a stereoscope, with each sorted sample placed into a well of a 96-well plate (Krehenwinkel, Wolf, et al., 2017). Larger animals yield more sequencing reads, and sorting to rough size categories allows an accurate recovery of relative abundances. The Collembola were processed separately, in parallel to the remaining arthropods, because they were in very high abundance to other taxa in the 0-2 mm category. Specimens from public and private collections were used to generate a 912 sequence DNA barcode reference library (Accession numbers XXX-XXX). Genomic DNA extraction of size sorted community samples was performed in 600 ul volumes using the Tissue protocol described in the Qiagen Puregene kit modified for automation. Each sample was amplified with a primer pair combination (ArF1/ Fol-degen-rev) (Gibson et al., 2014; Yu et al., 2012) that targets a 418 bp fragment in the barcode region of the Cytochrome Oxidase I (COI) gene. We previously have shown that by using a degenerate primer combination we can recover an accurate representation of Hawaiian taxonomic diversity (Krehenwinkel, Wolf, et al., 2017). Library preparation followed that described in Lange et al. (2014) with the modifications listed in Chapter 2. Indexed products were pooled, cleaned with SPRI beads, and quantified using qPCR, then sequenced on an Illumina® MiSeq using V3 (600 cycles) chemistry and 2 × 300 bp reads according to the manufacturer's protocol (Illumina, San Diego, USA). We aimed for a total of 30,000 reads per sample. Sequence libraries were demultiplexed, paired reads were merged (Zhang et al., 2014), quality filtered (≥90% of bases ≥Q30) and transformed into fasta files (Gordon & Hannon, 2010). The resulting fasta files were demultiplexed by amplicon primer and 6 bp inline barcode combination, using the forward and reverse primer sequences as indices with the grep command in UNIX and primer sequences were then trimmed using the UNIX stream editor.

Rarefaction, pseudogene removal, and ZOTU abundances

We rarefied each sample using a custom script that drew from the total reads of the metabarcoding analysis a number of reads that was equivalent to the numerical abundance of individual arthropods counted into each well of the 96-well plate, repeating the draw of sequences 100 X with replacement. The process of rarifying by repeating the random draw based on the expected individual specimen abundances, will help correct for any disproportionate abundance of sequences that accumulate for larger specimens compared to smaller specimens within each size class. We generated biologically relevant zero-radius OTUs (ZOTUs) from the rarefied raw reads with the unoise3 command (Edgar, 2016) following the recommended protocols in the USEARCH v11 pipeline (Edgar, 2010). We assigned taxonomy and removed non-Arthropod ZOTUs using a blastn search on a local nucleotide database downloaded May 2020 with BLAST+ (Camacho et al., 2009) and our custom DNA reference library for Hawaiian taxa. To remove putative pseudogenes from the ZOTU dataset we ran metaMATE (Andújar et al., 2021) with default specifications and the example specifications file. Using the output of metaMATE we applied the least stringent Numt removal strategy so that we could retain as many putatively true ZOTUs as possible (Graham et al., 2021). By rigorously rarefying and removing pseudogenes, molecular data measuring richness and abundances across sampled communities can be used for species abundance distributions comparisons under METE. To create a table with ZOTUs abundances (i.e. the relative sequence abundance values for each unique ZOTU) for community analyses we mapped a query set of raw reads to the filtered and taxonomically identified search database of ZOTUs in USEARCH v11 (Edgar, 2010) using the otutab command with the default 97% percent similarity mapping threshold. We dropped ZOTUs when they occurred with a sequence abundance of fewer than 5 reads, basing this cutoff from the negative control sequencing pool, given that storage of arthropods together likely carried minute amounts of DNA over between pools after they were sorted into size categories (Krehenwinkel, Fong, et al., 2018).

Separation of native and invasive taxa

To separate the species introduced to the islands through human activities we use a machine learning approach that can discriminate introduction status for ZOTUs based on sequence characteristics, according to the premise outlined in Anderson et al. (2019). This machine learning strategy was implemented using random forests in sklearn and packaged with multiple utilities and a graphical user interface in niclassify (https://github.com/tokebe/niclassify). The sequence characteristics show a higher amount of neutral (or otherwise) sequence variation among endemic taxa, as they have evolved from a common ancestor on the islands, when compared to non-native taxa that evolved elsewhere and have no sister species within the islands. By annotating the introduction status for sequences which are identifiable to species level (98% or above match to databases) niclassify can accurately assign status for the remaining sequences.

ZOTU abundance distributions

Data aggregation and statistical analyses were performed in R (Team, 2013). Given our interest in evaluating community evolution over extended time we separated the data into three groups 1) ‘native taxa only’; 2) ‘non-native taxa only’; and 3) ‘all taxa’. To obtain insight into community structuring we plotted rank abundance curves, for the ‘native only’ and ‘all taxa’ datasets, using the number of ZOTUs and the sequence abundance for each ZOTU at each replicated plot. We evaluated how the observed species abundance distribution (SAD) compared to a null model for each community based on expectations laid out under the theory of MaxEnt Theory of Ecology (METE, Harte 2011). Using the *meteR* package (Rominger and Merow 2017), we first calculated the ecosystem structure function, the core probability distribution from which all predictions arise, from the state variables describing arthropods, where total number of species, S, was the number of ZOTUs and the summed number of individuals, N, was the sequence abundance of each of those ZOTUs. METE’s predictions generally fail in ecosystems undergoing relatively rapid change, therefore, to evaluate model fit, we compared the observed likelihood of the data given the METE model to simulated likelihoods produced by simulating 100 communities from the fitted METE object and calculating their likelihoods. The simulated distribution represents hypothetical likelihoods for data sets obeying METE.

Overall discrepancy from METE predictions, depending on inclusion/exclusion of non-native species

To evaluate which stages of community formation were deviating the most from predictions of steady state we calculated the mean summed-over-rank squared deviation of data from prediction, or relative mean squared error (MSE), using the ‘mse’ function with type ‘rank’ and the arguments ‘relative’ set to true and ‘log’ set to false. To exemplify at what stage of community development the community is changing most rapidly including/excluding non-natives we visualized change in this ‘badness of fit’ measure over natural log transformed substrate age for the ‘native taxa only’, ‘non-native taxa only’, and ‘all taxa’ datasets.

Systematic deviations at tails of the species abundance distributions – most common taxa & rarest taxa

We then compared the fit of the predicted to the observed ZOTU abundance curves (for the ‘native taxa only’ and ‘all taxa’ datasets) for the most common species, least common and intermediate abundances. Specifically, for the most common species we judged if the observed or the predicted was highest in abundance on the curve. Because we use log(abundance) vs. species rank graphs to display SADs we can refer to the "length" of the horizontal line giving the number of ranks (i.e. species) for a given abundance. For the rarest species (singletons, doubletons, tripleton, quadrupletons, quintrupleton) we measured the length of the predicted values compared to the observed values. We visualized the discrepancy of the observed data from the SADs predicted under METE for the most common and rarest species by summarizing the differences per volcano by increasing community age.

Systematic deviations at the middle values of the species abundance distributions – intermediate abundance taxa

Last, we measured the fit of the predicted to the observed ZOTU abundance curves (for the 'native taxa only' and 'all taxa' datasets) for the intermediate abundances by recording the upper and lower limits of the abundances where the observed was flatter or steeper than the predicted. When the observed data is flatter there are more data points for those abundances, demonstrating that METE underpredicted for those abundances. When the observed data is steeper there are fewer data points for those abundances, demonstrating that METE overpredicted for those abundances. We visualized deviations from the log-series SADs predicted under METE for the intermediate abundance species by summarizing the differences per volcano by increasing community age.

RESULTS

Characterization of communities with DNA metabarcoding

The number of raw reads and number of ZOTUs before filtering for each size class are presented (Chapter 2, Figure S2). After all quality control filtering steps, the final number of ZOTUs was 3517, distributed across six classes: Collembola, Malacostraca, Insecta, Arachnida, Chilopoda, and Diplopoda. The barcode reference library increased taxonomic assignment to species or genus level for 401 ZOTUs. There were 2747 ZOTUs classified as native and 770 classified as non-native distributed across the archipelago, with the youngest sites having a greater relative proportion (>25%) of non-native taxa (Figure 1). Data availability DOI (zenodo.org or dryad.org).

ZOTU abundance distributions

Rank abundance curves are presented for each site replicate, separately for the dataset with native taxa only or all taxa (Figure S5). The rank of the ZOTU (i.e. how common it is at the site replicate) is on the x-axis and the log-abundance (here the log of sequence abundance for that ZOTU) is on the y-axis.

Overall discrepancy from METE predictions, depending on inclusion/exclusion of non-native species

Plotting MSE by log-transformed substrate age shows that the fit of observed data to METE predictions differs when including or excluding non-natives (Figure 2). When non-natives are excluded (i.e. the 'native taxa only' dataset) the deviation from model expectations is greatest in middle-aged communities (Kohala volcano on Big Island ~365,000 yo and Waikamoi on Maui ~545,000 yo). When natives are excluded (i.e. the 'non-native taxa only' dataset) the deviation from model expectations is greatest in the youngest communities, especially the ~50 yo community of 1973 lava flow. The fit is reasonably good for most aged communities when all taxa are included (i.e. the 'all taxa' dataset).

Systematic deviations at tails of the species abundance distributions – most common taxa & rarest taxa

For the 'all taxa' dataset METE very often underpredicts the most common taxa in youngest communities (< 20 kya), and there are very few instances where METE overpredicts or where theory and data agree for the all taxa dataset (Figure 3A). For the 'native taxa only' dataset METE most often underpredicts the most common taxa (actual observed abundance greater than predicted) in youngest communities (< 20 kya), but there are some site replicates where METE overpredicts or where theory and data agree (Figure 3B). In summary, the theory almost always underpredicts for the most common taxa when non-natives are included.

For both the 'native taxa only' dataset and the 'all taxa' dataset, METE always overpredicts for singletons and this deviation of observed to predicted for singletons is greatest in the communities on the middle-aged islands (Molokai, Maui) (Figure 4). For the other rare species, the observed data is close to matching the abundance values predicted under METE. There is more spread in the data for doubletons, quadrupletons, quintrupleton and the frequency of the data is more concentrated for singletons and tripletons. In summary, for the rare taxa, there is not much difference between the analysis that included non-native taxa (the 'all taxa' dataset) compared to analysis that excluded non-native taxa (the 'native taxa only' dataset), with systematic deviation from METE predictions of rare taxa occurring only for the singletons.

Systematic deviations at the middle values of the species abundance distributions – intermediate abundance taxa

For the 'all taxa' dataset, there are more species observed than predicted (flatter than log-series shape, METE underpredicts) for the lower abundances and fewer species observed than predicted for the higher abundances (steeper than log-series shape, METE overpredicts) (Figure 5, Figure 6). For the 'native taxa only' dataset, again, there are more species observed than predicted in the lower abundances (flatter, METE underpredicts) and there are fewer species observed than predicted in the higher abundances (steeper, METE overpredicts) (Figure 5, Figure 6). The abundances are much higher in the 'all taxa' dataset than in the 'native taxa only' dataset. In summary, for both datasets, the theory tends to predict fewer species than observed at the high abundances and more species than observed at the lower abundances, so that, in total, the distribution seems to veer from the predicted log-series shape in the direction of a lognormal.

DISCUSSION

Here, in the first high-throughput sequencing characterization of macroecological variables across an evolutionary time series, we examine the expectations of steady state in ecological communities under the principles of the maximum entropy theory of ecology (METE) (Harte et al., 2008; Harte, 2011). As predicted, the deviation of METE in communities in different stages of development depends on whether or not non-natives are included. When we analyzed the data with native taxa only, systematic deviation was greatest in the middle-aged communities. When we analyzed the combined data, including both native and non-native taxa, we found that the data fit the predictions of METE equally well across the archipelago, which conforms to our expectations that after the introduction of non-natives natural communities reach steady state more rapidly.

Expectations for 'naturally occurring' communities without non-native taxa

While our sites were entirely in high elevation native forest in the islands, with no evidence of invasion based on plant composition, we found a number of non-native arthropods at all sites (Figure 1). These taxa were removed bioinformatically (Andersen et al., 2019) in order to assess how exclusively native communities are changing. When non-natives are excluded the deviation from model expectations is greatest in middle-aged communities (Kohala volcano on Big Island ~365,000 yo and Waikamoi on Maui ~545,000 yo), matching our expectation that once enough time elapsed for evolutionary processes to influence species accumulation, the changing state-variables would cause METE to perform less well (Figure 2). This result agrees with a previous test of METE, generated using published data on arthropods censused from fogging experiments in Hawaiian tree canopy (Gruner, 2007), which showed that while METE better captured the central tendencies for younger and older sites predictions failed at the intermediate aged Kohala volcano site (Harte, 2011).

Given that the expectation is for METE to fail when a community is under "rapid" change, how can evolutionary processes lead to what we consider relatively rapid change? Such predictions rely on an understanding of the influx and efflux of species on the Hawaiian archipelago and how it relates to the process of community formation over time. If species richness follows a logistic-type curve, starting with initial barren colonizable youngest sites, and leveling off as species accumulation reaches equilibrium in large, rapidly radiating lineages, this would correlate with the deviation from METE. Multiple studies have shown that species diversity is far lower on the youngest island (e.g. Gillespie, 2004; Gillespie & Baldwin, 2009), a result supported by arthropod metabarcoding data in which richness of native taxa is low at young sites and increases with time for most lineages of arthropods, with many large, rapidly radiating arthropod lineages showing a peak in richness that at the middle-aged sites (Chapter 2). The slow accumulation of taxa at the youngest sites is related to the rate by which local ecological processes (e.g. colonization, environmental filtering) are adding species from within the island. Although the impact of isolation results in relatively slow ecological processes at younger sites, the same isolation leads to evolutionary processes (e.g. adaptation, speciation) that, after enough time has passed (on the slightly older islands), appear to result in relatively rapid changes in species accumulation, and in spectacular radiations of species (Gillespie & Baldwin, 2009; Givnish et al., 2009; Liebherr, 2015).

For the most common species METE sometimes underpredicts (more species are observed than predicted) in the youngest communities (Figure 3), potentially due to the unevenness that is prevalent in the arthropod communities across the archipelago, an effect that is especially pronounced at younger sites (Figure S5).

For the rarest species METE does reasonably well at predicting the abundances, but it always overpredicts (fewer species are observed than predicted) for singletons (Figure 4). The overprediction of singletons occurs at the intermediate aged sites, overlapping with the intermediate aged sites that deviate the most from METE predictions when we take the MSE of predicted to observed SADs. Hyper-rare species exist for tropical islands systems like the Hawaiian Islands. As the spatial scale of sampling increases, taxa that were singletons at a small scale will tend to have more than one individual at larger scale (Harte, 2011). At the smallest scale of our sampling plot, these predicted singletons are likely either newly formed species or the result of species (locally) going extinct. The deviation being greatest for singletons at the middle-aged sites appears to support our hypothesis that the most rapid change in middle-aged sites is correlated with rapid change due to the additive influence in community assembly from adaptation and speciation.

Anthropogenic influences and changing baselines

When we remove native taxa, and retain only non-native species, we find systematic deviation from theory in the youngest-aged sites, especially the 1973 lava flow, matching our hypothesis that METE would perform less well when these more ecologically open communities are infiltrated by highly abundant non-native taxa (Figure 2).

Expectations for 'infiltrated' communities containing both native and non-native taxa

The fit of the SAD to predictions of METE is reasonably good for most communities when all taxa, both native and non-native, are included (Figure 2). Given that the predictions of METE are based on the state-variables richness and abundance, it may be that turnover in these macroecological metrics is buffered by the presence both natives and non-natives. For example, rapid (ecological) change in the non-native taxa in young communities 'cancels out' the slow change for native taxa in young communities that is a result of slow colonization.

This equally good fit across communities of different age may imply that the infiltration of non-natives into evolutionary stable communities allows the abundance distributions to more quickly reach statistical expectations. This result is similar to that of Azorean arthropods sampled across sites with varying levels of land use, where there is no significant difference between the MSE measured when including/excluding non-natives, suggesting that the new arrivals have sufficiently integrated into appropriate equilibrium with taxa evolved *in situ* or naturally colonizing (M. Brush, pers. comm.). Importantly, the results show that METE can be useful in highlighting the dynamics of communities that are undergoing change, but only if (as in the case of the current study, or the study in the Azores) natives can be separated from non-natives so that the impact of non-natives can be understood.

For the most common species, as with the 'native taxa only' dataset, METE underpredicts (more species are observed than predicted) in the youngest communities; however, the effect is much more pronounced when non-natives are included (Figure 3). This underprediction at the common species end of the distribution is especially pronounced at younger sites. The combined dataset shows a strong tendency for the top-most common species to be at significantly higher abundance than the next ranked occurring taxa. This pattern is consistent with a reduction in SAD evenness and an increase in abundance of the most common species that has been previously recorded in more disturbed landscapes (Dyer et al., 2012; Oliveira et al., 2018; C. Xu et al., 2019). For example, with increasing grazing intensity there was a reduction of evenness as well as a reduction in richness of common species in plant SADs in a desert steppe of Inner Mongolia, China (C. Xu et al., 2019). Thus, when non-native taxa are present in a young community they establish in high-abundance, while the establishment of hyper-abundant species in later staged communities is likely tempered by the biotic resistance of increasingly diverse and ecologically filled (e.g. by monopolization and priority effects) communities.

For the abundances of the rarest species, the dataset including non-natives also shows a reasonably good fit with METE, as with the 'native taxa only' dataset, yet it still tends to overpredict for singletons at the intermediate aged sites (Figure 4). This result may reflect the natural dynamics of the native communities, as described above.

Value of DNA metabarcoding data for developing macroecological metrics

DNA metabarcoding provides rapid, accurate, comprehensive, and fine-scale measurements of the biotic component of ecosystems. In terms of its application for the assessment of SADs, we expect the DNA metabarcoding methodology will not be truncated for very rare taxa compared to traditional search methods for two reasons. First, the scale of community variation will be below the level of species, representative of genetic diversity, and species that are 'blinking in and out' locally during community development, and second, the census of a community will be more complete than traditional search methods. Given that METE's predictions are based on the input of a vector of ZOTUs and their related abundances, the performance of high-throughput sequencing along with METE predictions bodes well for biodiversity surveys, and for conservation biology, for which one of the important things to know about an ecosystem is the number of rare species, as these are the ones likely to be most at risk of extinction.

The input of state-variables for METE in the current study is ZOTU data and sequence abundances as a proxy for S0 (species) and N0 (count abundances). Sequence amplicon data may contain artefacts that would bias the shape of the SAD. However, care was taken during data processing to reduce or eliminate these biases. We reduced ZOTU inflation that is common in metabarcoding data with the removal of noise and nuclear mitochondrial like pseudogenes from the dataset with *metaMATE* (Andújar et al., 2021). By using zero-radius OTUs we removed any bias from differences in clustering thresholds (Edgar, 2016). Given that the sequence abundance of a ZOTU is not equivalent to the count abundance of a species, we rarefied the raw sequence abundances according to the count of organisms in each library, so that relative abundances can reliably be compared among sites. Previously we showed (Chapter 2) by species delimitation (Zhang et al., 2013) that ZOTUs are either equivalent to the level of species or representative of intraspecific diversity. In fact, a single ZOTU can represent one species or in other instances, anywhere from two to thirty ZOTUs can relate to a single species. As is made explicitly clear in Harte (2011, section 7.1) what we call a species does not matter, provided we use consistent criteria to define the units of analysis.

Concerning the completeness of a community census, DNA metabarcoding markedly improves on traditional search methods. Visual searching can often lead to incomplete sampling, which has implications for the predicted shape of SADs, and potentially biasing model comparisons across communities (Ulrich et al., 2010; Harte, 2011). A meta-analysis of SADs by Ulrich et al. (2010) highlights the importance of distinguishing between fully censused and incompletely sampled communities in the study of SADs, in that they found fully sampled terrestrial and freshwater communities tend to follow a lognormal distribution irrespective of species richness, spatial scale, and geographic position, rather than a log-series or power law type of distribution. A community that suffered from low coverage and higher evenness, with similar abundances across species, would not fit well to a log-series or lognormal shape. Some datasets, with census strategies that only include what is clearly an approximation of a community, such as trees above a threshold diameter or breeding bird censuses, are examples of experimental subsampling (Harte, 2011).

Here the characterization of Hawaiian arthropod communities using DNA metabarcoding can nominally be considered a near complete sampling of taxonomic diversity, at or below the scale of species. This represents a complete community of interacting biota. Any unsampled flighted, soil- and canopy-dwelling arthropods are interacting trivially, or during different life stages, with the part of the community sampled with the vegetation beat sampling methodology. The accurate and comprehensive sampling of biological communities using DNA metabarcoding provides an unparalleled opportunity to go beyond the limitations delimited by Preston's veil line (Preston, 1960). As the application of sequence data for macroecological studies increases, perhaps other studies will confirm the tendency for METE to overpredict in higher abundances and underpredict in lower abundances, for fully censused communities inclusive of genetic diversity.

Postulated effects of sampling scale and completeness on the predicted log-series shape

In both datasets for intermediate abundances, METE tends to overpredict (fewer species observed than predicted at the higher abundances) and underpredict (more species observed than predicted at the lower abundances) so that the distribution seems to follow more of a lognormal shape than the predicted log-series (Figure 5, Figure 6, Figure S5). Interestingly, the lognormal distribution provided the most accurate predictions for microorganisms that were also measured using high-throughput sequencing (Shoemaker et al., 2017). Given that ZOTUs represent genetic diversity, they offer a finer-scale representation of community diversity. ZOTUs are not, however, interacting (e.g. competing for resources, partitioning the environmental space) at the same scale as the macroorganisms typically sampled for input into METE or other macroecological metrics. With microorganisms, such as bacteria, there are also differences in the scale at which sampling occurs compared to the scale at which relevant biological interactions are taking place. Samples of microbial communities are likely to be lumping together ecologically distinct taxa that are utilizing the habitat in distinct ways (Fierer & Lennon, 2011). It may be that the discrepancy in model performance, and the tendency of the current SADs to follow more of a lognormal distribution, behaving more like microorganisms, is related to a difference in the sampling scale for the richness and turnover of Hawaiian communities using ZOTUs.

Discussions abound of the predicted shape of SADs in natural systems fitting the logseries (Fisher et al. 1943), the log-normal (Preston 1948), or alternative distributions (McGill et al. 2007), how much the fit is dependent on scale or sampling completeness, and to which extent the best fitting model reflects the biological processes underlying the distribution. Williamson and Gaston (2005) wrote a convincing paper on why the lognormal distribution is not an appropriate null model for SADs. They argued that it is unlikely to describe patterns in nature accurately because of some of its properties (e.g. skew, variance), and the fact that the central limit theorem, upon which the argument for the lognormal is based, predicts lognormality of abundances within species but not between them. Further, ter Steege et al. (2017) used simulations and empirical data from trees in Amazonia to compellingly argue that the rare tail of the log-series is likely there in the actual abundance distribution, but it is hidden by an inability to find all singletons. It is important to also note that the forms of ecological patterns predicted by METE could change depending on the constraints and state variables used. METE has been shown to predict a power law distribution if it constrains the SAD to N0/S0 while including a resource variable (Harte & Newman, 2014) and the MaxEnt procedure itself can give a lognormal (or any other function) by changing the limiting resources.

Conclusions

Here we use DNA metabarcoding for a near complete census of arthropod communities over extended geological time scales, to test predictions of macroecological metrics, with particular focus on the SAD using METE. The communities sampled were in native Hawaiian

Metrosideros polymorpha forest across an age gradient ranging from 48 years to 4.15 million years. We analyzed the deviations from expectations of METE, first with any non-native arthropods removed, with non-native arthropods only, and then with all arthropods (native and non-native) together. In the data set that included only native taxa, representative of the natural dynamics of communities prior to human arrival, we find that deviation from the expectations of theory is greatest in the middle-aged communities. This conforms to our expectation that on the archipelago new taxa accumulating by evolutionary processes at the middle-aged sites is causing relatively rapid change in state variables compared to new colonists arriving by ecological processes at the youngest sites. When we analyzed the fit of METE with the dataset containing only non-natives, the infiltration of very abundant non-native taxa appeared to disrupt steady state in ecological communities at younger stages of development, suggesting that older communities are more resistant to this type of anthropogenic change. The data set that includes native and non-native species together shows a much tighter agreement with the METE predictions, suggesting that non-natives allow the community to reach statistical steady state much more quickly. Our results support the utility of using deviations from METE predicted macroecological patterns to understand ecosystem change, but our results highlight the importance of separating non-native species to navigate the expectations for natural system versus modified system dynamics.

Figures

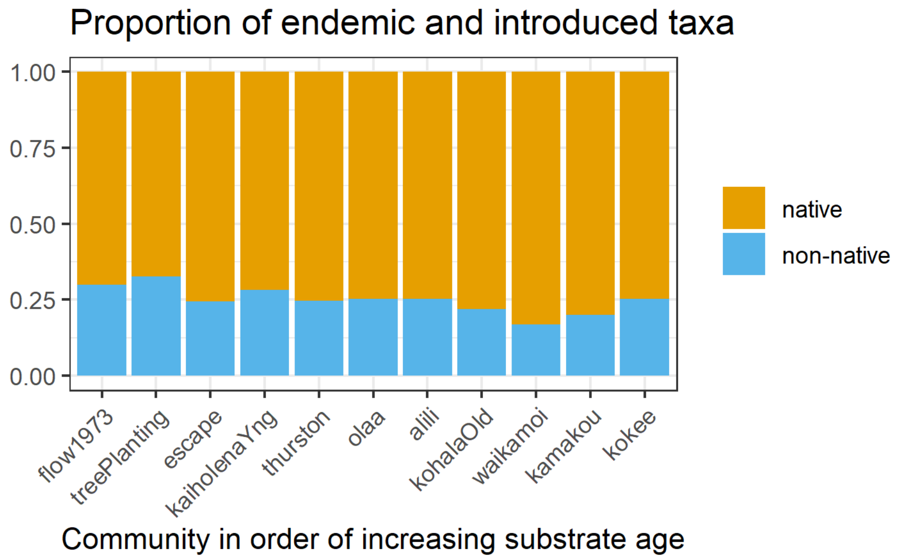

Figure 10. Comparing natives & non-natives. Proportion of non-native taxa compared to native taxa on the Hawaiian Islands at each community in order of increasing substrate age. Taxa were separated by introduction status using a machine learning algorithm.

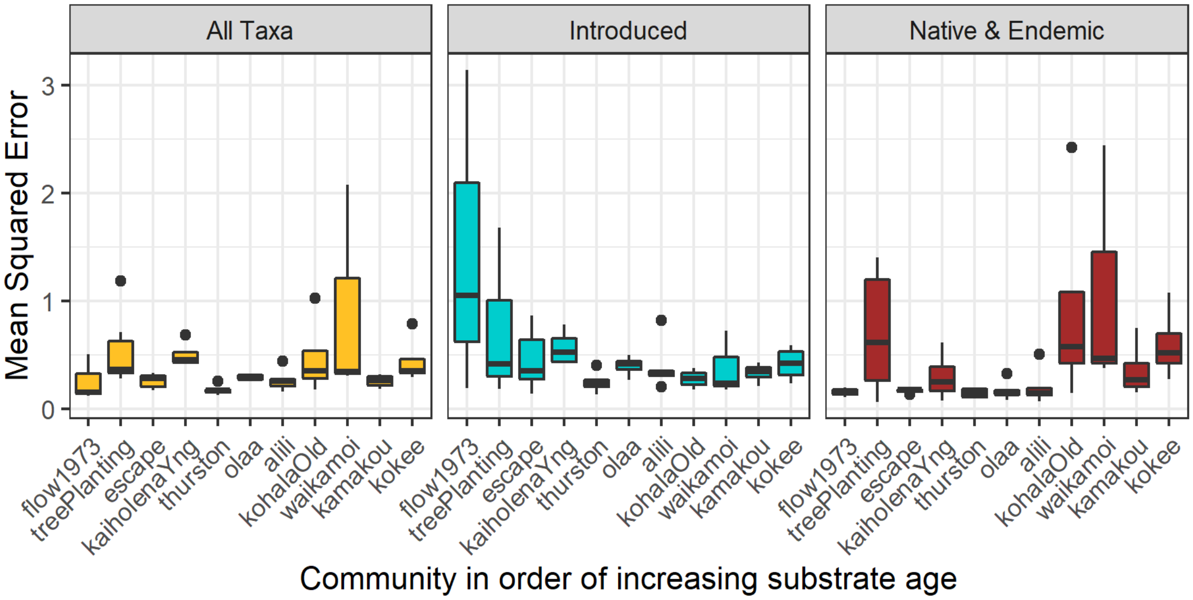

Figure 11. Deviation over time. Deviation from expectations of statistical steady state predicted under METE. Each site on the x-axis represents a community in a different stage of development from the youngest site sampled on Kilauea volcano to the oldest site sampled on Kauai. Plotted on the y-axis is the Mean Squared Error, which is a reliable and easily interpretable measure of the 'badness of fit' for observed data compared to theory. The youngest sites are the worst fit when only non-native species are included. The middle-aged sites are the worst fit when only native and endemic species are included. The model preforms reasonably well across different aged communities when all taxa are included.

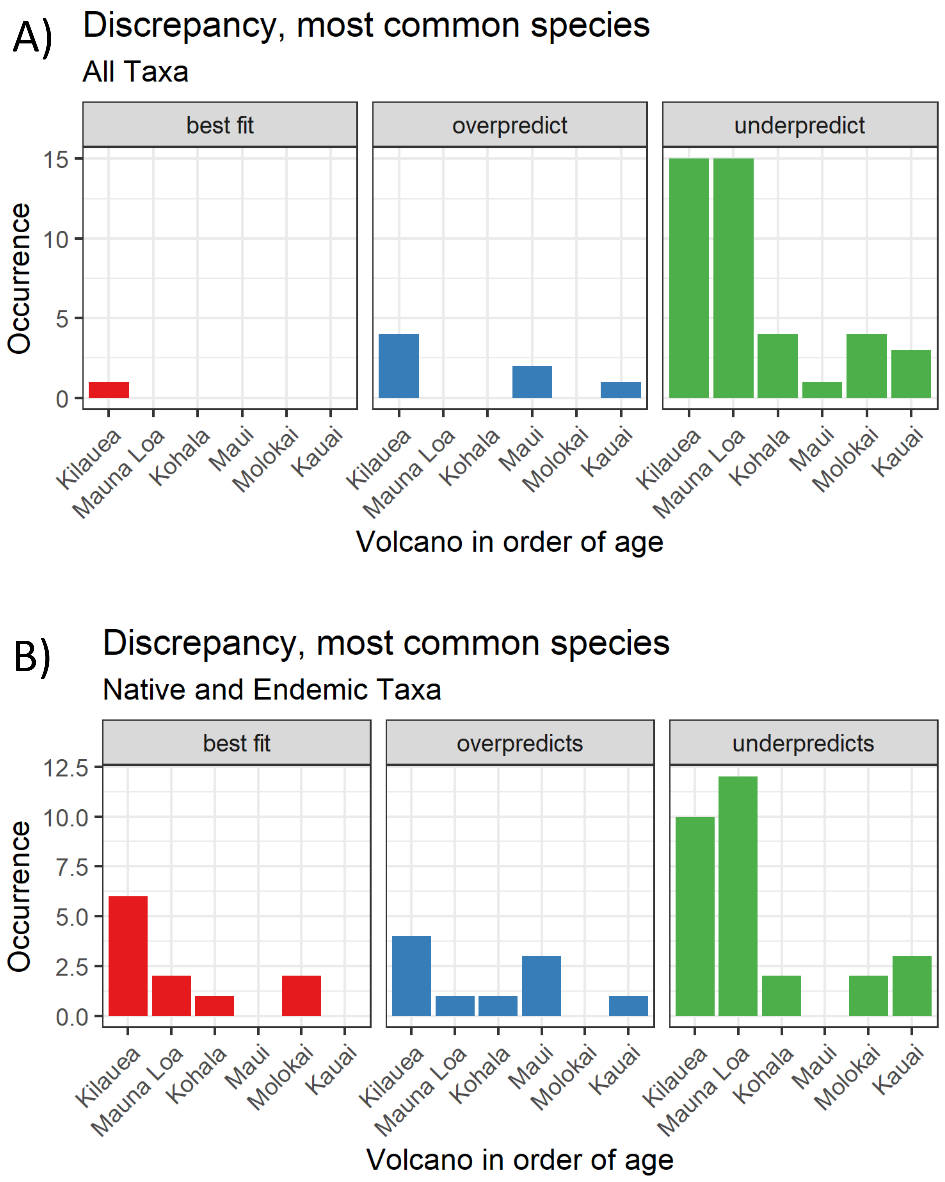

Figure 12. Discrepancy most common species. Discrepancy between observed data and predictions from METE for the most common species. Each site on the x-axis represents a community in a different stage of development from the youngest volcano Kilauea to the oldest volcano on Kauai. The occurrence of overprediction (predicted line extends above observed points) and underprediction (observed points extend above predicted line are plotted on the y-axis along with cases when predicted and observed where at the same abundance. METE almost always underpredicts for the most common species, especially when non-native taxa are included.

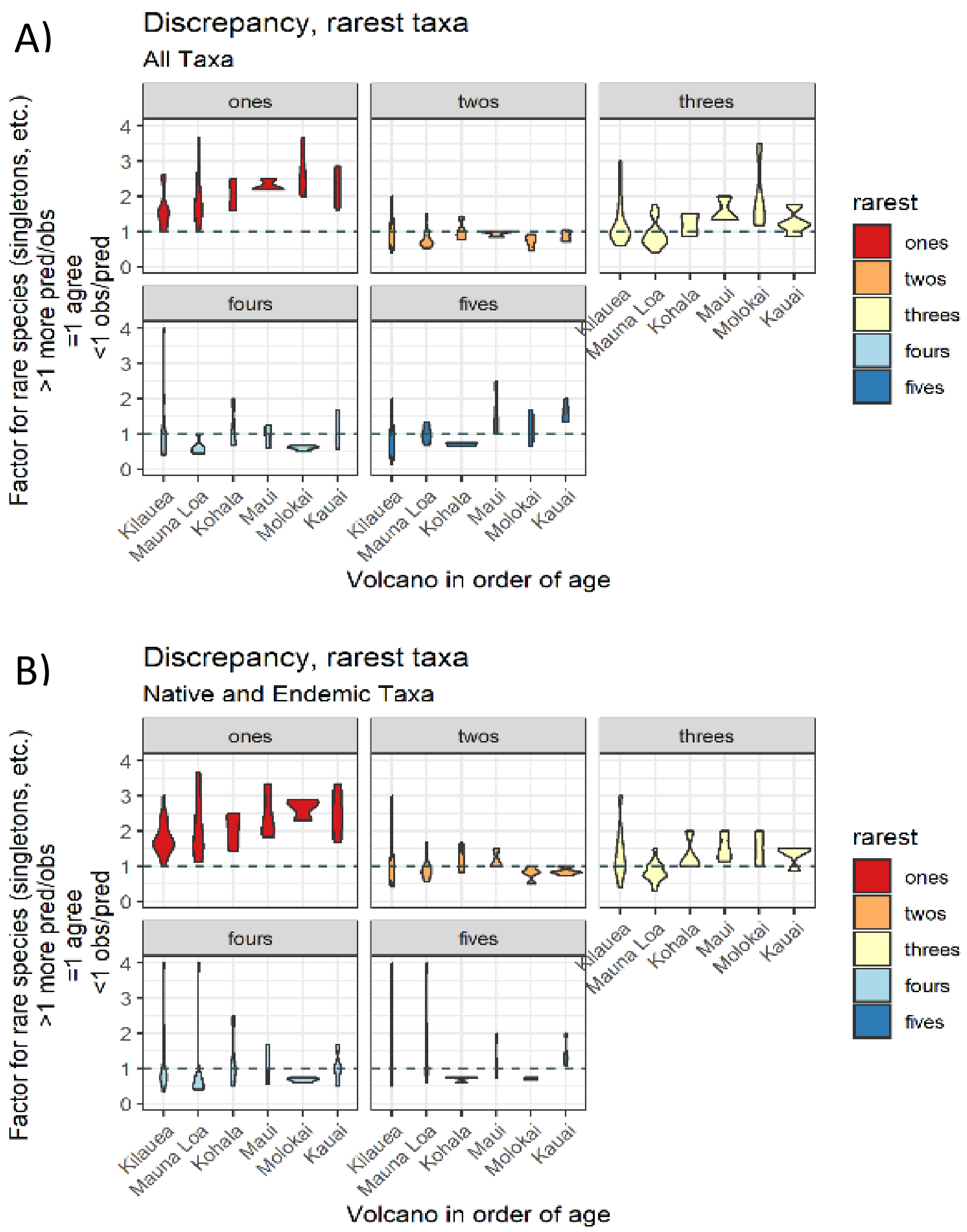

Figure 13. Discrepancy rarest species. Discrepancy between observed data and predictions from METE for the rare species (singletons, doubletons, tripletons, quadrupletons, quintrupletons). Each site on the x-axis represents a community in a different stage of development from the youngest volcano Kilauea to the oldest volcano on Kauai. On the y-axis is the measured ratio of the length of the predicted taxa at that rank compared to the length of the observed data at that rank. A ratio of greater than one indicated more predicted than observed. METE always overpredicts for singletons but does reasonably well for the other rare taxa.

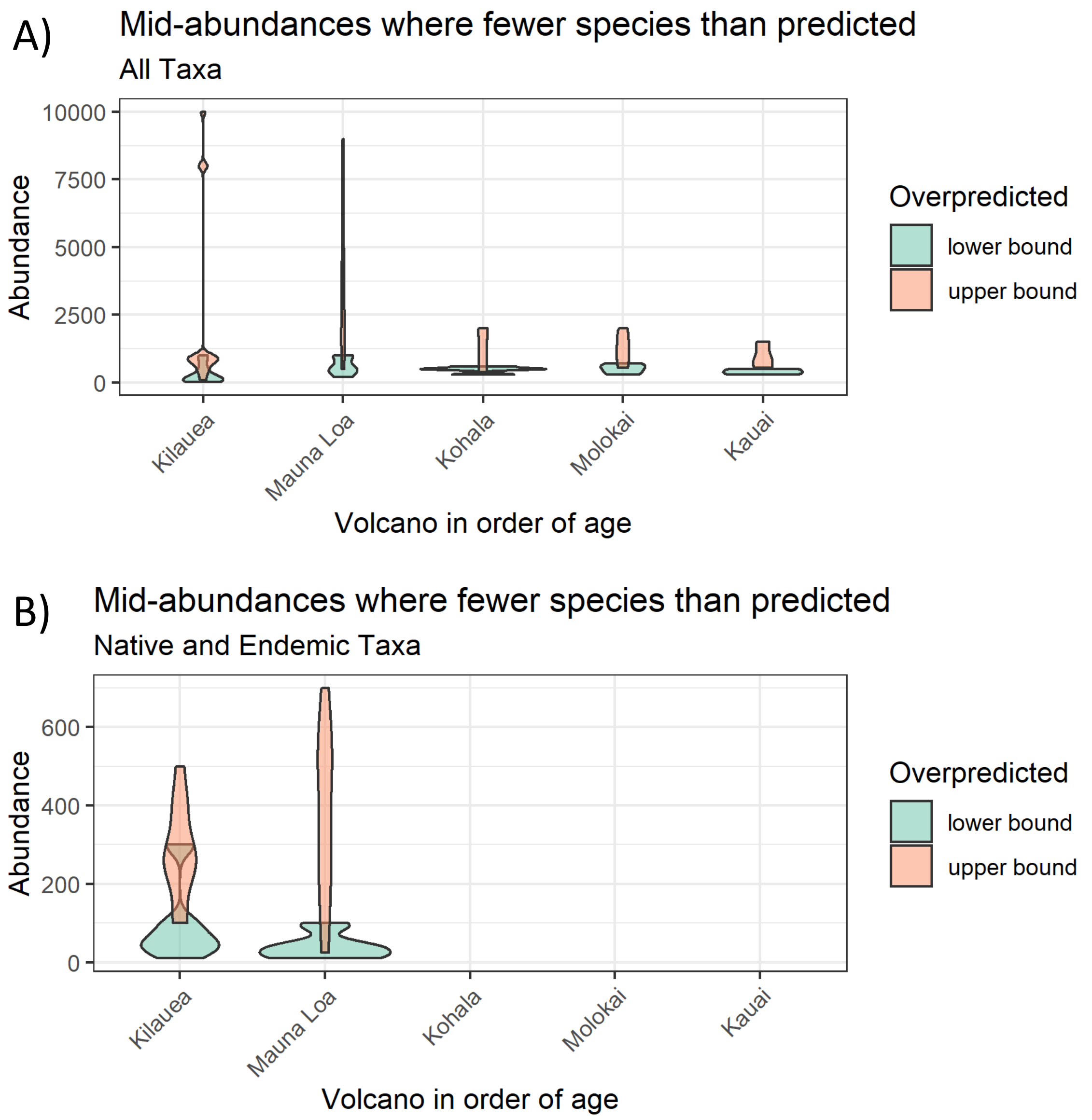

Figure 14. Discrepancy intermediate taxa, overpredicted. Discrepancy between observed data and predictions from METE for the intermediate abundance species. Each site on the x-axis represents a community in a different stage of development from the youngest volcano Kilauea to the oldest volcano on Kauai. When METE overpredicts the data are steeper than the predicted log-series. The lower bound represents the abundance where the steepness of the observed data begins and the upper bound is the abundance where the steepness of the observed data ended. METE only overpredicts for the youngest sites when the data includes native and endemic taxa only. METE also overpredicts for the youngest sites when non-natives are included, and the abundances where the overprediction occurs are much higher with non-natives present. The intermediate abundance where METE overpredicts are the higher abundances, while the intermediate abundances where METE underpredicts are the lower abundances, so that the rank abundance curve follows more of a lognormal distribution.

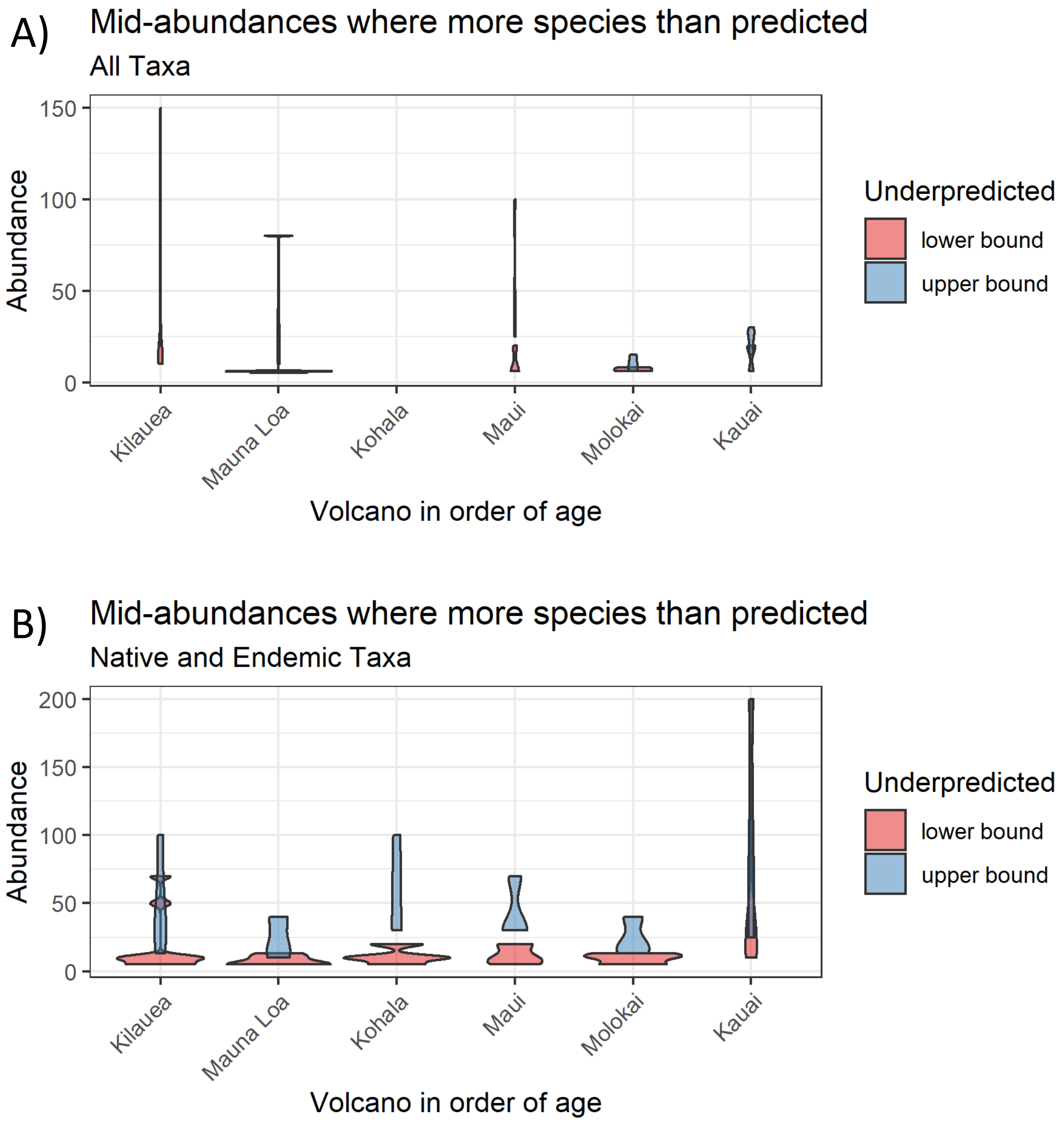

Figure 15. Discrepancy intermediate taxa, underpredicted. Discrepancy between observed data and predictions from METE for the intermediate abundance species. Each site on the x-axis represents a community in a different stage of development from the youngest volcano Kilauea to the oldest volcano on Kauai. When METE underpredicts the data are flatter than the predicted log-series. The lower bound represents the abundance where the flatness of the observed data begins and the upper bound is the abundance where the flatness of the observed data ended. METE very often underpredicts for all aged communities when the data includes native and endemic taxa only. The intermediate abundance where METE underpredicts are the lower abundances, while the intermediate abundances where METE overpredicts are the higher abundances, so that the rank abundance curve follows more of a lognormal distribution.

In the next chapter I examine how the architecture of species interaction networks changes in response to the dynamics of communities during assembly. I use quantitive network metrics to understand how the structure of species interaction networks in younger communities differs from that of older communities. This chapter marks the first research to examine how ecological networks change in response to community development over long-term temporal scales. With this work I show that younger communities are composed of more generalist species and that communities become more specialized over time.

Chapter 4

Evaluating ecological network structure in response to community assembly processes over evolutionary time

N. R. GRAHAM[1*], H. KREHENWINKEL[2], R. G. GILLESPIE[1]

[1] Department of Environmental Sciences Policy and Management, University of California Berkeley, Mulford Hall, Berkeley, California, USA
[2] Department of Biogeography, Trier University, Faculty of Regional and Environmental Sciences, Trier 54286, Germany

*Corresponding author
Email: n.graham@berkeley.edu

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INTRODUCTION

All species are linked in a complex network of interactions (Paine, 1988) that Darwin (1859) called an entangled bank. Early ecological research focused on negative interactions (e.g. competition, predation, parasitism) as the main factors structuring communities and regulating biological diversity (J. H. Connell, 1978; Grime, 1973) while more recent studies have highlighted the importance of positive interactions (e.g., pollination, frugivory) for diversity and community structure (Fricke et al., 2017; Thébault & Fontaine, 2010). However, many (perhaps most) interactions are neither positive nor negative as, for example, when organisms seek out particular plant species that facilitate communication (Mullet et al., 2017) or offer camouflage (Lindstedt et al., 2019; Stachowicz & Hay, 1999). Moreover, the interactions of a few species are embedded in larger networks of interactions that often exhibit a predictable structure (Olesen et al., 2007; Vázquez et al., 2009). These networks of interacting species play an instrumental role in community assembly (Pimm, 1979; Ponisio et al., 2019; Post & Pimm, 1983). Feedbacks between ecological and evolutionary processes during the process of community assembly result in changes in species traits (Kraft et al., 2007) and niche evolution (D. L. Warren et al., 2008), all leading to individual and community specialization (Emerson & Gillespie, 2008). However, what has not been well characterized is how the structure of ecological communities varies as species assemble, and how such changing network structure might influence the overall health of an ecosystem.

Insights into community dynamics have relied extensively on theoretical developments. Models of food web organization (Allesina et al., 2008; Cattin et al., 2004; Cohen et al., 2012; Stouffer, 2010) provide the basis for investigating how the architecture of ecological networks affects species persistence (Dunne & Williams, 2009; Gross et al., 2009) and ecosystem services (Montoya et al., 2003). For example, in some models, the extinction of a single species can set off a cascade of rapid local extinctions; and greater compartmentalization imparts greater persistence because it allows containment of perturbations (such as extinctions) within compartments (Stouffer & Bascompte, 2011). However, some key empirical studies have demonstrated how network architecture can be modified by environmental stressors, such as agricultural intensification (Albrecht et al., 2007; Tylianakis et al., 2007) or biological invasions (Aizen et al., 2008; Albrecht et al., 2014; Vacher et al., 2010). When invasive species become increasingly abundant or change their per capita interaction strength, a positive feedback that favors invasiveness is created, which weakens and erodes the structure of ecological networks (Aizen et al., 2008; Olesen et al., 2002). Overall, it appears that complimentary resource strategies and functional role redundancy can stabilize community wide interactions (Peralta et al., 2014). Clearly, the architecture of food webs acts to significantly increase ecosystem persistence and resilience against perturbations (Dunne & Williams, 2009; Montoya et al., 2006; Stouffer & Bascompte, 2011; Thébault & Fontaine, 2010). Yet, efforts to assess how interactions change through time have been limited because of the difficulty of generating any kind of holistic estimates across entire communities.

Holistic assessment of entire biological communities has been largely limited to microbiomes, in which it is possible to monitor how communities assemble over very short periods of time (Boon et al., 2014; Koskella et al., 2017; Koskella & Brockhurst, 2014; Venturelli et al., 2018). However, obtaining comprehensive sampling of organisms present within an ecosystem and characterizing the possible links among them presents considerable challenges. As such, relatively few well-resolved macro-scale ecological networks have been constructed (but see Lafferty et al., 2006; Melián et al., 2009) and there is little replication amongst those networks (Pocock et al., 2012). Next-generation sequencing (NGS; high-throughput sequencing) data make it possible to simultaneously assess thousands of species and species interactions rapidly, accurately, and for relatively small financial costs, offering enormous potential for reconstructing complex ecological networks (Clare, 2014; Hrček & Godfray, 2015; Vacher et al., 2016). Combining high-throughput sequencing with theoretical approaches, such as statistical modelling (Faust & Raes, 2012; Newman & Girvan, 2004) and machine learning (Bohan et al., 2011), shows considerable promise in helping to close the gap on the historical impediments for comprehensive quantification of interactions in ecological communities.

Despite the promise of new molecular approaches in the assessment of metrics that characterize entire communities (Kennedy et al., 2020), there are few systems within which it is possible to infer how communities change through space and time, which is essential for understanding network dynamics. To study how ecological network structure changes in the process of assembly, and whether it does so predictably, a system of communities in different stages of formation is needed. Because islands provide discrete communities they can be used for natural experiments in interaction dynamics (Brodie, 2017; Castro-Urgal & Traveset, 2014; Olesen et al., 2002). In particular, oceanic islands, because they were formed *in situ*, offer the opportunity to study species interactions over evolutionary timescales (Hembry et al., 2018; Ponisio et al., 2019; Trøjelsgaard et al., 2013). The Hawaiian Islands provide a particularly rich environment for examining ecological interactions and how these change over time for two reasons. First, the native montane forest system is relatively simple ecologically making it easy to capture and characterize whole communities. Second, it represents an environmental chronosequence due to the islands being formed over a geological hotspot, allowing for a "space-for-time" substitution (Vitousek, 2002; L. R. Walker et al., 2010). Thus, variables relevant to entire communities can be measured over the period over which the native communities formed (Rominger et al., 2017).

Here we use high-throughput sequencing, specifically DNA metabarcoding (Taberlet et al., 2012; Yu et al., 2012), to characterize arthropod associations with native Hawaiian forest plants. Importantly, we sampled across communities in different stages of development, from 48 years to 4.15 million years old, holding biotic (wet forest dominated by the canopy tree *Metrosideros polymorpha* and with no evidence of non-native plants) and abiotic (elevation, temperature, precipitation) characteristics at each site constant. In this way, we could investigate the effect of community assembly on network attributes between the arthropods and plants over the entire geological time period. Given that community assembly dynamics will play a role in the formation and resultant architecture of species interaction networks, we will assess how this process will affect network structure. Given that youngest communities will necessarily be colonized from the regional species pool, while older communities will show increasing specialization through adaptation, we make the following specific predictions. (1) The youngest communities will be characterized by more generalist species, with network metrics showing lower link density, higher connectance, lower generality, and lower vulnerability. Further, younger communities will exhibit a nested network pattern in which the interactions of the few specialist species will be a subset of the interactions of generalist species (Bascompte et al., 2003). (2) The older communities will be characterized by more specialized biotic interactions, with network metrics showing higher interaction evenness and a higher index of specialization values. Additionally, older communities will be more compartmentalized (Guimerà et al., 2010), and exhibit higher modularity in which species within particular subsets of the network interact more commonly among themselves than with species outside that module (Newman, 2006; Newman & Girvan, 2004). We test these hypotheses by measuring the network properties that describe arthropod-plant associations during each stage of community assembly by calculating quantitative metrics (Almeida-Neto et al., 2008; Bersier et al., 2002; Blüthgen et al., 2006; Dunne et al., 2002; Tylianakis et al., 2007) of resultant bipartite networks. Specifically, we calculate straightforward network statistics (e.g. connectance, interaction evenness) as well as more derived statistics (e.g nestedness, modularity) that inform on increasing network specialization that we expect to occur during the transition from younger communities to older communities.

METHODS

Site selection and collection protocol

Site selection and specimen collection was performed as described in Chapter 2 and 3. Briefly, a replicated series of fourteen sampling sites across the Hawaiian Islands was selected. Each site was within a narrow band of elevation (1000-1300 m) and hence temperature (14-17ºC), and precipitation (rainfall 2500-3000 mm), and all were within forests characterized by the dominant canopy tree in Hawaii, *Metrosideros polymorpha*. This selection of sites allowed us to hold abiotic and biotic factors affecting community formation as constant as possible and focus on the effect of age, with sites spanning a gradient of substrate ages from 48 years to 4.15 million years old (Chapter 2, Figure 3). We combined data layers on vegetation classification, satellite and airborne imagery including LiDAR, and land use and degradation, to classify and prioritize sites with differing geological histories. We excluded sites showing signs of invasive plant establishment. We estimated the percent cover for each plant in a site replicate and identified them to genus or species. We sampled the arthropods from individual plants in proportion to their relative abundance at the site, using quantitative vegetation beating at six replicate 15 m radius plots per site during May 2015 through January 2016. Each plant-associated arthropod community sample was transferred to a 2 ml vial containing 95% ethanol and stored at -20 °C. We dropped site replicates if the sampling time for the replicate was within one standard deviation of the mean replicate sampling time, resulting in a total of 50 replicates and 11 sites (Chapter 2, Figure S1).

DNA sequencing and analysis

The processes of DNA extraction, DNA sequencing, and DNA analysis were performed as described in Chapter 2 and Chapter 3. Importantly, because our approach required estimates of relative abundance (Blanchet et al 2020), it was necessary to sort specimens to size. Larger animals yield more sequencing reads, and sorting to rough size categories allows an accurate recovery of relative abundances. Briefly, each plant beating sample was sorted into four size categories (0-2 mm, 2-4 mm, 4-7 mm, 7 mm and up) in the lab under a stereoscope, with each sorted sample placed into a well of a 96-well plate (Krehenwinkel, Wolf, et al., 2017). The Collembola were processed separately, in parallel to the remaining arthropods, because they were in very high abundance relative to other taxa in the 0-2 mm category and may have affected accurate detection of the other 0-2 mm arthropods. Specimens from public and private collections were used to generate a 912 sequence DNA barcode reference library (Accession numbers XXX-XXX). Genomic DNA extraction of size sorted community samples was performed in 600 ul volumes using the Tissue protocol described in the Qiagen Puregene kit modified for automation. Each sample was amplified with a primer pair combination (ArF1/ Fol-degen-rev) (Gibson et al., 2014; Yu et al., 2012) that targets a 418 bp fragment in the barcode region of the Cytochrome Oxidase I (COI) gene. We previously have shown that by using a degenerate primer combination we can recover an accurate representation of Hawaiian taxonomic diversity (Krehenwinkel, Wolf, et al., 2017). Library preparation followed that described in Lange et al. (2014) with the modifications listed in Chapter 2. Indexed products were pooled, cleaned with SPRI beads, and quantified using qPCR, then sequenced on an Illumina® MiSeq using V3 (600 cycles) chemistry and 2 × 300 bp reads according to the manufacturer's protocol (Illumina, San Diego, USA). We aimed for a total of 30,000 reads per sample. Sequence libraries were demultiplexed, paired reads were merged (Zhang et al., 2014), quality filtered (≥90% of bases ≥Q30) and transformed into fasta files (Gordon & Hannon, 2010). The resulting fasta files were demultiplexed by amplicon primer and 6 bp inline barcode combination, using the forward and reverse primer sequences as indices with the grep command in UNIX and primer sequences were then trimmed using the UNIX stream editor.

Rarefaction, pseudogene removal, and ZOTU abundances

We rarefied each sample using a custom script that drew from the total reads of the metabarcoding analysis a number of reads that was equivalent to the numerical abundance of individual arthropods counted into each well of the 96-well plate, repeating the draw of sequences 100 X with replacement. The process of rarifying by repeating the random draw based on the expected individual specimen abundances helps correct for any disproportionate abundance of sequences that accumulate for larger specimens compared to smaller specimens within each size class. We generated biologically relevant zero-radius OTUs (ZOTUs) from the rarefied raw reads with the unoise3 command (Edgar, 2016) following the recommended protocols in the USEARCH v11 pipeline (Edgar, 2010). ZOTUs represent intraspecific variation for a particular species, as such, a species may be represented by a single ZOTU or a species may have multiple ZOTUs. We assigned taxonomy and removed non-Arthropod ZOTUs using a blastn search on a local nucleotide database downloaded May 2020 with BLAST+ (Camacho et al., 2009) and our custom DNA reference library for Hawaiian taxa. To remove putative pseudogenes from the ZOTU dataset we ran metaMATE (Andújar et al., 2021) with default specifications and the example specifications file. Using the output of metaMATE we applied the least stringent Numt removal strategy so that we could retain as many putatively true ZOTUs as possible (Graham et al., 2021). By rigorously rarefying and removing pseudogenes, molecular data measuring richness and abundances of arthropod communities can be used for recovery of (non-trophic) biotic associations in species interaction networks (SINs). To create a table with ZOTU abundances (i.e. the relative sequence abundance values for each unique ZOTU) for community analyses we mapped a query set of raw reads to the filtered and taxonomically identified search database of ZOTUs in USEARCH v11 (Edgar, 2010) using the otutab command with the default 97% percent similarity mapping threshold. We dropped ZOTUs when they occurred with a sequence abundance of fewer than 5 reads, basing this cutoff from the negative control sequencing pool, given that storage of arthropods together likely carried minute amounts of DNA over between pools after they were sorted into size categories (Krehenwinkel, Fong, et al., 2018).

Calculation of quantitative SIN metrics

Data aggregation and statistical analyses were performed in R (Team, 2013). We previously characterized the native and non-native composition for each aged community by separating taxa based on DNA sequence characteristics using a machine learning algorithm (Chapter 3, Figure 1). Here we will analyze the whole community, regardless of introduction status. We reason that our interest in understanding the associations of arthropods and plants across different stages of community development dictates that we measure the current anthropogenic conditions all potential biotic interaction partners. We aggregated the sequence abundance for each arthropod ZOTU according to its association with a particular plant genus within a site. For example, we found the sum of the sequence abundances for ZOTU 'X', a Hemiptera from genus *Nesodyne*, that was associated with (i.e. collected on) plants in the genus Coprosma. We configured the arthropod-plant abundance data as a matrix with arthropods as columns and plants as rows; there were 11 matrices, one for each site of different substrate age. As such, we measure the strength of an interaction as the sequence abundance of the arthropod that was collected on a particular plant species, as it is an aggregated assessment of the arthropod-plant association across multiple plants and multiple site replicates.

The information contained in species interaction networks can be summarized in various ways. Qualitative properties used to describe networks, which treat all interactions as equal irrespective of their magnitude or frequency, tend to be highly sensitive to variation in sampling effort (Goldwasser & Roughgarden, 1997; Martinez et al., 1999). Quantitative metrics that weight each taxon by the total amount of its incoming and outgoing biomass flows (Bersier et al., 2002) are more robust to sampling differences (Banašek-Richter et al., 2004) because they take into account the magnitude of individual trophic interactions. We calculated a variety of quantitative indices for our bipartite network of arthropods and associated plants, including: (1) connectance, (2) link density, (3) generality, (4) vulnerability, (5) interaction evenness, (6) h2', index of specialization. We also calculated two additional indices about network architecture that are more derived statistically: (1) weighted NODF and (2) modularity. We used the 'compute modules' (modularity) and 'networklevel' commands (all other indices) in the *bipartite* package (Dormann et al., 2008) to calculate the indices for each site matrix.

Link density, sometimes called complexity, is the average number of links (i.e. diversity of interactions) per species (Bersier et al., 2002). Species that colonize young communities are often generalist species, for reasons outlined in our hypotheses. As such, we expect that link density will increase over time, due to a higher average number of links per species being highest within communities with lots of generalists.

Connectance is defined as the realized proportion of all possible links (Dunne et al., 2002), or the link density divided by the number of species in the web. We expect that community assembly processes will lead to a decrease in connectance over time, because for many large radiations of Hawaiian arthropods, species richness accumulates linearly with time (Chapter 2, Figure 5), and thus the link density is divided by a greater number of taxa. Further, connectance should be highest in young communities with many generalist species.

Generality and vulnerability measure consumer-prey asymmetries. Generality is defined as the average number of prey taxa per consumer and vulnerability is defined as the average number of consumers per prey species (Schoener, 1989). We calculated the quantitive metrics for generality and vulnerability presented by Bersier et al. (2002). In our study, because we are measuring biotic associations and not trophic interactions, we consider the arthropods to be the higher level (consumers) and the plants to be the lower level (prey). During community assembly, species richness of both plants and arthropods, is increasing over time, as species accumulate due to immigration and speciation. We expect an increase in generality and an increase in vulnerability over time as evolutionary processes (e.g. diversification, adaptation) lead to more species and more specific interaction partners. One important factor is that arthropod taxa are proliferating (at the ZOTU level) much faster than the accumulation of plant taxa (at the genus level).

Interaction evenness is a measure of the uniformity of energy flows along different pathways (Tylianakis et al., 2007), and as such, can quantify how balanced the distribution of interactions is across species. We expect that community assembly processes will lead to an increase in interaction evenness over time. In younger communities there will be more generalists species, with many interaction partners, but in older communities, there will be more specialist species, and consequently more one-to-one interactions

Index of specialization (H2’) is a network-wide specialization index ranging between 0 (no specialization) and 1 (complete specialization) (Blüthgen et al., 2006). The index of specialization (H2'), which is based on information theory (Blüthgen et al., 2006), characterizes the degree of specialization or partitioning among two parties in the entire network. The heterogeneity of interaction frequencies is not evident in measures based on binary information such as number of links (L) or connectance (C). In contrast, the index of specialization is based on frequency data and can account for sampling intensity and problems of scale dependence. Thus, it is useful for comparisons across different interaction webs. We expect that community assembly processes will lead to an increase in index of specialization over time because older communities tend to be made up of more specialist species due to niche differentiation and co-evolution.

WNODF (weighted nestedness based on overlap and decreasing fill) is a weighted version of NODF (Almeida-Neto & Ulrich, 2011) and is one of the most popular metrics for calculating nestedness in binary bipartite networks. NODF is calculated by averaging values of N, a score calculated for each pairwise comparison between columns or between rows based on relative degree (number of interaction partners). Despite a widespread interest nestedness, no general consensus exists on how to measure it (Payrató-Borràs et al., 2020). Although small and eccentric networks measured with WNODF may deviate from null expectations (Payrató-Borràs et al., 2020), and despite criticism that it requires decreasing fill, which penalizes degree degeneracy (Staniczenko et al., 2013), WNODF should be a robust statistic when calculated from the relatively large arthropod-plant networks in our study, and allow easy comparisons among communities of different age. With a nested network, the diet of the most specialized species is a subset of the diet of the next more generalized species, and its diet a subset of the next more generalized one, and so on, so that the most generalized species may include most of the prey species present in its diet. Here we consider nestedness to be measure the relative network interaction partners, as it relates to arthropods and their associated plants. We expect younger communities to be more nested for two reasons. First, stochastic colonization from a regional species pool will result in colonists without adaptions to the environment and without specific interaction partners. Further, a strong environmental filter exists in the youngest communities that are establishing on bare lava that conducive to early successional species, which are often generalist species.

Modularity is a measure of the compartmentalization of a network, where species tend to interact more often with one another (in semi-autonomous modules), than with other species outside these modules (Newman, 2006). To calculate modularity in a bipartite network we used the DIRTLPAwb+ algorithm, developed by Beckett (2016), and implemented in the 'computeModules' command in the *bipartite* package. The DIRTLPAwb+ algorithm works by aggregating modules until no further improvement of modularity can be achieved. The value for modularity is a likelihood score, equivalent to Q (Newman, 2006). We expect that community assembly processes will lead to an increase in modularity over time because interaction partners will become adapted to each other over time, consequently leading to more compartmentalization in the networks of older communities.

Tests of correlation and plotting

We plotted the matrices using the 'plotweb' command in the *bipartite* package. We regressed the values for the network metric at each site against log-substrate age. The untransformed substrate age data departed significantly from normality, so comparisons were performed using linear regressions on log-transformed substrate age data, as is customary in the literature (Cowie, 1995; Peck et al., 1999; Gruner, 2007). We report the equation of the line, correlation significance values, and adjusted R squared values as overlays for the plot of each network metric over time.

RESULTS

Characterization of communities with DNA metabarcoding

The number of raw reads and number of ZOTUs before filtering for each size class are presented (Chapter 2, Figure S2). After all quality control filtering steps, the final number of ZOTUs was 3517, distributed across six classes: Collembola, Malacostraca, Insecta, Arachnida, Chilopoda, and Diplopoda. The barcode reference library increased taxonomic assignment to species or genus level for 401 ZOTUs.

Specialization through time and the accompanying predictable changes network indices

We present the bipartite networks of arthropods and associated plants, with one bipartite graph for each site, representing different stages in community development (Figure 1). The bipartite plots help to visualize the increasing richness of arthropods and plants over time and the accompanying increasing diversity of interactions over time. We report the linear regression equations, significance values and R squared values for changes in network indices over log-transformed substrate age (Figure 2). In each metric, there was a highly significant ($p < 0.001$) relationship between the change in network metric and the increase in community age. For link properties of networks, link density increased over time (Figure 2A, $p < 0.001$, $R^2 = 0.5299$) and connectance decreased over time (Figure 2B, $p < 0.001$, $R^2 = 0.6534$). For consumer-prey asymmetries, generality increased over time (Figure 2C, $p < 0.001$, $R^2 = 0.1441$) and vulnerability increased over time (Figure 2D, $p < 0.001$, $R^2 = 0.5$). Interaction evenness, a measure of the uniformity of energy flows along different pathways, increased over time (Figure 2E, $p < 0.001$, $R^2 = 0.4945$). Values of the index of specialization (H2') increased over time (Figure 2F, $p < 0.001$, $R^2 = 0.3142$). For the more derived statistical calculations for patterns in networks, WNODF decreased over time (Figure 2G, $p < 0.001$, $R^2 = 0.4131$) and the modularity likelihood score increased over time (Figure 2H, $p < 0.001$, $R^2 = 0.7034$).

DISCUSSION

We present the first empirical study to examine how the processes of community assembly affect the architecture of species interaction networks in macro-organisms over evolutionary time. We show that younger communities have a lower diversity of interactions and higher connectance, lower values for consumer-prey asymmetries (generality and vulnerability) and are more nested. These results are consistent with our first hypothesis that younger communities are assembled from the regional species pool, and contain more generalist species, given that colonists are less likely to immigrate and establish with specialist interaction partners. We show that older communities have higher interaction evenness, higher values for the index of specialization, and are more modular. These results agree with our second hypothesis that older communities are characterized by more specialized biotic interactions, likely because of both evolution among interaction partners and partitioning of geographic and/or niche space.

Ecological and evolutionary processes during community assembly lead to predictable changes in network structure

The plotted bipartite networks of the associations of arthropods and plants at different stages of community development show clear changes in network architecture during the sequence of community assembly (Figure 1). Further, there is a statistically significant linear relationship between network indices that quantify community organization and community age (Figure 2). Clearly there is a relationship between time and the network structure of ecological communities. Moreover, the pattern fits our hypothesis that the network structure for the youngest sites on Hawaii island will be the result of community assembly processes taking place over ecological time that favor generalist species. We report values of network indices, including link properties (link density, connectance) and consumer-prey asymmetries, that support this hypothesis. Our results show strong parallels to work on community assembly over ecological time that demonstrate that trophic specialists typically colonize later than trophic generalists (Piechnik et al., 2008), presumably because generalists are more able to consume early-colonizing prey, while specialist success depends on the prior presence of particular species (R. D. Holt et al., 1999; Piechnik et al., 2008). Likewise, connectance was higher in the early stages of colonization (Piechnik et al., 2008), again paralleling our results that showed the highest connectance for the two youngest sites on Kilauea volcano (1973 lava flow ~50 yo, Tree planting rd. ~133 yo) and decreasing connectance over time (Figure 2B). The 1973 flow is a nearly open bare lava site and Tree planting rd. is bare lava covered in ground ferns and small stature plants.

Our result of increasing values for consumer-prey asymmetries (generality and vulnerability) over time is consistent with findings from Ponisio et al. (2019) that showed niche overlap of plants and herbivores decreased and niche breadth of herbivores decreased with island age. Further, we report a pattern of decreasing nestedness and increasing modularity over time that parallels the results and predictions of the conceptual model presented in Ponisio et al. (2019), which combines ecological networks and island biogeography theory, as well as the principles of niche theory, to better understand the role of assembly processes in shaping patterns of biodiversity. Thus, our results match expectations that evolutionary processes during later stage community assembly lead to network specialization.

Younger communities are a result of colonization from the regional species pool

The community network metrics from the youngest sites support our hypothesis that younger communities reflect colonization from a regional species pool and are composed of more generalist species. Considering first the metrics that describe the link properties of the species interaction networks, we see that link density increases over time and connectance decreases over time. Link density, which is the average number of links per species (Bersier et al., 2002) and can be thought of as the diversity of interactions within a community, is less than 10 for Tree Planting Rd. from where it steadily climbs when measured at communities of increasing age. We should note, however, that link density is relatively high at the youngest site (1973 flow, 50 yo), although the expectation is that it should increase over time as more specialist species are added to the system, which is the general trend that is observed. We discuss this below in the section on specialization. Connectance, which measures the fraction of all possible trophic links realized and is calculated as link density divided by the number of species in the network (Bersier et al., 2002), is highest in the youngest communities and drops over time. This pattern is consistent with our predictions as higher connectance indicates a greater representation of generalists within a network (Dunne et al., 2002). In all, the link properties at the youngest communities seem to be influenced by the number of generalists. We expect that the generalist species are composed of both colonists native to the archipelago as well as non-native species introduced to the islands. We previously showed that there were some non-native taxa at all sites with the highest proportion in the youngest communities (Chapter 3, Figure 1).

Considering next the metrics that describe consumer-prey asymmetries, we find the lowest values for generality (the average number of effective plant taxa over all arthropod taxa) and vulnerability (the average number of effective arthropod taxa over all plant taxa) in the youngest communities. This same pattern is reflected in our plotted bipartite networks (Figure 1), in which strength of the interaction is represented by the width of the arrow leaving the arthropod and leading to the plant. From the bipartite network visualizations it is evident that a large proportion of interactions on the youngest sites (< 300 yo) belong to the associations of Hemiptera (Hem) and Collembola (Ent) species with the early successional plant species, *Metrosideros polymorpha* and *Dicranopteris linearis*. Further, looking at the bipartite networks for each next oldest stage of community development the interaction strength of any one arthropod order decreases. Interestingly, the correlation is stronger for vulnerability compared to generality. For the present ecological network, this can likely be explained by the difference in taxonomic resolution between arthropods and plants. While plants in our study are more highly aggregated taxonomically, being analyzed at the level of genus, the arthropods are resolved at the species level or below species level, given that we used ZOTUs to assemble the networks. As such, vulnerability, being a top down measure, is more sensitive to the changing degree of consumer-prey (arthropod-plant) asymmetries.

Among generalist species niche breadth can vary (Sexton et al., 2017) and a few species can sometimes dominate in interaction frequency (i.e. super generalists). For example, supergeneralists are a widespread phenomenon in island pollination networks, perhaps because the low density of island species leads to low interspecific competition, high abundance and ultimately wide niches and super generalization (Olesen et al., 2002). Endemic super-generalist species may improve establishment success of non-native species (Olesen et al., 2002) leading to increasing numbers and impacts of invasions (Simberloff, 2006; Simberloff & Von Holle, 1999). In summary, the values of link properties and consumer-prey asymmetries in younger communities suggest that colonization history leads to more generalist species. These generalist species result from immigration by native species, and increasingly, may be the result of alien species infiltrating the system.

We found nestedness to decrease over time (Figure 2G), which is consistent with our hypothesis that younger communities will be colonized from the regional species pool. We expected younger communities to be more nested, potentially because of stochastic colonization from a regional species pool, and possibly also a strong environmental filter imposed by the bare lava environment, resulting in colonization of a few specialist species and many generalist species without specific interaction partners. Nestedness is a property of interaction networks characterized by the interactions of any node forming a subset of the interactions of all nodes with higher degree. For example, a nested ecological network is composed of generalist and specialist species, with the generalists interacting with a large amount of interaction partners, while the specialist species are interacting with only generalists, so that there are few specialist-specialist interactions. This same pattern of decreasing nestedness over a much shorter timeframe was found for a study of succession of macroinvertebrate communities species in man-made wetlands, where highly nested structures in pioneering assemblages decreased, which was suggested to be due to a shift from active pioneering taxa to passive disperser ones (Ruhí et al., 2013).

Older communities are a result of specialization from ecological fitting and speciation

The values of network metrics at the older sites are consistent with our hypothesis that older communities will reflect increased network specialization. We see an increase in interaction evenness over time (Figure 2E). Parallel work over ecological time scales has shown similar patterns, with greater habitat stability being associated with greater evenness of interaction frequencies among naturally occurring bees and wasps and their associated natural enemies (e.g. parasitoids) (Tylianakis et al., 2007). Given that interaction evenness increases during the course of community assembly, it appears that the uniformity of energy flow increases over time as well. One noted exception in the evenness by time trend is found at the 1973 flow, which has higher interaction evenness than the other sites on Kilauea volcano. For this very young site the energy appears to flow along a greater variety of pathways compared to the rest of the early stages of community assembly. This corresponds with the higher link density and higher vulnerability at the 1973 site compared to the remaining sites at Kilauea. We suggest that this may be due to the fact that at this very early stage of community development there are a few limited early successional plants, and as such, perhaps a higher proportion of transient arthropods associated with the few plants at this site.

We report a statistically significant increase in the index of specialization over time (Figure 2F). The older community at Maui Waikamoi (~545,000 yo) has the highest network-wide specialization score of > 0.6, while the young community at Thurston lava tube (~ 575 yo) has the lowest score of < 0.2, with the index ranging between 0 (no specialization) and 1 (complete specialization). The community at Waikamoi is where richness peaks for many native arthropod lineages as has been shown in several studies (Chapter 2, Gillespie & Baldwin, 2009; Gruner, 2007). The community at Thurston is at the stage of community assembly when ecological sorting is occurring, but at just 575 years, there hasn't been enough time for *in situ* speciation to lead to specialization. Plant and animal traits (e.g. morphology, chemical composition, physiological abilities, behavior) are major drivers of species specialization, and thus trait matching can be very important for explaining interactions and levels of network specialization (Blüthgen et al., 2008). For instance, species-specific preferences may strengthen some links, while structural barriers or an avoidance of defenses may inhibit others. Strong structural importance of trait matching leads to a high level of specialization in the community, while the absence of structuring by trait matching implies maximum generalization (Vázquez & Aizen, 2006). However, the rate specialization and adaptation, such as occurs through trait matching, can vary among functional groups in a community. For example, in a study of detritivore networks successional changes over three years, community composition and network specialization changed differently among functional groups (e.g. xylophages, fungivores, predators) (Wende et al., 2017). Clearly there exists a strong rationale for the relationship between community assembly processes (e.g. ecological fitting, speciation/adaption) and expectations for network specialization. Future work to explore the differences among the pace of specialization, and various functional groups, during the process of community assembly over longer temporal scales would be an exciting avenue to explore.

As expected, due to network specialization over time, we found a statistically significant increase in modularity over time (Figure 2H) and report the highest R squared value for the correlation of modularity and community age out of all the network indices. Many networks of interest in the sciences, including social networks, computer networks, and metabolic and regulatory networks, are found to divide naturally into communities or modules (Newman, 2006). In ecological networks these modules (or compartments) can help to define niche. For example, in studies of food web structure, results demonstrate pelagic and benthic compartments (Krause et al., 2003), and radially symmetrical flowers with more connections within their modules than species with bilaterally symmetrical flowers (Chamberlain et al., 2014). The development of modules in a network is related to species and network specialization and has been shown to be correlated with the index of specialization across 22 pollination networks (Dormann et al., 2008). From an evolutionary perspective, natural selection favors high profit resource combinations, and as such, species form increasingly specialized network modules of interacting species over time, based on shared phenotypic traits such as flower shape and animal body size (Darwin, 1859). Thus, in our arthropod-plant association networks increasing modularity likely reflects the development of specialized compartments, representative of the refinement of ecological niche space in older communities.

Translating results into understanding ecosystem health and community resiliency to perturbations

Using multiple complementary network indices our results highlight that network structure changes predictably during community assembly (Figure 2). Clearly the structure of ecological networks provides clues to the processes shaping biodiversity. However, the architecture of ecological networks can also help to identify how environmental perturbations influence ecosystem health. For example, modules in networks theoretically help to increase stability, because the impact of a disturbance is contained within a single compartment (Krause et al., 2003; Olesen et al., 2007; Stouffer & Bascompte, 2011). Species that are highly linked within their own module (i.e. hub species), connectors linking different modules, or both, are key to the structural importance in a network. If these key species go extinct they can cause the breakup of modules or networks, and as such should receive conservation prioritization (Olesen et al., 2007). Using data from 32 empirical food webs, Stouffer et al. (2012) find that species act in specific roles and have dynamic importance in their community, again highlighting the importance of distinct species groups when attempting to conserve ecological communities.

Our results suggest that older communities, which exhibit greater interaction diversity, higher network specialization, and a pattern of modularity, are more stable and resilient. Specialization allows species to utilize a single resource more effectively or share the same resources in different locations or times, resulting in resource complementarity. As communities age and species accumulate some redundancy in biotic interactions will also develop, for example, a number of species may feed on the same resource. Both resource complementarity and redundancy can minimize variability in the functioning of an ecosystem, for example when some consumer species decline in number (Peralta et al., 2014). A few species may have greater impact on ecosystem stability compared to others, depending on their functional roles. For example, theoretical work shows that a greater numbers of engineers can facilitate colonization and limit competitive exclusion, thereby reducing primary extinctions and the magnitude of extinction cascades, making a community more stable and persistent (Yeakel et al., 2020).

Our results also highlight that younger communities are at greater risk from environmental perturbations. Given that younger communities have lower link density and higher connectance they are less resilient to the removal of species, because high connectance implies that more pathways are available for the effects of primary extinctions to propagate across the network (Vieira & Almeida-Neto, 2015). The links between community architecture and stability can depend on the stability metric used (Krause et al., 2003) and can differ depending on whether the network is mutualistic or antagonistic (Thébault & Fontaine, 2010). Nonetheless, there is a clear relationship between network architecture and ecosystem health, and our results provide the opportunity to detect the shifting structure of ecological communities over time.

Caveats

High throughput sequencing and eDNA methodologies are greatly advancing our ability to assess community dynamics for macroorganisms (Kennedy et al., 2020), environmental microbiology (Rocca et al., 2019), and microbiome research (Levy & Borenstein, 2013). Our study uses relative abundances captured using DNA metabarcoding data to assess the biotic associations of thousands of species across a temporal sequence congruent with the stages of community assembly. However, there are three major caveats in this work that should be addressed:

Can co-occurrence data be used as evidence of ecological interactions eDNA detection of cooccurrence data offers an exciting avenue for capturing biotic associations for difficult to detect relationships (e.g. endangered species) and time series data (Bálint et al., 2018). Our data provide information on arthropod co-occurrences with specific species of plants. There have been multiple studies that question whether information on co-occurrence can indicate interactions when we have only presence -absence data, and there is general agreement that such data cannot inform on interactions (Blanchet et al., 2020). However, by including abundance data, we can achieve a signature of interaction strength (Popovic et al., 2019). Thus, in our study, we are capturing associations resulting from complex community interactions including acoustic signaling (Mullet et al., 2017), predator avoidance (Lindstedt et al., 2019; Stachowicz & Hay, 1999), and gregarious plant-feeding insects (Hunter, 2000). Certainly, there will be instances where we record transient associations that do not reflect any kind of interaction. However, the low frequency of such events will mean that they have little effect on the network.

Can metabarcoding data provide information on relative abundance? Inferring abundance from metabarcoding data is challenging because of biases introduced by specimen body size and primer binding affinity (Elbrecht & Leese, 2015; Fonseca, 2018; Yu et al., 2012). However, we have developed and demonstrated optimized protocols to indirectly obtain relative abundance estimates with minimized amplification bias (Kennedy et al., 2020; Krehenwinkel, Fong, et al., 2018; Krehenwinkel, Kennedy, et al., 2017; Krehenwinkel, Wolf, et al., 2017). This protocol includes sorting individual specimens into body size categories, both to reduce bias and to produce trait data, as we did. It is thus possible to use sequence abundance data from high throughput sequencing studies to provide reliable relative abundances of taxa (Giner et al., 2016).

Do the network metrics adequately account for changes in species diversity through time? We know that species diversity changes across the island chronosequence, and the diversity per unit areas is much lower on the youngest island than any of the older islands (Gillespie & Baldwin, 2009; Gruner, 2007). The question then is whether our results are simply reflecting the change in diversity rather than changes in the network metrics themselves. However, quantitive network metrics have been developed to accommodate for the diversity of species within a site (Almeida-Neto & Ulrich, 2011; Banašek-Richter et al., 2004; Beckett, 2016; Bersier et al., 2002; Blüthgen et al., 2006), so we are satisfied that the changes are in the network properties themselves.

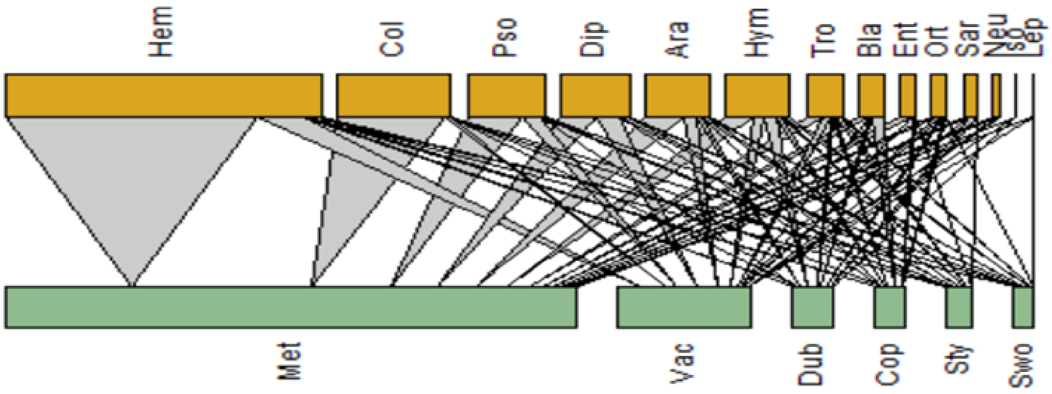
Thus, we are confident that our results are a reasonable representation of how interactions change through time. By employing eDNA and high throughput sequencing, it is clear that we can make inferences as to why a species occurs at a specific location, including fine scale spatial and temporal variation, and construct ecological networks to develop a holistic view of community architecture.

Conclusions

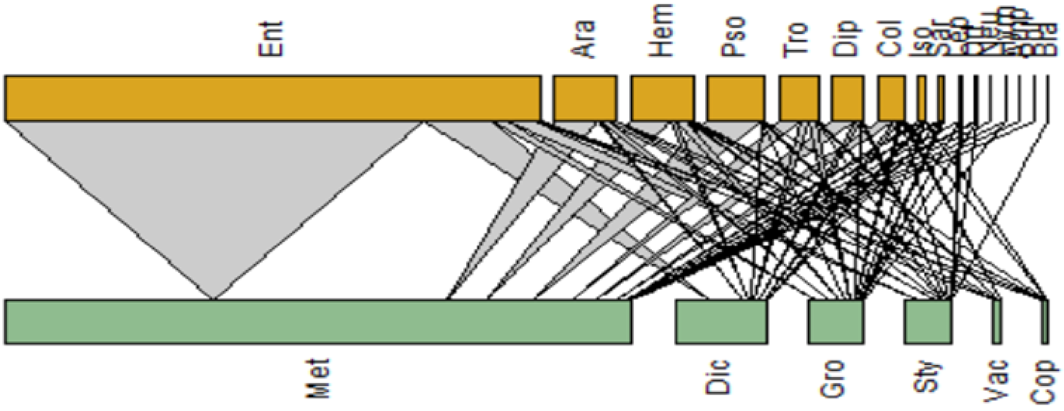
Here we present how network structure changes predictably with the stages of community assembly using high throughput sequencing of arthropod communities and their associated plants across a chronosequence in the Hawaiian Islands. We find that the youngest communities are more nested and have link properties as well as consumer-prey asymmetries that reflect their lack of specialization. Statistically significant linear relationships between network indices and community age help demonstrate that network specialization increases over both ecological and evolutionary timescales. The degree of specialization does not appear to level off based on the values of the oldest site (Kauai ~4.15 my) compared to the next oldest; however, the peak of many indices measuring specialization (e.g. H2', modularity) or the flow of energy (e.g. vulnerability, evenness) is at Maui, an intermediate aged island. This peak in network statistics corresponds to the peak in richness found for many native arthropods that diversified on the archipelago. The architecture of networks is linked to community stability and there is evidence that younger communities may be less resistant to environmental perturbations. Information about the fragility of communities during the early stages of community assembly can be helpful for restoration planning. By providing the first empirical evidence of the shifting architecture of ecological communities during distinct stages of community assembly over deep time we highlight how the temporal dynamics of biodiversity play a central role in characterizing community functioning and ultimately ecosystem health.

Figures

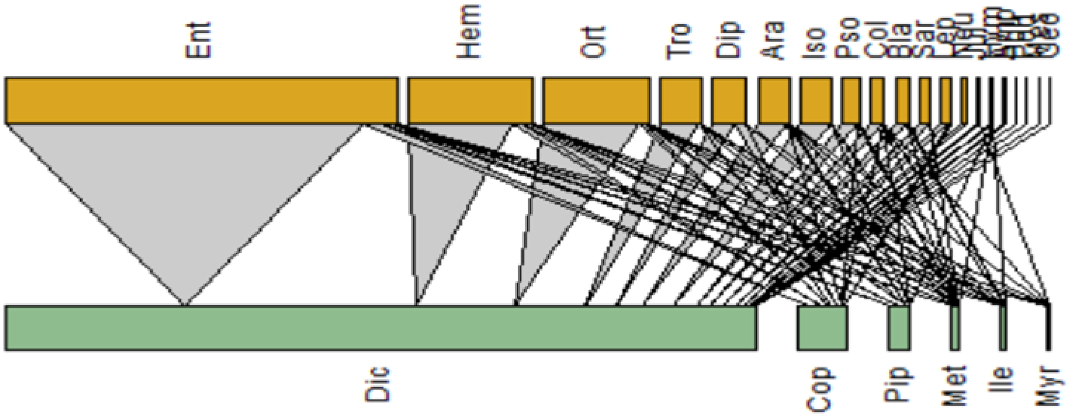
1973 Lava Flow ~ 48 yo



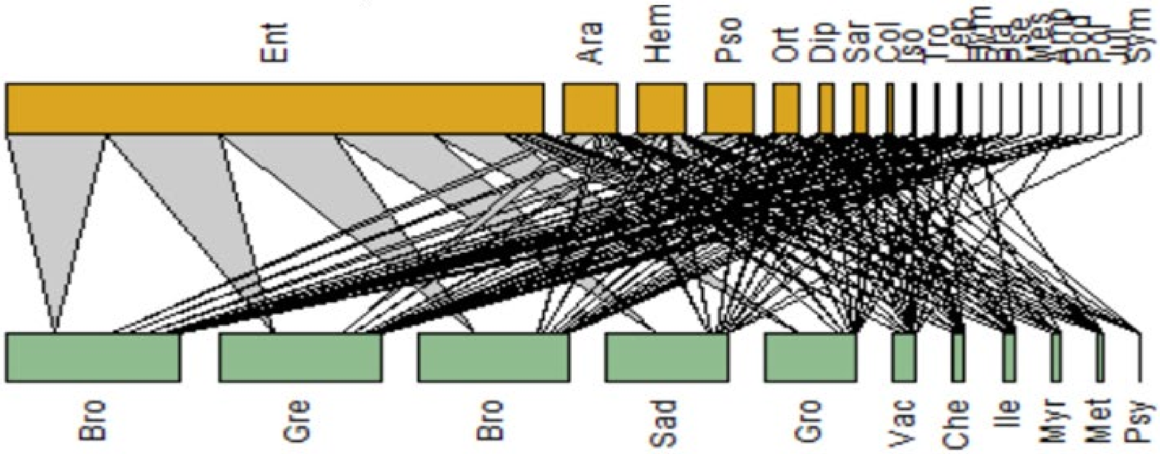
Tree Planting Road ~ 133 yo



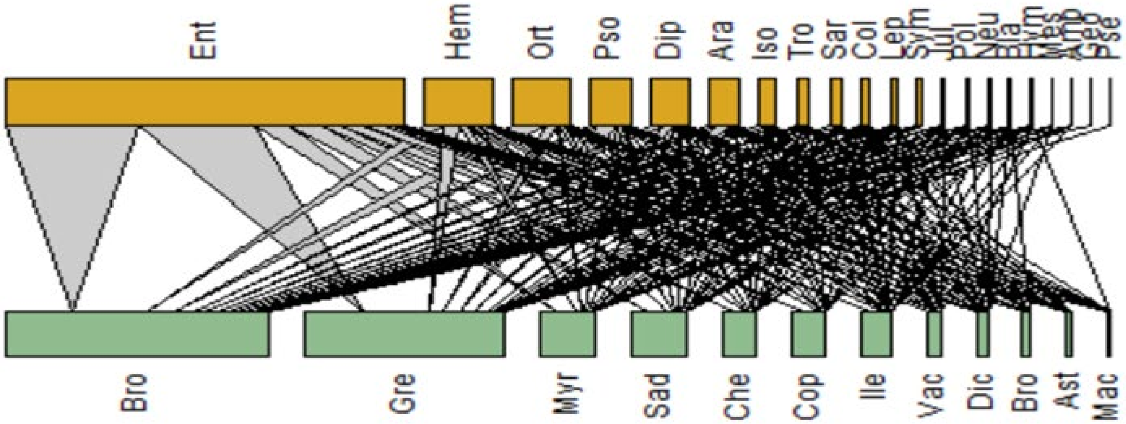
Escape Road ~ 300 yo



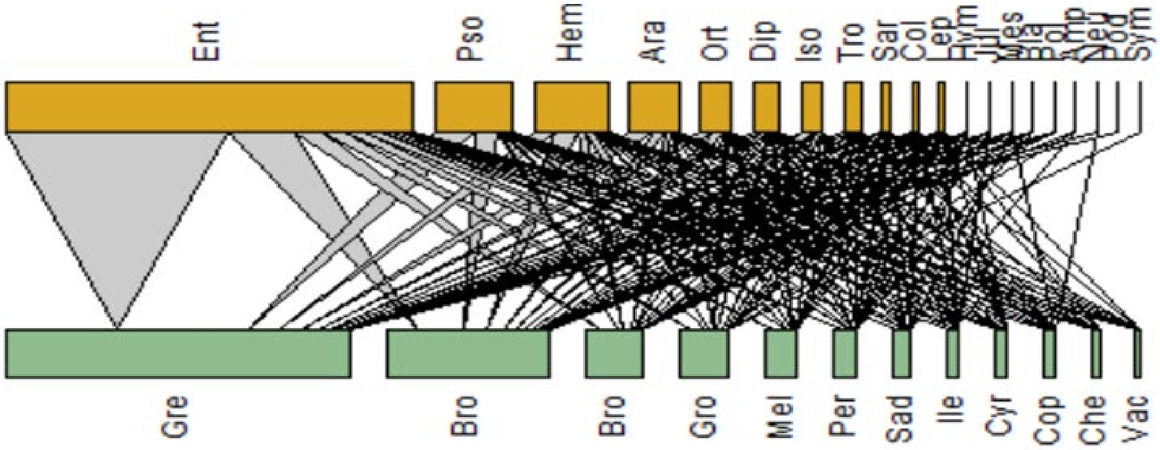
Kaiholena TNC ~475 yo



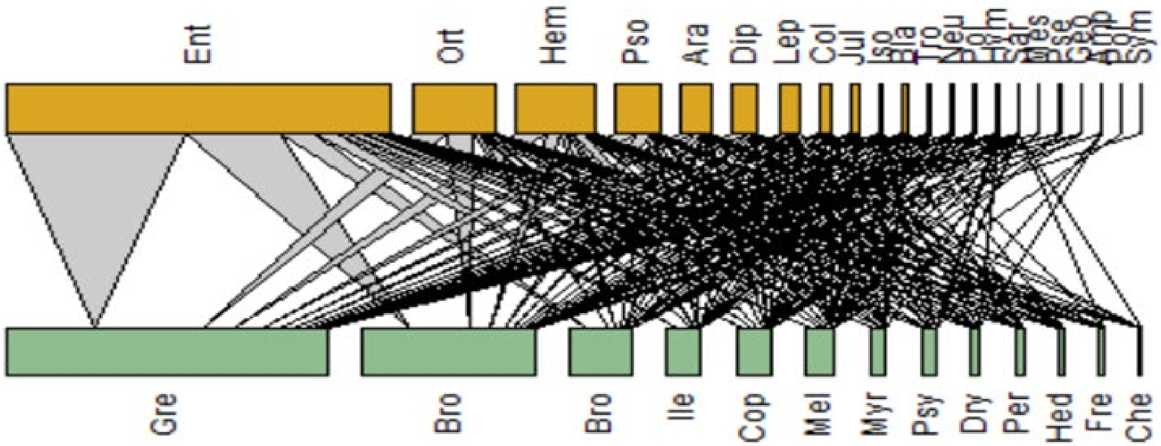
Thurston Lava Tube ~575 yo



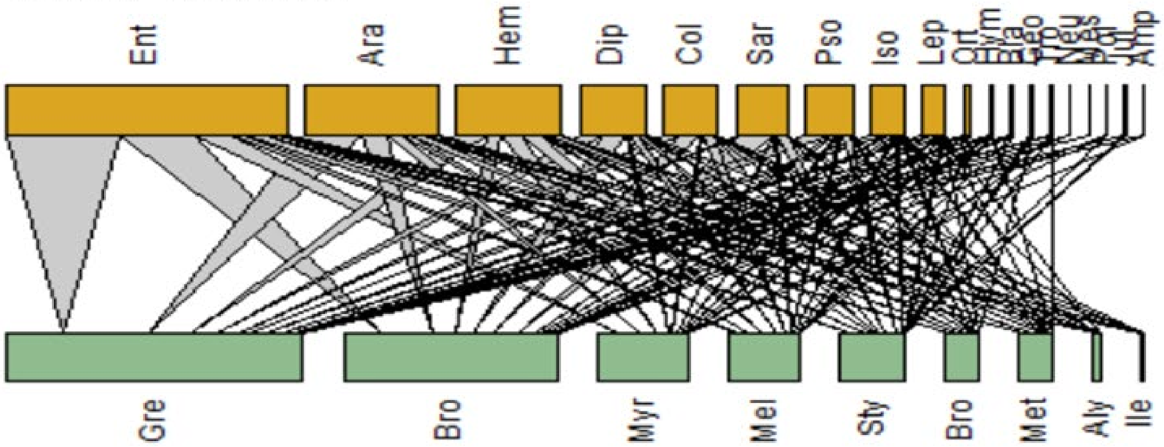
Olaa FR 7,500 yo



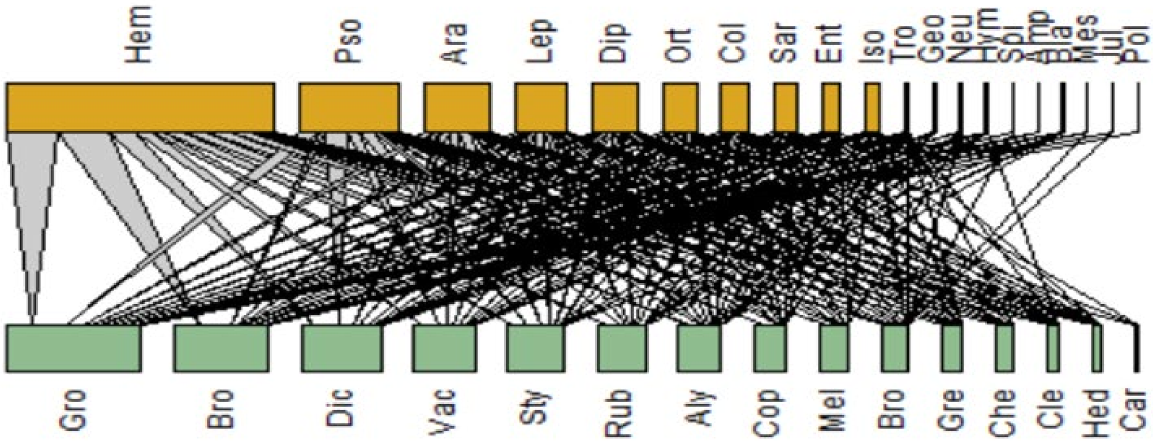
Alili Spring ~20,500 yo



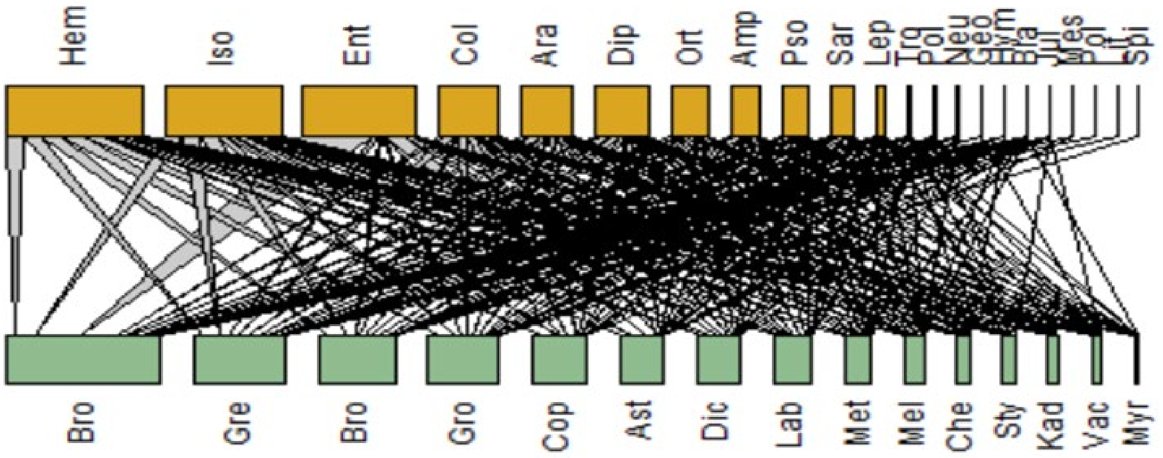
Kohala ~365,000 yo



Waikamoi TNC ~545,000



Kamakou TNC ~1.4 myo



Kokee SP ~4.15 myo

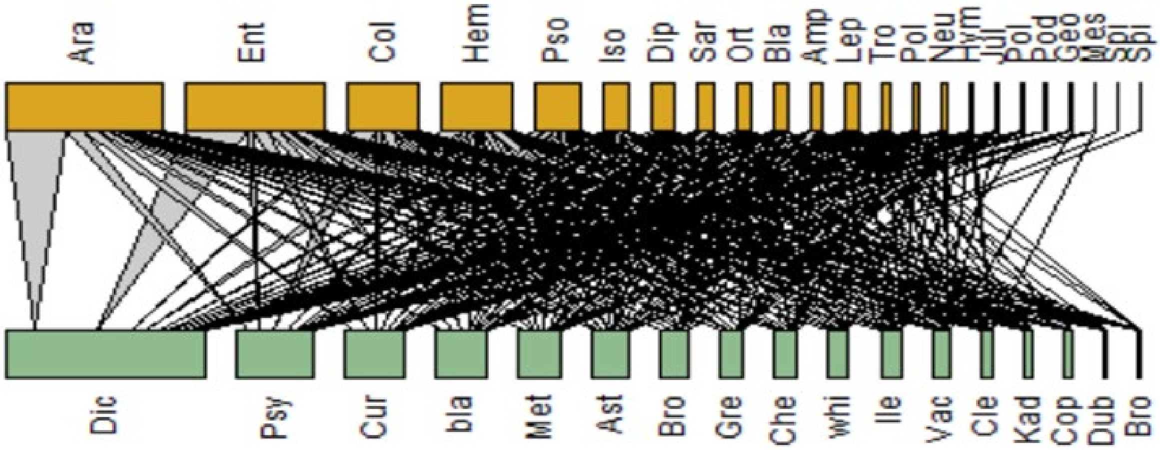


Figure 16. Bipartite networks arthropods-plants. Plotted bipartite networks of community architecture with arthropods on top and plants on bottom. Interaction strengths, represented by the arrows between arthropods and associated plants, show the strength of each association, and are estimated by the total cumulative sequence abundance for each arthropod collected from multiple representatives of each plant at multiple site replicates.

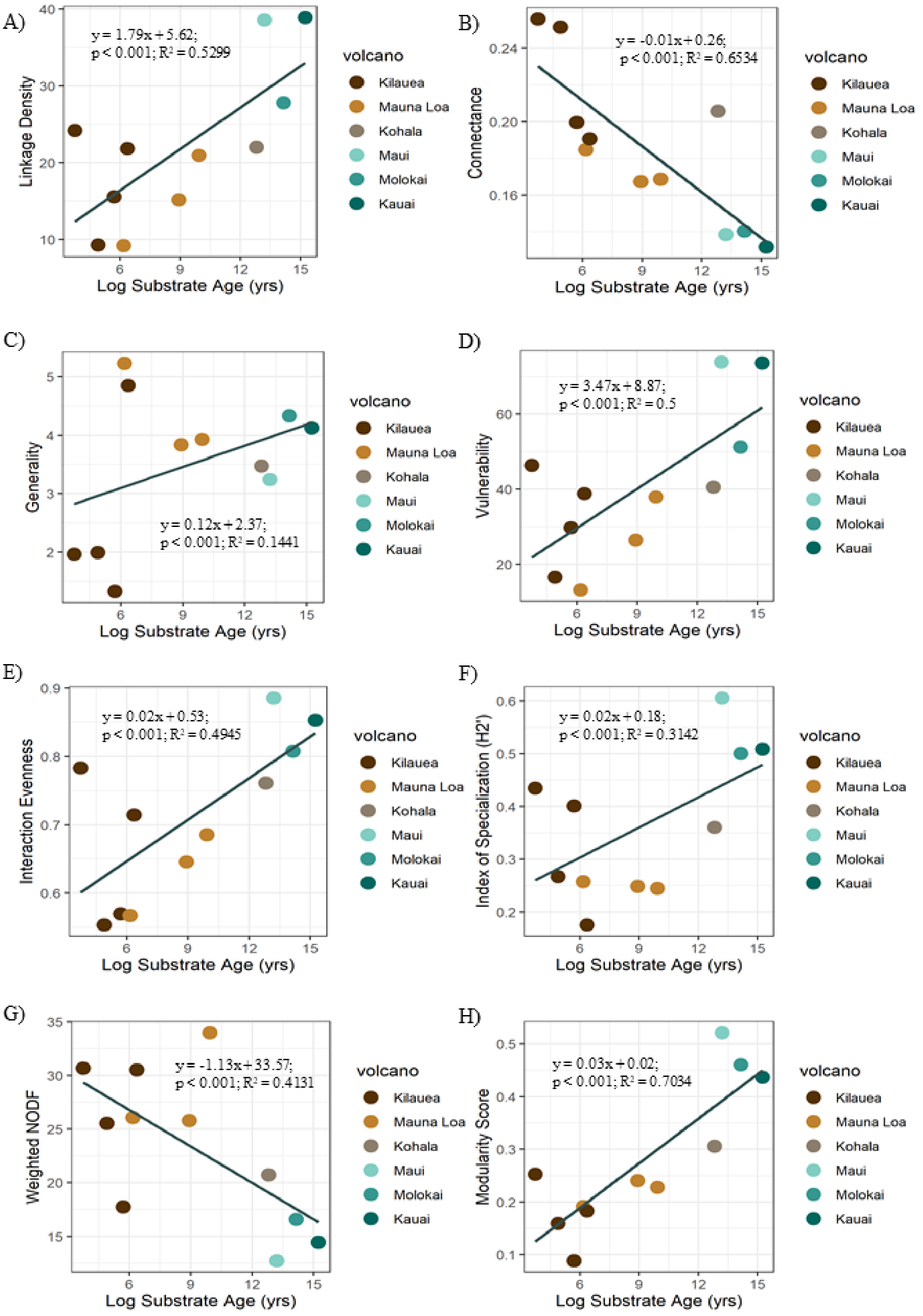


Figure 17. Regression of network metrics by community age. Simple linear regression models of the relationship of the values of quantitative network indices measured for communities of

different age (measured by log-substrate age), representing various stages of community assembly.

Conclusions

Here I have assembled both a literature review and empirical dataset that showcase the utility of island systems for understanding basic questions in ecological and evolutionary biology. I focus on the ecological and evolutionary processes that influence community assembly. I show that ecological processes are more prominent during the earlier stages of community development and that gradually with time evolutionary processes become more influential. I demonstrate this with lines of evidence from multiple scales of biodiversity, from the genetic level, the species level, and the ecosystem level. The data presented here are the first to comprehensively sample communities using environmental DNA across long-term temporal scales. By combining this comprehensive dataset with analyses that capture a hierarchy of biodiversity organization I provide the first examination of biodiversity dynamics during community evolution. Finally, I explore how natural communities are changing due to global change phenomena and I discuss the implications biodiversity conservation.

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Appendices

APPENDIX I

Supplementary materials Chapter 2: Ecological and evolutionary processes during community assembly evaluated using DNA metabarcoding of arthropods on islands

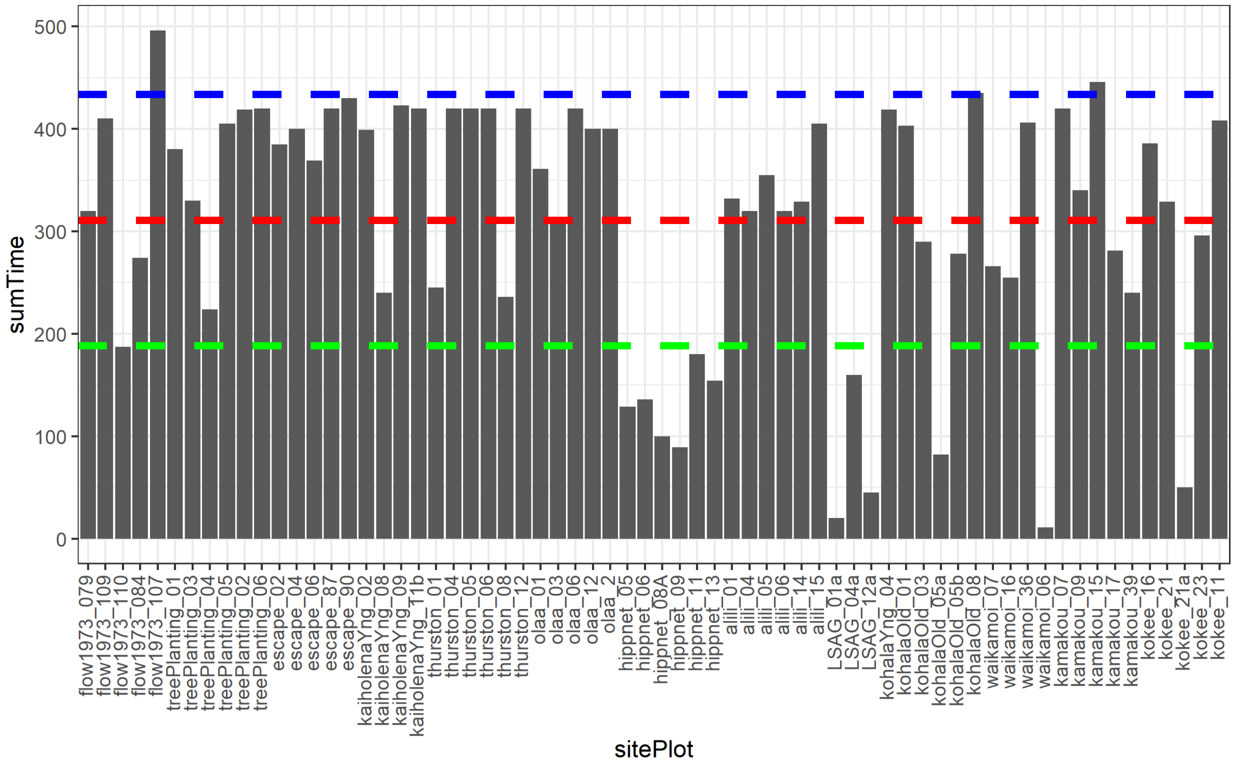


Figure S1. Sampling effort per site replicate. To normalize sampling effort sites were dropped from analysis if they were less than one standard deviation below (green line) or above (blue line) the mean sampling time (red line). Site replicates on the x-axis are in order of increasing substrate age.

Table 1. Taxonomic coverage of sequencing. Number of ZOTUs per arthropod order followed by the number of taxonomic level assignments based on DNA voucher matching with percent similarity to searches >=98 species, >=94 and <98 genus, >=88 and <94. ZOTUs represent both native and non-native species.

order	zotus	species	genus	families
Amphipoda	29		1	1
Araneae	539	58	9	8
Blattodea	12	2	1	
Coleoptera	421	18	4	12
Diptera	454	27	8	19
Entomobryomorpha	295	8	3	1
Geophilomorpha	9			
Hemiptera	774	37	11	9
Hymenoptera	98	12	3	5
Isopoda	59	2	2	1
Julida	4	3		1
Lepidoptera	290	18	16	15
Lithobiomorpha	1			
Mesostigmata	9	1		
Neuroptera	30	2	1	2
Orthoptera	197	4	2	2
Poduromorpha	7		1	1
Polydesmida	1	1		
Polyxenida	1			
Pseudoscorpiones	4			
Psocoptera	266	4	1	
Sarcoptiformes	262	1	2	
Spirobolida	1			
Spirostreptida	4			
Symphypleona	4	1	1	
Trombidiformes	14	1		1

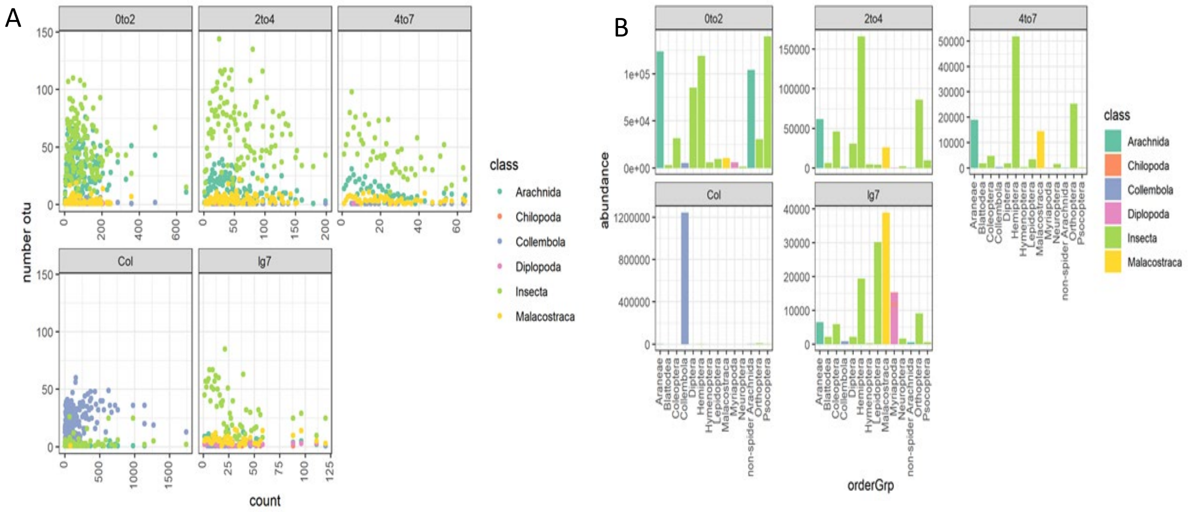


Figure S2. Sequencing results by size class. A) Number of ZOTUs per count of individuals in each sequencing pool. Color indicates Class of arthropod. B) Raw sequence abundances for arthropod Order in each sequencing pool. Collembola were sequenced seperately. A large number of sequence reads belonging to Class Arachnida in the 0-2 mm category are residual DNA from the soft tissue of spiders when community samples were stored together in ethanol.

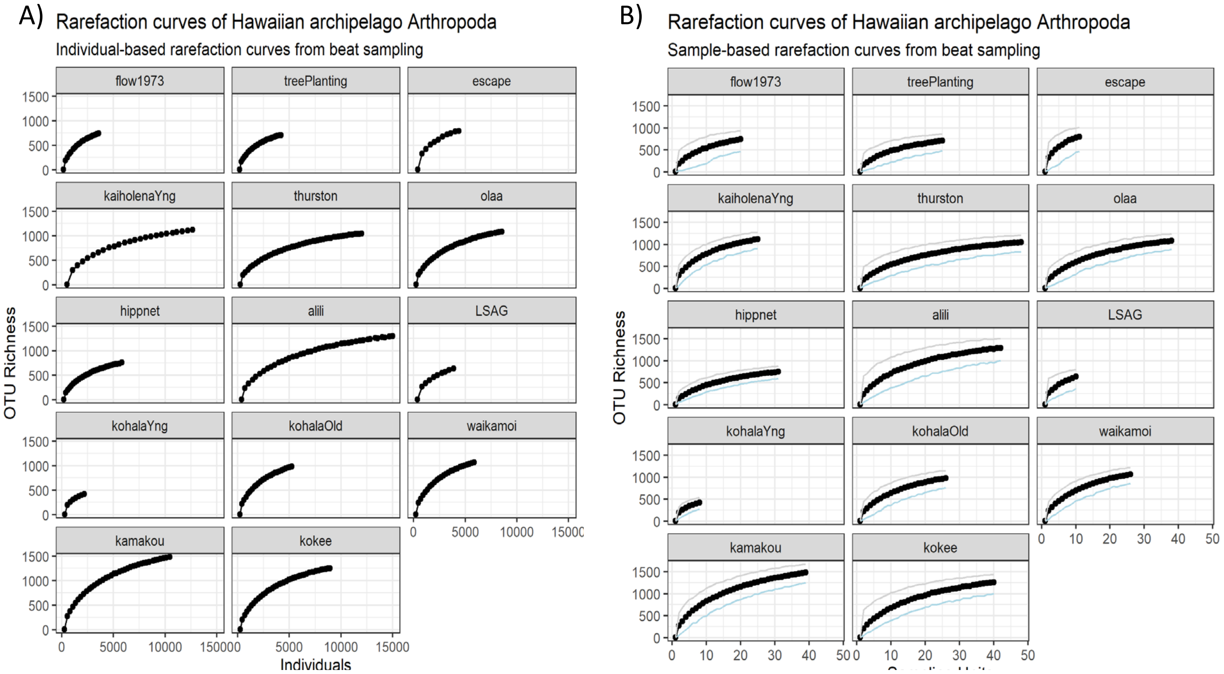


Figure S3. By site sampling completeness. A) Individual and B) sampling unit-based rarefaction curves. Sampling unit is all the arthropods on a plant genus within a replicated plot at a site.

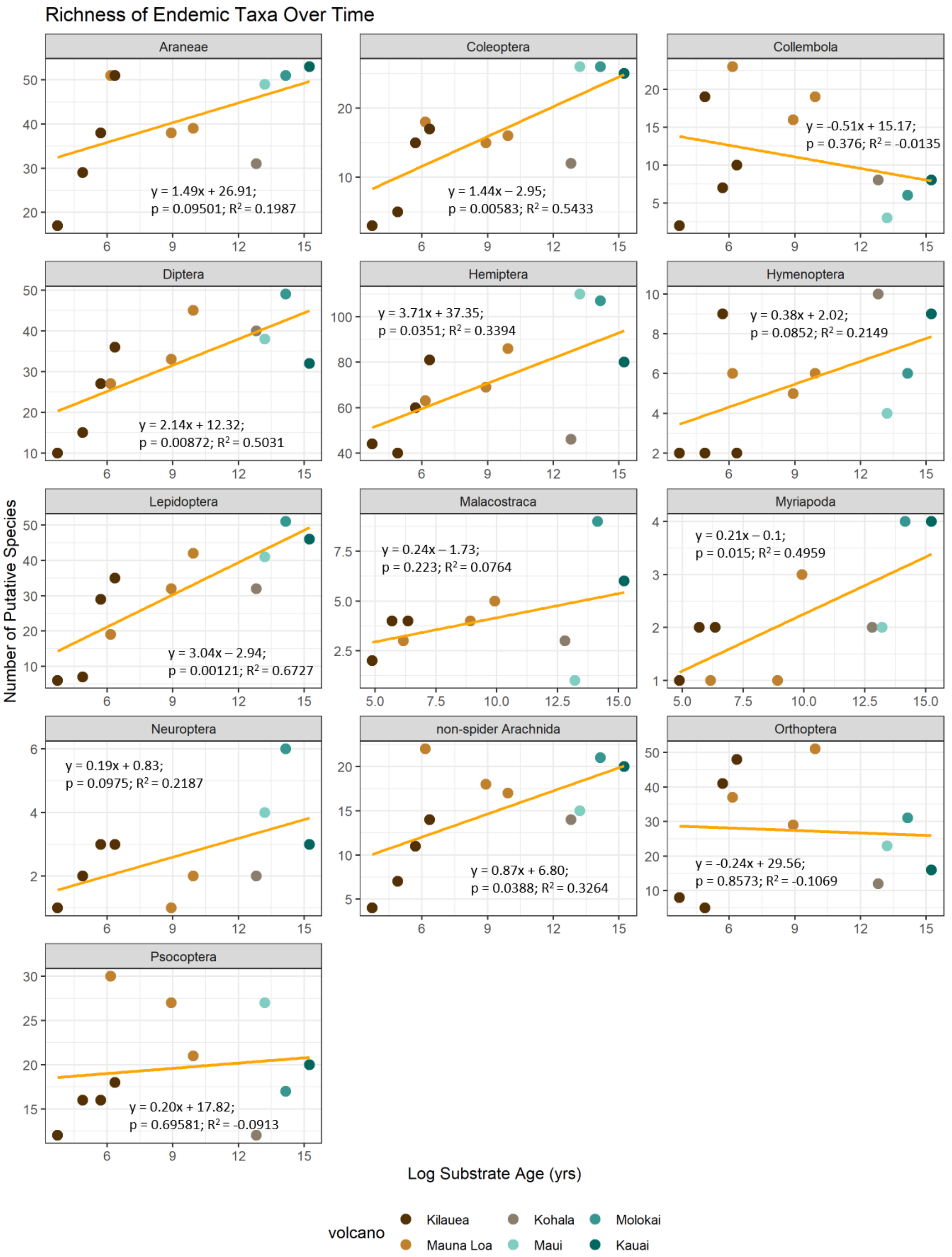
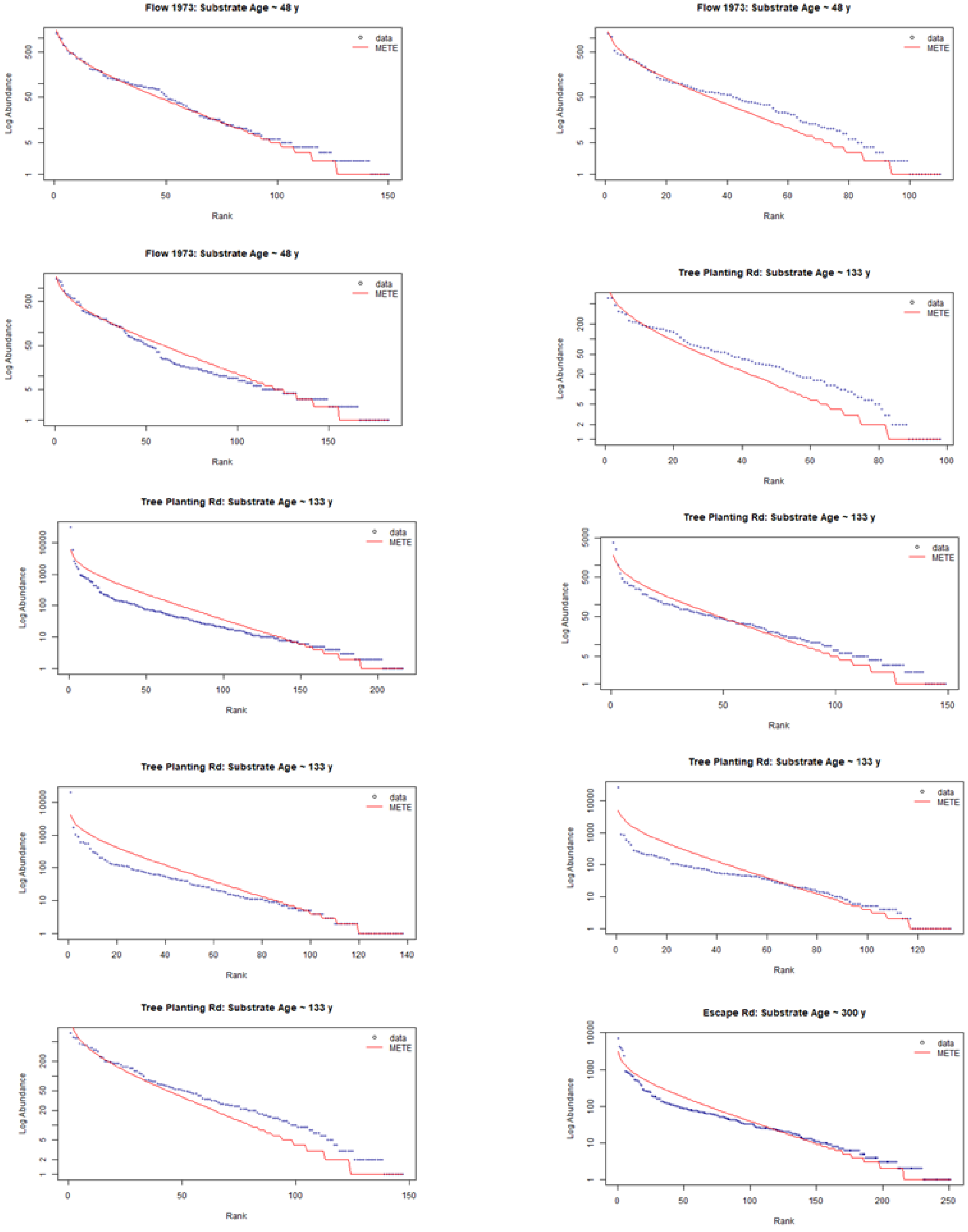


Figure S4. Richness over time. Correlation of taxon richness, measured as the number of unique Zotus from each order at each site, and the log-transformed substrate age of each community. Linear equations with significance values for regressions are presented along with adjusted R^2 values. Many arthropod orders (Araneae, Coleoptera, Diptera, Hemiptera, Lepidoptera, Myriapoda, non-spider Arachnida) show a significant increase of richness with community age ($p<0.05$). Richness in other groups (Collembola, Hymenoptera, Malacostraca, Neuroptera, Orthoptera, Psocoptera) does not accumulate linearly with time.

APPENDIX II

Supplementary materials Chapter 3: An empirical test of steady state in ecological communities over evolutionary time

All taxa ZOTU abundance curves



Flow 1973: Substrate Age ~ 48 y

Flow 1973: Substrate Age ~ 48 y

Flow 1973: Substrate Age ~ 48 y

Tree Planting Rd: Substrate Age ~ 133 y

Tree Planting Rd: Substrate Age ~ 133 y

Tree Planting Rd: Substrate Age ~ 133 y

Tree Planting Rd: Substrate Age ~ 133 y

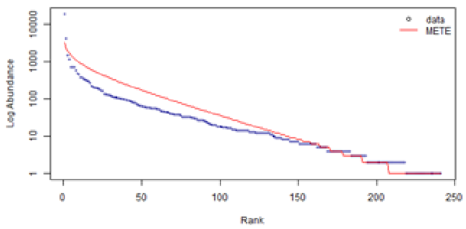
Tree Planting Rd: Substrate Age ~ 133 y

Tree Planting Rd: Substrate Age ~ 133 y

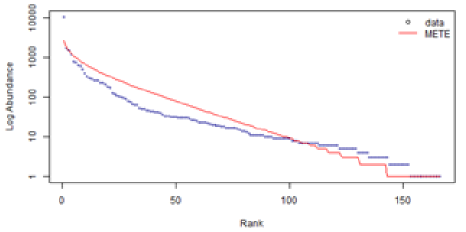
Escape Rd: Substrate Age ~ 300 y

All taxa ZOTU abundance curves

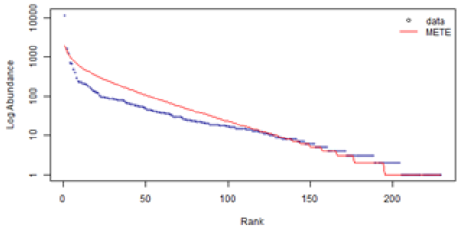
Escape Rd: Substrate Age ~ 300 y



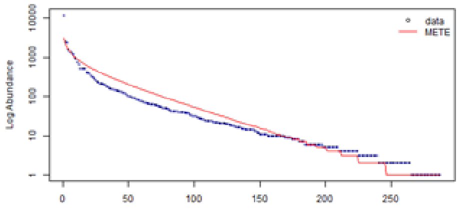
Escape Rd: Substrate Age ~ 300 y



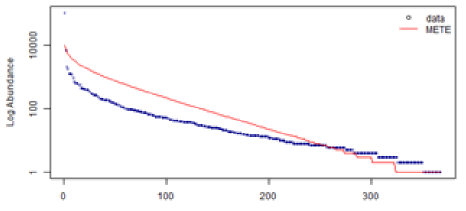
Escape Rd: Substrate Age ~ 300 y



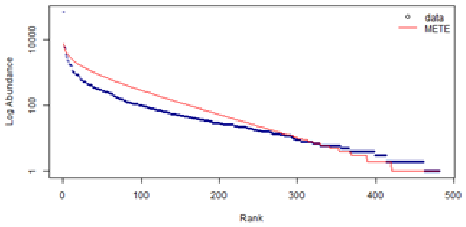
Escape Rd: Substrate Age ~ 300 y



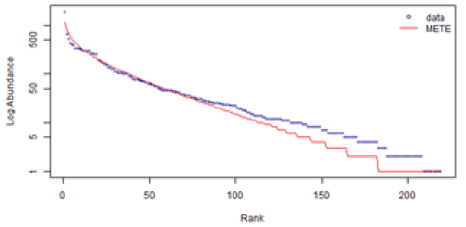
Kaiholena TNC: Substrate Age ~ 475 y

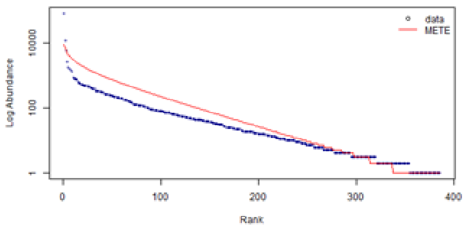


Kaiholena TNC: Substrate Age ~ 475 y

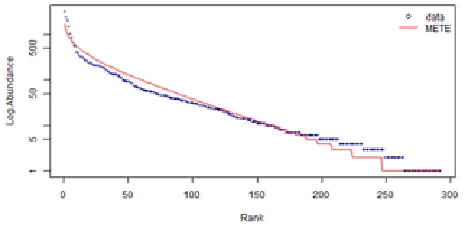


Kaiholena TNC: Substrate Age ~ 475 y

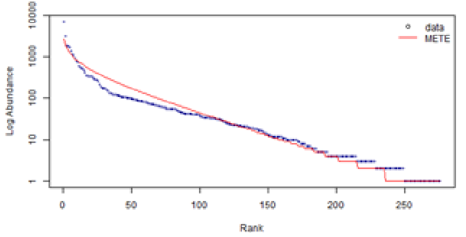




Thurston Lava Tube: Substrate Age ~ 575 y

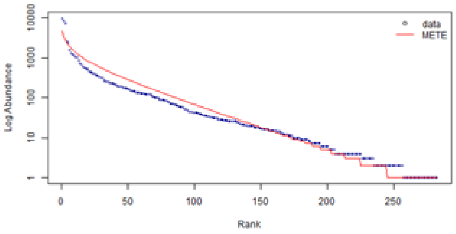


Thurston Lava Tube: Substrate Age ~ 575 y

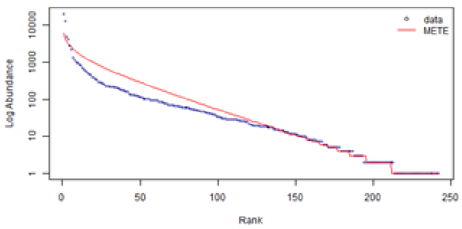


All taxa ZOTU abundance curves

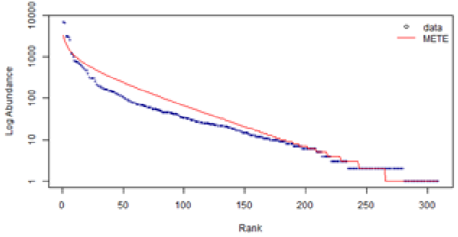
Thurston Lava Tube: Substrate Age ~ 575 y



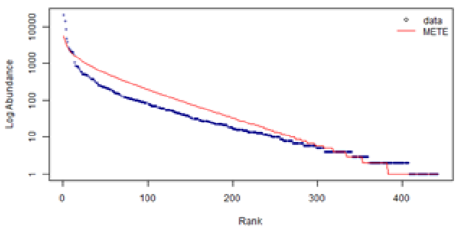
Thurston Lava Tube: Substrate Age ~ 575 y



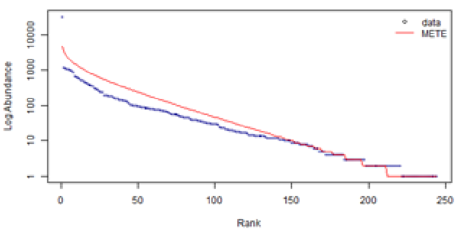
Thurston Lava Tube: Substrate Age ~ 575 y



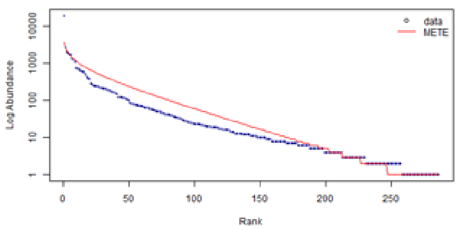
Thurston Lava Tube: Substrate Age ~ 575 y



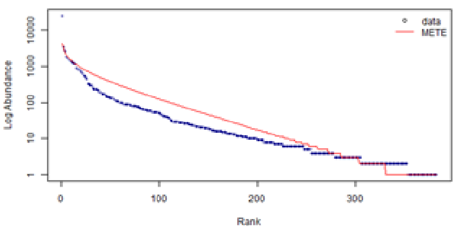
Olaa Forest: Substrate Age ~ 7500 y



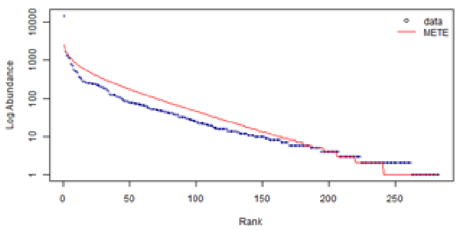
Olaa Forest: Substrate Age ~ 7500 y



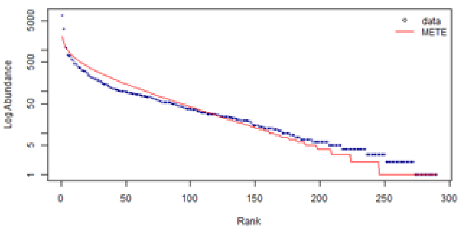
Olaa Forest: Substrate Age ~ 7500 y



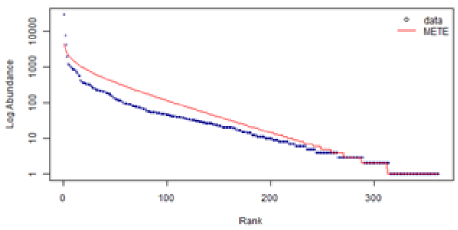
Olaa Forest: Substrate Age ~ 7500 y



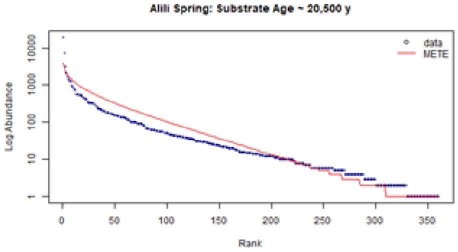
Olaa Forest: Substrate Age ~ 7500 y

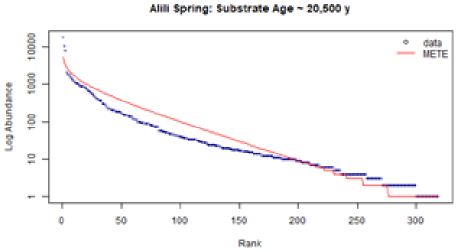


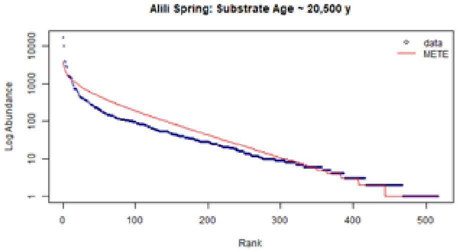
Alili Spring: Substrate Age ~ 20,500 y

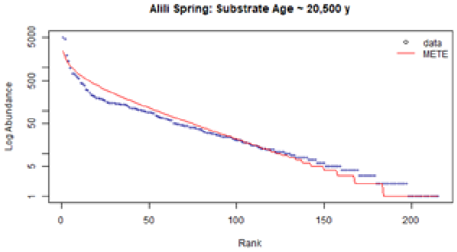


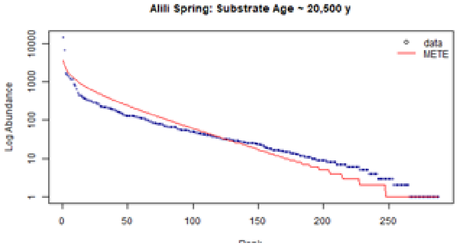
All taxa ZOTU abundance curves

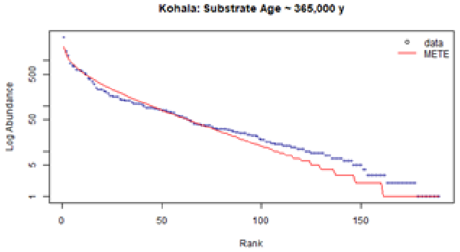

Alili Spring: Substrate Age ~ 20,500 y

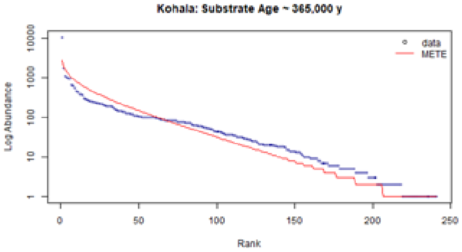

Alili Spring: Substrate Age ~ 20,500 y

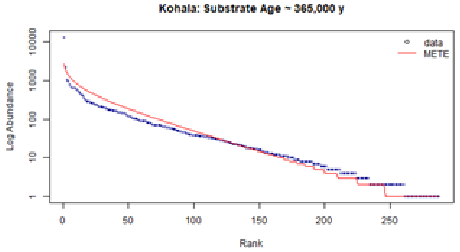

Alili Spring: Substrate Age ~ 20,500 y

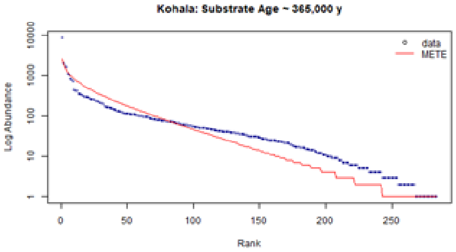

Alili Spring: Substrate Age ~ 20,500 y

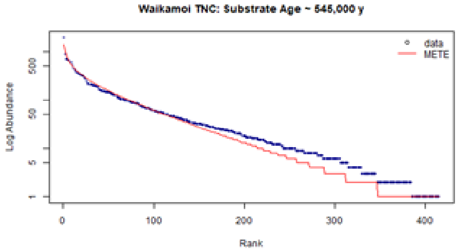

Alili Spring: Substrate Age ~ 20,500 y


Kohala: Substrate Age ~ 365,000 y

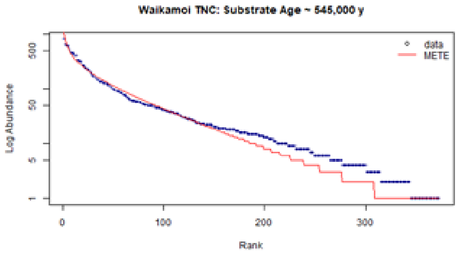

Kohala: Substrate Age ~ 365,000 y

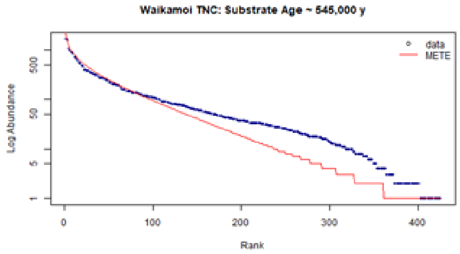

Kohala: Substrate Age ~ 365,000 y

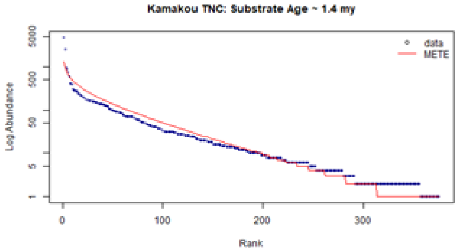

Kohala: Substrate Age ~ 365,000 y

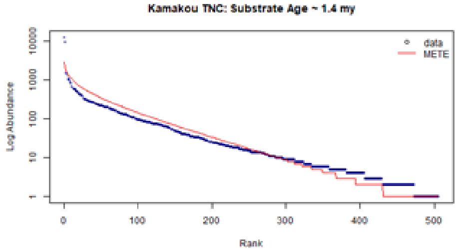

Waikamoi TNC: Substrate Age ~ 545,000 y

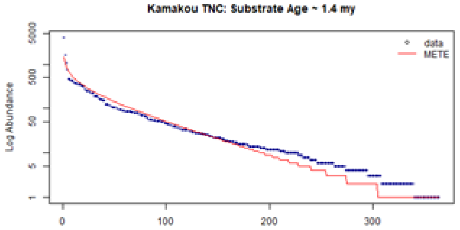
All taxa ZOTU abundance curves

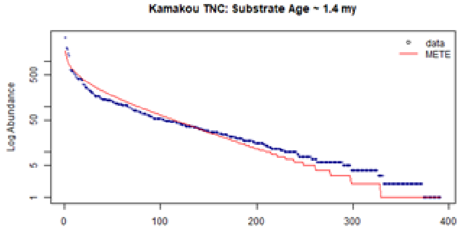

Waikamoi TNC: Substrate Age ~ 545,000 y

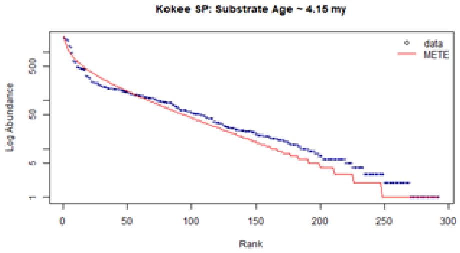

Waikamoi TNC: Substrate Age ~ 545,000 y

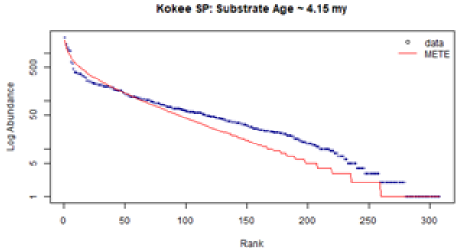

Kamakou TNC: Substrate Age ~ 1.4 my


Kamakou TNC: Substrate Age ~ 1.4 my


Kamakou TNC: Substrate Age ~ 1.4 my


Kamakou TNC: Substrate Age ~ 1.4 my


Kokee SP: Substrate Age ~ 4.15 my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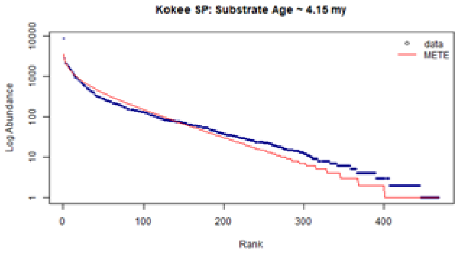
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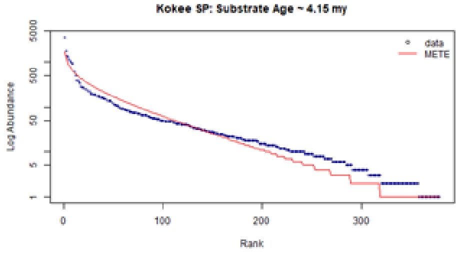
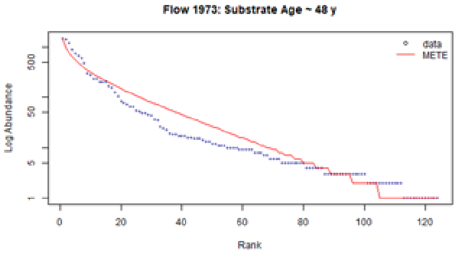
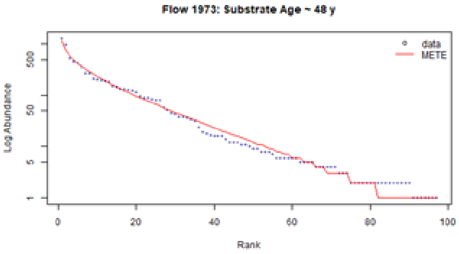
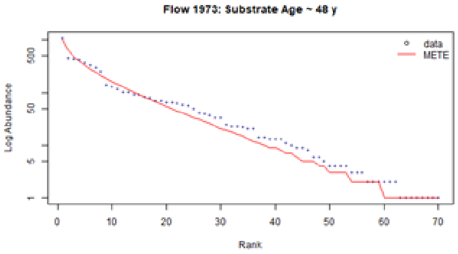
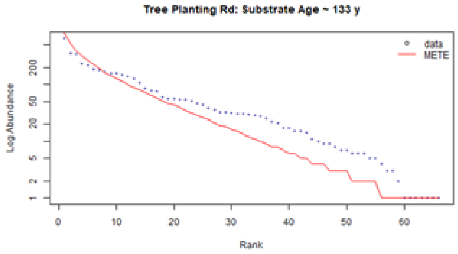

Kokee SP: Substrate Age ~ 4.15 my

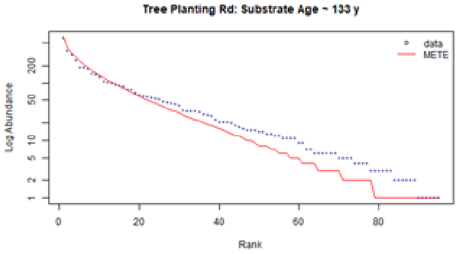
Native and Endemic taxa ZOTU abundance curves


Flow 1973: Substrate Age ~ 48 y


Flow 1973: Substrate Age ~ 48 y


Flow 1973: Substrate Age ~ 48 y


Tree Planting Rd: Substrate Age ~ 133 y


Tree Planting Rd: Substrate Age ~ 133 y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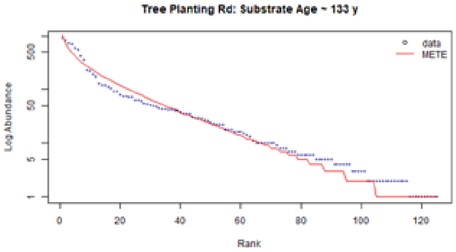
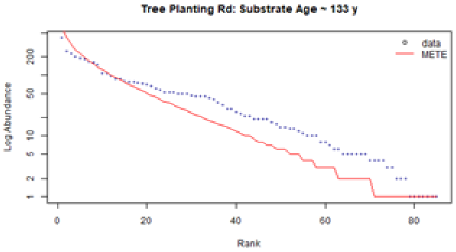
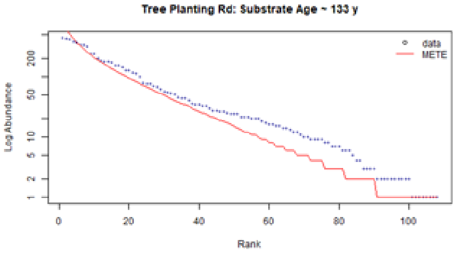
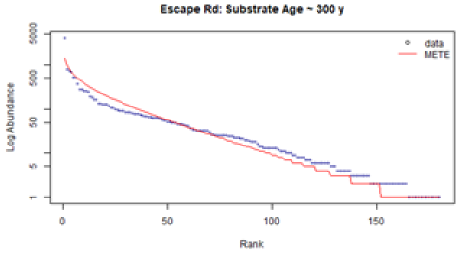
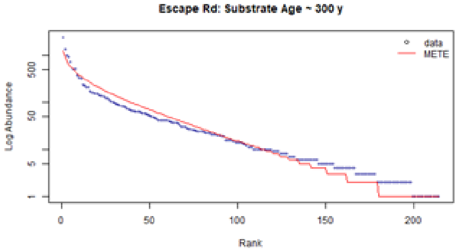

Tree Planting Rd: Substrate Age ~ 133 y

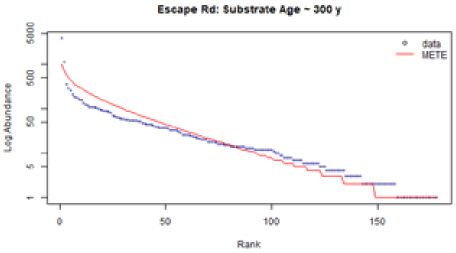

Tree Planting Rd: Substrate Age ~ 133 y

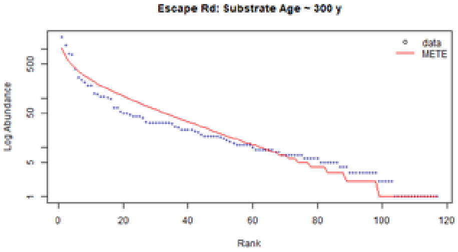

Tree Planting Rd: Substrate Age ~ 133 y

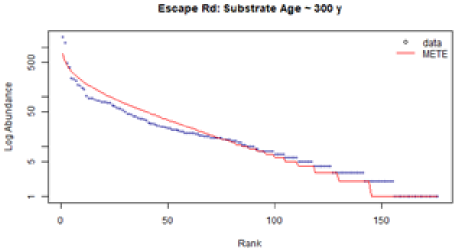

Escape Rd: Substrate Age ~ 300 y

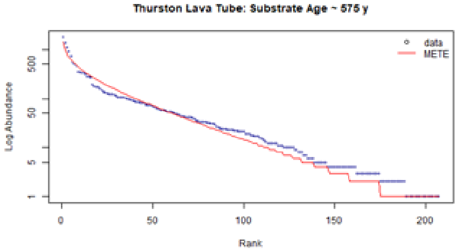
Native and Endemic taxa ZOTU abundance curves

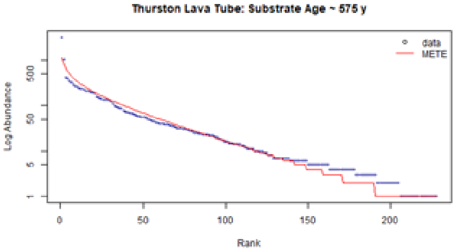


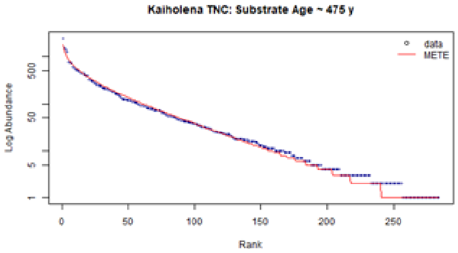


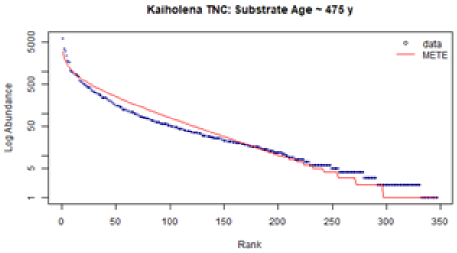


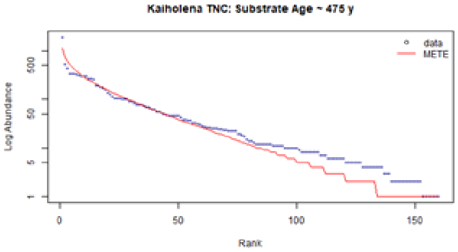


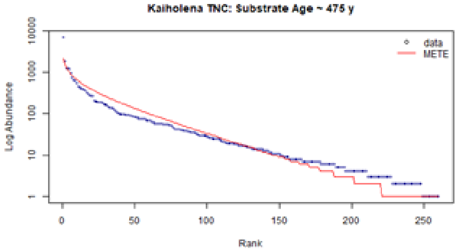




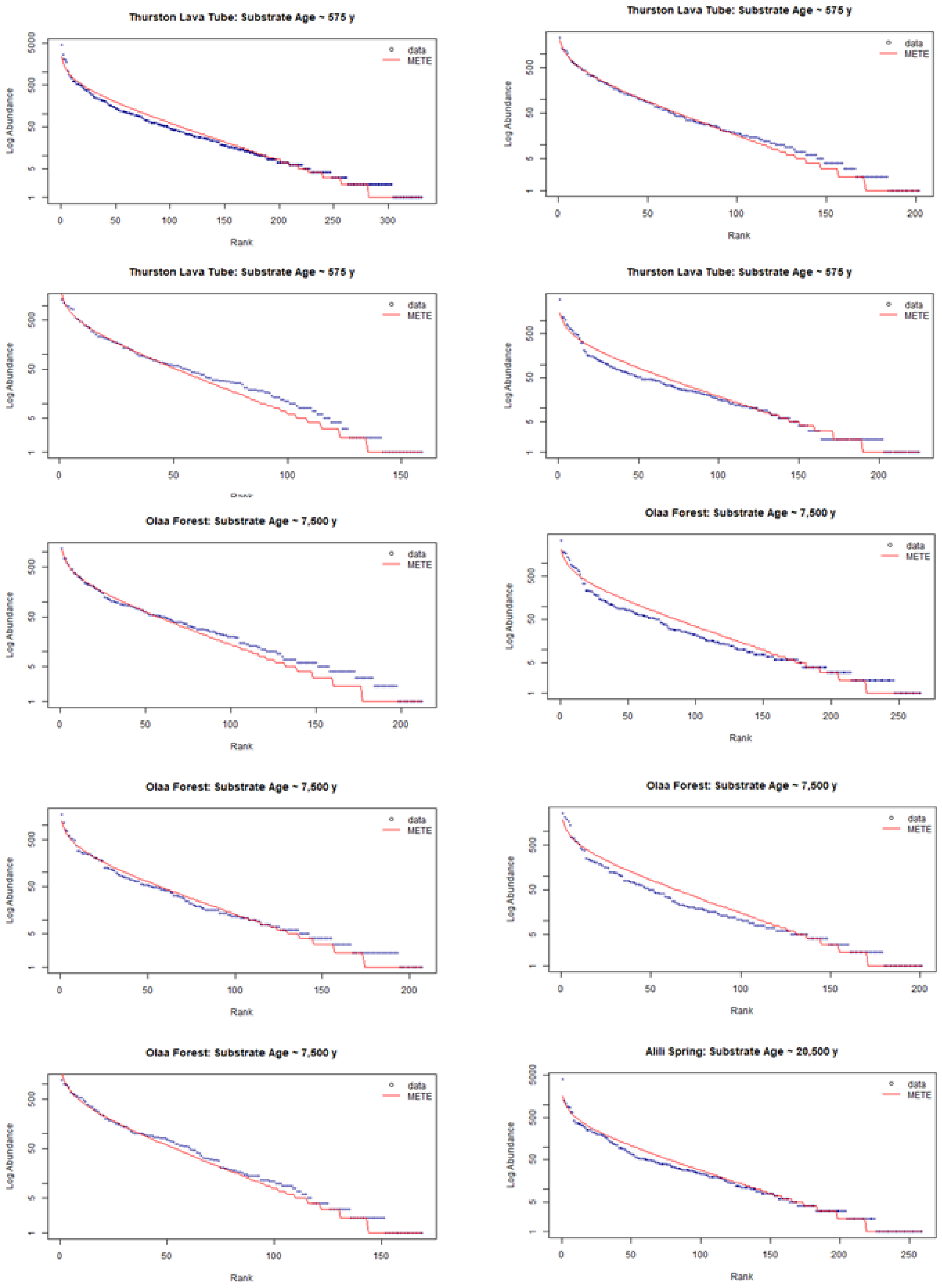




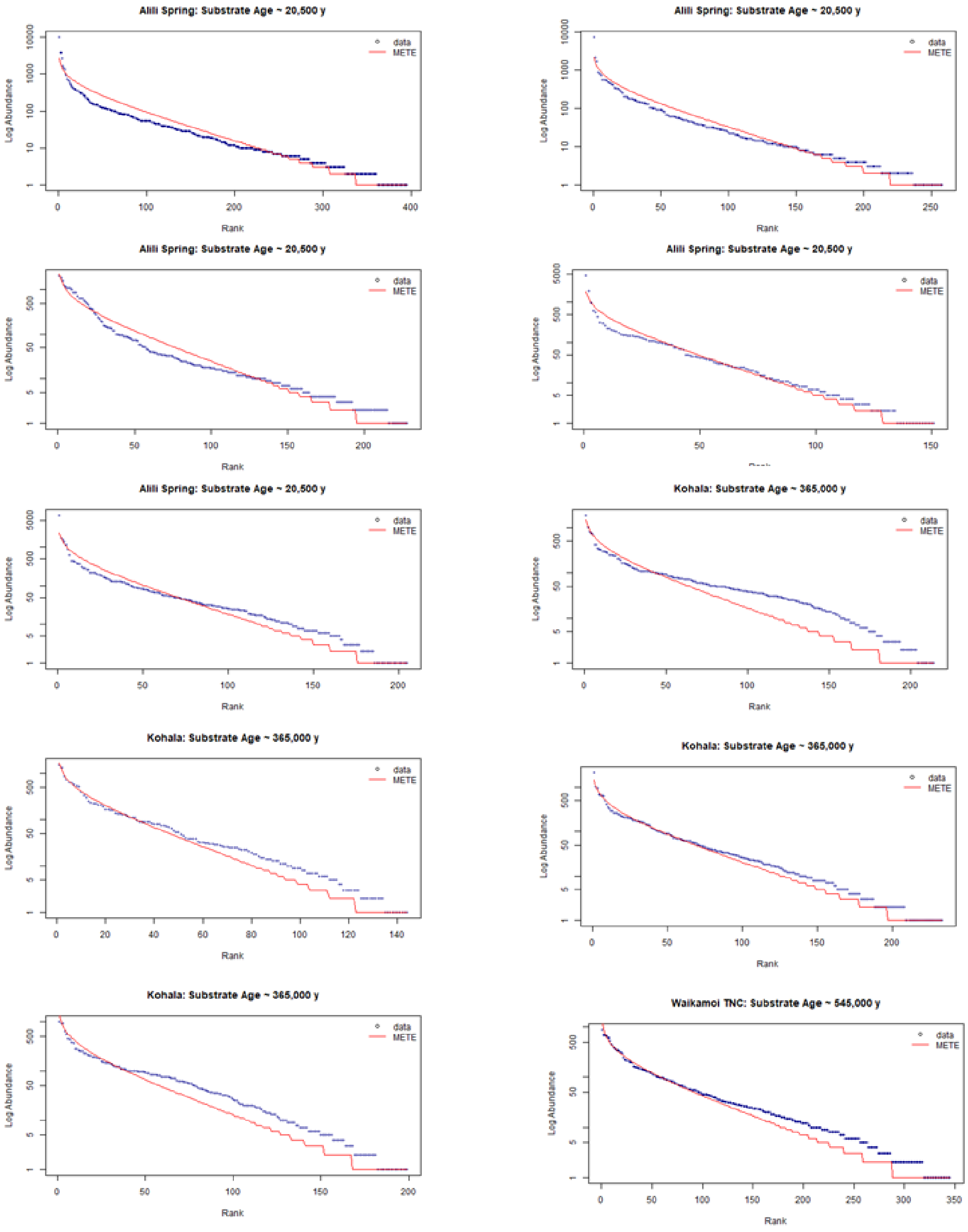




Native and Endemic taxa ZOTU abundance curves



Native and Endemic taxa ZOTU abundance curves



Alili Spring: Substrate Age ~ 20,500 y

Alili Spring: Substrate Age ~ 20,500 y

Alili Spring: Substrate Age ~ 20,500 y

Alili Spring: Substrate Age ~ 20,500 y

Alili Spring: Substrate Age ~ 20,500 y

Kohala: Substrate Age ~ 365,000 y

Kohala: Substrate Age ~ 365,000 y

Kohala: Substrate Age ~ 365,000 y

Kohala: Substrate Age ~ 365,000 y

Waikamoi TNC: Substrate Age ~ 545,000 y

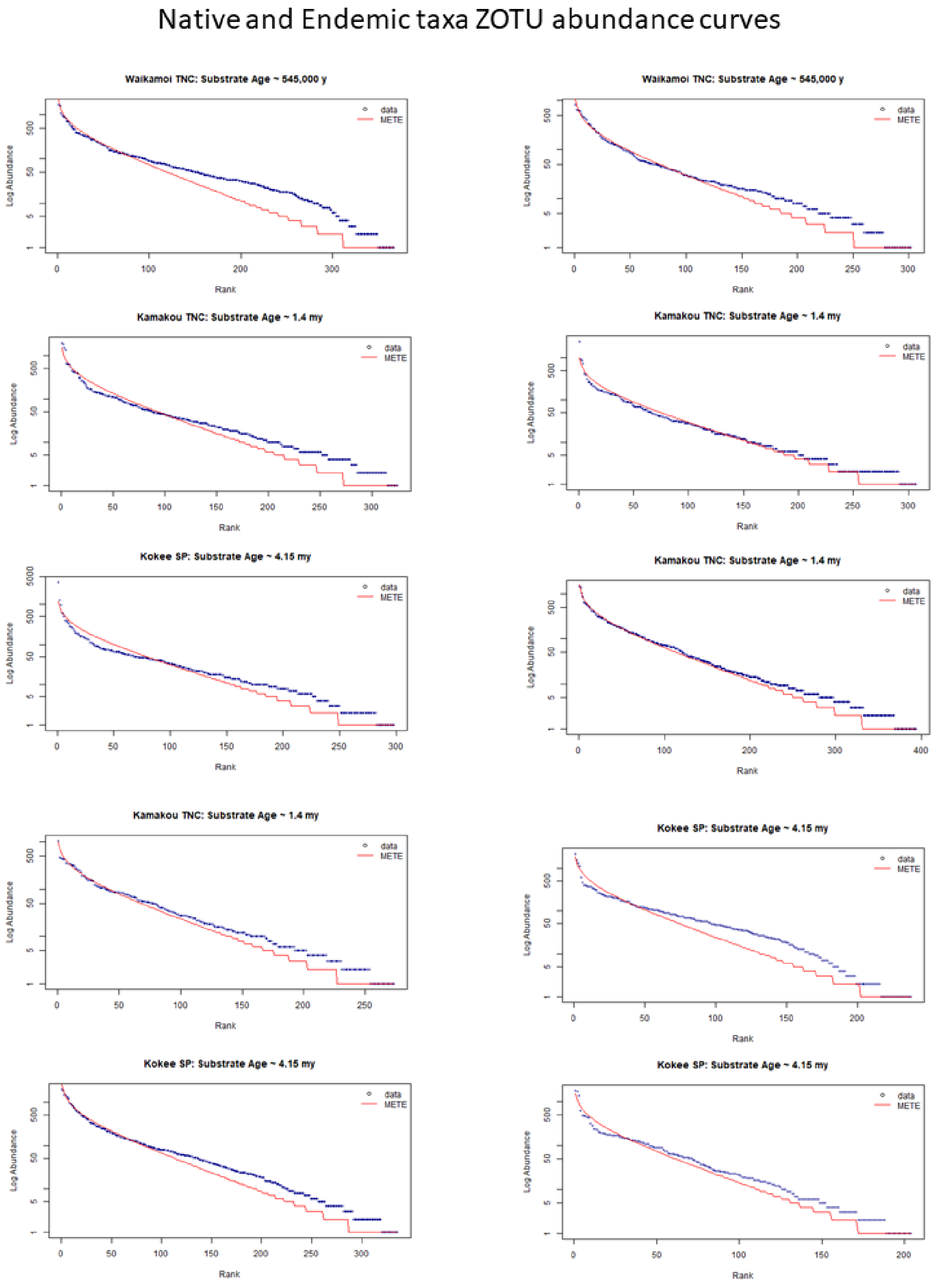


Figure S5. Species Abundance Distributions by community age. Rank abundance curves for each site replicate.