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Ecological network structure in response to community assembly processes over evolutionary time

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Abstract

The dynamic structure of ecological communities results from interactions among taxa that change with shifts in species composition in space and time. However, our ability to study the interplay of ecological and evolutionary processes on community assembly remains relatively unexplored due to the difficulty of measuring community structure over long temporal scales. Here, we made use of a geological chronosequence across the Hawaiian Islands, representing 50 years to 4.15 million years of ecosystem development, to sample 11 communities of arthropods and their associated plant taxa using semiquantitative DNA metabarcoding. We then examined how ecological communities changed with community age by calculating quantitative network statistics for bipartite networks of arthropod–plant associations. The average number of interactions per species (linkage density), ratio of plant to arthropod species (vulnerability) and uniformity of energy flow (interaction evenness) increased significantly in concert with community age. The index of specialization H_2' has a curvilinear relationship with community age. Our analyses suggest that younger communities are characterized by fewer but stronger interactions, while biotic associations become more even and diverse as communities mature. These shifts in structure became especially prominent on East Maui (~0.5 million years old) and older volcanos, after enough time had elapsed for adaptation and specialization to act on populations in situ. Such natural progression of specialization during community assembly is probably impeded by the rapid infiltration of non-native species, with special risk to younger or more recently disturbed communities that are composed of fewer specialized relationships.

KEYWORDS
community assembly, DNA metabarcoding, ecological networks, oceanic islands

1 | INTRODUCTION

Biodiversity is organized into complex ecological networks of interacting species that change through time in response to ecological and evolutionary processes. Understanding these changes is important for predicting the impacts of global change on higher multispecies organization (Dell et al., 2019; Smith-Ramesh et al., 2017; Staniczenko et al., 2017). A suite of analytical tools (Delmas et al., 2019) exist to quantify changing community structure in response to a variety of perturbations (Aizen et al., 2008;

Fricke et al., 2017; Vacher et al., 2010). A major challenge remaining is to understand the configuration of ecological networks in a predictive context over long spatiotemporal scales (Poisot et al., 2015; Trøjelsgaard & Olesen, 2016; Yeakel et al., 2014). Consequently, the effect of community assembly processes on the structure of interaction networks describing ecological communities remains poorly understood (Ponisio et al., 2019; Rominger et al., 2016).

Early research on community assembly often ignored ecological interactions due to their complexity. Notably, neutral models for community assembly are even agnostic to organismal identity (Hubbell, 2001; Rosindell et al., 2011). As species identity and interactions began to be incorporated into models, the initial "assembly rules" of Diamond (1975) highlighted "forbidden species combinations" and nonrandom patterns of co-occurrence. A growing recent theme focuses on the effect of abiotic and biotic filters on a regional species pool (Münkemüller et al., 2020) with varying temporal and spatial filters dictating network structure (Peralta et al., 2019). However, much of this work ignores the role of evolution in shaping interactions through time. The extent of adaptation, and possible speciation, in shaping interactions as communities assemble depends on the isolation of the community from the source pool (Gillespie et al., 2020; Rosindell & Phillimore, 2011). At the extreme, evolution will have shaped interactions among every member of a community and the effects of filtering from a regional species pool might thus appear relatively weak (Ponisio et al., 2019). While most communities will include the role of both ecological filtering and evolutionary adaptation, our ability to thread complex ecological questions of network structure into an evolutionary framework has presented a major obstacle.

Recognizing this impediment, recent work has examined avenues to approach the problem. In particular, models of trait evolution on phylogenies provide a means to understand how eco-evolutionary feedbacks shape interactions as communities assemble (Segar et al., 2020). Likewise, based on theory showing how change across short timescales affects longer-term evolutionary dynamics, clade-level phylogenetic comparative approaches can be applied to community data to understand the dynamics of network structure (Weber et al., 2017). Both these approaches focus on the lineages that make up communities, asking how interacting sets of lineages affect each other. However, another approach is to focus explicitly on the community rather than individual lineages, connecting large-scale understanding of community interactions at a given time in a spatially variable environment with the understanding of how the integrated structure of biodiversity changes through time. Such an approach attempts to address a major gap in the field by bridging macroecology and macroevolution (McGill et al., 2019) and hence showing how network structure changes across scales of space and time within a whole-community context (Weber et al., 2017).

While theory indicates a clear role for biotic interactions leading to individual and community specialization over long-term community development, empirical evaluation has been challenging. One difficulty is in obtaining measures of community composition and interactions at relevant spatial scales, and another obstacle is the vast time frame over which evolutionary phenomena occur. With their short generation times that are amenable to laboratory studies, microbial systems provide exceptional cases that document community assembly over evolutionary timescales (Boon et al., 2014; Koskella et al., 2017; Koskella & Brockhurst, 2014; Venturelli et al., 2018). In particular, studies of the plant phyllosphere showed a more prominent role of non-neutral selection over time and an increase in the strength of biotic interactions and community cohesion (Morella et al., 2020). However, to infer the role of interactions in community assembly of longer-lived macroorganisms requires very particular systems. Here, we make use of two sets of circumstances that, together, provide an extraordinary opportunity to assess the nexus of ecological and evolutionary community assembly in the context of interaction networks.

First, we use the system provided by the Hawaiian Islands. Islands in general provide discrete communities that can be used for natural experiments in interaction dynamics (Brodie, 2017; Castro-Urgal & Traveset, 2014; Olesen et al., 2002). In particular, oceanic archipelagos formed in situ over millions of years offer the opportunity to study species interactions over evolutionary timescales (Hembry et al., 2018; Ponisio et al., 2019; Rominger et al., 2016; Trøjelsgaard et al., 2013). Moreover, the geological series of islands in the Hawaiian archipelago represents a chronosequence (Vitousek, 2002; Walker et al., 2010); each substrate age represents communities of different ages, ranging from ~50 years to ~5 million years (Myr) (Shaw & Gillespie, 2016). Notably, the native montane forest of Hawaii is dominated by just two canopy tree species (*Metrosideros polymorpha* and *Acacia koa*), making it relatively simple ecologically and hence more amenable to capturing and characterizing whole communities.

Second, we make use of the emerging field of DNA metabarcoding (Krehenwinkel, Wolf, et al., 2017; Yu et al., 2012), which makes a comprehensive analysis of taxonomic composition possible, offering the opportunity to simultaneously assess thousands of species rapidly, and offering enormous potential for reconstructing complex ecological networks (Clare, 2014; Hrček & Godfray, 2015; Vacher et al., 2016). Relative sequence abundances offer a proxy for interaction strength (Lim et al., 2021), providing greater reliability for co-occurrence studies to measure biotic associations (Bálint et al., 2018; Mata et al., 2021). 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.

Here we use semiquantitative DNA metabarcoding to build networks of arthropod–plant associations at 11 sites across the Hawaiian chronosequence, using the substrate age at a site as a measure of community age, and then use those networks to test a range of expectations on how ecological and evolutionary processes shape community structure over long timescales (e.g., Table 1). We expect network size—both the number of nodes and number of links—will increase with community age, but disproportionately, as younger communities gain taxa through colonization only and older

TABLE 1 Some expectations for network structure in response to ecological and evolutionary factors influencing community assembly through time.

Process	Richness?	Specialization?	For example, metric change	Expected change in metric through extended period of community assembly
Immigration	↑	—	Connectance	Connectance should increase in response to immigration when generalist colonists take advantage of weak resource defences, increasing the number of associations that form out of all possible associations [1]
Environmental filtering	↓	↑	Linkage density	Linkage density should be higher in novel (young or recently disturbed) networks due to generalists with a greater number of interactions per species [1]
Increasing abundance	↑↓	—	Interaction evenness	Interaction evenness should decrease with dynamics in taxon abundance because skewed frequency distributions (i.e., a few species with many links and many species with few links) are largely driven by species abundance [2]
Antagonistic interactions	↑↓	↑	Vulnerability	Vulnerability is high in highly modified systems [3] because the average number of consumers per resource is high. Thus, we expect vulnerability to be high in the very youngest community forming on bare lava and go down. However, we expect vulnerability will increase over long temporal scales with reduced competition, predation, parasitism, etc., leading to some level of resource redundancy; for example, several species feed on the same resource
Beneficial interactions	↑	↑	Generality	Generality should increase with increasing pollination, frugivory, camouflage, etc., because species can share the same resources in different locations or times resulting in resource complementarity [4]
Increasing niches	↑	↑	Generality, Vulnerability	The narrowing of interactions to a subset of resources increases specialization. [5] As niches proliferate the fraction of species associations per interaction partner will go down; for example, niche breadth of herbivores decreased with island age [6]
Trait diversification	↑	↑	Linkage density, H_2'	Evolution and network structure are linked [7]. For example, traits with a phylogenetic signal (such as flower symmetry and pollinator size) can accurately predict interaction partners [8]. If species traits diversify together (co-evolve) then more one-to-one relationships will result in lower linkage density and higher specialization (H_2')
Speciation	↑	↑	Interaction evenness	Where food webs are dominated by a single link interaction evenness is lowest [3]. With the addition of species through in situ speciation, the number of links will increase with greater interaction evenness
Extinction	↓	↓	Connectance	A specialist species may go extinct from the loss of an essential resource. Species interaction networks composed of many specialist species should have low connectance, and primary extinctions are expected to propagate quickly [9]

Note: Specialization should change at the network level to depict the changes at the taxon level of more evolved associations. Since the associations of each species are becoming more specialized there is an expectation that an individual taxon will have fewer interactions and the makeup of the whole community will have more interactions overall. [1] Bufford et al. (2020), [2] Vázquez et al. (2007), [3] Tylianakis et al. (2007), [4] Peralta et al. (2014), [5] Coux et al. (2016), [6] Ponisio et al. (2019), [7] Segar et al. (2020), [8] Chamberlain et al. (2014), [9] Blüthgen et al. (2006), [10] Dunne and Williams (2009).

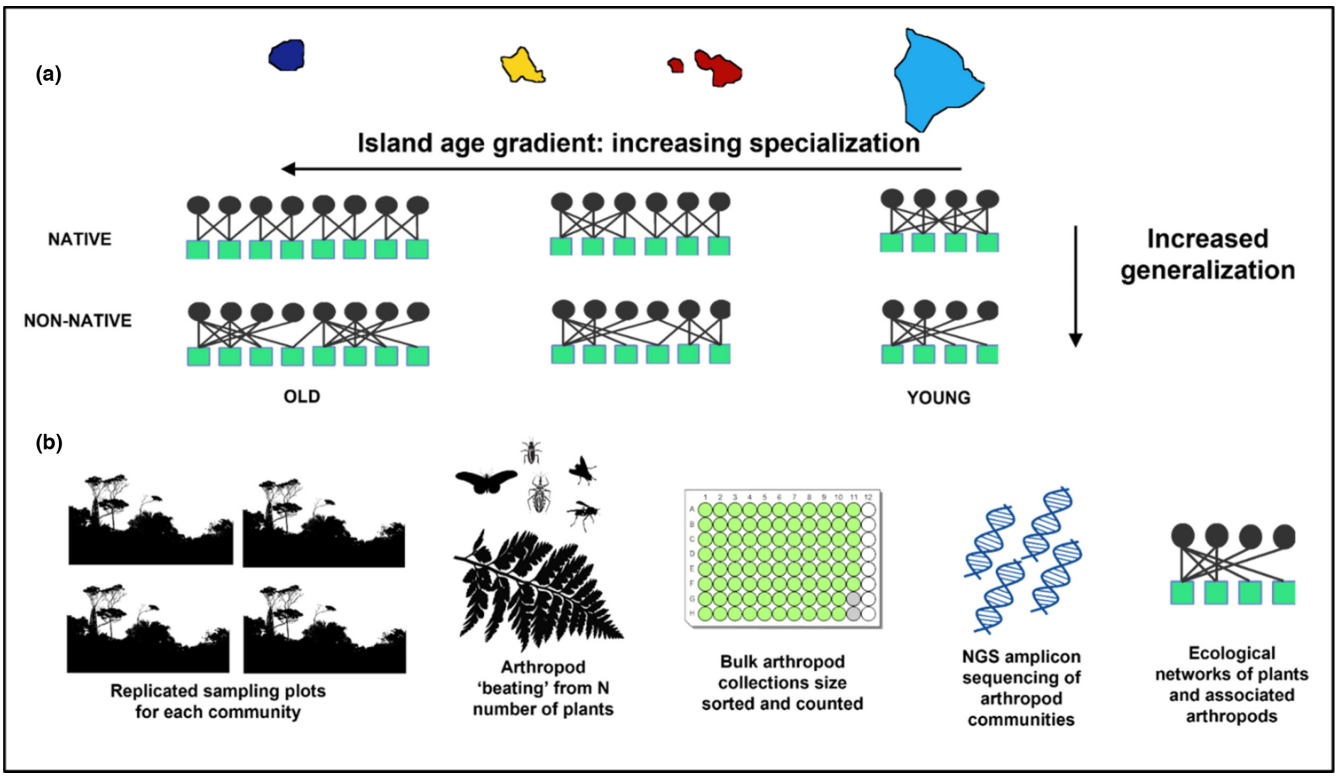

FIGURE 1 Study overview. (a) Study aims. As communities assemble over time species will be added through ecological and evolutionary processes. Network size will increase over time. There will be a trend towards greater specialization as relationships among species are modified through ecological fitting and evolutionary adaptation over extended time, young to old sites (top panel). Recently introduced species (i.e., non-natives) evolved elsewhere and have not adapted in place to biotic and abiotic factors, thus limiting their specialization within communities at all stages of development (bottom panel). (b) Study design. Within multiple 15-m-radius plots at 11 communities from ages 50 years to 4.15 million years, plant species were sampled for associated arthropods by vegetation beating according to their relative abundance. Each sample of plant-associated arthropods was size sorted, counted and placed into a well of a 96-well plate, such that well "A1" contained sample 1, size category 0–2 mm, and well "A2" contained sample 1, size category 2–4 mm, and so on. DNA extraction and PCR amplification with dual-indexing was used to prepare the size-sorted samples into amplicon libraries which were sequenced on an Illumina Miseq for the cytochrome oxidase I locus. Ecological networks were constructed from the arthropod–plant associations for each community age. [Colour figure can be viewed at wileyonlinelibrary.com]

communities assemble through colonization and evolutionary processes. This allows tests of the following hypotheses for evolutionary assembly of networks (Figure 1). (H1) Starting from bare lava, early successional communities offer low resource diversity yet are necessarily composed of assemblages from nearby species pools. Therefore, younger communities will have a high proportion of generalists—a subset of the nearby species pool most likely to persist without particular interaction partners—but greater interaction frequency on fewer interaction pathways because of resource heterogeneity. (H2) The set of biotic interactions that a given taxon will experience will decrease with community age, and the evenness of the interactions among resources will increase, resulting in greater network specialization (Ponisio et al., 2019; Rominger et al., 2016). One ideal component of this study is the large temporal span of time for community assembly. We can assume that younger communities will gain taxa only through colonization given that they are not established long enough for in situ speciation to take place. Of course, older communities will assemble through colonization and evolutionary processes. We cannot tease apart the effects of both processes at the oldest sites, but we can compare the youngest to oldest sites and their related ecological networks for signatures of assembly after evolutionary processes have taken effect. With an increasing number of taxa that have evolved together in a community it follows there will also be an increase in the specialization of the interactions among these species that may be detectable at the network architecture level.

2 | MATERIALS AND METHODS

2.1 | Site selection methods

The Hawaiian Islands are formed as the Pacific plate moves northwestward across a stationary volcanic hotspot, and therefore the archipelago represents a chronosequence of geological age from the youngest island (Hawaii, ~0–0.5 Myr), to the oldest high island of Kauai (~5 Myr) (Clague, 1996). Discrete volcanoes within islands present additional contrasts in geological age, and the underlying substrate age has been mapped in fine detail (Wolfe & Morris, 1996). *Metrosideros polymorpha* (Myrtaceae) is the dominant canopy tree

in these forests across islands, with patches of subdominant *Acacia koa* (Fabaceae) and numerous associated understorey trees, shrubs, herbs and ferns (Gagne & Cuddihy, 1990).

We selected 14 sites of varying geological age, ranging from 50 to 4.15×10^6 years old, across four islands of the archipelago: Hawaii, Maui, Molokai, Kauai (Figure S1; Table S1). To control for climatic differences and disturbance across sites, sites were constrained to ranges of elevation (1000–1300 m) and precipitation (average annual precipitation 2500–3000 mm) and within accessible protected forest lands (Gap Analysis Project | U.S. Geological Survey, 2019; Giambelluca et al., 2013).

For each potential site, spatial polygons were created based on the intersections of these layers and initial field reconnaissance to confirm remotely sensed data and feasibility of access. Within these potential site polygons, airborne high-resolution laser scanning from the Global Airborne Observatory (GAO; formerly named Carnegie Airborne Observatory; Asner et al., 2012) was used to generate forest canopy height profiles using a physical model described in Asner et al. (2008). The ground digital elevation model was also generated using the method of Asner et al. (2007). The data were collected at four laser shots per square metre, processed to height profiles at 5-m resolution and then averaged at a grid cell spacing of 30 m (Figure S2; Table S2).

Twenty randomized candidate plots were generated for each site, with the intention of ultimately selecting six, 15-m-radius plots. These 20 randomized candidate plots were constrained to be a minimum distance of 200 m from all other plots and to be within the top 40% quantile of LiDAR-estimated canopy heights. Candidate plot generation was achieved with custom scripts in the R programming language (R Core Development Team, 2019) using a simple rejection sampling algorithm: random sets of spatial locations are generated within pixels of sufficient canopy height until a set of locations is found which meet the requirement of being 200 m distant. The minimum distance of 200 m was a constraint to maximize independence among sampling areas while capturing more spatial heterogeneity within sites.

At each site, each of the 20 candidate plots were ground-truthed to confirm the plot was dominated by native vegetation and minimally impacted by human use and/or invasive vertebrates. This ground-truthing process eliminated a variable number of the initial 20 candidate plots. If fewer than six final plots remained after ground-truthing, another set of candidate plots were generated and ground-truthed to find a final set of six plots. If more than six plots remained after ground-truthing, the final six plots were selected by randomly selecting from the ground-truthed plots.

2.2 | Collection protocol

We collected arthropods using vegetation beating at six 15-m-radius plots per site during May 2015 to January 2016, with plots sampled randomly to avoid seasonality effects on arthropod composition. To ensure equal sampling effort across sites, sampling was limited to a total of 420 s in each plot. If after all arthropod collection processing steps (described below) the total vegetation beating time for a plot was not within one standard deviation of 420 s sampling effort then that plot was dropped from further analyses, resulting in a total of 50 plots from 11 sites (Table S1). As we were interested in characterizing plant–arthropod associations, we sampled plant genera in each plot proportional to their relative abundance. Percentage cover of each understorey plant genus was estimated visually. Where plants could not be identified to the genus level, we grouped them into morphotaxa and sampled them accordingly. Vegetation beating was performed by placing 1 × 1-m white beating sheets under individual plants and gently agitating the foliage using a 1-m-long PVC pole for timed second intervals. Arthropods dislodged by the agitation which drop onto the beating sheet are aspirated 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, labelled and transported to the laboratory where it was stored at −20°C.

2.3 | Specimen sorting and DNA extraction

To reduce bias due to differently sized individuals contributing disproportionate amounts of DNA (Elbrecht & Leese, 2015) specimens were sorted following procedures described in Lim et al. (2021). Each plant beating sample was sorted in Petri dishes on 1-mm graph paper under a stereoscope into four size categories (0–2, 2–4, 4–7, and ≥7 mm) based on the body size distribution found in a common Hawaiian ecosystem. Individuals in each size category were counted and placed with fresh ethanol into a single well in a 96-well plate. Thus, all individuals from a particular plant genus at a particular plot have their DNA extracted together and are prepared together using a dual-indexing strategy described below into next-generation sequencing (NGS) amplicon libraries for sequencing. The Collembola had considerably higher abundance than the remaining arthropods in the small size categories, and therefore Collembola were separated into 1.5-mL Eppendorf tubes and processed for DNA extraction and sequencing parallel to the remaining arthropod community samples.

Specimens from public and private collections were also used to generate a DNA barcode reference library for 57 species. We used whole bodies of species from private collections where available because these were easiest to generate sequences from preserved material (86% barcode generation success). Genomic DNA extraction of size-sorted arthropod–plant community samples was performed in 600-μL volumes using the Tissue protocol described in the Qiagen Puregene kit modified for automation (Lim et al., 2021). DNA was eluted in 50 μL DNA Hydration Solution.

2.4 | Sequence analysis

Each size-sorted sample and a polymerase chain reaction (PCR)-negative for each 96-well plate (containing no template DNA) was amplified with a primer 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. This primer pair has been suggested as the most appropriate for capturing arthropod diversity in DNA metabarcoding studies (Elbrecht & Leese, 2015) and has been shown to reliably amplify the Hawaiian arthropod community (de Kerdrel et al., 2020). PCRs were run in 10-μL volumes using the Qiagen Multiplex PCR kit at an annealing temperature of 46°C, with 1 μL 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 can be pooled together. PCR products were cleaned of residual primer using a 1× ratio of SPRI beads (Sera-Mag) and pooled together based on band intensity (i.e., DNA concentration) 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 six cycles was performed with the pooled amplicons to introduce dual indexes and Illumina TruSeq sequencing adapters to 5′-tails of the locus-specific PCR primers (Lange et al., 2014), with a final 5′–3′ layout as Illumina adapter, 6-bp inline barcode and PCR primer as described in de Kerdrel et al. (2020). The indexed products were cleaned again with SPRI beads, quantified by electrophoresis, and then pooled in equal amounts into a single tube. The samples were then sequenced on an Illumina MiSeq using V3 (600 cycles) chemistry according to the manufacturer's protocol (Illumina). We aimed for a total of 30,000 reads per sample. Each PCR negative was sequenced with each plate of specimen libraries regardless of the absence of detectable PCR product on a gel.

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 with no mismatches allowed. 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 PCR primer and 6-bp inline barcode combination, using the forward and reverse primer sequences as indices with the grep command in UNIX, and the primer sequences were then trimmed using the UNIX stream editor.

2.5 | Rarefaction and pseudogene removal

We rarefied each sample using a custom unix command 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× with replacement. The process of rarifying by repeated 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 (Lim et al., 2021).

We generated zero-radius operational taxonomic units (zOTUs), from the rarefied raw reads with the unoise3 command (Edgar, 2016) following the recommended protocols in the USEARCH version 11 pipeline (Edgar, 2010). Specifically, the quality trimmed reads were dereplicated and clustered into zOTUs using the unoise3 command in USEARCH. Chimeras were removed de novo in USEARCH. The resulting zOTUs were compared against the NCBI GenBank database and our custom-made DNA reference library for Hawaiian taxa using BLASTN with a maximum of 10 target sequences. All nonarthropod zOTUs were removed after which 5046 zOTUs remained. We aligned these 5046 zOTUs using default settings in CLUSTAL OMEGA (Sievers et al., 2011). To remove putative pseudogenes from the zOTU data set we ran METAMATE with default specifications and the example specifications file to detail how per-zOTU read frequencies should be assessed (Andujar 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.

2.6 | Taxonomic matching and abundance estimates

About a quarter of the zOTUs (n = 901) were matched to the BLAST or voucher DNA reference library with less than 85% similarity. To validate the taxonomic identification for each zOTU at higher taxonomic levels (e.g., order, family) we compared the top 10 BLAST and reference library hits with phylogenetic clustering from a maximum-likelihood (ML) tree. An ML tree with bootstop autoMRE bootstrap support was generated by running RAXML-HPC version 8 on XSEDE on the Cipres science gateway (Miller et al., 2010) under the GTR model with a gamma distribution plus invariant sites. For 28 zOTUs, taxonomic order could not be determined via sequence similarity to databases or phylogenetic clustering and were thus removed from downstream analysis. Taxonomic assignment was considered trustworthy if the percentage similarity of the metabarcoding sequence to the NCBI GenBank or DNA reference voucher was: 88%–94% for family, 94%–98% for genus and >98% similarity for species, while matches below 88% similarity were made only to order. These threshold values were arbitrarily chosen based on previous investigations using mock communities or photo voucher integrative taxonomy of selected taxa from the same high-elevation wet forest communities of Hawaiian arthropods and amplified using the same COI marker (Krehenwinkel, Kennedy, et al., 2017; Krehenwinkel, Wolf, et al., 2017, de Kerdrel et al., 2020).

To create a table with OTU abundances for community analyses we mapped a query set of raw reads to the filtered and taxonomically identified search database of zOTUs in USEARCH version 11 (Edgar, 2010) using the otutab command with the default 97%

similarity mapping threshold. After OTU mapping and read removal based on the PCR-negative control sequencing pool, the number of unique sequences was reduced by 133 zOTUs to 4197 OTUs.

To use relative sequence abundance of arthropod OTUs as an approximation of arthropod–plant associations, we adopted a semi-quantitative processing pipeline (Lim et al., 2021) to ameliorate differences in sampling effort, body size of specimens and genomic procedures. To review: (i) for each site (community) there were six plots, (ii) within each plot each plant taxon was sampled by seconds of time corresponding to its relative abundance in the plot, (iii) we sorted bulk arthropod samples by size and counted the individuals, (iv) sequences were generated using a DNA region with demonstrated success for Hawaiian arthropod taxa (de Kerdrel et al., 2020) and false reads (pseudogenes) were removed (Graham et al., 2021), (v) we randomly sampled the sequencing reads based on the count of individuals in each size class and (vi) sequence reads were summed across size classes and plots (Figure 1b).

2.7 | Calculation of quantitative ecological network metrics

Using bipartite networks of arthropod–plant associations at each community age, we tested our hypotheses by calculating quantitative (weighted) and qualitative (binary) network metrics (Table 2) expected to occur in the transition from younger communities to older communities (Table 1). Data processing and statistical analyses were performed in R version 4.0.2. To distinguish between taxa that have colonized the archipelago historically or more recently, we characterized the probable native and non-native composition for each aged community based solely on sequence characteristics as outlined in Andersen et al. (2019). The approach considers both the evolutionary distances between species and the genetic diversity within species. Sequence characteristics of OTUs 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. The approach was implemented into a machine learning strategy using random forests in SKLEARN and packaged with multiple utilities and a graphical user interface in NICLASSIFY (https://github.com/tokebe/niclassify). By annotating the nativeness status for sequences which are identifiable to species level (98% or above match to databases), NICLASSIFY can accurately assign status for the remaining sequences. As part of the NICLASSIFY classifications an output of accuracy is obtained by withholding species with known statuses during the training, and then comparing the results for those samples based on the classifications. These samples are randomly selected by the program, so biases with regard to well- vs. under-sampled taxa are not expected to influence the training.

We aggregated the sequence abundance for each arthropod OTU according to its association with a particular plant genus within a site. For example, we found the sum of the sequence abundances for OTU "X," a Hemiptera from the 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 plots within a site. We also graphed quantitative and qualitative metrics for matrices of arthropod–plant interactions at each plot within a site. However, we constrain our discussion to the aggregated network data because our confidence in the network statistics increases with the size of the networks. This was a particular issue for some plots at the youngest and most depauperate sites, where fewer than plant species were sampled within the plot radius and networks would be small.

We plotted the ecological network matrix for each community age using the "plotweb" command in the R package BIPARTITE. For each network, lower bars represent plant abundance based on sampling time and upper bars represent arthropod abundance based on OTU frequency. For visual simplicity, we grouped upper bars by arthropod order. As described above, link width represents relative read abundance of arthropod OTUs collected on each plant taxon (Alberdi et al., 2019); in other words, link width corresponds to the relative frequency of each association.

The information contained in ecological 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). Using the "networklevel" commands in the R package BIPARTITE (Dormann et al., 2008) we calculated six quantitative indices for our bipartite networks of arthropods and associated plants: (i) linkage density, (ii) connectance, (iii) generality, (iv) vulnerability, (v) interaction evenness and (vi) the index of specialization H_2' (Table 2), that we reasoned would be associated with network specialization (Table 1). We converted each matrix to a binary presence—absence matrix and calculated the qualitative equivalent of: (i) linkage density, (ii) connectance, (iii) generality and (iv) vulnerability. We additionally calculated the ratio of resource species to consumers for the qualitative matrices, which is the ratio of plant genera to arthropod OTUs. These metrics represent the most fundamental biological and ecological properties of a community. We reasoned that the simplest metrics are a reasonable starting point given the limited understanding of how evolution shapes network structure, which would be necessary to justify the application of more involved network metrics. Further, these metrics have values that are interpretable with respect to their effect on specialization over time.

TABLE 2 Binary (B) and weighted (W) network summary statistics.

Summary statistic		Equation or notation	Description
Number of nodes	B	S = R + C	Total number of species (S) or "nodes" is equal to the number of prey or resource species (R; lower-level) plus the number of consumer species (C; upper-level)
Number of links	B	L	Total number of interactions or "links"
Ratio resource: consumers	B	R/C [1]	Average number of resource species per consumer species
Diversity of inflows	W	$H_{N,k}$, (5) [1]	Shannon entropy of weights for a given consumer sp.
Diversity of outflows	W	$H_{P,k}$, (6) [1]	Shannon entropy of weights for a given resource sp.
Log-reciprocal of (5)	W	$n_{N,k}$, (7) [1]	Effective number of resource spp. for a given consumer sp.
Log-reciprocal of (6)	W	$n_{p,k}$, (8) [1]	Effective number of consumer spp. for a given resource sp.
Link density	B	LD = L/S [1]	Average number of interactions per species
	W	LD_q, (14) [1]	Weighted version
Connectance	B	Conn = L/(R × C) [1]	Proportion of realized links
	W	$Conn_q = LD_q/S$ [1]	Weighted version
Generality	B	G = L/C [1]	Average number of resource sp. per consumer sp.
	W	G_q, (25) [1]	Weighted version
Vulnerability	B	V = L/R [1]	Average number of consumer sp. per resource sp.
	W	V_q, (27) [1]	Weighted version
Interaction evenness	W	I.E. [2]	Shannon entropy of interaction weights
Index of specialization	W	H'_2 [3]	Ranges between 0 and 1.0 for extreme generalization and specialization, respectively

Note: Metrics calculated from binary (i.e. unweighted, presence–absence) matrices are easily interpretable but sensitive to sampling differences (Banašek-Richter et al., 2004). Quantitative versions based on information theory are more conservative when comparing differences among sites. Each metric incorporates the diversity of individuals comprising the resource (H_N, the diversity of inflows) and of that going to the consumers (H_P, the diversity of outflows) for each species *k*. The quantitative metrics are then based on the reciprocals of these Shannon entropy values ($n_{N,k}$, and $n_{P,k}$, respectively). The notation q is applied to the quantitative version of that metric. All equations and notations reference [1] Bersier et al. (2002), [2] Tylianakis et al. (2007) and [3] Blüthgen et al. (2006).

2.8 | Tests of network metric significance and correlation between network properties

We used null models (Vázquez & Aizen, 2006) to test the statistical significance of empirical network metric values for the weighted data. For each weighted empirical network, we generated 1000 synthetic networks so that the total number of interactions and the identity of interaction partners is maintained while the weight associated with each interaction is shuffled (Staniczenko et al., 2013). With this simple quantitative null model, the distribution of interaction weights is conserved, along with the pattern of binary interactions, but not the identities of which interaction partners are associated with which weights. In terms of biological reasoning, the null model assumes that the identities of any two species involved in a nonforbidden interaction are unimportant for explaining network metrics. We calculated *p*-values and *z*-scores for each combination of empirical network and metric by comparing the observed metric value calculated from the empirical network to the distribution of metric values calculated from synthetic matrices generated by the null model; that is, the *p*-value quantifies how unlikely the observed, empirical metric value is to have been generated by the null model.

To compare the effect of community assembly on network size, arthropod diversity and network metrics, we regressed the

dependent variables by mean substrate age for each collection site. The untransformed substrate age data departed significantly from normality, so comparisons were performed using regressions on natural log-transformed substrate age data (Cowie, 1995; Gruner, 2007). We tested the significance of the correlation between network size and community age, each network metric and community age, and each network metric and network size, using Spearman's correlation tests. Additionally, we fitted a second-degree polynomial equation for the curvilinear relationship between the index of specialization H_2' and community age.

3 | RESULTS

3.1 | Composition of communities

Sites were selected using climatic and lidar data to restrict abiotic and biotic variation between sites so the effect of community age on ecological network structure could best be explored (Tables S1 and S2). There was some variation in forest structure as would be expected with sites during primary succession (e.g., forest height and density changes; Figure S2). Our ecological networks document 34 plant genera and 3517 arthropod OTUs, distributed across six classes: Entognatha, Crustacea (Amphipods and Isopods), Insecta, Arachnida, Chilopoda and Diplopoda. The arthropod–plant associations in our networks represent many kinds of trophic and nontrophic biotic interactions that capture functional differences among species of the understorey of the Hawaiian native forest. The barcode reference library increased taxonomic assignment from low taxonomic resolution to genus or species for 401 OTUs. Confident assignment was accomplished for a percentage of OTUs at each taxonomic level: Order 99.9%, Family 67.3%, Genus 38.1% and Species 24.9% (Table S3).

There were 2747 OTUs classified as native and 770 classified as non-native using NICLASSIFY. The overall accuracy for our data set predictions of nativeness using NICLASSIFY was 99.9%. Of the native OTUs, Hemiptera were the dominant order (652 OTUs), followed by Araneae (467 OTUs), Diptera (327 OTUs) and Coleoptera (266 OTUs). We found a highly significant (Table S4) increase in network size with community age for both nodes and links, with a disproportionate increase in the number of links (interactions) after several hundred years of community development (Figure 2a). The number of native arthropod species increases dramatically over both ecological and evolutionary time while the number of non-native arthropod species remains relatively steady (Figure 2b). The abundance of native and non-native arthropods peaks in the middle-aged communities but the proportion of non-native taxon abundance is highest in younger communities (Figure 2c). Plant diversity increased with community age (Figure 2d).

3.2 | Arthropod–plant association networks

Arthropod OTU richness, plant diversity and number of interactions increased with the geological age of the site. Bipartite networks of younger communities contain linkage widths between the few dominant taxa (e.g., Hemiptera and *Metrosideros*) while older communities contain smaller linkage widths representative of the many more

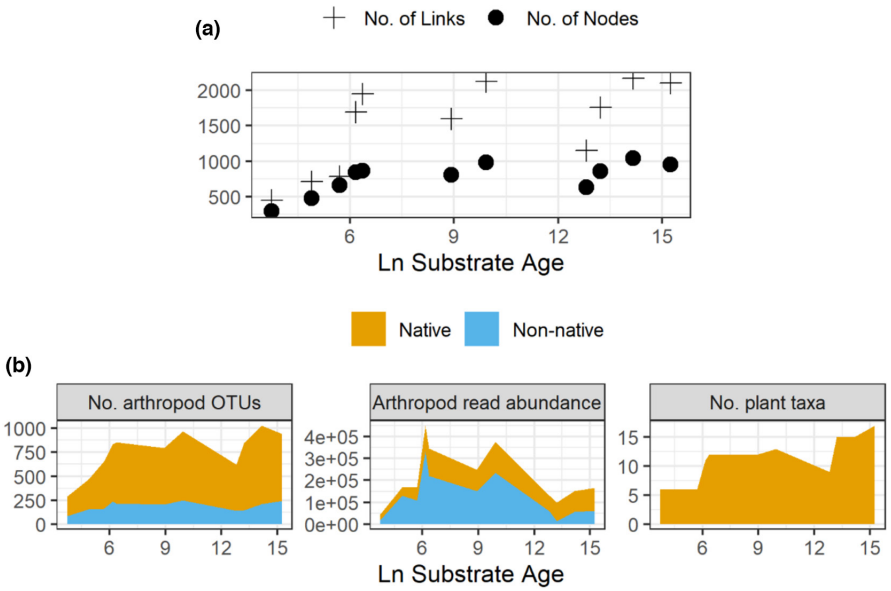

FIGURE 2 Effect of community assembly over evolutionary time on network size and diversity of native and non-native taxa. (a) The number of nodes (arthropod and plant richness) and the number of links (arthropod–plant associations) significantly increase in concert with community age. Spearman's correlation test values are given in Table S4. (b) Native arthropod richness increases, while non-native richness does not increase, with community age. (c) Abundance of native and non-native arthropod species peaks at middle-aged communities but the abundance of non-native taxa is proportionately higher in the youngest communities. (d) Native plant richness increases with community age. [Colour figure can be viewed at wileyonlinelibrary.com]

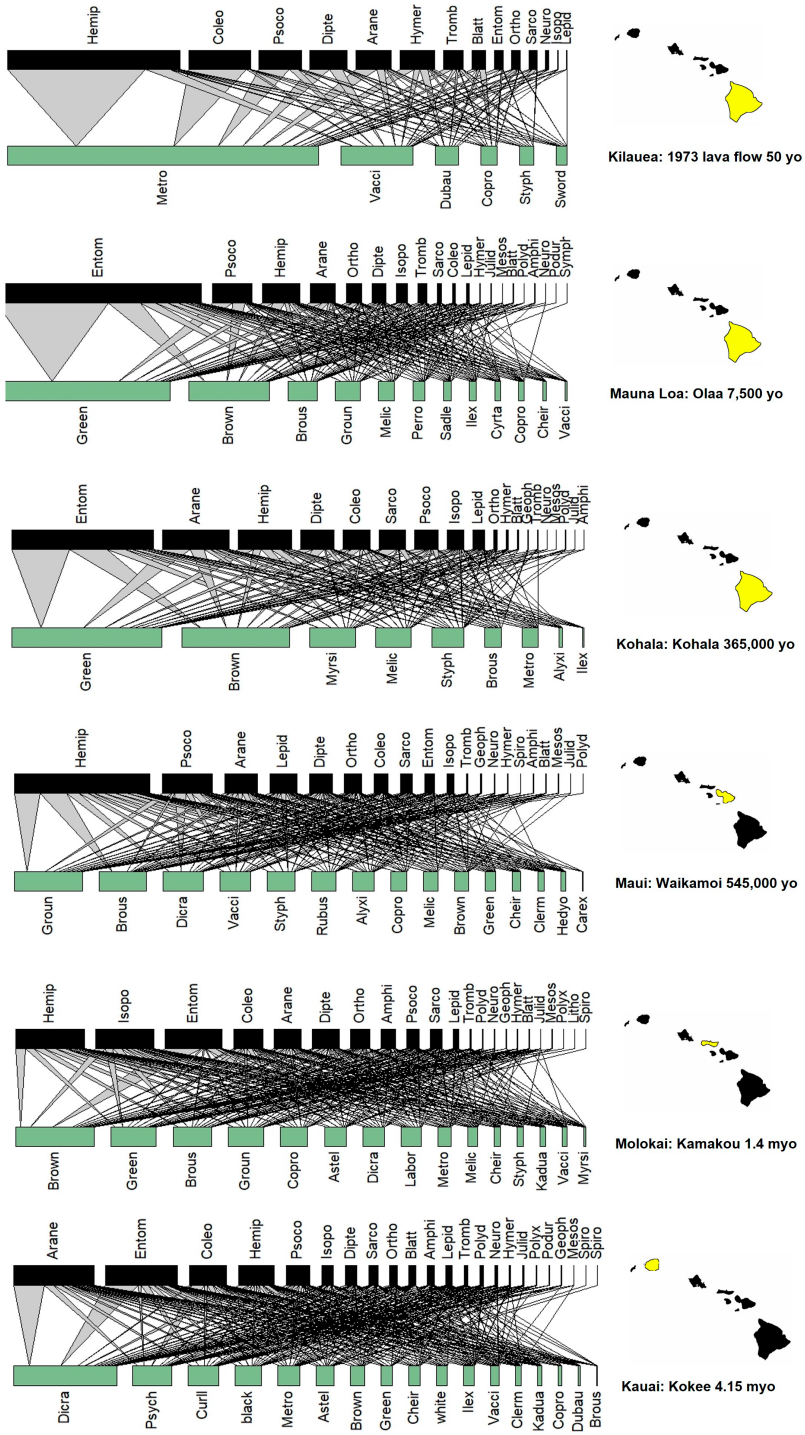

FIGURE 3 Quantitative arthropod–plant networks along a gradient of increasing community assembly (top to bottom). For each network, lower bars represent plant abundance based on sampling time and upper bars represent arthropod abundance based on OTU frequency. Each network is plotted in order of the most abundant taxa from left to right so that the turnover in arthropod–plant association can be seen for each community. Linkage width indicates the frequency of each association as measured using arthropod read abundance. As a summary, the networks show interaction data pooled across all plots for each community age with OTUs pooled by arthropod order, but analyses were performed at the OTU per plant genus level. The bipartite graphs from each of the 11 sampled sites are given in Figure S3. Arthropod and plant ID codes are given in Table S3. [Colour figure can be viewed at wileyonlinelibrary.com]

associations distributed among the greater diversity of both higher and lower level taxa (Figure 3; Figure S3).

For the null model analyses of the weighted matrices, some observed network metric values were not significantly different ($p < .05$) from metric values produced from the synthetic matrices (Table S5; Figure S6).

Results of the Spearman's correlation tests show linkage density (average number of interactions per species), network vulnerability (a measure of the ratio of plant generic richness to arthropod OTU richness) and interaction evenness (a measure of the uniformity of energy flows along different pathways) increased significantly with community age (Figure 4; Table 3). Both generality (a measure of the ratio of arthropod OTU richness to plant generic richness) and the index of specialization H_2' increased with community age but were not significantly positively correlated. The index of specialization H_2' has a curvilinear relationship with community age, first decreasing then increasing. A second-degree polynomial provides the best approximation of the relationship between H_2' and community age ($F = 6.85$, $R^2 = .5392$, $p < .05$). By beat sampling and sequencing all plant-associated arthropods, our

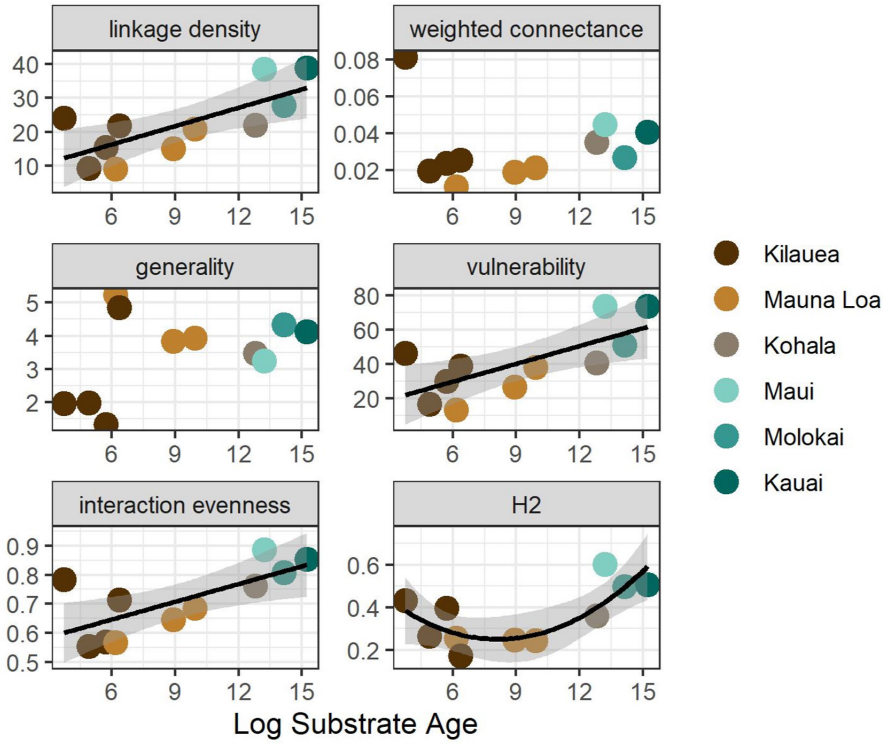

FIGURE 4 The effect of community age on quantitative ecological network metrics. Statistical measures of network architecture indicating changes in arthropod–plant associations in concert with community age. Each network was weighted with the read abundance of the arthropod OTU associated with the plant genus it was collected from, across all plots for a community age. Three metrics show significant relationships with community assembly, increasing over time: linkage density, vulnerability and interaction evenness. Spearman's correlation test values are given in Table 3. Results of the null model analysis for the quantitative ecological networks metrics are presented in Figure S6 and Table S5. A graph of the results when analysed for each of the sampled plots within a community age site is presented in Figure S7. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Spearman's correlation tests for network metrics and community age.

	S	*p*-Value	Spearman's rho
Quantitative (weighted) × community age			
Linkage density	76	.033	0.65
Weighted connectance	154	.371	0.30
Generality	124	.183	0.44
Vulnerability	80	.040	0.64
Interaction evenness	70	.025	0.68
Index of specialization H'_2	134	.237	0.39

Note: Spearman's correlation tests were used to determine the significance of the relationship between each quantitative network metric value and ln substrate age (community age). Graphs of regressions are shown in Figure 4.

sampling of arthropod taxa is at finer taxonomic resolution than that of plants. As a result, generality (links/arthropods) is very close arithmetically to linkage density (links/arthropods + plants) in our data set because the number of arthropod OTUs is many times greater than the number of plant genera for all communities. Connectance (proportion of realized interactions) was not significantly correlated with increasing community age, but instead is highest at the youngest site, and relatively constant for the remainder of the sites.

For the qualitative metrics calculated from the binary matrices, linkage density (links/species), connectance links/(arthropods*plants) and generality (links/arthropods) were significantly correlated with community age, while vulnerability (links/plants) and the ratio of resource species to consumers (plants/arthropods) was not (Figure S4; Table S4). The results from plot-level analysis are consistent with the site-level data and the variance among plots at the same sites is minimal (Figure S7). These results help corroborate the trend of increasing specialization over time.

For the regressions of network metrics against network size, with the exception of generality, quantitative network metrics were not significantly correlated with network size (Figure S5A; Table S4). By contrast, qualitative metrics were significantly correlated with network size with the exception of the ratio of resource species to consumers, and vulnerability (Figure S5B; Table S4).

4 | DISCUSSION

Using a data set of biotic associations during the course of community assembly, we present strong evidence of increasing specialization within arthropod communities through evolutionary time. Our DNA metabarcoding data have allowed us to collect a large sample of the arthropods from the understorey of Hawaiian forests, representing a broad swathe of trophic and nontrophic arthropod–plant associations. As expected, the qualitative metrics were strongly biased by network size (Banašek-Richter et al., 2004; Goldwasser & Roughgarden, 1997) and showed higher linkage density, generality, vulnerability, interaction evenness and lower connectance in older communities, because the diversity of plants and arthropods was higher in these communities (Figure S4; Table S4). Our null model analysis helped to demonstrate that the distribution of link weights was itself an important feature of the

observed network structure (i.e., not which species they are between). We present clear signatures of change in quantitative, weighted network metrics with community age (Figure 4; Table 3) that resulted from changing community composition and ecological dynamics.

4.1 | Ecological processes dominate younger communities

Theory suggests that the composition of the youngest communities is shaped through colonization from a regional species pool. This expectation is supported by our results, with the younger communities having significantly lower linkage density, vulnerability and interaction evenness (Figure 4). These results indicate that species in younger communities are interacting with greater frequency along less uniform interaction pathways, compared to species assemblages at older sites. However, notably the very youngest site, the 1973 lava flow, is an outlier. At the 1973 lava flow, linkage density is high (LD = 24.2), probably reflecting strong environmental filtering and an opportunistic community of generalist species (Bufford et al., 2020; Kortsch et al., 2015) suited for survival during primary succession. At other young sites, linkage density is low, from <10 (Tree Planting Rd.), whereas it peaks and levels off at Maui (LD = 38.6) and Kauai (LD = 38.9), respectively. Thus, linkage density was low at young sites with low resource diversity while the diversity of interactions increased over evolutionary time in step with increasing community complexity.

Interaction evenness was low, as expected, on the youngest sites, again with the exception of the 1973 flow. As a measure of the uniformity of energy flows along different pathways, we expected interaction evenness to be low in young communities because some interaction partners would dominate the associations in the network. For example, a large proportion of interactions on the youngest sites (<300 years old) belong to the associations of Hemiptera and Collembola species with early successional plant species, *Metrosideros polymorpha* and *Dicranopteris linearis* (Figure 3; Figure S3). Low interaction evenness has also been demonstrated among bees and wasps and their associated natural enemies (e.g., parasitoids) under conditions of intensive management (Staniczenko et al., 2017; Tylianakis et al., 2007). The early successional communities in Hawaii are ecologically similar to highly modified sites, due to the recent disturbance from lava and the paucity of resource diversity. We suggest that the higher interaction evenness at the 1973 lava flow is due to the extremely limited resources (plants) on the sparsely vegetated lava substrate. At this site colonists may be joined by a relatively large representation of transient arthropods, which may be less host-specific and appear randomly associated with the available plants, increasing interaction evenness. Connectance also peaked in the youngest community (1973 lava flow) probably due to the greater representation of generalists within this network (Kortsch et al., 2015; Ponisio et al., 2019).

The network metric values are less consistent among the youngest sites compared to the older sites (Figure 4). This is probably due to the relatively rapid changes in community composition in early primary succession (Atkinson, 1970; Roderick et al., 2012) compared to older established sites. An alternative explanation is that change in the composition of the understorey plants (Figure 3; Figure S3) and canopy structure (Figure S2; Table S2) results in the network metric variation at the youngest sites. The higher variation among network values at the youngest sites may also point to the different rates of specialization and adaptation among different lineages of arthropods. Among functional groups of beetles (e.g., xylophages, fungivores, predators), community composition and network specialization changed differently during early succession (Wende et al., 2017).

4.2 | Specialization increases through evolutionary time

For a given taxon on average, the number of biotic interactions it is involved in decreases with community age, resulting in greater network specialization. This is reflected in the increased linkage density with community age, as early colonizing species gave way to a greater diversity of associations (Figure 4). However, weighted connectance stabilized at around the same level for the remainder of the communities after the 1973 lava flow. This may be explained by the "constant connectance" hypothesis (Martinez, 1992) that posits that species are linked to a fixed fraction of species in a network, independent of the number of species in a community. A similar pattern of constant connectance and community age was found in arthropods recolonizing defaunated mangrove islands (Piechnik et al., 2008). For the Hawaiian Islands, several factors probably produce constant connectance over long-term community development. First, resource availability limits specialists at early stages; for example, Escape Road (~300 years) is dominated by a single species of fern. Next, over evolutionary time, the Hawaiian fauna is characterized by a remarkably high rate of lineage diversification (Gillespie et al., 2020; Gillespie, 2016; Zimmerman, 1970) that has added novel species and associations. Finally, at more recent timescales (after human arrival) immigration of non-natives has been sufficiently high so as to add generalist taxa across all stages of community development (Figure 2b).

A previous study which used an island chronosequence to examine how pollinator interactions change through extended time (Trøjelsgaard et al., 2013) also found connectance was poorly explained by age. However, contrary to our results, the Canary Islands study showed hump-shaped relationships of interaction richness and specialization with island age. One reason for the different results is that we used a natural log scale for the skewness of island age. For the Hawaiian islands, values for linkage density, vulnerability, interaction evenness and index of specialization H_2' were especially high on the volcano of East Maui. The islands of Maui Nui are also where richness peaks for many native arthropod lineages (Gillespie

& Baldwin, 2009; Gruner, 2007). However, unlike the Canary Island pollinators, our values of linkage density were highest on the oldest island, and values for interaction evenness, vulnerability and index of specialization H_2' were nearly as high, indicating that the overall changes in network structure were more linear than hump-shaped. An alternative explanation for the difference in the results is that the older islands of the Canary archipelago have environments that are very different from the younger islands. Although the Canary Island study focused on communities that were characterized by the plant species *Euphorbia balsamifera*, the abiotic environment changes significantly across their chronosequence, with the older islands being much lower and drier (Juan et al., 2000). Thus, the finding of a hump-shaped relationship in the Canary Islands is associated with the combined effects of time, island geomorphological transitions and associated change in climate regimes. In contrast, the current study in the Hawaiian Islands aimed to standardize environments (elevation, precipitation and forest cover, with sampling from standardized plots across the islands). Therefore, any confounding environmental differences were minimized and changes in network properties should largely reflect the influence of community age.

Both vulnerability and generality show positive correlations with community age (Figure 4; Table 3), and thus the average number of arthropods per plant species (vulnerability) and the average number of plant species per arthropod (generality) are increasing over time. This is consistent with our expectation that specialization increases resource overlap when a reduction in antagonistic interactions leads to some level of resource redundancy and an increase in diversity of beneficial interactions leads to greater resource complementarity (Table 1). In other words, over evolutionary time, if two species are in direct competition for resources, they can evolve traits that allow them to coexist. One result of trait matching between interaction partners is decreasing niche breadth (i.e., decreasing diversity of resources used). Thus, our results are consistent with decreasing niche breadth with island age found previously from literature for herbivores (Ponisio et al., 2019). Moreover, although the rate of specialization and adaptation, such as occurs through trait matching and decreasing niche breadth, can vary among functional groups in a community, our data show that community specialization is happening at the network level, averaging over the high variation in rates of specialization.

The network-level specialization index H_2' is largely unaffected by network size, network architecture or total number of interactions for a fixed matrix size (Blüthgen et al., 2006), making it an ideal metric compare between different networks for understanding specialization over time. We find that the index of specialization increases over time but is better fit by a second-degree polynomial equation. In early-stage communities from 50 to 575 years the index of specialization is decreasing. This drop in specialization in the first several hundred years is followed by an increase over the next tens of thousands of years. For random associations H_2' is usually close to zero. On Maui it reaches a value of 0.6 then levels out to 0.5 on Molokai and Kauai. This pattern is consistent with other metrics in our analysis suggesting that very young communities are organized by assembly rules making them appear specialized. After ecological sorting and the impact of in situ evolution in later stage communities we see organization at a secondary, evolutionary stage of development.

4.3 | Resilience of communities increases through time

While communities sampled from the youngest sites are composed primarily of native species from the regional pool (Figure 2b), younger communities have proportionally higher abundances of non-native species infiltrating the system (Figure 2c). Thus, it appears that young communities are more invasive, which is consistent with previous studies showing that communities composed of endemic generalist taxa are more vulnerable to infiltration by non-natives (Olesen et al., 2002). By increasing connectance and lowering network specialization, higher numbers of alien species may in turn facilitate increasing numbers and impacts of invasions (Simberloff & Von Holle, 1999; Simberloff., 2006).

However, this result runs counter to work suggesting that higher-connectance food webs tend to host fewer invaders and exert stronger biotic resistance compared to low-connectance webs (Smith-Ramesh et al., 2017). Further, community resistance to invasion is known to increase with native species diversity (Gallien & Carboni, 2017) and network complexity (Wei et al., 2015). Considering the results from our study within the context of this previous work, older communities, which are characterized by low connectance and high specialization, may be more resistant to invasion; however, individual taxa may be more susceptible to extinction. From an individual species level, because all species are linked together either directly or indirectly (Montoya et al., 2006), individual species with high specialization and low connectance are susceptible to extinction because of secondary extinctions occurring when specialized consumers lose their only prey (Dunne et al., 2002; Staniczenko et al., 2010). From a network level, as communities age, several species may be associated with the same resource (resource redundancy) or utilize a single resource more effectively (resource complementarity), minimizing variability in the functioning of an ecosystem, for example when some consumer species decline in number (Peralta et al., 2014).

Although ecological processes, such as interspecific interactions or disturbance, are often attributed to the geographical differences in exotic species richness (Lockwood et al., 2013) an alternative explanation for the apparent reduced biotic resistance to invasion of younger communities may be that they experience increased propagule pressure (Lockwood et al., 2005). The younger sites on Kilauea volcano are accessed more frequently by tourists compared to the older sites, which require greater on foot distances to reach or special access permits. Furthermore, while our study directly assesses arthropod–plant associations, it only indirectly measures the effect of higher trophic associations. Differential top-down pressure (e.g., predator turnover) during community

assembly probably also changes biotic resistance to invasion; for example, generalist insectivorous birds reduced infiltration of an invasive species of spider at the 133-year-old Tree Planting Rd. community (Gruner, 2005).

4.4 | Conclusions and outlook

Our study uses whole-community DNA metabarcoding data to assess the biotic associations of thousands of arthropod OTUs on plants across a geological chronosequence. By including relative abundance data, we achieve a signature of interaction strength (Popovic et al., 2019) not captured for co-occurrences with presence–absence observations (Blanchet et al., 2020). Although DNA metabarcoding can be used for observation of trophic interactions (Alberdi et al., 2019; Krehenwinkel, Kennedy, et al., 2017), our analysis instead includes all biotic associations between arthropod–plant communities, including those that can be difficult to detect (e.g., involving cryptic species, new non-natives, endangered species, juveniles). Thus, we are able to include complex community interactions including substrates chosen for acoustic signalling (Mullet et al., 2017), predator avoidance (Lindstedt et al., 2019; Stachowicz & Hay, 1999) and gregarious plant-feeding insects (Hunter, 2000) that are often overlooked in traditional network studies. Compared to the limitations of small, unweighted early food web studies (Cohen et al., 1993; Hall & Raffaelli, 1991), DNA metabarcoding offers exciting avenues forward for capturing community complexity.

This research revealed a strong association between the network structure of ecological communities and community development over evolutionary time. Quantitative network metrics demonstrate that younger communities are composed of more generalist species that interact with greater frequency along fewer interaction pathways, with individual and network specialization increasing with community age. Our data highlight the utility of DNA metabarcoding for understanding longstanding questions of ecology and evolutionary biology that remain time consuming (e.g., keying out morphological species) or impossible (e.g., identification of juveniles) to assess with traditional methods. From a conservation perspective, our results indicate that habitat disturbance erodes a complex web of biotic associations, far greater than the sum of the community metrics of richness and abundance, that have evolved in situ over thousands to millions of years.

AUTHOR CONTRIBUTIONS

Natalie R. Graham, Daniel S. Gruner, Rosemary G. Gillespie, Henrik Krehenwinkel and Jun Ying Lim designed the research, Natalie R. Graham and Henrik Krehenwinkel performed molecular processing of the samples, Natalie R. Graham and Phillip Staniczenko analysed the data, Jackson Callaghan and Jeremy C. Andersen contributed new analytical tools, and Natalie R. Graham and Rosemary G. Gillespie wrote the manuscript with input and comments from all coauthors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.6078/D1DX4T.

DATA AVAILABILITY STATEMENT

Data are deposited in a Dryad data repository at https://doi.org/10.6078/D1DX4T and code is hosted on Zenodo at https://zenodo.org/record/7349067 including: (i) DNA sequence data (fasta files) from whole organism community metabarcoding and the DNA barcode reference library, and (ii) processed data used in these analyses including the OTU table, phylogenetic species IDs, NICLASSIFY predictions and geographical information.

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