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

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

3 Ecological networks and community assembly

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20

21 **Abstract**

22

23 The dynamical structure of ecological communities results from interactions among taxa that change with
24 shifts in species composition in space and time. However, our ability to study the interplay of ecological
25 and evolutionary processes on community assembly remains relatively unexplored due to the difficulty of
26 measuring community structure over long temporal scales. Here, we made use of a geological
27 chronosequence across the Hawaiian Islands, representing 50 years to 4.15 million years of ecosystem

28 development, to sample 11 communities of arthropods and their associated plant taxa using semi-
29 quantitative DNA metabarcoding. We then examined how ecological communities changed with
30 community age by calculating quantitative network statistics for bipartite networks of arthropod-plant
31 associations. The average number of interactions per species (linkage density), ratio of plant to arthropod
32 species (vulnerability), and uniformity of energy flow (interaction evenness) increased significantly in
33 concert with community age. The index of specialization H_2' has a curvilinear relationship with community
34 age. Our analyses suggest that younger communities are characterized by fewer but stronger
35 interactions, while biotic associations become more even and diverse as communities mature. These
36 shifts in structure became especially prominent on East Maui (~0.5 my) and older volcanos, after enough
37 time had elapsed for adaptation and specialization to act on populations *in situ*. Such natural progression
38 of specialization during community assembly is likely impeded by the rapid infiltration of non-native
39 species, with special risk to younger or more recently disturbed communities that are composed of fewer
40 specialized relationships.

41

42 **Keywords** community assembly, ecological networks, DNA metabarcoding, oceanic islands

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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.

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

84

85 While theory indicates a clear role for biotic interactions leading to individual and community
86 specialization over long-term community development, empirical evaluation has been challenging. One
87 difficulty is in obtaining measures of community composition and interactions at relevant spatial scales,
88 and another obstacle is the vast timeframe over which evolutionary phenomena occur. With their short
89 generation times that are amenable to laboratory studies, microbial systems provide exceptional cases
90 that document community assembly over evolutionary time scales (Boon et al., 2014; Koskella et al.,
91 2017; Koskella & Brockhurst, 2014; Venturelli et al., 2018). In particular, studies of the plant phyllosphere
92 showed a more prominent role of nonneutral selection over time and an increase in the strength of biotic
93 interactions and community cohesion (Morella et al., 2020). However, to infer the role of interactions in
94 community assembly of longer-lived macroorganisms requires very particular systems. Here, we make
95 use of two sets of circumstances that, together, provide an extraordinary opportunity to assess the nexus
96 of ecological and evolutionary community assembly in the context of interaction networks.

97

98 First, we use the system provided by the Hawaiian Islands. Islands in general provide discrete
99 communities that can be used for natural experiments in interaction dynamics (Brodie, 2017; Castro-Urgal
100 & Traveset, 2014; Olesen et al., 2002). In particular, oceanic archipelagos formed *in situ* over millions of
101 years offer the opportunity to study species interactions over evolutionary timescales (Hembry et al.,
102 2018; Ponisio et al., 2019; Rominger et al., 2016; Trøjelsgaard et al., 2013). Moreover, the geological
103 series of islands in the Hawaiian archipelago represents a chronosequence (Vitousek, 2002; Walker et
104 al., 2010); each substrate age represents communities of different ages, ranging from ~ 50 years to ~ 5
105 Myr (Shaw & Gillespie, 2016). Notably, the native montane forest of Hawaii is dominated by just two
106 canopy tree species (*Metrosideros polymorpha* and *Acacia koa*), making it relatively simple ecologically
107 and hence more amenable to capturing and characterizing whole communities.

108

109 Second, we make use of the emerging field of DNA metabarcoding (Kreherwinkel, Wolf, et al., 2017; Yu
110 et al., 2012), which makes a comprehensive analysis of taxonomic composition possible, offering the
111 opportunity to simultaneously assess thousands of species rapidly, offering enormous potential for
112 reconstructing complex ecological networks (Clare, 2014; Hrček & Godfray, 2015; Vacher et al., 2016).
113 Relative sequence abundances offer a proxy for interaction strength (Lim et al., 2021), providing greater
114 reliability for co-occurrence studies to measure biotic associations (Bálint et al., 2018; Mata et al., 2021).
115 Combining high-throughput sequencing with theoretical approaches, such as statistical modeling (Faust &
116 Raes, 2012; Newman & Girvan, 2004) and machine learning (Bohan et al., 2011), shows considerable
117 promise in helping to close the gap on the historical impediments for comprehensive quantification of
118 interactions in ecological communities.

119

120 Here we use semi-quantitative DNA metabarcoding to build networks of arthropod-plant associations at
121 11 sites across the Hawaiian chronosequence, using the substrate age at a site as measure of
122 community age, and then use those networks to test a range of expectations on how ecological and
123 evolutionary processes shape community structure over long timescales (e.g., Table 1). We expect
124 network size - both the number of nodes and number of links - will increase with community age, but
125 disproportionately, as younger communities gain taxa through colonization only and older communities

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

143 **Materials and Methods**

144 *Site selection methods*

145 The Hawaiian Islands are formed as the Pacific plate moves northwestward across a stationary volcanic
146 hotspot, therefore the archipelago represents a chronosequence of geological age from the youngest
147 island (Hawaii, ~0–0.5 Myr), to the oldest high island of Kauai (~5 Myr), (Clague, 1996). Discrete
148 volcanoes within islands present additional contrasts in geological age, and the underlying substrate age
149 has been mapped in fine detail (Wolfe & Morris, 1996). *Metrosideros polymorpha* (Myrtaceae) is the
150 dominant canopy tree in these forests across islands, with patches of sub-dominant *Acacia koa*
151 (Fabaceae) and numerous associated understory trees, shrubs, herbs, and ferns (Gagne & Cuddihy,
152 1990).

153
154 We selected 14 sites of varying geologic age, ranging from 50 to 4.15×10^6 years old, across four islands
155 of the archipelago: Hawaii, Maui, Molokai, Kauai (SI Figure 1, SI Table 1). To control for climatic
156 differences and disturbance across sites, sites were constrained to ranges of elevation (1000-1300 m)
157 and precipitation (average annual precipitation 2500-3000 mm) and within accessible protected forest
158 lands (*Gap Analysis Project* | *U.S. Geological Survey*, 2019; Giambelluca et al., 2013).

159
160 For each potential site, spatial polygons were created based on the intersections of these layers and
161 initial field reconnaissance to confirm remotely sensed data and feasibility of access. Within these
162 potential site polygons, airborne high-resolution laser scanning from the Global Airborne Observatory
163 (GAO; formerly named Carnegie Airborne Observatory; Asner et al. (2012)) was used to generate forest
164 canopy height profiles using a physical model described in Asner et al. (2008). The ground digital
165 elevation model was also generated using the method of Asner et al. (2007). The data were collected at
166 4 laser shots per square meter, processed to height profiles at 5 m resolution and then averaged at a grid
167 cell spacing of 30 m (SI Figure 2 and SI Table 2).

168
169 20 randomized candidate plots were generated for each site, with the intention of ultimately selecting six,
170 15 m-radius plots. These 20 randomized candidate plots were constrained to be a minimum distance of
171 200 m from all other plots and to be within the top 40% quantile of LiDAR-estimated canopy heights.
172 Candidate plot generation was achieved with custom scripts in the R programming language (R Core
173 Development Team, 2019) using a simple rejection sampling algorithm: random sets of spatial locations
174 are generated within pixels of sufficient canopy height until a set of locations is found which meet the
175 requirement of being 200 m distant. The minimum distance of 200 m was a constraint to maximize
176 independence among sampling areas while capturing more spatial heterogeneity within sites.

177
178 At each site, each of the 20 candidate plots were ground-truthed to confirm the plot was dominated by
179 native vegetation and minimally impacted by human use and/or invasive vertebrates. This ground-truthing
180 process eliminated a variable number of the initial 20 candidate plots. If fewer than six final plots

181 remained after ground-truthing, another set of candidate plots were generated and ground-truthed to find
182 a final set of six plots. If more than six plots remained after ground-truthing, the final six plots were
183 selected by randomly selecting from the ground-truthed plots.

184

185 *Collection protocol*

186 We collected arthropods using vegetation beating at six 15 m radius plots per site during May 2015
187 through January 2016, with plots sampled randomly to avoid seasonality effects on arthropod
188 composition. To ensure equal sampling effort across sites, sampling was limited to a total of 420 seconds
189 in each plot. If after all arthropod collection processing steps (described below) the total vegetation
190 beating time for a plot was not within one standard deviation of 420 seconds sampling effort then that plot
191 was dropped from further analyses, resulting in a total of 50 plots from 11 sites (SI Table 1). As we were
192 interested in characterizing plant-arthropod associations, we sampled plant genera in each plot
193 proportional to their relative abundance. Percentage cover of each understory plant genus was estimated
194 visually. Where plants could not be identified to the genus-level, we grouped them into morphotaxa and
195 sampled them accordingly. Vegetation beating was performed by placing 1 m x 1 m white beating sheets
196 under individual plants and gently agitating the foliage using a one meter length pvc pole for timed second
197 intervals. Arthropods dislodged by the agitation which drop onto the beating sheet are aspirated into a vial
198 containing 95% ethanol. Each plant associated arthropod community sample was transferred to one or
199 more 2 ml vials containing fresh 95% ethanol, labeled, and transported to the lab where it was stored at -
200 20 °C.

201

202 *Specimen sorting and DNA extraction*

203

204 To reduce bias due to differently sized individuals contributing disproportionate amounts of DNA (Elbrecht
205 & Leese, 2015) specimens were sorted following procedures described in Lim et al. (2021). Each plant
206 beating sample was sorted in petri dishes on 1 mm graph paper under a stereoscope into four size
207 categories (0-2 mm, 2-4 mm, 4-7 mm, 7 mm and up) based on the body size distribution found in a
208 common Hawaiian ecosystem. Individuals in each size category were counted and placed with fresh

209 ethanol into a single well in a 96-well plate. Thus, all individuals from a particular plant genus at a
210 particular plot have their DNA extracted together and are prepared together using a dual-indexing
211 strategy described below into NGS amplicon libraries for sequencing. The Collembola had considerably
212 higher abundance than the remaining arthropods in the small size categories, therefore Collembola were
213 separated into 1.5 ml Eppendorf tubes and processed for DNA extraction and sequencing parallel to the
214 remaining arthropod community samples.

215

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

222

223 *Sequence analysis*

224 Each size sorted sample and a PCR negative for each 96 well plate (containing no template DNA) was
225 amplified with a primer combination (ArF1/ Fol-degen-rev) (Gibson et al., 2014; Yu et al., 2012) that
226 targets a 418 bp fragment in the barcode region of the Cytochrome Oxidase I (COI) gene. This primer
227 pair has been suggested as the most appropriate for capturing arthropod diversity in DNA metabarcoding
228 studies (Elbrecht & Leese, 2015) and has been shown to reliably amplify the Hawaiian arthropod
229 community (de Kerdrel et al., 2020). PCRs were run in 10 μ l volumes using the Qiagen Multiplex PCR kit
230 at an annealing temperature of 46 °C, with 1 μ l of DNA and 0.5 μ l of each 10 μ M primer. A first round of
231 PCR consisted of 32 cycles using tailed primers; each primer additionally had a unique 6 bp inline
232 barcode so that multiple plates of the same primer can be pooled together. PCR products were cleaned
233 of residual primer using a 1x ratio of SPRI beads (Sera-Mag™) and pooled together based on band
234 intensity (i.e., DNA concentration) on an agarose gel relative to a DNA ladder (NEB) and using the Gel
235 Doc XR System with the Quantity One software (Bio-Rad). A second indexing PCR of 6 cycles was
236 performed with the pooled amplicons to introduce dual indexes and Illumina® TruSeq sequencing

237 adapters to 5'-tails of the locus-specific PCR primers (Lange et al., 2014), with a final 5'-3' layout as
238 Illumina adapter, 6 bp inline barcode, PCR primer as described in de Kerdrel et al. (2020). The indexed
239 products were cleaned again with SPRI beads, quantified by electrophoresis, and then pooled in equal
240 amounts into a single tube. The samples were then sequenced on an Illumina® MiSeq using V3 (600
241 cycles) chemistry according to the manufacturer's protocol (Illumina, San Diego, USA). We aimed for a
242 total of 30,000 reads per sample. Each PCR negative was sequenced with each plate of specimen
243 libraries regardless of the absence of detectable PCR product on a gel.

244
245 We generated 2276 metabarcoding libraries with each library representing the total arthropods collected for
246 each plant genus for each plot (a sampling event), sorted into one of four size categories (a sequencing
247 pool). Sequences were demultiplexed on Illumina® BaseSpace by sample well based on the two 8-bp
248 indexes with no mismatches allowed. We merged paired reads using PEAR (Zhang et al., 2014) with a
249 minimum overlap of 50 bp and a minimum quality of Q20. Merged reads were quality filtered ($\geq 90\%$ of
250 bases $\geq Q30$) and transformed into fasta files using the FastX Toolkit (Gordon & Hannon, 2010). The
251 resulting fasta files were demultiplexed by PCR primer and 6 bp inline barcode combination, using the
252 forward and reverse primer sequences as indices with the grep command in UNIX, and the primer
253 sequences were then trimmed using the UNIX stream editor.

254

255 *Rarefaction and pseudogene removal*

256 We rarefied each sample using a custom unix command that drew from the total reads of the
257 metabarcoding analysis a number of reads that was equivalent to the numerical abundance of individual
258 arthropods counted into each well of the 96-well plate, repeating the draw of sequences 100 X with
259 replacement. The process of rarifying by repeated random draw based on the expected individual
260 specimen abundance should correct the disproportionate abundance of sequences that accumulate for
261 larger specimens compared to smaller specimens, due to the amplification bias that is inherently caused
262 by differential starting tissue amounts (Lim et al., 2021).

263

264 We generated zero-radius OTUs (zOTUs), from the rarefied raw reads with the unoise3 command (Edgar,
265 2016) following the recommended protocols in the USEARCH v11 pipeline (Edgar, 2010). Specifically,
266 the quality trimmed reads were dereplicated and clustered into zOTUs using the unoise3 command in
267 USEARCH. Chimera were removed *de novo* in USEARCH. The resulting zOTUs were compared against
268 the NCBI Genbank database and our custom-made DNA reference library for Hawaiian taxa using
269 BLASTn with a maximum of 10 target sequences. All non-arthropod zOTUs were removed after which
270 5,046 zOTUs remained. We aligned these 5046 zOTUs using default settings in Clustal Omega (Sievers
271 et al., 2011). To remove putative pseudogenes from the zOTU dataset we ran metaMATE with default
272 specifications and the example specifications file to detail how per-zOTU read frequencies should be
273 assessed (Andujar et al., 2021). Using the output of metaMATE we applied the least stringent Numt
274 removal strategy so that we could retain as many putatively true zOTUs as possible (Graham et al.,
275 2021), this reduced the number of zOTUs from 5046 to 4330.

276

277 *Taxonomic matching and abundance estimates*

278 About a quarter of the zOTUs ($n = 901$) were matched to the Blast or voucher DNA reference library with
279 less than 85 percent similarity. To validate the taxonomic identification for each zOTU at higher taxonomic
280 levels (e.g. order, family) we compared the top 10 blast and reference library hits with phylogenetic
281 clustering from a ML tree. A ML tree with bootstop autoMRE bootstrap support was generated by running
282 RAxML-HPC v.8 on XSEDE on the Cipres science gateway (Miller et al., 2010) under the GTR model with
283 a gamma distribution plus invariant sites. For 28 zOTUs taxonomic order could not be determined via
284 sequence similarity to databases or phylogenetic clustering and were thus removed from downstream
285 analysis. Taxonomic assignment was considered trustworthy if the percent similarity of the metabarcoding
286 sequence to the NCBI GenBank or DNA reference voucher was: between 88-94% for family, between
287 94%-98% for genus and greater than 98% percent similarity for species, while matches below 88%
288 similarity were made only to order. These threshold values were arbitrarily chosen based on previous
289 investigations using mock communities or photo voucher integrative taxonomy of select taxa from the
290 same high-elevation wet forest communities of Hawaiian arthropods and amplified using the same COI
291 marker (Krehenwinkel et al. 2017, de Kerdrel et al. 2020).

292
293 To create a table with OTU abundances for community analyses we mapped a query set of raw reads to
294 the filtered and taxonomically identified search database of zOTUs in USEARCH v11 (Edgar, 2010) using
295 the otutab command with the default 97% percent similarity mapping threshold. After OTU mapping and
296 read removal based on the PCR negative control sequencing pool the number of unique sequences was
297 reduced by 133 zOTUs to 4197 OTUs.

298
299 In order to use relative sequence abundance of arthropod OTUs as an approximation of arthropod-plant
300 associations, we adopted a semi-quantitative processing pipeline (Lim et al., 2021) to ameliorate
301 differences in sampling effort, body size of specimens, and genomic procedures. To review, (1) for each
302 site (community) there was six plots, (2) within each plot each plant taxon was sampled by seconds of
303 time corresponding to its relative abundance in the plot, (3) we sorted bulk arthropod samples by size and
304 counted the individuals, (4) sequences were generated using a DNA region with demonstrated success
305 for Hawaiian arthropod taxa (de Kerdrel et al., 2020) and false reads (pseudogenes) were removed
306 (Graham et al., 2021), (5) we randomly sampled the sequencing reads based on the count of individuals
307 in each size class, and finally, (6) sequence reads were summed across size classes and plots (Figure
308 1b).

309
310 *Calculation of quantitative ecological network metrics*

311
312 Using bipartite networks of arthropod-plant associations at each community age, we tested our
313 hypotheses by calculating quantitative (weighted) and qualitative (binary) network metrics (Table 2)
314 expected to occur in the transition from younger communities to older communities (Table 1). Data
315 processing and statistical analyses were performed in R version 4.0.2. To distinguish between taxa that
316 have colonized the archipelago historically or more recently, we characterized the probable native and
317 non-native composition for each aged community based solely on sequence characteristics as outlined in
318 Andersen et al. (2019). The approach considers both the evolutionary distances between species and the
319 genetic diversity within species. Sequence characteristics of OTUs show a higher amount of neutral (or

320 otherwise) sequence variation among endemic taxa, as they have evolved from a common ancestor on
321 the islands, when compared to non-native taxa that evolved elsewhere and have no close relatives. The
322 approach was implemented into a machine learning strategy using random forests in sklearn and
323 packaged with multiple utilities and a graphical user interface in NIClassify
324 (<https://github.com/tokebe/niclassify>). By annotating the nativeness status for sequences which are
325 identifiable to species level (98% or above match to databases), NIClassify can accurately assign status
326 for the remaining sequences. As part of the NIClassify classifications an output of accuracy is obtained by
327 withholding species with known statuses during the training, and then comparing the results for those
328 samples based on the classifications. These samples are randomly selected by the program, so biases in
329 regards to well- versus under-sampled taxa are not expected to influence the training.

330 We aggregated the sequence abundance for each arthropod OTU according to its association with a
331 particular plant genus within a site. For example, we found the sum of the sequence abundances for OTU
332 'X', a Hemiptera from genus *Nesodyne*, that was associated with (i.e., collected on) plants in the genus
333 *Coprosma*. We configured the arthropod-plant abundance data as a matrix with arthropods as columns
334 and plants as rows; there were 11 matrices, one for each site of different substrate age. As such, we
335 measure the strength of an interaction as the sequence abundance of the arthropod that was collected on
336 a particular plant species, as it is an aggregated assessment of the arthropod-plant association across
337 multiple plants and multiple plots within a site. We also graphed quantitative and qualitative metrics for
338 matrices of arthropod-plant interactions at each plot within a site. However, we constrain our discussion to
339 the aggregated network data because our confidence in the network statistics increases with the size of
340 the networks. This was a particular issue for some plots at the youngest and most depauperate sites,
341 where < 4 plant species were sampled within the plot radius and networks would be small.

342 We plotted the ecological network matrix for each community age using the 'plotweb' command in the R
343 package bipartite. For each network, lower bars represent plant abundance based on sampling time and
344 upper bars represent arthropod abundance based on OTU frequency. For visual simplicity, we grouped
345 upper bars by arthropod order. As described above, link width represents relative read abundance of

346 arthropod OTUs collected on each plant taxon (Alberdi et al., 2019); in other words, link width
347 corresponds to the relative frequency of each association.

348 The information contained in ecological networks can be summarized in various ways. Qualitative
349 properties used to describe networks, which treat all interactions as equal irrespective of their magnitude
350 or frequency, tend to be highly sensitive to variation in sampling effort (Goldwasser & Roughgarden,
351 1997; Martinez et al., 1999). Quantitative metrics that weight each taxon by the total amount of its
352 incoming and outgoing biomass flows (Bersier et al., 2002) are more robust to sampling differences
353 (Banašek-Richter et al., 2004). Using the 'networklevel' commands in the R package bipartite (Dormann
354 et al., 2008) we calculated six quantitative indices for our bipartite networks of arthropods and associated
355 plants: (1) linkage density, (2) connectance, (3) generality, (4) vulnerability, (5) interaction evenness, and
356 (6) the index of specialization H_2' (Table 2), that we reasoned would be associated with network
357 specialization (Table 1). We converted each matrix to a binary presence-absence matrix and calculated
358 the qualitative equivalent of: (1) linkage density, (2) connectance, (3) generality, and (4) vulnerability. We
359 additionally calculated the ratio of resource species to consumers for the qualitative matrices, which is the
360 ratio of plant genera to arthropod OTUs. These metrics represent the most fundamental biological and
361 ecological properties of a community. We reasoned that the simplest metrics are a reasonable starting
362 point given the limited understanding of how evolution shapes network structure, which would be
363 necessary to justify the application of more involved network metrics. Further, these metrics have values
364 that are interpretable with respect to their effect on specialization over time.

365 *Tests of network metric significance and correlation between network properties*

366 We used null models (Vázquez & Aizen, 2006) to test the statistical significance of empirical
367 network metric values for the weighted data. For each weighted empirical network, we generated 1000
368 synthetic networks so that the total number of interactions and the identity of interaction partners is
369 maintained while the weight associated with each interaction is shuffled (Staniczenko et al., 2013). With
370 this simple quantitative null model, the distribution of interaction weights is conserved, along with the
371 pattern of binary interactions, but not the identities of which interaction partners are associated with which
372 weights. In terms of biological reasoning, the null model assumes that the identities of any two species

373 involved in a non-forbidden interaction are unimportant for explaining network metrics. We calculated p-
374 values and z-scores for each combination of empirical network and metric by comparing the observed
375 metric value calculated from the empirical network to the distribution of metric values calculated from
376 synthetic matrices generated by the null model, i.e., the p-value quantifies how unlikely the observed,
377 empirical metric value is to have been generated by the null model.

378 To compare the effect of community assembly on network size, arthropod diversity, and network metrics,
379 we regressed the dependent variables by mean substrate age for each collection site. The untransformed
380 substrate age data departed significantly from normality, so comparisons were performed using
381 regressions on natural log-transformed substrate age data (Cowie, 1995; Gruner, 2007). We tested the
382 significance of the correlation between network size and community age, each network metric and
383 community age, and each network metric and network size, using Spearman's correlation tests.
384 Additionally, we fit a second-degree polynomial equation for the curvilinear relationship between the index
385 of specialization H_2' and community age.

386 **Results**

387 *Composition of communities*

388 Sites were selected using climatic and lidar data to restrict abiotic and biotic variation between sites so
389 the effect of community age on ecological network structure could best be explored (SI Table 1 and SI
390 Table 2). There was some variation in forest structure as would be expected with sites during primary
391 succession (e.g., forest height and density changes) (SI Figure 2). Our ecological networks document 34
392 plant genera and 3517 arthropod OTUs, distributed across six classes: Entognatha, Crustacea
393 (Amphipods and Isopods), Insecta, Arachnida, Chilopoda, and Diplopoda. The arthropod-plant
394 associations in our networks represent many kinds of trophic and non-trophic biotic interactions that
395 capture functional differences among species of the understory of the Hawaiian native forest. The
396 barcode reference library increased taxonomic assignment from low taxonomic resolution to genus or
397 species for 401 OTUs. Confident assignment was accomplished for a percentage of OTUs at each
398 taxonomic level: Order 99.9%, Family 67.3%, Genus 38.1% and Species 24.9% (SI Table 3).

399

400 There were 2747 OTUs classified as native and 770 classified as non-native using NIClassify. The overall
401 accuracy for our dataset predictions of nativeness using NIClassify was 99.9%. Of the native OTUs,
402 Hemiptera were the dominant order (652 OTUs), followed by Araneae (467 OTUs), Diptera (327 OTUs),
403 and Coleoptera (266 OTUs). We found a highly significant (SI Table 4) increase in network size with
404 community age for both nodes and links, with a disproportionate increase in the number of links
405 (interactions) after several hundred years of community development (Figure 2a). The number of native
406 arthropod species increases dramatically over both ecological and evolutionary time while the number of
407 non-native arthropod species remains relatively steady (Figure 2b). The abundance of native and non-
408 native arthropods peaks in the middle-aged communities but the proportion of non-native taxon
409 abundance is highest in younger communities (Figure 2c). Plant diversity increased with community age
410 (Figure 2d).

411

412 *Arthropod-plant association networks*

413

414 Arthropod OTU richness, plant diversity and number of interactions increased with the geologic age of the
415 site. Bipartite networks of younger communities contain linkage widths between the few dominant taxa
416 (e.g., Hemiptera and *Metrosideros*) while older communities contain smaller linkage widths representative
417 of the many more associations distributed among the greater diversity of both higher and lower level taxa
418 (Figure 3, SI Figure 3).

419

420 For the null model analyses of the weighted matrices, some observed network metric values were not
421 significantly different ($p < 0.05$) from metric values produced from the synthetic matrices (SI Table 5, SI
422 Figure 6).

423

424 Results of the Spearman's correlation tests show linkage density (average number of interactions per
425 species), network vulnerability (a measure of the ratio of plant generic richness to arthropod OTU
426 richness) and interaction evenness (a measure of the uniformity of energy flows along different pathways)
427 increased significantly with community age (Figure 4, Table 3). Generality (a measure of the ratio of

428 arthropod OTU richness to plant generic richness) and the index of specialization H_2' both increased with
429 community age but were not significantly positively correlated. The index of specialization H_2' has a
430 curvilinear relationship with community age, first decreasing then increasing. A second-degree polynomial
431 provides the best approximation of the relationship between the H_2' and community age ($F = 6.85$, $R^2 =$
432 0.5392 , $p < 0.05$). By beat sampling and sequencing all plant-associated arthropods, our sampling of
433 arthropod taxa is at finer taxonomic resolution than plants. As a result, generality (links/arthropods) is very
434 close arithmetically to linkage density (links/arthropods + plants) in our dataset because the number of
435 arthropod OTUs is many times greater than the number of plant genera for all communities. Connectance
436 (proportion of realized interactions) was not significantly correlated with increasing community age, but
437 instead is highest at the youngest site, and relatively constant for the remainder of sites.

438

439 For the qualitative metrics calculated from the binary matrices, linkage density (links/species),
440 connectance links/(arthropods*plants), and generality (links/arthropods) were significantly correlated with
441 community age, while vulnerability (links/plants) and the ratio of resource species to consumers
442 (plants/arthropods) was not (SI Figure 4, SI Table 4). The results from plot level analysis are consistent
443 with the site level data and the variance among plots at the same sites is minimal (SI Figure 7). These
444 results help corroborate the trend of increasing specialization over time.

445

446 For the regressions of network metrics against network size, with the exception of generality, quantitative
447 network metrics were not significantly correlated with network size (SI Figure 5A, SI Table 4). By contrast,
448 qualitative metrics were significantly correlated with network size with the exception of the ratio of
449 resource species to consumers, and vulnerability (SI Figure 5B, SI Table 4).

450

451 **Discussion**

452 Using a dataset of biotic associations during the course of community assembly here we present strong
453 evidence of increasing specialization within arthropod communities through evolutionary time. Our DNA
454 metabarcoding data have allowed us to collect a large sample of the arthropods from the understory of
455 Hawaiian forests, representing a broad swath of trophic and non-trophic arthropod-plant associations. As

456 expected, the qualitative metrics were strongly biased by network size (Banašek-Richter et al., 2004;
457 Goldwasser & Roughgarden, 1997) and showed higher linkage density, generality, vulnerability,
458 interaction evenness, and lower connectance in older communities, because the diversity of plants and
459 arthropods was higher in these communities (SI Figure 4, SI Table 4). Our null model analysis helped to
460 demonstrate that the distribution of link weights was itself an important feature of the observed network
461 structure (i.e., not which species they are between). We present clear signatures of change in
462 quantitative, weighted network metrics with community age (Figure 4, Table 3) that resulted from
463 changing community composition and ecological dynamics.

464

465 *Ecological processes dominate younger communities*

466

467 Theory suggests that the composition of the youngest communities is shaped through colonization from a
468 regional species pool. This expectation is supported by our results, with the younger communities having
469 significantly lower linkage density, vulnerability, and interaction evenness (Figure 4). These results
470 indicate that species in younger communities are interacting with greater frequency along less uniform
471 interaction pathways, compared to species assemblages at older sites. However, notably the very
472 youngest site, the 1973 lava flow, is an outlier. At the 1973 lava flow, linkage density is high ($LD = 24.2$),
473 likely reflecting strong environmental filtering and an opportunistic community of generalist species
474 (Bufford et al., 2020; Kortsch et al., 2015) suited for survival during primary succession. At other young
475 sites, linkage density is low, from < 10 (Tree Planting Rd.) whereas it peaks and levels off at Maui ($LD =$
476 38.6) and Kauai ($LD = 38.9$), respectively. Thus, linkage density was low at young sites with low resource
477 diversity while the diversity of interactions increased over evolutionary time in step with increasing
478 community complexity.

479

480 Interaction evenness was low, as expected, on the youngest sites, again with the exception of the 1973
481 flow. As a measure of the uniformity of energy flows along different pathways, we expected interaction
482 evenness to be low in young communities because some interaction partners would dominate the
483 associations in the network. For example, a large proportion of interactions on the youngest sites (< 300

484 years old) belong to the associations of Hemiptera and Collembola species with early successional plant
485 species, *Metrosideros polymorpha* and *Dicranopteris linearis* (Figure 3, SI Figure 3). Low interaction
486 evenness has also been demonstrated among bees and wasps and their associated natural enemies
487 (e.g. parasitoids) under conditions of intensive management (Staniczenko et al., 2017; Tylianakis et al.,
488 2007). The early successional communities in Hawaii are ecologically similar to highly modified sites, due
489 to the recent disturbance from lava and the paucity of resource diversity. We suggest that the higher
490 interaction evenness at the 1973 lava flow is due to the extremely limited resources (plants) on the
491 sparsely vegetated lava substrate. At this site colonists may be joined by a relatively large representation
492 of transient arthropods, which may be less host-specific and appear randomly associated with the
493 available plants, increasing interaction evenness. Connectance also peaked in the youngest community
494 (1973 lava flow) likely due to the greater representation of generalists within this network (Kortsch et al.,
495 2015; Ponisio et al., 2019).

496

497 The network metric values are less consistent among the youngest sites compared to the older sites
498 (Figure 4). This is likely due to the relatively rapid changes in community composition in early primary
499 succession (Atkinson, 1970; Roderick et al., 2012) compared to older established sites. An alternative
500 explanation is that change in the composition of the understory plants (Figure 3 and SI Figure 3) and
501 canopy structure (SI Figure 2 and SI Table 2) results in the network metric variation at the youngest sites.
502 The higher variation among network values at the youngest sites may also point to the different rates of
503 specialization and adaptation among different lineages of arthropods. Among functional groups of beetles
504 (e.g., xylophages, fungivores, predators), community composition and network specialization changed
505 differently during early succession (Wende et al., 2017).

506

507 *Specialization increases through evolutionary time*

508

509 For a given taxon on average, the number of biotic interactions it is involved in decreases with community
510 age, resulting in greater network specialization. This is reflected in the increased linkage density with
511 community age, as early colonizing species gave way to a greater diversity of associations (Figure 4).

512 However, weighted connectance stabilized at around the same level for the remainder of the communities
513 after the 1973 lava flow. This may be explained by the 'constant connectance' hypothesis (Martinez,
514 1992) that posits that species are linked to a fixed fraction of species in a network, independent of the
515 number of species in a community. A similar pattern of constant connectance and community age was
516 found in arthropods recolonizing defaunated mangrove islands (Piechnik et al., 2008). For the Hawaiian
517 Islands, several factors likely produce constant connectance over long-term community development.
518 First, resource availability limits specialists at early stages; e.g., Escape Road (~ 300 yrs) is dominated by
519 a single species of fern. Next, over evolutionary time, the Hawaiian fauna is characterized by a
520 remarkably high rate of lineage diversification (Gillespie, 2016; Gillespie et al., 2020; Zimmerman, 1970)
521 that has added novel species and associations. Finally, at more recent timescales (after human arrival)
522 immigration of non-natives has been sufficiently high so as to add generalist taxa across all stages of
523 community development (Figure 2b).

524
525 A previous study which used an island chronosequence to examine how pollinator interactions change
526 through extended time (Trøjelsgaard et al 2013) also found connectance was poorly explained by age.
527 However, contrary to our results, the Canary Islands study showed hump-shaped relationships of
528 interaction richness and specialization with island age. One reason for the different results is that we used
529 a natural log scale for the skewness of island age. For the Hawaiian islands, values for linkage density,
530 vulnerability, interaction evenness, and index of specialization H_2' were especially high on the volcano of
531 East Maui. The islands of Maui Nui are also where richness peaks for many native arthropod lineages
532 (Gillespie & Baldwin, 2009; Gruner, 2007). However, unlike the Canary Island pollinators, our values of
533 linkage density were highest on the oldest island, and values for interaction evenness, vulnerability, and
534 index of specialization H_2' were nearly as high, indicating that the overall changes in network structure
535 were more linear than hump-shaped. An alternative explanation for the difference in the results is that the
536 older islands of the Canary archipelago have environments that are very different from the younger
537 islands. Although the Canary Island study focused on communities that were characterized by the plant
538 species *Euphorbia balsamifera*, the abiotic environment changes significantly across their
539 chronosequence, with the older islands much lower and drier (Juan et al., 2000). Thus, the finding of a

540 hump-shaped relationship in the Canary Islands is associated with the combined effects of time, island
541 geomorphological transitions, and associated change in climate regimes. In contrast, the current study in
542 the Hawaiian Islands aimed to standardize environments (elevation, precipitation, and forest cover, with
543 sampling from standardized plots across the islands). Therefore, any confounding environmental
544 differences were minimized and changes in network properties should largely reflect the influence of
545 community age.

546

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

560

561 The network-level specialization index H_2' is largely unaffected by network size, network architecture or
562 total number of interactions for a fixed matrix size (Blüthgen et al. 2006), making it an ideal metric
563 compare between different networks for understanding specialization over time. We find that the index of
564 specialization increases over time but is better fit by a second-degree polynomial equation. In early stage
565 communities from 50 to 575 years the index of specialization is decreasing. This drop in specialization in
566 the first several hundred years, is followed by an increase over the next tens of thousands of years. For
567 random associations H_2' is usually close to zero. On Maui it reaches a value of 0.6 then levels out to 0.5

568 on Molokai and Kauai. This pattern is consistent with other metrics in our analysis suggesting that very
569 young communities are organized by assembly rules making them appear specialized. After ecological
570 sorting and the impact of *in situ* evolution in later stage communities we see organization at a secondary,
571 evolutionary stage of development.

572

573 *Resilience of communities increases through time*

574

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

582

583 However, this result runs counter to work suggesting higher-connectance food webs tend to host fewer
584 invaders and exert stronger biotic resistance compared to low-connectance webs (Smith-Ramesh et al.,
585 2017). Further, community resistance to invasion is known to increase with native species diversity
586 (Gallien & Carboni, 2017) and network complexity (Wei et al., 2015). Considering the results from our
587 study within the context of this previous work, older communities, which are characterized by low
588 connectance and high specialization, may be more resistant to invasion; however, individual taxa may be
589 more susceptible to extinction. From an individual species level, because all species are linked together
590 either directly or indirectly (Montoya et al., 2006), individual species with high specialization and low
591 connectance are susceptible to extinction because of secondary extinctions occurring when specialized
592 consumers lose their only prey (Dunne et al., 2002; Staniczenko et al., 2010). From a network level, as
593 communities age, several species may be associated with the same resource (resource redundancy) or
594 utilize a single resource more effectively (resource complementarity), minimizing variability in the

595 functioning of an ecosystem, for example when some consumer species decline in number (Peralta et al.,
596 2014).

597

598 Although ecological processes, such as interspecific interactions or disturbance, are often attributed to
599 the geographical differences in exotic species richness (Lockwood et al., 2013) an alternative explanation
600 for the apparent reduced biotic resistance to invasion of younger communities may be that they
601 experience increased propagule pressure (Lockwood et al., 2005). The younger sites on Kilauea volcano
602 are accessed more frequently by tourists compared to the older sites, which require greater on foot
603 distances to reach or special access permits. Furthermore, while our study directly assesses arthropod-
604 plant associations, it only indirectly measures the effect of higher trophic associations. Differential top-
605 down pressure (e.g., predator turnover) during community assembly likely also changes biotic resistance
606 to invasion, for example, generalist insectivorous birds reduced infiltration of an invasive species of spider
607 at the 133 years old Tree Planting Rd. community (Gruner, 2005).

608

609 *Conclusions and outlook*

610

611 Our study uses whole-community DNA metabarcoding data to assess the biotic associations of
612 thousands of arthropod OTUs on plants across a geological chronosequence. By including relative
613 abundance data, we achieve a signature of interaction strength (Popovic et al., 2019) not captured for co-
614 occurrences with presence-absence observations (Blanchet et al., 2020). Although DNA metabarcoding
615 can be used for observation of trophic interactions (Alberdi et al., 2019; Krehenwinkel, Kennedy, et al.,
616 2017), our analysis instead includes all biotic associations between arthropod-plant communities,
617 including those that can be difficult to detect (e.g. involving cryptic species, new non-natives, endangered
618 species, juveniles). Thus, we are able to include complex community interactions including substrates
619 chosen for acoustic signaling (Mullet et al., 2017), predator avoidance (Lindstedt et al., 2019; Stachowicz
620 & Hay, 1999) and gregarious plant-feeding insects (Hunter, 2000) that are often overlooked in traditional
621 network studies. Compared to the limitations of small, unweighted early food web studies (Cohen et al.,

622 1993; Hall & Raffaelli, 1991) DNA metabarcoding offers exciting avenues forward for capturing
623 community complexity.

624
625 This research revealed a strong association between the network structure of ecological communities and
626 community development over evolutionary time. Quantitative network metrics demonstrate that younger
627 communities are composed of more generalist species that interact with greater frequency along fewer
628 interaction pathways, with individual and network specialization increasing with community age. Our data
629 highlight the utility of DNA metabarcoding for understanding longstanding questions of ecology and
630 evolutionary biology that remain time consuming (e.g., keying out morphological species) or impossible
631 (e.g., identification of juveniles) to assess with traditional methods. From a conservation perspective, our
632 results indicate that habitat disturbance erodes a complex web of biotic associations, far greater than the
633 sum of the community metrics of richness and abundance, that have evolved *in situ* over thousands to
634 millions of years.

635

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637

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652

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959 **Data Accessibility**

960 Data are deposited in a Dryad data repository at <https://doi.org/10.6078/D1DX4T> and code is hosted on
 961 Zenodo at <https://zenodo.org/record/7349067> including: 1) DNA sequence data (fasta files) from whole
 962 organism community metabarcoding and the DNA barcode reference library, and 2) processed data used
 963 in these analyses including the OTU table, phylogenetic species ids, NIClassify predictions, and
 964 geographic information.

965

966 **Author Contributions**

967 N.R.G., D.S.G, R.G.G., H.K., J.Y.L. designed the research, N.R.G. and H.K. performed molecular
968 processing of the samples, N.R.G. and P.S. analyzed the data, J.C. and J.C.A. contributed new analytical
969 tools, N.R.G. and R.G.G. wrote the manuscript with input and comments from all coauthors.

970

971 **Tables and Figures**

972 **Table 1: Some expectations for network structure in response to ecological and evolutionary**
 973 **factors influencing community assembly through time.** Specialization should change at the network
 974 level to depict the changes at the taxon level of more evolved associations. Since the associations of
 975 each species are becoming more specialized there is an expectation that an individual taxon will have
 976 fewer interactions and the makeup of the whole community will have more interactions overall. [1]
 977 (Bufford et al., 2020) [2] (Vázquez et al., 2007) [3] (Tylianakis et al., 2007) [4] (Peralta et al., 2014) [5]
 978 (Coux et al., 2016) [6] (Ponisio et al., 2019) [7] (Segar et al., 2020) [8] (Chamberlain et al., 2014) [9]
 979 (Blüthgen et al. 2006) [10] (Dunne & Williams, 2009)
 980

Process	Richness?	Specialization?	e.g., 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 defenses, 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, e.g., 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 <i>in situ</i> 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]

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983 **Table 2. Binary and Weighted network summary statistics.** Metrics calculated from binary (i.e.
 984 unweighted, presence-absence) matrices are easily interpretable but sensitive to sampling differences
 985 (Banašek-Richter et al., 2004). Quantitative versions based on information theory are more conservative
 986 when comparing differences among sites. Each metric incorporates the diversity of individuals comprising
 987 the resource (H_N , the diversity of inflows) and of that going to the consumers (H_P , the diversity of outflows)
 988 for each species k . The quantitative metrics are then based on the reciprocals of these Shannon Entropy
 989 values ($n_{N,k}$, and $n_{P,k}$, respectively). The notation q is applied to the quantitative version of that metric. All
 990 equations and notations reference [1] (Bersier et al., 2002) [2] (Tylianakis et al., 2007) [3] (Blüthgen et al.
 991 2006).
 992

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] W LD_q , (14) [1]	Average number of interactions per species weighted version
Connectance	B $Conn = L/(R \times C)$ [1] W $Conn_q = LD_q/S$ [1]	Proportion of realized links weighted version
Generality	B $G = L/C$ [1] W G_q , (25) [1]	Average number of resource sp. per consumer sp. weighted version
Vulnerability	B $V = L/R$ [1] W V_q , (27) [1]	Average number of consumer sp. per resource sp. weighted version
Interaction evenness	W I.E. [2]	Shannon entropy of interaction weights
Index of specialization	W $H2'$ [3]	Ranges between 0 and 1.0 for extreme generalization and specialization, respectively

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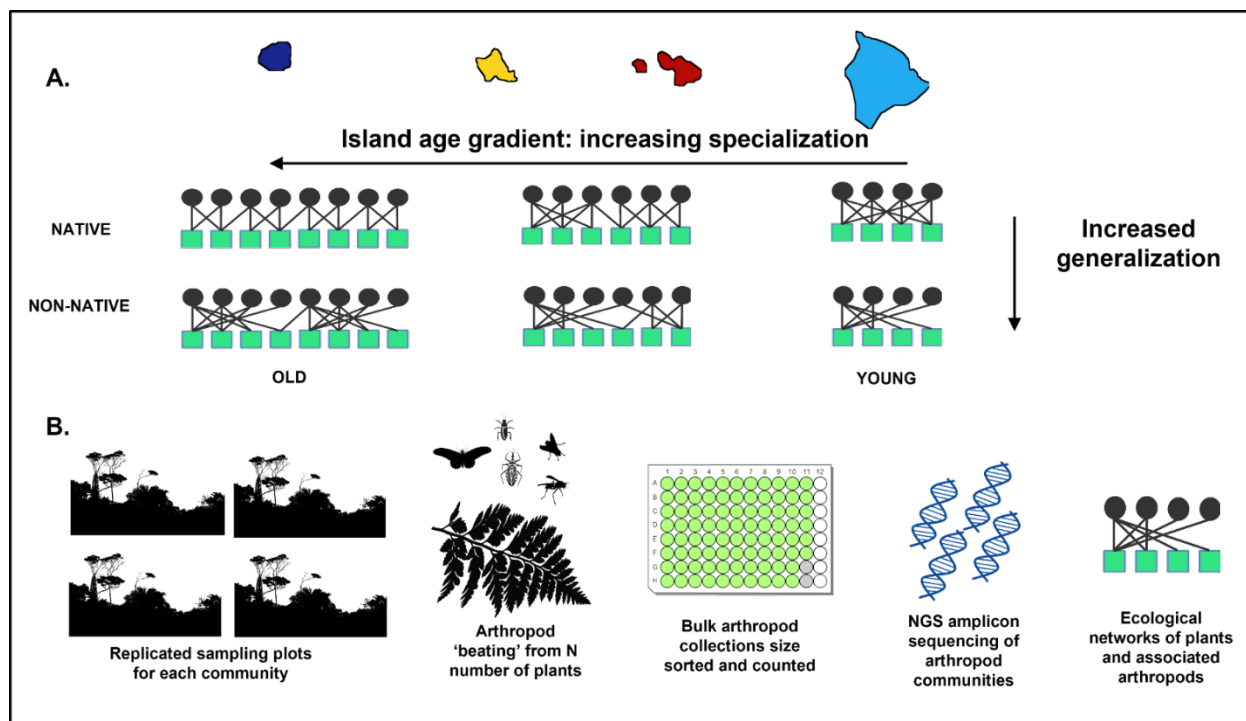
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995 **Table 3. Spearman's correlation tests for network metrics and community age.**
 996 Spearman's correlation tests were used to determine the significance of the relationship between each
 997 quantitative network metric value and ln substrate age (community age). Graphs of regressions in Figure
 998 4.

	S	p-value	Spearman's rho
<i>Quantitative (weighted) x community age</i>			
linkage density	76	0.033	0.65
weighted connectance	154	0.371	0.30
generality	124	0.183	0.44
vulnerability	80	0.040	0.64
interaction evenness	70	0.025	0.68
index of specialization H_2'	134	0.237	0.39

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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 y to 4.15 Myr 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 2-4mm, 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.

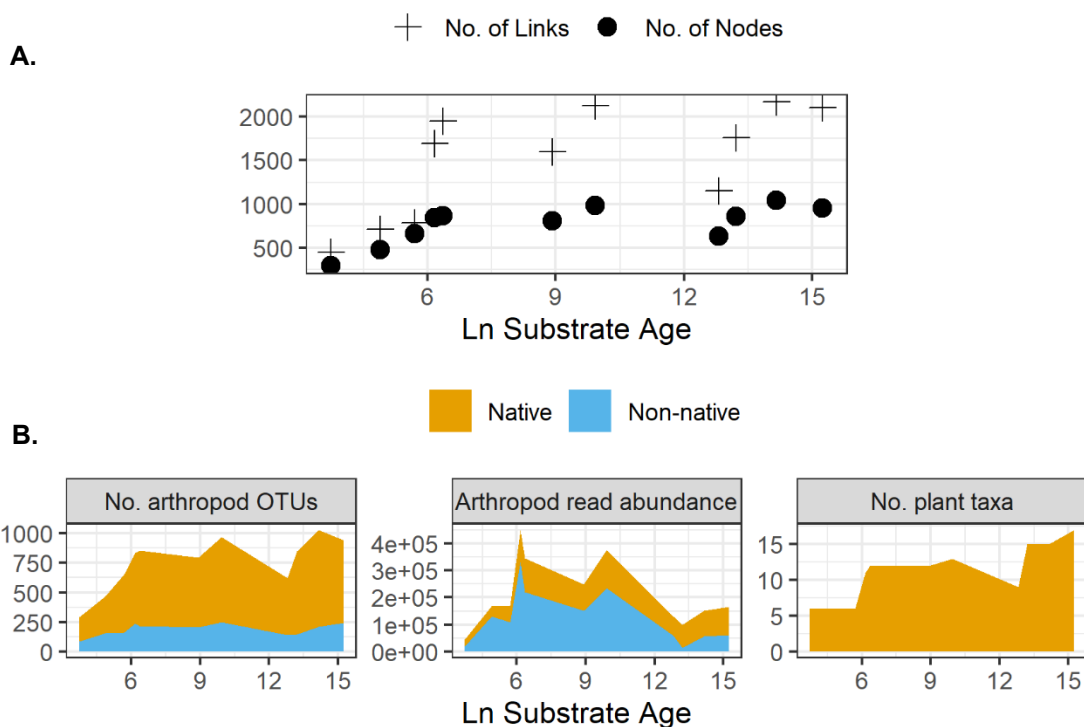
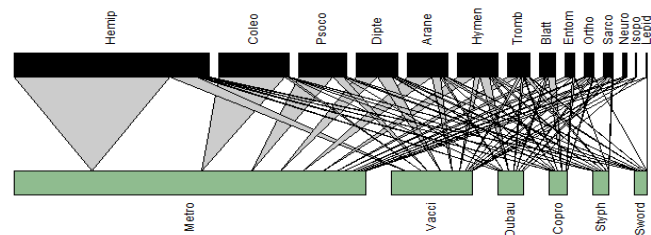
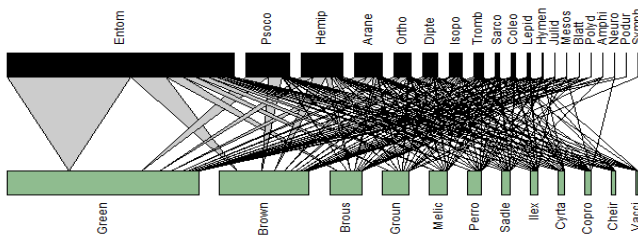


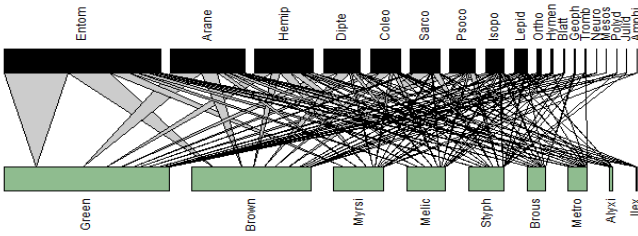
Figure 2. Effect of community assembly over evolutionary time on network size and diversity of native and non-native taxa. A) The number of nodes (arthropod and plant richness) and the number of links (arthropod-plant associations) significantly increase in concert with community age. Spearman's correlation test values SI Table 4. 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.



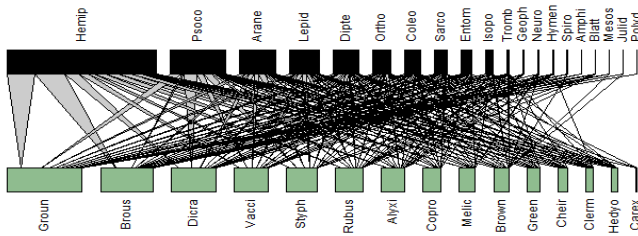
Kilauea: 1973 lava flow 50 yo



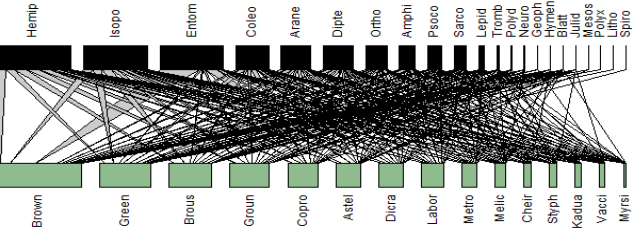
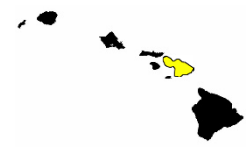
Mauna Loa: Oiaa 7,500 yo



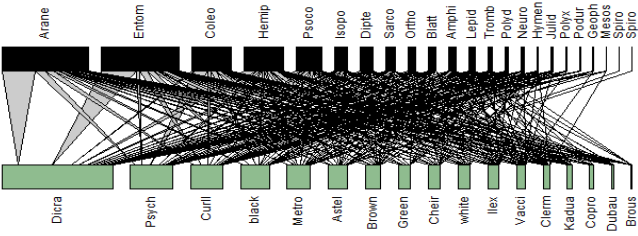
Kohala: Kohala 365,000 yo



Maui: Waikamoi 545,000 yo



Molokai: Kamakou 1.4 myo

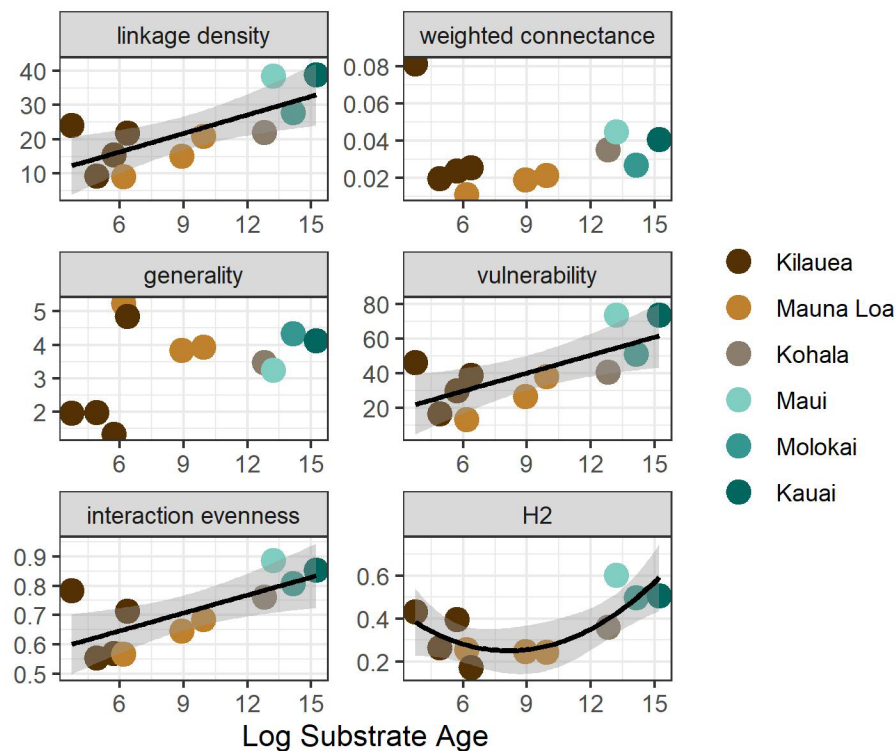


Kauai: Kokee 4.15 myo



1028 **Figure 3. Quantitative arthropod–plant networks along a gradient of increasing community**
1029 **assembly (top to bottom).** For each network, lower bars represent plant abundance based on sampling
1030 time and upper bars represent arthropod abundance based on OTU frequency. Each network is plotted in
1031 order of the most abundant taxa from left to right so that the turnover in arthropod-plant association can
1032 be seen for each community. Linkage width indicates the frequency of each association as measured
1033 using arthropod read abundance. As a summary, the networks show interaction data pooled across all
1034 plots for each community age with OTUs pooled by arthropod order, but analyses were performed at the
1035 OTU per plant genus level. The bipartite graphs from each of the 11 sampled sites are in SI Figure 3.
1036 Arthropod and plant id codes are given in SI Table 3.
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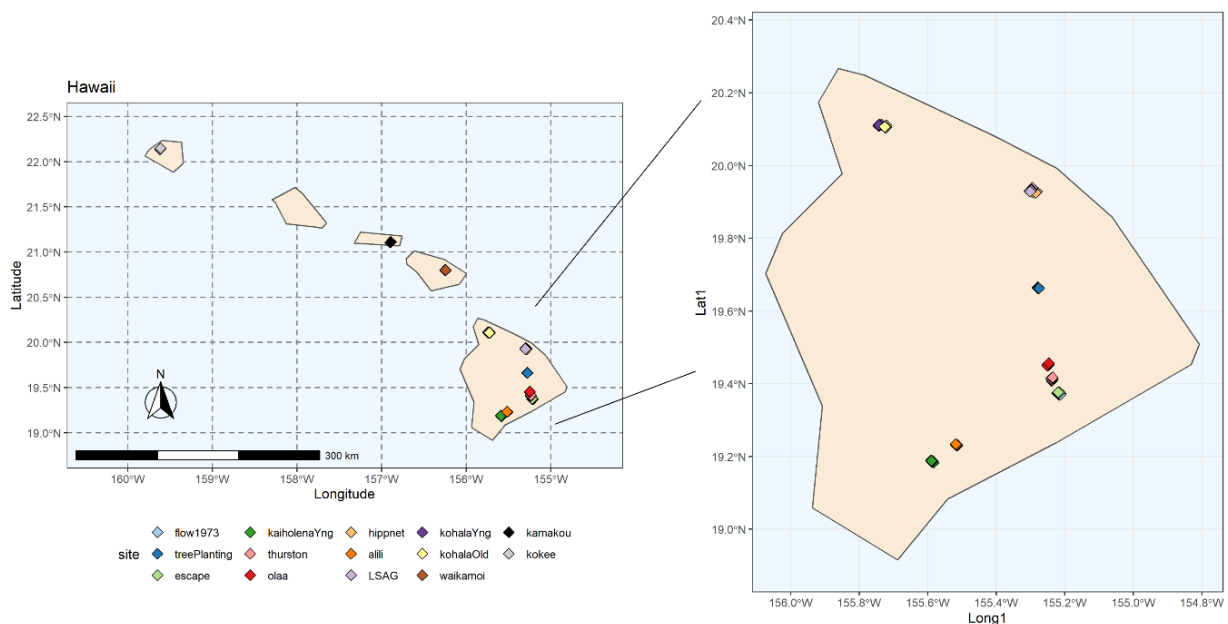
Figure 4. The effect of community age on quantitative ecological network metrics.

1041 Statistical measures of network architecture indicating changes in arthropod-plant associations in concert
 1042 with community age. Each network was weighted with the read abundance of the arthropod OTU
 1043 associated with the plant genus it was collected from, across all plots for a community age. Three metrics
 1044 show significant relationships with community assembly, increasing over time: linkage density,
 1045 vulnerability, interaction evenness. Spearman's correlation test values Table 3. Results of the null model
 1046 analysis for the quantitative ecological networks metrics are presented in SI Figure 6 and SI Table 5. A
 1047 graph of the results when analyzed for each of the sampled plots within a community age site is
 1048 presented in SI Figure 7.

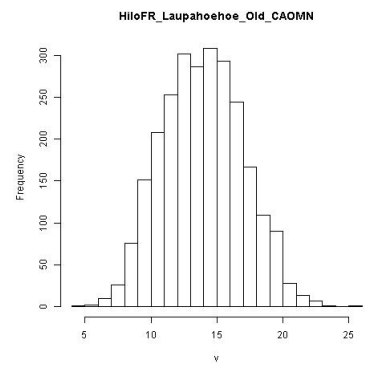
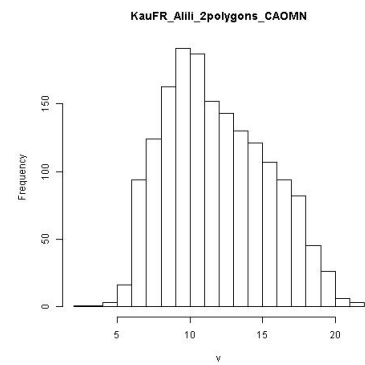
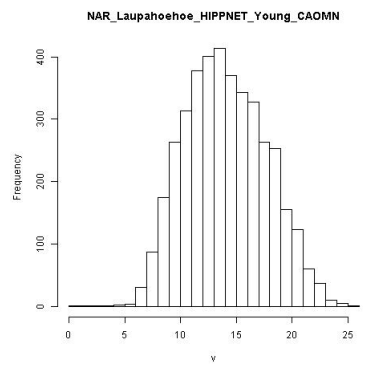
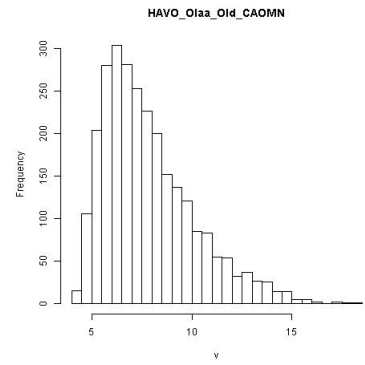
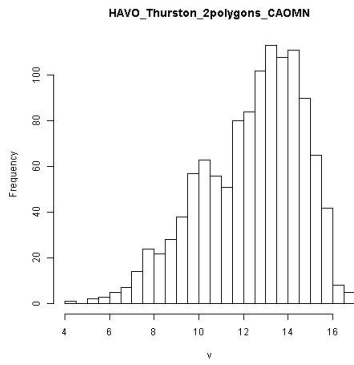
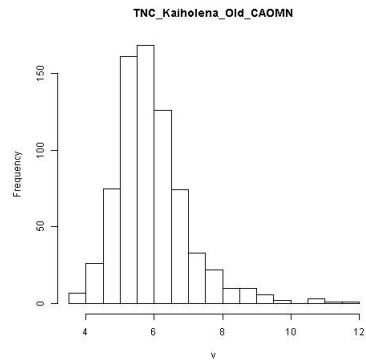
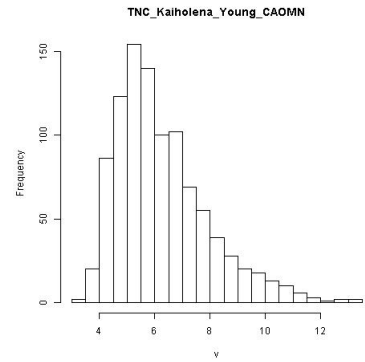
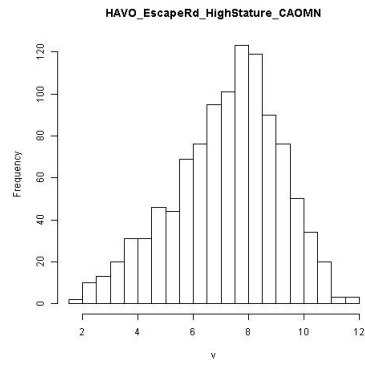
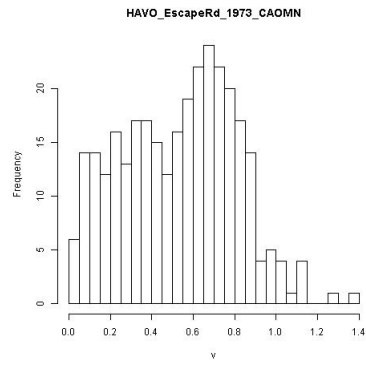
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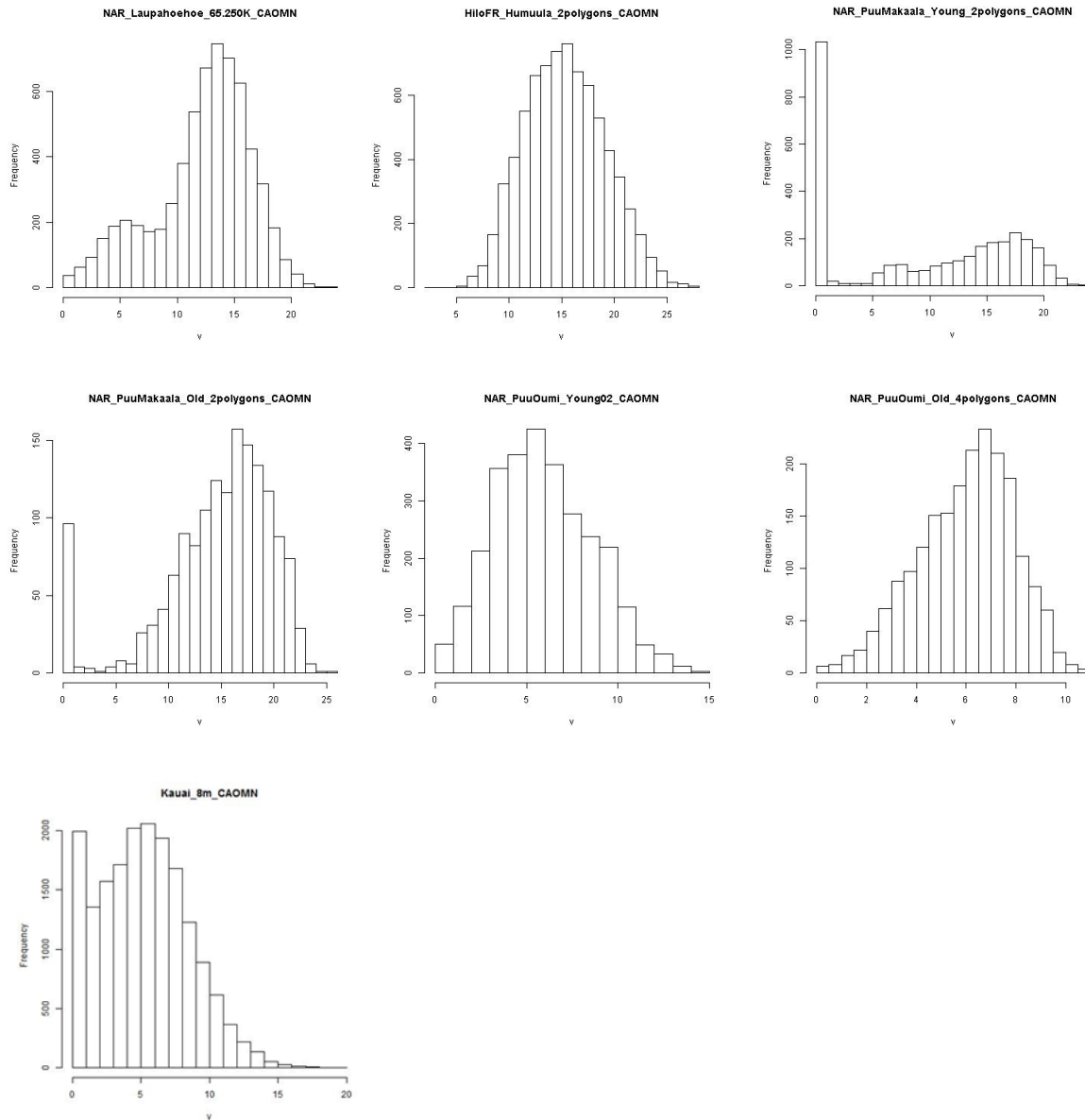
Ecological network structure in response to community assembly processes over evolutionary time

Natalie R. Graham, Henrik Krehenwinkel, Jun Ying Lim, Phillip Staniczenko, Jackson Callaghan, Jeremy C. Andersen, Daniel S. Gruner, Rosemary G. Gillespie



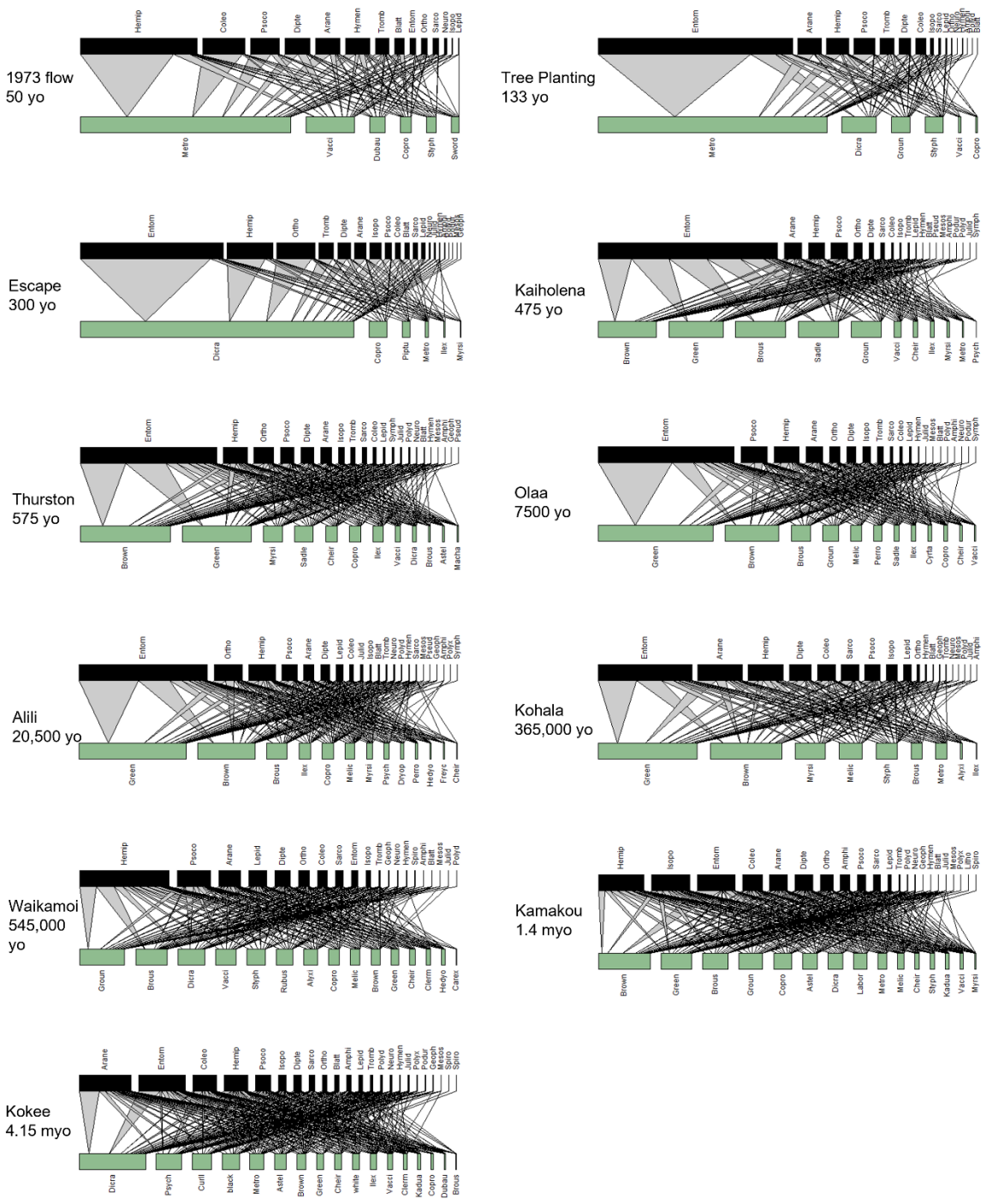
SI Figure 1. Map of sampling sites. 14 sites of varying geologic age were selected, ranging from 50 to 4.15×10^6 years old, across four islands of the archipelago: Hawaii, Maui, Molokai, Kauai. To control for climatic differences and disturbance across sites, sites were constrained to ranges of elevation (1000-1300 m) and precipitation (average annual precipitation 2500-3000 mm) and within accessible protected forest lands. See SI Table 2 for substrate age and other site characteristics.





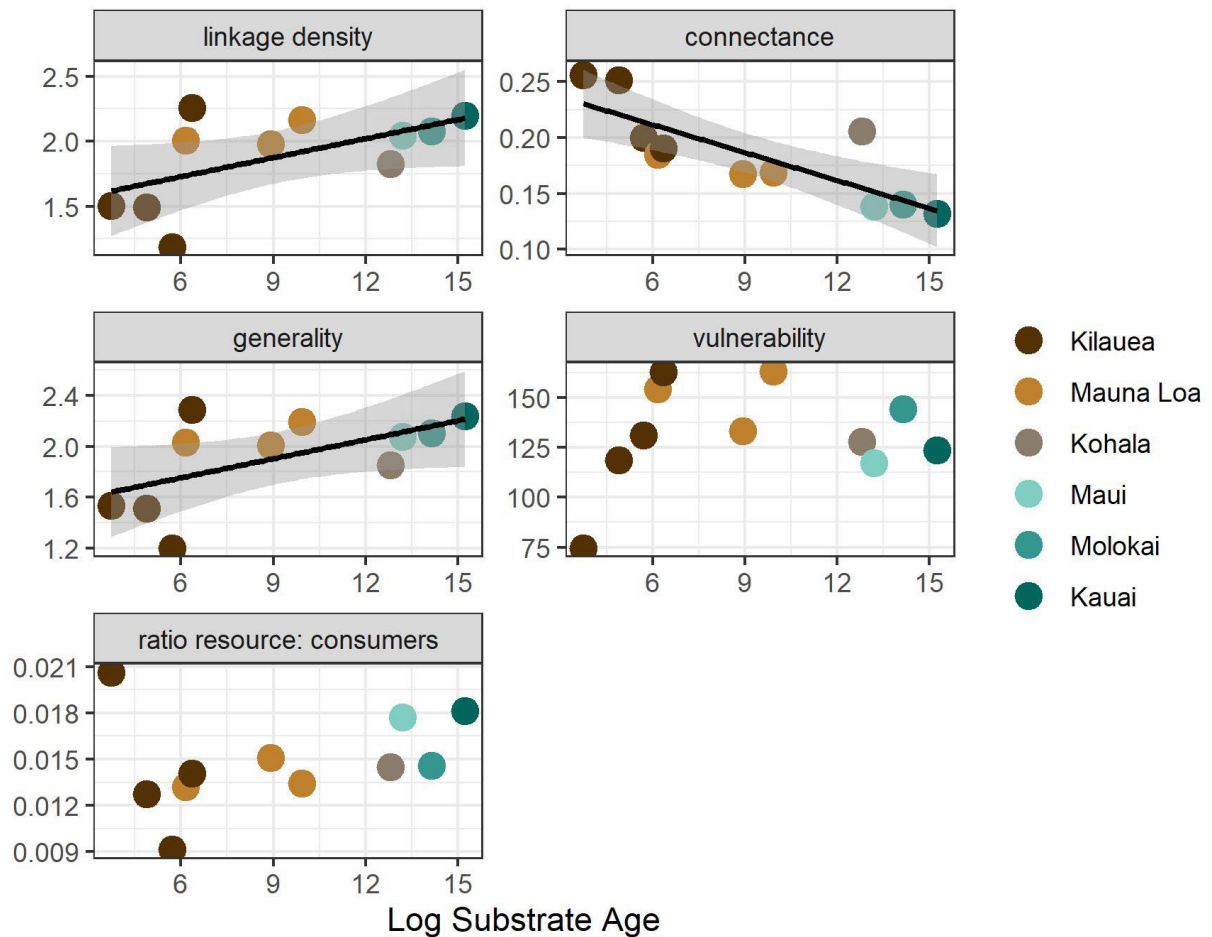
SI Figure 2. Histograms of height profiles from lidar flyover data. Each of the potential sites for the study was assessed for site suitability including these lidar data to evaluate the forest structure. Grain size for lidar was 20 m for all sites except Kauai for which it was 8 m grain size. We used 40% quantile for site comparison because the tails of the distribution of heights was long for some sites, and it would have been difficult to find sites with overlapping height profiles with a more restrictive choice.

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SI Figure 3. Additional bipartite network plots. For each network, lower bars represent plant abundance based on sampling time and upper bars represent arthropod abundance based on OTU

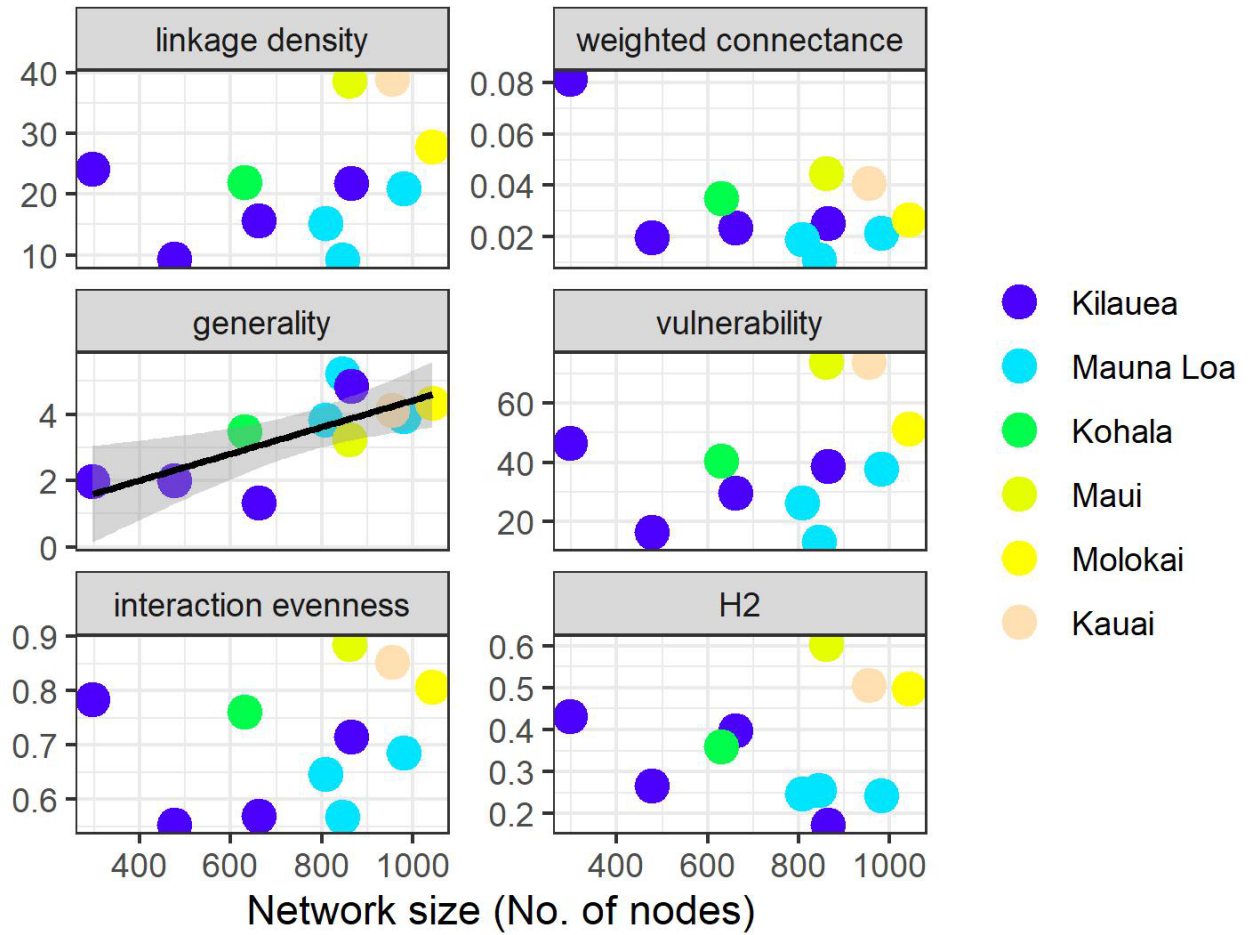
frequency. Each network is plotted in order of the most abundant taxa from left to right so that the turnover in arthropod-plant association can be seen for each community. Linkage width indicates the frequency of each association as measured using arthropod read abundance. As a summary, the networks show interaction data pooled across all plots for each community age with OTUs pooled by arthropod order. However, analyses were performed at the OTU per plant genus level. Arthropod and plant id codes are given in Supplementary Table 1.

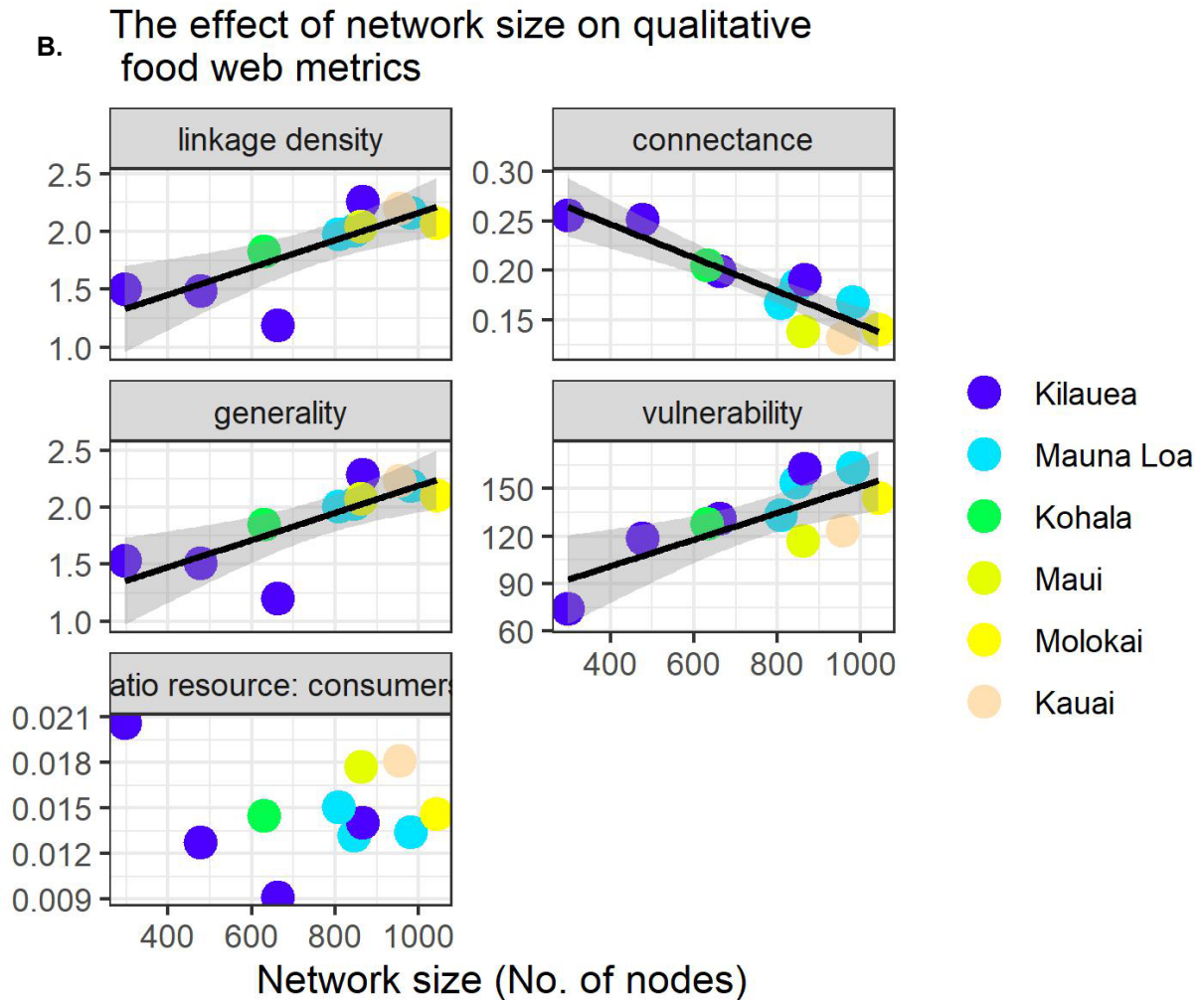


SI Figure 4. The effect of community age on qualitative ecological network metrics. Statistical measures of network architecture were performed using qualitative measures on presence-absence matrices. Three metrics show significant relationships with community age, increasing linkage density and generality and decreasing connectance. Spearman rho and p-values in SI Table 4.

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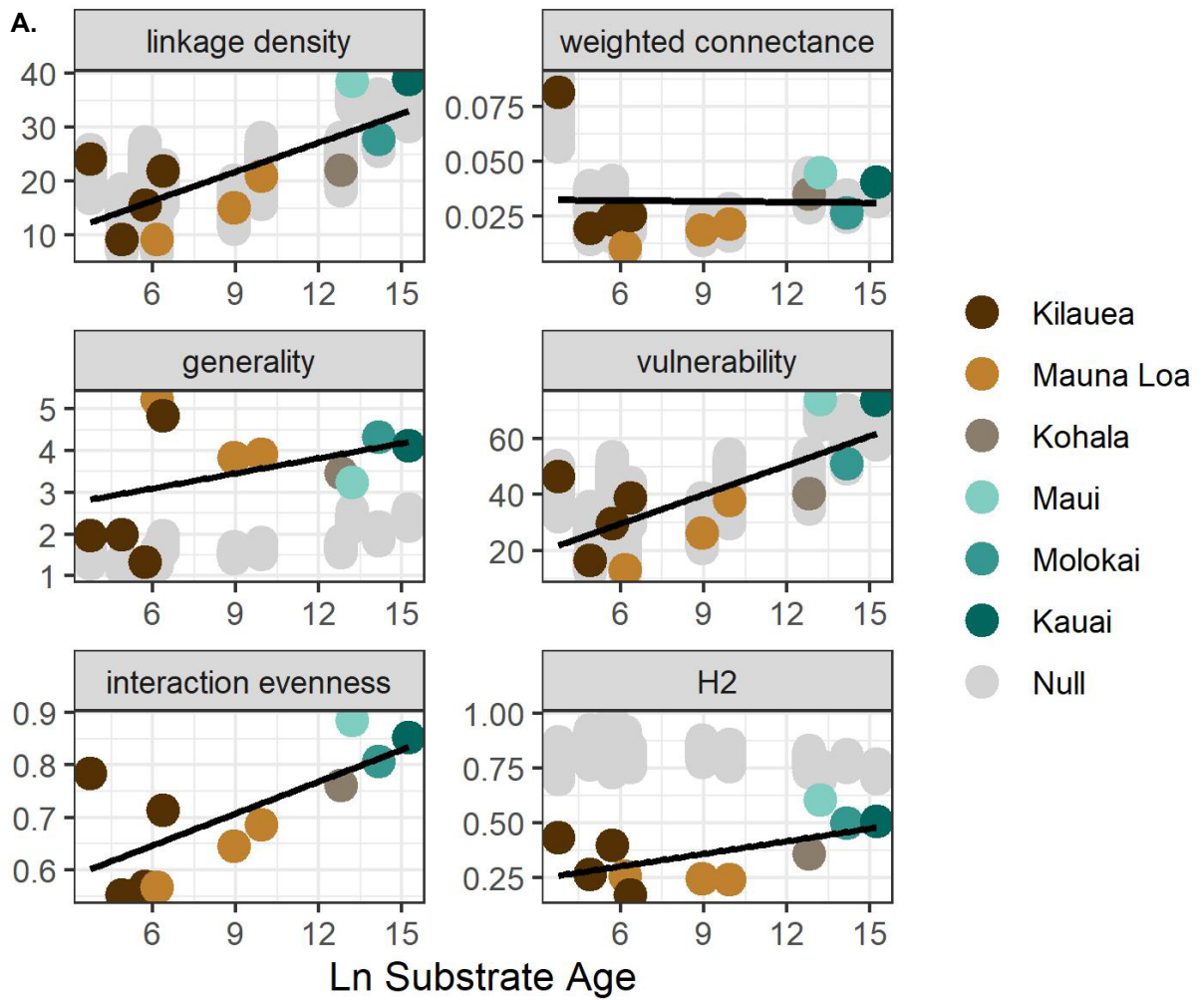
A. The effect of network size on quantitative food web metrics

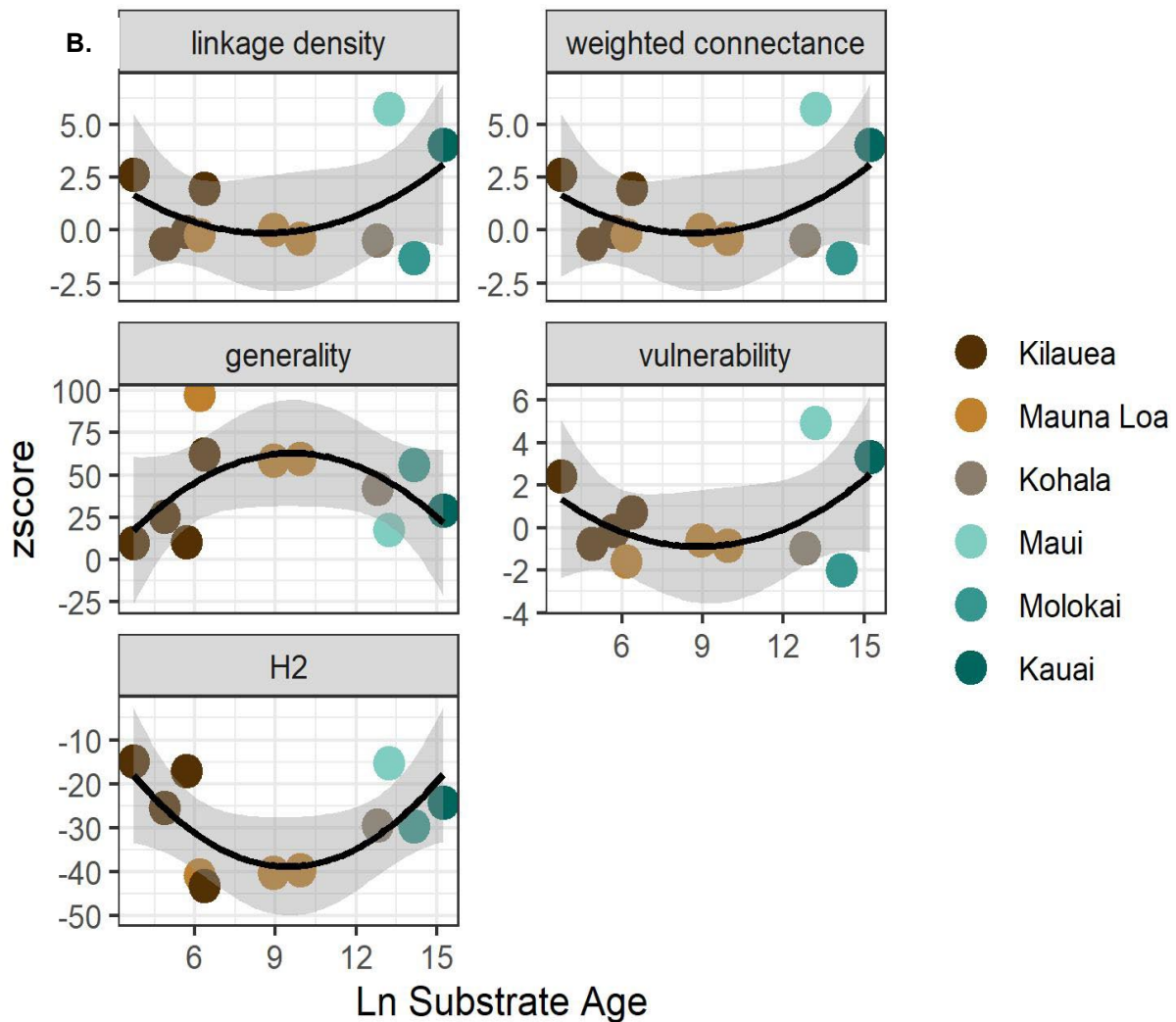




SI Figure 5. The effect of network size on ecological network metrics. Each quantitative and qualitative metrics was regressed against the number of nodes (representing network size). A) Quantitative metrics were not significantly correlated with network size with the exception of generality. B) Qualitative metrics were significantly correlated with network size, with the exception of the ratio of resource:consumers. Spearman rho and p-values in SI Table 4.

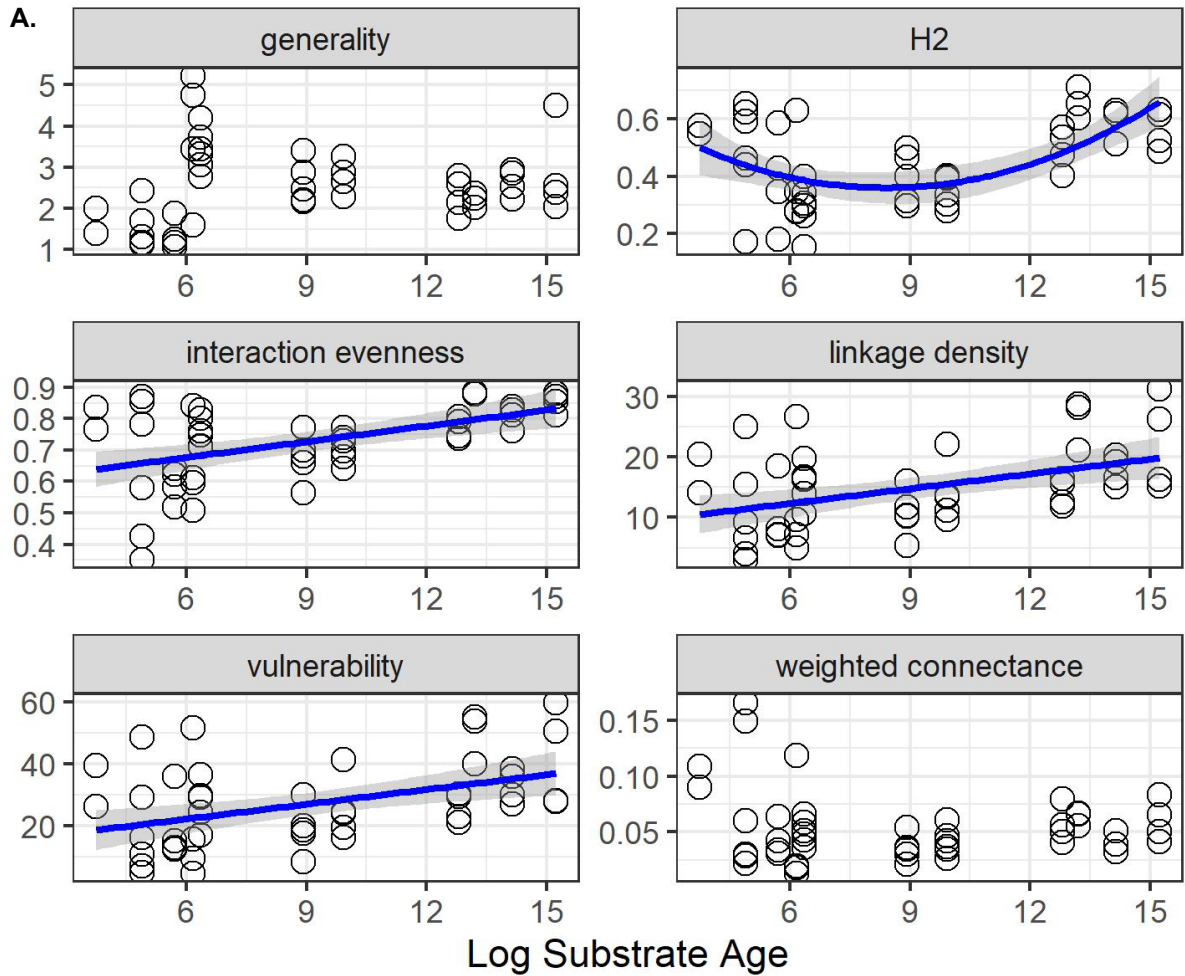
MOLECULAR ECOLOGY

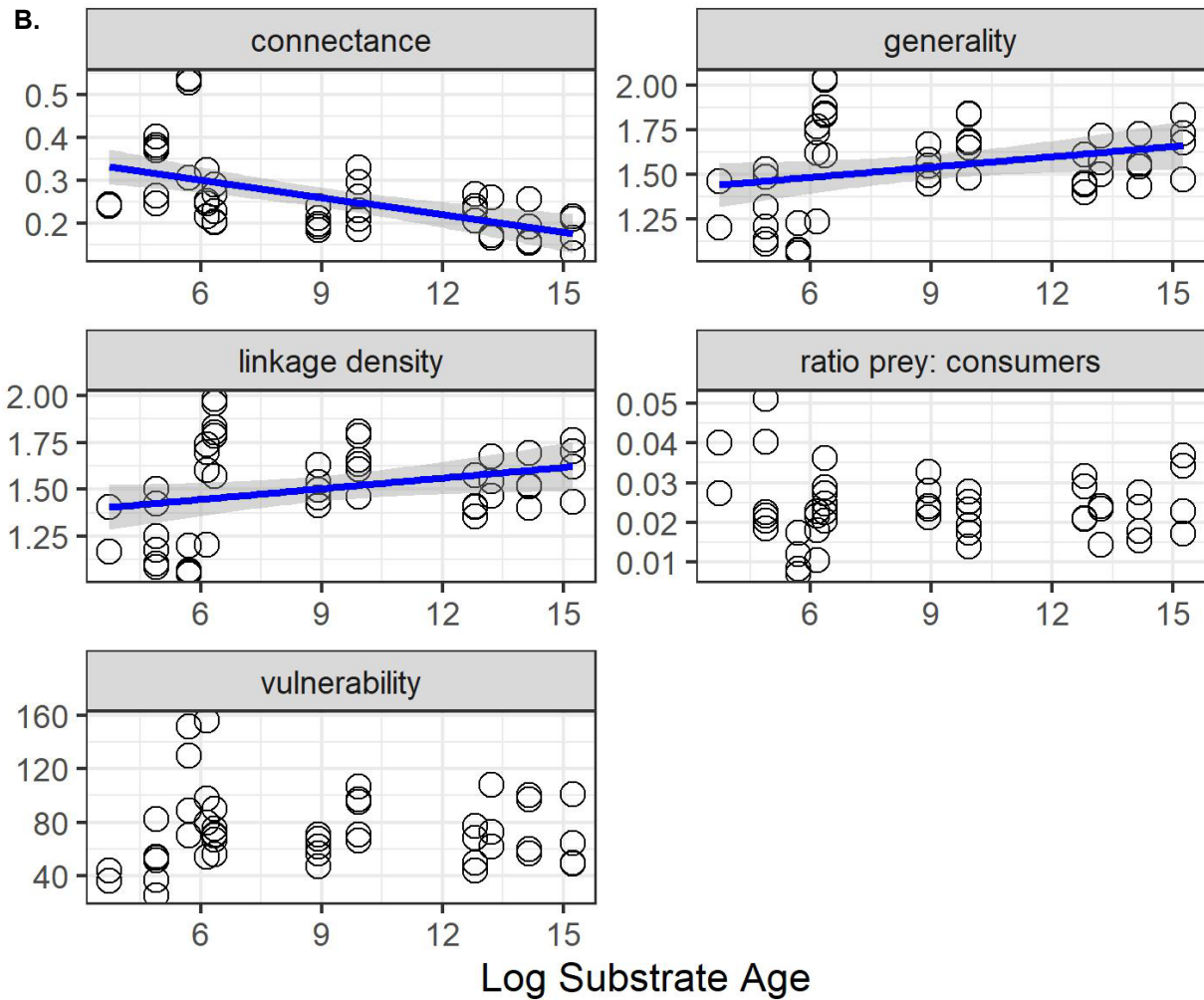




SI Figure 6. Null model analysis for weighted networks. A. Graphs of all 1000 null matrices metric values (gray) and observed values (color) by community age. B. Z-scores from null model analysis of weighted metrics are plotted by community age. There are no null values or z-score for interaction evenness because the null model we used wasn't appropriate given that interaction evenness is based on the empirical distribution of weights, regardless of which species those weights are between.

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SI Figure 7. Plot level. A. Quantitative and B. Qualitative metrics shown for matrices of arthropod-plant interactions at each plot within a site. In the main text, one network was created per site 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 < 4 plant species were sampled within the plot radius and networks would be small. The results from plot level analysis are consistent with the site level data. The variance among plots at the same sites is minimal. These results help corroborate the trend of increasing specialization over time.

SI Table 1. Site information. Information for the sampling plots within each age community. Six plots were sampled in fourteen sites across the island. Plots with greater or less than 1 standard deviation of sample beating time were dropped from analysis. This left a total of 11 sites and 50 plots (highlighted).

Island	Site	Plot	Elev m	AAP m	Lat	Long	Flow age min	Flow age max	Flow age mean
Hawaii	flow1973	flow1973_079	975	2661	19.37	-155.21	42	42	42
Hawaii	flow1973	flow1973_109	975	2613	19.37	-155.22	42	42	42
Hawaii	flow1973	flow1973_084	975	2756	19.37	-155.21	42	42	42
Hawaii	flow1973	flow1973_081	975	2661	19.37	-155.22	42	42	42
Hawaii	flow1973	flow1973_107	975	2570	19.37	-155.22	42	42	42
Hawaii	flow1973	flow1973_110	975	2657	19.37	-155.21	42	42	42
Hawaii	treePlanting	treePlanting_01	1209	4515	19.66	-155.28	133	133	133
Hawaii	treePlanting	treePlanting_03	1204	4495	19.66	-155.28	133	133	133
Hawaii	treePlanting	treePlanting_04	1196	4719	19.66	-155.28	133	133	133
Hawaii	treePlanting	treePlanting_05	1198	4700	19.66	-155.28	133	133	133
Hawaii	treePlanting	treePlanting_02	1198	4515	19.66	-155.28	133	133	133
Hawaii	treePlanting	treePlanting_06	1192	4700	19.66	-155.28	133	133	133
Hawaii	escape	escape_02	997	2658	19.38	-155.22	200	400	300
Hawaii	escape	escape_04	1012	2565	19.37	-155.22	200	400	300
Hawaii	escape	escape_06	1007	2610	19.37	-155.22	200	400	300
Hawaii	escape	escape_87	1004	2748	19.38	-155.22	200	400	300
Hawaii	escape	escape_90	1013	2647	19.38	-155.22	200	400	300
Hawaii	escape	escape_89	1003	2748	19.38	-155.21	200	400	300
Hawaii	kaiholenaYng	kaiholenaYng_02	966	2888	19.18	-155.58	200	750	475
Hawaii	kaiholenaYng	kaiholenaYng_08	1003	2947	19.19	-155.59	200	750	475
Hawaii	kaiholenaYng	kaiholenaYng_09	1045	2937	19.19	-155.59	200	750	475
Hawaii	kaiholenaYng	kaiholenaYng_04	1012	2909	19.19	-155.59	200	750	475
Hawaii	kaiholenaYng	kaiholenaYng_10	1029	2932	19.19	-155.59	200	750	475
Hawaii	kaiholenaYng	kaiholenaYng_11	1045	2926	19.19	-155.59	200	750	475
Hawaii	thurston	thurston_01	1177	2610	19.41	-155.24	400	750	575
Hawaii	thurston	thurston_04	1181	2880	19.41	-155.24	400	750	575
Hawaii	thurston	thurston_05	1189	2838	19.41	-155.24	400	750	575
Hawaii	thurston	thurston_06	1195	2709	19.42	-155.24	400	750	575
Hawaii	thurston	thurston_08	1195	2797	19.42	-155.24	400	750	575
Hawaii	thurston	thurston_12	1189	2850	19.42	-155.24	400	750	575
Hawaii	olaa	olaa_01	1170	3011	19.45	-155.25	5000	10000	7500
Hawaii	olaa	olaa_03	1169	3071	19.45	-155.25	5000	10000	7500
Hawaii	olaa	olaa_06	1177	2957	19.45	-155.25	5000	10000	7500
Hawaii	olaa	olaa_12	1183	2908	19.46	-155.25	5000	10000	7500

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Hawaii	olaa	olaa_10	1183	2915	19.45	-155.25	5000	10000	7500
Hawaii	hippnet	hippnet_05	1131	3538	19.93	-155.28	4000	14000	9000
Hawaii	hippnet	hippnet_06	1152	3506	19.93	-155.29	4000	14000	9000
Hawaii	hippnet	hippnet_08	1126	3654	19.93	-155.28	4000	14000	9000
Hawaii	hippnet	hippnet_09	1212	3318	19.93	-155.29	4000	14000	9000
Hawaii	hippnet	hippnet_11	1231	3295	19.93	-155.29	4000	14000	9000
Hawaii	hippnet	hippnet_13	1177	3479	19.93	-155.29	4000	14000	9000
Hawaii	alili	alili_01	917	2460	19.23	-155.51	11000	30000	20500
Hawaii	alili	alili_04	960	2573	19.23	-155.52	11000	30000	20500
Hawaii	alili	alili_05	917	2504	19.23	-155.52	11000	30000	20500
Hawaii	alili	alili_06	942	2541	19.23	-155.51	11000	30000	20500
Hawaii	alili	alili_14	969	2597	19.23	-155.52	11000	30000	20500
Hawaii	alili	alili_15	985	2583	19.23	-155.52	11000	30000	20500
Hawaii	laupLSAG	laupLSAG_01	1102	3433	19.94	-155.3	14000	65000	39500
Hawaii	laupLSAG	laupLSAG_04	1158	3235	19.94	-155.3	14000	65000	39500
Hawaii	laupLSAG	laupLSAG_07	1155	3235	19.93	-155.3	14000	65000	39500
Hawaii	laupLSAG	laupLSAG_10	1181	3291	19.93	-155.3	14000	65000	39500
Hawaii	laupLSAG	laupLSAG_12	1251	3080	19.93	-155.3	14000	65000	39500
Hawaii	laupLSAG	laupLSAG_15	1242	3112	19.93	-155.3	14000	65000	39500
Hawaii	kohalaYng	kohalaYng_03	1285	2779	20.11	-155.74	120000	230000	175000
Hawaii	kohalaYng	kohalaYng_04	1303	2863	20.11	-155.74	120000	230000	175000
Hawaii	kohalaYng	kohalaYng_07	1274	3004	20.11	-155.74	120000	230000	175000
Hawaii	kohalaYng	kohalaYng_08	1266	3177	20.11	-155.73	120000	230000	175000
Hawaii	kohalaYng	kohalaYng_10	1305	2779	20.11	-155.74	120000	230000	175000
Hawaii	kohalaYng	kohalaYng_20	1281	2918	20.11	-155.74	120000	230000	175000
Hawaii	kohalaOld	kohalaOld_01	1294	3568	20.11	-155.72	230000	500000	365000
Hawaii	kohalaOld	kohalaOld_03	1273	3704	20.11	-155.72	230000	500000	365000
Hawaii	kohalaOld	kohalaOld_08	1313	3402	20.11	-155.73	230000	500000	365000
Hawaii	kohalaOld	kohalaOld_05	1318	3501	20.11	-155.72	230000	500000	365000
Hawaii	kohalaOld	kohalaOld_06	1309	3501	20.11	-155.72	230000	500000	365000
Hawaii	kohalaOld	kohalaOld_07	1335	3419	20.1	-155.72	230000	500000	365000
Maui	waikamoi	waikamoi_07	1591	2518	20.8	-156.25	140000	950000	545000
Maui	waikamoi	waikamoi_16	1545	3040	20.8	-156.24	140000	950000	545000
Maui	waikamoi	waikamoi_36	1564	2765	20.8	-156.25	140000	950000	545000
Maui	waikamoi	waikamoi_06	1547	2834	20.8	-156.25	140000	950000	545000
Maui	waikamoi	waikamoi_18	1552	3107	20.8	-156.24	140000	950000	545000
Maui	waikamoi	waikamoi_32	1524	2598	20.8	-156.25	140000	950000	545000
Molokai	kamakou	kamakou_07	1191	2718	21.11	-156.9	1300000	1500000	1400000
Molokai	kamakou	kamakou_09	1223	3007	21.11	-156.9	1300000	1500000	1400000
Molokai	kamakou	kamakou_17	1213	3007	21.11	-156.89	1300000	1500000	1400000
Molokai	kamakou	kamakou_39	1182	2800	21.11	-156.89	1300000	1500000	1400000

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Molokai	kamakou	kamakou_15	1245	3276	21.11	-156.89	1300000	1500000	1400000
Molokai	kamakou	kamakou_41	1159	2532	21.11	-156.9	1300000	1500000	1400000
Kauai	kokee	kokee_16	1116	2251	22.14	-159.62	4000000	4300000	4150000
Kauai	kokee	kokee_21	1200	2860	22.15	-159.62	4000000	4300000	4150000
Kauai	kokee	kokee_23	1210	2877	22.15	-159.61	4000000	4300000	4150000
Kauai	kokee	kokee_11	1147	2487	22.14	-159.62	4000000	4300000	4150000
Kauai	kokee	kokee_09	1172	2413	22.14	-159.62	4000000	4300000	4150000
Kauai	kokee	kokee_22	1202	2724	22.15	-159.62	4000000	4300000	4150000

SI Table 2. Summary statistics from lidar data during site selection. Here we show the basic forest structure characteristics for each of the sites that was characterized as a possible area for arthropod community assessment, The highlighted sites were included for arthropod community assessment using DNA metabarcoding. The sites with NA we did not have lidar data availability.

Site	MEAN	MEDIAN	MIN	MAX	SD	Q40	Kurtosis	KurtSE	Skewness	SkewSE
HAVO_EscapeRd_1973	0.530168	0.563037	0.0099	1.364975	0.274771	0.631655	-0.61762	0.271608	0.044372	0.137784
Tree Planting Road	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
HAVO_EscapeRd_HighStature	7.21131	7.457712	1.78265	11.91813	1.905806	7.93115	-0.25263	0.14969	-0.42313	0.075164
TNC_Kaiholena_Young	6.254615	5.85335	3.378	13.40897	1.664489	6.35712	1.333989	0.154296	1.105489	0.077498
TNC_Kaiholena_Old	5.928797	5.7883	3.774975	11.6454	1.070454	5.99883	3.697232	0.180073	1.37507	0.090596
HAVO_Thurston	12.37064	12.76153	4.408525	16.77472	2.261814	13.35568	-0.1841	0.141771	-0.61303	0.071156
HAVO_Olaa_Old	7.830925	7.329275	4.076075	18.0546	2.272184	7.9105	0.922662	0.093658	1.041491	0.046906
NAR_Laupahoehoe_HIPPNET	14.05824	13.8389	0.030125	25.2793	3.653928	14.90676	-0.47155	0.077027	0.153058	0.038557
KauFR_Alili	11.86238	11.4214	2.585675	21.81295	3.492847	12.57993	-0.73541	0.118676	0.311218	0.059496
HiloFR_Laupahoehoe_Old	13.94185	13.91757	4.52675	25.16357	3.045798	14.75297	-0.37097	0.096224	0.125453	0.048196
NAR_Laupahoehoe_65-250K	12.23722	13.00572	0.1083	23.588	4.243211	13.84491	-0.04895	0.061824	-0.66387	0.030934
HiloFR_Humuula	15.33543	15.18775	2.23905	27.6691	3.801326	16.22402	-0.40367	0.056136	0.17102	0.028085
NAR_PuuMakaala_Young	5.951452	5.722412	0.14485	14.82202	2.678494	6.496805	-0.37364	0.091493	0.30729	0.045819
NAR_PuuMakaala_Old	14.71206	15.72586	0	25.28247	5.365967	16.84449	1.253179	0.123676	-1.16721	0.062017
NAR_PuuOumi_Young02	5.951452	5.722412	0.14485	14.82202	2.678494	6.496805	-0.37364	0.091493	0.30729	0.045819
NAR_PuuOumi_Old	5.999913	6.243275	0.3082	10.97857	1.918523	6.687105	-0.25813	0.10721	-0.3717	0.053721
Kamakou Molokai	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Waikamoi Maui	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kauai 8m	5.248667	5.121489	0.010311	19.3347	3.227693	6.003569	-0.26206	0.036622	0.38	0.018316

SI Table 3. Taxonomic coverage. Taxonomic coverage of arthropod OTUs as determined by percent match to GenBank accessions and a 57 species reference collection. Taxonomy of plants as determined by morphological identification in the field. Plants that were unidentified were grouped by morphotaxa. The bipartite web id is the 5-letter code to match the bipartite plots in Figure 3 and SI Figure 3.

Arthropod Order or Plant Genus/Morphotaxon	otus	species	genus	families	bipartite web id
<i>arthropod molecular taxonomy</i>					
Amphipoda	29		1	1	Amphi
Araneae	539	58	9	8	Arane
Blattodea	12	2	1		Blatt
Coleoptera	421	18	4	12	Coleo
Diptera	454	27	8	19	Dipte
Entomobryomorpha	295	8	3	1	Entom
Geophilomorpha	9				Geoph
Hemiptera	774	37	11	9	Hemip
Hymenoptera	98	12	3	5	Hymen
Isopoda	59	2	2	1	Isopo
Julida	4	3		1	Julid
Lepidoptera	290	18	16	15	Lepid
Lithobiomorpha	1				Litho
Mesostigmata	9	1			Mesos
Neuroptera	30	2	1	2	Neuro
Orthoptera	197	4	2	2	Ortho
Poduromorpha	7		1	1	Podur
Polydesmida	1	1			Polyd
Polyxenida	1				Polyx
Pseudoscorpiones	4				Pseud
Psocoptera	266	4	1		Psoco
Sarcoptiformes	262	1	2		Sarco
Spirobolida	1				Spiro
Spirostreptida	4				Spiro
Symphyleona	4	1	1		Symph
Trombidiformes	14	1		1	Tromb
<i>plant morphological taxonomy</i>					
Metrosideros					Metro
Styphelia					Styph
Vaccinium					Vacci

MOLECULAR ECOLOGY

Swordfern	Sword
Coprosma	Copro
Dubautia	Dubau
Dicranopteris	Dicra
Groundfern	Groun
Myrsine	Myrsi
Pipturus	Piptu
Ilex	Ilex
Broussaisia	Brous
BrownCibotium	Brown
GreenCibotium	Green
Sadleria	Sadle
Cheirodendron	Cheir
Psychotria	Psych
Astelia	Astel
Machaerina	Macha
Melicope	Melic
Perrottetia	Perro
Cyrtandra	Cyrta
Dryopteris	Dryop
Freycinetia	Freyc
Hedyotis	Hedyo
Alyxia	Alyxi
Rubus	Rubus
Clermontia	Clerm
Carex	Carex
Kadua	Kadua
Labordia	Labor
Curleaf	Curll
whitemid	white
blackvelvet	black

SI Table 4. Spearman's correlation tests. Spearman's correlation tests were used to determine significance for the: A) regression against log substrate age (community age) of qualitative network metric values calculated from binary matrices at each site. B) regression against number of nodes (community size) of quantitative network metric values calculated from weighted matrices at each site C) regression against number of nodes (community size) of qualitative network metric values calculated from binary matrices at each site and D) regression of size of network (number of nodes, number of links) by log flow age (community age).

	S	p-value	Spearman's rho
<i>Qualitative (binary) x community age</i>			
linkage density	78	0.037	0.65
connectance	404	0.003	-0.84
generality	78	0.037	0.65
vulnerability	188	0.670	0.15
ratio prey:consumers	132	0.225	0.40
<i>Quantitative (weighted) x number of nodes</i>			
linkage density	140	0.273	0.36
weighted connectance	226	0.946	-0.03
generality	68	0.023	0.69
vulnerability	144	0.300	0.35
interaction evenness	134	0.237	0.39
index of specialization H ₂ '	218	0.989	0.01
<i>Qualitative (binary) x number of nodes</i>			
linkage density	34	0.002	0.85
connectance	396	0.005	-0.80
generality	34	0.002	0.61
vulnerability	86	0.051	0.61
ratio prey:consumers	202	0.818	0.08
<i>number of nodes x log flow age</i>	3741	0.000	0.74
<i>number of links x log flow age</i>	3096	0.000	0.78

SI Table 5. p-value table weighted metrics null model analysis. The network metrics of 1000 null matrices were compared to the network metrics of the empirical matrix. The null hypothesis is said to be true if the empirical values are within the null model distribution. This is a two tailed distribution. A "1" indicates that every null model value that we compared to our observed was greater than or equal to the observed value. A "0" indicates that no null model value that we compared to our observed was greater than or equal to the observed value. A value of greater than 0 and less than 1 is simply the number values that the null metric was greater than the empirical metric.

site	pval.Connectance	pval.LD	pval.Gen	pval.Vul	pval.H2
flow1973	0.001	0.001	0	0.006	1
treePlanting	0.518	0.518	0	0.553	1
escape	0.283	0.283	0.002	0.293	1
kaiholenaYng	0.571	0.571	0	0.96	1
thurston	0.031	0.031	0	0.225	1
olaa	0.492	0.492	0	0.645	1
alili	0.596	0.596	0	0.749	1
kohalaOld	0.653	0.653	0	0.825	1
waikamoi	0	0	0	0	1
kamakou	0.889	0.889	0	0.973	1
kokee	0	0	0	0	1