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# Ecosystem consequences of plant genetic divergence with colonization of new habitat

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**Abstract.** When plants colonize new habitats altered by natural or anthropogenic disturbances, those individuals may encounter biotic and abiotic conditions novel to the species, which can cause plant functional trait divergence. Over time, site-driven adaptation can give rise to population-level genetic variation, with consequences for plant community dynamics and ecosystem processes. We used a series of 3000-yr-old, lava-created forest fragments on the Island of Hawai`i to examine whether disturbance and subsequent colonization can lead to genetically differentiated populations, and where differentiation occurs, if there are ecosystem consequences of trait-driven changes. These fragments are dominated by a single tree species, *Metrosideros polymorpha* (Myrtaceae) or ʻōhiʻa, which have been actively colonizing the surrounding lava flow created in 1858. To test our ideas about differentiation of genetically determined traits, we (1) created rooted cuttings of ʻōhiʻa individuals sampled from fragment interiors and open lava sites, raised these individuals in a greenhouse, and then used these cuttings to create a common garden where plant growth was monitored for three years; and (2) assessed genetic variation and made $Q_{ST}/F_{ST}$ comparisons using microsatellite repeat markers. Results from the greenhouse showed quantitative trait divergence in plant height and pubescence across plants sampled from fragment interior and matrix sites. Results from the subsequent common garden study confirmed that the matrix environment can select for individuals with 9.1% less shoot production and 17.3% higher leaf pubescence. We found no difference in molecular genetic variation indicating gene flow among the populations. The strongest $Q_{ST}$ level was greater than the $F_{ST}$ estimate, indicating sympatric genetic divergence in growth traits. Tree height was correlated with ecosystem properties such as soil carbon and nitrogen storage, soil carbon turnover rates, and soil phosphatase activity, indicating that selection for growth traits will influence structure, function, and dynamics of developing ecosystems. These data show that divergence can occur on centennial timescales of early colonization.

**Key words:** colonization; divergence; functional plant traits; kīpuka; *Metrosideros polymorpha;* selection.

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## Introduction

The movement of plant species into new habitats can have important evolutionary and ecological consequences. For example, when species colonize new created habitats, as a result of natural or anthropogenic disturbances, they may encounter novel biotic and abiotic factors that can (1) affect their success or failure (Hobbs et al. 2006); (2) influence the evolutionary dynamics of populations (Holt 2003, Parmesan 2006); and (3) alter functional phenotypes (Whitham et al. 2006, Bailey et al. 2014, Read et al. 2014, Schweitzer et al. 2014, Kinnison et al. 2015). To better predict the consequences of colonization of new habitats from the influences of natural disturbance and global environmental change, understanding how selection operates during colonization is critical to correctly interpreting ecosystem consequences of phenotypic divergence.

The evolution of colonizing plant species has been examined in the context of local adaptation (Clausen et al. 1947), coevolution (Carroll et al. 2005), and exotic species invasions (Zenni et al. 2014*a*). It has become apparent that individuals colonizing unique ecotypes are phenotypically different from those occurring in source populations within the previous core range of the species (Phillips et al. 2006, Eckert et al. 2008, Felker-Quinn et al. 2013). For example, invasive pine trees in Brazil had distinct phenotypes in colonized vs. source portions of their range (Zenni et al. 2014*b*), suggesting that selective pressures during colonization favored certain traits. Such effects clearly have important implications for managing invasive species, but beyond the study of invasive species (see reviews by Buswell et al. 2011, Felker-Quinn et al. 2013, Moran and Alexander 2014), surprisingly few studies have examined the evolutionary consequences of colonization by native species (Foster et al. 2007, Schwarzer et al. 2013, Hargreaves et al. 2014). This knowledge gap is notable because such colonization events are extensive and arguably the most important process in primary and secondary succession, as well as recovery of the Earth's degraded landscapes (Sarrazin and Lecomte 2016).

Invasion biology provides important empirical and theoretical analogs for understanding the evolutionary consequences of range shifts and the colonization of new habitat. Dispersal and colonization are most often considered demographic processes driven by propagule pressure, frequently modeled on simulated landscapes assumed to be homogenous (Travis et al. 2009, Burton et al. 2010). Because few studies have examined native species dynamics, we know much less about how altered environments drive selection for specific traits and contribute to species persistence in newly created habitats (Jump and Peñuelas 2005, Hargreaves et al. 2014). Evidence of rapid evolution in plants is widespread (Jump and Peñuelas 2005, Lau 2008, Strauss et al. 2008, Buswell et al. 2011, Felker-Quinn et al. 2013), suggesting that successful colonization of novel habitats may result in trait selection and genetically based functional trait shifts relative to the source population.

After the introduction of cane toads in Australia, subsequent colonization of uninvaded habitat led to the evolution of increased dispersal ability along the front of the invasion (Phillips et al. 2006). In the previously described Brazilian system, Zenni et al. (2014*a*, *b*) demonstrated rapid evolution of non-native pine trees that escaped silviculture plantations and colonized into native Brazilian ecosystems. These examples point to wide-ranging capacity for rapid evolution at the colonizing front, and the rapid evolutionary changes that accompany some invasions can have consequences that alter ecosystems. With the invasive tallow tree (*Sapium sebifera*), new populations of invasive individuals have evolved lower foliar tannins in the invaded range, altering plant herbivore interactions and chemical inputs to soils (Siemann and Rogers 2003). It is changes in traits such as these that can have large subsequent consequences for ecosystem processes.

Plants possess traits that structure belowground communities and influence ecosystem dynamics (Vitousek et al. 1987, Ohtonen et al. 1999, Bardgett and Wardle 2010, Cutler et al. 2014). For example, Ohtonen et al. (1999) found that different plant species colonizing a bare soil drastically changed the belowground microbial community. Many studies have shown how invasive species can alter their surroundings by disrupting mycorrhizal relationships (Wolfe et al. 2008), changing soil chemistry (Vitousek et al. 1987, Gómez-Aparicio and Canham 2008), and altering carbon uptake and pool size (see review

by Peltzer et al. 2010). Aboveground traits, such as leaf lignin concentrations, can influence belowground nutrient cycles by altering decomposition rates and mineralization of organic matter (Melillo et al. 1982) including within species (Hobbie et al. 2006). Furthermore, intraspecific variation in plant functional traits can alter ecosystem processes (Whitham et al. 2006). For example, leaf litterfall and subsequent decomposition rates were dependent on variation in leaf source within the species *Alnus rubra* (Jackrel and Wootton 2014). Thus, when colonizing trees enter new locations, their traits can alter belowground ecosystem processes differently than expected from plants growing in established portions of the range (Wardle et al. 2004, Bardgett and Wardle 2010).

*Metrosideros polymorpha* is considered a foundation species because it is one of the few large trees native to the Hawaiian archipelago and plays an important role in early colonization of lava (Percy et al. 2008, Flaspohler et al. 2010). *Metrosideros polymorpha* is known to have high genetic and phenotypic variation and respond strongly to environmental gradients, such as those that occur across elevation or substrate age (Vitousek 2004, Morrison and Stacy 2014, Stacy et al. 2014). Additionally, genetically based phenotypic variation in *M. polymorpha* has been shown to influence litter decomposition rates and soil nutrient dynamics (Vitousek 2004). The ability to respond to strong environmental gradients and known links between genetically based traits and ecosystem function makes *M. polymorpha* an ideal focal plant species for the study of the evolutionary and ecological consequences of colonization (Cordell et al. 1998, Treseder and Vitousek 2001, Martin et al. 2007). To understand the evolutionary and ecological consequences of colonization, we examined how adjacent populations of *M. polymorpha* varied in functional plant traits and ecosystem processes along a colonization front and strong edaphic and environmental variation in Hawai`i. Using a field and common garden approach, we tested two related hypotheses: (1) Functional plant traits within *M. polymorpha* have diverged in newly colonized sites relative to source populations resulting in differential establishment and growth and (2) variation in traits in *M. polymorpha* in newly colonized sites results in changes to soil processes.

## Methods

### *Study system and sites*

The Island of Hawai`i is an ideal location to test the consequences of plant evolution on contemporary timescales. A well-constrained post-volcanic colonization front allows examination of how plant traits differ in newly colonized areas and how these traits influence soil nutrient dynamics (sensu Treseder and Vitousek 2001). In 1854–1855, the Mauna Loa volcano erupted, resulting in lava flows that fragmented forests on its eastern face (19.67° N, −155.3° E). More than 1000 fragments ("kīpuka" in Hawai`ian) were created by the eruption, and range in size from 0.01 to over 100 ha, with large abiotic and biotic environmental differences between the bare substrate of the matrix and the well-developed, 3000- to 5000-yr substrates of the kīpuka (Raich et al. 1997, Flaspohler et al. 2010, Vaughn et al. 2014, Vannette et al. 2016). These forest fragments persist because primary succession onto new lava is slow—resulting in continuing colonization of the matrix.

The kīpuka–matrix comparison is an ideal field system for studying colonization as an evolutionary process. It is a simple and uniform flora dominated by a single canopy tree species (*M. polymorpha*), which comprises >85% of the basal area across kīpuka as well as nearly all saplings in the lava flow matrix (Flaspohler et al. 2010, Vaughn et al. 2014). The kīpuka and the lava flow matrix are adjacent to one another, thus making these sites ideal for understanding selection of plant functional phenotypes. All kīpuka and matrix sites in this study occur within a narrow geographic area between 1509 and 1637 m above sea level, and so share similar annual ambient temperatures (14.0–16.5°C) and precipitation (2400–2900 mm; Western and Juvik 1983, Vaughn et al. 2014), with lava age in this field system not correlated with temperature or rainfall (Tsujii et al. 2016). Lastly, the primary succession of trees onto barren basalt lava flows allows for an examination of the direct effects of plants on organic matter formation and associated soils.

### *Functional plant traits*

To determine whether there is variation in functional plant traits and the ecosystem processes they mediate for trees in the colonizing matrix and kīpuka populations, 14 kīpuka sites

surrounded by the adjacent matrix were selected along the Mauna Loa 1855 lava flow. Within each kīpuka site, and also nearby in the surrounding matrix, 10 randomly selected individual trees were sampled and measured in the field. Specific leaf area (SLA), leaf pubescence, and tree height were measured on all trees in the field. Dry leaf mass was determined by oven drying samples at 70°C for 48 h before weighing. Specific leaf area of all kīpuka and matrix trees was determined by calculating the surface area and the mass of three to five leaves per individual (collected from terminal shoots from multiple locations on a tree). Leaf pubescence was estimated in the field with a standardized scale (1–5) by which glabrous leaves were given a score of 1 and the most pubescent leaves were scored 5. Tree heights were estimated to the closest half meter.

### *Experimental common garden*

To determine whether phenotypic differences observed in the field were a plastic response to the environment or resulted from genetic divergence, we established a common garden with rooted cuttings to separate the genetic and environmental components of functional plant traits that vary in response to underlying substrate properties of the kīpuka/lava matrix system (Anderson et al. 2014). Distinct *M. polymorpha* phenotypes were collected from kīpuka and the surrounding lava matrix in June 2012. These cuttings were taken from the same kīpuka sites as the field measurements. However, cuttings from trees on the lava matrix were sampled along a transect spanning the elevation of the kīpuka study site (1509–1637 m above sea level), and located between kīpuka (therefore kīpuka and lava cuttings were not paired). Ten 15-cm terminal cuttings from 110 individuals on the lava matrix and 108 individuals in kīpuka were collected in the field and kept moist and cool until planting. Tree cuttings were collected from terminal branch tips, and there was no significant difference in cutting diameter between sites or soil substrates (data not shown). Cuttings were scored with pruning shears, and dipped in Hormodin (indole-3-butyric acid; Hormodin 2; OHP, Mainland, Pennsylvania, USA). The lower leaves were removed and remaining leaves were cut in half (to reduce water loss), inserted into 1.5-L pots with a standard potting mix (equal parts of peat, perlite, and vermiculite), and placed under a misting bench (misted every 20 min during the day) in a greenhouse facility at the Institute of Pacific Islands Forestry in Hilo, Hawaii. To decrease any potential variation in stored nutrients in the cut branches, successfully rooted cuttings were replanted in the same potting mix plus an addition of 3 g of 13:13:13 nitrogen/phosphorus/potassium slow-release fertilizer pellets one year prior to measuring traits. The greenhouse trees were randomized into four blocks and rotated periodically to avoid any positional environmental effects. In June of 2013, stem diameter and length, SLA, and leaf pubescence were measured on the new growth. In June of 2014, tree height was measured as the trees were being planted into a common garden in the field at the Institute of Pacific Island Forestry's Laupāhoehoe Science and Education Center (LSEC, Laupāhoehoe, Hawaii, USA) and measured again in 2015. To determine whether underlying genetic variation was responsible for differences in tree height, only the individuals who survived to the 2015 measurement were used in the analysis of the 2014 height data. The common garden was designated into four random blocks on a site where slope varies from 2° to 15°. Trees were spaced three meters from each other in a grid and each grid was surrounded by an outer row of edge trees. Within each block, a single replicate of each genotype was planted at a random location with ~10 g of NPK 20:20:20 fertilizer in each tree hole.

### *Plant molecular analyses*

To examine genetic structure and gene flow between kīpuka and matrix trees in this system, we used 11 microsatellite markers targeted at repeat regions of the genome (Crawford et al. 2008). Leaves from 168 tree genotypes from the common garden were successfully extracted and genotyped (74 matrix and 94 kīpuka genotypes). Powdered samples of leaf tissue were used to extract genomic DNA (gDNA). Tissues were ground to a fine powder using a ball mill (Spex mixer/mill 8000D; Spex Sample Prep, Metuchen, New Jersey, USA). Approximately 0.2 g of leaf powder was used to extract gDNA with the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions, except the first incubation step was conducted overnight (a minimum of 12 h). Although gDNA yields were low (some <5 ng/μL),

samples were diluted 1/10 (one part gDNA into nine parts molecular grade water) to minimize the effects of polymerase chain reaction (PCR) inhibitors for downstream reactions. We generated multi-locus genotypes for each sample using 11 presumably neutral microsatellite markers that were selected from Crawford et al. (2008; Appendix S1: Table S1). All PCRs were carried out in 10 μL volumes containing the following reagents (given in final concentrations): 1–5 ng of DNA template, 1× PCR buffer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 0.1 U Platinum *Taq* polymerase (Invitrogen, Carlsbad, California, USA), and 0.4 μmol/L of each primer, except one marker (MePo514), which was changed to 0.1 μmol/L. A final concentration of 0.5 μg/μL bovine serum albumin (BSA) was added to increase target specificity and PCR yield for four markers (MePo504, MePo507, MePo509, and MePo510). PCRs were thermocycled according to the following conditions: 10 min at 95°C to release the Platinum *Taq* antibody, followed by 40 cycles of 60 s at 94°C, 30 s at the annealing temperature ($T_a$), and 30 s at 72°C. The $T_a$, mixing strategy (multiplex vs. singleplex loci and/or pooling scheme), dilution, and forward primer dye for each locus are provided in Appendix S1: Table S1. Diluted PCR products were electrophoresed on an ABI3130 sequencer with LIZ-1200 size standard and analyzed using the software GENEMAPPER v4.0 (Applied Biosystems, Foster City, California, USA). All genotypes were manually checked for accuracy, and positive controls were included on all runs. We did not observe amplification in our negative control reactions (water as template). We also ran independent PCR replicates on 10% of the trees to check for genotyping errors, and no errors were observed.

### *Soil processes*

To determine the variation in soils, we sampled along the constrained colonization sites where macro-environmental conditions are similar (Flaspohler et al. 2010, Tsujii et al. 2016). Soils were collected to a depth up to 20 cm under each of the *M. polymorpha* trees in the kīpuka and matrix that were measured for field traits. Soils were collected within 0.25 m from the trunk, placed in plastic bags, and stored on ice in a cooler until the end of a day in the field when soils were transferred to a 4°C refrigerator. These soils were almost entirely organic matter, especially in the matrix sites, where soil collection involved scraping a layer of organic material off of basalt bedrock (see Appendix S1: Fig. S1 for detailed explanation and photos of the sites). Samples were shipped on dry ice by two-day mail to a laboratory at the University of Tennessee where they were stored at 4°C until processed the following day. Processing included sieving soils through a 4-mm mesh and dividing samples for multiple analyses. Soil subsamples were used for fluorometric enzyme assays, soil gravimetric water content, pH, total soil carbon (C) and nitrogen (N), and laboratory incubations to assess C decomposition rates.

Soils were assayed for activities of the following enzymes: β-1,4-glucosidase (EC 0.2.1.21), α-1, 4-glucosidase (EC 3.2.1.20), β-1,4-*N*-acetylglucosaminidase (EC 3.1.6.1), acid phosphatase (EC 3.1.3.2), phenol oxidase (EC 1.10.3.2), and peroxidases (EC 1.11.1.7; Stritar et al. 2010). For these analyses, 1 g of each soil was diluted with 125 mL of 50 μmol/L sodium acetate buffer (pH = 5) and mixed on a stir plate for 2 min, thoroughly suspending the soil in buffer. Enzyme assays were undertaken with eight analytical replicates for β-glucosidase, α-glucosidase, β-1, 4-*N*-acetylglucosaminidase, and phosphatase. Phenol oxidase/peroxidases activities were measured with 16 analytic replicates. The β-glucosidase, α-glucosidase, *N*-acetylglucosaminidase, and phosphatase activities were determined by fluorometric response of the 4-methylumbelliferyl (MUB) substrate (excitation at 365 nm, emission at 450 nm, on a BioTek Synergy HT microplate reader; BioTek, Winooski, Vermont, USA). Phenol oxidase/peroxidase activities were determined by colorimetric analysis of the L-3,4-dihydroxyphenylalanine substrate (DOPA; Absorbance at 460 nm, SpectraMax Plus$^{384}$ spectrophotometer; Molecular Devices Corp., Sunnyvale, California, USA). *N*-acetylglucosaminidase and phosphatase were incubated for 30 min prior to being read, β-glucosidase and α-glucosidase were incubated for 2 h, while phenol oxidase and peroxidase were incubated for 24 h.

Soil C and N concentrations were determined on finely ground subsamples (mortar and pestle) by dry combustion (Flash EA 1112 CNH analyzer; Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Gravimetric water content was assayed by drying soil samples in a Thermo

Isotherm soil oven at 105°C for 48 h and comparing wet and dry masses. Soil pH was determined using a 0.1 mol/L $CaCl_2$ extraction and measured with a Denver Instruments pH probe and reader (Sartorius AG, Goettingen, Germany).

Laboratory incubations were conducted over a 30-d period to determine differences in soil C use by microorganisms, and to examine variation in soil C decomposition rates between kīpuka and matrix soils under common conditions (following methods in Schweitzer et al. 2004). Each sieved soil sample was split into two 10 g subsamples and placed into 125-mL specimen cups and soils were brought up to field capacity (based on field GWC measurements) with the addition of deionized water. All cups were placed into 0.994-L glass jars also containing ~30 mL of deionized water to maintain humidity. The jars were left sealed to incubate in the dark at 22°C. Carbon decomposition rates (g $CO_2$-C·$g^{-1}$ soil C·$d^{-1}$) were measured by comparing carbon dioxide ($CO_2$) in all jars to a reference at days 1, 2, 4, 8, 14, 22, 27, and 30 by direct injection using an infrared gas analyzer (LI-6400XT; LI-COR, Lincoln, Nebraska, USA). Presented carbon decomposition rates represent 30-d cumulative totals.

### *Statistical analysis*

To address whether functional plant phenotypes in the field varied in response to substrate type, we took a restricted maximum-likelihood approach, predicting height, pubescence, and SLA with substrate location (kīpuka or matrix) as a fixed effect and site as a random effect. Common garden data for these three traits were analyzed separately using a nested analysis of variance with genotype nested within substrate type (kīpuka or matrix). Foliar nitrogen and foliar carbon were also examined as a response to substrate type in the field with a mixed-effect model like the one above. Hypothesis testing on all of these mixed-effect models was done using a likelihood ratio test between full and null models with an alpha value of 0.05. The R statistical package was used for all analyses (R Core Team 2014). The lme4 package (Bates et al. 2015) was used for building statistical models using random effects (varying intercepts).

To understand the potential role of gene flow and natural selection, the common garden quantitative trait differences were compared to microsatellite loci. To determine whether the variation in plant phenotype is due to non-random genetic factors, we compared trait variation ($Q_{ST}$) and genetic variation ($F_{ST}$), calculated by comparing variation among kīpuka and matrix populations to variation within these populations using the following equation:

$$Q_{ST} = \frac{\sigma B}{\sigma B + 2\sigma W}$$

where $\sigma B$ is the variance measured among populations and $\sigma W$ is the variance within populations. A significant difference ($\alpha = 0.05$) in average common garden traits between kīpuka plants and matrix plants would allow us to reject the null hypothesis that all variation observed in the field is due to phenotypic plasticity. The quantitative variation was also calculated for each trait to examine the variance accounted for by kīpuka and matrix substrate ($Q_{ST}$). Secondly, an analysis of molecular variance (AMOVA) was conducted on the 11 microsatellite loci to determine the genetic structuring of the populations. Low variance between populations would suggest that there is little random genetic variance (the null expectation of testing for genetic drift). Comparison of molecular variance at neutral loci ($F_{ST}$) and quantitative trait variance ($Q_{ST}$) can discriminate between selection and drift as the driving evolutionary force (Leinonen et al. 2013). For example, a $Q_{ST}$ value greater than an $F_{ST}$ would suggest that trait variation between populations is higher than would be expected by random processes alone. This would suggest that selection on the measured traits is occurring. Paired with a significant difference in the trait value means, we would be able to infer directional selection on plant traits.

To determine whether soils vary in response to phenotypic differences among plants, we used a mixed-effect model predicting soil C, soil N, soil pH, soil C mineralization with substrate as a fixed effect and site as a random effect. Soil potential enzyme activity data were standardized by soil C before being analyzed between kīpuka and matrix soils like above with a mixed-effect model. All of these soil properties were also compared to tree height using an analysis of covariance, where tree height in the field was a continuous variable, substrate type was a categorical variable, and the soil chemistry data were continuous responses.

## Results

Observations from both the field site and common garden plantings show differences in plant growth traits. Field observations show kīpuka trees were 111.8% taller than matrix trees (Table 1, Fig. 1a). Shoot length, measured in the greenhouse, was consistent with field height observations, with shoot lengths in kīpuka-derived plants 53% greater than matrix-derived plants (Fig. 1b). This pattern was maintained in the common garden where kīpuka trees were 9.2% taller in 2014 (Fig. 1c) and 9.3% taller than matrix trees in 2015 (Fig. 1d). These results highlight a consistent pattern of genetic divergence in plant height between the kīpuka and matrix individuals.

Similarly, in the field, we found that other traits differed between trees growing on the two substrate types. Matrix trees were 28.5% more pubescent (Table 1) than trees in the kīpuka. Greenhouse pubescence on newly emerged leaves was 17.3% higher in the matrix populations. However, leaf pubescence in the field and greenhouse was weakly correlated, suggesting greater plasticity for this trait. Although in situ SLA was observed to be 27.9% greater in kīpuka vs. matrix trees, there were no significant differences observed in the common garden after one year of growth, although there appeared to be a trend in this direction. Overall, our quantitative trait analyses support the hypothesis that colonizing trees have unique growth phenotypes, but other traits may be more plastic.

Table 1. Variation in plant traits in the field and common garden.

| Response | *P*-value | % Change in matrix |
|---|---|---|
| Plant functional traits | | |
| Pubescence | **<0.0001** | 25.8 |
| Height | **<0.0001** | −111.8 |
| Specific leaf area | **<0.0001** | −27.9 |
| Chemistry | | |
| Foliar nitrogen | 0.99 | 0 |
| Foliar carbon | **<0.0001** | 1.6 |
| Soil total nitrogen | **<0.0001** | −18.2 |
| Soil total carbon | **0.0071** | −4.0 |
| Soil pH | **<0.0001** | 7.2 |
| Soil $CO_2$ incubation | **0.0495** | 10.8 |
| β-glucosidase | 0.0941 | 33.8 |
| α-glucosidase | 0.4565 | 33.6 |
| *N*-acetylglucosaminidase | 0.8838 | 0.67 |
| Phosphatase | **0.0192** | 47.3 |
| Phenol oxidase | 0.0603 | 159.4 |
| Plant functional traits | | |
| Pubescence 2013 | **<0.0001** | 17.3 |
| Specific leaf area 2013 | 0.0917 | −2.5 |
| Stem diameter 2013 | 0.1383 | −3.2 |
| Shoot length 2013 | **<0.0001** | −46.9 |
| Height 2014 | **0.0178** | −9.2 |
| Height 2015 | **0.0424** | −9.3 |

*Notes:* Analysis of variance for field observations (Field 2012) and related common garden traits (CG 2013–2015). *P*-values (*P*) are shown along with the percent difference between the traits from trees from the kīpuka and surrounding lava matrix. Negative percent differences represent situations where the trait or response is greater in the kīpuka and positive percent differences represent higher matrix values. Growth in the field was estimated with height, while shoot length was used to estimate growth in the greenhouse. Growth measurements were taken in a common environment in Hawai`i for three consecutive years; 2013 in the greenhouse and 2014 and 2015 in the outplanted common garden. Bolded *P*-values represent significant effects at $\alpha = 0.05$.

Microsatellite data were used to determine whether there was any population genetic structure between the kīpuka and matrix sites. We found no difference in population genetic structure among the kīpuka/matrix pairs ($Phi_{PT} = 0.001$, $P = 0.46$), indicating extensive genetic exchange between the sites (indeed, kīpuka trees likely are the source of matrix populations), and a lack of genetic divergence at neutral loci. Allelic richness was high at many microsatellites (range of 5–49 alleles, mean = 18 alleles per locus) and observed heterozygosity was accordingly high in both kīpuka and matrix populations ($H_O = 0.67$ for both).

As shown above, the kīpuka and matrix populations are genetically indistinguishable at 11 microsatellite loci, suggesting that kīpuka and matrix populations belong to a single interbreeding population. The quantitative trait variance ($Q_{ST}$) for stem growth in 2013 is 0.323, 0.343 for tree height in 2014, and 0.333 for the tree height in 2015, showing high levels of differentiation between the populations (similar to Alberto 2013) that is much greater than the $F_{ST}$ estimate.

### *Soil properties*

There were five key differences in soil properties associated with trees from kīpuka and matrix sites. Because there was no soil before colonization of the matrix (i.e., the matrix is covered by bare basalt; see Appendix S1: Fig. S1), we make the assumption that effects on soil organic matter

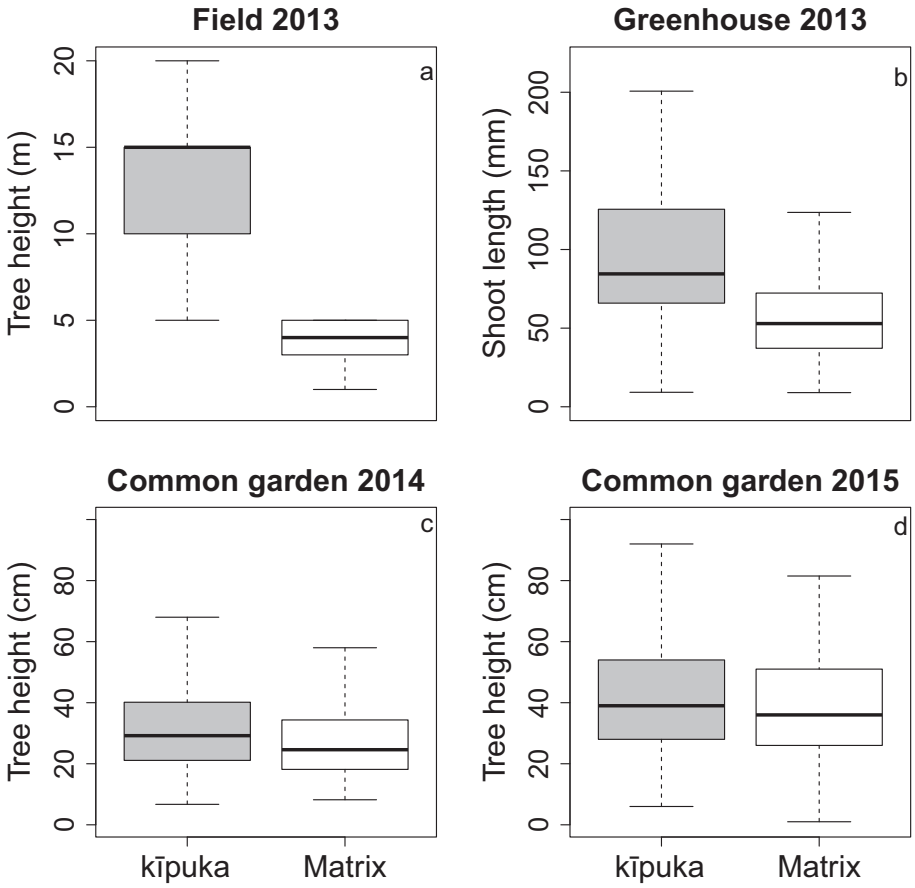


Fig. 1. Colonization results in variation in plant growth traits. In both the field (a) and a common garden experiment across years (b–d), trees that colonized the lava matrix have significantly reduced growth (i.e., shorter total heights in field/common garden and shoot length in the greenhouse). Boxes represent the distribution of the first to third quartiles of the data, while tails represent 95% confidence intervals. Each panel is showing a significant difference between means at an alpha value $\alpha = 0.05$.

in the matrix are due to plant inputs and thus differences in soil properties are due to plants inputs as well as variation in environmental conditions (e.g., temperature, light) that can alter soil microbial activities. Soils collected under trees from kīpuka had higher total soil N (Δ18.2%, Table 1, Fig. 2a) and total soil C (Δ4.0%, Fig. 2b) and a 7.2% lower pH (Fig. 2c). Kīpuka soils were also 10.8% slower at mineralizing C in the laboratory (Fig. 2e) that along with the total soil C data indicate higher C storage in kīpuka soils relative to those in the colonizing matrix. Phosphatase activity, when standardized by total soil C, was 47.3% greater in soils collected from under lava matrix plants (Fig. 2d), likely to utilize the phosphorus (P) bound in the young soils. Gravimetric water content and all other enzyme activities (β-glucosidase, α-glucosidase, *N*-acetylglucosaminidase, and phenol oxidase) were not significantly different between the matrix and kīpuka soils. These data suggest that overall kīpuka and matrix phenotypes and environmental conditions have differential impacts on belowground processes.

The range of phenotypic variation in growth across all field sites is correlated with variation in soil properties. Despite the coarse estimates of plant growth in the field, changes in soil processes based on tree height independent of substrate were also observed (Table 2, Fig. 2f–j), suggesting that tree phenotypes associated with colonization are altering soils. Soil N,

phosphatase activity, and C decomposition rates significantly increased with tree height, but did not vary significantly with location, suggesting that plant traits were more important than site conditions in altering these particular soil variables. Soil pH was the only soil trait measured that was significantly affected by the interaction between tree height and location, increasing as trees grew taller in the matrix, but not changing in the kīpuka.

## Discussion

Our results show that strong edaphic and environmental filters can drive plant genetic divergence and shifts in associated soil processes, despite strong gene flow. Colonization of the new lava matrix substrate resulted in significant genetically based phenotypic changes in functional plant traits, including 9% shorter plants and 17% more pubescence on leaves. A high $Q_{ST}$ value relative to a low $F_{ST}$ for stem growth and height supports the hypothesis that directional selection of colonizing phenotypes is leading to evolution of plant traits on the lava matrix (Storz 2002, Frei et al. 2014). The reduction in aboveground growth in the matrix is correlated with changes in the belowground ecosystem relative to kīpuka trees, leading to significant decreases in total soil C, N, and acidity, whereas phosphorus availability and carbon decomposition increased. Variation in soil chemistry and microbial function along tree size gradients suggests that tree growth has some level of control over belowground communities, likely via the amount of carbon allocated belowground.

The variation in growth (of new shoots) between kīpuka and matrix trees was consistent in a common garden over two years, suggesting a genetic basis to plant height differences. The alternative explanation of different starting conditions is minimized because all cuttings were the same length and basal diameter, and thus began growing with the same starting conditions. Although we cannot eliminate other hormonal maternal effects, the fact that the surviving trees showed little variation across time, when measuring traits on new growth, suggests that this effect is minimal or has been stabilized. If the pattern were driven entirely by unequal starting conditions, and not underlying genetic variation, we would expect the difference in heights to continue to decrease.

Soils are a consistent selective filter on plant populations, always interacting with plants, and potentially having dramatic consequences for their evolution. The most obvious examples are serpentine soils, which are hotspots of plant diversity that often contain more trait variation than surrounding locations (Brady et al. 2005, Harrison et al. 2006). Soil gradients such as those of mine tailings and serpentine soils are strong selective filters along which evolution of distinct plant phenotypes occurs. The difficulty of growing in toxic soils not only leads to novel traits (Brady et al. 2005), but also alters the rates of future evolution of serpentine endemics (Anacker et al. 2010). However, plant evolution across soil gradients is not limited to cases of extreme toxicity, and the evolution of locally adapted phenotypes is commonly due to soil gradients with different available resources (Chapin et al. 1993, Treseder and Vitousek 2001). For example, across a gradient of soil nitrogen in Hawai`i, *M. polymorpha* showed distinct genetic separation among sites along with variation in traits associated with nitrogen cycling (Treseder and Vitousek 2001). Furthermore, a recent meta-analysis has shown that species growth response to soil N is better predicted by a phylogenetic approach that incorporates natural selection into models than only incorporating genetic drift (Wooliver et al. 2016). Soils, therefore, can have lasting evolutionary effects on plant traits, not just in extreme examples of toxicity but also along common ecological gradients.

The data shown here show genetically based functional trait variation in an admixed population with complete gene flow, suggesting that sympatric colonization/expansion can also lead to quantitative genetic change. Although invasion is largely an allopatric process (Felker-Quinn et al. 2013), colonization of novel habitats can be sympatric, as seen in expansion fronts (Phillips et al. 2006, Eckert et al. 2008). Unlike allopatric processes, when populations are close geographically, the potential for genetic exchange is high and therefore, natural selection would need to be strong to drive differences among populations. In the examples that exist, it is clear that colonizing individuals are genotypically and phenotypically different than those individuals that exist in the core of the species range (Phillips et al. 2006, Eckert et al. 2008) and that evolution

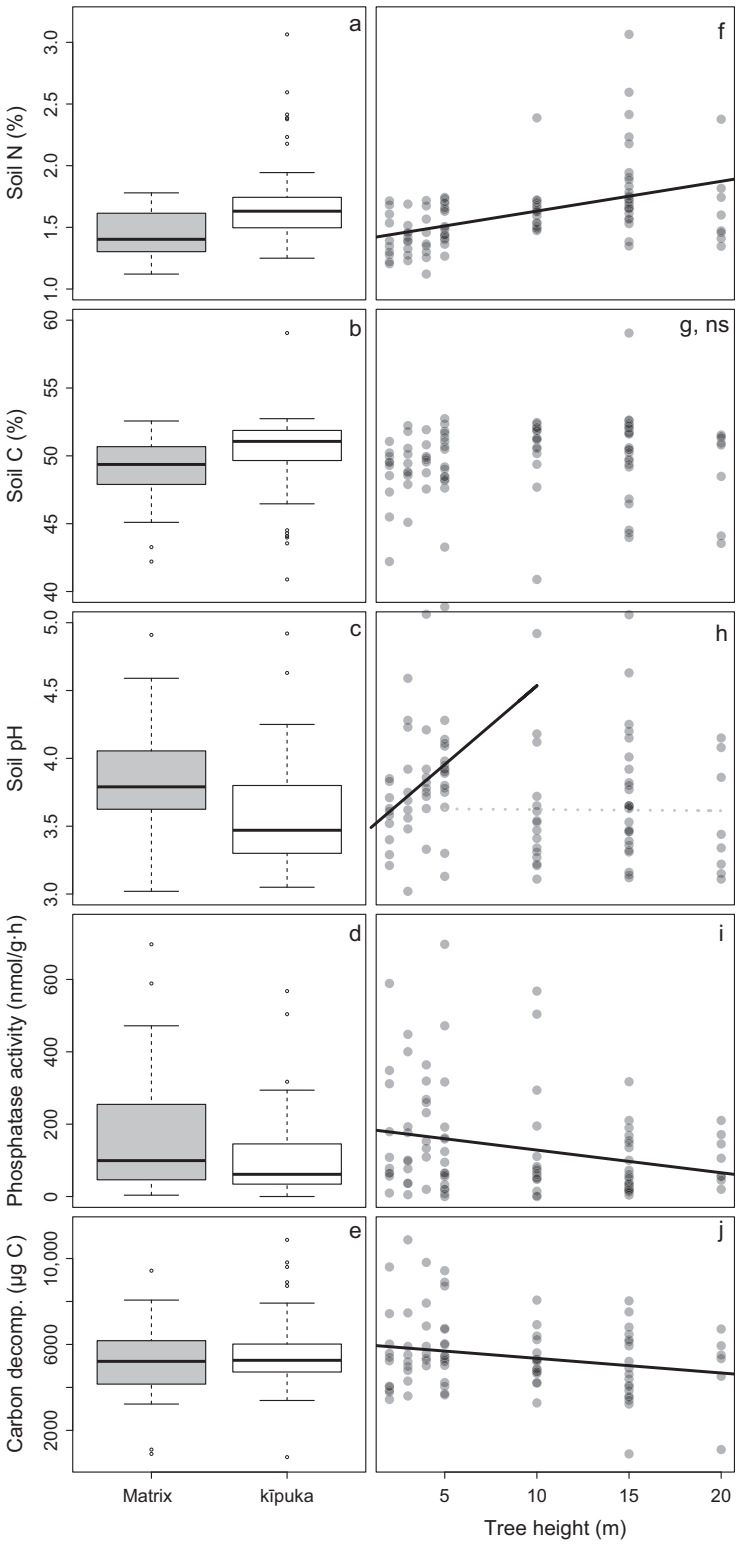


Fig. 2. Variation in soil properties between kīpuka and matrix substrates. Soils in the older substrate had

(*Fig. 2. Continued*)
higher concentrations of (a) carbon (C), (b) nitrogen (N), and (c) were more acidic. Soils in the younger substrate had (d) higher phosphatase activities and (e) higher C decomposition. Increases in tree height and shoot length significantly increase (f) soil N found beneath these trees, but not (g) carbon. Soil pH (h, black solid line) significantly increases with height in the matrix, without (h, gray dashed line) changing in the kīpuka. Increases in the (i) extracellular enzyme activity of phosphatase were found in soils of shorter trees, and soils under shorter trees also have increased (j) C decomposition (i.e., reduced soil C storage), when measured during a 30-d laboratory incubation.

in sympatry may be more common than currently appreciated. For example, a meta-analysis by Eckert et al. (2008) shows that populations at range edges are likely to be more genetically differentiated.

The colonizing individuals in this study demonstrate variation in leaf pubescence and growth for three years in a common garden environment, showing that colonization during primary succession is an evolutionary force even in the face of gene flow. As the $Q_{ST}/F_{ST}$ comparisons in growth traits show, the quantitative trait variation is proportionally greater than neutral genetic variation, indicating directional selection on growth (Storz 2002, Leinonen et al. 2013, Frei et al. 2014). With ranges shifting due to natural and anthropogenic causes, there are multiple ways selection can occur. Many studies have shown the evolution of increased growth rate of invaders in invaded habitats (Matlaga et al. 2012, Liao et al. 2013). Increased growth rates at range edges have also been found due to natural range expansion and poleward migration of species (Evans et al. 2013, Kilkenny and Galloway 2013, Schwarzer et al. 2013). Trait evolution on the leading edge of a continuously moving colonization front may maintain a viable colonizing phenotype. The maintenance of distinct ecotypes on a landscape arisen through local adaptation within a species can be attributed to strong edaphic and environmental gradients, and sympatric isolations such as flowering time. For example, dwarf ecotypes of *Eucalyptus globulus* have evolved independently multiple times on rocky cliff outcrops (Foster et al. 2007), but these populations remain much more genetically isolated by distance and phenology, than the rocky colonists *M. polymorpha* studied here.

### *Ecosystem consequences of trait evolution during colonization*

Variation in aboveground plant traits, caused by underlying genetic variation, has been shown to change community and ecosystem processes.

Table 2. Variation in tree height and substrate influence mortality in the common garden and soil properties in the field.

| Response | Height | Substrate | Height × Substrate |
|---|---|---|---|
| Soil chemical data | | | |
| Soil total nitrogen | **25.14** (<0.0001) | 1.1902 (0.28) | 0.3473 (0.55) |
| Soil total carbon | 0.9459 (0.33) | 1.6304 (0.20) | 0.1137 (0.74) |
| Soil pH | 2.3870 (0.16) | 2.5363 (0.11) | **5.1746** (0.03) |
| Soil enzyme activities | | | |
| Phosphatase | **6.3877** (0.01) | 1.3947 (0.24) | 0.0769 (0.78) |
| β-glucosidase | 0.5874 (0.44) | 2.3661 (0.13) | 0.0229 (0.88) |
| α-glucosidase | 1.0004 (0.32) | 0.0503 (0.82) | 0.0116 (0.91) |
| NAG | 1.6434 (0.20) | 0.0172 (0.90) | 0.1146 (0.74) |
| Phenol oxidase | *3.1962* (0.08) | 1.5699 (0.21) | 0.3323 (0.57) |
| Soil $CO_2$ incubation | | | |
| 30 d decomposition | **4.3479** (0.04) | 0.5959 (0.44) | 0.1534 (0.70) |

*Notes:* Analysis of covariance for field soil response variables to tree height (Height) and the soil age (Substrate). *F* ratios for each parameter are listed followed by the *P* value in parentheses. Bolded *F* ratios represent significant ($\alpha = 0.05$) effects and italicized *F* ratios represent $P < 0.1$.

Genetic variation within a species can influence associated arthropods (Keith et al. 2010) and soil microbial communities (Schweitzer et al. 2008, Bardgett and Wardle 2010) leading to changes in ecosystem function (Hobbie et al. 2006). Furthermore, whether under direct control from plants or indirectly through associated communities, genotypic variation in plant traits has been shown to influence soil respiration (Lojewski et al. 2012) and total soil C and N (Pregitzer et al. 2013) and annual rates of N mineralization (Schweitzer et al. 2012). At broader scales, it has been demonstrated that plant traits influence decomposition rates globally, often being just as important as climate (Cornwell 2008). It is clear that shifts in plant functional traits due to evolution in a novel range can alter ecosystem processes, potentially feeding back on global C and N cycles.

The data reported here suggest that divergence of plant traits, in addition to variation in environmental factors that can alter microbial communities, in matrix trees is significantly changing soil processes in these unique and nutrient-poor areas. Variation in soil chemistry such as pH and total N, which are correlated with tree height, as well as differences in light and temperature can alter the microbial communities present and the soil processes they mediate. These different soil communities are acquiring phosphorus at different rates and utilize soil C substrates to different efficiencies. Matrix soils utilized more recalcitrant C substrates effectively and leading to more C storage in kīpuka beyond the effect of longer storage times. The faster turnover of recalcitrant soil C in the matrix could be due to lower litter quality and the need for specialist microorganisms (Keeler et al. 2009). If this is the case, nutrient limitation in colonizing phenotypes and their associated microbial communities may be decreasing long-term C storage within the ecosystem. It is critical to realize that these patterns cannot be separated from the underlying differences in substrate age. However, the soils sampled in this study are very young and comprised primarily of organic inputs. For this reason, we assume that a substantial proportion of the among-site variation in soil chemistry is due to unique plant traits. With potential decreases in tropical C storage in coarse woody debris (Iwashita et al. 2013), increased litter decomposition rates (Bothwell et al. 2014), and belowground process rates (Giardina et al. 2014) as climates warm, understanding how tree genotypes influence C process rates in tropical fragmented systems will be important to understanding belowground feedbacks to global climate.

## Conclusions

Colonization into novel environments occurs constantly in both natural and anthropogenically driven contexts, with colonizing species evolving due to biotic and abiotic filters encountered in the new habitats. Our results from the field, the greenhouse, and common garden measurements show that divergence in growth occurs, despite strong gene flow, and divergence can lead to variation in growth and other functional traits. Moreover, these data show that phenotypic differences, in combination with environmental differences across the sites, may alter soil properties and ecosystem processes. These data support previous work showing that plant colonization may lead to niche construction, creating distinct soil conditions that influence soil C and nutrient dynamics. Foundation species can rapidly evolve and the ecosystem consequences of these colonizing phenotypes are critical to understanding the full effects of plant species migration under both natural and anthropogenic circumstances.

The Hawai`ian Islands provide globally unique model study systems for testing ecological theory (Vitousek 2004), and understanding evolutionary change, including species divergence (Freed et al. 1987), rapid evolution (Carson and Johnson 1975), and phenotypic plasticity (Cordell et al. 1998). Re-colonization of lava by *M. polymorpha* post-eruption provides another model study system for understanding the long-term evolutionary consequences of colonization, especially as this tree species is both an early colonizer and a long-lived canopy dominant. This implies that the strong abiotic changes that are encountered during colonization can act as a selective agent. This study supports the idea that evolutionary processes in land plants can be rapid along soil gradients (Chapin et al. 1993, Treseder and Vitousek 2001, Brady et al. 2005, Buswell et al. 2011). The drastic change in the potential fitness between the kīpuka and lava matrix has likely led to traits being selected for during colonization of the lava

matrix. Colonization is a subset of species movement, similar to invasion, in both how plants evolve in novel locations, and how evolved phenotypes alter ecosystems. Drawing on both of these ideas in a broad framework of species movement is necessary for understanding ecosystem consequences in a changing world.

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## Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1743/full