# Isoscapes of remnant and restored Hawaiian montane forests reveal differences in biological nitrogen fixation and carbon inputs

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Deforestation and subsequent land-use conversion has altered ecosystems and led to negative effects on biodiversity. To ameliorate these effects, nitrogen-fixing ($N_2$-fixing) trees are frequently used in the reforestation of degraded landscapes, especially in the tropics; however, their influence on ecosystem properties such as nitrogen (N) availability and carbon (C) stocks are understudied. Here, we use a 30-y old reforestation site of outplanted native $N_2$-fixing trees (*Acacia koa*) dominated by exotic grass understory, and a neighboring remnant forest dominated by *A. koa* canopy trees and native understory, to assess whether restoration is leading to similar N and C biogeochemical landscapes and soil and plant properties as a target remnant forest ecosystem. We measured nutrient contents and isotope values ($\delta^{15}N$, $\delta^{13}C$) in soils, *A. koa*, and non-$N_2$-fixing understory plants (*Rubus* spp.) and generated $\delta^{13}C$ and $\delta^{15}N$ isoscapes of the two forests to test for (1) different levels of biological nitrogen fixation (BNF) and its contribution to non-$N_2$-fixing understory plants, and (2) the influence of historic land conversion and more recent afforestation on plant and soil $\delta^{13}C$. In the plantation, *A. koa* densities were higher and foliar $\delta^{15}N$ values for *A. koa* and *Rubus* spp. were lower than in the remnant forest. Foliar and soil isoscapes also showed a more homogeneous distribution of low $\delta^{15}N$ values in the plantation and greater influence of *A. koa* on neighboring plants and soil, suggesting greater BNF. Foliar $\delta^{13}C$also indicated higher water use efficiency ($WUE_i$) in the plantation, indicative of differences in plant-water relations or soil water status between the two forest types. Plantation soil $\delta^{13}C$ was higher than the remnant forest, consistent with greater

contributions of exotic $C_4$-pasture grasses to soil C pools, possibly due to facilitation of non-native grasses by the dense *A. koa* canopy. These findings are consequential for forest restoration, as they contribute to the mounting evidence that outplanting $N_2$-fixing trees produces different biogeochemical landscapes than those observed in reference ecosystems, thereby influencing plant-soil interactions which can influence restoration outcomes.

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## Author contribution statement

CBW, NAH, GG designed the experiment; CBW, NAH, GG, SIOS collected data and performed field work; CBW, GG analyzed data; all authors wrote the manuscript

**Abstract**

Deforestation and subsequent land-use conversion has altered ecosystems and led to negative effects on biodiversity. To ameliorate these effects, nitrogen-fixing ($N_2$-fixing) trees are frequently used in the reforestation of degraded landscapes, especially in the tropics; however, their influence on ecosystem properties such as nitrogen (N) availability and carbon (C) stocks are understudied. Here, we use a 30-y old reforestation site of outplanted native $N_2$-fixing trees (*Acacia koa*) dominated by exotic grass understory, and a neighboring remnant forest dominated by *A. koa* canopy trees and native understory, to assess whether restoration is leading to similar N and C biogeochemical landscapes and soil and plant properties as a target remnant forest ecosystem. We measured nutrient contents and isotope values ($\delta^{15}N$, $\delta^{13}C$) in soils, *A. koa*, and non-$N_2$-fixing understory plants (*Rubus* spp.) and generated $\delta^{13}C$ and $\delta^{15}N$ isoscapes of the two forests to test for (1) different levels of biological nitrogen fixation (BNF) and its contribution to non-$N_2$-fixing understory plants, and (2) the influence of historic land conversion and more recent afforestation on plant and soil $\delta^{13}C$. In the plantation, *A. koa* densities were higher and foliar $\delta^{15}N$ values for *A. koa* and *Rubus* spp. were lower than in the remnant forest. Foliar and soil isoscapes also showed a more homogeneous distribution of low $\delta^{15}N$ values in the plantation and greater influence of *A. koa* on neighboring plants and soil, suggesting greater BNF. Foliar $\delta^{13}C$ also indicated higher water use efficiency ($WUE_i$) in the plantation, indicative of differences in plant-water relations or soil water status between the two forest types.

Plantation soil $\delta^{13}C$ was higher than the remnant forest, consistent with greater contributions of exotic $C_4$-pasture grasses to soil C pools, possibly due to facilitation of non-native grasses by the dense *A. koa* canopy. These findings are consequential for forest restoration, as they contribute to the mounting evidence that outplanting $N_2$-fixing trees produces different biogeochemical

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49 interactions which can influence restoration outcomes.

## Introduction

Forests provide essential ecosystem services including carbon storage and nutrient cycling. However, human-induced disturbances, deforestation, land conversion, and invasive species have led to devastating losses in biodiversity and potentially irreversible alterations to biogeochemical processes (Cramer et al., 2004; Asner et al., 2008; Handa et al., 2014; Veldkamp et al., 2020). While active forest restoration has been touted as a tool to spur secondary succession and regain desirable ecosystem states, it remains unclear if this practice will lead to the recuperation of the biogeochemical properties provided by primary forest reference ecosystems (Aerts & Honnay, 2011; Sullivan et al., 2019; Yelenik et al., 2021). As land management strategies focused on restoration are developed to reconcile losses of ecosystem services, there is a need to understand the spatial extent and long-term impacts of these restoration strategies on restoring biogeochemical properties and carbon and nutrient cycling.

Nitrogen (N) is a key component of biogeochemical cycles, and N is often one of the most limiting nutrients for plant growth and photosynthesis – especially in early successional or secondary tropical forests (Vitousek & Farrington, 1997). Furthermore, the conversion of tropical forests to pasture and other agricultural crops can lead to significant changes in soil properties such rates of N cycling and N availability, as well as carbon sequestration and storage (Veldkamp et al., 2020). Trees capable of atmospheric nitrogen ($N_2$) fixation via symbiotic interactions with root nodule inhabiting bacteria (ex. *Rhizobia* spp.) can be integral to restoring forests by catalyzing ecological succession, reversing the effects of destructive land-use practices on soil N availability, and aiding global efforts to mitigate climate change through increasing C sequestration (Chazdon, 2003; Batterman et al., 2013; Chazdon et al., 2016; Levy-Varon et al., 2019; but see, Kou-Giesbrecht & Menge, 2019). Specifically, as the density of $N_2$-fixing trees,

such as leguminous species in the genus *Acacia* (Resh, Binkley & Parrotta, 2002) increases, N in the soil and neighboring plants can also increase (Sitters, Edwards & Olde Venterink, 2013). Therefore, BNF has the capacity to positively influence the performance and N-budgets of both $N_2$-fixing and non-$N_2$-fixing plants.

In the tropics, $N_2$-fixing trees are often used as ecosystem engineers for the restoration of degraded landscapes (Fisher, 1995; Scowcroft, Haraguchi & Hue, 2004). However, the outcomes of these efforts are mixed, with some restored areas stalled in apparent alternative stable states (Yelenik, 2017), while others progress toward ecosystem targets and restoration goals (Fisher, 1995; Rhoades, Eckert & Coleman, 1998; Koutika et al., 2021). These differing effects of $N_2$-fixing trees may be owed to heterogeneous N inputs across landscapes (Dixon et al., 2010; Sullivan et al., 2014), as well as ecosystem-specific biotic and abiotic factors (Pearson & Vitousek, 2002; Staddon, 2004; Wynn & Bird, 2007; Dixon et al., 2010; Barron, Purves & Hedin, 2011; Sitters, Edwards & Olde Venterink, 2013). For example, active restoration that relies on establishing forests of $N_2$-fixing trees may have unintended consequences, such as promoting weedy or invasive species (Funk & Vitousek, 2007), thereby altering successional trajectories toward non-target states (Stinca et al., 2015). A better understanding of the spatiotemporal effects of $N_2$-fixing trees on ecosystems is needed, especially in the context of forest restoration.

Stable isotopes are time-integrating markers that can provide insight into biogeochemical processes and shifts in ecosystem services occurring across multiple landscapes and spatiotemporal scales (Cheesman & Cernusak, 2016). Spatially explicit, geo-referenced isotope landscapes (termed ‘isoscapes’) allow for the measured isotope values of individual replicates (i.e., within or among species, or sample types) to be interpolated to landscape scales (Bowen,

2010). While only a few studies have used isoscapes in plant ecology (Hellmann et al., 2011; Rascher et al., 2012), their application may provide new and important insights into plant-microbe and plant-soil feedbacks, such as biological nitrogen fixation (BNF) by $N_2$-fixing trees, which can vary at relatively smaller spatial scales.

BNF results in nitrogen stable isotope values ($\delta^{15}N$) that are more similar to the atmosphere (~0‰) relative to nitrogen assimilated from soil nitrogen pools (Craine et al., 2015). Soil $\delta^{15}N$ values represent an integration of nitrogen inputs and outputs in an ecosystem, and are influenced by processes that lead to isotope fractionation (i.e., nitrification, denitrification, ammonia volatilization), atmospheric deposition, leaching, as well as the pedogenic and environmental factors that shape these processes (Natelhoffer & Fry, 1988; Austin & Vitousek, 1998; Martinelli et al., 1999; Burnett et al., 2022). Variability in soil $\delta^{15}N$ can complicate the interpretation of leaf $\delta^{15}N$ values, especially for non-$N_2$-fixing plants (Robinson, 2001). However, the low $\delta^{15}N$ values associated with BNF are distinct enough to be traced through soils and vegetation (Hellmann et al., 2011). For instance, $\delta^{15}N$ isoscapes revealed the contribution of an invasive, non-native $N_2$-fixing shrub (*Acacia longifolia*) to the nitrogen pools of surrounding plants and the corresponding modification of nutrient cycling and community function due to this invasive species (Rascher et al., 2012).

Along with standard comparisons of C to N concentrations, leaf and soil C stable isotope values ($\delta^{13}C$) can provide additional evidence on the rates of nutrient cycling, the relative contribution of plant functional types (i.e., $C_3$ vs $C_4$ photosynthesis) to soil C pools, and the integrated plant water use efficiency ($WUE_i$) of plants in an ecosystem (Cernusak et al., 2013; Driscoll et al., 2020). Combined, $\delta^{13}C$ and $\delta^{15}N$ values sampled across a landscape can provide spatially explicit information on plant performance and ecosystem processes, which can be

compared among ecological community members or between communities (Hellmann et al., 2016). While it is clear that isoscapes provide new perspectives on the spatial relationships of biochemical processes and biological interactions fundamental to ecology (Cheesman & Cernusak, 2016; McCue et al., 2020), they have yet to be widely applied to understand plant-soil feedbacks in the context of restoration.

Native Hawaiian montane mesic and wet forests have significantly declined due to over a century of deforestation and extensive human- and livestock-mediated disturbance (Pau, Gillespie & Price, 2009; McDaniel et al., 2011; Yelenik, 2017). These forests provide are dominated by two foundational endemic tree species – $N_2$-fixing *Acacia koa* (koa, Fabaceae) and the non-$N_2$-fixing *Metrosideros polymorpha* (‘ōhi‘a lehua, Myrtaceae) – and provide critical ecosystem services, including habitat for many endangered bird species in Hawai‘i (Paxton et al., 2018). To restore montane mesic forest habitats on Hawai‘i Island, over 390,000 nursery-grown *A. koa* trees (i.e., outplants) have been planted in the Hakalau Forest National Wildlife Refuge since 1987 (hereafter, Hakalau; Fig. 1). This effort started in the greenhouse, where individual trees (one per pot) were grown in 15-cm-long cone-tainers, reaching a height of 30-cm in <6 months. In the plantation, a bulldozer with a miniblade scoured the soil to remove grass competitors and make plots (1 × 1 m) every 3 m, with rows every 4 m a part. Individual greenhouse-grown koa were planted in holes created by an auger power planter (the same size as the root plug) with fertilizer added to the auger hole (see Jeffrey and Horiuchi, 2003).

*Acacia koa* outplants were introduced to 5,000 acres of fenced, abandoned pastureland (previously grazed by cattle, pigs, goats) in an effort to restore forested habitats and support the recovery of native plants and endangered bird populations (Jeffrey & Horiuchi, 2003; McDaniel & Ostertag, 2010). Forest patches are critical in supporting biodiversity (Wintle et al. 2019), and

patchiness and/or fragmentation can lead to dramatic differences in forest climate that support rates litter decomposition and nutrient cycling (Crockatt & Bebber, 2014). Therefore, the active outplanting of *A. koa* in discrete corridors was leveraged to facilitate the passive restoration and expansion of koa recruitments and root-suckers, with the goal of ultimately turning isolated patches into intact, contiguous forests habitat (Scowcroft and Yeh, 2013). However, after 30 years the understory of *A. koa* plantations remain grass-dominated, native woody plant recruitment is low, and endangered birds are sparse (Yelenik, 2017; Paxton et al., 2018). The causes of this stalled forest recovery are uncertain, but they likely include the influence of *A. koa* BNF on soil chemistry, nutrient concentrations, and nutrient cycling (Scowcroft, Haraguchi & Hue, 2004). As a $N_2$-fixing tree, *A. koa* produces abundant low C:N leaf litter (high N), which can lead to greater supply of N to the soil surface under *A. koa* relative to *M. polymorpha*, which has high litter C:N (Scowcroft, 1999; Yelenik, Rehm & D'Antonio, 2022). These differences in litter N can increase rates of decomposition and ultimately affect the return of nutrients to soil N pools (Baker, 2009; Zhou et al., 2018). Differences in canopy composition and associated litter decomposition can have important implications for forest nutrient cycling and the composition of forest understories. In the plantations, the ability for *A. koa* to increase soil nutrient, coupled with the high densities of this canopy tree in monoculture corridors, may be favoring the growth of exotic nitrophilous grasses from the historic pastures and inhibiting native seedling establishment (Yelenik, 2017).

To examine the interactions between BNF, soil and leaf N content as well as C inputs at the ecological community scale, we applied a spatially explicit sampling approach within a mixed *M. polymorpha*/*A. koa* native montane forest (hereafter, the "remnant forest") and an *A. koa* plantation (hereafter, "koa plantations"). The remnant forest is largely dominated by native

165 understory plants, whereas the koa plantation understory is dominated by $C_4$ exotic grasses
166 interspersed with a $C_3$ non-native shrub (*Rubus argutus*). While the land-use history and present-
167 day plant communities of these two forest types differ, they occupy the same climate and parent
168 soil types (http://rainfall.geography.hawaii.edu, https://gis.ctahr.hawaii.edu/SoilAtlas). We used
169 $\delta^{15}N$ and $\delta^{13}C$ values of soil and foliar samples to construct isoscapes and examine the degree of
170 BNF between the two forest types and test the influence of BNF by *A. koa* on $\delta^{15}N$ values in
171 soils and neighboring plants (*Rubus* spp.). We hypothesized that the higher density and more
172 even distribution of *A. koa* in the plantation compared to the remnant forest will result in lower
173 $\delta^{15}N$ values in soil and plants from the plantations with greater and spatially more homogeneous
174 BNF inputs than the remnant forests. In addition, we expected higher foliar $\delta^{13}C$ values in the
175 plantation as a result of greater water demand in the young, dense *A. koa* forests driving higher
176 $WUE_i$ (Kagawa et al., 2009). Introduced $C_4$ grasses are abundant in the understory of the
177 plantation but are uncommon in the remnant forest where there are no known native $C_4$ plants
178 (Yelenik 2017). Therefore, we also predicted that soil $\delta^{13}C$ would be higher in the plantation
179 relative to the remnant forest, indicating greater contributions of $C_4$ grasses to soil C pools. The
180 results of this study will clarify the effects of tropical $N_2$-fixing monoculture plantations on the
181 distribution of N and soil nutrient cycling and how these may differ from the target ecosystem
182 for restoration.
183
184 **Materials and Methods**
185 *Site description and sample collection*
186 Two sections of remnant forest and koa plantation were identified within the Hakalau
187 Forest National Wildlife Refuge on the Island of Hawai‘i (19°49’12”N, 155°19’22”W) (Fig. 1).

Remnant forests are characterized by a mixed canopy of *Acacia koa* (koa) and *Metrosideros polymorpha* (‘ōhi‘a lehua) along with native understory woody plants *Cheirodendron trigynum* (‘ōlapa), *Coprosma rhynchocarpa* (pilo), *Leptecophylla tameiameia* (pukiawe), *Myrsine lessertiana* (kōlea), *Rubus hawaiensis* (‘ākala), and *Vaccinium calycinum* (‘ōhelo). In contrast, the plantations are largely monoculture stands of *A. koa,* with non-native grass understories of *Cenchrus clandestinus* (kikuyu, a $C_4$ grass), along with *Ehrharta stipoides* ($C_3$), with sporadic occurrences of the non-native shrub *Rubus argutus* and ferns.

Each sampling area – koa plantation (KP) or remnant forest (RK) – had similar montane forest climates, elevations (~1800 m), and soils from the same parent material (Scowcroft et al., 2007), and were selected due their proximity to transects used in previous studies (Paxton et al., 2017). Both the plantation and the remnant forest were dominated by *A. koa*, with koa representing 100% of the canopy tree species in the plantation. ‘Ōhi‘a was absent in the koa plantation, and only one mature ‘ōhi‘a was observed in the remnant forest area. Each 700 $m^2$ sampling area was representative of the vegetation found throughout the remnant and “restored” plantation forests of Hakalau. Samples were collected at a spatial resolution (0-5 m) relevant for detection of BNF and changes in C cycling at the landscape scale in soil, $N_2$-fixing and non-$N_2$-fixing foliage. Individual samples were also collected at a spatial resolution that balanced considerations of spatial autocorrelation and the ability to extrapolate via kriging (see methods section below) to the landscape scale (Hellmann et al., 2011; Rascher et al., 2012).

In each forest plot of 20 × 35 m (Fig. S1), we collected soil samples at 4 or 5-m intervals ($n$ = 48 soil samples per forest) using a sterilized (70% ethanol) soil borer that sampled the upper 10 cm of the soil after surface leaf litter was removed. Soil samples were placed into paper envelopes for future processing. Within the remnant forest and the plantation, we collected foliar

samples from all mature *A. koa* canopy trees and understory *Rubus* spp. (detailed below). Juvenile *A. koa* have bipinnately-compound true leaves that develop into phyllodes during maturation. Hereafter, we will refer to all foliar samples (i.e., *A. koa* phyllodes, *Rubus* true leaves) as 'leaves'. Grasses were not sampled due to grasses being rare in the remnant forest. Similar to other plant derived isoscapes (Hellmann et al., 2011; Rascher et al., 2012), this sampling scheme was designed to capture any signature of $N_2$-fixation from *A. koa* on understory plants as well as the soil environment.

In both forests, all mature *A. koa* larger than 2 m tall were marked (plantation [$n$ = 18] and remnant [$n$ = 10], 257 and 142 trees per hectare, respectively) and their relative position mapped (Fig. 1, Fig. S1). Three leaves were collected and pooled from each individual *A. koa* tree in the mid-exterior forest canopy using pole pruners at a consistent height (ca. 6 m). The pooled leaf sample for each *A. koa* was placed into a paper envelope until further processing for isotope analyses (detailed below). Due to the distance between individual koa trees and their lack of clonal reproduction, each pooled leaf sample per tree is considered an independent replicate. Each tagged *A. koa* was measured for diameter at breast height (dbh) using a diameter tape, measured at 1.5 m from the ground. When multiple trunks were found on the same tree, dbh was represented as the square root of the sum of squared dbh measurements of individual trunks (Meeuwig et al., 1981). Canopy area ($m^2$) was measured for each *A. koa* in the forest type using a laser to measure distance from the trunk to the canopy edge; measurements were taken at four bearings (0°, 90°, 180°, 270°). Individual *A. koa* canopy area (canopy $m^2$) was determined using the formula for the area of an ellipse (area = $\pi ab$), where $a$ and $b$ are the average distances at the major radii (0° and 180°) and minor radii (90° and 270°).

Two Rosaceae species (*Rubus* spp.) were present in the two sampling areas and were used as indicator plants for the influence of BNF on neighboring plants based on their $\delta^{15}N$ values: the native *Rubus hawaiensis* in the understory of the remnant forest ($n$ = 14 plants [200 plants hectare$^{-1}$) and the invasive *Rubus argutus* in the plantation ($n$ = 28 plants [400 plants hectare$^{-1}$]). Since the two *Rubus* species tended to separate by forest type, it was not possible to collect both species in the two forests. When a *Rubus* individual was present in a quadrat (20 m$^2$), three leaves were collected from the top of up-to three adult stems and pooled per individual plant and placed in a paper envelope. The spatial location of the *Rubus* samples was standardized to the centroid of the quadrat where they were collected (Fig. S1). Within 6 h of collection, soil and foliar samples were transported to the University of Hawai‘i-Hilo Hakalau Forest Biological Field Station and oven dried (60°C, ca. 5 h) in paper envelopes. The dried samples were transported to the BayCEER Laboratory of Isotope Biogeochemistry at the University of Bayreuth for processing.

*Stable isotope analyses*

Prior to final analysis, foliar and soil samples were again oven dried (105°C) to a constant weight (Gebauer & Schulze, 1991) and homogenized to a powder using a ball mill (Retsch Schwingmühle MM2, Haan, Germany). While many studies use 60°C for foliar samples, extensive internal laboratory testing of drying procedures and temperatures (60°, 80°, 105°C) have shown negligible drying effects on foliar and soil isotope values (within ± 0.2 ‰ measurement error), while drying at higher temperatures ensures complete sample desiccation. Where present, root material was removed from soil samples prior to homogenization. A subset of each sample was placed in a tin capsule and weighed (foliar [2.8 - 5.5 mg], soil [3.5 - 4.5

mg]). A standard material (acetanilide [0.4 - 1.5 mg]) with known C and N stable isotope abundance and concentration was also analyzed for quality control and calculation of C and N concentrations in our samples. Standard materials were run at least six times within each batch of 50 samples following protocols previously described (Gebauer & Schulze, 1991). Molar concentrations of C and N and their ratio (C:N), as well as natural abundance isotope values ($\delta^{13}C$, $\delta^{15}N$), were measured using an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS) coupling, combining an elemental analyzer (Carlo Erba Instruments 1108, Milano, Italy) with a continuous flow isotope ratio mass spectrometer (delta S, Finnigan MAT, Bremen, Germany) via a ConFlo III open-split interface (Thermo Fisher Scientific, Bremen, Germany). Natural abundance isotopic values were reported in delta values ($\delta$) using permil (‰) notation relative to standard materials (Vienna-Peedee Belemnite [V-PDB] and atmospheric $N_2$ standards [air] for C and N, respectively). Reproducibility of isotope abundance measurements was always within ± 0.2‰. All standard gases (Riessner, Lichtenfels, Germany) were calibrated versus the international standards using reference substances provided by the International Atomic Energy Agency, Vienna, Austria (CH-3, CH-6 and NBS-18 for the carbon isotopes and N-1 and N-2 for the N isotopes).

*Isoscape kriging*

Spatially explicit sampling of soil, *A. koa*, and indicator plant taxa (*Rubus* spp.) allowed for the generation of maps of $\delta^{15}N$ and $\delta^{13}C$ values across the two forest types (i.e., isoscapes). The spatial coordinates for each sample were determined by its location within the sampling grid (i.e., quadrat corners [soil], centroid [*Rubus* spp.], or relative position [*A. koa*]), which was used to generate a spatial grid and points for interpolation using the functions *coordinates* and

279 *spsample* in the R package sp (Pebesma & Bivand, 2005; Bivand, Pebesma & Gómez-Rubio,
280 2013). To visualize the stable isotope landscape, we used kriging: a common statistical method
281 that uses variograms to calculate the spatial autocorrelation between points and distance and
282 interpolate isotope values across a continuous surface (Bowen, 2010). We generated $\delta^{15}N$ and
283 $\delta^{13}C$ isoscapes for soil-only and foliar-only samples. A foliar $\delta^{13}C$ isoscape was not generated
284 since samples were limited to $C_3$ plants of similar $\delta^{13}C$ values. Data interpolation was performed
285 using ordinary kriging to a continuous surface, with variogram models generated by the function
286 *autoKrige* in the R package automap (Hiemstra et al., 2009). *AutoKrige* performs iterative model
287 selection fitting variogram models to the collected data (the experimental variograms). Best-fit
288 variogram models (i.e., those with lowest residual sum of squares) have four main model
289 parameters: *the nugget effect*, the estimate of experimental error inherent in measurements,
290 sampling design, and environmental variability; *the sill,* the spatial pattern intensity and the
291 semivariance asymptote; *the spatial range*, the distance or lag of sample correlation where
292 samples are influenced by same underlying process; and *the sserr* (sum of squares between
293 experimental and fitted variogram model) (Fortin, Dale & Ver Hoef, 2012). These model
294 parameters describe the autocorrelation between the semivariance (y-axis) and distance or lag
295 parameter (x-axis). With distance between samples, semivariance increases and correlation
296 decreases; therefore, model saturation (i.e., *sill*) indicates a point where semivariance no longer
297 increases with increasing distance (i.e., *range*) and points no longer show autocorrelation. We
298 used variogram models and their predicted values ($\delta^{15}N_{predicted}$ and $\delta^{13}C_{predicted}$) to generate
299 isoscapes using the function *spplot* in the R package sp. Where model variograms showed poor
300 fit to experimental variogram data, we used *autoKrige* to run iterations of user-defined, fixed
301 values for variogram parameters (*nugget*, *range*, *sill*). Predicted values from model variograms

were then compared between the two forest types over a continuous surface with statistical inferences made on the distributions of predicted values using non-parametric tests.

*Statistical analyses*

Data normality and heteroscedasticity were inferred from graphical inspection of residuals and quantile-quantile plots. Where data failed to meet assumptions of parametric models, non-parametric tests were used. Differences in *A. koa* dbh and canopy area between forest types (koa plantation or remnant forest) were analyzed using Mann-Whitney *U*-tests using the function *mwu* in the package sjstats (Lüdecke, 2021). Isotope data ($\delta^{15}N$, $\delta^{13}C$), total C and total N concentration (mmol g/dry weight [gdw]), and molar C:N were analyzed using a two-way linear model with sample type (*A. koa*, *Rubus* spp., soil) and forest type (remnant forest, koa plantation) as fixed effects. Analysis of variance tables were generated with Type-II sum of squares in the *car* package (Fox et al., 2019). Post-hoc slice-tests were used in *a priori* contrasts to test for significant differences among sample types within a forest (*A. koa* vs. *Rubus* spp.) and between forest types using the *emmeans* package (Lenth, 2022). Differences between the $\delta^{15}N$ and $\delta^{13}C$ isoscapes in the remnant forest and koa plantation were assessed visually, through Mann-Whitney *U*-tests using density plots of interpolated/predicted isotope values ($\delta^{15}N$ or $\delta^{13}C$), and the characteristics of the model variogram.

While we recognize our study is limited to two areas (20 × 35 m) each within a different forest type (plantation and remnant forest), the utility of an isoscape approach is that it allows for spatial interpolation so that biogeochemical patterns at community and ecosystem levels can be quantified. Experimental designs such as ours, where a single large area representative of the community types to be compared, are common and accepted approaches among isoscape studies

assuming that sampling is sufficient to capture natural variation in isotope values within the community of interest (Bowen, 2010; Hellmann et al., 2011; Rascher et al., 2012). Here, the unit of replication is individual sample points used to generate isoscape maps (plants [$n$ = 24 – 36], soil [$n$ = 48]), with statistical relationships determined using linear models. All data analyses were performed in R (version 4.2.1) (R Core Team, 2022). Archived data and code for analyses can be found at GitHub (https://github.com/cbwall/Hakalau) and are published at Zenodo (Wall et al., 2023).

## Results

*Vegetation structure*

Differences between *A. koa* trees in the two forest types were observed, notably dbh was greater in the plantation ($p$=0.010) (Fig. S2A), but the *A. koa* canopy area was not different between the two forests ($p$=0.666) (Fig. S2B, Table S1). In the remnant forest, 80% of *A. koa* were single-stemmed, whereas in the plantation multi-stemmed *A. koa* dominated with only 20% of trees being single-stemmed. The total basal area of mature *A. koa* cover in the plantation and remnant forest (18 and 10 trees, respectively) was 283 and 258 $m^2$, covering ~40% of the 700$m^2$ sampling areas in each forest.

*Soil and foliar isotope values*

$\delta^{15}N$ values differed according to sample type ($p$<0.001) and forest ($p$<0.001), with overall lower $\delta^{15}N$ values in the plantation (Table S2). $\delta^{15}N$ values of soils were not different among the forests ($p$ = 0.085). *Acacia koa* and *Rubus* spp. $\delta^{15}N$ values were both significantly closer to zero (less $^{15}N$-enriched by ~1‰) in the plantation compared to remnant forest samples

(post-hoc: $p \leq 0.029$) (Fig. 2A). As expected, $\delta^{15}N$ values of the $N_2$-fixing *A. koa* were significantly lower compared to *Rubus* spp. in both the plantation (post-hoc: $p=0.020$) and remnant forest (post-hoc: $p=0.029$). Overall, there was a greater range and standard deviation in $\delta^{15}N$ values in the remnant forest *A. koa* (range: 2.19‰, SD: 1.03‰) and *Rubus* spp. (range: 3.54‰, SD: 0.89‰) compared to plantation *A. koa* (range: 0.85‰, SD: 0.65‰) and *Rubus* spp. (and 2.55‰, SD: 0.75‰). This larger range and variance in plant $\delta^{15}N$ values is consistent with heterogeneous N sources in the remnant forest, which is being expressed in the leaves of canopy $N_2$-fixing trees and understory plants. Soil $\delta^{15}N$ values were ~3‰ higher than those in foliar samples, but similar among the plantation and the remnant forest (post-hoc: $p=0.085$) (Fig. 2A).

$\delta^{13}C$ values were different among sample types ($p<0.001$) and between forests ($p<0.001$), with significantly lower $\delta^{13}C$ values in all sample types from the remnant forest relative to those from the plantation (Fig. 2B; Table S2). However, when compared within the same forest, the $\delta^{13}C$ values of *A. koa* and *Rubus* spp. were not different from each other in the remnant forest (post-hoc: $p=0.057$) or the plantation (post-hoc: $p=0.281$).

*Isoscapes and model variograms*

Variograms provided insights into the spatial correlation within isoscapes and the semivariance-by-distance relationship. $\delta^{15}N$ and $\delta^{13}C$ isoscape variograms for both soil and leaves showed equivalent ranges in spatial autocorrelation in both forests (~3-5 m) (Table S3). These results indicate similar relationships between semivariance-by-distance decay (i.e., less autocorrelation with increasing distance) in the koa plantation and the remnant forest at the scale of our experimental sampling. However, these estimates should be interpreted with caution since (1) the minimum sampling distance between samples for soils was 4 m and (2) in several

371 modeled variograms the increased *nugget* values coupled with low *sill* values may indicate
372 greater influence of random variation. Nevertheless, we found significantly different
373 distributions of $\delta^{15}N$ and $\delta^{13}C$ isotope values between forest types for both soil and foliar
374 isoscapes ($p<0.001$) (Figs. 3, 4 & 5; Table S1).

375 In both soil and foliar $\delta^{15}N$ isoscapes, we observed lower $\delta^{15}N_{predicted}$ values in the koa
376 plantation ($p<0.001$) (Table S1), with mean $\delta^{15}N$ values for all interpolated data points lower by
377 ~0.5‰ (soil isoscape) and 1.2‰ (foliar isoscape) in the plantation relative to the remnant forest
378 (Fig. 3A, C). Both forests showed hot spots of low $\delta^{15}N$ values (<4‰ soil [Fig. 4A-B], <2‰
379 foliar [Fig. 5A-B]) that tended to be in areas proximate to *A. koa* or sampling points representing
380 *A. koa* leaves (and to a lesser extent *Rubus* spp.) (Figs. 4 & 5). However, low $\delta^{15}N$ areas were
381 most pronounced in the plantation, where *A. koa* density was greater than the remnant forest (18
382 [KP] vs. 10 [RK] mature trees), particularly through the middle of the plantation where *A. koa*
383 trees are numerous and planted in parallel rows. Similarly, in the $\delta^{15}N$ soil-isoscape, the koa
384 plantation had more uniform low $\delta^{15}N$ areas within this central *A. koa* corridor (Fig. 4A). The
385 influence of *A. koa* on soils in the remnant forest was more variable, with a localized region of
386 low $\delta^{15}N$ values in the southern portion of the forest plot (Fig. 4B).

387 $\delta^{13}C$ isoscapes of soil samples showed lower $\delta^{13}C$ values throughout the remnant forest
388 relative to the plantation (Fig. 4C-D), with the distribution of $\delta^{13}C_{predicted}$ values for soil samples
389 (Fig. 3B) being lower in the remnant forest ($p<0.001$) (Table S1). These differences represent
390 ~1.5‰ lower mean $\delta^{13}C$ values for interpolated values in the remnant forest compared to the
391 plantation. Hotspots of low $\delta^{13}C$ values in both $\delta^{13}C$ soil isoscapes corresponded to areas within
392 the *A. koa* corridor in the plantation and areas where juvenile *A. koa* were located (Fig. 4C-D &
393 Fig. S1).

394

395 *Nitrogen and carbon concentration patterns*

396 N concentration (mmol/gdw) differed according to sample types ($p$<0.001) (Fig. 2C),
397 being highest in *A. koa*, followed by *Rubus* spp., and lowest in soil samples ($p$<0.001) but did not
398 differ between forests ($p$=0.107) (Table S2). Total C concentrations (mmol/gdw) differed among
399 sample types ($p$<0.001) and were overall lower in the koa plantation ($p$=0.003), driven by small
400 differences in C concentrations of soil and *Rubus* sp. (<2%) (Fig. 2D). N and C concentrations
401 were greater in *A. koa* compared to *Rubus* spp. in the plantation (post-hoc: $p$<0.001) and the
402 remnant forest (post-hoc: $p$≤0.022). Molar C:N values differed by sample types ($p$<0.001), but
403 not forests ($p$=0.621), and were lowest in soil (16), followed by *A. koa* (19), and *Rubus* spp. (21)
404 (pooled means ± 0.3 SE, $n$=14-96).

405

406 **Discussion**

407 Assessments of biogeochemical landscapes can provide insight into key ecosystem
408 properties, plant-soil feedback, and plant-plant interactions. As the degree of BNF increases,
409 there is a well-documented decrease in foliar $\delta^{15}N$ in $N_2$-fixing plant species (Craine et al.,
410 2015). Differences in BNF between ecological communities often have corresponding effects on
411 other ecosystem properties such as soil and neighboring non-$N_2$-fixing plant $\delta^{15}N$ values and
412 nutrient status, as well as rates of photosynthesis and nutrient cycling. In addition to $\delta^{15}N$
413 analyses, $\delta^{13}C$ values coupled with information on C and N concentrations can aid in inferences
414 about each of these ecosystem-level processes occurring at different spatial scales (Moyer-Henry
415 et al., 2006; Rascher et al., 2012; Hoogmoed et al., 2014; Craine et al., 2015). The use of large
416 “super-plots” in discrete habitats, which are sampled at small spatial scales and/or grids to

417 perform spatial interpolation, is common in isoscape experimental designs (Rasher et al., 2012;
418 Hellmann et al., 2016). Earlier studies sampled *A. koa* across elevation and rainfall gradients on
419 Hawai‘i Island, mapping $\delta^{15}N$ and $\delta^{13}C$ values to determine environmental impacts on plant
420 performance and suitability of sites for forest restoration (Lawson & Pike 2017). We find that
421 spatially-explicit sampling at smaller spatial scales is equally useful in identifying difference is
422 plant-soil-microbe interactions and the influence of BNF. Here, using $\delta^{15}N$ isoscapes to
423 determine differences in BNF between two forest patches that differ in their land use histories
424 and management, we found strong evidence for greater, and more homogeneous contribution of
425 newly-fixed N in the soil and plants of the afforested koa plantation compared to the remnant
426 native forest. While these forests plots represent but a subset of the plantation and remnant
427 forests habitats, each plot covered a significant area (700 $m^2$) and were specifically chosen
428 because the vegetation is representative of each unique forest type, while the abiotic conditions
429 are similar (climate, slope, aspect, soil parent material). Therefore, the results of our study should
430 be considered representative of forest community-level dynamics. However, increasing plot-
431 level replication in future studies may allow for greater biogeochemical heterogeneity to be
432 quantified within and among forest types (plantations and remnant forests) and the influence of
433 environmental factors (such as elevation, rainfall, volcanic organic gases) on BNF and forest
434 restoration.

435 In the koa plantation, $\delta^{15}N$ soil and foliar isoscapes revealed a more evenly distributed
436 signature of low $\delta^{15}N$ values in the plantation relative to the remnant forest, indicative of a
437 greater contribution of BNF in the plantation that could be related to the demography and density
438 of *A. koa* in the plantation (Figs. 4 & 5). The finding that both *A. koa* and understory *Rubus* spp.
439 also had lower $\delta^{15}N$ values in the plantation relative to the remnant forest (Fig. 2) further

suggests greater BNF by *A. koa* and a greater contribution of newly-fixed N to neighboring plants. While soil $\delta^{15}N$ values did not statistically differ significantly between forest types, the $\delta^{15}N$ soil isoscape showed overall lower $\delta^{15}N$ values in the plantation compared to the remnant forest (Fig. 4). The marginal differences in soil $\delta^{15}N$ between the two forest types may be due in part to differences in water availability between forest types (Austin & Vitousek, 1998), but further efforts are needed to determine if the soil water status differs among these forests (Bothwell et al., 2014). In both forests, however, the high soil $\delta^{15}N$ values indicate significant losses of N relative to the size of the nitrogen pool, possibly from rapid N turnover and fractionation or leaching (Natelhoffer & Fry, 1988; Austin & Vitousek, 1998; Burnett et al., 2022). Nevertheless, our suite of biogeochemical metrics ($\delta^{15}N$, $\delta^{13}C$, N and C concentrations) provide new insights on plant-soil-water relations, nutrient and carbon cycling, and offer clues as to why the plantations continue to foster a non-native grass dominated state even after three decades post reforestation (Yelenik, 2017; Yelenik et al., 2021).

In addition to higher density of *A. koa* in the plantation, the demographics of *A. koa* outplants and saplings in the plantation (<30 y old) compared to the remnant forest may be influencing C and N cycling in this system. Nodule biomass and N fixation rates are life-stage dependent, with younger stands (6 y) exhibiting an order of magnitude higher nodule biomass and $N_2$-fixation rates than older stands (20 y) (Pearson & Vitousek, 2001). The growth rate of *A. koa* across the Hawaiian Islands is variable, with dbh-based growth rates of 10-15 mm/y in sunlit areas where the crown is exposed and 6-7 mm/y estimated across a range of sites in the Hawai‘i Department of Forestry and Wildlife long-term forest plots (Baker, Scowcroft, & Ewel, 2009). In remnant forests of Hakalau, growth rates of 4 mm/y for *A. koa* have been observed (Hart 2010), but no dbh-age estimates exist for the koa plantation. If we assume *A. koa* in the sunlit plantation

have growth rates similar to other sunlit areas across Hawai‘i (~ 12 mm/y) and *A. koa* in the remnant forest are 4 mm/y, we estimate mean (±SE) ages of trees from the plantation to be significantly younger ($p$=0.049, Table S1) than the remnant forest (30 ± 2 [KP] and 55 ± 12 years [RK]) (Fig. S3), with two trees from the remnant forest being > 110 years old. These estimates agree with the known age of the planting of the plantation (~ 1990) and support the hypothesis that forest demographics may be important to affecting the degree of $N_2$-fixation in *A. koa* and its contribution to plants and soils of Hakalau.

In our study system, the high densities *A. koa* in the plantation, along with immature *A. koa* recruits in thickets adjacent to planted trees, may be contributing to the higher rates of $N_2$-fixation we detected based on lower $\delta^{15}N$ values (Figs. 2A, 4 & 5). The high density of *A. koa* has the potential to contribute more leaf litter of lower C:N to the forest floor relative to the mixed-canopy remnant forest. The greater number of multi-stemmed *A. koa* in the plantation (80% in KP vs. 20% RK) – which may be due to the spacing of outplants and the lack of competition in the plantation – also emphasizes the differing growth patterns in the outplanted *A. koa* relative to naturally recruited trees in remnant forests. These conditions may shape litterfall to the forest floor, which can be dynamic in both abundance and nutrient concentrations and reflective of shifts in plant community composition (Lanuza et al., 2018). Taken together, we suggest the greater densities, faster growth rates, and younger demographics of *A. koa* in the plantation provide a context for low C:N litterfall (and greater N contributions) in the early stages of reforestation to lead to persistent changes in nutrient cycling.

Despite a strong signal of significantly higher BNF in the plantation (Figs. 2A, 4A & 5A), we found no statistically significant differences in foliar or soil N concentrations (Fig. 2C-D). While this result is puzzling, one likely explanation is that the plantation may have relatively

more well drained soils, and plantation *A. koa* may have higher water demands compared to remnant forests (Meinzer, Fownes & Harrington, 1996; Brauman, Freyberg & Daily, 2015). We note *A. koa* foliar $\delta^{15}N$ values reported here are high relative to other studies of *A. koa* (Burnett et al., 2022; Lawson and Pike, 2017); however, these values are in-line with $N_2$-fixing plants from dry-forests and grasslands (Heaton, 1986), suggesting water limitations may be influencing *A. koa* $\delta^{15}N$ in these forests. Even though we did not measure water holding capacity of each soil type, the significantly higher foliar $\delta^{13}C$ values in the plantation – coupled with the lack of differences in soil and foliar N despite evidence of higher BNF – is indicative of water being an important factor limiting *A. koa* productivity and affecting soil N properties (Ares & Fownes, 1999; Burnett et al., 2022).

Assuming water is more limited in the plantation, higher $\delta^{13}C$ foliar values among *A. koa* and *Rubus* sp. individuals in the plantation may indicate higher rates of photosynthesis (reduced $^{13}CO_2$ discrimination) and/or higher $WUE_i$ relative to the remnant forest (Farquhar, Ehleringer & Hubick, 1989; Cernusak et al., 2013). Considering the similarity in soil and foliar N in the two forests, we expect the differences in $\delta^{13}C$ values are more likely to relate to $WUE_i$ and not increased rates of photosynthesis. In support of this, (Ares & Fownes, 1999) found *A. koa* foliar $\delta^{13}C$ values increased across a gradient of decreasing rainfall, and $WUE_i$ increased in greenhouse grown seedlings experiencing drought-stress. Furthermore, it has been documented that remnant native Hawaiian forests conserve more water (Kagawa et al., 2009) and contribute more to aquifer recharge compared to plantation forests (Brauman, Freyberg & Daily, 2015). More stable water availability and less draw down of water resources in the remnant forest is consistent with patterns of greater stomatal conductance, higher ci/ca, and lower $WUE_i$ (Farquhar *et al.* 1989). Therefore, lower foliar $\delta^{13}C$ values in *A. koa* and *Rubus* sp. in the remnant forest may be an

effect of less water demand in native/remnant forests, better soil water holding capacity, or both (Fig. 2). This finding is important, as the greater abundance of $C_4$ grasses in the understory of the plantation (Yelenik, 2017) may relate to the greater competitive ability of these grasses for water resources compared to native understory plants.

Photosynthetic fractionation is less in $C_4$ than $C_3$ plants, resulting in $^{13}C$-enrichment and higher leaf $\delta^{13}C$ values ranging from -10‰ to -15‰ compared to $C_3$ plants (-21‰ to - 30‰) (Farquhar, Ehleringer & Hubick, 1989; Ehleringer, Buchmann & Flanagan, 2000). In the plantation soils, a clear pattern emerges of higher soil $\delta^{13}C$ values, consistent with a greater contribution of $C_4$-derived C to soils (Staddon, 2004). This finding is also supported by the soil $\delta^{13}C$ isoscape (Fig. 4), where we observed increasing soil $\delta^{13}C$ values in areas where grasses were dominant. N availability also influences rates of decomposition and has a direct influence on soil C pools (Averill & Waring, 2018); therefore, greater BNF in the plantation could accelerate soil C turnover, which may result in soils enriched in $^{13}C$ (Choi et al., 2005). Accordingly, future studies should examine isotope values in understory grasses and how low C:N leaf litter such as *A. koa*, is assimilated into soil and understory plant biomass, especially in the nitrophilous kikuyu grass (*Cenchrus clandestinus*). Follow up studies are on-going to determine rates of soil nutrient cycling in Hakalau, especially in order to disentangle how soil C, N, and leaf litter C:N influence soil $\delta^{13}C$ values.

## Conclusion

Recuperating biogeochemical properties in secondary tropical forests is vital to supporting plant growth and post-disturbance recovery (Sullivan et al., 2019). In some cases $N_2$-fixing plants may therefore be useful in facilitating nutrient and plant community recovery

(Chaer et al., 2011). However, our data suggest that monoculture plantations of a native $N_2$-fixing tree can lead to an increase in BNF and a more homogeneous distribution of fixed N in plants and soils that is unlike the distribution of N in a primary forest – the target habitat for restoration (Figs. 4 & 5). If this "plantation-effect" promotes undesirable species such as the non-native grasses in our plantation site, then creating a homogeneous area of high BNF may hinder restoration goals. Therefore, restoration efforts should consider how the abundance and density of $N_2$-fixing trees may influence plant-plant and plant-soil interactions and the potential for restoration to produce ecosystem states that differ from reference ecosystem counterparts. Our study represents a subset of the larger remnant forest and koa plantations in Hakalau and many more restoration areas and forest reserves exist across Hawai'i Island (Lawson & Pike, 2017). Considering the wide application of $N_2$-fixing trees in tropical forest restoration, both in Hawai'i and abroad, there remains a need to better understand how BNF by canopy trees varies within-and-among forest areas, across environmental conditions and habitat types, and its role in affecting nutrient cycling and forest communities. Addressing this uncertainty will support effective restoration strategic planning (i.e., outplanting density, multiple species planting) and management goals (i.e., habitat restoration, seedling and avian recruitment). Based on our findings in Hakalau, we suggest future restoration efforts might include a greater diversity of $N_2$-fixing and non-$N_2$-fixing canopy tree species to generate greater variation in understory and soil conditions more typical of remnant or intact forests.

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**Data accessibility**

All data and code are available at Github (https://github.com/cbwall/Hakalau) and are published at Zenodo (Wall et al, 2023).

**Conflict of interest**

The authors declare no conflict of interest

573 **Figure legends**

574 **Figure 1.** Site map of (**A**) the Hawaiian Archipelago highlighting the location of Hakalau Forest
575 National Wildlife Refuge (red diamond) on the island of Hawai‘i; (**B**) Koa plantation and
576 remnant forest sampling areas (KP, RK); (**C, D**) layouts (20 × 35 m), with plot corners in red
577 circles and *Acacia koa* trees in green triangles; (**E**) images of the koa plantation and (**F**)
578 perimeter of the remnant forest. Image credit: (A-D) GoogleMaps from R package ggmap, (E-F)
579 L. Kersting.

580

581 **Figure 2.** Soil and foliar (*Acacia koa, Rubus* spp.) (**A**) $\delta^{15}N$, (**B**) $\delta^{13}C$, (**C**) total nitrogen, and (**D**)
582 total carbon in koa plantation (KP) and remnant (RK) Hakalau forests. Asterisks indicate
583 significant differences between forests within a sample type ($p < 0.05$). Values are mean±SE,
584 $n$=48 (soil), 18 and 10 [*Acacia koa*], 28 and 14 [*Rubus* spp.] in KP and RK forests, respectively.

585

586 **Figure 3.** (**A**, **B**) Density plot of soil $\delta^{15}N_{predicted}$ (*left*), soil $\delta^{13}C_{predicted}$ (*middle*), and (**C**) foliar
587 $\delta^{15}N_{predicted}$ samples (*Acacia koa* and *Rubus* spp., *right*) from isoscapes in the koa plantation (KP)
588 and remnant (RK) Hakalau forests. Dashed lines indicate mean values in each forest.

589

590 **Figure 4.** (**A**, **B**) Soil $\delta^{15}N$ and (**C**, **D**) $\delta^{13}C$ isoscapes for Hakalau koa plantation (KP, *top*) and
591 remnant forests (RK, *bottom*). Color bar represents $\delta^{15}N$ and $\delta^{13}C$ values. Symbols represent
592 locations where soil (open circles), *Acacia koa* (filled triangles), and *Rubus* spp. (open triangles)
593 samples were collected; only soil samples were used in spatial interpolation.

594

595 **Figure 5.** (**A**) Foliar $\delta^{15}N$ for Hakalau koa plantation (KP, *top*) and (**B**) remnant forests (RK,
596 bottom). Color bar represents $\delta^{15}N$ and $\delta^{13}C$ values. Symbols represent locations where soil
597 (open circles), *Acacia koa* (filled triangles), and *Rubus* spp. (open triangles) samples were
598 collected; only foliar samples were used in spatial interpolation.
599

600 **References**

601 Aerts R, Honnay O. 2011. Forest restoration, biodiversity and ecosystem functioning. *BMC*
602 *ecology* 11:29. DOI: 10.1186/1472-6785-11-29.
603 Ares A, Fownes JH. 1999. Water supply regulates structure, productivity, and water use
604 efficiency of *Acacia koa* forest in Hawaii. *Oecologia* 121:458–466. DOI:
605 10.1007/s004420050952.
606 Asner GP, Hughes RF, Vitousek PM, Knapp DE, Kennedy-Bowdoin T, Boardman J, Martin RE,
607 Eastwood M, Green RO. 2008. Invasive plants transform the three-dimensional structure of
608 rain forests. *Proceedings of the National Academy of Sciences of the United States of*
609 *America* 105:4519–4523. DOI: 10.1073/pnas.0710811105.
610 Austin AT, Vitousek PM. 1998. Nutrient dynamics on a precipitation gradient in Hawai'i.
611 *Oecologia* 113:519–529. DOI: 10.1007/s004420050405.
612 Averill C, Waring B. 2018. Nitrogen limitation of decomposition and decay: How can it occur?
613 *Global change biology* 24:1417–1427. DOI: 10.1111/gcb.13980.
614 Baker PJ, Scowcroft PG, Ewel JJ. 2009. *Koa (Acacia Koa) Ecology and Silviculture*. United
615 States Department of Agriculture, Forest Service, Pacific Southwest Research Station.
616 Barron AR, Purves DW, Hedin LO. 2011. Facultative nitrogen fixation by canopy legumes in a
617 lowland tropical forest. *Oecologia* 165:511–520. DOI: 10.1007/s00442-010-1838-3.
618 Batterman SA, Hedin LO, van Breugel M, Ransijn J, Craven DJ, Hall JS. 2013. Key role of
619 symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* 502:224–227.
620 DOI: 10.1038/nature12525.
621 Bivand RS, Pebesma E, Gómez-Rubio V. 2013. *Applied Spatial Data Analysis with R*. Springer
622 New York. DOI: 10.1007/978-1-4614-7618-4.

623 Bothwell LD, Selmants PC, Giardina CP, Litton CM. 2014. Leaf litter decomposition rates
624 increase with rising mean annual temperature in Hawaiian tropical montane wet forests.
625 *PeerJ* 2:e685. DOI: 10.7717/peerj.685.
626 Bowen GJ. 2010. Isoscapes: Spatial pattern in isotopic biogeochemistry. *Annual review of earth*
627 *and planetary sciences* 38:161–187.
628 Brauman KA, Freyberg DL, Daily GC. 2015. Impacts of land-use change on groundwater
629 supply: ecosystem services assessment in Kona, Hawaii. *Journal of Water Resources*
630 *Planning and Management* 141:A4014001. DOI: 10.1061/(ASCE)WR.1943-5452.0000495.
631 Burnett MW, Bobbett AE, Brendel CE, Marshall K, von Sperber C, Paulus EL, Vitousek PM.
632 2022. Foliar $\delta^{15}N$ patterns in legumes and non-N fixers across a climate gradient, Hawai'i
633 Island, USA. *Oecologia* 198:229–242. DOI: 10.1007/s00442-021-05089-1.
634 Cernusak LA, Ubierna N, Winter K, Holtum JAM, Marshall JD, Farquhar GD. 2013.
635 Environmental and physiological determinants of carbon isotope discrimination in terrestrial
636 plants. *The New phytologist* 200:950–965. DOI: 10.1111/nph.12423.
637 Chaer GM, Resende AS, Campello EFC, de Faria SM, Boddey RM. 2011. Nitrogen-fixing
638 legume tree species for the reclamation of severely degraded lands in Brazil. *Tree*
639 *physiology* 31:139–149. DOI: 10.1093/treephys/tpq116.
640 Chazdon RL. 2003. Tropical forest recovery: legacies of human impact and natural disturbances.
641 *Perspectives in Plant Ecology, Evolution and Systematics* 6:51–71. DOI: 10.1078/1433-
642 8319-00042.
643 Chazdon RL, Broadbent EN, Rozendaal DMA, Bongers F, Zambrano AMA, Aide TM,
644 Balvanera P, Becknell JM, Boukili V, Brancalion PHS, Craven D, Almeida-Cortez JS,
645 Cabral GAL, de Jong B, Denslow JS, Dent DH, DeWalt SJ, Dupuy JM, Durán SM, Espírito-

Santo MM, Fandino MC, César RG, Hall JS, Hernández-Stefanoni JL, Jakovac CC, Junqueira AB, Kennard D, Letcher SG, Lohbeck M, Martínez-Ramos M, Massoca P, Meave JA, Mesquita R, Mora F, Muñoz R, Muscarella R, Nunes YRF, Ochoa-Gaona S, Orihuela-Belmonte E, Peña-Claros M, Pérez-García EA, Piotto D, Powers JS, Rodríguez-Velazquez J, Romero-Pérez IE, Ruíz J, Saldarriaga JG, Sanchez-Azofeifa A, Schwartz NB, Steininger MK, Swenson NG, Uriarte M, van Breugel M, van der Wal H, Veloso MDM, Vester H, Vieira ICG, Bentos TV, Williamson GB, Poorter L. 2016. Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. *Science advances* 2:e1501639. DOI: 10.1126/sciadv.1501639.

Cheesman AW, Cernusak LA. 2016. Isoscapes: a new dimension in community ecology. *Tree physiology* 36:1456–1459. DOI: 10.1093/treephys/tpw099.

Choi W-J, Chang SX, Allen HL, Kelting DL, Ro H-M. 2005. Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. *Forest ecology and management* 213:90–101. DOI: 10.1016/j.foreco.2005.03.016.

Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E, Wang L. 2015. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and soil* 396:1–26. DOI: 10.1007/s11104-015-2542-1.

Cramer W, Bondeau A, Schaphoff S, Lucht W, Smith B, Sitch S. 2004. Tropical forests and the global carbon cycle: impacts of atmospheric carbon dioxide, climate change and rate of deforestation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359:331–343. DOI: 10.1098/rstb.2003.1428.

Crockatt ME, Bebber DP. 2015. Edge effects on moisture reduce wood decomposition rate in a temperate forest. *Global change biology* 21:698–707. DOI: 10.1111/gcb.12676.

669 Dixon ER, Blackwell MSA, Dhanoa MS, Berryman Z, de la Fuente Martinez N, Junquera D,
670 Martinez A, Murray PJ, Kemp HF, Meier-Augenstein W, Duffy A, Bol R. 2010.
671 Measurement at the field scale of soil $\delta^{13}C$ and $\delta^{15}N$ under improved grassland. *Rapid*
672 *communications in mass spectrometry: RCM* 24:511–518. DOI: 10.1002/rcm.4345.
673 Driscoll AW, Bitter NQ, Sandquist DR, Ehleringer JR. 2020. Multidecadal records of intrinsic
674 water-use efficiency in the desert shrub *Encelia farinosa* reveal strong responses to climate
675 change. *Proceedings of the National Academy of Sciences of the United States of America*
676 117:18161–18168. DOI: 10.1073/pnas.2008345117.
677 Ehleringer JR, Buchmann N, Flanagan LB. 2000. Carbon isotope ratios in belowground carbon
678 cycle processes. *Ecological applications: a publication of the Ecological Society of America*
679 10:412–422. DOI: 10.2307/2641103.
680 Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and
681 photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* 40:503–
682 537. DOI: 10.1146/annurev.pp.40.060189.002443.
683 Fisher RF. 1995. Amelioration of degraded rain forest soils by plantations of native trees. *Soil*
684 *Science Society of America Journal. Soil Science Society of America* 59:544–549. DOI:
685 10.2136/sssaj1995.03615995005900020039x.
686 Fortin M-J, Dale MRT, Ver Hoef JM. 2012. Spatial Analysis in Ecology. In: *Encyclopedia of*
687 *Environmetrics*. Wiley,. DOI: 10.1002/9780470057339.vas039.pub2.
688 Fox J, Weisberg S, Price B, Adler D, Bates D, Baud-Bovy G, Bolker B, Others. 2019. *car:*
689 *Companion to Applied Regression. R package version 3.0-2*. Sage.
690 Funk JL, Vitousek PM. 2007. Resource-use efficiency and plant invasion in low-resource
691 systems. *Nature* 446:1079–1081. DOI: 10.1038/nature05719.

692 Gebauer G, Schulze E-D. 1991. Carbon and nitrogen isotope ratios in different compartments of
693 a healthy and a declining *Picea abies* forest in the Fichtelgebirge, NE Bavaria. *Oecologia*
694 87:198–207. DOI: 10.1007/BF00325257.
695 Handa IT, Aerts R, Berendse F, Berg MP, Bruder A, Butenschoen O, Chauvet E, Gessner MO,
696 Jabiol J, Makkonen M, McKie BG, Malmqvist B, Peeters ETHM, Scheu S, Schmid B, van
697 Ruijven J, Vos VCA, Hättenschwiler S. 2014. Consequences of biodiversity loss for litter
698 decomposition across biomes. *Nature* 509:218–221. DOI: 10.1038/nature13247.
699 Hart PJ. 2010. Tree growth and age in an ancient Hawaiian wet forest: vegetation dynamics at
700 two spatial scales. *Journal of Tropical Ecology* 26:1–11. DOI:
701 10.1017/S0266467409990320.
702 Heaton THE. 1987. The $^{15}N^{14}N$ ratios of plants in South Africa and Namibia: Relationship to
703 climate and coastal/saline environments. *Oecologia* 74:236–246.
704 Hellmann C, Rascher KG, Oldeland J, Werner C. 2016. Isoscapes resolve species-specific spatial
705 patterns in plant-plant interactions in an invaded Mediterranean dune ecosystem. *Tree*
706 *physiology* 36:1460–1470. DOI: 10.1093/treephys/tpw075.
707 Hellmann C, Sutter R, Rascher KG, Máguas C, Correia O, Werner C. 2011. Impact of an exotic
708 $N_2$-fixing *Acacia* on composition and N status of a native Mediterranean community. *Acta*
709 *Oecologica* 37:43–50. DOI: 10.1016/j.actao.2010.11.005.
710 Hiemstra PH, Pebesma EJ, Twenhöfel CJW, Heuvelink GBM. 2009. Real-time automatic
711 interpolation of ambient gamma dose rates from the Dutch radioactivity monitoring
712 network. *Computers & Geosciences* 35:1711–1721. DOI: 10.1016/j.cageo.2008.10.011.
713 Hoogmoed M, Cunningham SC, Baker P, Beringer J, Cavagnaro TR. 2014. N-fixing trees in
714 restoration plantings: Effects on nitrogen supply and soil microbial communities. *Soil*

715 *Biology & Biochemistry* 77:203–212. DOI: 10.1016/j.soilbio.2014.06.008.
716 Jeffrey J, Horiuchi B. 2003. Tree planting at Hakalau Forest National Wildlife Refuge —the
717 right tool for the right stock type. *Native Plants Journal* 4:30–31. DOI: 10.3368/npj.4.1.30.
718 Kagawa A, Sack L, Duarte K'eo, James S. 2009. Hawaiian native forest conserves water relative
719 to timber plantation: species and stand traits influence water use. *Ecological Applications: a*
720 *Publication of the Ecological Society of America* 19:1429–1443. DOI: 10.1890/08-1704.1.
721 Kou-Giesbrecht S, Menge D. 2019. Nitrogen-fixing trees could exacerbate climate change under
722 elevated nitrogen deposition. *Nature Communications* 10:1493. DOI: 10.1038/s41467-019-
723 09424-2.
724 Koutika L-S, Taba K, Ndongo M, Kaonga M. 2021. Nitrogen-fixing trees increase organic
725 carbon sequestration in forest and agroforestry ecosystems in the Congo basin. *Regional*
726 *Environmental Change* 21:109. DOI: 10.1007/s10113-021-01816-9.
727 Lanuza O, Casanoves F, Zahawi RA, Celentano D, Delgado D, Holl KD. 2018. Litterfall and
728 nutrient dynamics shift in tropical forest restoration sites after a decade of recovery.
729 *Biotropica* 50:491–498. DOI: 10.1111/btp.12533.
730 Lawson S, Pike C. 2017. Stable isotope ratios and reforestation potential in *Acacia koa*
731 populations on Hawai'i. *Annals of Forest Research* 60:279–295. DOI:
732 10.15287/afr.2017.805.
733 Lenth RV. 2022. Estimated marginal means, aka least-squares means [R package emmeans
734 version 1.8.1-1]. :1–3.
735 Levy-Varon JH, Batterman SA, Medvigy D, Xu X, Hall JS, van Breugel M, Hedin LO. 2019.
736 Tropical carbon sink accelerated by symbiotic dinitrogen fixation. *Nature Communications*
737 10:5637. DOI: 10.1038/s41467-019-13656-7.

738 Lüdecke D. 2021. sjstats: Statistical functions for regression models (Version 0.18.1). DOI:
739 10.5281/zenodo.1284472.
740 Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, Mcdowell W, Robertson
741 GP, Santos OC, Treseder K. 1999. Nitrogen stable isotopic composition of leaves and soil:
742 Tropical versus temperate forests. In: Townsend AR ed. *New Perspectives on Nitrogen*
743 *Cycling in the Temperate and Tropical Americas: Report of the International SCOPE*
744 *Nitrogen Project*. Dordrecht: Springer Netherlands, 45–65. DOI: 10.1007/978-94-011-4645-
745 6_3.
746 McCue MD, Javal M, Clusella-Trullas S, Le Roux JJ, Jackson MC, Ellis AG, Richardson DM,
747 Valentine AJ, Terblanche JS. 2020. Using stable isotope analysis to answer fundamental
748 questions in invasion ecology: Progress and prospects. *Methods in ecology and evolution*
749 11:196–214. DOI: 10.1111/2041-210X.13327.
750 McDaniel S, Loh R, Dale S, Yanger C. 2011. *Experimental restoration of mesic and wet forests*
751 *in former pastureland, Kahuku Unit, Hawaii Volcanoes National Park*. Pacific Cooperate
752 Studies Unit, UH Manoa.
753 McDaniel S, Ostertag R. 2010. Strategic light manipulation as a restoration strategy to reduce
754 alien grasses and encourage native regeneration in Hawaiian mesic forests. *Applied*
755 *Vegetation Science* 13:280–290. DOI: 10.1111/j.1654-109X.2009.01074.x.
756 Meeuwig RO, Cooper SV, Forestry Sciences Laboratory (Missoula M)., Service. USF, Forest I,
757 Range Experiment Station (Ogden U. 1981. *Stand estimates of biomass and growth on*
758 *pinyon-juniper woodlands in Nevada*. Ogden, Utah, U.S. Dept. of Agriculture, Forest
759 Service, Intermountain Forest and Range Experiment Station, 1981.
760 Meinzer FC, Fownes JH, Harrington RA. 1996. Growth indices and stomatal control of

761 transpiration in Acacia koa stands planted at different densities. *Tree Physiology* 16:607–
762 615. DOI: 10.1093/treephys/16.7.607.
763 Moyer-Henry KA, Burton JW, Israel DW, Rufty TW. 2006. Nitrogen transfer between plants: A
764 $^{15}N$ natural abundance study with crop and weed species. *Plant and Soil* 282:7–20. DOI:
765 10.1007/s11104-005-3081-y.
766 Natelhoffer KJ, Fry B. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest
767 soil organic matter. *Soil Science Society of America Journal* 52:1633–1640. DOI:
768 10.2136/sssaj1988.03615995005200060024x.
769 Pau S, Gillespie TW, Price JP. 2009. Natural history, biogeography, and endangerment of
770 Hawaiian dry forest trees. *Biodiversity and Conservation* 18:3167. DOI: 10.1007/s10531-
771 009-9635-1.
772 Paxton EH, Yelenik SG, Borneman TE, Rose ET, Camp RJ, Kendall SJ. 2018. Rapid
773 colonization of a Hawaiian restoration forest by a diverse avian community: Bird
774 colonization of tropical restoration forest. *Restoration Ecology* 26:165–173. DOI:
775 10.1111/rec.12540.
776 Pearson HL, Vitousek PM. 2001. Stand dynamics, nitrogen accumulation, and symbiotic
777 nitrogen fixation in regenerating stands of *Acacia koa*. *Ecological Applications* 11:1381–
778 1394. DOI: 10.1890/1051-0761(2001)011[1381:SDNAAS]2.0.CO;2.
779 Pearson HL, Vitousek PM. 2002. Soil phosphorus fractions and symbiotic nitrogen fixation
780 across a substrate-age gradient in Hawaii. *Ecosystems* 5:587–596. DOI: 10.1007/s10021-
781 002-0172-y.
782 Pebesma EJ, Bivand RS. 2005. Classes and methods for spatial data in R. *R News* 5:9–13.
783 Rascher KG, Hellmann C, Máguas C, Werner C. 2012. Community scale $^{15}N$ isoscapes: tracing

784 the spatial impact of an exotic $N_2$-fixing invader. *Ecology Letters* 15:484–491. DOI:
785 10.1111/j.1461-0248.2012.01761.x.
786 R Core Team. 2022. *R: A language and environment for statistical computing*. Vienna, Austria:
787 R Foundation for Statistical Computing.
788 Resh SC, Binkley D, Parrotta JA. 2002. Greater soil carbon sequestration under nitrogen-fixing
789 trees compared with *Eucalyptus* species. *Ecosystems* 5:217–231. DOI: 10.1007/s10021-001-
790 0067-3.
791 Rhoades CC, Eckert GE, Coleman DC. 1998. Effect of pasture trees on soil nitrogen and organic
792 matter: Implications for tropical montane forest restoration. *Restoration Ecology* 6:262–270.
793 DOI: 10.1046/j.1526-100x.1998.00639.x.
794 Robinson D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution*
795 16:153–162. DOI: 10.1016/S0169-5347(00)02098-X.
796 Scowcroft PG, Friday JB, Idol T, Dudley N, Haraguchi J, Meason D. 2007. Growth response of
797 *Acacia koa* trees to thinning, grass control, and phosphorus fertilization in a secondary forest
798 in Hawai‘i. *Forest Ecology and Management* 239:69–80. DOI:
799 10.1016/j.foreco.2006.11.009.
800 Scowcroft PG, Haraguchi JE, Hue NV. 2004. Reforestation and topography affect Montane soil
801 properties, nitrogen pools, and nitrogen transformations in Hawaii. *Soil Science Society of*
802 *America Journal* 68:959. DOI: 10.2136/sssaj2004.0959.
803 Sitters J, Edwards PJ, Olde Venterink H. 2013. Increases of soil C, N, and P pools along an
804 *Acacia* tree density gradient and their effects on trees and grasses. *Ecosystems* 16:347–357.
805 DOI: 10.1007/s10021-012-9621-4.
806 Staddon PL. 2004. Carbon isotopes in functional soil ecology. *Trends in Ecology & Evolution*

807 19:148–154. DOI: 10.1016/j.tree.2003.12.003.
808 Stinca A, Chirico GB, Incerti G, Bonanomi G. 2015. Regime shift by an exotic nitrogen-fixing
809 shrub mediates plant facilitation in primary succession. *PloS one* 10:e0123128. DOI:
810 10.1371/journal.pone.0123128.
811 Sullivan BW, Nifong RL, Nasto MK, Alvarez-Clare S, Dencker CM, Soper FM, Shoemaker KT,
812 Ishida FY, Zaragoza-Castells J, Davidson EA, Cleveland CC. 2019. Biogeochemical
813 recuperation of lowland tropical forest during succession. *Ecology* 100:e02641. DOI:
814 10.1002/ecy.2641.
815 Sullivan BW, Smith WK, Townsend AR, Nasto MK, Reed SC, Chazdon RL, Cleveland CC.
816 2014. Spatially robust estimates of biological nitrogen (N) fixation imply substantial human
817 alteration of the tropical N cycle. *Proceedings of the National Academy of Sciences of the*
818 *United States of America* 111:8101–8106. DOI: 10.1073/pnas.1320646111.
819 Veldkamp E, Schmidt M, Powers JS, Corre MD. 2020. Deforestation and reforestation impacts
820 on soils in the tropics. *Nature Reviews Earth & Environment*. DOI: 10.1038/s43017-020-
821 0091-5.
822 Vitousek PM, Farrington H. 1997. Nutrient limitation and soil development: Experimental test of
823 a biogeochemical theory. *Biogeochemistry* 37:63–75. DOI: 10.1023/A:1005757218475.
824 Wintle BA, Kujala H, Whitehead A, Cameron A, Veloz S, Kukkala A, Moilanen A, Gordon A,
825 Lentini PE, Cadenhead NCR, Bekessy SA. 2019. Global synthesis of conservation studies
826 reveals the importance of small habitat patches for biodiversity. *Proceedings of the National*
827 *Academy of Sciences of the United States of America* 116:909–914. DOI:
828 10.1073/pnas.1813051115.
829 Wynn JG, Bird MI. 2007. C4-derived soil organic carbon decomposes faster than its C3

counterpart in mixed C3/C4 soils. *Global Change Biology* 13:2206–2217. DOI: 10.1111/j.1365-2486.2007.01435.x.

Yelenik SG. 2017. Linking dominant Hawaiian tree species to understory development in recovering pastures via impacts on soils and litter: Canopy tree effects on understory regeneration. *Restoration Ecology* 25:42–52. DOI: 10.1111/rec.12377.

Yelenik SG, D'Antonio CM, Rehm EM, Caldwell I. 2021. Multiple feedbacks due to biotic interactions across trophic levels can lead to persistent novel conditions that hinder restoration. In: *Plant Invasions: The role of biotic interactions*. CABI, 402–420.

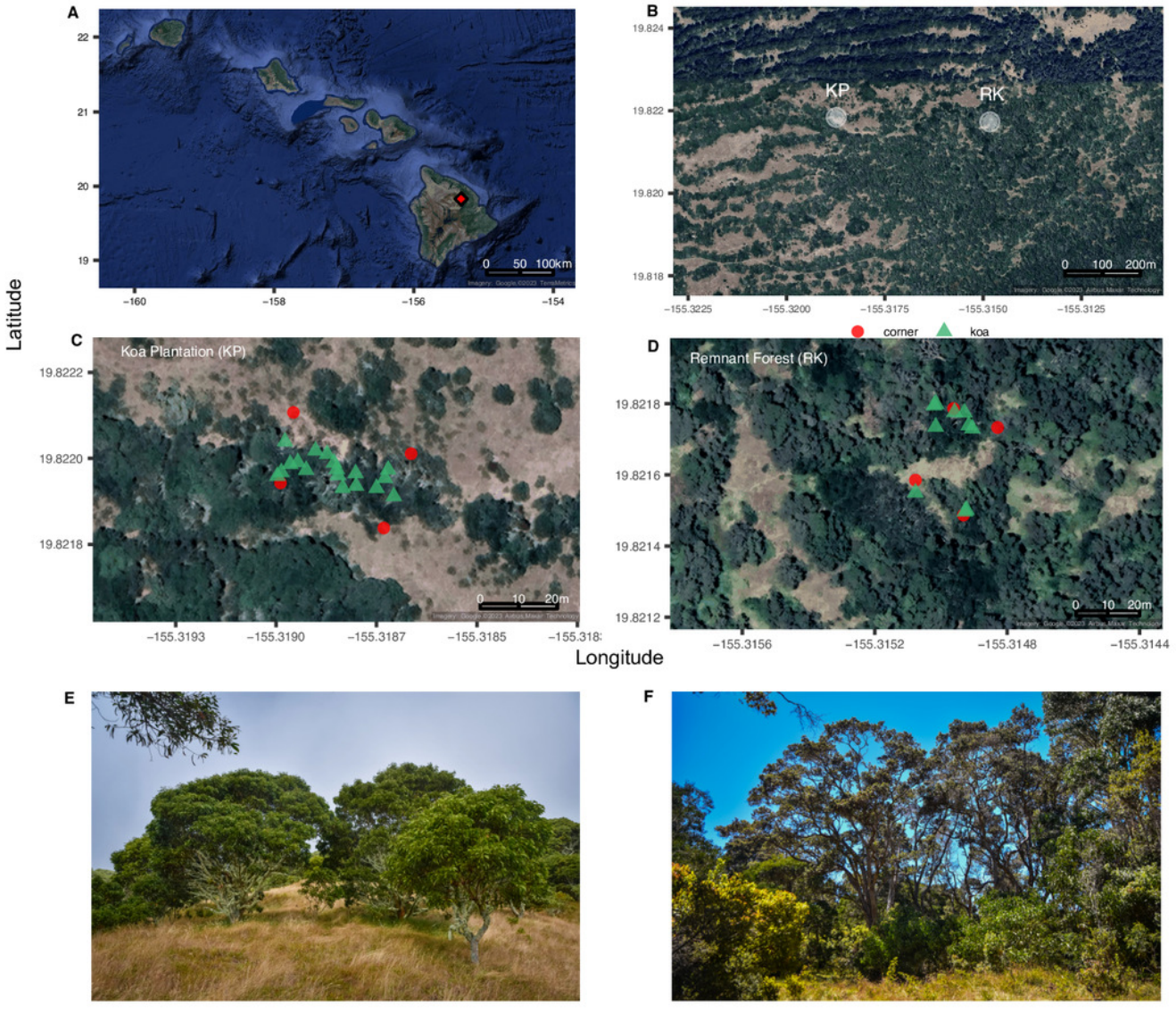
Yelenik SG, Rehm EM, D'Antonio CM. 2022. Can the impact of canopy trees on soil and understory be altered using litter additions? *Ecological applications: a publication of the Ecological Society of America* 32:e02477. DOI: 10.1002/eap.2477.

Zhou G, Zhang J, Qiu X, Wei F, Xu X. 2018. Decomposing litter and associated microbial activity responses to nitrogen deposition in two subtropical forests containing nitrogen-fixing or non-nitrogen-fixing tree species. *Scientific Reports* 8:12934. DOI: 10.1038/s41598-018-30666-5.

# Figure 1

The Hawaiian Archipelago and sampling sites.

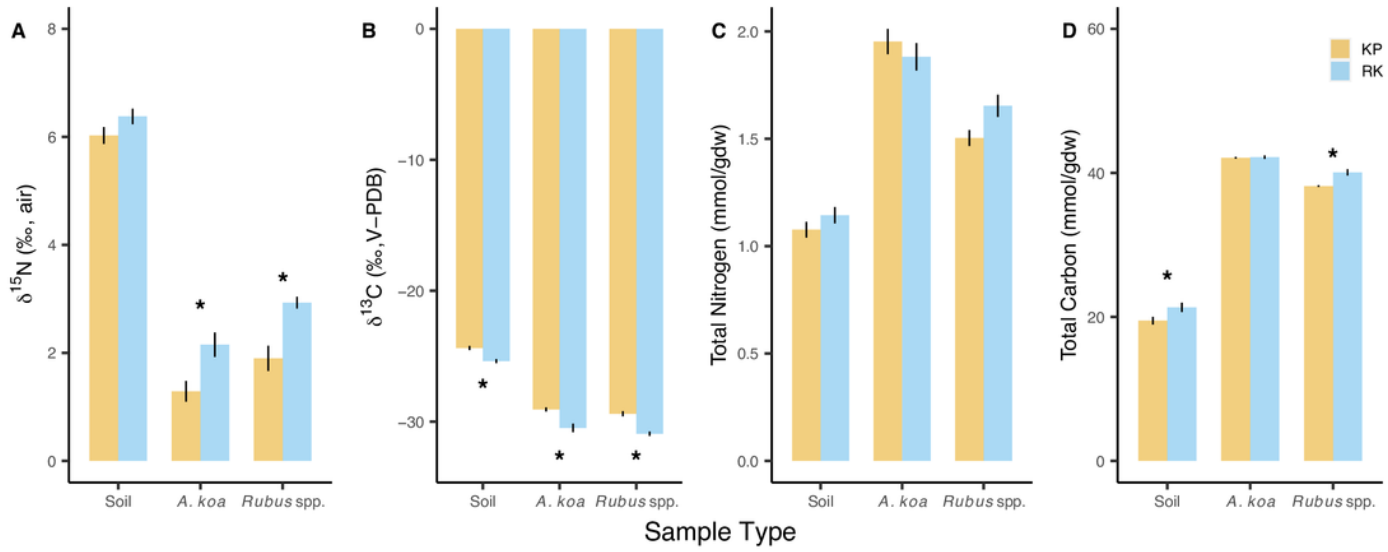
Site map of (**A**) the Hawaiian Archipelago highlighting the location of Hakalau Forest National Wildlife Refuge (red diamond) on the island of Hawai'i; (**B**) Koa plantation and remnant forest sampling areas (KP, RK); (**C, D**) layouts (20 × 35 m), with plot corners in red circles and *Acacia koa* trees in green triangles; (**E**) images of the koa plantation and (**F**) perimeter of the remnant forest. Image credit: (A-D) GoogleMaps from R package ggmap, (E-F) L. Kersting.



# Figure 2

Soil and foliar isotope values and elemental concentrations.

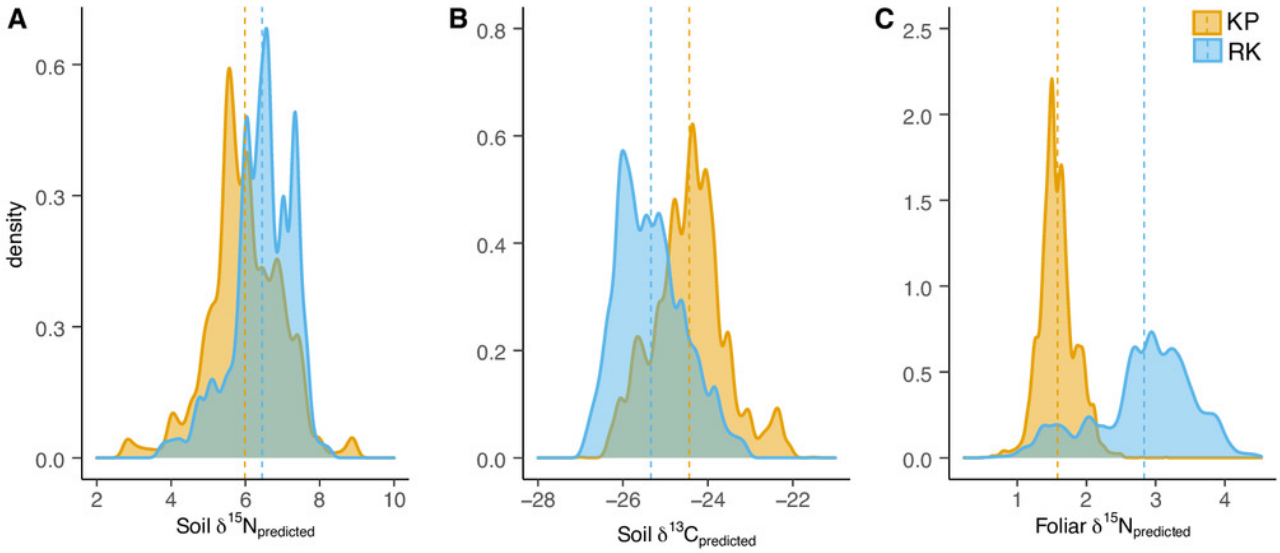
Soil and foliar (*Acacia koa, Rubus* spp.) (**A**) $\delta^{15}N$, (**B**) $\delta^{13}C$, (**C**) total nitrogen, and (**D**) total carbon in koa plantation (KP) and remnant (RK) Hakalau forests. Asterisks indicate significant differences between forests within a sample type ($p$ <0.05). Values are mean±SE, $n$=48 (soil), 18 and 10 [*Acacia koa*], 28 and 14 [*Rubus* spp.] in KP and RK forests, respectively.



# Figure 3

Soil and foliar isoscape density plots

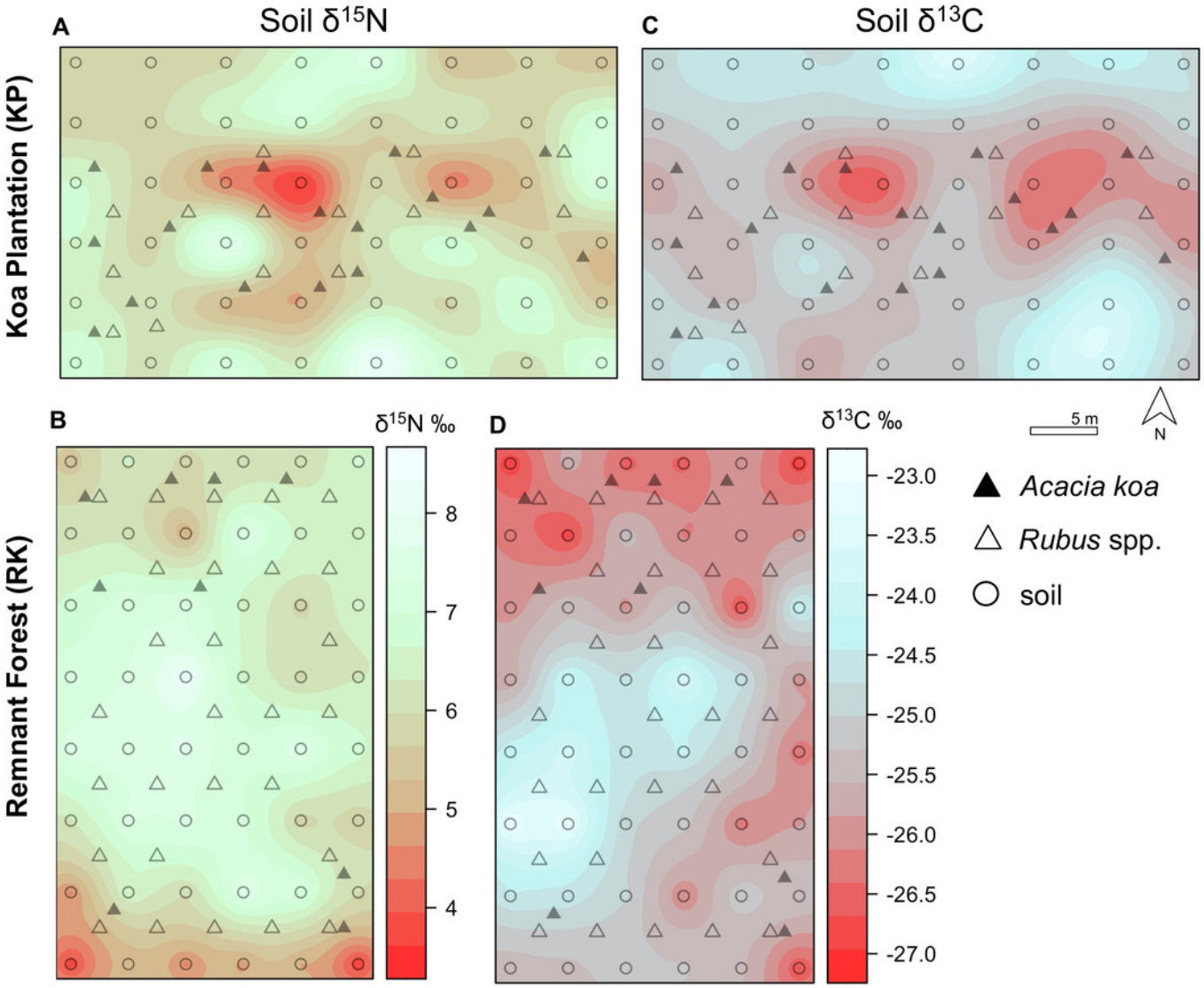
(**A**, **B**) Density plot of soil $\delta^{15}N_{predicted}$ (*left*), soil $\delta^{13}C_{predicted}$ (*middle*), and (**C**) foliar $\delta^{15}N_{predicted}$ samples (*Acacia koa* and *Rubus* spp., *right*) from isoscapes in the koa plantation (KP) and remnant (RK) Hakalau forests. Dashed lines indicate mean values in each forest.



# Figure 4

Soil $\delta^{15}N$ and $\delta^{13}C$ isoscapes

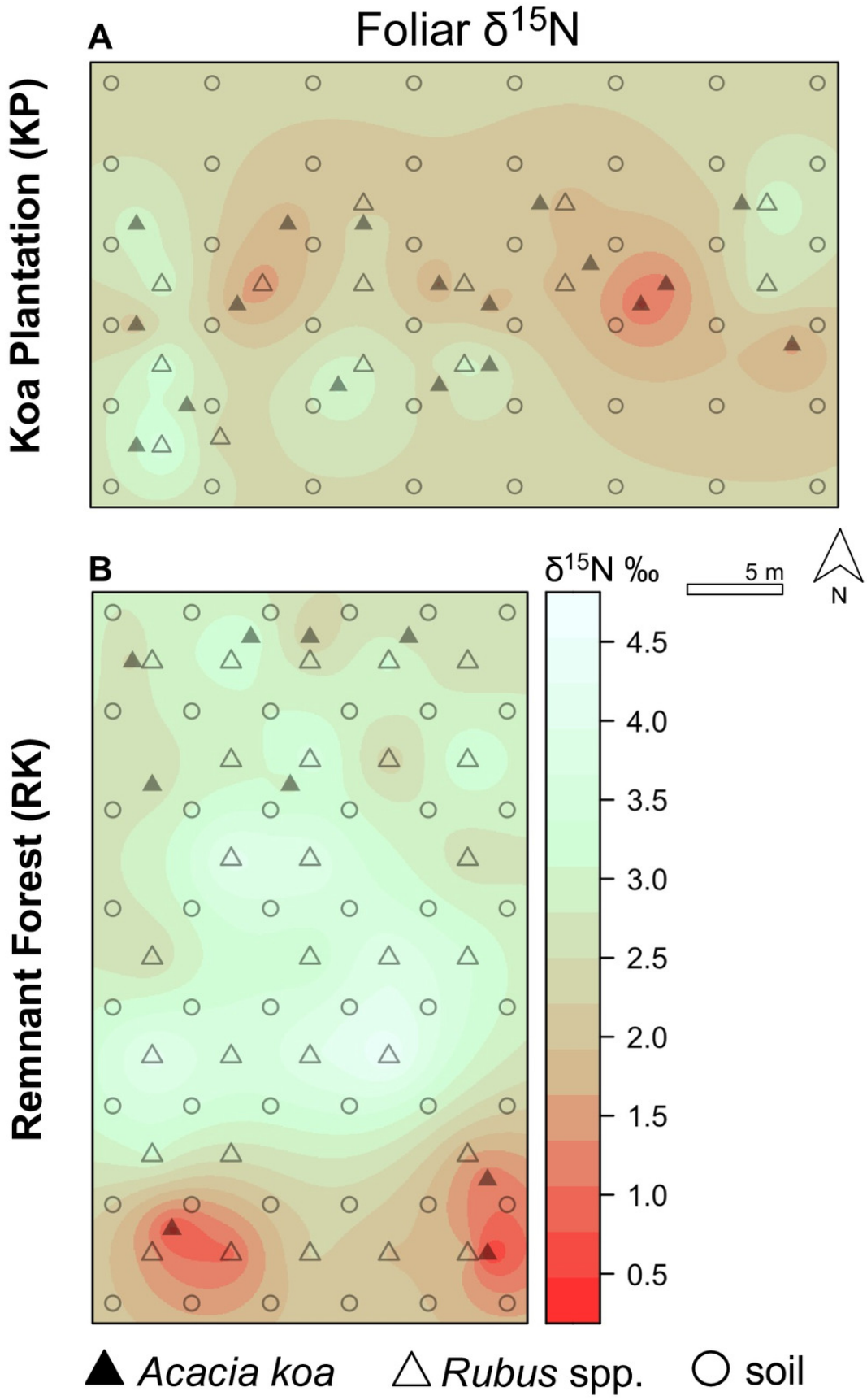
(**A**, **B**) Soil $\delta^{15}N$ and (**C**, **D**) $\delta^{13}C$ isoscapes for Hakalau koa plantation (KP, *top*) and remnant forests (RK, *bottom*). Color bar represents $\delta^{15}N$ and $\delta^{13}C$ values. Symbols represent locations where soil (open circles), *Acacia koa* (filled triangles), and *Rubus* spp. (open triangles) samples were collected; only soil samples were used in spatial interpolation.



# Figure 5

Foliar $\delta^{15}N$ isoscapes

(**A**) Foliar $\delta^{15}N$ for Hakalau koa plantation (KP, *top*) and (**B**) remnant forests (RK, bottom). Color bar represents $\delta^{15}N$ and $\delta^{13}C$ values. Symbols represent locations where soil (open circles), *Acacia koa* (filled triangles), and *Rubus* spp. (open triangles) samples were collected; only foliar samples were used in spatial interpolation.


A
Foliar δ15N
Koa Plantation (KP)
B
Remnant Forest (RK)
δ15N ‰
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
5 m
N
Acacia koa
Rubus spp.
soil