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Impacts of endangered seabirds on nutrient cycling in montane forest ecosystems of Hawai‘i

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Abstract

Allochthonous nutrient flow from marine sources via seabirds to the terrestrial habitats where they nest can impact resident organisms and neighboring ecosystems. Seabird populations are decreasing both in Hawai‘i and globally, yet little is known about what is being lost from the ecosystems where they traditionally nested in large numbers. Given the marked decline in seabirds, we hypothesized that the current sparsely populated seabird colonies in wet montane ecosystems of Hawai‘i contribute minimally to nutrient availability but that this small contribution should still be reflected in vegetative uptake of soil N and in plant community composition. Soil nutrient availability on Kaua‘i was assessed using ion-exchange resin probes. Plant and soil uptake of marine-derived nitrogen was determined using $\delta^{15}N$ values in soil and foliage of the two dominant species using a two-end member N isotope mass balance mixing model. To determine if the added nutrients impacted the plant community, we also compared canopy cover (total and by dominant species), and species richness between treatments. Soil in seabird areas had more available ammonium, while nitrate and total inorganic N did not differ between sites. The dominant canopy tree, *Metrosideros polymorpha*, derived 28% of foliar N from marine sources, while this value was 15% for the dominant understory plant, *Dicranopteris linearis*. Plant species composition was not influenced by the presence or absence of seabirds. Because N plays a large role in net primary productivity, the use of marine-derived N by native plants under even limited seabird populations is likely important to the functioning of these ecosystems.

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Introduction

Globally, seabirds can be important drivers of nutrient cycling via allochthonous deposition of nutrients such as nitrogen (N) and phosphorus (P) that influence all trophic levels in the ecosystems where they breed and nest (Anderson and Polis 1999, Mulder and Keall 2001, Whelan *et al.* 2008, Towns *et al.* 2009, Grant-Hoffman *et al.* 2010). Guano naturally contains N and P, nutrients which commonly limit ecosystem processes in forest ecosystems globally (Hutchinson 1950, Elser *et al.* 2007). Guano composition for White-capped Noddies (*Anous minutus*) and Wedge-tailed Shearwaters (*Puffinus pacificus*) is approximately 7.3% N and 1.5% P (Staunton Smith and Johnson 1995), but N content of guano increases to 14.9–28.6% for pelagic seabirds such as Northern Fulmar (*Fulmarus glacialis*) and Manx Shearwater (*Puffinus puffinus*) (Burger *et al.* 1978, Bird *et al.* 2008). As such, seabird defecation can increase available pools of N and P in terrestrial ecosystems by 100 and 400 times, respectively (Mulder *et al.* 2011). In turn, marine-derived nutrient subsidies affect a suite of processes including primary productivity (Mulder and Keall 2001), plant community composition (Anderson *et al.* 2008, Mizota 2009), and the population size of top predators (Rose and Polis 1998).

The role of seabird guano in the transport of nutrients from marine sources to land has been studied in many coastal ecosystems, but tropical montane ecosystems where many burrowing seabirds nest have been poorly studied (Mulder *et al.* 2011). Nutrient levels in highly weathered tropical ecosystems can be low, particularly for P, and with high rainfall and steep slopes N and P can be readily lost through leaching and erosion (Ehleringer *et al.* 1986, Posada and Schuur 2011). In these nutrient poor ecosystems, the addition of N and P rich guano may be vital to maintaining or enhancing plant communities and ecosystem process rates (Mizota 2009).

Seabirds face many challenges including habitat loss, introduction of predators, collision with man-made structures, light pollution, toxins, change in prey availability, and poisoning

(Millenium Ecosystem Assessment 2005, Hebshi *et al.* 2008, Duffy 2010, Griesemer and Holmes 2011, Loss *et al.* 2012, 2015, Wiley *et al.* 2013). These challenges have typically led to severe population declines and in some cases extinctions, resulting in reduced nutrient inputs to the terrestrial habitats that seabirds traditionally occupied. In Hawaiʻi, seabirds that nest in montane forest ecosystems have experienced severe population declines, with some seabird species extirpated from the Hawaiian Islands and others remaining in greatly reduced ranges and numbers (Olson and James 1994, Burney *et al.* 2001, Hearty *et al.* 2005). The decline of nutrient flux from marine to terrestrial ecosystems due to the reduction of seabirds on the main Hawaiian Islands may have significant effects on plant community dynamics and ecosystem processes. As such, understanding how native plants utilize nutrient subsidies is important to inform the conservation and restoration of native habitats.

Two seabird species that have experienced drastic population declines in the montane regions of Hawaiʻi historically, as well as in the last 25 years, are the Newell's Shearwater (*Puffinus newelli*) and Hawaiian Petrel (*Pterodroma sandwichensis*), which are federally listed as threatened and endangered, respectively (James 1991, Burney *et al.* 2001, Griesemer and Holmes 2011). Formerly numerous and widespread, these two species are currently limited to remote colonies in hard to access locations due to loss of habitat and an increase in introduced predators. We sought to determine whether Newell's shearwaters and Hawaiian Petrels in low numbers still influence soil nutrient availability and plant nutrient uptake. Specifically, we sought to answer three primary research questions. First, do low numbers of seabirds increase the availability of macronutrients and micronutrients in wet montane forest soils? Second, do the dominant plants in wet montane ecosystems utilize marine-derived N, and if so to what extent? Third, does avian nutrient subsidy influence plant species composition? We hypothesized that: 1) soil micronutrient and macronutrient availability would be higher around the seabird colonies than in areas without seabirds, but only minimally given the greatly reduced seabird populations (Wainright *et al.* 1998, Liu *et al.* 2006); 2) $\delta^{15}N$ values would be higher in soil and foliage at seabird nesting sites, reflecting a marine-derived nutrient subsidy (Caut *et al.* 2012, Kazama *et al.* 2013), and; 3) the plant community composition in seabird plots would be biased towards nitrophilic plants adapted to high nutrient levels (Vitousek and Farrington 1997, Martinelli *et al.* 1999, Bond *et al.* 2010).

Materials and Methods

Study Site

Though greatly reduced from historic levels, the Island of Kauaʻi is home to the densest populations of montane nesting seabirds in the Hawaiian archipelago. Study sites were located in the montane forests of Upper Limahuli Preserve and Hono O Nā Pali, Kauaʻi. We considered two treatment types in each of these areas: active seabird colonies and non-seabird areas (areas without current seabird colonies and with no evidence of recent nesting) (Fig. 1). Notably, it is likely that seabirds historically nested in most, if not all, montane areas in the past, but the control sites contained no burrows (new or old), bird sign (e.g., feathers or guano), or records of bird activity since 2006 when the Kauaʻi Endangered Seabird Restoration Project began working in the area. Furthermore, the density of seabirds in the most heavily used areas is only 0.04 burrows m^{-2}, which is low for colonial nesting seabirds such as shearwaters and petrels which are known to nest up to 0.76 burrows m^{-2} for Grey-faced Petrel (*Pterodroma macroptera gouldi*; Whitehead *et al.* 2014) and 0.08 burrows m^{-2} for Cook's Petrel (*Pterodroma cookie;* Rayner *et al.* 2007).

We established 24 plots on ridge tops: nine seabird and four non-seabird plots in Upper Limahuli Preserve and eight seabird and three non-seabird plots in Hono O Nā Pali (Fig. 1). On the geologically older Hawaiian island of Kauaʻi, ecosystem processes are typically limited by P availability (Crews *et al.* 1995) which is expected to apply to our study sites as well. Seabird and non-seabird plots were selected opportunistically in areas with and without seabirds, respectively. Steep slopes, lack of helicopter landing locations, and low seabird numbers made random or uniform plot selection unrealistic. Sample size for isotopic comparisons was based on an *a priori* power analysis (G*Power version 3, 2012; Erdfelder *et al.* 1996). Effect size was set at 1.27 based on published soil and *Metrosideros polymorpha* δ^{15}Nvalues (Vitousek *et al.* 1989); error probability (α) was set at 0.05 and power (1-β error probability) was 0.95; and total sample size was calculated n=24. Each plot was 5 m in diameter and established in different seabird sub-colony clumps. The Upper Limahuli Preserve ranges in elevation from 750 to 980 m with plots located above 800 m. Hono O Nā Pali is located above 1,200 m and plots were situated between 1,210 and 1,287 m.

All soils in the two study areas were surveyed by reconnaissance survey. The soils in Upper Limahuli are classified as Alakai mucky peat and Waialeale mucky silty clay loam, while the soils in Hono O Nā Pali are classified as rough mountainous land (Soil Report for Island of Kauaʻi, Hawaiʻi, 2014). Alakai mucky peat taxonomic classification is clayey, ferrihumic, dysic, isomesic Terric Haplosaprists with pH values typically less than 4.0. Waialeale mucky silty clay loam is classified as very-fine, isotic, isothermic Typic Epiaquods with pH values commonly less than 4.4. Based on similar topography and rainfall it is likely that the rough mountainous land in Hono O Nā Pali has the same classification as the soils in Upper Limahuli.

All plots were located in wet montane forest with the majority of vegetation being native and dominated by *Metrosideros polymorpha* (ohia) in the canopy and the staghorn fern *Dicranopteris linearis* (uluhe) in the understory. No nitrogen fixing plants were present, although there is evidence of N-fixation in the litter of *D. linearis* (Russell and Vitousek 1997). Mean annual precipitation at both sites ranges between 2,500 and 3,000 mm with rain occurring throughout the year (Giambelluca *et al.* 2013). Mean annual temperature is 13°C, with warmest temperatures occurring in August and September (Juvik and Juvik 1998). Both study sites are remote with relatively intact forests exposed to introduced pigs (*Sus scrofa*), cats (*Felis catus*), mice (*Mus musculus*), and two rat species (*Rattus exulans* and *Rattus rattus*). In recent years, control and restoration measures have been implemented, including ungulate-proof fencing and pig removal in Upper Limahuli, and trapping for invasive animals and nonnative plant removal in both sites (Jon-Carl Watson, Limahuli Preserve Operations Manager at National Tropical Botanical Garden *pers. comm.*).

Available Soil Nutrients

To assess the availability of inorganic soil macronutrients and micronutrients, plant root simulator (PRS) probes (Western Ag Innovations Inc., Saskatchewan, Canada) were deployed in the top 5–10 cm of mineral soil. The PRS probes consist of separate anion and cation exchange membranes that assess nutrient supply rates by continuously absorbing charged ions over the period that they are in the soil. Nutrients indexed represent the bioavailable, labile, inorganic pools in the forms of NO_3^-, NH_4^+, $H_2PO_4^{3-}$, SO_4^{2-}, K^+, Mg^{2+} and Ca^{2+} (Johnson *et al.* 2005, Meason and Idol 2008, Beyene and Katzensteiner 2011). To account for soil heterogeneity, eight pairs of PRS probes were deployed per plot (192 total) during peak to late seabird breeding season (September 8–October 9 2013). The probes were retrieved after four weeks, when they were presumed to have reached a dynamic equilibrium (Meason and Idol 2008, Beyene and Katzensteiner 2011). Probes were rinsed with deionized water to remove roots and soil, and shipped to Western Ag Innovations Inc. for extraction and analysis. Nutrients were extracted by shaking the probe in 35 mL of 0.5 mol L^{-1} HCl for 1 hour to remove > 95% of sorbed ions from the membrane. Concentrations of NH_4^+, NO_3^-, and PO_4 were then analyzed using colormetic analysis with a Technicon AutoAnalyzer, while K^+, Ca_2^+, and Mg_2^+ were determined using an inductively coupled plasma spectrometer (PerkinElmer Optima 3000-DV ICP, PerkinElmer, Norwalk, CT)(Johnson *et al.* 2005, Meason and Idol 2008).

Isotopic analysis

Nitrogen isotopic ratios were determined for the top 10 cm of mineral soil, sunlit foliage of *M. polymorpha* and *D. linearis* from seabird and non-seabird plots at both sites as well as seabird guano. Samples were composited (5 soil samples, and 5 samples each of *M. polymorpha* and *D. linearis* foliage) in each plot. Soil samples were collected using a 1.27 cm diameter soil corer and sunlit, live leaves were collected from the newest fully mature cohort. Hawaiian Petrel and Newell's Shearwater guano samples were opportunistically collected from field sites during the 2014 breeding season. Twelve relatively fresh guano samples were placed in Ziploc bags and kept in a cooler on ice until they could be frozen. Organic materials were hand picked out of guano samples prior to analysis. Freshness could not be determined, but the high rainfall at these sites should preclude guano from remaining on the ground for more than two days of rain. The mean carbon: nitrogen ratio for the sampled seabird guano was 1.09 ± 0.21 (n = 8), and $\delta^{15}N$ = 8.23‰ ± 1.68‰ (n = 8).

Soil and foliar samples were dried at 70°C, sieved through a 2 mm mesh, homogenized, and powdered in a ball mill (Carter and Gregorich 2006). Guano samples were freeze dried and ground using a mortar and pestle. The isotopic composition of all samples was analyzed at the University of Hawai‘i at Manoa Biogeochemical Stable Isotope Facility using a continuous flow mass spectrometer (ThermoFinnigan Deltaplus XP) coupled with an elemental analyzer (Costech ECS 4010) via a Conflo IV interface. Nitrogen isotopic compositions are expressed as $\delta^{15}N$ values in ‰ relative to Air:

$$\delta^{15}N = [(R_{sample}/R_{standard})-1] \times 1000$$

where R_{sample} is the isotopic ratio ($^{15}N/^{14}N$) of the sample and $R_{standard}$ is the $^{15}N/^{14}N$ of Air. Accuracy and precision of $\delta^{15}N$ values were < 0.2‰, as determined from reference materials analyzed every 10 samples (glycine and a tuna muscle homogenate with $\delta^{15}N$ values of 11.25 ± 0.04‰ and 12.97 ± 0.06‰ respectively, which were determined by extensive characterization using National Institute of Standards and Technology certified reference materials and their $\delta^{15}N$ values were verified independently in other laboratories). Percent N was determined for soil and foliage samples from the results of isotopic analysis.

A two-component N isotope mass balance mixing model was used to determine the proportion of marine-derived nutrients in the top 10 cm of soil and the foliage of the two dominant plants in the plots (Phillips, Newsome, and Gregg 2005; West *et al.* 2006). As such, we used the mixing model equation from Dawson *et al.* (2002):

$$\delta T = ʆA\delta A + (1-ʆA)\delta B$$

$$1 = ʆA+ʆB$$

where δT is the total sampled isotopic value, δA and δB are the two source values and ʆA is the portion of the total value that is provided by source A (Dawson *et al.* 2002). The non-seabird plot data were used to establish terrestrial δ15N end member and the δ15N value of seabird guano was used as the marine end member. Solutions from the mixing model provide the percent of total N in soil and foliage of *M. polymorpha* and *D. linearis* in the seabird plots that was marine-derived (Fry 2006; Phillips 2012; Dawson *et al.* 2002; Boecklen *et al.* 2011). Uncertainty in the marine-derived fraction of total N was determined by propagation of error using the analytical solution of the partial differentiation of general Taylor series approximation determined using the two-component stable isotope mixing model (see Gelwicks and Hayes 1990, Phillips and Gregg 2001).

Vegetation community assessment

Data were collected in the same 5 m plots discussed above in Upper Limahuli and Hono O Nā Pali in collaboration with Kaua'i Endangered Seabird Restoration Project. The following variables were quantified: % cover for each plant species taller than 2 m, % cover for each species shorter than 2 m, average vegetation height, and canopy cover. Species richness was measured by number of species per plot. To measure proportional diversity we used the Shannon index (H'): H' = ∑ (pl)\ln plI; where (pl) is the proportion of the total number of individuals in the population that are in species "l"(Stirling *et al.* 2001). Percent cover of each species was used in lieu of number of individuals.

Statistical Analyses

Statistical analyses were performed in SPSS 22 (SPSS Inc. 2007). Levine's test was used to assess homogeneity of variance in soil nutrient concentration as well as the plant community composition. Of all nutrients analyzed (%N, total inorganic N, NO_3^-, NH_4^+, SO_4^{2-}, PO_4^{3-}, and Ca^{+2}), only NH_4^+ did not pass Levine's test and these data were $\log_{10}$ transformed for analysis. All plant community composition data passed Levine's test. One-way ANOVA, with significance set at $\alpha = 0.05$, was used to test for differences in available soil nutrients as well as relative differences in $\delta^{15}N$ values in soil and *M. polymorpha* and *D. linearis* foliage between treatments. All results are presented as means ± SE, unless otherwise noted. We used t-tests to determine differences between treatments for % N in *M. polymorpha* and *D. linearis.* For plant community composition; we analyzed canopy cover, average vegetation height, total *Metrosideros* cover, and total number of species present to look for differences between treatments also using *t*-tests.

Results

Available soil nutrients

Across all measured inorganic soil nutrients, only the concentration of NH_4^+ showed higher values in the seabird plots compared to non-seabird plots ($F_{1,21} = 4.74$, $p = 0.04$) (Table 1). Total inorganic N availability did not differ between treatments, largely because the availability of NO_3^- was nearly identical between seabird and non-seabird plots. In addition, PO_4^{3-}, Ca^{+2}, and SO_4^{2-} were slightly, but not significantly, lower in seabird plots (Table 1).

<<Table 1 near here>>

Isotopic analysis

Foliage of *M. polymorpha* had significantly higher $\delta^{15}N$ values in the seabird than in the non-seabird plots ($F_{1,21} = 5.07$, $p = 0.036$; Table 2). Although $\delta^{15}N$ values in soil and *D. linearis* leaves between the two treatments were not statistically different ($F_{1,21} = 2.78$, $p = 0.11$; $F_{1,21} = 2.82$, $p = 0.11$, respectively), there was a positive trend towards increasing $\delta^{15}N$ values in the seabird plots compared to the non-seabird plots (Table 2). Results from the mixing model indicated that 32% of the total soil N was derived from seabirds in the seabird plots. Foliar N of *M. polymorpha* in seabird plots was 27.9% (8% SE) from marine source, while *D. linearis* foliage contained 16.9% (0.08 SE) N from a marine source (Table 3). However, % N did not differ between seabird and non-seabird plots (Table 4) for soil ($t_{32} = 0.81$, $p = 0.43$), *M. polymorpha* foliage ($t_{20} = 0.17$, $p = 0.26$), or *D. linearis* foliage ($t_{21} = 0.92$, $p = 0.37$).

<<Tables 2-4 near here>>

Plant Community Composition

Seabird and non-seabird plots had similar species composition and vegetation structure. Specifically, canopy cover ($t_{22} = -0.13$, $p = 0.21$), average vegetation height ($t_{22} = -0.20$, $p = .84$), total *M. polymorpha* cover ($t_{22} = -1.35$, $p = 0.19$), total species recorded ($t_{22} = 0.48$, $p = 0.96$), and H' ($t_{22} = 0.038$, $p = 0.97$) were all similar between treatments.

Discussion

We found support for the first hypothesis in that more inorganic N in the form of NH_4^+ was found where seabirds were present. In support of the second hypothesis, 32% of soil nitrogen was of a marine source, and that marine-derived N accounted for 17–28% of foliar N in the two dominant native plants in the study system. However, we did not find evidence to support the third hypothesis that plant community composition would differ with and without allochthonous input of nutrients by seabirds.

Microbial processes can affect the nitrogen isotopic composition of plant-soil systems (Amundson 2003, Szpak 2014). Nitrogen in guano is deposited primarily as uric acid ($C_5H_4O_3N_4$; Bird *et al.*, 2008) and microbes mineralize this organic N into NH_4^+ and NO_3^-. Inorganic nitrogen as NH_4^+ and NO_3^- is available to most plants in soil solution, making this a potentially important addition to the ecosystem. Ammonium can be lost from the system through oxidation (the first step in soil nitrification), which can lead to N loss as nitrates are leached from the soil or through ammonia volatilization. Nitrogen transformations such as ammonia volatilization and denitrification can affect the $\delta^{15}N$ values of plants and soil because there are large nitrogen isotope fractionations associated with these processes. We do not however believe that nitrogen loss in the studied systems studied was the main cause of ^{15}N enrichment in seabird sites. The soil types in these areas are acidic (pH less than 4.5) and under these acidic conditions ammonia would be protonated so that the dominant form of reduced N would be ammonium. In Hawaiian rainforests in regions with mean annual rainfall exceeding ~2,500 mm, soil microbial denitrification completely consumes nitrate in local soil environments, preventing expression of the isotope effect associated with denitrification (Houlton *et al.* 2006). Under these conditions $\delta^{15}N$ values of soils converge on the $\delta^{15}N$ values of the N input. We anticipate that these potential losses of N may make seabird subsidies more critical to the ecosystem than currently understood.

The impacts of seabirds on soil and plant characteristics vary across systems and depend at least partially on the life history of the seabirds in question. Durrett *et al.* (2014) found that trees and shrubs differed in their response to the addition of marine nutrients with trees increasing slowly in foliar %N and $\delta^{15}N$ values with increasing population density while shrubs showed a strong positive response at low densities and negative responses at higher densities. In ecosystems that are N and/or P limited, the addition of guano may increase primary productivity and select for fast growing plants that can take advantage of periodic resource subsidies, or seabirds that nest in high density may make the soils toxic to plants with the excessive addition of nutrients (Wainright *et al.* 1998, Anderson and Polis 1999, Kolb *et al.* 2010). In similar studies based on coastal seabird colonies, nutrient pulses caused changes that were observed though the food web and even back into the marine environment. These changes included increased plant productivity, increased plant predation, increased arthropod and lizard density, and increased coastal nutrient influx into the nearshore environment leading to increased plankton growth (Barrett *et al.* 2005, Spiller *et al.* 2010, McCauley *et al.* 2012). On Kaua‘i, Newell’s Shearwater and Hawaiian Petrel nest colonially, but in low densities in burrows. As burrow nesting birds are not observed to reach densities that cause nutrient toxicity to plants, seabirds likely played a larger supporting role historically in nutrient cycling in this ecosystem when population numbers were higher.

We estimated the quantity of nutrients potentially added to our study sites by guano input. The two sites total about 200 ha, 160 ha in Upper Limahuli and 40 ha in Hono O Nā Pali, including non-seabird areas as well as areas where the seabirds are nesting. We have no official estimates of seabird population numbers in these areas, but using Griesemer's island wide estimates for Newell's Shearwater as well as estimates from the field, we estimated 500 pairs (± 250) of Newell's Shearwater and Hawaiian Petrel combined between the two sites with proportionally more Newell's Shearwater in Upper Limahuli and proportionally more Hawaiian Petrels in Hono O Nā Pali (Griesemer and Holmes 2011). No studies have been conducted to indicate how much Newell's Shearwater or Hawaiian Petrel eat or excrete per day. However, the wandering albatross weighs approximately 10 kg and was found to consume two kilograms of food per day (Salamolard and Weimerskirch 1993). The average weight for Newell's Shearwater and Hawaiian Petrel is 0.4 kg (Judge *et al.* 2014; Ainley *et al.* 1997). Therefore, since small organisms require more food per unit body mass than larger organisms, we used the allometric relationship:

$$\text{Intake(g)} \sim \text{Mass(g)}^{0.72}$$ (Schneider 2002)

to estimate food intake, assuming birds are in homeostasis. Doing this we estimated that seabirds in our study sites consume 0.2 kg of marine-based food per day. Based on the seabird intake and calculations of guano production in dovekies (Gabrielsen *et al.* 1991), we estimated that 500 seabirds could produce 98.5 kg of guano day^{-1}, or 73.9 kg ha^{-1} y^{-1}. Not all of the guano would end up in the montane ecosystem as one bird of a pair would likely be out to sea, so half of this estimate is 37 kg guano ha-1 y-1. Estimating the nitrogen content of the guano at 22% yields 16 kg N ha-1 y-1 (Bird *et al.* 2008). For comparison, total N deposition from precipitation was measured as 1 kg N ha-1 y-1 at a site on Hawai'i Island (P. Vitousek 2004). *Acacia koa*, a dominant native symbiotic N fixing tree, was not present in our sites but estimates of N-fixation in dense regenerating *A. koa* stands range from 23 kg N ha-1 y-1 in 5-year-old stands to 1.5 kg N ha-1 y-1 in 20 year old stands (Pearson and Vitousek 2002).

Isotopic analysis

Seabirds have been shown to increase N levels in soil and surrounding organisms via marine-derived N (Wainright *et al.* 1998, Wait *et al.* 2005, Mizota 2009). However, it was previously unknown if this is also the case in wet tropical montane regions characterized by high rainfall, warm temperatures, and low current population densities of seabirds (Martinelli *et al.* 1999, Garcia *et al.* 2002). While marine N was clearly higher in *M. polymorpha*, the effect of seabird added nitrogen may be masked in *D. linearis* by nitrogen fixation that may occur in the litter (Russell and Vitousek 1997). It should also be kept in mind that although the non-seabird sites had no current evidence for nesting, they were likely colonized in the past and may have a legacy of high $\delta^{15}N$ values in soil. Thus, using these non-seabird sites likely resulted in an overestimation of terrestrial $\delta^{15}N$ end member values in the isotope mass balance mixing model, and thus systematically underestimated the proportion of marine-derived N available in the soil and incorporated into the foliage of both studied species.

Vitousek *et al.* (1989) and Martinelli *et al.* (1999) measured $\delta^{15}N$ of *M. polymorpha* foliage in non-seabird areas in Hawai‘i across multiple islands and found mean $\delta^{15}N$ values of -3.3 (± 2.3‰ SD) and -2.8 (± 2.6‰ SD), respectively. A comparison of their values with this study are complicated by difference in substrate age between sampling sites in these prior studies and ours on Kaua‘i. Martinelli *et al.* (1999) also measured a $\delta^{15}N$ value of -0.5‰ from a single *M. polymorpha* on Kaua‘i from a non-seabird area. It is unknown if the $\delta^{15}N$ value of -0.5‰ is an outlier or representative of *M. polymorpha* on Kaua‘i. However, the archipelago averages of -3.3‰ and -2.8‰ in non-seabird areas are slightly lower than the non-seabird values that we measured for *M. polymorpha* (-2.3‰), and this also indicates a potential underestimate of the importance of current marine-derived N as presented here.

Results of the isotopic mixing models indicate that dominant plants in this ecosystem utilize at least some N derived from a marine source, and soil and foliage of both plant species showed marine influence. Although standard error was high, the amount of marine sourced N was higher across all sampled substrates in seabird plots, and $\delta^{15}N$ values were significantly higher in *M. polymorpha* foliage in seabird plots compared to control plots.

Plant community composition

None of the plant species composition measures indicated differences between seabird and non-seabird plots. This is likely due to not only the limited amount of nitrogen being added to the seabird sites, but also the species depauperate nature of the islands and isolation of the study sites. There are a limited number of native species on the islands to populate these areas and the isolated nature of the study sites means less influence of the nonnative and invasive plant species that occur in high densities in more disturbed areas in Hawaiʻi. Invasive plant species were actively managed in Upper Limahuli and pulled opportunistically in Hono O Nā Pali, though the density of invasive plants is low in both sites due to the remote locations. Another reason for the lack of differences in some of the measured variables is that other drivers besides the addition of nutrients by seabirds may be more influential, particularly in low density seabird sites such as ours. Other researchers found that on high and medium density seabird islands, seabirds drove ecosystem properties such as $\delta^{15}N$ values (soil and leaf), soil and leaf N, NH_4^+, and NO_3. However, in low density colonies other ecosystem processes drove these ecosystem properties more than the seabirds (Durrett *et al.* 2014).

Conclusion

Despite being at historically low population densities, seabirds contribute to the ecosystems where they still nest in montane Hawai‘i via marine-derived nutrient deposition. These study sites in Kaua‘i contain some of the last relatively intact tropical montane ecosystem with native seabirds, yet very little research has occurred there. Studies in the arctic and in coastal systems are abundant (Polis and Hurd 1996, Mulder *et al.* 2011, Gagnon *et al.* 2013), and generally show seabirds to increase nutrient availability and biodiversity in the arctic (Keatley *et al.* 2009, Zmudczyńska *et al.* 2012), and to fertilize or even create toxic conditions in coastal ecosystems depending on seabird density (Kolb *et al.* 2010, VanderWerf *et al.* 2014). However, comprehensive studies about how seabirds and their nutrient subsidies impact tropical montane ecosystems are notably lacking (Hawke and Holdaway 2009).

Our control plots may have a historical legacy of seabirds and thus may still contain nutrients from seabirds. Therefore, our estimate of allochthonous nutrient input by seabirds is likely conservative. The influx of N and P may be more important to the resiliency of these ecosystems, especially in the face of climate change and other stressors, than is currently understood (Perry, Goerge *et al.* 2010, Doughty *et al.* 2015). Historically seabirds in the Hawaiian Islands may have played a major role in controlling soil fertility in areas where they nested. The montane forest seabirds in the Hawaiian Islands have been greatly reduced both in number and in range, yet even in vastly reduced numbers these birds impact soil and vegetation nutrient content. The decrease in nutrient flow and its impacts on the ecosystem are vital for restoration project managers to understand as they attempt to rebuild the ecosystem and restore endemic plants and wildlife. While we know that seabird declines have sweeping affects throughout the food web in warm temperate New Zealand (Fukami *et al.* 2006), the consequences of seabird declines on tropical vegetation dynamics and ecosystem processes are largely unknown.

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Table 1. Mean and standard error (SE) inorganic soil nutrient availability for seabird and non-seabird plots (µg/10 cm^{-2}/4 weeks). Results in bold indicate significant differences between seabird and non-seabird plots at $p < 0.05$. Total N refers to total inorganic N.

		mean	SE	F	df	p
Total N	Seabird	59.4	14.7	0.595	1/21	0.449
	NonSeabird	36.5	17.6			
NO_3^-	Seabird	23.5	11.2	0	1/21	0.988
	NonSeabird	23	15.8			
NH_4^+	**Seabird**	**36**	**0.1**	**4.74**	**1/21**	**0.041**
	NonSeabird	**13.8**	**0.1**			
SO_4^{2-}	Seabird	47.15	11.4	0.046	1/21	0.833
	NonSeabird	52.4	22.6			
PO_4^{3-}	Seabird	5.3	1.6	0.272	1/21	0.607
	NonSeabird	7.2	3.6			
Ca^{2+}	Seabird	155.1	26.7	0.019	1/21	0.891
	Non-Seabird	146.8	138.5			

Table 2. Mean and standard error (SE) of $\delta^{15}N$ values of soil and two dominant plant species in seabird and non-seabird plots on Kaua'i. Results in bold indicate significant differences between seabird and non-seabird plots at $p < 0.05$.

		mean	SE	F	df	*p*
Soil	Seabird	1.23	0.31	2.82	1	0.108
	Non-seabird	-0.183	0.62			
M. polymorpha	**Seabird**	**0.65**	**0.76**	**5.07**	**1**	**0.036**
	Non-seabird	**-2.26**	**0.46**			
D. linearis	Seabird	6.06	0.46	2.77	1	0.109
	Non-seabird	5.04	0.56			

Table 3. Mean proportions and propagated error for a two source, one isotope ($\delta^{15}N$) model for soil and two dominant plant species in seabird and non-seabird plots. Values reported are means and standard error, calculated as per Phillips and Gregg (2001). Input values for the seabird substrates and source 1 and source 2 are also listed.

	Seabird			Source 1 – plots with no seabird nests			Source 2- guano
	Soil	*M. polymorpha* foliage	*D. linearis* foliage	Soil	*M. polymorpha* foliage	*D. linearis* foliage	
$\delta^{15}N$ (‰) (SE)	6.1 (0.30)	0.7 (0.76)	1.2 (0.46)	5.0 (0.63)	-2.3 (0.46)	-0.2 (0.56)	8.2 (1.7)
Sample Size	19	16	17	11	6	6	8
Proportion of N from marine source (SE)	32% (0.0.18)	27.9% (0.08)	16.9% (0.078)				
95% Confidence Limits	0–69%	11–45%	0–34%				

Table 4. Mean %N in soil, *M. polymorpha*, and *D. linearis*.

		mean ± SD	t	*p*
soil	seabird	0.54 (0.21)	$t_{32} = 0.81$	0.43
	non-seabird	0.48 (0.25)		
D. linearis	seabird	1.2 (0.19)	$t_{20} = 0.17$	0.26
	non-seabird	1.28 (0.22)		
M. polymorpha	seabird	0.66 (0.09)	$t_{21} = 0.92$	0.37
	non-seabird	0.61 (0.10)		

Figure 1. Location of plots in Upper Limahuli Preserve (ULP) and Hono O Na Pali (Pihea), Kaua'i, HI, seabird plots are represented with a triangle and non-seabird plots are represented with a circle.