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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Foliar $\delta^{15}N$ patterns in legumes and non-N fixers across a climate gradient, Hawai'i Island, USA

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Abstract

Recent studies from the Hawaiian Islands showed that pedogenic thresholds demarcate domains in which rock-derived nutrient dynamics remain similar across wide variations in rainfall. These thresholds appear related to certain aspects of N cycling, but the degree to which they correspond to patterns of biological N fixation (BNF)—the dominant input of N into less-managed ecosystems—remains unclear. We measured aboveground plant biomass, foliar nutrient concentrations, and foliar $\delta^{15}N$ along a climate gradient on ~ 150,000-year-old basaltic substrate to characterize foliar N sources and spatially relate them to soil nutrients. Patterns in legume $\delta^{15}N$ correspond to known pedogenic thresholds along the rainfall gradient, with low $\delta^{15}N$ values (~ 0 to − 2‰) occurring in the dry, biologically inactive domain and the wet, highly weathered domain. Elevated $\delta^{15}N$ in the middle, fertile domain suggests a greater reliance of legumes on soil N where it has accumulated over time. Non-legume face N deficiencies throughout most of the gradient while legumes maintain low C:N ratios via symbiotic BNF. However, legume abundance declines outside the fertile domain, limiting ecosystem N inputs. Breakpoints in legume $\delta^{15}N$ data suggest that P (and potentially other nutrients) limits BNF and, by extension, legume abundance in wet region. Nutrients may also constrain legume abundance in the dry domain, but pedogenic effects could not be isolated from climatic constraints at the dry sites. We conclude that pedogenic thresholds defined by climate can be informative of foliar $\delta^{15}N$ patterns in cases where legumes are not directly constrained by climate, land use, or other external factors.

Keywords Stable isotopes · Climate gradient · Ndfa · Weathering · N cycling

Introduction

Nitrogen (N) plays a unique role in the development of Earth's ecosystems: it is essential for all life, abundant on Earth yet frequently inaccessible to organisms, and its cycling through ecosystems is mediated largely by biological rather than geochemical processes. Despite the presence of an immense and well-mixed N supply in the atmosphere, new ecosystem inputs of N are relatively small and (in less-managed ecosystems) occur almost exclusively via biological N fixation (BNF), in which prokaryotic microorganisms (diazotrophs) reduce atmospheric dinitrogen (N_2) to bioavailable forms (Vitousek et al. 1997; Canfield et al. 2010). BNF is an energy- and resource-intensive process (Gutschick 1981; Vitousek and Field 1999; O'Hara 2001), so while N is crucial for survival and abundant throughout Earth's atmosphere, only a limited set of organisms within the bacterial and archaeal domains have evolved the ability to access the atmospheric N pool directly (Young 1992; Reed et al.

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2011; Vitousek et al. 2013). Because of its high mobility, its integral role in the biochemistry of photosynthesis, and its reliance on BNF to enter ecosystems, N frequently limits productivity in the terrestrial and marine domains alike (Vitousek and Howarth 1991; Sterner and Elser 2002; Elser et al. 2007; LeBauer and Treseder 2008).

In light of N's control on primary productivity, a growing body of research seeks to understand connections between N cycling and the climatic forces that shape ecosystem development (Amundson et al. 2003; Houlton et al. 2007; Craine et al. 2009, 2015; Wang et al. 2014; Liu et al. 2017). The concept of pedogenic thresholds and associated soil process domains can be a useful tool for characterizing the influence of climate on many aspects of soil development: pedogenic thresholds occur along continuous environmental gradients where soil properties change abruptly and substantially, and soil process domains are the regions between thresholds in which soil properties exhibit relatively small changes despite large variations in the independent environmental drivers (Muhs 1984; Chadwick and Chorover 2001; Vitousek and Chadwick 2013; Vitousek et al. 2016; Bateman et al. 2019).

Prior work in the Hawaiian archipelago has demonstrated the usefulness of examining pedogenic thresholds and soil process domains on basalt-derived soils, finding (for instance) abrupt transitions from high to low base cation saturation over narrow ranges of increasing rainfall (Chadwick et al. 2003; Vitousek and Chadwick 2013; Lincoln et al. 2014). Vitousek and Chadwick (2013) classified 150,000 year-old Hawī volcanic soils on Kohala Mountain into three soil process domains: below 700 mm/yr of rainfall, wind erosion and a lack of biological uplift leads to low and variable soil nutrient levels; from 700 to 1700 mm/yr, high rates of weathering and biological uplift create a zone of relatively high soil fertility; and above 1700 mm/yr nutrient mobilization begins to outpace rates of weathering and uplift, resulting in generally low fertility above 2100 mm/yr.

While the study of such thresholds and domains is useful for understanding patterns of ecosystem development that emerge under different climatic conditions, research has largely neglected N due to its regulation by processes often considered independent of the pedogenic system (Vitousek et al. 2019). Nonetheless, recent findings suggest that N-cycling patterns do correspond to pedogenic thresholds in certain circumstances (von Sperber et al. 2017), highlighting the need for detailed studies of N cycling along environmental gradients. Studying how N cycling relates to the pedogenic thresholds that arise from differences in climate should help illuminate the processes that control ecosystem development across the world, for such processes frequently depend on N availability.

Recently, certain components of the N cycle have been analyzed across the same Hawaiian climatic gradients to search for correlations between dominant soil process domains and N-cycling regimes: von Sperber et al. (2017) returned to the Hawī volcanics on Kohala and reported elevated rates of potential net N mineralization in the domain of abundant rock-derived nutrients defined by Vitousek and Chadwick (2013). von Sperber et al. concluded that N cycling correlates with pedogenic thresholds defined by soil characteristics independent of N. However, another recent study from > 4 million year-old basalts on Kaua'i determined that N mineralization peaked in a wetter pedogenic domain than would be expected in such highly weathered, low-nutrient soils (Vitousek et al. 2019). This alternative pattern in N availability was apparently driven by the distribution of legumes along the climate gradient, which was itself controlled by factors (e.g. land use, biological invasion, rainfall) other than rock-derived nutrients.

Thus, from previous studies of N mineralization, the utility of pedogenic thresholds and soil process domains for understanding N cycling remains unclear. Additional insight may be gleaned from studies of BNF—the dominant pathway through which N enters terrestrial ecosystem N pools—along similar climatic gradients. As a biological process, BNF is controlled by a variety of factors including the distribution of plants able to fix N symbiotically, the regulatory strategies of such plants when N is available in the soil (Menge et al. 2015; Sheffer et al. 2015), the abundance of free-living diazotrophs (Reed et al. 2011), climatic conditions (Gei et al. 2018), and the availability of essential resources other than N (e.g. energy, P, Fe, K, and Mo; Vitousek et al. 2013). This set of drivers may seem too complex to allow soil process domains defined by rock-derived nutrient levels to categorize BNF. However, BNF's significant resource requirements (Gutschick 1981; O'Hara 2001) and the immense influence of climate on soil nutrient levels in basaltic substrates (Vitousek and Chadwick 2013; Bateman et al. 2019) imply that pedogenic thresholds along climate gradients could delineate regions of limited or sizeable BNF fluxes.

The BNF activity of N-fixing plants can be studied by sampling the stable isotope ratios of N present in plant biomass. $\delta^{15}N$ represents the per-mille ratio of ^{15}N to ^{14}N in each sample relative to the atmospheric standard ratio, which is zero by definition (Mariotti 1983). Soils tend to accumulate the heavier ^{15}N isotope due to kinetic isotope fractionation effects, leading to positive $\delta^{15}N$ values; plants that lack the ability to fix atmospheric N_2 may thus be expected to feature greater $\delta^{15}N$ than N-fixing plants that derive their N from the atmosphere as well as the soil (Shearer and Kohl 1986). The percentage of N derived from the atmosphere by an N-fixing plant can be estimated by measuring the $\delta^{15}N$ of the plant in question alongside reference values from plants restricted to only pedogenic or atmospheric N, but these requirements are difficult to realize in large-scale field studies and myriad uncertainties can hamper the approach's precision and interpretability (Unkovich et al.

1994; Rose et al. 2018). We instead focus on the broad-scale patterns in soil and foliar $\delta^{15}N$ that emerge along an intensively sampled climate gradient—sudden breakpoints and changes in isotopic trends can shed light on broad regimes of variable BNF activity in sampled legumes, even if exact BNF fractions are difficult to quantify.

To evaluate the relevance of pedogenic thresholds to patterns in symbiotic BNF, we returned to the Kohala climate gradient in which N mineralization rates were previously found to correspond with pedogenic thresholds in rock-derived nutrients (von Sperber et al. 2017). The consistent rangeland use of the Kohala transect and presence of pasture legumes at all rainfall levels avoid some of the confounding factors found on the Kaua'i transect used by Vitousek et al. (2019). While von Sperber et al.'s results highlight the variable potential of leeward Kohala's soils to provide N through mineralization, we examine whether spatial patterns in $\delta^{15}N$, legume abundance, and foliar N content likewise correspond to the region's distinct pedogenic thresholds. These variables directly relate to symbiotic BNF and will furnish a more holistic understanding of how pedogenic thresholds interact with the terrestrial N cycle.

We investigate (a) how legume abundance and, by extension, the capacity for symbiotic inputs of N into the ecosystem vary between soil process domains; (b) whether Kohala's soil process domains resemble patterns in the isotopic composition of foliar N in legumes, which can reflect BNF's contribution to plant N; and (c) whether foliar nutrient variations across the rainfall gradient imply nutrient limitations to BNF or legume growth in any of Kohala's domains. We hypothesize that isotopic compositions will reveal legumes' significant reliance on BNF across the rainfall gradient, with perhaps a lower reliance in the fertile domain where N mineralization is relatively high. When taking legume abundance into account, we further hypothesize that ecosystem BNF capacity in Kohala corresponds with thresholds in rock-derived nutrients essential for legumes and their symbioses, and that the low correspondence between N mineralization and parent-derived nutrient availability found by Vitousek et al. (2019) in Kaua'i may therefore be due to external controls (e.g. climate, land use) on legume abundance which affected soil N pools over long time scales. These findings would suggest that pedogenic thresholds may be best applied to the study of N cycling where leguminous species are present across rainfall levels and not regulated by land use or other factors external to the pedogenic system.

Methods

Study site

Plant samples were collected from 46 sites along a ~ 17-km transect on the leeward slope of Kohala Volcano, Hawai'i Island (Fig. 1). The transect captures a strong climate gradient, with rainfall ranging from ~ 250 mm/yr at the lowest sites to ~ 3200 mm/yr at the windward end (Giambelluca et al. 2013). Mean annual temperature negatively correlates with rainfall along the sample transect, ranging from 23.5 °C at the driest site to 16 °C at the wettest site (Giambelluca et al. 2014). While temperature is known to directly affect BNF processes (Gibson 1963), the temperature variation on this gradient is much less drastic than the variation in rainfall and occurs in a range of temperatures at which the BNF symbiotic system has been shown to function relatively unimpeded (Bordeleau and Prévost 1994). When estimated using the Priestly-Taylor method, potential evapotranspiration (PET) exceeds precipitation below ~ 1600 mm of annual rainfall (Giambelluca et al. 2014; von Sperber et al. 2017). The elevation of the lowest and most arid site is about 50 m above sea level, and the highest and wettest site is situated at about 1000 m. All sample points are located on the same ~ 150,000-year-old Hawī volcanic formation (Chadwick et al. 2003; Sherrod et al. 2007), feature the same coarse-scale slope and aspect, and have experienced the same land use (cattle grazing) for over 100 years (Kagawa and Vitousek 2012). These characteristics allow climate to be isolated as a driver of soil and ecosystem development. Soils along the gradient are volcanic Andisols, ranging from Typic Haplotorrands in the driest sites to Typic Fulvudands in the wettest sites (Chadwick et al. 2003). The 46 sampling sites used in this study are the same as those evaluated by von Sperber et al. (2017) and Peay et al. (2017).

Most of the transect lies within the *ahupua'a* of Puanui, a traditional Hawaiian land division that stretches from Kohala's leeward coast to its crest (Kagawa and Vitousek 2012; Marshall et al. 2017). Puanui is one of the 32 *ahupua'a* that contained the Leeward Kohala Field System, a vast dryland agricultural system that occupied the middle elevations of leeward Kohala until it was abandoned and replaced by the ranching industry in the nineteenth century (Tomonari-Tuggle 1988; Ladefoged et al. 1996; Ladefoged and Graves 2011). The field system's location was ultimately determined by a zone of elevated soil fertility where rainfall was sufficient to weather basaltic parent materials and support an abundance of plants, but not great enough that most nutrients have been leached from the soil (Vitousek et al. 2004).

Our sampling points vary in species composition due to the wide range of environmental conditions present along the transect. The plant communities on leeward Kohala have also undergone extensive changes in the past two centuries, so the flora we sampled do not necessarily resemble the biological regime that shaped the area's nutrient dynamics for millennia. However, the high mobility of N in terrestrial ecosystems suggests that these shifts have had ample time to shape ecosystem N pools (Vitousek and Howarth 1991). Pre-human vegetation in leeward Kohala likely consisted of

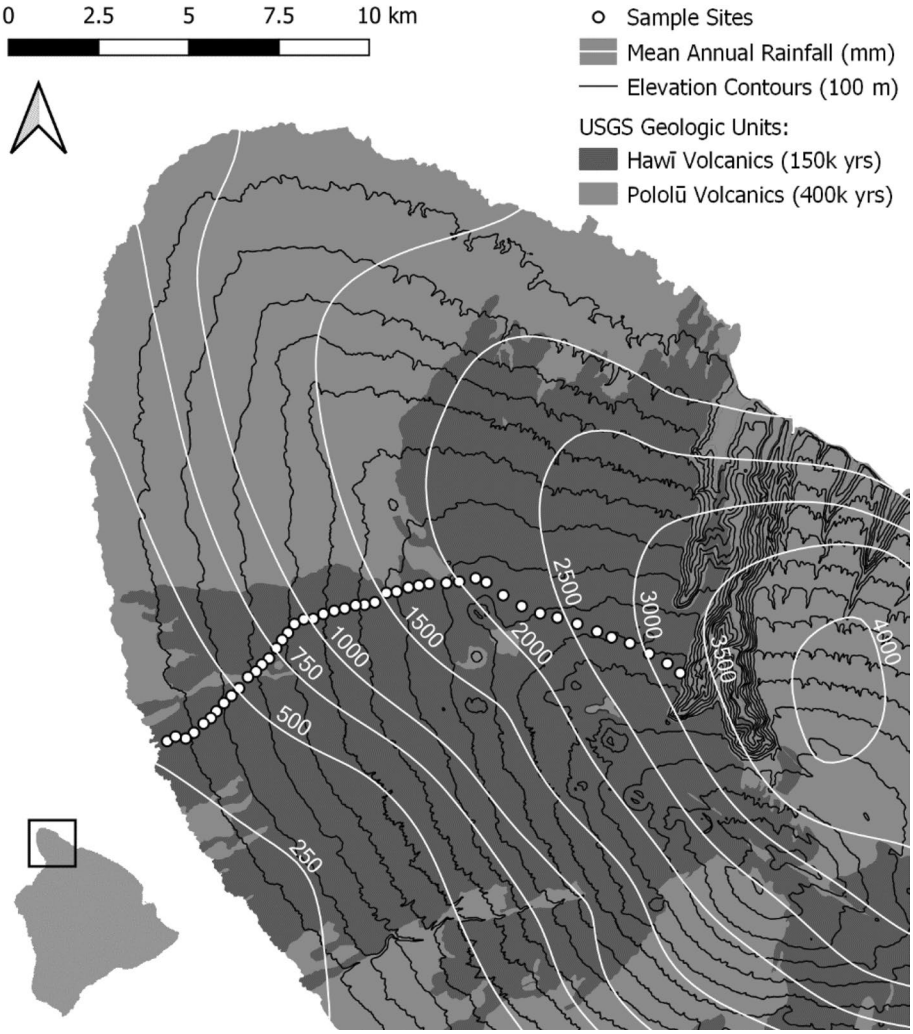

Fig. 1 Location of sample sites on Kohala, Hawai'i Island, USA. Thin dark lines are elevation contours, and white lines are mean annual rainfall isohyets from Giambelluca et al. (2013). The dark gray shading shows the distribution of ~ 150,000 year-old Hawī lava flows, and lighter shading shows ~ 400,000 year-old Pololū volcanics (Sherrod et al. 2007)

wet closed-canopy forests above 2000 mm/yr of rainfall, dry C_4-dominated grasslands/shrublands below 500 mm/yr of rainfall, and a broad area of mixed woodlands, shrublands, and mesic forests in between (Chadwick et al. 2007). Most legumes present today in leeward Kohala are introduced species, but various indigenous legumes occurred throughout the region's rainfall gradient in prehistory (Price et al. 2012).

Today, the exotic C_4 pasture grasses *Pennisetum clandestinum* (kikuyu grass) and *Cenchrus ciliaris* (buffel grass) dominate Puanui. *P. clandestinum* occupies sites receiving over 1250 mm/yr of rainfall and *C. ciliaris* occupies the arid portion of the gradient with little overlap in range (von Sperber et al. 2017). These grasses are closely related, and recent phylogenetic reviews suggest they may be considered congeneric (Hanselka et al. 2004; Chemisquy et al. 2010). The indigenous grass *Heteropogon contortus* (pili) also occurs sporadically in the dry region. The pasture grasses are intermixed with the exotic herbaceous legumes *Neonotonia wightii* (glycine or perennial soybean), *Trifolium repens* (white clover), and *Desmodium incanum* (ka'imi or Spanish clover) where water is sufficient. The invasive non-leguminous herb *Polygonum capitatum* was collected at the wettest sites. Common in drier regions are the introduced woody legumes *Prosopis pallida* (kiawe) and *Leucaena leucocephala* (koa haole), as well as a few remnant individuals of the endemic leguminous tree *Erythrina sandwicensis* (wiliwili) and various indigenous shrubs including *Sida fallax* ('ilima) and *Waltheria indica* ('uhaloa). Some stands of the indigenous, non-leguminous tree *Metrosideros polymorpha* ('ōhi'a) remain from the montane forests that once occupied the wet region at the upper edge of and beyond Puanui. Much of the pre-contact mesic forest was likely composed of the endemic woody legume *Acacia koaia* (koai'a), which today is present only in small numbers on Kohala.

Field sampling

Vegetation samples were primarily collected in July and August 2015, with some additional samples collected in July 2017 and December 2018. All aboveground herbaceous

matter was cut and collected from four randomly distributed quadrats within 9 m of the GPS-located sample sites. The samples from each 25 × 25 cm quadrat were sorted to species and oven-dried immediately after removal from the field. For most sites, all collected plant matter was weighed after drying to obtain standardized aboveground biomass data. We also collected foliar samples from trees (which were only present in the wet and dry ends of the sample transect) by randomly selecting an individual within 20 m of each sample site. These samples targeted mature, sun-exposed leaves from *P. pallida* and *L. leucocephala* (both introduced legumes) at dry sites and from *M. polymorpha* (an endemic non-legume) at wet sites. Woody legume abundance was sampled by counting all individuals in a 400 m^2 area centered about the sample point.

Soil stable isotope data were derived from integrated 30-cm samples collected in July 2014 from the same 46 sample points (von Sperber et al. 2017).

Laboratory analyses

Oven-dried foliar samples were shipped to the Environmental Measurements 1 (EM-1) and Stable Isotope Biogeochemical Laboratory (SIBL) facilities at Stanford University and ground in a Wiley mill. Leaf and pasture grass samples were analyzed for C, N, and $\delta^{15}N$ on a Carlo Erba 1500 CN Analyzer coupled to a Thermo Finnigan Delta Plus mass spectrometer. The analytical precision of the $\delta^{15}N$ measurements was 0.3‰ (von Sperber et al. 2017). Pasture grasses and the leaves of legumes were also analyzed to determine mass fractions of Ca, Fe, K, Mg, Mn, and P using a combination of inductively coupled plasma optical emission spectrometry (ICP-OES) and X-ray fluorescence (XRF). These six elements are all essential nutrients for symbiotic BNF (O'Hara 2001). For ICP-OES, ~ 500 mg of each sample were digested in 68% nitric acid and analyzed using a Thermo Scientific ICAP-6300 Duo spectrometer. Several samples with insufficient mass for ICP-OES were instead analyzed with a Spectro XEPOS ED-XRF spectrometer.

Statistics

To locate climatic thresholds in plant N dynamics, we developed segmented linear regressions with 95% confidence intervals between $\delta^{15}N$ and rainfall data in R (Muggeo 2003, 2008). We avoided overfitting by attempting regressions with different numbers of breakpoints until the model with the highest adjusted R^2 (a modified form of the coefficient of determination which penalizes the inclusion of additional explanatory terms) was identified. The statistical significance of all breakpoints were then tested using Davies' Test (Davies 2002; Muggeo 2008).

The foliar $\delta^{15}N$ patterns of *P. capitatum* diverged from those of other non-legumes. Present only above 2100 mm/yr average rainfall, *P. capitatum* leaves featured $\delta^{15}N$ values about 4‰ lower than those of other non-legume species—these near-zero $\delta^{15}N$ values are comparable to those measured in soil samples (median difference of − 0.48‰ from 17 samples). Because all other non-legumes followed a consistent and plausible pattern, we treated *P. capitatum* as an outlier and did not include it in regression models. The heavier stable isotope ratios found in this species could result from less reliance on soil nitrate pools, which are small and depleted in ^{15}N where *P. capitatum* was found (von Sperber et al. 2017).

Results

Plant abundance and aboveground biomass

Aboveground biomass and tree abundance data illustrate the prevalence of legumes relative to the rest of the vegetation community at different rainfall levels (Fig. 2). Herbaceous legumes generally were abundant from about 1150–1800 mm of annual rainfall, but their populations abruptly dropped off in wetter and drier conditions (Fig. 2b). In contrast, herbaceous non-legumes and pasture grasses featured abundant biomass above ~ 1250 mm/yr, where sparse *C. ciliaris* clumps give way to thick mats of *P. clandestinum* (Fig. 2a). In the arid region below ~ 1150 mm/yr, herbaceous biomass declined as deeper-rooted woody legumes continued to thrive due to groundwater access (Fig. 2b) (Dudley et al. 2014). These xeric legumes were much less common at the driest sites below ~ 500 mm/yr. Even where leguminous biomass is greatest, legumes never dominate the herbaceous community anywhere along the rainfall gradient.

Stable isotope patterns

Non-legume $\delta^{15}N$ follows soil $\delta^{15}N$ throughout the climate gradient with a consistent ~ − 4‰ offset (Fig. 3a), while legume $\delta^{15}N$ remains much closer to the atmospheric value of 0‰ at all rainfall levels (Fig. 3b). Segmented linear regressions highlight very similar patterns in soil and non-legume $\delta^{15}N$ data (Table 1): above ~ 2000 mm/yr of rainfall, $\delta^{15}N$ values remain nearly constant (3–5‰ for soil and − 2–2‰ for non-legumes) until the wet end of the gradient at ~ 3200 mm/yr; below ~ 2000 mm/yr, both soil and non-legume samples become enriched in ^{15}N with decreasing rainfall (trendlines peak at ~ 14‰ and ~ 10‰ for soil and non-legumes, respectively), until $\delta^{15}N$ values plummet by ~ 4‰ from 350 to 250 mm/yr. The segmented function for legume $\delta^{15}N$ features three breakpoints and can be interpreted as representing three domains: $\delta^{15}N$ remains

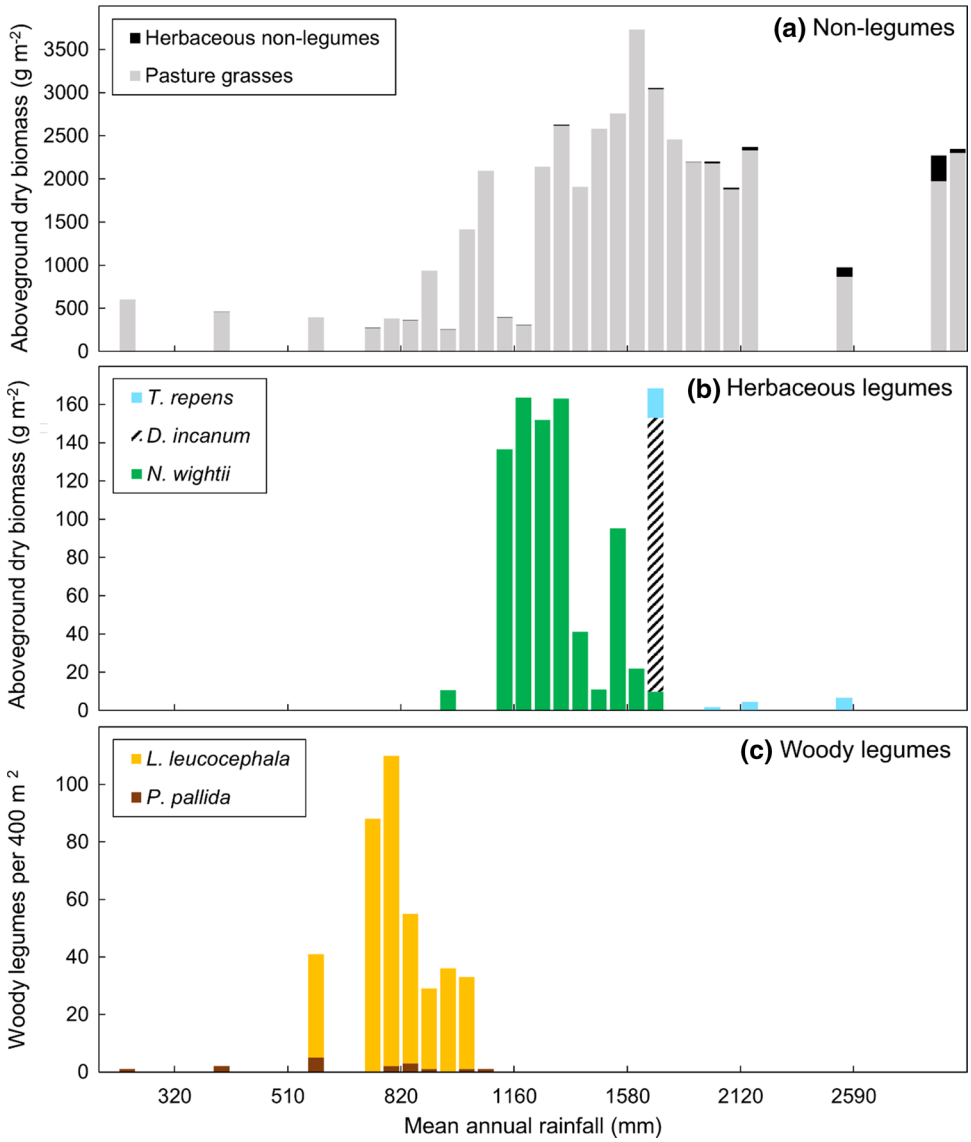

Fig. 2 **a** Aboveground dry non-leguminous biomass per m^2, **b** aboveground dry biomass of herbaceous legumes per m^2, and **c** abundance of woody legumes per 400 m^2. The horizontal mean annual rainfall (mm) scales are only roughly linear as each bar represents one of 46 sample points. Points with no data displayed in panel (**a**) were not sampled

relatively stable between − 2‰ and 0‰ above ~ 1600 mm/yr and below ~ 750 mm/yr of rainfall (although $\delta^{15}N$ becomes slightly enriched to positive values at the very driest sites), and a middle domain displays a rise and fall of $\delta^{15}N$, peaking around 1000−1150 mm/yr at nearly 4‰.

Foliar chemistry

Non-legumes show significant variability in C:N across the rainfall gradient as well as at individual sample points, but legumes at all rainfall levels can consistently maintain C:N ratios near or below 20 (Fig. 4). Measured C:N from legumes on the Kohala transect are consistently close to values derived from previous studies of the same or similar species in non-N-limited contexts (Arnold 1976; Ruschel et al. 1979; Sandli et al. 1993; Campo and Dirzo 2003), suggesting legumes can acquire sufficient N throughout the sample transect (Table 2). Conversely, non-legume C:N ranges from ~ 20 to nearly 200 and only converges to values below 40 around 1300 mm/yr of rainfall, where herbaceous legume biomass is relatively great (Fig. 2b) and high soil N availability is evident from N mineralization data (von Sperber et al. 2017). Here and throughout the rainfall gradient, grass N contents are less than half of published values from N-fertilized systems (Table 2) (Mears 1970; Colman and O'Neill 1978; Mannetje and Jones 1990).

To examine other nutrient dynamics along the rainfall gradient, concentrations of Ca, Fe, K, Mg, Mn, and P were measured in foliar samples of legumes and non-leguminous pasture grasses (Fig. 5). Foliar Ca concentrations are generally greater at dry sample points, whereas foliar Fe follows a concave trend with depleted Fe levels between ~ 1000 and ~ 2000 mm/yr. Foliar K peaks in both legumes and non-legumes from approximately ~ 750

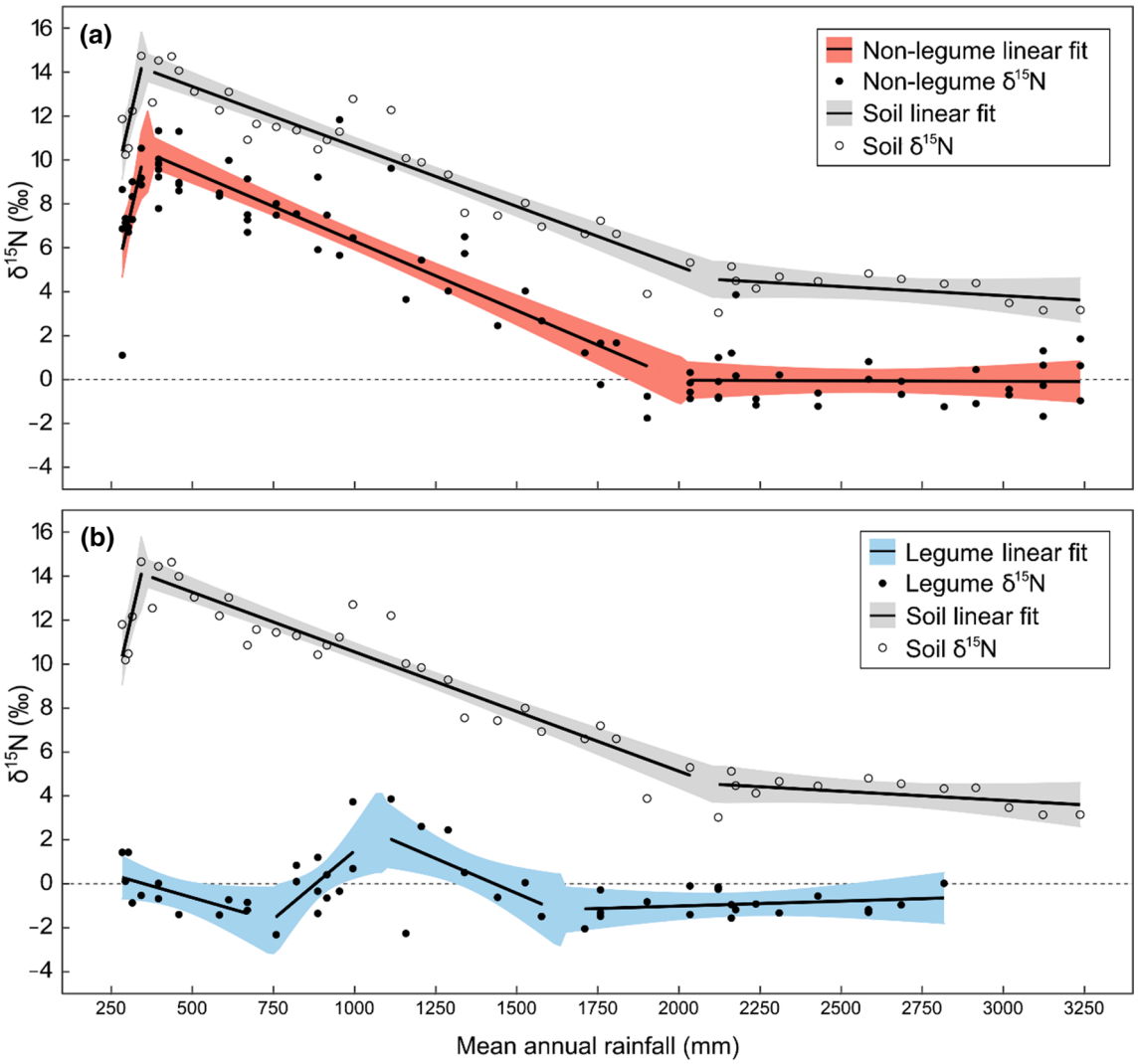

Fig. 3 **a** Segmented regressions of soil and non-legume $\delta^{15}N$ (‰) versus mean annual rainfall (mm). **b** The soil segmented regression from (**a**) plotted alongside legume $\delta^{15}N$ (‰) data and a corresponding segmented regression. Regression statistics and breakpoints are provided in Table 1. Shaded regions are 95% confidence intervals for the regression line in each segment

Table 1 Segmented regression statistics

$\delta^{15}N$ regression	Sample size	Adjusted R^2	Breakpoints (mm/yr)	*P* (Davies' test)
Soil	46	0.9409	343.9 ± 12.6	1.86e–4
			2109.7 ± 124.9	1.49e–5
Non-legumes	88	0.8837	354.1 ± 14.0	1.35e–6
			2004 ± 108.9	4.77e–10
Legumes	51	0.3339	746.0 ± 64.9	0.012
			1067.1 ± 62.2	0.26
			1619.4 ± 140.4	2.00e–5

to ~ 1750 mm/yr and decreases towards the extremes of the gradient. Mg and Mn concentrations are elevated below ~ 1000 mm/yr in legumes, and to a lesser degree in the region of high rainfall (although data there are sparser). Foliar P is markedly decreased below 1000 mm/

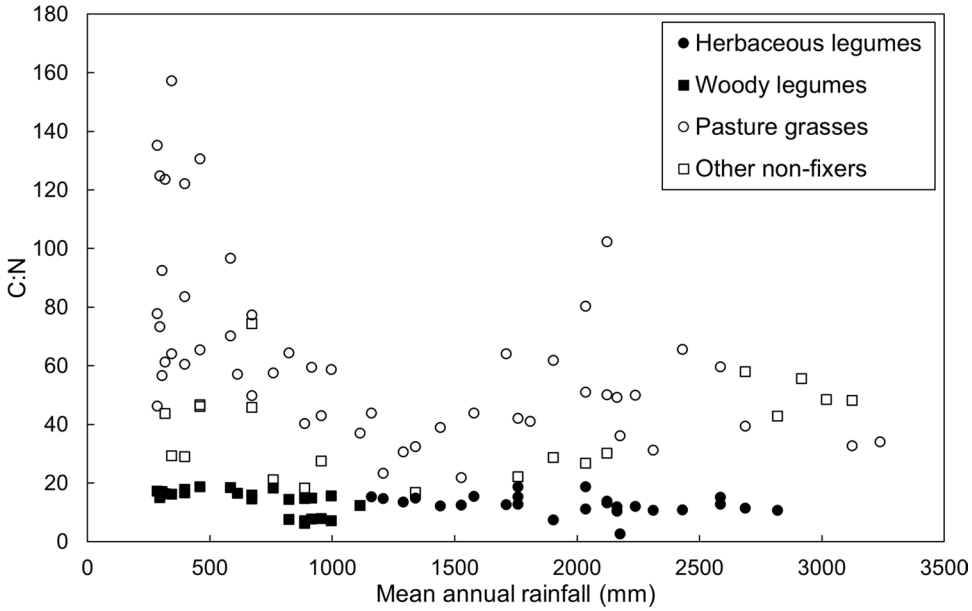

Fig. 4 C:N mass ratios from herbaceous legume, woody legume, pasture grass, and other non-legume leaves across the rainfall gradient

Table 2 Foliar C:N ratios from Kohala and existing literature

Species	Kohala means			Non-N-limited systems		
Legumes	% N	% C	C:N	% N	C:N*	Source
T. repens	3.8	42.0	11.1	~4	10.5	Sandli et al. (1993)
N. wightii	3.3	46.5	14.1	3.3	14.1	Ruschel et al. (1979)[a]
L. leucocephala	5.8	48.7	8.4	3.1	15.7	Campo and Dirzo (2003)
P. pallida	3.0	47.5	15.8	3.3	14.4	Arnold (1976)[b]
Non-legumes	% N	% C	C:N	% N	C:N	Source
C. ciliaris	0.7	44.6	63.7	2.6	17.2	Mannetje and Jones (1990)
P. clandestinum	1.1	46.4	42.2	~3–4	13.3	Colman and O'Neill (1978)

*C:N from previous studies estimated using mass % C means from Kohala

[a]*N. wightii* is represented here by close relative *Glycine max*

[b]*P. pallida* is represented here by close relative *Prosopis glandulosa*

yr in legumes and grasses, but does not appear to drop off significantly between the fertile and wet domains.

Discussion

Patterns and drivers of legume abundance

Previous research from leeward Kohala has described pedogenic thresholds that manifest in the availability of soil nutrients across variations in rainfall. Vitousek and Chadwick (2013) classified Hawī volcanic soils into three soil process domains: below 700 mm/yr of rainfall, wind erosion and a lack of biological uplift leads to low and variable soil nutrient levels; from 700 to 1700 mm/yr, high rates of weathering and biological uplift create a zone of relatively high soil fertility; above 1700 mm/yr, nutrient mobilization begins to outpace rates of weathering and uplift, resulting in generally low soil fertility above 2100 mm/yr. von Sperber et al. (2017) reported potential net N mineralization thresholds largely consistent with Vitousek and Chadwick (2013)'s soil process domains, with moisture-normalized potential net N mineralization peaking at a rainfall level statistically similar to the peaks of resin-extractable P and exchangeable Ca (~ 1200 mm/yr). Potential N mineralization decreases linearly below ~ 1000 mm/yr and is low above ~ 1700 mm/yr, although slightly elevated mineralization rates were detected at the driest and wettest extremes. In summary, soils are relatively enriched in both rock-derived nutrients and N in the moderate-rainfall region that roughly matches the bounds of the ancient Leeward Kohala Field System (Marshall et al. 2017). Below this moderate-rainfall region, N, P, Ca, and other nutrients decline due to low rates of weathering and biological activity; above 1700 mm/yr, accelerated leaching of nutrients leads to depleted soils and low levels of bioavailable N.

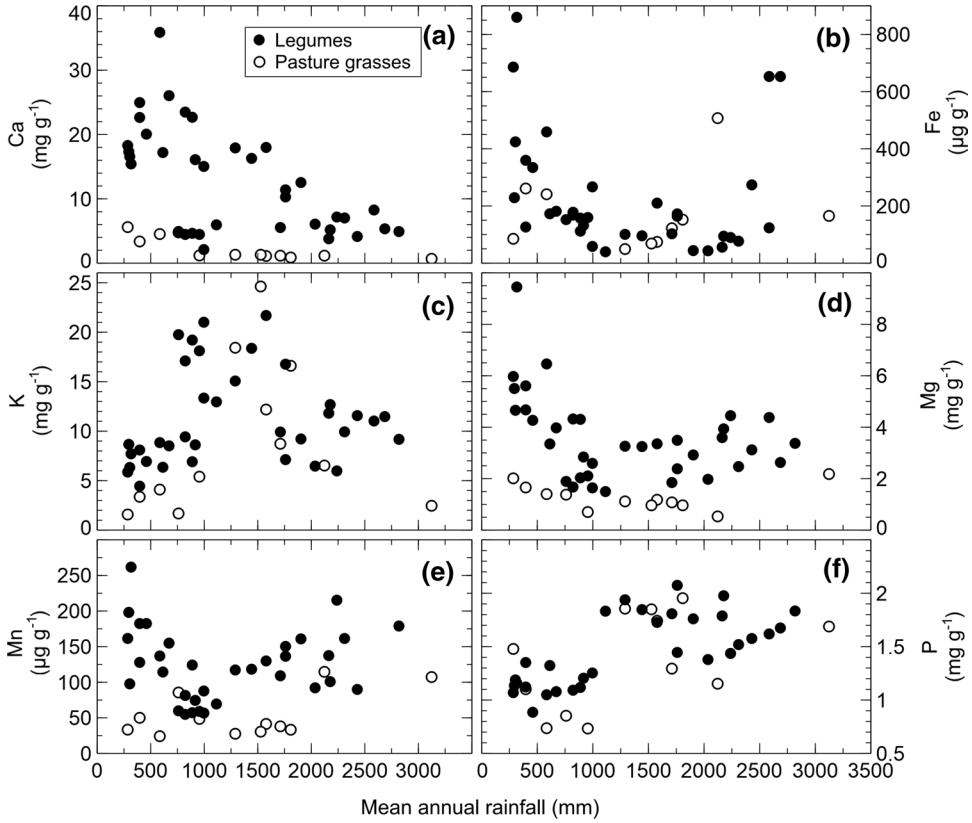

Fig. 5 Foliar concentrations of **a** calcium in mg g^{-1}, **b** iron in µg g^{-1}, **c** potassium in mg g^{-1}, **d** magnesium in mg g^{-1}, **e** manganese in µg g^{-1}, and **f** phosphorus in mg g^{-1}. Legumes are represented by filled circles and pasture grasses by open circles

Biomass data from Kohala show that legume abundance drastically declines once rainfall exceeds ~ 1800 mm/yr (Fig. 2b), consistent with the onset of nutrient mobility and depletion (Vitousek and Chadwick 2013). Legume density also decreases around 700 mm/yr (Fig. 2c), the driest point at which Vitousek and Chadwick (2013) detected the signal of biological nutrient uplift (Jobbágy and Jackson 2001), but the decline in legume abundance here may not be as abrupt as at the pedogenic threshold separating the fertile and wet domains. Legume abundance at the driest sites could be limited by aridity as well as the low availability of rock-derived nutrients; the two factors cannot be easily disentangled in this study due to lower sampling density at the driest sites and the inherent correlation of very dry conditions with low fertility in Kohala's moderately-aged basalts (Bateman et al. 2019). The abrupt transition from high to low herbaceous legume biomass at the wetter pedogenic threshold, however, implicates rock-derived-nutrients as the immediate driver of legume abundance where water is plentiful. Legume aboveground biomass falls from 163 to 0 g m^{-2} where rainfall increases by just 50 mm/yr but various rock-derived nutrients feature precipitous declines (Vitousek and Chadwick 2013; von Sperber et al. 2017). This pattern suggests that legume abundance (and, by extension, the magnitude of N inputs into the soil) is controlled at least in part by essential nutrients whose distributions are themselves determined by the dynamics of weathering, leaching, and biological uplift.

Prior work on Hawaiian rainforest canopy species is consistent with this hypothesis. The N-fixer *Acacia koa* (and dryland relative *Acacia koaia*) and non-fixing *Metrosideros polymorpha* both occupy a wide altitudinal/climatic range and together dominate most native Hawaiian forests. Their abundance and relative dominance in different climates varies depending on the age of the basaltic substrate: very young forests feature abundant *A. koa* at cold, high elevations with moderate-to-high rainfall; older substrates like Mauna Kea and Kohala produce a new area of abundant N fixers in the warm mesic zone (which aligns well with the fertile domain of high legume growth found by this study) while *A. koa* becomes dominant in the cold subalpine environment (not captured by this study); and *A. koa* dominates forests on the oldest substrates in Kaua'i only in dry and mesic areas (Mueller-Dombois 1987; Baker et al. 2009). The climatic distribution of these highly adaptable, widely ranged N fixers is heavily modulated by substrate age in Hawaiian systems, implicating rock-derived nutrient supply (itself a long-term integration of climatic forcing) as an immediate determinant of legume abundance on Hawaiian basaltic soils in all but the wettest and driest climate extremes.

Foliar nutrient trends

Of the rock-derived nutrients that could limit legume growth, P is the most likely candidate, as it is an essential (and frequently limiting) nutrient for BNF (Vitousek et al. 2002) and its abundance is strikingly elevated in soils from 700 to 1700 mm/yr (Vitousek and Chadwick 2013; Vitousek et al. 2016). Foliar P concentrations (Fig. 5f) do not capture any signal clearly implying P limitation, in part because the foliar response to P availability can vary significantly between plant species (Mo et al. 2019), but P has also been implicated as the primary limiter of BNF along this rainfall gradient by biogeochemical modeling (Vitousek et al. 2021).

Foliar K concentrations in both legumes and pasture grass are greater in the fertile domain than in the wetter and drier domains (Fig. 5c). This pattern may reflect soil K availability, as soils above ~ 1700 mm/yr are almost completely depleted of their K content (Chadwick et al. 2003; reported rainfall level adjusted according to Marshall et al. 2017) while soils in the dry domain may not be weathered enough to allow plants to fully access the large amounts of K still present there. It is possible that the scarcity of soil K inhibits BNF and legume growth in the wet and dry domains, but more research is needed to evaluate such a hypothesis.

Foliar Ca in legumes negatively correlates with rainfall (Fig. 5a), mirroring soil Ca levels (Chadwick et al. 2003; Vitousek and Chadwick 2013), but this trend is probably caused by the deposition of Ca in foliage by plants' transpiration streams (Hanger 1979)—in arid climates, high evaporative demand drives high transpiration rates that transport large amounts of Ca to leaves, where the Ca accumulates. The woody legumes present in leeward Kohala's dry domain feature high transpiration rates (Miyazawa et al. 2016), while the C_4 grass inhabiting those sites is adapted to minimize transpiration (Osborne and Sack 2012), possibly explaining the stark differences in foliar Ca concentrations between pasture grasses and legumes observed there.

Foliar Fe, Mg, and Mn concentrations all appear somewhat elevated at the ends of the climate gradient (Fig. 5); no clear sign of nutrient limitation is visible in these data. Fe, which is known to limit symbiotic BNF in some natural systems (Vitousek et al. 2013), roughly matches soil Fe availability across the climate gradient (Vitousek and Chadwick 2013). Mo is another resource frequently found to limit BNF (Schwenke and Kerridge 2000; O'Hara 2001; Vitousek et al. 2013), but we were unable to analyze foliar Mo in this study.

Despite the drop-offs in legume biomass outside of Kohala's fertile region, foliar C:N ratios (Fig. 4) indicate that the legumes present in those regions can still acquire sufficient N throughout the sample transect. In contrast, pasture grass and other non-fixer C:N ratios are elevated on either side of the peak in soil N availability from 1000 and 1500 mm/yr of rainfall (von Sperber et al. 2017), implying N deficiency in these regions. Foliar nutrient data also suggest that, despite low soil P supplies, sampled legumes above 1700 mm/yr are not P deficient (Fig. 5f). Thus, the few legume individuals (all *T. repens*) that survive in the wet domain appear to have acquired sufficient N and P. Yet their scarcity implies the lack of a competitive advantage over the non-legumes that remain abundant above ~ 1250 mm/yr, suggesting P limitation of BNF could still be occurring at the community scale even if sampled foliage contained normal P levels (Vitousek et al. 2021).

^{15}N patterns and N dynamics across pedogenic thresholds

The isotope data in Fig. 3 illustrate that legume $\delta^{15}N$ is largely decoupled from soil $\delta^{15}N$ across the gradient, probably due to a significant contribution of atmospheric N_2 ($\delta^{15}N = 0‰$) to legume N pools, while non-legume $\delta^{15}N$ closely tracks soil $\delta^{15}N$ with a consistent offset of ~ −4‰. The similarity of non-legume and soil $\delta^{15}N$ trends reflects the plants' sole reliance on soil N pools, while the offset likely reflects fractionation effects during the uptake, transformation, and storage of N (Evans 2001).

The consistently low C:N ratios (Fig. 4) but variable isotopic signatures (Fig. 3b) observed in legume foliage throughout the sample transect are most likely due to legumes sourcing varying proportions of N from the soil and atmosphere—that is, legumes derive most of their N from the atmosphere where soil N pools are depleted and access soil N wherever available. This fact is readily apparent from the elevated $\delta^{15}N$ values in the middle domain where von Sperber et al. (2017) measured peak soil N mineralization, and from the slight $\delta^{15}N$ increases at the dry extreme of the sample transect where slightly elevated N mineralization rates were measured. While legume $\delta^{15}N$ patterns clearly diverge from soil and non-legume $\delta^{15}N$ patterns in the dry and fertile domains, all three isotopic trends remain essentially flat above ~ 2000 mm/yr of rainfall (although the legume isotope ratios are slightly more negative on average than the non-legume ratios). This similarity means that a high reliance of legumes on BNF cannot be directly inferred from the isotope data here, but the consistently low C:N ratios of legumes relative to non-legumes (Fig. 4) suggest BNF is indeed important to legumes in the wet domain.

The correspondence of legume $\delta^{15}N$ breakpoints (Table 1) with previously described thresholds in rock-derived nutrients is striking: the two statistically significant breakpoints at ~ 750 mm/yr and ~ 1600 mm/yr of rainfall are very close (within 95% confidence intervals) to the boundaries of the fertile domain presented by Vitousek and Chadwick (2013). Within this domain, legume $\delta^{15}N$ values rise higher than anywhere else on the climate gradient. A lesser overall reliance of legumes on BNF at these fertile

sites most readily explains the observed peak in legume $\delta^{15}N$—other fractionating effects in the plant-soil system would be unlikely to cause such drastic fluctuations in legume $\delta^{15}N$ while leaving no visible effects on non-legume $\delta^{15}N$, and inter-species physiological differences are unlikely to account for the observed pattern given the continuity of the trend and the mix of species involved.

The fertile domain contains three legume species (the woody *L. leucocephala* and *P. pallida* and herbaceous *N. wightii*), one of which (*P. pallida*) is present throughout the dry domain as well. *P. pallida* displays distinct yet continuous $\delta^{15}N$ trends in each domain, and the foliar $\delta^{15}N$ trend where woody legumes give way to herbaceous legumes (at ~ 1100 mm/yr of rainfall) likewise features no obvious discontinuity. These results indicate that the elevated foliar $\delta^{15}N$ observed in legumes in the fertile domain is not simply caused by species- or functional group-level physiological differences in the processing, fractionation, and storage of N, but rather reflects a non-species-specific link between legume N sources and the climate gradient.

While elevated foliar $\delta^{15}N$ indicates legumes are deriving a greater proportion of N from the soil in the fertile domain, actual trends in BNF rates remain unclear: BNF may be down-regulated at these sites as a facultative response to soil N availability, or BNF rates may be unaffected if the legumes are obligate N fixers but still derive a larger proportion of their N from the soil due to its high availability (Menge et al. 2015). BNF regulation strategies can vary greatly even in closely related plants, but the fraction of N derived from the soil (and thus foliar $\delta^{15}N$, in this system) should generally increase provided enough soil N is available (Menge et al. 2015).

Even where individual legumes appear able to derive a significant portion of their N from the atmosphere in the dry and wet domains (based on near-zero $\delta^{15}N$ values and consistently low C:N ratios in both domains), legumes remain scarce (Fig. 2): the aboveground dry biomass of *T. repens* never exceeds 10 g m^{-2} in the wet domain, while the dry biomass of legumes in the fertile domain exceeds 160 g m^{-2} at several sites. A study of *T. repens* in a wet pasture environment found that ecosystem-scale BNF rates were highly correlated with the legume's dry matter yields (which were much greater than observed in the wet domain on Kohala), indicating symbiotic inputs of N into the ecosystem are small in the wet domain (Goh and Bruce 2005). N fixers are similarly scarce below 700 mm/yr of rainfall, represented only by *P. pallida* growing at very low density.

Thus a picture of ecosystem development on this climatic gradient begins to emerge: legumes grow best where rainfall integrated over geologic time scales provides the right balance of water availability, chemical weathering, nutrient mobility, and biological nutrient uplift; over time, legumes build large soil N pools at these rainfall levels. Once established, these soil N pools allow legumes to rely in part on soil N, meaning the long-term magnitude of these pools likely depends on the BNF regulation strategies of the legumes present (Menge et al. 2015) as well as various loss pathways (Vitousek et al. 2021). Individual legumes remain highly reliant on BNF for N acquisition at other rainfall levels where resource limitations (e.g. P and water) prevent legumes from growing in abundance, as evidenced by low $\delta^{15}N$ values and relatively low C:N ratios in the dry and wet domains. N inputs that occur in such sites are far exceeded by N demand, causing these ecosystems to remain proximally N-limited (Vitousek et al. 2021).

Soil $\delta^{15}N$ generally increases with decreasing rainfall across the sample transect, as has been observed in many studies elsewhere (Wang et al. 2014; Craine et al. 2015; von Sperber et al. 2017; Vitousek et al. 2019). This is most likely caused by increased fractionating gaseous losses of N in dry sites, especially ammonia volatilization (Vitousek et al. 2019). The sudden decline in soil $\delta^{15}N$ at arid sites that has been observed both here and in northern China (Wang et al. 2014) is clearly not attributable to a significant increase in BNF—elevated legume $\delta^{15}N$ at the driest sites despite lower soil $\delta^{15}N$ could indicate lower reliance on BNF, and the very low legume abundance at these rainfall levels would sharply limit ecosystem-level BNF inputs. Rather, it may reflect a shift to abiotic pathways of gaseous N loss following soil wetting events, which could lead to lower isotopic fractionation at these very dry sites (von Sperber et al. 2017).

Known pedogenic thresholds and soil process domains appear consistent with some, but not all, patterns of $\delta^{15}N$ in leeward Kohala. Observed trends in legume biomass, $\delta^{15}N$, and foliar chemistry correspond to the thresholds presented by Vitousek and Chadwick (2013), although some $\delta^{15}N$ patterns at the extremes of the climate gradient are not explained by their framework. For instance, the aforementioned N dynamics at the dry extreme of the gradient are not clearly associated with any soil process domains known from Hawī volcanics. Further study of the wet and dry extremes of ecosystem N cycles is needed to understand observed dynamics and to determine whether they may be attributed to pedogenic processes or to climatic controls.

A recent study of N dynamics on the geologically older substrates of Kaua'i found that peak long-term BNF activity (as manifested by high potential net N mineralization levels) in both Kohala and Kaua'i corresponds roughly to the same long-term net water balance, suggesting that BNF rates may be more readily linked to the direct effects of climate on legume activity than to soil nutrient patterns (Vitousek et al. 2019). However, in the Kaua'i study one legume species, *A. koa*, dominated the sites with the highest N mineralization rates while land use and biological invasions could have eliminated N-fixing plants from the drier domain more analogous to Kohala's fertile middle domain in terms

of rock-derived nutrient levels. On Kohala, nutrient scarcity cannot easily be disentangled from aridity as the primary limiter of legume growth in the dry domain, but the sharp decrease in legume abundance above the pedogenic threshold at 1,700 mm/yr indicates that soil process domains are indeed informative of BNF patterns where a diverse array of legume species can grow across the relevant rainfall levels. In conclusion, pedogenic thresholds appear to hold significant explanatory value in characterizing symbiotic BNF and its contribution to ecosystem development where legume distributions are not drastically altered by human land use or climatic factors.

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