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Organic matter and nitrogen accumulation and nitrogen fixation during early ecosystem development in Hawaii

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Abstract. We used a chronosequence comprised of 10 y, 52 y and 142 y old ‘a’a lava flows on Mauna Loa, Hawaii, to determine the accumulation of organic matter and nitrogen and rates of nitrogen fixation through time. The mass of organic matter (live and dead biomass and soil organic matter) on the 1984, 1942 and 1852 lava flows was 0.6, 2.2 and 7.6 kg m^{-2}, respectively, while total N was 4.8, 10.9 and 85.7 g m^{-2}.

We estimated the total rates of nitrogen fixation for the three different aged ecosystems using an acetylene reduction assay calibrated with ^{15}N incubations. While mean rates of total N fixation remained largely constant across the three sites – between 2.0 and 3.1 kg ha^{-1} y^{-1} – the most important sources of N fixation changed. On the 10 y flow, the most important fixer was the pioneering cyanolichen, *Stereocaulon vulcani*. After 52 years of ecosystem development, the most important N fixer was a cyanoalga, while after 142 years, the predominant N fixers were heterotrophic bacteria associated with leaf litter, twigs and detritus. The total amount of N accumulated after 52 years of ecosystem development was equivalent to cumulative inputs through biological N fixation. After 142 years, however, cumulative inputs from N fixation could only account for between 27–59% of the total nitrogen accrued.

We used fertilizer additions of all essential nutrients other than N to test whether the availability of lithophilic nutrients regulated rates of N fixation in early ecosystem development. Rates of nitrogen fixation by the lichen, *S. vulcani*, approximately doubled when fertilized on the 1984 and 1942 flows. Rates of N-fixation by heterotrophic nitrogen fixing bacteria on leaf litter of *Metrosideros polymorpha* also increased significantly when fertilized with lithophilic nutrients. These findings suggest that weathering rates of lava in part regulate rates of nitrogen fixation in these young ecosystems.

Introduction

Globally, rates of organic matter and nitrogen accumulation in primary succession vary widely in response to the climate, slope, parent material, organisms and age of particular sites (Jenny 1941). Since primary succession is a slow process by human standards, many researchers have used chronosequences of land forms to infer the changes in ecological communities and their underlying processes through time (Stevens & Walker 1970). Geomorphological formations that have lent themselves to chronosequence studies include glacial till deposits (Crocker & Major 1955; Tisdale et al. 1966; Bormann & Sidle 1990; Chapin et al. 1994), sand dunes (Olson 1958) and lava flows (Eggler 1971; Raich et al. 1997). Here we use lava flows selected from the Mauna Loa Environmental Matrix in Hawaii (Vitousek et al. 1992). The surface of Mauna Loa is comprised of many different aged lava flows that span a wide range of elevations and precipitation regimes. This matrix of climate and soil age offers the opportunity to hold the soil forming factors of parent material, climate, slope and organisms relatively constant while examining changes in processes that occur during primary succession.

In their classic review of chronosequence studies, Stevens and Walker (1970) reported that nearly all chronosequece studies documented the presence of N-fixing vascular plants at one or more seral stages early in primary succession. These pioneer nitrogen-fixing plants can dramatically increase the amount of N cycling in developing ecosystems (Chapin et al. 1994). The montane rainforest in Hawaii is unusual in that there are no native N-fixing vascular plants in young primary successional communities (Vitousek 1994). The exotic, actinorhizal plant *Myrica faya* has been successful at colonizing some young substrates on the island of Hawaii, but its range is still restricted (Vitousek & Walker 1989). In this study, we evaluated how organic matter and nitrogen accumulate in primary succession when the only nitrogen fixers are cyanobacteria and free-living heterotrophic bacteria.

Plant biomass and species composition have been determined on Mauna Loa. Not surprisingly, biomass and soil depth increase as a function of lava flow age (Aplet & Vitousek 1994; Kitayama et al. 1995). In the present study, we used a short chronosequence comprised of three sites which were also a part of the studies by Aplet and Vitousek (1994) and Kitayama et al. (1995) – the 1984, 1942 and 1852 ‘a’a lava flows at 1130 m. This chronosequence allowed us to address the following questions: 1) what are the patterns of organic matter and nitrogen accumulation associated with the increase in plant biomass?; 2) how do rates of N fixation by different cyanobacteria and heterotrophic nitrogen fixers change as the overall community changes through time?; 3) how do measured rates of N fixation compare with N accumulation? and 4) are rates of symbiotic and heterotrophic nitrogen fixation limited by the availability of lithophilic nutrients weathered from rock substrates?

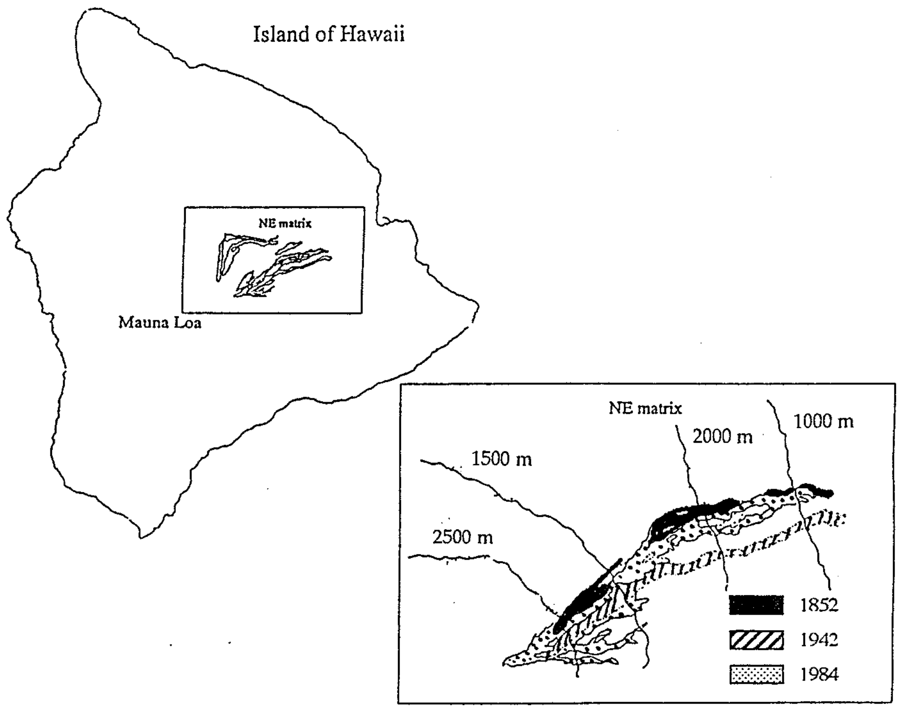

Figure 1. Location of chronosequence sites on Mauna Loa Volcano, Hawaii.

Study sites

We located study sites at 1130 m on three recent lava flows on the NE, windward flank of the volcano Mauna Loa on the Island of Hawaii (Figure 1). The lava flows originated in 1852, 1942 and 1984, so that at the time of the study the parent material ages were 142, 52 and 10 years old. All sites had a northeastern aspect and a grade of <4%. The average annual rainfall at the three sites is 430 cm (Juvik & Nullet 1994), which for the most part is distributed evenly throughout the year, as it is associated with the relatively constant northeast Trade Winds (Carlquist 1980). Average monthly rainfall at the sites exceeds evaporative demand during all months of the year (Juvik & Nullet 1994). The mean temperature of the warmest month is 18 °C in September and the mean temperature of the coolest month is 13 °C in February (Juvik & Nullet 1994). All of the sites were in a stage of early primary succession and had not been significantly disturbed by burning, clearing, wind or other impacts. Feral pigs (*Sus scofra*) were present in the general region, but their impact on these particular sites was not discernible.

The volcanic parent material at the three sites was ‘a’a lava – a coarse, frothy rock that forms a rugged and irregular surface. The chemical composition of closely related lava flows (i.e., flows that originated from the same volcano within a short time) is generally quite similar (Wright & Helz 1987). Some chemical variation can exist, however, as in the case of the 1852 flow in this study, which has a relatively high proportion of olivine (Tilling et al. 1987). The overall physical and textural properties of the three flows were similar when compared to other types of volcanic rocks, but we did observe some differences in texture with the 1942 ‘a’a being the most friable and the 1852 ‘a’a the most consolidated.

The three lava flows supported a range of primary successional communities from early colonizing lichens and bryophytes, to small statured rainforest. The surface of the 1984 ‘a’a was covered almost entirely by the lichen *Stereocaulon vulcani* and the moss *Rhacomitrium lanuginosum*. No soil organic matter had accumulated where the lichens grew, while only thin films were found under the mosses. On the 1942 flow, some of the lava surface was still colonized by *Stereocaulon vulcani* with little apparent organic matter buildup in those areas. Other surfaces were covered with a variety of live mosses and liverworts, and/or a 1–10 cm thick layer of decomposing detritus with recognizable vestiges of *S. vulcani* and *R. lanuginosum*. This flow also was extensively colonized by vascular plants, including the tree *Metrosideros polymorpha*. The same vascular plant species were present at greater density and stature on the 1852 ‘a’a flow, with the tree *M. polymorpha* dominating the overstory at an average height of 4.1 m. Bryophytes and lichens (mainly *Cladonia skottsbergii* as opposed to *S. vulcani*) overlaid a 1–10 cm histic horizon and largely blanketed the surface of the 1852 flow. The soil at the 1852 site has been classified as a poorly developed isothermic, Lithic Tropofolist (Raich et al. 1996).

Using a cluster analysis of species composition across their chronosequence sites (except the lichen and moss dominated 1984 flow), Kitayama et al. (1995) found vascular plant community similarities to cluster into four groups which they designated as four phases of primary succession. According to the designation by Kitayama et al. (1995), the 1942 and 1852 sites of our chronosequence fall into phase one of vascular plant succession. This phase is distinguished from the older phases by the high cover of the fern *Dicranopteris linearis* and the absence or low cover of the fern *Cibotium glaucum*. Further description of the vegetation at each of the three sites can be found in Aplet and Vitousek (1994), Kitayama et al. (1995) and Drake and Mueller-Dombois (1993).

Methods

Nitrogen and biomass accumulation

At the 1984 site, we randomly selected ten 0.25 m^2 plots across the width of the flow. We harvested all vegetation from these plots. The biomass was separated by species *S. vulcani* and *R. lanuginosum*, weighed wet, and then sub-samples were taken for dry weight conversion. We dried these and all other vegetation sub-samples referred to below for a minimum of 48 hours at 70 °C. Sub-samples of the dried materials were analyzed for total C and N on a Carlo Erba CHN analyzer. No measurements of soil organic matter (SOM) were made on the 1984 flow, as the accumulation of SOM at this early stage of ecosystem development was negligible.

At the 1942 site, we established two 50 m transects towards the middle of this relatively narrow flow so as to minimize edge effects with the older adjacent flows. At 10 m intervals on the 50 m transects, we established perpendicular 5 m lines, and used the terminal 1 m sections to locate square meter plots. We harvested, weighed and took sub-samples for dry weight conversion of all live vegetation, with the exception of the shrubs *Vaccinium* spp., and young *M. polymorpha* trees. We estimated woody vegetation biomass of these species using allometric conversions reported by Aplet and Vitousek (1994). All recognizable litter and stems were collected, separated by species and dried. The total N and C of live biomass and litter were determined using a Carlo Erba CHN analyzer. We collected soil organic matter in two ways; first by scraping surface accumulations of the soil surface, and 2) by digging the 1 m^2 plots to a depth of 1 m, and sieving and weighing the loose material that was $<$1 cm. We sub-sampled loose material, and brought it back to the lab where it was sieved to separate particles into $>$2 mm, 1–2 mm and $<$1 mm size classes. Sub-samples from each of these size classes as well as samples of the soil organic matter scraped from the lava surface were dried at 105 °C for 48 hours and analyzed for C and N on a Carlo Erba CHN Analyzer. On the 1942 flow, we estimated SOM by multiplying soil organic carbon values by 1.9 (Nelson & Sommers 1982).

At the 1852 site, we established one 50 m transect at a location 20 m to the north of the ‘a’a experimental fertilization plots established by Raich et al. (1996). We established five 1 m^2 plots in the same fashion as described for the 1942 site. We harvested all bryophytes and lichens, leaf litter, dead stems, and soil organic matter from the plots and processed the biomass in the same manner as materials from the 1942 site. Estimates of live, standing vascular plant biomass at the 1852 site were derived from Aplet and Vitousek (1994). To estimate total N and C in plant biomass, we sampled plant tissues of all species, dried the materials at 70 °C for a minimum of 48 hours, ground them in a Wiley mill and analyzed them on a CHN analyzer; tissue nutrient concentrations were then multiplied by the biomass values of Aplet and Vitousek (1994) for estimates of biomass-N.

Nitrogen fixation

We estimated nitrogenase activity for all of the substrates sampled from the three lava flows using the acetylene reduction (AR) assay (Hardy et al. 1968). When significant levels of nitrogenase activity were detected on a substrate, we repeated the measurement on 2–10 sampling dates (4–5 replicate samples per time) (Table 1) to calculate an average rate of nitrogenase activity. Included in our initial sampling survey were roots of *M. polymorpha* and other common plant species at the sites. No nitrogenase activity associated with the rhizosphere of these species was detected.

We sampled substrates from each site at least once every other month throughout the spring and summer of 1994, and analyzed them for nitrogenase activity at field moisture levels. Extrapolating short-term estimates of nitrogenase activity measured by acetylene reduction to annual rates of N fixation has been justifiably criticized, in part due to the sensitivity of nitrogen fixing organisms to diurnal, seasonal and other variability in the environment. However, these criticisms are most important in environments with extreme variation in light, temperature or moisture such as in desert or arctic ecosystems. The windward slopes of Hawaii have relatively constant conditions of temperature, moisture and light which reduces the error associated with environmental variation in extrapolating acetylene reduction assays to annual rates of N fixation.

Depending on the substrate, we placed between 5–15 g wet weight of materials in 500 mL mason canning jars. The jars were modified for the AR assay by installing gas-tight, rubber septa in the lids. We injected 50 mLs of high purity acetylene (C_2H_2) into each jar, gently shook them, and inserted a needle into the septa to normalize atmospheric pressure with the outside. The substrates were incubated in the 9% C_2H_2 atmosphere for 3 hours, and then 10 mL samples were removed from the jar atmospheres, and 5mL was injected into a gas chromatograph (Shimadzu model GC-8A fitted with a Porapak N column) to measure ethylene (C_2H_4). After incubations, we dried the substrates at 70 °C for at least 24 hours to determine sample dry weights. All substrates were screened for ethylene consumption and production before acetylene reduction analyses were carried out.

To normalize acetylene reduction rates to rates of nitrogen fixation, we conducted parallel acetylene and ^{15}N incubations (Giller & Wilson 1991) of the substrates that had demonstrated the greatest nitrogenase activity. For the ^{15}N assay, we evacuated one-half atmosphere of air from the 500 mL

Table 1. Nitrogen fixation (acetylene reduction) associated with various substrates at 10, 52 and 142 years of ecosystem development.

Lava flow	Substrate	% contribution to total N fixed in ecosystem	nmols C_2H_4 gdw^{-1} hr^{-1} mean (SE)	# sampling dates[1]	gdw substrate m^{-2}	%N in substrate
1984	*Stereocaulon vulcani*	69	9.2[2]	–	369	0.64
	Rhacomitrium lanuginosum	27	5.2 (4.2)	3	254	0.86
	Dubautia scabra litter	4	16.0[3]	–	11	0.83
1942	Rock colonizing "cyanoalgae"	32	45.0 (22)	4	51	2.43
	Live bryophytes	28	5.4 (2.5)	3	380	0.86
	Stereocaulon vulcani	15	9.2[2]	–	114	0.68
	Surface soil organic matter	10	3.1 (1.5)	3	241	0.91
	Dubautia scabra litter	9	16.0 (8.6)	4	42	1.15
	Dicranopteris linearis litter	4	3.76 (2.1)	3	72	0.44
	Metrosideros polymorpha litter	1	3.6 (1.1)	8	16	0.35
	Machaerina angustifolia litter	1	0.66 (–)	1	73	0.35
	Cibotium glaucum litter and stems	<1	3.43 (2.1)	4	7	0.39
	Hedyotis centranthoides litter	<1	1.5 (–)	1	6	0.68
	Vaccinium spp. litter	<1	4.0 (–)	1	2	–
	Psilotum nudum	<1	6.2 (–)	1	1	0.69
1852	Live bryophytes	43	5.4 (2.5)	3	449	0.70
	Dicranopteris linearis litter	24	3.8 (2.1)	3	354	0.66
	Metrosideros polymorpha litter	10	3.9 (0.8)	10	137	0.42
	Mixed detritus	9	4.3 (–)	1	113	–
	Machaerina angustifolia litter + corum	8	0.7 (0.03)	2	631	0.27
	Lycopodium cernuum litter	4	11.5 (5.7)	4	20	0.68
	Vaccinium spp. litter	<1	4.2 (–)	1	3	0.52
	Styphelia tameiameiae	<1	4.0 (–)	1	1	0.71
	Cladonia skottsbergii	<1	3.5 (0.15)	3	1	0.25

[1] 4–5 replicates per substrate type were conducted on each date.

[2] Estimate of N fixation for *S. vulcani* interpolated from Kurina (1998).

[3] Acetylene reduction was not carried out on *Dubautia scabra* litter from the 1984 flow so the mean value from the 1942 flow is used here.

incubation chambers and replaced it with a 20%–O_2/80%–N_2 gas; the N_2 had a purity of 99% ^{15}N. Substrates were incubated in the enriched atmosphere for 24 hours after which time they were dried, ground and analyzed for ^{15}N by mass spectroscopy by the lab of J. Ehleringer. The parallel acetylene reduction assay was carried out in the same manner as the AR assays described above, except the substrates were sealed and incubated in air for 21 hours before the acetylene was injected; the ^{15}N substrates and the AR substrates were therefore incubated in sealed atmospheres for the same period of time. When checked against our standard 3-hour AR incubation method, we measured no difference in the nitrogenase activities of substrates that were 'pre-incubated' for 21 hours before the addition of acetylene. The average C_2H_4:N_2 molar ratio we measured for all substrates was 2.59, which is close to the theoretical ratio that has been estimated at 3:1 (Hardy et al.

1968). We used our empirically derived value to convert rates of acetylene reduction to annual nitrogen fixation.

To extrapolate annual fixation rates from hourly rates we assumed that N fixation took place 11 hours/day for 365 days per year for autotrophs and 24 hours/day for 365 days/year for heterotrophs. We did not display error bars for our estimates of annual fixation rates as we do not know of a legitimate approach to propagating sampling errors associated with multiple, unrelated sampling regimes; i.e., acetylene reduction, biomass and ^{15}N incubation measurements. However, in order to see how the variation in acetylene reduction measurements affected our extrapolations of annual N fixation at the three sites, we carried out a sensitivity analysis using the high and low estimates of standard error for each substrate to calculate annual N fixation.

To estimate nitrogen fixation rates by the lichen *Stereocaulon vulcani* for the 1984 and 1942 flows, we used values of fixation per unit biomass from a detailed study by Kurina (1998). She estimated N fixation of *S. vulcani* at 1500 m and 900 m on the 1984 flow to be 0.15 and 0.50 mg N gdw^{-1} yr^{-1} respectively by combining N-fixation data from growth chamber and hydration experiments with microclimate measurements made near her study sites. We interpolated between Kurina's (1998) two estimates to obtain the value of 0.33 mg N gdw^{-1} yr^{-1} for our 1130 m site. Kurina did not find differences in acetylene reduction rates per unit lichen thallus dry weight across lava flow ages, and thus we used the same per unit rate to estimate fixation on the 1984 and 1942 flows.

Nutrient addition experiments

We designated study patches of *S. vulcani* by placing 80 plastic rings that were 21 cm in diameter on both the 1984 and 1942 flows. Every week for approximately 7 weeks, we sprayed forty of the eighty rings with 10 mL of a dilute nutrient solution containing all of the essential elements except nitrogen. The nutrient solution consisted of 0.156 g L^{-1} $CaHPO_4$, 0.226 g L^{-1} KH_2PO_4, 0.720 g L^{-1} $MgSO_4$-7H_2O, 2.0 mg L^{-1} $FeSO_4$-7$H_2$0, 1.37 mg L^{-1} $MnSO_4$-$H_2$0, 2.86 mg L^{-1} H_3BO_3, 0.22 mg L^{-1} $ZnSO_4$-7$H_2$0, 0.08 mg L^{-1} $CuSO_4$-5$H_2$0, 0.094 mg L^{-1} Na_2MoO_4, 0.56 μg L^{-1} $CoSO_4$; at the same time we sprayed the other 40 rings with 10mL of deionized water. At the end of the fertilization period, the lichens were harvested from the rock surfaces, taken to the laboratory, hydrated with deionized water, and tested for nitrogenase activity using the AR assay.

To examine fertilization effects on heterotrophic N-fixing bacteria, we collected *Metrosideros polymorpha* leaf litter from every plot in a full factorial fertilization experiment on the 1852 flow. The fertilization treatments included N, P, T (all essential nutrients other than N and P) and all combinations of these primary treatments (for a detailed description of rates and timing of fertilization, see Raich et al. 1996). Leaf litter from the different plots was taken to the laboratory and analyzed for nitrogenase activity using the AR assay.

We calculated significant differences between fertilized and control patches of *S. vulcani* using two-tailed *t*-tests. We analyzed the data from the factorial fertilization experiment using an ANOVA design with four blocks and three treatments. Assumptions of constant variance and normality of the data were satisfied for the *t*-test and ANOVA analyses.

Results

Biomass and nitrogen accretion

Live, aboveground biomass increased across the chronosequence from 634 to 1101 to 2162 gdw m^{-2} at the 1984, 1942 and 1852 flows respectively. The species that contributed the most to total biomass shifted from the lichen, *Stereocaulon vulcani* after 10 years of ecosystem development, to the tree *Metrosideros polymorpha* from 52 years on (Table 2). *M. polymorpha* was by far the greatest component of total biomass at the 1852 site and remains the dominant tree in the montane rainforest through long-term ecosystem development in Hawaii (Kitayama & Mueller-Dombois 1995). In total biomass, bryophytes were second in importance at all three sites, and their abundance increased through time (Table 2).

Total ecosystem organic matter, including live biomass, detritus and soil organic matter also increased through time. There was a significant shift, however, in where the majority of organic matter was found in the ecosystems – on the 1984 flow it was held in live biomass while on the 1852 flow it was found in soil organic matter (Figure 2(a)). Total nitrogen accretion paralleled increases in organic matter across the chronosequence (Figure 2(b)), but the increase from the 1942 flow to the 1852 flow was greater for N than it was for organic matter as a whole. This relative increase in N mainly occurred in the soil organic matter pool – the percent N in SOM increased from 0.4% on the 1942 flow to 1.4% on the 1852 flow.

Nitrogen fixation

We measured considerable variation in N fixation rates across different substrate types, and within a given substrate on different sampling events (Table 1). In spite of this variation, the summed rates of nitrogen fixation for

Table 2. Live, aboveground plant biomass accumulation and tissue nitrogen and phosphorus concentrations at 10, 52 and 142 y of ecosystem development. Plants that made up 1% or more of the total biomass are included.

Lava flow	Plant	% of total aboveground biomass	Aboveground biomass gdw m^{-2}	%N[2]	%P[2]
1984	*Stereocaulon vulcani* (l)[1]	58	369	0.64	0.025
	Rhacomitrium lanuginosum (b)	40	254	0.86	0.024
	Dubautia scabra (s)	2	11	0.83	0.109
1942	*Metrosideros polymorpha* (t)	39	428	0.37	0.104
	Bryophytes[3]	34	380	0.86	0.024
	Stereocaulon vulcani (l)	10	114	0.68	0.030
	Dicranopteris linearis (f)	5	54	0.69	0.046
	"Cyanoalga"	5	51	2.43	0.025
	Dubautia scabra (s)	3	31	0.83	0.109
	Machaerina angustifolia (m)	2	17	0.72	0.052
	Cibotium glaucum (f)	1	13	0.75	0.097
	Hedyotis centranthoides (s)	1	7	0.67	0.104
	Polypodium pellucidum (s)	1	6	0.59	0.048
1852	*Metrosideros polymorpha* (t)	63	1379	0.33	0.070
	Bryophytes[4]	18	449	0.36–0.71	0.031–0.065
	Dicranopteris linearis (f)	6	134	0.48	0.045
	Lycopodium cernuum (cm)	2	53	0.42	0.031
	Vaccinium spp. (s)	2	46	0.71	0.060
	Machaerina angustifolia (m)	2	42	0.74	0.053
	Coprosma spp. (s)	2	33	1.02	0.107
	Styphelia tameiameiae (s)	1	26	0.60	0.059

[1] Letters in parenthsis following plant names indicate the following: cm = club moss, f = fern, l = lichen, m = monocot, s = shrub, t = tree.

[2] Tissue N and P concentrations for trees are for stem materials only, while shrub values are the average tissue nutrient concentrations of leaves and stems.

[3] The dominant bryophyte at the 1942 lava flow was *Rhacomitrium lanuginosum* although others were present. The nutrient values listed are for *R. lanuginosum*.

[4] The bryophyte community of the 1852 lava flow community was comprised of numberous species including *R. lanuginosum*, *Leucobryum* sp. and *Plagiochila* sp. The range of tissue nutrient concentrations listed is for these three species.

all substrates remained remarkably stable through time with 0.212 (±0.051), 0.315 (±0.130) and 0.238 (±0.092) g N fixed m^{-2} y^{-1} on the 1984, 1942 and 1852 flows, respectively. The values in parentheses are the results of a sensitivity analysis where high and low N fixation estimates were calculated based on the standard errors of acetylene reduction assays of each individual substrate.

While total ecosystem N fixation rates remained constant through time, the most important N-fixing substrates changed. After 10 years of ecosystem development, the most important source of N fixation was the lichen *S. vulcani*, which accounted for 69% of total N fixation (Table 1, Figure 3).

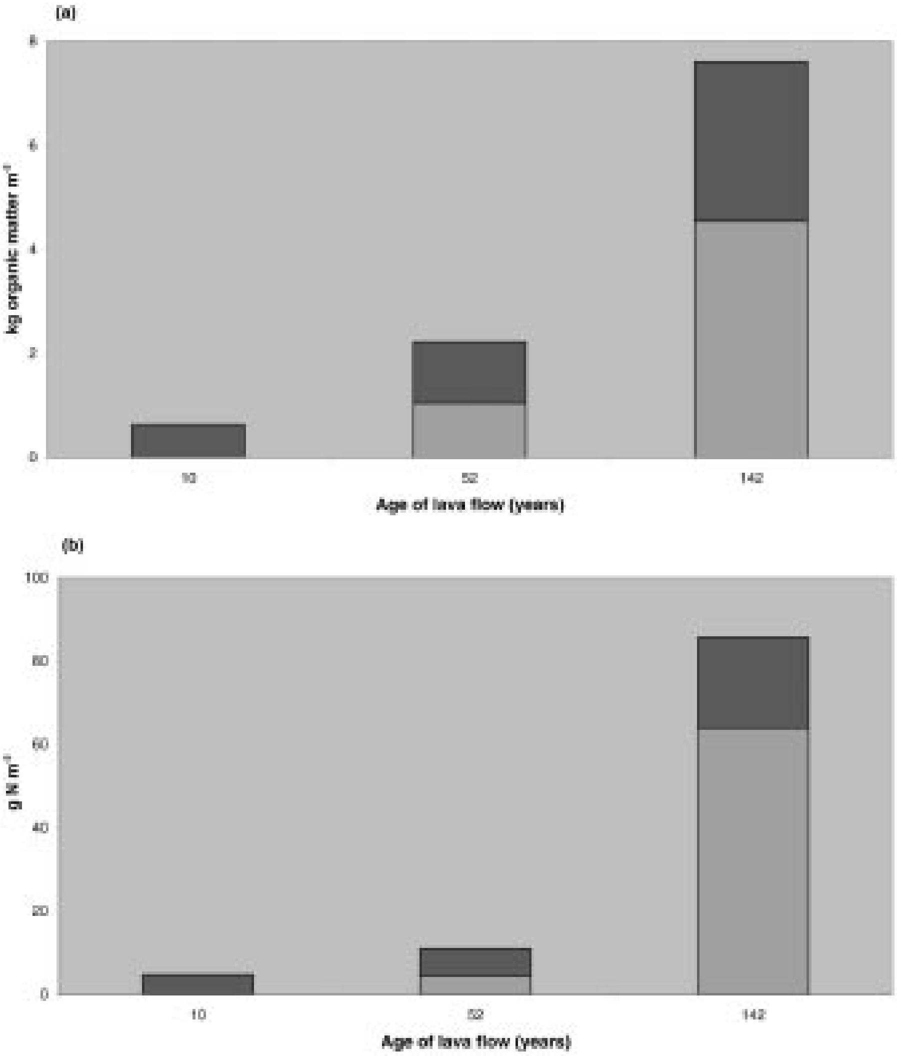

Figure 2. (a) Total organic matter and (b) total nitrogen of the 1984, 1942 and 1852 ‘a’a lava flows at 1130 m on Mauna Loa. Dark bars represent live and dead biomass and light bars represent soil.

On the 1942 flow, the most active N fixer was a small alga that grew on rock surfaces; it contained cyanobacteria with heterocysts (Chris Smith, personal communication). These ‘cyanoalga’, which was only found on the 1942 flow, had the greatest mean rates of N fixation gdw^{-1} measured of any substrate from all three flows – 45 nmols hr^{-1} – and also contained the highest tissue N concentrations (Table 1). Nitrogen fixation by the cyanoalga comprised about

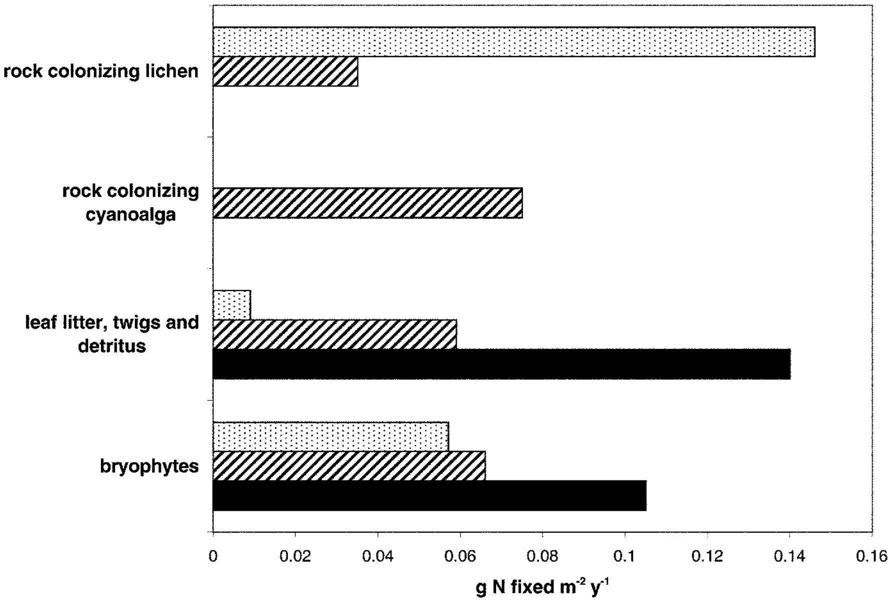

Figure 3. Nitrogen fixation by different categories of substrates on the 1984, 1942 and 1852 ‘a’a lava flows on Mauna Loa. Stippled bars = 1984 flow; hatched bars = 1942 flow; solid bars = 1852 flow.

32% of the total rate of N fixation we measured on the 1942 flow. Nitrogen fixation by cyanobacteria associated with bryophytes was important across the chronosequence, but it grew in relative importance with time as bryophyte biomass increased (Figure 3). N fixation by free-living, heterotrophic bacteria took place at low, constant levels on virtually all detritus measured (Table 1). As detritus increased during succession, so did the importance of this N fixation source (Figure 3) to where on the 1852 flow, heterotrophic fixers became more important as a category than autotrophic fixers. One notable exception to the low rates of nitrogenase activities measured on detritus substrates were the relatively high rates measured on the litter of *Dubautia scabra* (Table 1).

Regulation of N fixation by weathered elements

When fertilized with a dilute solution of all essential nutrients other than nitrogen, nitrogenase activity of the lichen *Stereocaulon vulcani* approximately doubled on both the 1984 and 1942 flows (Figure 4).

On the 1852 flow, we found nitrogenase activity of heterotrophic bacteria to be significantly greater on *Metrosideros polymorpha* leaf litter collected from plots fertilized with P and T (all essential elements other than N and P) than on leaf litter collected from the control plots (Figure 5). We also found

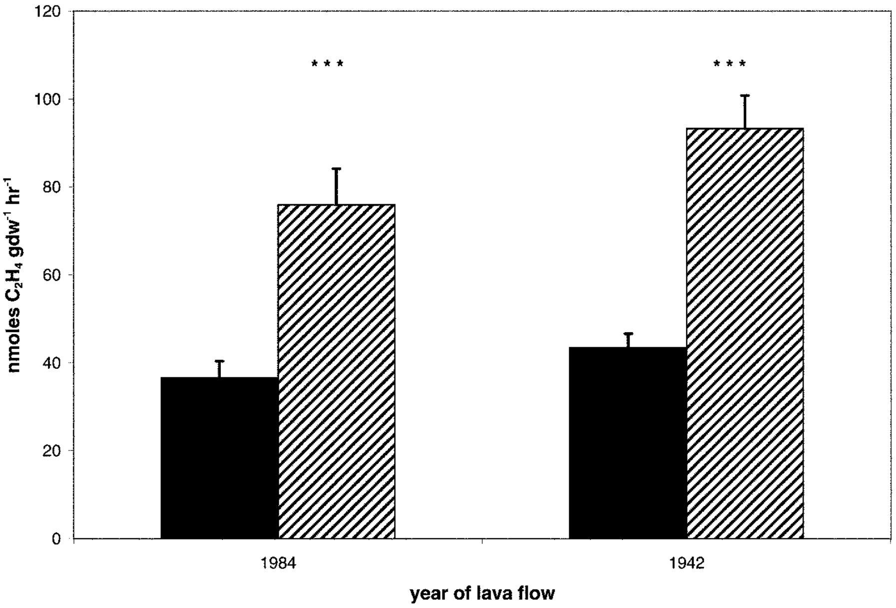

Figure 4. Comparison of rates of acetylene reduction by *Stereocaulon vulcani* with and without nutrient additions. The nutrient treatment consisted of all essential nutrients other than N. Solid bars = controls (deionized water additions only); Hatched bars = nutrient addition treatments. $^{***}P < 0.001$.

significant interactive effects of P∗T and N∗P on acetylene reduction rates with maximum rates of nitrogenase activity in the combined NP and T treatments. Although this increase appears to be mainly due to the additive effects of the NP and PT treatments, it is noteworthy that nitrogenase activity was suppressed by N in plots where N alone was added, whereas the addition of N increased activity when added in combination with the P and PT treatments (Figure 5).

Discussion

Biomass and N accumulation and the role of N-fixation

Live, aboveground biomass accumulated very quickly (63 gdw m^{-2} y^{-1}) in the first decade of primary succession. During the subsequent 132 years, live, aboveground biomass accumulations slowed considerably to an average rate of 11–12 gdw m^{-2} y^{-1}. Accumulation of soil organic matter initially lagged behind biomass, but became the dominant pool of organic matter at the oldest site (Figure 2(a)). On the 1984 flow there was essentially no buildup of SOM,

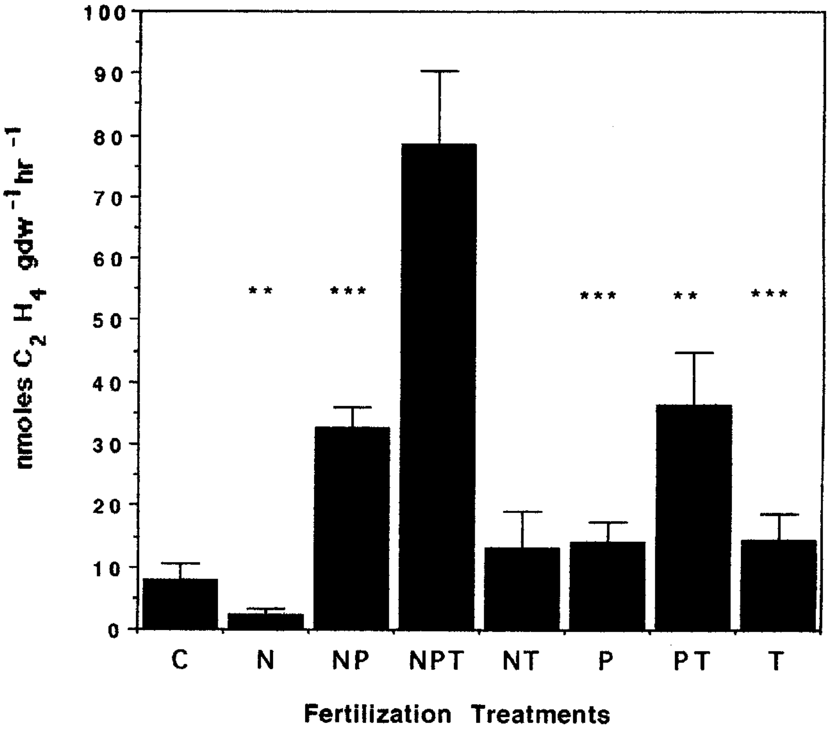

Figure 5. Heterotrophic N-fixation (acetylene reduction) on *Metrosideros polymorpha* litter collected from long-term, full factorial fertilization plots on the 1852 'a'a lava flow. N = nitrogen fertilizer additions; P = phosphorus fertilizer additions; T = fertilizer additions of all essential nutrients other than N and P. Bars with asterisks are significantly different. $^{**}P < 0.01$, $^{***}P < 0.001$.

whereas on the 1942 and 1852 flows we measured 1.05 and 4.54 kg m^{-2} respectively. Assuming a ratio of SOM: organic C of 1.9 (Nelson & Sommers 1982) organic C was accumulating at a rate of ~13 g m^{-2} y^{-1} between years 10–52 and at a rate of ~27 g m^{-2} y^{-1} between years 52 and 142. These rates of organic C accumulation are comparable to the rate of 17 g m^{-2} y^{-1} over 136 years measured by Raich et al. (1997) for the 1855 pahoehoe lava flow that is in close proximity to our 1852 'a'a site, but considerably lower than those reported by Schlesinger et al. (1998) who found organic C to accumulate at rates of 45–127 g C m^{-2} y^{-1} in a 110 y volcanic ash soil on Krakatua. These differences are reasonable based on the Krakatua site having several attributes that probably contribute to a greater rate of ecosystem development: a finer textured parent material (ash), higher temperatures and dominance by symbiotic N fixers (Schlesinger et al. 1998).

Patterns of nitrogen accumulation were similar to those of total organic matter except that the increase in soil-N between 52 years and 142 years of ecosystem development was proportionately greater than the overall increase in the soil organic matter pool (Figures 2a & 2b); during this time SOM increased by 4.3$\times$ whereas soil N increased by 14.9$\times$. This increase in SOM-N represents a transition from an SOM pool dominated by carbon-rich litter substrates, to a slow cycling SOM pool with a lower C:N composition.

While the most prominent N-fixer differed at each of the three stages of ecosystem development, the aggregate rates of N fixation by all substrates remained relatively constant between 2 and 3.1 kg N ha^{-1} y^{-1}. On a chronosequence of volcanic cinder in Hawaii dated at 27, 200 and 2000 years old, Vitousek (1994) found a similar range of potential, total ecosystem N fixation associated with a similar set of organisms – the rates he measured were 0.3, 1.2 and 2.8 kg N-fixed ha^{-1} y^{-1} by non-symbiotic bacteria and bryophytes.

On the 1984 flow, we estimated the lichen *S. vulcani* to fix 1.5 kg N ha^{-1} y^{-1}. This rate is well within the range of 0.5–3.5 kg N fixed ha^{-1} y^{-1} reported by Millbank (1984) in a review of N fixation by cyanolichens in a variety of ecosystems. Free living cyanobacteria associated with bryophytes were responsible for approximately 0.6 kg N-fixed ha^{-1} y^{-1} on the 1984 and 1942 flows, and 1.1 kg N-fixed ha^{-1} y^{-1} on the 1852 flow, at the low end of other rates reported for non-symbiotic cyanobacteria. In prairie ecosystems, where fire plays a role in maintaining light and nutrient resources for cyanobacteria mats on the soil surface, estimates of N fixation range from 10–21 kg ha^{-1} y^{-1} (DuBois & Kapustka 1983; Eisele et al. 1989). The cyanoalgae that was present only on the 1942 flow fixed about 0.7 kg N ha^{-1} y^{-1} – we were unable to find reports of fixation by a similar association for comparison.

On the 1852 flow, 59% of the total N fixed, or 1.4 kg ha^{-1} y^{-1} was by heterotrophic bacteria on leaf litter. At a 200 y and 2000 y site developed on cinders on the Hawaiian volcano Kileaua, Vitousek (1994) estimated heterotrophic N-fixation associated with wood and leaf litter to be 0.83 and 2.4 kg ha^{-1} y^{-1} respectively. Heterotrophic bacteria in the topsoil of coniferous and deciduous forests in Sweden were estimated to fix between 0.4 and 1.4 kg ha^{-1} y^{-1} (Nohrstedt 1985). In Oregon, two estimates were made of potential rates of heterotrophic N fixation associated with litter of *Pseudotsuga menziesii*, they were $\sim$1.4 kg ha^{-1} y^{-1} (Silvester et al. 1982) and 1.08 kg ha^{-1} y^{-1} (Heath et al. 1988). The similarity between all of these measurements suggests that 'background' rates of 0.5–2.0 kg N fixed ha^{-1} y^{-1} associated with decomposing biomass may be common in wet forests of both tropical and temperate regions.

Our findings suggest that in the initial decade of ecosystem development, N accumulation rates exceed the cumulative inputs from N fixation (Figure

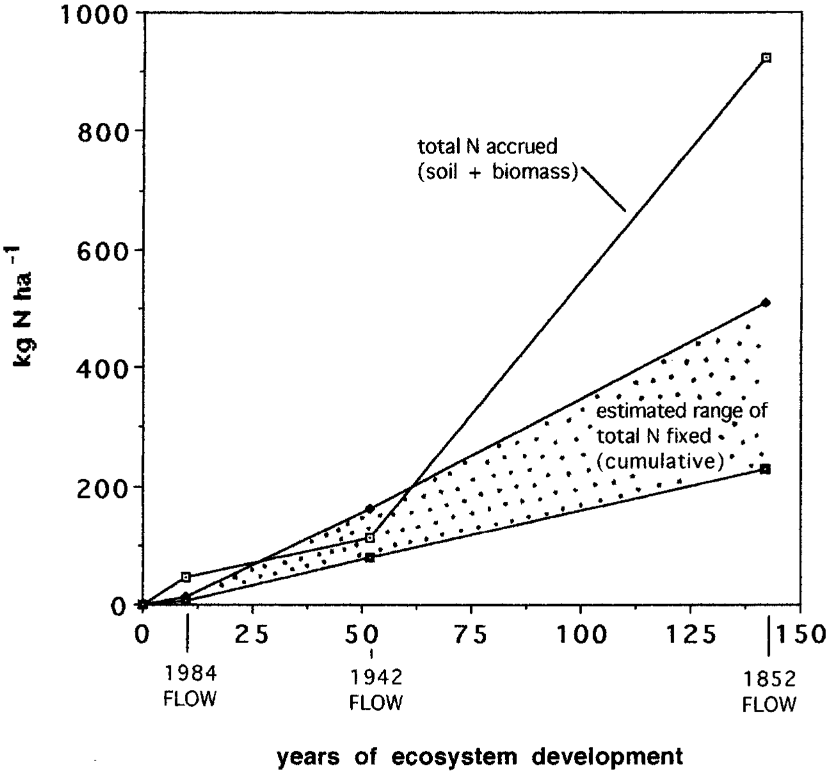

Figure 6. Nitrogen accumulation and range of cumulative nitrogen fixation at 10, 52 and 142 year stages of ecosystem development. Stippled area = cumulative N fixed; Solid squares = total N accrued.

6). Estimates by Kurina (1998) further support this view. She found that on the 1984 flow at 1500 m (300 m in elevation higher than our site), N-fixation by the lichen *S. vulcani* could only account for about 14% of the total N in the lichen biomass. This percentage increased with decreasing elevation – N-fixation by *S. vulcani* at 900 m accounted for 42% of the total N in the lichen biomass – but a majority of N inputs still originated from sources other than N fixation. Between 10 and 52 years of ecosystem development, we found that rates of N accumulation paralleled rates of N fixation and that total N accumulation on the 1942 flow could be accounted for by N fixation alone. Between years 52 and 142, however, rates of N accumulation far exceeded cumulative potential inputs from N fixation (Figure 6).

The discrepancy between rates of N accretion and cumulative N fixation at the 1852 site underscores the probable importance of nitrogen inputs from atmospheric deposition. Heath and Huebert (1999) estimated the N in atmospheric deposition for a $\sim$300 y and younger rainforest near the caldera of Kilauea Volcano, at the same elevation as our study sites and approximately 25 km to the southwest. Measuring both NO_3^- and NH_4^+, they estimated N inputs to be 0.6 and 0.15 kg ha^{-1} y^{-1} in rainfall and dry deposition, and 4–9 kg ha^{-1} y^{-1} in cloud water deposition that is not influenced by active volcanoes. The rate of N accumulation not accounted for by N fixation that we measured over 142 years of ecosystem development averages 3.9–4.9 kg ha^{-1} y^{-1} which coincides with the atmospheric deposition estimates made by Heath and Huebert (1999). Assuming there was no influence of abiotic volcanic N fixation (see Heath & Huebert 1999), the N deposition from atmospheric sources across our chronosequence was likely to be 2× to 4× greater than N inputs from biological N-fixation.

While there appears to be a significant concentration of N in rainfall at these sites, we speculate that the pioneer lichen and moss community is less efficient than more developed communities at capturing N delivered in rainfall as the vegetation lacks roots and soil (including soil organic matter). As organic matter accumulates with ecosystem development and roots of vascular plants take hold, it is possible that rainfall N is increasingly appropriated by the community allowing for N accumulation to substantially exceed cumulative rates of N fixation after 142 years (Figure 6).

Regulation of N-fixation by geologically cycled elements

Walker and Syers (1976) suggested that because parent materials contain their maximum phosphorus contents and essentially no nitrogen during early stages of soil development, sources of N inputs such as nitrogen fixing plant/bacterial symbioses tend to play a central role in primary succession. Phosphorus availability is only seen as having a pronounced, direct effect on productivity and/or indirect effects on ecosystem nitrogen dynamics after primary mineral P has been exhausted, and a substantial fraction of soil P has been lost or bound in unavailable organic and inorganic forms (Walker & Syers 1976).

Numerous researchers have relied on this model of Walker and Syers' to explain N limitation in early stages of ecosystem development (Chapin et al. 1991; Chapin 1993; Vitousek et al. 1993). In the present study we show that the availability of one or more geologically cycled elements (P, K, Ca, Mg, + micronutrients) significantly limits rates of nitrogen fixation by autotrophic lichens after 10 and 52 years of ecosystem development (Figure 4), and by heterotrophic bacteria after 142 years (Figure 5). Although new parent materials contain relatively high levels of essential geologically cycled nutrients, most of these nutrients are not available to the biota early in pedogenesis.

The environmental conditions for nutrient release from rock weathering are nearly optimal on Mauna Loa in Hawaii. The basalt parent material is among the most ‘weatherable’ of igneous rocks (Carroll 1970). This combined with a climate that is wet and relatively warm creates conditions that are favorable to relatively high rates of rock dissolution (Gorham et al. 1979). Thus, even under conditions where we would expect the rate of nutrient release from rock weathering to be high, we found that the availability of rock-derived nutrients is still an important factor regulating nitrogen inputs into the young ecosystems studied.

The actual mechanism by which nutrients regulate heterotrophic N fixation remains unclear. In many cases, populations of heterotrophic bacteria are primarily limited by carbon availability (Gibson et al. 1988; Alexander 1977). Was the increase in N-fixation measured on the *M. polymorpha* leaf litter in the 1852 fertilized plots due to a direct response by the heterotrophic nitrogen fixers to the elements added, or did the elements added in the P and T fertilization treatments result in *M. polymorpha* leaf litter with better tissue quality (i.e., more labile C)? Vitousek (1998) found that additions of P reduced lignin concentrations and increased P concentrations in senesced leaf litter. In a decomposition experiment, the P-enriched leaves collected from the fertilized plots decomposed more readily than did unfertilized leaves (Vitousek 1998). These findings suggest that the heterotrophic bacteria (including N-fixers) respond to improved carbon availability caused by P additions, however it is not possible to rule out a direct, positive effect of P on N-fixing bacteria.

Vitousek (1998) did not find any relationship between the T fertilizer additions (all essential elements other than P and N) and lignin content of *M. polymorpha* leaf litter, nor did he find leaves collected from the T plots to decompose more readily than leaves from the control plots. That carbon availability to decomposers does not appear to be affected by the T nutrient additions points to enhanced nutrient availability as a possible explanation for greater N fixation rates in the T fertilization plots. *M. polymorpha* foliage collected from the T plots had significantly elevated concentrations of K and Mg (Vitousek 1998). While concentrations of Mo have been found to be elevated in leaf litter from the T plots (Vitousek, unpublished data), more analyses will be required to see if concentrations of other elements known to be important to N fixation are elevated as well. An alternative to either increased nutrient content, or carbon availability to explain greater heterotrophic N fixation on leaf litter in the P and T plots could be fertilizer-induced changes in the soil environment (such as pH) that enhance rates of N fixation.

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