Biogeochemistry **55:** 179–194, 2001.

Nitrogen fixation rates of *Stereocaulon vulcani* on young Hawaiian lava flows

LIANNE M. KURINA[1,2,*] & PETER M. VITOUSEK[2]
[1]*Unit of Health-Care Epidemiology, Department of Public Health, University of Oxford, Old Road, Headington, OX3 7LF, U.K.;* [2]*Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, U.S.A. (*Author for correspondence, e-mail: lianne.kurina@uhce.ox.ac.uk)*

Key words: nonsymbiotic nitrogen fixation, primary succession, tropics

Abstract. Previous research has suggested that nitrogen-fixing lichens can play an important role in the nitrogen cycle of early primary successional systems and other extreme environments. In this study, we estimate rates of nitrogen fixation by a nitrogen-fixing lichen, *Stereocaulon vulcani*, at 1500 m on the northeast slope of Mauna Loa volcano. Using microclimate measurements and a climate-driven model of nitrogen fixation, we estimate that *S. vulcani* fixes between 0.2 and 0.45 kg N ha^{-1} yr^{-1}. We calculate that *S. vulcani* could have derived 40% of its nitrogen content from biological fixation.

Introduction

Nitrogen-fixing cyanobacteria are among the first colonists in most primary successional seres – whether in free-living form, in loose associations with algae, or in lichen symbioses (Eggler 1963; Henriksson et al. 1972; Sheridan 1991; Sprent 1993). It has been suggested that nitrogen-fixing organisms should have a competitive advantage early in succession, since most new substrates contain almost no nitrogen (Vitousek & Walker 1987). Moreover, colonization by nitrogen-fixers could increase nitrogen availability and facilitate colonization by non-nitrogen fixing plants (Vitousek & Howarth 1991). Research addressing the role of nitrogen-fixing lichens in the nitrogen cycle of early primary successional systems, and in extreme environments more generally, suggests that while lichens' rates of nitrogen fixation are low relative to those of nodulated vascular plants, their contribution of nitrogen is important, given the profound nitrogen limitation of their environment, and the absence of nodulated fixers in many such systems (Snyder & Wullstein 1973; Alexander et al. 1978; Skujins & Klubek 1978; Rhoades 1981; Van Cleve & Alexander 1981; Chapin & Bledsoe 1992; Line 1992).

However, these estimates are often extrapolations of acetylene reduction activity (ARA) measurements that take only a cursory account of climate-driven variation in nitrogenase activity. Studies that have incorporated microclimate measurements into estimates of annual nitrogen fixation have yielded much lower nitrogen contributions (e.g. Gunther 1989). Lichens are poikilohydric organisms, whose physiological activity depends on both present and past climate conditions (Huss-Danell 1977; Crittenden & Kershaw 1978). Accurately assessing the role of lichens in the nitrogen cycle of their environment, therefore, demands a consideration of these climate conditions, and an understanding of their effect on lichen nitrogenase activity.

Stereocaulon vulcani (Bory) Ach. is a tripartite, nitrogen-fixing lichen that dominates the first stage of succession on a'a'-texture lava flows in Hawaii (Skottsberg 1941; Uhe 1988; Smith 1991; Kurina & Vitousek 1999). *S. vulcani* is the most widespread nitrogen-fixing organism in a system where biomass accumulation in young sites is profoundly nitrogen-limited (Vitousek et al. 1993; Raich et al. 1996). In Hawaii, *S. vulcani* is particularly abundant in wet, low elevation sites on young lava flows, where it can accumulate up to 600 g m^{-2} of biomass (Kurina & Vitousek 1999).

In this study, we estimate rates of nitrogen fixation by *S. vulcani* using a climate-driven model. We measured the microclimate conditions experienced by *S. vulcani* in the field, and constructed a model of *S. vulcani* acetylene reduction activity based on laboratory-derived responses of *S. vulcani* ARA to thallus temperature, ambient light level, and thallus water content. We used this model, together with the microclimate measurements and measurements of lichen biomass (Kurina & Vitousek 1999) to estimate lichen N fixation on three different-aged flows. These estimates were then used to calculate the proportion of nitrogen in the tissue of *S. vulcani* samples from a young lava flow that could have been derived from biological fixation versus from other sources.

Sites

The northeast slope of Mauna Loa volcano (19°32′N, 155°35′W) consists of a matrix of different-aged lava flows, many crossing thousands of feet in elevation. *S. vulcani* is found on young flows on the NE slope of Mauna Loa from sea level to over 2400 m. Rates of lichen colonization and accumulation are highest at low elevation, where conditions are warm and wet, but lichens persist longest in the colder, drier, high elevation sites (Kurina & Vitousek 1999). In this study, we estimate lichen N fixation on a 10 year-old, 52 year-old and 142 year-old flow at mid-elevation (1500 m). Mean annual temperature and precipitation at 1500 m are 14.1 °C and 2750 mm

yr^{-1}, respectively (Giambelluca et al. 1986; Juvik & Nullet 1994; Nullet et al. 1995). *S. vulcani* abundance on the three flows at this elevation has been carefully documented (Kurina & Vitousek 1999) and a climate station at 1640 m recorded air temperature, PAR, and rainfall data from September 1991 through September 1996 (though not always continuously).

Results

Microclimate measurements

Lichen temperature was measured at 1640 m on the NE slope of Mauna Loa for 12 days in the spring (March–April) and 21 days in the summer (June–September) of 1996. We measured the temperatures of five separate lichen thalli using copper-constantan thermocouples. The 5-minute temperature averages were recorded on a Campbell 21x datalogger (Campbell Scientific, Logan, Utah). Lichen temperature and air temperature followed the same temporal pattern, but lichen temperature exceeded air temperature by a few degrees during the day and was slightly lower than air temperature at night. The months during which we sampled had weather typical of the Island of Hawaii (NOAA records, Hilo, Hawaii).

The lichen temperature data were used with air temperature data collected simultaneously by the climate station to calculate hourly air-to-lichen temperature conversion factors (Kurina 1998). We calculated hourly air temperature averages for each hour from 700–1800 using the climate station data (J. Juvik & D. Nullet, personal communication). Mean hourly lichen temperature was calculated as the sum of the hourly air temperature average and the hour-appropriate air-to-lichen temperature conversion factor. Variance in lichen temperature is the sum of the air temperature and conversion factor variances. Estimated mean hourly lichen temperature at 1640 m ranged from 11.1 °C at 700 hours to 21.3 °C at 1200 hours (Figure 1a).

We estimated mean hourly photosynthetically active radiation (PAR) in these sites using climate station data. Mean hourly PAR at 1640 m ranged from 76 μmol photons m^{-2} sec^{-1} at 700 hours to 1253 μmol photons m^{-2} sec^{-1} at 1200 hours (Figure 1b).

We measured lichen water content for varying lengths of time (2 to 7 hours) for 7 days in March 1996, and for 7 days in the summer of 1996 (July–September), for a total of 60 hours sampled. Any given hourly lichen water content average is the result of 3 to 10 hours sampled. During the 14 field sampling periods, we recorded the weight of a single lichen sample (attached to a small piece of lava) every five minutes on a Mettler BD202 scale (Mettler-Toledo, Inc., Columbus, Ohio), specially modified to minimize the area of the

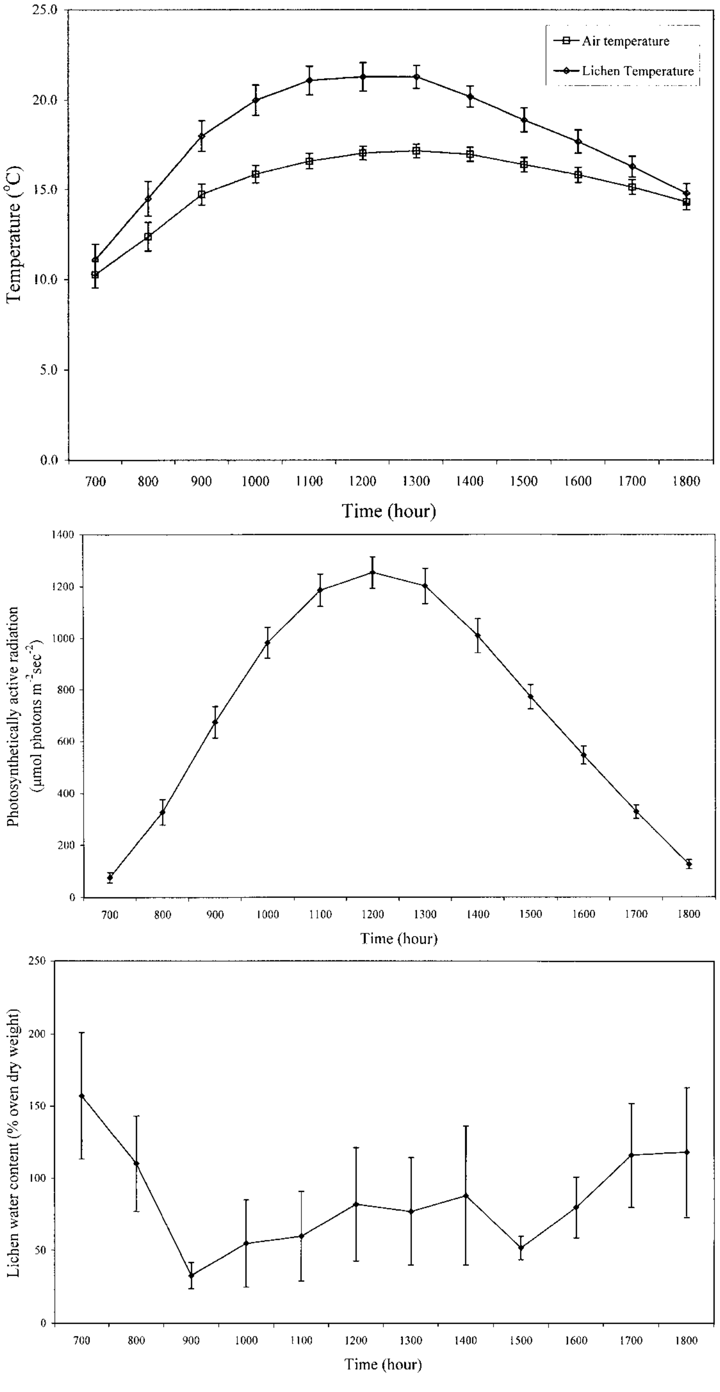

Figure 1. Mean hourly lichen and air temperature, ambient PAR, and lichen water content at 1640 m on the NE slope of Mauna Loa. Bars represent standard errors.

weighing surface. By leaving the lichens on the scale continuously, we were able to detect slight weight differences caused by cloud or mist events, in addition to the more obvious weight changes caused by rainfall or drying. The lichen samples were then oven-dried at 70 °C for 48 hours. After correcting the weight measurements for water accumulated by the lava substrate or the weighing pan, we calculated an average lichen water content for each hour sampled, and then calculated an hourly mean from these averages. Mean hourly lichen water content at 1640 m ranged from 158% (expressed as % oven dry weight) at 700 hours to 33% at 1000 hours (Figure 1c).

Model of S. vulcani acetylene reduction activity

ARA responses to temperature and light

We measured *S. vulcani* ARA in a variety of temperature and light conditions in order to model its response to different climate conditions in the field. We collected *S. vulcani* from the 1942 flow at 1500 m on the northeast slope of Mauna Loa volcano in September 1996. The samples were shipped to Arizona State University and stored air-dried at –18 °C until the acetylene reduction activity experiments were completed, within 2 months of sample collection. Freezing has no detectable effect on *S. vulcani* ARA (Kurina, unpublished data).

Two days prior to an ARA experiment, the lichens were transferred from the freezer to a growth chamber to acclimate to standard conditions of 12 hours light (200 μmol photons m^{-2} sec^{-1}) and 10, 15, 20, or 25 °C for the temperature response experiments. For the light response experiments, the temperature was set at 15 °C and light levels were 50, 100, 200, 400, or 800 μmol photons m^{-2} sec^{-1}. Six replicates per temperature or per light level were run. One day prior to the experiment, the lichen samples were saturated with deionized water to stimulate metabolic activity, and then allowed to dry. On the day of the experiment, the lichens were wet to saturation with deionized water two hours prior to beginning measurements.

We measured *S. vulcani* ARA using the traditional assay (Stewart et al. 1967) as modified by Jeffries (1989) and Thomas (1994). *S. vulcani* samples were placed in 30 ml airtight chromatography vials. 3 ml ($\sim$10%) of the air volume were removed from the cuvettes and replaced with a like volume of acetylene. Samples were incubated in the growth chambers under conditions appropriate to the experiment for 2 to 4 hours, depending on sample activity. Concentrations of acetylene and ethylene in the vial headspace were measured with a Perkin-Elmer Sigma 4 GC (PerkinElmer LLC, Norwalk, Connecticut) equipped with a Flame Ionization Detector and a 3 m × 2 mm ID stainless steel column with a Porapak R column (80–100 mesh). The

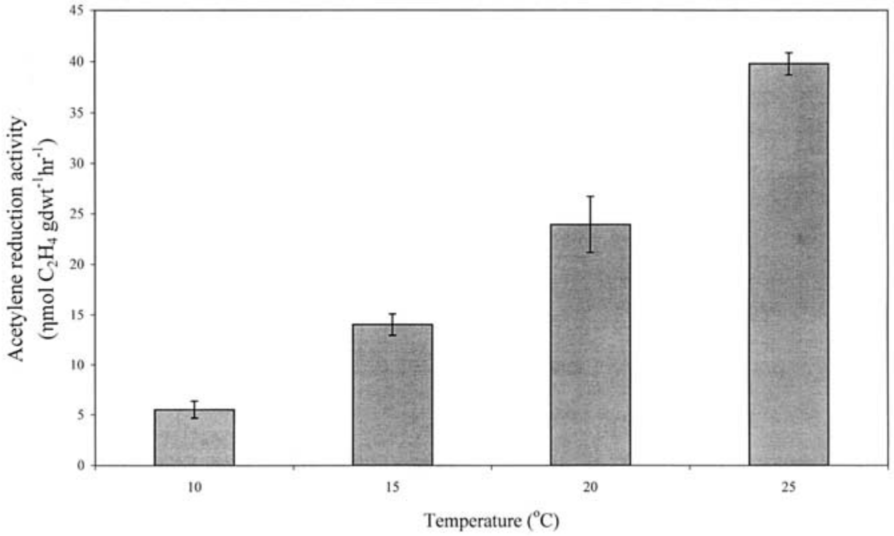

Figure 2. S. vulcani acetylene reduction activity vs. temperature (n = 6 at each temperature). Bars represent standard errors.

column temperature was set at 56 °C. Lichens were weighed before and after each experiment and then oven-dried at 70 °C for 24 hours.

We used SPSS (version 9.0) to test the relationships between ARA and temperature and ARA and light level. A single-factor ANOVA showed that *S. vulcani* ARA increased significantly with temperature between 10 and 25 °C (Figure 2, F = 110.97, df = 3, $p < 0.0001$). Bonferroni post-hoc tests showed that ARA at any given temperature was significantly different from ARA at any other temperature ($p \leq 0.002$ for all of the comparisons). We related *S. vulcani* ARA (expressed as a fraction of the maximum activity) to temperature (T) using a linear regression model (n = 23, F = 245.30, $p \ll 0.0001$).

$$\text{T scalar} = 0.05\text{T} - 0.417 \qquad r^2 = 0.92$$

A single-factor ANOVA showed that ARA changed significantly with light level (Figure 3, F = 16.62, df = 4, $p < 0.0001$). ARA was highest at the two lowest levels of PAR tested, 50 and 100 μmol photons m^{-2} sec^{-1}; Bonferroni post-hoc tests showed no difference in ARA between these two light levels but did show that ARA at either 50 or 100 μmol photons m^{-2} sec^{-1} was significantly different ($p < 0.05$) from ARA at any of the 3 higher light levels (200, 400, 800 μmol photons m^{-2} sec^{-1}). Assays done in Hawaii yielded comparable rates of ARA at 25 μmol photons m^{-2} sec^{-1}. ARA was significantly lower at the three higher PAR levels tested (200, 400,

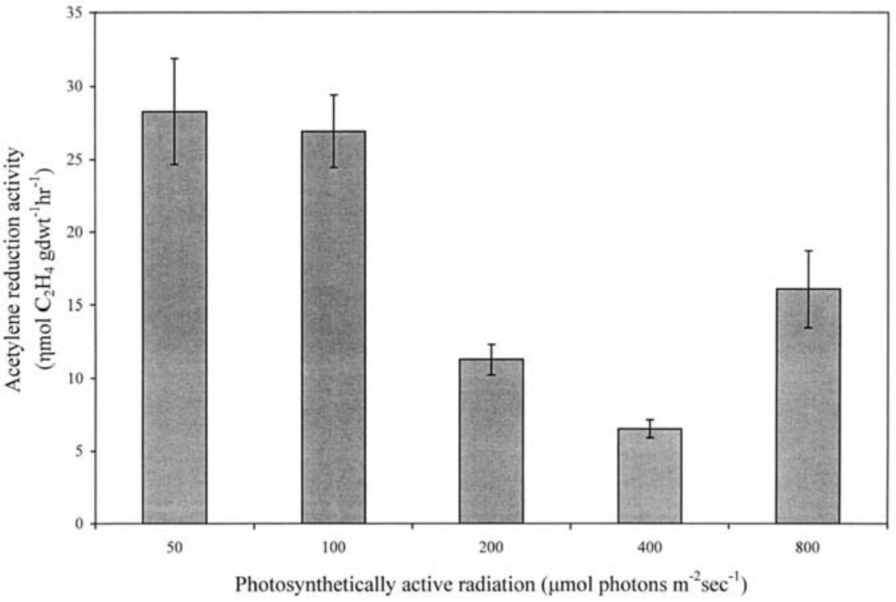

Figure 3. *S. vulcani* acetylene reduction activity vs. photosynthetically active radiation (n = 6 at each light level). Bars represent standard errors.

800 μmol photons m^{-2} sec^{-1}); again Bonferroni post-hoc tests showed no significant differences among these values. For simplicity's sake in the model, we assumed that ARA would be zero at values less than 25 μmol photons m^{-2} sec^{-1}, maximal at values between 25 and 100 μmol photons m^{-2} sec^{-1}, and half maximal at values greater than 100 μmol photons m^{-2} sec^{-1}.

ARA response to water content

Three separate experiments were done in Hawaii to test the response of *S. vulcani* ARA to water content. Samples for the first experiment were collected from the 1984 flow at 1200 m. Samples for the latter two experiments came from the 1984 flow at 1200 m and the 1942 flow at 1500 m. On the day of the collection, we brought the samples (which were saturated from rainy field conditions in all three experiments) back to the lab and placed approximately 2 g (wet weight) in open 250 ml glass canning jars, allowing them to air dry. The following morning, five sets of lichen samples (5 replicates each) were sprayed with different amounts of deionized water to generate a variety of lichen water contents among samples. The lichens were kept hydrated for one hour under low light levels (25–50 μmol photons m^{-2} sec^{-1}). The rate of ARA in *S. vulcani* recovers fully after one hour of rehydration (Kurina, unpublished data). The samples incubated in the 10% C_2H_4 atmosphere for 2 hours; 5 ml samples were then injected

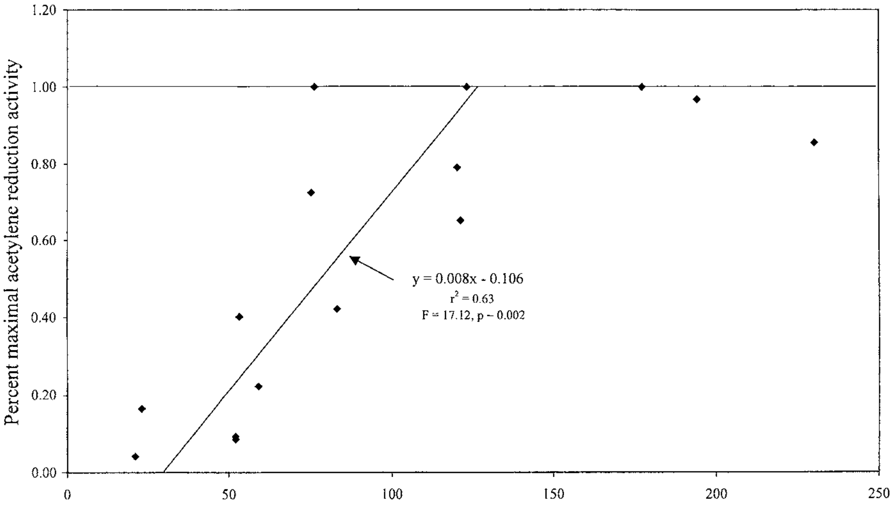

Figure 4. S. vulcani acetylene reduction activity vs. lichen water content.

into a gas chromatograph for ethylene (C_2H_4) analysis using a Shimadzu GC-8A (Scientific Instruments Inc., Columbia, Maryland) with a flame ionization detector at 140 °C and a Porapak N column at 80 °C. Acetylene blanks were run to determine the background level of ethylene. Neither ethylene production in the absence of acetylene nor ethylene consumption by lichens had been detectable in previous analyses of *S. vulcani* (T. Crews, personal communication). The lichen samples were then weighed and oven-dried at 70 °C to determine the water content and dry weights of the lichens.

S. vulcani ARA increased linearly with water content to values of approximately 125% (Figure 4). We regressed the fraction of *S. vulcani's* maximal ARA against water content, excluding the three points above 125%. This regression yielded the following scalar for water content (WC) (n = 12, F = 17.12, $p = 0.002$):

$$\text{WC scalar} = 0.008\text{WC} - 0.106 \qquad r^2 = 0.63$$

We used this equation to calculate that ARA would be maximal at water contents of ≈ 130%. In the model, we used the linear equation to calculate the scaling factor when lichen water content was less than 130% and assumed maximal ARA when the water content was equal to or greater than 130%.

Model of S. vulcani ARA

The overall model of *S. vulcani* ARA is:

$$\mathrm{ARA} = 90\,\eta\mathrm{mol\ C_2H_4\ gdwt^{-1}\,hr^{-1}}\,(0.05\mathrm{T}-0.417)\,(\mathrm{PAR\ scalar})\,(\mathrm{WC\ scalar})$$

90 ηmol C_2H_4 gdwt^{-1} hr^{-1} was the 95th percentile value of *S. vulcani* acetylene reduction activity in the distribution of the 44 means of ARA assays done between 1993 and 1996 (Kurina, unpublished data); we treated this as the maximum value of ARA. T equals temperature; the PAR scalar equals either 0 (at values less than 25 μmol photons m^{-2} sec^{-1}), or 1 (at values between 25 and 100 μmol photons m^{-2} sec^{-1}), or $^{1}/_{2}$ (at values over 100 μmol photons m^{-2} sec^{-1}); and the WC (water content) scalar equals 0.008 (WC) – 0.106 when WC $<$ 130% and 1 when WC $\geq$ 130%.

The estimates of annual lichen nitrogen fixation using this model entail a number of assumptions (see Appendix for a summary of these assumptions) discussed in detail elsewhere (Kurina 1998). In particular, we assumed zero lichen N fixation at night and a fixed daylength of 12 hours throughout the year.

Estimates of S. vulcani nitrogen fixation

Hourly rates of *S. vulcani* ARA between 700 and 1800 hours were calculated using the model above, together with the microclimate data. These hourly rates were then converted into hourly N fixation rates using the ratio 1 mol N_2 reduced/3.1 mol C_2H_2 reduced determined for *S. vulcani* from an ^{15}N calibration experiment (Vitousek 1994). The hourly N fixation rates between 700 and 1800 hours were summed into a daily rate. Annual *S. vulcani* N fixation rates on the lava flows were calculated by multiplying the daily N fixation rate by 365 days/year and by the lichen biomass per unit area on the 1984, 1942, and 1852 flows (97 g/m^2, 99 g/m^2 and 53 g/m^2, respectively). We estimate that, at mid-elevation, *S. vulcani* fixes 0.45, 0.45 and 0.2 kg N ha^{-1} yr^{-1} on the 1984, 1942, and 1852 flows, respectively.

The three climate factors each constrained hourly ARA by approximately one half, on average. Temperature, however, reduced ARA most strongly in the morning and evening, while water content limited ARA between 900 and 1500 hours. PAR reduced ARA by one half every hour beginning at 800 hours.

Percent of nitrogen derived from lichen through fixation

We used the values for *S. vulcani* abundance and nitrogen content on the 1984 flow, together with the N fixation estimates calculated above, to determine how much lichen nitrogen could have come from biological fixation. We assumed that lichen biomass accumulated at a constant, linear rate over the ten years since the flow was initiated and that lichen senescence was negligible over this time period.

We estimate that *S. vulcani* fixed approximately 2.4 kg N_2 ha^{-1} between 1985 and 1994 at 1500 m. The total amount of nitrogen in *S. vulcani* tissue on the 1984 flow is approximately 6 kg N_2 ha^{-1}. Biological N fixation, then, could account for 40% of the nitrogen in *S. vulcani* tissue at 1500 m.

Discussion

A recent evaluation of global patterns of biological N fixation estimated that nonsymbiotic N fixation contributes anywhere from 0.3 to 9.5 kg N ha^{-1} yr^{-1}, depending on the ecosystem and on the substrate or type of fixer being studied (Cleveland et al. 1999). Although N fixation rates were highest in the tropics and lower in arid and high latitude ecosystems, an interesting feature of these estimates is their similarity. In all of the ecosystems considered but one, estimates of nonsymbiotic N fixation were at least 1 kg N ha^{-1} yr^{-1} and most systems were thought to have inputs exceeding 2 kg N ha^{-1} yr^{-1} from nonsymbiotic N fixation. We suggest that the consistency and magnitude of these estimates of nonsymbiotic N fixation may result from a disregard of the role of climate in constraining levels of nonsymbiotic N fixation.

Although tripartite lichens are symbiotic organisms, the nitrogen-fixing component (the cyanobacterial population) is metabolically independent of the major carbon-fixing component (the algal population); that is, the cyanobacteria use their own photosynthesis to power nitrogen fixation (Rai 1988). The analogy, then, between nitrogen-fixing cyanobacteria in lichens and free-living nitrogen fixing organisms, is fairly straightforward.

If we assumed that measured rates of fixation under good conditions were sustained (as many studies do), we would calculate annual rates of lichen N fixation between 1 and 9 kg N ha^{-1} yr^{-1} on Mauna Loa's northeast slope. Taking climate conditions and an understanding of how those conditions affect *S. vulcani* ARA into account reduced our estimate of lichen N fixation on a young lava flow at mid-elevation considerably, to 0.45 kg N ha^{-1} yr^{-1}. Nitrogen inputs from lichen N fixation are easily exceeded by annual inputs of inorganic nitrogen in atmospheric deposition in a nearby site – estimated at approximately 1.0 kg N ha^{-1} yr^{-1} in precipitation and substantially more in cloudwater (Heath & Huebert 1999). Earlier field studies with other *Stereocaulon* spp. (*S. alpinum, S. paschale, S. vesuvianum*) in high-latitude ecosystems also yielded quite low estimates of annual lichen N fixation (Crittenden 1975, Alexander 1981, Huss-Danell 1977). *S. vulcani* nitrogenase activity, like that of all cyanolichens, depends on lichen temperature, light availability, and water content (Kallio et al. 1972; Kershaw 1974; Kelly & Becker 1975; Crittenden & Kershaw 1979; Fritz-Sheridan & Coxson 1988). Conditions at mid-elevation on Mauna Loa's NE slope often preclude maximum lichen nitrogenase activity. At low elevation, in contrast, where the climatic constraints would be substantially relaxed, there could be much more fixation, as much as 9 kg N ha^{-1} yr^{-1}, if water is rarely limiting.

The assumption that nonsymbiotic N-fixers are successful colonists in primary successional seres because of their ability to fix nitrogen implies that these organisms are largely independent of their nitrogen environment, deriving the majority of their nitrogen from biological fixation (Denison 1973). Nitrogen fixation, however, is an energetically expensive process – on average, for free-living diazotrophic bacteria, the figures vary between 10 and 50 mg N fixed/g carbon utilized (Marschner 1995); the nitrogen demand of N-fixing lichens must be relatively low, given their slow growth rate (Nash 1996); and many lichens are efficient at harvesting inorganic nitrogen from rainfall (Crittenden 1983; Knops et al. 1991).

We estimate that lichens on the 1984 flow at mid-elevation can only have derived 40% of their nitrogen from biological fixation. This suggests that the dominance of *S. vulcani* on young lava flows at mid-elevation on Mauna Loa's NE slope is not primarily a function of its ability to fix nitrogen, in accord with earlier work on cyanolichens in diverse systems (Hitch and Stewart 1973; Becker et al. 1977).

Comparing the response surfaces of carbon and nitrogen assimilation in *S. vulcani* suggests a possible explanation for why *S. vulcani* is so widespread at mid- and high elevation on the NE slope of Mauna Loa, even given its relatively low rates of biological N fixation. Rates of net CO_2 uptake in *S. vulcani* saturate at a lower temperature and water content than do rates of nitrogen fixation (Kurina 1998). N fixation rates, in contrast, saturate at a much lower light level (25 μmol photons m^{-2} sec^{-1}) than carbon fixation rates (200 μmol photons m^{-2} sec^{-1}), and are adversely affected by high light levels. These results support other lichenological studies which have shown that, first, cyanobacterial photobionts require liquid water in order to be metabolically active – in contrast to green algae, which can be active in conditions of high relative humidity (Lange et al. 1986; Lange et al. 1993) and, second, that cyanobacterial symbionts are often more sensitive to high light than green algal symbionts (Demmig-Adams et al. 1990). The climate conditions at mid-elevation on Mauna Loa which mitigate against *S. vulcani* N fixation may support relatively high levels of carbon assimilation.

Lichens have long been thought of in terms of their contribution to the surrounding system – initiating soil formation by weathering the rock substrate (but see Cooper & Rudolph 1953), and, for cyanolichens, bringing nitrogen into N-scarce environments, whether early in primary succession or in desert, tundra, or Arctic systems (Denison 1973; Snyder & Wullstein 1973; Alexander et al. 1978; Boring et al. 1988; Chapin & Bledsoe 1992; Line 1992; Eldridge & Green 1994). However, a recent geochemical study of the basalt surface *S. vulcani* inhabits found no weathering effects attributable to *S. vulcani's* presence (Cochran & Berner 1993). Further, our results suggest that its importance as a source of nitrogen in this young system is limited both temporally, to a relatively brief period of time during which lichen abundance is high, and spatially, to the warmest and wettest locations on Mauna Loa's NE slope. *S. vulcani* apparently fulfills neither of its anticipated ecosystem-level roles. Rather than conceptualizing lichens in terms of these roles, it may be more accurate to think of them as organisms well-suited to inhabiting conditions ill-tolerated by vascular plants.

Acknowledgements

We thank Jim Juvik and Dennis Nullet for access to their climate stations and data and for the use of microclimate measurement equipment. Chris Lund modified the scale used for water content measurements in the field. Tom Nash and the members of his laboratory at Arizona State University were generous with their equipment and their time. Jonathan Pritchard advised on the analysis of the microclimate data. Comments by Christopher Field, Sharon Long and Harold Mooney improved the manuscript. This research was supported by National Science Foundation grants BSR-8918382 and DEB-9628803, and a grant from the Andrew W. Mellon foundation.

Appendix

Assumptions used for the N fixation estimate include the following:

1. ***S. vulcani* doesn't fix nitrogen in the dark.**
   Although some cyanolichens do fix N at low levels in the dark (MacFarlane et al. 1976, Kershaw et al. 1977), field assays with *S. vulcani* showed that nitrogenase activity was zero one to two hours before sunrise (Kurina, unpublished data); whether *S. vulcani* fixes nitrogen shortly after sunset is unknown. Restricting the calculation of *S. vulcani* nitrogen fixation to daylight hours may underestimate *S. vulcani's* true nitrogen fixation, but not by much.
2. **The restrictive effects of water content, temperature, and light availability on *S. vulcani* ARA are multiplicative.**
   This model makes good intuitive sense, but we don't have a complete matrix of ARA responses to water content, temperature, and light, to support this assumption. A simple alternative model is that *S. vulcani* ARA is reduced to the level dictated by the climate

factor most limiting at that time and is not restricted further by the other climate factors. N fixation estimates under this model do increase somewhat, to 1.25 kg N ha^{-1} yr^{-1} on the 1984 and 1942 flows and 0.7 kg N ha^{-1} yr^{-1} on the 1852 flow.

3. **Using hourly averages of temperature, light, and water content doesn't bias the estimate, despite the nonlinear response of ARA to these parameters.**
   Estimates of N fixation based on ARA estimates calculated for every 5 minute period of the microclimate measurements were essentially identical to our original estimates using hourly averages (Kurina 1998).
4. **Hourly temperature, light, and water content averages are a good representation of the microclimate conditions *S. vulcani* typically experiences and it is therefore reasonable to extrapolate up from a value for nitrogen fixation in a typical day to a yearly nitrogen fixation estimate.**
   Extrapolating upwards from a typical day of lichen N fixation to a yearly N fixation estimate neglects day-to-day variation in weather. However, N fixation estimates recalculated using hourly values of the climate factor in question that were either one standard deviation above or below the mean had little material effect on the estimates (Kurina 1998). We also calculated rates of lichen N fixation in an extremely dry year and in an extremely wet year to explore the effect of yearly climate variations on the N fixation estimate. Our estimate of N fixation in a extremely dry year at mid-elevation dropped to 0.2 kg N ha^{-1} yr^{-1} on the 1984 and 1942 flows and to 0.09 kg N ha^{-1} yr^{-1} on the 1852 flow. In an extremely wet year, our estimate of *S. vulcani* N fixation doubled to 0.9 kg N ha^{-1} yr^{-1} on the 1984 and 1942 flows and to 0.5 kg N ha^{-1} yr^{-1} on the 1852 flow. Finally, we explored the effect on the N fixation estimate of incorporating a variable day length across the year. We calculated mean hourly lichen temperatures for November to April and for May to October, assuming a day length of 11 hours and 13 hours for these two time periods, respectively, and re-calculated lichen N fixation. Although lichen N fixation does vary across the year under these assumptions, from 0.32 kg N ha^{-1} yr^{-1} for the winter/spring months to 0.61 kg N ha^{-1} yr^{-1} for the summer/fall months, mean annual lichen N fixation is essentially unchanged – 0.46 kg N ha^{-1} yr^{-1} on the 1984 flow, 0.48 kg N ha^{-1} yr^{-1} on the 1942 flow, and 0.26 kg N ha^{-1} yr^{-1} on the 1852 flow.
5. ***S. vulcani* ARA doesn't vary among flows.**
   ARA rates per unit thallus dry weight do not vary appreciably across lava flow ages or elevations (Kurina, unpublished data).

References

Alexander V (1981) Nitrogen-fixing lichens in tundra and taiga ecosystems. In: Gibson AH & WE Newton (Eds) Current Perspectives in Nitrogen Fixation (p 257). Elsevier/North-Holland Biomedical Press, Amsterdam.

Alexander V, Billington M & Schell DM (1978) Nitrogen fixation in Arctic and Alpine tundra. In: Tieszen LL (Ed) Vegetation and Production Ecology of an Alaskan Arctic Tundra: Ecological Studies, v. 29 (pp 539–558). Springer-Verlag, New York

Becker VE, Reeder J & Stetler R (1977) Biomass and habitat of nitrogen fixing lichens in an oak forest in the North Carolina Piedmont. Bryologist 80: 93–99

Boring LR, Swank WT, Waide JB & Henderson GS (1988) Sources, fates, and impacts of nitrogen inputs to terrestrial ecosystems: review and synthesis. Biogeochemistry 6: 119–159

Chapin DM & Bledsoe CS (1992) Nitrogen fixation in Arctic plant communities. In: Chapin III FS (Ed) Arctic Ecosystems in a Changing Climate (pp 301–319). Academic Press, San Diego

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JD, Elseroad A & Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. Global Biogeochemical Cycles 13: 623–645

Cochran MF & Berner RA (1993) Reply to the comments on "Weathering, plants, and the long-term carbon cycle". Geochimica et Cosmochimica Acta 57: 2147–2148

Cooper R & Rudolph ED (1953) The role of lichens in soil formation and plant succession. Ecology 34: 805–807

Crittenden PD (1975) Nitrogen fixation by lichens on glacial drift in Iceland. New Phytologist 74: 41–49

Crittenden PD (1983) The role of lichens in the nitrogen economy of subarctic woodlands: nitrogen loss from the nitrogen-fixing lichen, *Stereocaulon paschale* during rainfall. In: Lee JA, McNeill S & Rorison IH (Eds) Nitrogen as an Ecological Factor. Blackwell Publishers. Oxford

Crittenden PD & Kershaw KA (1979) Studies on lichen-dominated systems. XXII. The environmental control of nitrogenase activity in *Stereocaulon paschale* in spruce-lichen woodland. Canadian Journal of Botany 57: 236–254

Crittenden PD & Kershaw KA (1978) Discovering the role of lichens in the nitrogen cycle in boreal-arctic ecosystems. Bryologist 81: 258–267

Demmig-Adams B, Maguas C, Adams III WW, Meyer A, Kilian E & Lange OL (1990) Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. Planta 180: 400–409

Denison WC (1973) Life in tall trees. Scientific American 228: 78–80

Eggler WA (1963) Plant life of Paricutin volcano, Mexico, eight years after activity ceased. American Midland Naturalist 69: 38–69

Eldridge DJ & Greene RSB (1994) Microbiotic soil crusts: a review of their role in soil and ecological processes in the rangelands of Australia. Aust. J. Soil Res. 32: 389–415

Fritz-Sheridan RP & Coxson DS (1988) Nitrogen fixation on a tropical volcano, La Soufriere (Guadeloupe): the interaction of temperature, moisture, and light with net photosynthesis and nitrogenase activity in *Stereocaulon virgatum* and response to periods of insolation shock. Lichenologist 20: 63–81

Giambelluca TW, Nullet MS & Schroeder TA (1986) Rainfall Atlas of Hawaii. Department of Land and Natural Resources, Honolulu, HI

Gunther AJ (1989) Nitrogen fixation by lichens in a subarctic Alaskan watershed. Bryologist 92: 202–208

Heath JA & Huebert BJ (1999) Cloudwater deposition is a source of fixed nitrogen in a Hawaiian montane forest. Biogeochemistry 44: 119–134

Henriksson E, Henriksson LE & Pejler B (1972) Nitrogen fixation by blue-green algae on the Island of Surtsey, Iceland. Surtsey Research Report Reykjavik 6: 66–68

Hitch CJB & Stewart WDP (1973) Nitrogen fixation by lichens in Scotland. New Phytologist 72: 509–524

Huss-Danell K (1977) Nitrogen fixation by *Stereocaulon paschale* under field conditions. Canadian Journal of Botany 55: 585–592

Jeffries DL (1989) The Vegetation, Soil, and Cryptogamic Crusts of Blackbrush Communities in the Kaiparowits Basin. Doctoral Dissertation. Arizona State University, Tempe, AZ

Juvik JO & Nullet D (1994) A climate transect through tropical montane rain forest in Hawaii. Applied Meteorology 33: 1304–1312

Kallio P, Suhonen S & Kallio H (1972) The ecology of nitrogen fixation in *Nephroma arcticum* and *Solorina crocea*. Reports of the Kevo Subarctic Research Station 9: 7–14

Kelly BB & Becker VE (1975) Effects of light intensity and temperature on nitrogen fixation by *Lobaria pulmonaria*, *Sticta weigelii, Leptogium cyanescens* and *Collema subfurvum*. Bryologist 78: 350–355

Kershaw KA, MacFarlane JD & Tysiaczny MJ (1977) Physiological-environmental interactions in lichens. V. The interaction of temperature with nitrogenase activity in the dark. New Phytologist 79: 409–416

Kershaw, KA (1974) Dependence of the level of nitrogenase activity on the water content of the thallus in *Peltigera canina*, *P. evansiana, polydactyla* and *P. praetextata*. Canadian Journal of Botany 52: 1423–1427

Knops JMH, Nash III TH, Boucher VL & Schlesinger WH (1991) Mineral cycling and epiphytic lichens: implications at the ecosystem level. Lichenologist 23: 309–321

Kurina LM (1998) Controls over the abundance and nitrogen-fixing activity of *Stereocaulon vulcani*, a primary lichen lichen colonist on young Hawaiian lava flows. PhD Dissertation. Stanford University, Stanford, California

Kurina LM & Vitousek PM (1999) Controls over the accumulation and decline of a nitrogen-fixing lichen, *Stereocaulon vulcani*, on young Hawaiian lava flows. Journal of Ecology 87: 784–799

Lange OL, Budel B, Meyer A & Kilian E (1993) Further evidence that activation of net photosynthesis by dry cyanobacterial lichens requires liquid water. Lichenologist 25: 175–189

Lange OL, Kilian E & Ziegler H (1986) Water vapor uptake and photosynthesis of lichens: performance differences in species with green and blue-green algae as phycobionts. Oecologia 71: 104–110

Line MA (1992) Nitrogen fixation in the sub-Antarctic Macquarie Island. Polar Biology 11: 601–606

MacFarlane JD, Maikawa E, Kershaw KA & Oaks A (1976) Environmental-physiological interactions in lichens. I. The interaction of light/dark periods and nitrogenase activity in *Peltigera polydactyla*. New Phytologist 77: 705–711

Marschner H (1995) Mineral Nutrition of Higher Plants. Academic Press, London

Nash III TH (1996) Lichen Biology. Cambridge University Press, Cambridge

Nullet D, Juvik, JO & Wall A (1995) A Hawaiian mountain climate cross-section. Climate Research 5: 131–137

Rai AN (1988) Nitrogen metabolism. In: Margalith Galun (Ed) CRC Handbook of Lichenology, vol. I (pp 201–237). CRC Press, Boca Raton

Raich JW, Russell AE, Crews TE, Farrington H & Vitousek PM (1996) Both nitrogen and phosphorus limit plant production on young Hawaiian lava flows. Biogeochemistry 32: 1–14

Rhoades FM (1981) Biomass of epiphytic lichens and bryophytes on *Abies lasiocarpa* on a Mt. Baker lava flow, Washington. The Bryologist 84: 39–47

Sheridan RP (1991) Nitrogenase activity by *Hapalosiphon flexuosus* associated with *Sphagnum erythrocalyx* mats in the cloud forest on the volcano La Soufriere, Guadeloupe, French West Indies. Biotropica 23: 134–140

Skottsberg C (1941) Plant succession on recent lava flows in the Island of Hawaii. Goteborgs Kungl. Vetenskaps- och Vitterhets-Samhalles Handlingar. Sjatte Foljden. Ser. B. Band 1. No: 8

Skujins J & Klubek B (1978) Nitrogen fixation and cycling by blue-green algae-lichen-crusts in arid rangeland soils. Ecological Bulletin (Stockholm) 26: 164–171

Smith CW (1991) Lichen conservation in Hawaii. In: Galloway DJ (Ed) Tropical Lichens: Their Systematics, Conservation, and Ecology (pp 35–45). Clarendon Press, Oxford

Snyder JM & Wullstein LH (1973) The role of desert cryptogams in nitrogen fixation. American Midland Naturalist 90: 257–265

Sprent JI (1993) The role of nitrogen fixation in primary succession on land. In: Miles J & Walton DWH (Eds) Primary Succession on Land. Blackwell Scientific Publications. Oxford

Stewart WDP, Fitzgerald GP & Burris RH (1967) In situ studies on N_2 fixation using the acetylene reduction technique. Proceedings of the National Academy of Science 58: 2071–2078

Thomas MA (1994) Cryptogamic Crusts: Implications on Soil Nitrogen Patterns in a Pinyon-Juniper Woodland. Masters Thesis. Arizona State University, Tempe, AZ

Uhe G (1988) The composition of the plant communities inhabiting the recent volcanic deposits of Maui and Hawaii, Hawaiian Islands (U.S.A.). Tropical Ecology 298: 48–54

Van Cleve K & Alexander V (1981) Nitrogen cycling in tundra and boreal ecosystems. Ecological Bulletin (Stockholm) 33: 375–404

Vitousek PM (1994) Potential nitrogen fixation during primary succession in Hawaii Volcanoes National Park. Biotropica 26: 234–240

Vitousek PM, Walker LR, Whiteaker LD & Matson PA (1993) Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. Biogeochemistry 23: 197–215

Vitousek PM & Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13: 87–115

Vitousek PM & Walker LR (1987) Colonization, succession and resource availability: ecosystem-level interactions. In: Gray AJ, Crawley MJ & Edwards PJ (Eds) Colonization, Succession and Sustainability. Blackwell Scientific, Oxford