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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Bryophyte and lichen biomass and nitrogen fixation in a high elevation cloud forest in Cerro de La Muerte, Costa Rica

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Abstract

Cloud forests have been found to lose more nitrogen in stream discharge than they gain from atmospheric deposition. They also support a large diversity and biomass of tree epiphytes, predominately composed of cryptogams. Since cryptogam epiphytes harbor nitrogen fixing cyanobacteria, they may help make up for the nitrogen loss from ecosystems. We assessed cryptogam biomass on the ground, boles and branches in *Quercus costaricensis* dominated stands near the tree line in the Cordillera de Talamanca, Costa Rica. Nitrogen fixation was assayed using $^{15}N_2$ uptake. Total cryptogam biomass was 2 977 kg ha^{-1}, with 67% being found on the lower branches. Bryophytes and chlorolichens made up 53% and 44%, respectively, of the biomass. Half of the bryophyte mass was composed of the liverwort *Plagiochila heterophylla,* and 66% of the chlorolichen of *Lobariella pallida.* There were no significant differences in nitrogen fixation rates between the cryptogam species, with a mean rate of 5.04 µg N g^{-1} day^{-1} during the predominantly wet condition in the forest. The overall nitrogen input from fixation was 6.1 kg N ha^{-1} year^{-1}, of which 78% came from bryophytes, 18% from chlorolichens, and 4% from cyanolichens. Only 2.0% of the fixation occurred in cryptogams on the ground, whereas 67%, 24%, and 7% occurred on the lower branches, boles, and upper branches, respectively. These results show that tree epiphytes constitute a significant source of nitrogen for these forests, due to the trees' large surface area, and can make up for the nitrogen lost from these ecosystems.

Keywords Liverworts · Associative nitrogen fixation · Chlorolichens · Montane forest · Oak forest epiphytes · *Quercus costaricensis*

Introduction

Most high-latitude and high-altitude ecosystems are nitrogen-limited due to reduced rates of decomposition (Tamm 1991; Benner et al. 2007). In tropical regions, soil nitrogen mineralization decreases with elevation (Mars et al. 1988). Consequently, tree growth in montane tropical forests is nitrogen-limited, or nitrogen and phosphorus co-limited (Cavelier et al. 2000). Therefore, it was an unexpected discovery that montane tropical forests, especially cloud forests, have high rates of nitrate discharge from streams (Brookshire et al. 2012). Also, the total amount of nitrogen transported out of the ecosystem in stream water exceeds the amount entering via atmospheric deposition by 2–7.5 kg N ha^{-1} year^{-1}.

Although plant symbiotic nitrogen fixation can provide ecosystems with large inputs of fixed nitrogen (Andrews et al. 2011; Vitousek et al. 2002), nitrogen fixing plants tend to only be abundant in early successional communities in temperate and high-altitude regions (Galloway et al. 2004). Free-living nitrogen fixers on leaf surfaces (the phyllosphere) and in the soil provide a limited amount of fixed nitrogen to terrestrial ecosystem (Favilli and Messini 1990). Bryophytes and lichens (cryptogams) can also provide a fixed nitrogen source by harboring nitrogen-fixing cyanobacteria.

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Some lichens (cyanolichens) do so in specialized symbiotic structures, i.e., the cephalopodia (Brodo et al. 2001). Other lichens and bryophytes are associative nitrogen fixers, where they are simply colonized on their surfaces or internal tissues and lack and symbiotic structures (Adams and Duggan 2008; DeLuca et al. 2002). In many forests, cryptogam biomass and diversity increase throughout forest succession into the old-growth stage (Bergeron and Fenton 2012). This potentially allows cryptogams to make an increasingly important contribution to a forests' nitrogen budget. Across the globe, studies usually find the input of nitrogen to ecosystems from cryptogams is around 2 kg N ha^{-1} $year^{-1}$ (Gavazov et al. 2010; Markham 2009; Zackrisson et al. 2004). This is low compared to the ca. 200 kg N ha^{-1} $year^{-1}$ levels that can be achieved by plant symbiotic nitrogen fixation when the plants are abundant (Torrey 1978). On a tissue mass basis, nitrogen fixation rates in cryptogams tend to differ between the cryptogam species and the form of association with the nitrogen-fixing organism. Associative fixation per tissue mass in mosses and chlorolichens tends to be in the range of about 0.3–3 µg N g^{-1} day^{-1} (Lagerström et al. 2007; Sheridan 1991). This is about ten times lower than the rates sometimes reported in symbiotic nitrogen-fixing cyanolichens (Millbank and Olsen 1986; Darnajoux et al. 2017), and is likely due to a higher concentration of nitrogen-fixing cells in cyanolichen cephalodia (Henskens et al. 2012). However, in many ecosystems, the contribution of cyanolichens to ecosystem-level nitrogen inputs is often low due to the low biomass of cyanolichens (Asplund and Wardle 2017). Since mosses tend to have a higher cover and biomass than cyanolichens, they can potentially contribute more nitrogen to ecosystems than cyanolichens.

Cloud forests are found mainly in montane regions where much of the precipitation they receive comes from horizontal cloud movement (Bruijnzeel et al. 2010). They are hotspots of biodiversity, often containing many endemic species, and are also vulnerable to climate change (Still et al. 1999). Tree epiphytes in cloud forests are mostly composed of cryptogams, and their mass can be highly variable, ranging from 400 to 44 000 kg ha^{-1} (Köhler et al. 2007). This mass can represent a large proportion of a cloud forest's photosynthetic biomass (Nadkarni et al. 2004). Epiphyte biomass, especially bryophytes, tends to increase in older forests and at higher elevations (Freiberg and Freiberg 2000). This mass is known to store large quantities of water, having a major influence on forest hydrology and potentially on other ecosystem properties (Coxson and Nadkarni, 1995; Liu et al. 2002). Oak highland forests in Costa Rica have a massive cryptogam coverage in the canopy and tend to have little cover of ground-dwelling cryptogams (Holz and Gradstein 2005). However, compared to ground-dwelling cryptogams, we have very little information about nitrogen fixation by tree epiphytic cryptogams, especially in the tropics (Cusack et al. 2009; Lindo and Whiteley 2010). Although tree surfaces may be more limited in the phosphorus and molybdenum needed for nitrogen fixation (Stanton et al. 2019), in cloud forests, the large mass of epiphytic cryptogams may compensate for a reduced rate of fixation on a tissue mass basis. Our hypothesis, therefore, is that cloud forest epiphytic cryptogams contribute substantial amounts of nitrogen to their ecosystems, given their biomass, rather than their fixation rates per mass. They may therefore be able to make up for the nitrogen lost from these systems in stream discharge. The purpose of this study was to quantify the biomass of cryptogams in a tropical cloud forest and their contribution of fixed nitrogen to the ecosystem.

Materials and methods

The study took place at the Los Nímbulos Field station, in a high-elevation cloud forest, just below the tree line, which occurs at ca. 3300 m asl in the Talamanca Mountain Range, Costa Rica (9° 33′42 N, 83° 44′27 W). There is no evidence of human disturbance in the forest we studied. Mean monthly precipitation at a weather station 1.4 km from the site is 185 mm, with the lowest amount falling in February (20.4 mm) and the highest in April. From 2016 to 2018, the mean daily temperature (± standard deviation) measured at the site was 8.95 ± 0.93 °C, and mean daily relative humidity was 93 ± 6.1%, with 97.5% of the humidity readings being greater than 74%. There was also only an average of 7 days in a year when the daily maximum relative humidity did not exceed 95%, due to clouds reaching the ground level. The forest stands from ca. 3100 m up to the tree line have a single-layer canopy composed exclusively of *Quercus costaricensis,* accounting for 93% of the tree basal area, with *Weinmannia pinnata*, *Didymopanax pittieri* making up the remainder of the tree composition but never reaching the forest canopy. Compared to the lower-elevation mixed forest (mainly *Q. costaricensis* and *Quercus bumelioides*), this relatively simple forest structure allowed us to estimate the tree surface area for cryptogams. The understory is primarily composed of a thick layer of *Chusquea talamancensis*. The leaves of the trees show little colonization by cryptogams, consistent with the sampling of Holz et al. (2002) in a nearby *Quercus* forest at ca. 2300 m elevation. Leaves were, therefore, not sampled for cryptogam cover. The canopy of the trees is quite shallow, being an average of 3 m from the top to the lowest leaf.

Sampling was done in three stands at 3237, 3309, and 3323 m elevation (stands 1, 2, and 3, respectively) within one km of one another. In each stand, ground-dwelling tree epiphytic cryptograms were sampled in a 20 × 20 m plot. Tree density and basal area were estimated by measuring the diameter at breast height of all trees in the plots. In each plot

tree, the surface area that supported cryptogams was estimated on 10 trees. Trees were selected at random within the plots. Some selected trees were not sampled if we could not visually assess all tree parts, in which case another tree was selected (all trees were *Q. costaricensis* in this random selection). Trees were divided into 3 sections: boles, lower (sub canopy) branches, and upper (canopy) branches. Boles were defined as the main stem from the ground to the first large branch (10 cm or greater in diameter). All lower branches were always 10 cm in diameter or larger. The upper branches were 10 cm in diameter or less and in the canopy zone or up to 1 m below it. The lower branches are equivalent to zones 2 and 3, and the upper branches equivalent to zone 4 in Francisco et al. (2019). Surface areas were estimated from the lengths and circumferences (at breast height for boles and mid-way along the length for branches) of each tree part, assuming they were cylindrical in shape. Boles and large branch lengths were visually estimated from the ground using a hypsometer to estimate their heights. Their lengths were then calculated by accounting for their vertical angle, estimated with a clinometer. Long branches were divided into sections based on a visual assessment of their diameter, in 10 cm diameter increment classes. These visual assessments of branch diameter class were confirmed by visually assessing and then measuring branches cut from trees, or on recently fallen trees outside the plots. We also compared the visual estimates of the surface area of tree parts on standing trees to estimates of surface areas on the cut and fallen branches (Supplementary Fig. 1). The total tree surface per plot was calculated by multiplying the mean surface area of each tree's part by the number of trees per plot, expressed per hectare of land. Estimating each tree's surface area based on allometric equations of the measured tree diameter at breast height versus total surface area yielded similar estimates of total tree surface area per plot.

Cryptogam mass per tree and ground surface area was estimated using two approaches. In each plot, the cover of cryptogams was assessed on the ground and boles. Cover on the ground was estimated in 28 randomly placed 0.25×0.25 m quadrats. Cover on the boles was estimated in 16×16 cm quadrats at breast height in up to 28 randomly selected trees per plot (all of the 23 trees in stand one and 28 trees in stand two and three). For a subset of these samples (56 quadrats from the three stands), we also estimated each species' mass by collecting all cryptogams within the quadrats and drying them to a constant mass. The cover-to-mass relationship was used to convert cover values to mass estimates for all other samples. Above breast height, mass estimates per 16×16 cm quadrat were made on 14 boles, 3–10 m above the ground, 16 lower branches 5–13 m above the ground, and 8 branches that had broken from trees during a wind storm. The sample locations of intact branches were determined by the ease of access to the surfaces. Trees used for these samples fell within and outside the plots. The mass of cryptogams on upper branches was made on six branches cut from trees and 16 branches from two trees outside the plots that had fallen in a windstorm during the study. We measured the dry weight of all cryptogams in 30 cm sections of branches of different diameter classes for these upper branches. Each species' mass was compared between the different surfaces (ground, boles, lower and upper branches), averaged across plots, using Kruskal–Wallis non-parametric rank tests followed by Dunns (i.e., Bonferroni adjusted) multiple comparison tests.

$^{15}N_2$ uptake assays were performed on eight days between February 2 and 24, 2016. On each $^{15}N_2$ uptake sampling date, 15 assays were performed on samples of 12 of the most common cryptogam species. Samples of ca. 170 mg fresh mass (ca. 85 mg dry mass) were incubated in an atmosphere containing 16.7% ^{15}N, as N_2 (calculated from the volume of $^{15}N_2$ gas injected), in clear air-tight acrylic containers ($15\ cm \times 10\ cm \times 0.8\ cm$), with three different species in each of five containers on each sample day. The chambers were suspended from tree branches in the forest understory for 24 h to match natural light and temperature conditions. A subsample of each specimen was collected before the incubation to determine its natural ^{15}N abundance. The $^{15}N_2$ added to the chamber was generated according to Diocares et al. (2006) just before it was injected into the chamber. After the incubation, samples were dried to a constant mass at the field site using a microwave oven. Samples were ground in a ball mill and analyzed for ^{15}N content at the UC Davis Stable Isotope Facility. N uptake was calculated from the difference in ^{15}N in incubated and pre-incubated samples, relative to the amount of ^{15}N available in the incubation atmosphere according to the equation:

$$\frac{A_{\text{final}} - A_{\text{initial}}}{A_{\text{N2 labelled}} - A_{\text{N2 unlabelled}}} \times [N]$$

where A is the abundance ratio (atom%) of ^{15}N before incubation (initial), after incubation (final), in the (labeled) atmosphere, and in the air (unlabelled), and $[N]$ is the concentration of N in the sample (Montoya et al. 1996). Species were considered to fix nitrogen if the mean difference between the post and pre-incubation tissue ^{15}N values for a species was greater than zero. Rates of N fixation were expressed on a per tissue dry mass basis.

Sampling was partly conducted during the short annual dry period in the region. Therefore, we also conducted a separate set of cryptogam moisture content assays (the amount of water relative to the dry mass) to determine how cryptogam moisture content changed with precipitation and cloud interception. The moisture content of cryptogams was monitored every 1–3 days, with cryptogams of the most abundant species harvested from lower branches or boles and placed

in individual polyethylene bags. After recording their fresh mass, they were dried in a microwave oven to a constant mass and re-weighed. Precipitation outside and inside the forest canopy was measured with rain gauges (five 50 ml centrifuge tubes placed randomly in stand 1 and two rain gauges in the open). Hours of ground-level cloud cover during daylight were noted and compared with weather station relative humidity levels.

Differences in log-transformed nitrogen fixation rates per mass of tissue were tested using restricted maximum likelihood models. We compared nitrogen fixation rates between cryptogam types (liverworts, mosses, chlorolichens, and cyanolichens), nesting species within cryptogam type (using JMP Pro 14, SAS Institute). Models included date and assay chamber as random variables and the number of hours of cloud cover in the previous 24 h (as an index of cryptogam moisture content). We used AIC to select the best model.

The overall nitrogen fixation rate was derived as the fixation rate per year of each cryptogam type multiplied by their mass per unit land area on each surface and summed over all surfaces. Since the site is wet most of the year (see "Results"), we assumed the nitrogen fixation rate would be constant over the wet part of the year. We determined the number of days in a year cryptogams would not be moisture-limited. This was done using weather station data to calculate estimate daily hours of cloud interception by the forest. Cryptogam nitrogen fixation per year was then calculated as the daily fixation rate during the wet period when $^{15}N_2$ assays were run multiplied by the number of days in the year the cryptograms were wet. Each cryptogam species' biomass was calculated as biomass per surface, multiplied by the surface area to land area of the surface type. We assumed that species not assayed for ^{15}N uptake (which made up 7.8% of the total cryptogam biomass) would fix nitrogen at the same rate as other species in the same cryptogam group. The mass of the cryptogam type was the sum of the species in each cryptogam group.

To account for error propagation, for each cryptogam type on each surface, a bootstrapping approach was used to estimate the biomass and fixation rate and then take their product (Blukacz et al. 2005). The resampling was performed 10,000 using the boot package (version 1.3–25) in R (3.6.2). For the biomass estimates on the ground and boles, we used the mean biomass per quadrat in each stand for the resampling. For the lower and upper branch, biomass estimates with the individual sample estimates. We used the 68% confidence interval (equivalent to a standard deviation) from the resampled data as a measure of variation in the estimates of nitrogen fixation per land area per year.

Results

Tree density varied threefold among the plots, and the total area of tree surfaces varied twofold (Table 1). Although stand 1 had half the tree density of stand 2 and 3, the trees were larger, having higher bole and branch surface areas per tree. Stand 3 had less surface area of lower branches because the average branch length per tree was shorter (2.2 ± 0.5 m) than the branch length in the other two stands (3.78 ± 0.47 m). Taken together, the different tree parts had a surface area 1.9–3.6 times the surface area of the land the trees occupied.

Lower branches had almost four times more total cryptogam mass per surface area than any other tree surface, and 24 times more mass per area than the cryptogams on the ground (Table 2). The high variability in biomass of species occurred as the result of samples not containing a particular species. 21 taxa of cryptogams were found on sampled surfaces, identified to the species or genus level. In total, liverworts, mosses, chlorolichens, and cyanolichens made up 30.0%, 23.3%, 44.6%, and 2.1% of the biomass of cryptogams on a surface area basis, respectively. Twelve of the taxa showed differences in mass between the sampled surfaces, according to Kruskal–Wallis tests. The two most abundant taxa were a liverwort, *Plagiochila heterophylla,* and a chlorolichen, *Lobariella pallida,* which combined made up 58% of the total biomass. Also, they both had a higher biomass on lower branches than on any other surface. The next most abundant cryptogam was the moss *Hypnum cupressiforme,* which made up 10.5% of the total cryptogam biomass and was almost exclusively found on lower branches. Another moss, *Thuidium delicatulum,* was the only species found exclusively on the ground, making up

Table 1 Tree density, basal area, mean height and circumference at breast height (± standard deviation), and total surface areas of tree parts in three *Quercus costaricensis* stands

Stand	Density (ha^{-1})	Basal area ($m^2\ ha^{-1}$)	Height (m)	Circumference (cm)	Surface area ($m^2\ ha^{-1}$)			
					Boles	Lower branches	Upper branches	Total
1	575	80.0 (100)	23.0 ± 2.3	118 ± 47	9142	11,960	4255	25,358
2	1425	50.6 (82)	17.4 ± 1.6	75 ± 22	12,118	14,535	9191	35,844
3	1300	54.6 (92)	18.2 ± 0.7	76 ± 18	8801	4628	5265	18,694

Values in brackets are the percentage of basal area composed of *Q. costaricensis,* the remainder being made up of *Weinmannia pinnata*

Table 2 Mass per surface area (g m^{-2}) of cryptogams on the ground and tree parts (with sample size in brackets)

Taxa	Ground (84)	Boles (93)	Lower branches (24)	Upper branches (30)
Liverworts	1.28 ± 0.77B	30.30 ± 4.82 A	49.18 ± 12.64 AB	9.29 ± 4.98 B
Plagiochilla heterophylla	0.42 ± 0.23 C	27.42 ± 4.67 A	49.15 ± 12.65 AB	3.48 ± 1.19 C
Plagiochila arbuscula	0*	1.50 ± 1.32	0	0
Plagiochila asplenoides	0.62 ± 0.62	0.76 ± 0.74	0	0
Frullania convoluta	0.01 ± 0.01 AB	0.03 ± 0.02 B	0.03 ± 0.02 AB	7.61 ± 4.89 A
Lepidozia cupressina	0.22 ± 0.22	0.53 ± 0.51	0	0
Mosses	1.81 ± 0.61 B	8.93 ± 2.96 B	54.97 ± 19.28 A	3.77 ± 1.66 B
Dicranum rhabdocarpum	0.00* ± 0.00 B	0.41 ± 0.21 B	2.63 ± 1.61 A	0 B
Hypnum cupressiforme	0.01 ± 0.01 B	1.07 ± 0.57 B	28.44 ± 18.07 A	0.33 ± 0.29 B
Macromitrium cirrosum	0	0.90 ± 0.57	2.11 ± 2.11	0.21 ± 0.21
Pleurozium sp.	0 B	0.53 ± 0.34 AB	1.08 ± 0.96 AB	1.77 ± 1.20 A
Sematophyllum swartzii	0.03 ± 0.02	0.03 ± 0.02	5.56 ± 19.245	1.46 ± 1.22
Thuidium delicatulum	1.77 ± 0.61 A	0 B	0 B	0 B
Tomentypnum nitens	0.01 ± 0.01 B	6.00 ± 2.55 A	15.15 ± 9.69 A	0 B
Chlorolichens	0.67 ± .27 D	18.11 ± 3.74 C	87.01 ± 25.21 A	41.09 ± 8.97 B
Bryoria kokiana	0	0.00* ± 0.00	0	0
Bunodophoron melanocarpum	0	0.22 ± 0.12	0	0
Everniastrum catawbiense	0	0.07 ± 0.03	2.15 ± 2.15	0.10 ± 0.08
Hypotrachyna pulvinata	0	0.30 ± 0.21	0	0.28 ± 0.28
Hypotrachyna sp.	0 C	4.05 ± 1.32 B	0 C	11.71 ± 4.52 A
Lobariella pallida	0.32 ± 0.19 B	8.30 ± 2.85 B	77.79 ± 27.17 A	1.51 ± 0.88 B
Lobaria sp.	0 B	4.62 ± 2.41 A	0 B	0 B
Usnea subfloridana	0.35 ± 0.19 B	0.60 ± 0.29B	4.92 ± 3.58 B	27.39 ± 6.87 A
Cyanolichens				
Peltigera membranacea	0.70 ± 0.67 B	1.34 ± 0.72 AB	1.35 ± 0.95 AB	3.32 ± 1.90 A
Total mass	4.65 ± 1.56 C	58.70 ± 6.01 B	190.35 ± 23.58 A	59.16 ± 10.87 B

Values are means ± standard error across all stands. For each species and category of cryptogam, values on different surfaces with the same letters are not significantly different according to a Dunn's multiple comparison test performed when a KS test indicated significant differences in rankings in mass. Values with no letters indicated the species did not differ in mass between surfaces. 0 indicates species not present

*Taxa present but less than 0.00 g m^2

55.2% of the cryptogam mass on this surface. The lichens *Usnea subfloridana*, a species of *Hypotrachyna,* and *Peltigera membranacea,* as well as the liverwort *Frullania convoluta,* were more commonly found on upper branches than on other surfaces, making up 46.3%, 19.7%, 5.6%, and 12.9% of the mass on upper branches, respectively. Accounting for the tree surfaces per unit land area, trees had 98.5% of all the cryptogam biomass (a total of 2 977 kg ha^{-1}), with 67%, 20%, and 13% found on the lower branches, boles, and upper branches, respectively (Supplementary Table 1).

Ground-level cloud cover caused relative humidity to exceed 95% (Supplementary Fig. 2). From January 28 to February 7, daily cloud cover never exceeded 2.5 h and never resulted in measurable precipitation in the forest's rain gauges. After February 8, there were never more than two consecutive days where there were not four or more hours of cloud cover per day, and at least 5 mm of precipitation in rain gauges in the forest. Data from the field station weather monitoring system showed that between 2016 and 2018, there were only 23 days in the year where cloud cover was less than 4 h per day (i.e., relative humidity was less than 96%).

As a consequence of the dry period, for the 1st week of February, the mean cryptogam moisture content was 23 ± 15% compared to a 149 ± 78% for the rest of the study period (Supplementary Fig. 3). Previous cloud cover hours had a significant effect on cryptogam moisture content ($p = 0.0355$ for the interaction between cryptogam type and hours of cloud cover; Supplemental Statistical Analyses 1).

Assays of $^{15}N_2$ uptake during the dry period showed an average rate of 0.40 µg N g^{-1} day^{-1} with all samples being less than 2 µmol N g^{-1} day^{-1}. After February 8, all of the taxa assayed showed evidence of nitrogen fixation with an average rate of fixation of 5.04 ± 10.1 µg g^{-1} day^{-1}. Given the short period during the year when cryptogams are dry, we excluded the $^{15}N_2$ uptake assays from the dry period in

early February from further analysis. The best restricted maximum likelihood model showed no differences between cryptogam type ($p = 0.0956$) on log-transformed rates of N fixation per tissue mass (Supplementary data analysis 2), with sampling date, species, and assay chamber accounting for 30, 14, and 7% of the variation, respectively. Another model showed that the past day's cloud cover had no effect on fixation rates on assays conducted after February 8, suggesting that once the forest starts intercepting clouds, cryptogram nitrogen fixation is not moisture-limited. Some taxa showed high variability in their rate of nitrogen fixation (Table 3). The chlorolichens as a group showed lower variability in fixation than the other cryptogams. Three chlorolichen species (*Hypotrachyna pulvinata*, *Everniastrum catawbiense,* and *Usnea subfloridana* showed consistently low levels of fixation, never exceeding 3 µg N g^{-1} day^{-1}.

Assuming that cryptogams would fix nitrogen at the same rate as we measured after February 8, and would do so every day, except for 23 days a year, we estimated the total annual amount of nitrogen fixed was 6.0 ± 1.9 kg ha^{-1} $year^{-1}$ (mean ± standard deviation). Liverworts accounted for 50% of the total nitrogen fixed, with the most abundant liverwort *P. heterophylla,* accounted for 43% of the total fixation (Fig. 1). Mosses accounted for 28.4% of the fixed nitrogen, chlorolichens accounted for 17.7%, and cyanolichens 4.0%. Cryptogams on the ground contributed only 2% to the amount of nitrogen fixed, the rest coming from trees: 63% from the lower branches, 24% from the boles, and 7% from the upper branches. These differences reflect differences in the biomass of cryptogam rather than their rate of N fixation. The nine taxa that were not assayed for $^{15}N_2$ uptake were estimated to contribute 4% of the total nitrogen fixed.

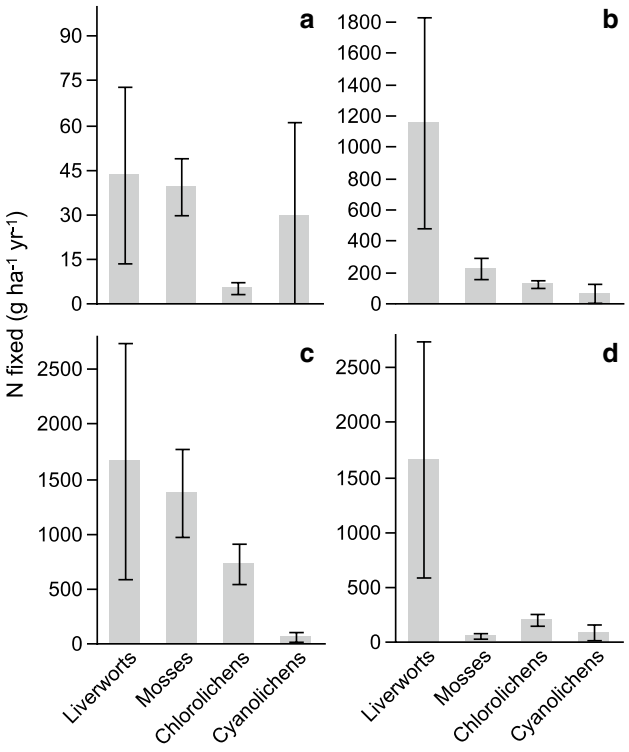

Fig. 1 Nitrogen fixation by cryptogam groups on the **a** ground, **b** boles, **c** lower branches and, **d** upper branches, per unit land area on an annual basis. Bars are means with ± one standard deviation error bars from bootstrap estimates of nitrogen fixation

Table 3 Nitrogen fixation per tissue mass estimated from $^{15}N_2$ uptake for the most abundant cryptogam species (sample size in brackets)

Taxa	N fixed µg g^{-1} day^{-1}
Liverworts	
Plagiochilla heterophylla (7)	9.1 ± 18.2
Frullania convolute (6)	9.1 ± 6.2
Mean	9.1 ± 17.1
Mosses	
Hypnum cupressiforme (6)	2.6 ± 2.8
Pleurozium sp. (6)	2.9 ± 1.1
Tomentypnum nitens (6)	10.1 ± 7.0
Mean	4.7 ± 5.2
Chlorolichens	
Everniastrum catawbiense (6)	1.6 ± 1.7
Hypotrachyna pulvinata (6)	0.9 ± 0.4
Hypotrachyna sp. (8)	2.7 ± 2.3
Lobariella pallida (8)	3.9 ± 1.8
Usnea subfloridana (5)	1.7 ± 0.8
Mean	2.3 ± 2.0
Cyanolichens	
Peltigera membranacea (8)	12.0 ± 21.1

Discussion

Our results show that nitrogen fixed by cryptogams on trees is high enough to offset the loss of nitrogen known to occur in these ecosystems. Brookshire et al. (2012) predicted that the long-term export of nitrogen in streams from tropical montane forests would have to be offset by nitrogen fixation. There are few nitrogen-fixing plants in cloud forests, but our data show epiphytes can supply the lost nitrogen. Trees create additional surface area for epiphyte colonization, allowing a larger mass than would be possible on the ground alone. In this forest, the ground supports a very small mass of cryptogams. This was likely due to the thick cover of *Chusquea talamancensis*, and the simple canopy structure resulting in a heavily shaded understory. In the nearby lower-elevation *Quercus* forests, the canopy and understory are more complex, and the ground is the major habitat for bryophyte abundance and diversity (Holz et al. 2002). As with

other studies, we found most of the tree epiphyte cryptogam biomass was on the lower branches (McCune 1990; Cusack et al. 2009; Chen et al. 2010; Werner et al. 2012). Consequently, lower branches were also the most important source of fixed nitrogen. Our estimate of total cryptogam biomass was similar to what has been estimated in the other montane cloud forests (reviewed in Köhler et al. 2007). In this highland forest, chlorolichens and bryophytes were about equally abundant on the trees, with a much smaller biomass of cyanolichens. This pattern does not seem to be typical of lower-elevation montane cloud forests, in that several other studies have found bryophytes to be the most abundant epiphyte (Freiberg and Freiberg 2000; Ellyson and Sillet 2003; Köhler et al. 2007; Chen et al. 2010). On the other hand, a study in a Columbian montane forest (Forman 1975) with a more diverse canopy structure estimated that cyanolichens made up 76% of the 6.91 kg ha^{-1} of the mass of epiphytic lichens (bryophytes biomass was not assessed). We also found about half the species specializing on different tree parts. Many of the patterns we found were consistent with the more extensive cryptogam survey in a lower-elevation *Quercus* forest by Holz and Gradstein (2005).

The relatively simple structure of this forest makes estimates of cryptogam biomass and nitrogen fixation relatively straight forward. In other forest types, tree size and age can be more heterogeneous, with epiphytes having a much higher mass on the larger and older trees (Lindo and Whitely 2010). Epiphyte biomass also tends to increases with stand age (Nadkarni et al. 2004), which likely makes old forests functionally different from secondary forests. At the lower-elevation limit of cloud forests, bryophyte diversity also tends to decrease, probably due to drying conditions (Wolf 1993; Sim-Sim et al. 2011). This likely changes their contribution to nitrogen fixation through decreased biomass and reduced fixation rates during periods of dryness. Forests with more heterogeneous tree sizes and canopy openness are likely to create a greater challenge in determining cryptogam contributions to nitrogen fixation.

The total nitrogen fixation rate we found is on the high side of estimates for epiphytic cryptogams, even though the site is relatively cool. Studies on epiphytic cryptogam nitrogen fixation generally find rates of about 1 kg N ha^{-1} $year^{-1}$ (Lindo and Whitely 2011; Benner et al. 2007). However, Cusack estimated mosses fix 3.5 kg N ha^{-1} $year^{-1}$ on a lower montane forest, and Forman (1975) estimated a rate of 8 kg N ha^{-1} $year^{-1}$ in a high-elevation forest with an exceptionally large biomass of cyanolichens. Our fixation rates are partly due to a minimal moisture limitation throughout the year in this system; the interception of clouds by trees results in few days throughout the year when cryptogams are dry. Cryptogam physiological activity and associative nitrogen fixation are generally moisture-limited (Gundale et al. 2012). Liu (2017) found that when the moisture content of cyanolichens in montane forests falls below 150%, nitrogen fixation is reduced. Epiphytic cryptogams may be particularly prone to dry conditions in most habitats, accounting for their high biomass in cloud forests. As with other studies, we found some cryptogam species showed highly variable rates of fixation (Markham 2009; Deluca et al. 2002). The consistently low level of fixation in the chlorolichens is consistent with Cusack et al. (2009), who found little nitrogen fixation associated with tree lichens in Puerto Rican forests. Other potential sources of nitrogen fixation in forests include the phyllosphere (Favilli and Messini 1990) and heterotrophic soil diazotrophs, especially in rotting wood (Matzek and Vitousek 2003; Pérez et al. 2010). However, the phyllosphere is not a major contributor to nitrogen fixation to montane forests and is unlikely to contribute a significant amount of nitrogen to most ecosystems (Cusack et al. 2009). For instance, Fürnkranz et al. (2008) sampled a lowland wet tropical forest where nitrogen fixation on leaves was about 4 µmol m^{-2} day^{-1} for the species showing high rates of fixation. With a leaf area index of 1, this would contribute only 0.2 kg N ha^{-1} $year^{-1}$. Tree surface may be particularly nutrient-limited, explaining this low level of nitrogen fixation, which could temporarily be alleviated by nutrient inputs from dust (Stanton et al. 2019). Across a range of forest site ages in Hawaii, Matzek and Vitousek (2003) found the rotting wood provides 27–120% of the nitrogen fixation contributed by the dominant mosses and lichens. Due to its large mass, the soil can provide a substantial amount of fixed nitrogen via heterotrophic diazotrophs in montane forests (Cusack et al. 2009), as well as other systems (Sullivan et al. 2014). However, decreasing temperatures with increased elevation and canopy cover are likely to limit all soil physiological processes in these forests. It is, therefore, likely that epiphytic cryptogams are the major contributor of fixed nitrogen in tropical mature highland forests.

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