Does global stoichiometric theory apply to bryophytes? Tests across an elevation × soil age ecosystem matrix on Mauna Loa, Hawaii

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Summary

1. General hypotheses have related nutrient stoichiometry to physiology and structure for tracheophytes across global resource gradients. These hypotheses have not been tested on bryophytes despite their importance in numerous ecosystem processes.

2. Twelve bryophyte species were sampled across an elevation × soil age matrix representing a dramatic range of climates on Mauna Loa, Hawaii.

3. We tested six major hypotheses for the relationship of tissue nutrients to environment and morphology, and for differences in composition between bryophytes and tracheophytes.

4. Results supported stoichiometric theory. Mass-based nutrient concentrations increased with soil nutrient availability; area-based nutrient concentrations increased with irradiance as mediated by bryophyte canopy mass per area; N and P followed the general scaling shown previously for tracheophytes; and P increased and N:P decreased with elevation consistent with increasing cold tolerance.

5. *Synthesis.* These findings extend the generality of stoichiometric theory, pointing to convergent physiological responses across distantly related lineages, operating across local and global resource gradients.

Key-words: bryophytes, ecophysiology, ecosystem, Hawaii, irradiance, latitudinal gradient, moss, nitrogen, phosphorus, stoichiometry

Introduction

Decades of research on a wide range of organisms and communities has shown the importance of elemental composition for explaining and predicting ecological patterns and processes. These studies led to theoretical syntheses for aquatic systems (Redfield 1958), and recently for terrestrial tracheophytes (Gusewell 2004; Reich & Oleksyn 2004; Ordonez *et al.* 2009; Elser *et al.* 2010). Thus, the stoichiometry, or relative abundances, of elements such as N and P correlate across species with plant functional type (Reich, Wright & Lusk 2007), climate (Reich & Oleksyn 2004) and soil fertility (Gusewell 2004). However, very few studies have focused on bryophyte composition or physiology (Cornelissen *et al.* 2007). Bryophytes present an excellent system for testing theories established for tracheophytes, since they represent an ancient lineage, reflecting the condition of primitive plants and with relatively simple organization, and as such are becoming a

model for genetics, development and function (Cove *et al.* 2006). Bryophytes contrast with typical tracheophytes in taking up water and nutrients primarily via the leaf surface from precipitation and dry deposition, respectively (Bates 2009). Bryophytes typically have leaves only one cell thick except at the midrib but the bryophyte *colony* may act as a functional unit like the tracheophyte leaf (Proctor 2000), with canopy mass per area (CMA) analogous to the leaf mass per area (LMA) of tracheophytes (Waite & Sack 2010). Bryophytes tend to grow slowly, under limited water, nutrient and/or light availability (Grime, Rincon & Wickerson 1990; During 1992), but are morphologically and ecologically diverse along resource gradients (Slack 1990; Waite & Sack 2010), and contribute importantly to carbon fluxes (Swanson & Flanagan 2001).

This study provides a novel case to test the generality of stoichiometric hypotheses and to extend the principles to a more ancient lineage. We focused on Hawaiian bryophytes as a model system including species evolved and assembled across dramatic gradients of soil age and climate. We tested global

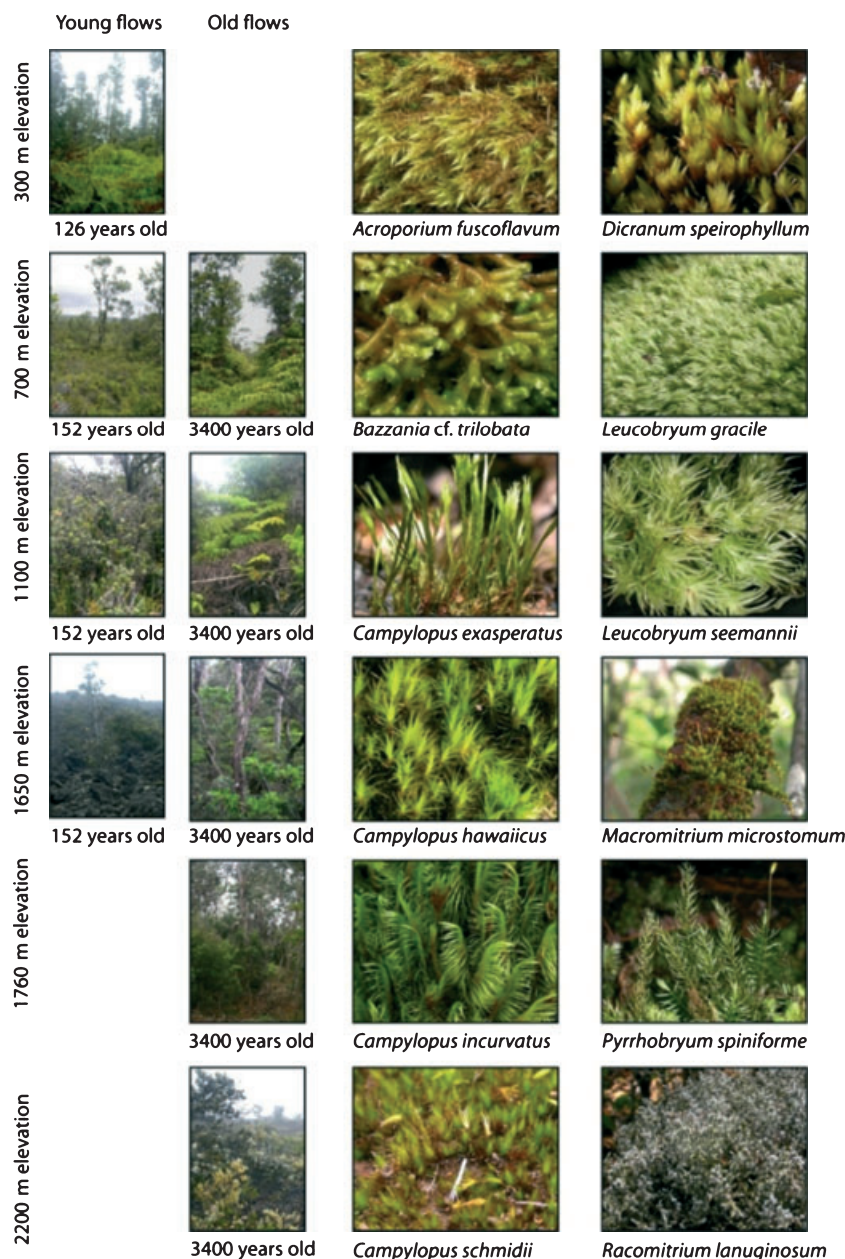*Corresponding author. E-mail: lawrensack@ucla.edu

Young flows Old flows

300 m elevation

126 years old

Acroporium fuscoflavum

Dicranum speirophyllum

700 m elevation

152 years old 3400 years old

Bazzania cf. *trilobata*

Leucobryum gracile

1100 m elevation

152 years old 3400 years old

Campylopus exasperatus

Leucobryum seemannii

1650 m elevation

152 years old 3400 years old

Campylopus hawaiicus

Macromitrium microstomum

1760 m elevation

3400 years old

Campylopus incurvatus

Pyrrhobryum spiniforme

2200 m elevation

3400 years old

Campylopus schmidii

Racomitrium lanuginosum

Fig. 1. Views of the vegetation on the young and old lava flows at the sampled elevations, and of the 12 bryophyte species sampled on Mauna Loa, Hawaii. The ca. 400 year old lava flow at 1750 m is not pictured.

theories using 12 species from seven genera on Mauna Loa, Hawaii, distributed across sites 300–2200 m in elevation (Fig. 1 and see Fig. S1 in Supporting Information), varying fourfold in mean annual precipitation (MAP), and twofold in mean annual temperature (MAT; Table 1). The Mauna Loa matrix consists of lava flows ca. 150 and 3000 years old, where the older soils have accumulated greater N and P availability, especially at lower elevation (Raich, Russell & Vitousek 1997). We tested expectations derived from six partially overlapping hypotheses based on previous work for tracheophytes and other organisms, for concentrations and stoichiometric ratios of N and P within and among bryophyte species spanning the matrix. (i) Tissue nutrient concentrations and stoichiometric ratios reflect the general substrate nutrient availability ('plants reflect the soil'; Elser *et al.* 2010). (ii) Tissue nutrient concentrations relate to shifts in leaf morphology for improved perfor-

mance in response to environmental conditions ('leaf structure influences composition'; Niinemets 1999; Shipley *et al.* 2006). Tracheophytes are predicted to have higher mass-based concentrations of N and P (N_{mass} and P_{mass}, respectively) in leaves that are thinner or of lower density, i.e. leaves of lower LMA (the product of thickness and density), and thus, for bryophytes, with the canopy analogous to the tracheophyte leaf (Waite & Sack 2010), N_{mass} and P_{mass} may be higher in canopies with lower CMA (the product of canopy height and density). (iii) Stoichiometric constraints on biochemistry mandate relatively constant proportions of tissue nutrient concentrations ('stoichiometric homeostasis'; Elser *et al.* 2010) such that N_{mass} and P_{mass} are correlated, and further, (iv) N_{mass} increases with the 2/3 power of P_{mass}, as found in a canonical analysis for tracheophytes ('general scaling of N and P'; Elser *et al.* 2010; Reich *et al.* 2010). (v) Variation in nutrient

Table 1. Bryophyte species and substrates sampled from young and/or old lava flows at seven elevations on Mauna Loa, Island of Hawaii. Species nomenclature follows Staples *et al.* (2004); family nomenclature follows Tropicos (http://www.tropicos.org/), Missouri Botanical Garden. Lava age is designated in the table as 'new' for 1855–1881 lava flows and 'old' for the 3400 year old lava flow (and 400 year old lava flow at 1760 m site). At most sites, overstorey cover (OC) ranged 58–63% (45–47% at 1750 m and 2200 m; although the 2200 m site was mostly open with few trees, the moss *D. speirophyllum* was only found under trees)

Species sampled	Family	Substrate	300 m	700 m	1100 m	1650 m	1750 m	1760 m	2200 m
Acroporium fusco-flavum	Sematophyllaceae	Leaf litter - bark	New	New, old	New, old	Old	Old	–	–
Bazzania cf. trilobata	Lepidoziaceae	Bark - humus	–	New, old	New, old	Old	Old	Old	–
Campylopus exasperatus	Dicranaceae	Rock	–	New	–	–	–	–	–
Campylopus hawaiicus	Dicranaceae	Humus - bark	–	–	Old	Old	Old	Old	–
Campylopus incurvatus	Dicranaceae	Bark	–	Old	–	–	–	–	–
Campylopus schmidii ssp. schimidii	Dicranaceae	Rock	–	–	–	–	Old	–	–
Dicranum speirophyllum	Dicranaceae	Leaf litter - humus	–	–	New	New, old	Old	Old	Old
Leucobryum gracile	Dicranaceae	Bark - humus	New	Old	Old	Old	–	Old	–
Leucobryum seemannii	Dicranaceae	Humus	New	Old	–	–	–	–	–
Macromitrium microstomum	Orthotrichaceae	Bark	New	New, old	New, old	New, old	Old	Old	–
Pyrrhobryum spiniforme	Rhizogoniaceae	Bark - humus	–	Old	Old	Old	–	Old	–
Racomitrium lanuginosum	Grimmiaceae	Rock	New	New	New	New	Old	Old	–
Modelled climate									
Mean annual temperature (ºC)			21.0	18.1	15.4	12.8	12.3	12.2	9.7
Mean annual precipitation (mm)			4514	5840	4320	2840	2420	2360	1510
Mean annual vapour pressure deficit (kPa)			0.573	0.369	0.318	0.352	0.365	0.366	0.430
Average species-site overstorey cover (%)			66	46, 68	53, 72	37, 66	47	61	45

concentrations and stoichiometry are associated with physiological cold adaptation ('cold tolerance'; Reich & Oleksyn 2004). Finally, (vi) stoichiometry is related to growth rate, as faster growth requires higher relative amounts of P-rich RNA ('growth rate-RNA'; Elser *et al.* 2010), which would be expected to occur with greater nutrient supplies and moisture.

Given the fundamental nature of these processes for tracheophytes globally, we expected similar patterns among the bryophytes along the strong gradients in soil age and elevation of the Mauna Loa matrix. These principles led to a series of key predictions for the nutrient concentrations and stoichiometry of bryophytes arrayed on Mauna Loa (see Table S1 in Supporting Information).

Materials and methods

STUDY SITE AND SPECIES

Bryophyte species were sampled from native communities on Mauna Loa, Hawaii Island, where soil development and nutrient availability is greater on older flows than younger flows and at lower elevations on a given flow (Raich, Russell & Vitousek 1997). Sampling was conducted at six elevations (287–2239 m) on young (ca. 150 years) and old lava flows (3000–5000 years except at Puu Oo flow, where age is ca. 400 years; Wolfe & Morris 1996; Fig. 1 and Fig. S1; Table 1).

The sampled locations differed in species composition and abundance. Eleven moss species from seven genera, including the most common species, and one liverwort species were collected (Table 1). Four of the species were distributed from 300 to 1700 m in elevation, and *Racomitrium lanuginosum* was found across the entire 300–2200 m gradient (although not sampled at 2200 m because of difficulty distinguishing live from dead tissue); by contrast, three of four *Campylopus* species were collected at single sites. Three canopy samples of 4–7 cm diameter were taken at each sampling location for each species when possible (in six of 53 cases only a single colony was found for a given species) and stored in individual plastic bags, transported to the lab on ice and transferred to a refrigerator until processing.

MEASUREMENTS – CLUMP STRUCTURE AND TISSUE NUTRIENT CONCENTRATION

The height of the green canopy (canopy height; CH) was measured in the field to the nearest mm by ruler at three random positions. Canopy projected area was measured by tracing the outline on clear plastic in the field and later scanning and measuring the area using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). Upper, green stem portions were separated from the older, brown tissue using fine scissors or a razor blade, oven-dried at 80 °C for at least 48 h in tin foil packets, which were open during drying, but were sealed on removal from the oven to prevent uptake of

atmospheric moisture, before weighing for dry mass (Mettler-Toledo AB204S∕FACT). Canopy mass per area was determined as dry mass divided by canopy projected area (i.e. the traced outline), and canopy density as CMA divided by CH. Nitrogen per mass (N_{mass}) and carbon per mass (C_{mass}) were measured using high-temperature combustion in an elemental analyser (Costech ECS 4010; Valencia, CA, USA). Phosphorus per mass (P_{mass}) was measured from samples dry ashed in glass vials (Miller 1998), dissolved in 1 N HCl and analysed using inductively coupled plasma-optical emission spectrometry (Varian Vista MPX Instrument, Varian, Palo Alto, CA, USA; Porder, Paytan & Vitousek 2005). Concentrations of nitrogen and phosphorus per area (N_{area} and P_{area}) were determined by multiplying N_{mass} and P_{mass} by CMA.

CLIMATE AND OVERSTOREY CLOSURE

We estimated MAT, precipitation (MAP) and relative humidity (MARH) for each sampling location from its coordinates using a Geographic Information System model based on climate station data (Cao *et al.* 2007; T.W. Giambelluca & L. Cao, unpubl. data). Mean annual vapour pressure deficit (VPD) was calculated from MAT and MARH (Campbell & Norman 1998). Vapour pressure deficit is a measure of atmospheric drought, the driving force for evaporation, and can be quantified as an absolute pressure difference (in kPa), or as a mole fraction (i.e. normalized by atmospheric pressure). Across the Mauna Loa matrix study sites, the two VPD measures were highly correlated ($r = 0.92$; $P < 0.001$); we present results for both but discuss in the text correlations with absolute VPD. Overstorey cover (%; OC), the proportion of the sky obscured from view (equivalent to canopy closure *sensu* Jennings, Brown & Sheil 1999), was visually assessed to the nearest 10%. Visual canopy cover estimates correlate with measurements using a densiometer or hemispherical photography, especially given training, as was undertaken previously to this study to achieve a correspondence of ±5% relative to hemispherical photos (Korhonen *et al.* 2006; Paletto & Tosi 2009).

STATISTICAL ANALYSES

Statistical procedures were applied using R 2.6.1 (http://www.r-project.org). We tested differences in traits across elevations, soil ages and species using analyses of variance (ANOVAs) after log-transformation of data to improve normality and homoscedasticity (Sokal & Rohlf 1995). To account for the over-representation of old-soil sites at higher elevation the ANOVA was repeated for the three middle elevations where both soil ages were sampled. Pearson correlations were calculated for trait–trait and trait–climate relationships, using data averaged across individuals of each species for each site. Correlations were tested using both raw data and log-transformed data, for 'all means' (i.e. all species-site averages), and for data of 'young' and 'old' soil separately, and for data of each taxon separately, for those taxa found at six or more sampling sites (*A. fuscoflavum, Bazzania* cf. *trilabata, Campylopus* spp., *D. speirophyllum, M. microstomum* and *R. lanuginosum*; the four *Campylopus* spp. were here analysed together to attain six sampling sites for the genus). When a relationship was significant for 'all means', and also for both individual soil ages or for two or more individual taxa, we tested for similarity of the slopes and intercepts between the soil ages, or among the taxa, using raw or log-transformed data according to which showed the stronger relationship across all data (using SMATR; http://www.bio.mq.edu.au/ecology/SMATR; Warton *et al.* 2006). Standard major axes were used to compare relationships between

independent variables (i.e. structural and composition traits), whereas least squares regressions were used for relationships between a dependent and independent variable (i.e. between traits and climate or OC; Sokal & Rohlf 1995). Significant correlations among inter-correlated variables were further resolved with partial correlation analysis (Sokal & Rohlf 1995), indicating the relationship between two variables holding other variables constant (*corpcor* package; R; Schaefer, Opgen-Rhein & Strimmer 2007).

Results

VARIATION IN BRYOPHYTE NUTRIENT CONCENTRATIONS, STOICHIOMETRY AND MORPHOLOGY

Bryophytes varied strongly in nutrient composition, stoichiometry and canopy morphology across the matrix (Table 2; see Table S2a, b). Averaging for given species at each site, we found variation of 16- to 44-fold in CH, density (CD) and CMA; of three- to sevenfold in N_{mass} and P_{mass}; of 20-fold in N_{area} and P_{area}; and of three- to sixfold in N:P, C:N and C:P. These differences were strongly associated with species, elevation and soil age. In general, bryophytes at higher elevation had greater CMA, CD, P_{mass}, P_{area} and C:N, and lower N_{mass}, N:P and C:P. When analysing the six sites including both soil ages at the three elevations, bryophytes on older soil had higher N_{mass} and P_{mass}, and lower CMA, N_{area}, P_{area}, N:P and C:P (Table 2; Table S2b). Notably, elevation and species were the most important sources of variation, with soil age much less, and the interactions among elevation, species and soil age were often significant (Table S2).

Across the bryophytes, CH and CD were negatively correlated ($r = -0.80$, $P < 0.001$; Table 3), and both correlated positively with CMA ($r = 0.42–0.47$, $P < 0.001–0.003$).

CORRELATION OF NUTRIENT CONCENTRATIONS, STOICHIOMETRY AND MORPHOLOGY ACROSS THE MATRIX

Across the matrix, nutrient concentrations and stoichiometry were inter-related (Table 3). N_{mass} correlated positively with P_{mass} ($r = 0.51$, $P < 0.001$), and N_{area} with P_{area} ($r = 0.88$, $P < 0.001$). The allometric slope relating N_{mass} to P_{mass} was 0.61 (95% CIs were 0.48–0.80). The strong variation in N and P drove their respective negative correlations with C:N and with C:P ($r = -0.49$ to -0.98, $P < 0.001$). N:P was negatively correlated with P_{mass} and P_{area} and positively with C:P ($|r| = 0.67–0.83$; $P < 0.001$) but not with N_{mass} or N_{area} ($|r| = 0.10–0.22$, $P = 0.12–0.46$), probably due to the greater variation in P_{mass} than N_{mass} across the matrix (coefficients of variation were 40 and 24%, respectively; Table 2).

Nutrient concentrations were also related to bryophyte canopy morphology. We note that the concentration of a nutrient per canopy area was determined as tissue concentration per mass × CMA, where CMA = CH × CD. N_{area} was weakly, non-significantly related to N_{mass} ($r = 0.24$, $P = 0.08$), whereas P_{area} was strongly driven by P_{mass} ($r = 0.68$,

Table 2. Ranges of variation and results of analyses of variance (ANOVAs) for canopy structure and composition traits for bryophytes across the Mauna Loa matrix, Island of Hawaii. For each trait, the minimum and maximum species-site means are presented, the coefficient of variation (CV), the mean value for the lowest and highest elevation that had multiple species (and the value for the highest elevation with only a single species represented), the mean values for 'young' and 'old' soil, and the minimum and maximum species-means. Results of a three-way ANOVA with factors species (Sp), elevation (E), and soil age (S); *** $P \leq 0.001$, ** $P \leq 0.01$. * $P < 0.05$; % of variation explained presented beneath; no three-way interactions were significant

Trait (minimum, maximum)	CV (%)	Elevation low, upper (highest with single species)	Soil age (Young, Old)	Soil age, paired elevations (Young, Old)	Species (minimum, maximum)	E×S	E×Sp	S×Sp
Canopy height (cm) (0.23–4.10)	58	1.44, 1.59 (1.67)	1.47, 1.62*** 2%	1.49, 1.64	0.32, 3.30*** 90%	* 0.4%	* 2%	
Canopy mass per area (g m⁻²) (41–641)	62	97, 213 (194)*** 22%	177, 168*** 7%	208, 136***	86, 641*** 38%		*** 14%	
Canopy density × 100 (g m⁻³) (16–710)	108	107, 203 (126)*** 7%	205, 162*** 6%	259, 133	32, 535*** 74%	* 1%		* 1%
Nitrogen per mass (%) (0.36–1.02)	24	0.727, 0.661(0.395)*** 32%	0.593, 0.580** 2%	0.541, 0.607**	0.418, 0.757*** 26%	* 2%		
Nitrogen per area (g m⁻²) (21–427)	67	71, 141 (77) 24%	103, 95*** 3%	115, 80**	52, 427*** 34%	** 3%	*** 17%	
Phosphorus per mass (%) (0.0130–0.0886)	40	0.0384, 0.0581 (0.0316)*** 40%	0.0386, 0.0478*** 3%	0.0386, 0.0479***	0.0331, 0.0633*** 19%	*** 6%		
Phosphorus per area (g m⁻²) (1.6–33.6)	80	3.6, 11.9 (6.1)*** 41%	7.63, 7.94 3%	9.17, 6.49	3.8, 33.6*** 27%	*** 4%	* 8%	* 2%
Nitrogen: Phosphorus (6.4–29.3)	37	20.0, 11.9 (12.6)*** 30%	18.5, 13.6	17.9, 14.7**	10.3, 21.8*** 26%	** 4%	* 11%	
Carbon: Nitrogen (44–116)	24	57.6, 68.5 (109)*** 32%	76.1, 77.2** 3%	82.2, 72.4**	59.2, 99.8*** 27%	* 2%		
Carbon: Phosphorus (523–3170)	45	1160, 790 (1380)*** 40%	1430, 1030*** 3%	1530, 1050***	729, 1550*** 18%	*** 6%		

Table 3. Correlations of nutrient concentrations, stoichiometric ratios and climate for bryophytes across the Mauna Loa matrix, Island of Hawaii. The lower triangle contains Pearson correlation coefficients across all data averaged by species per site; bold type indicates significance at $P \leq 0.05$, and italics indicates that correlations were derived from log-transformed data because these were significant and stronger than those from raw data. The upper triangle contains trait relationships that remained significant when considering bryophytes only on young or old soil age (Y and O respectively), and/or when considering relationships within taxa for those with at least six collection sites (a = *A. fuscoflavum*, b = *Bazzania* sp., c = *Campylopus* genus, d = *D. speirophyllum*, m = *M. microstomum*, r = *R. lanuginosum*). Trait symbols: CH = canopy height, CMA = canopy mass per area, CD = canopy density, N_{mass} = nitrogen concentration per mass, N_{area} = nitrogen concentration per canopy projected area, P_{mass} = phosphorus per mass, P_{area} = phosphorus per projected canopy area, N:P = nitrogen to phosphorus mass ratio, C:N = carbon to nitrogen mass ratio, C:P = carbon to phosphorus mass ratio, OC = overstory cover, MAT = mean annual temperature, MAP = mean annual precipitation, VPD = absolute vapour pressure deficit, molarVPD = mole fraction vapour pressure deficit.

Trait	CH	CMA	CD	N_{mass}	N_{area}	P_{mass}	P_{area}	N:P	C:N	C:P	OC	Elevation	MAT	MAP	VPD	MolarVPD
CH		O	YO[m]				O			YO[br]	YO					
CMA	0.42		YO[abmr]				YO[abcm] O[abm]	Y[am]		Y[a]	YO[ac]					
CD	-0.80	0.47								YO[abcdmr]	YO[a]					
N_{mass}	-0.20	-0.14	0.05		YO[abcm] YO[ab]	YO[br]			YO[abcdmr]	YO[abcdm]	Y[c]	m			Y	Y
N_{area}	0.28	0.91	0.48	0.24		Y[c]	YO[abcmr]			YO[abdmr]		m				
P_{mass}	0.10	0.13	0.17	0.51	0.36		YO[abcdm]	YO[abcdmr]	YO[r]	YO[br]	Y[c]		m	Y[m]	Y	YO
P_{area}	0.36	0.86	0.45	0.10	0.88	0.68		YO[abcmr]					O[m]	O[r]		
N:P	-0.16	-0.28	-0.20	0.10	-0.22	-0.81	-0.67					Y[m]	Y[m]	Y[mr]	Y	Y
C:N	0.31	0.20	-0.13	-0.98	-0.17	-0.49	-0.07	-0.04				m	m	Y[m]		
C:P	0.00	-0.10	-0.24	0.18	-0.32	-0.98	-0.64	0.83	0.55						Y	
OC	0.47	-0.46	-0.66	-0.12	-0.37	0.00	-0.35	0.08	-0.18	-0.08						
Elevation	0.00	0.17	0.14	0.14	0.13	0.35	0.34	-0.51	0.20	-0.32	-0.21		YO	YO	Y	YO
MAT	0.00	-0.28	-0.15	-0.03	-0.13	-0.34	-0.40	0.52	-0.21	0.34	0.22	-1.00		YO	Y	O
MAP	0.04	-0.07	-0.11	0.32	-0.07	-0.37	-0.36	0.48	-0.05	0.39	0.16	-0.89	0.92		Y	YO
VPD	-0.02	-0.12	-0.09	0.30	-0.04	-0.02	-0.11	0.16	-0.34	-0.09	0.09	-0.68	0.53	0.11		YO
Molar VPD	-0.02	-0.05	-0.04		0.01	0.13	0.00	-0.05	-0.29	-0.24	0.00	-0.31	0.16	-0.34	0.92	

$P < 0.001$). Both N_{mass} and P_{mass} were independent of CMA ($|r| = 0.13$–0.14, $P = 0.31$–0.36), but both N_{area} and P_{area} correlated positively with CMA ($r = 0.86$–0.91, $P < 0.001$). These relationships of N_{area} and P_{area} with CMA were due to relationships with both CD and CH ($r = 0.28$–0.48, $P = 0.05$ to $P < 0.001$). Across the matrix, N:P correlated negatively with CMA ($r = -0.28$, $P < 0.05$; Table 3), due to its negative correlation with P_{area} which correlated with CMA (see above).

Of the 24 trait–trait correlations described above, 20 were significant when considered only for bryophytes on old soil ($|r| = 0.35$–0.98, $P < 0.001$ to $P = 0.04$), and 20 for young soil ($|r| = 0.50$–0.99, $P < 0.001$ to $P = 0.03$); 17 were significant for both (Table 3). For 11 of those 17 relationships, the soil types shared similar slopes and intercepts ($P = 0.11$–0.96; Table S3); for the other six relationships the soil types shared similar slopes ($P = 0.06$–0.98) and differed in intercepts ($P = 0.006$–0.049). For the relationships N_{area} versus P_{area}, and N_{mass} versus P_{mass}, the older soil had lower intercepts, indicating lower N:P. Of the 24 trait–trait correlations, 21 were significant for individual taxa, and 17 for two or more individual taxa (Table 3). For two of these 17 correlations, the taxa shared similar slopes and intercepts ($P = 0.06$–0.45); for another, the taxa differed in slope ($P = 0.04$) and for 14, the taxa had similar slopes ($P = 0.052$–0.85) but differed in intercepts ($P < 0.001$ to $P = 0.03$; Table S3).

CORRELATION OF NUTRIENT COMPOSITION, STOICHIOMETRY AND MORPHOLOGY WITH CLIMATE

Climate variables were inter-related across the Mauna Loa matrix. Site MAT, MAP and VPD correlated negatively with elevation ($r = -0.68$ to -0.999, $P < 0.001$), and MAT with MAP ($r = 0.92$, $P < 0.001$) and VPD ($r = 0.53$, $P < 0.001$). Notably, MAP was independent of VPD ($r = 0.11$, $P = 0.45$). While OC differed among species, elevations and soil ages (three-way ANOVA; $P < 0.001$), with significant interactions among factors ($P < 0.001$–0.004), OC did not correlate with elevation or the climate variables ($|r| < 0.001$ to 0.22, $P = 0.12$–0.99). Young and old soil sites had average OC values of 52% and 62%, respectively.

Across the matrix, tissue P but not N correlated with elevation and climate (Fig 2) (Fig. S2). Thus, P_{mass} and P_{area} increased with elevation ($r = 0.34$–0.35, $P = 0.01$), and related negatively to MAT ($r = -0.34$ to -0.40, $P = 0.01$–0.03), whereas N_{mass} and N_{area} were independent of elevation and of MAT ($|r| = 0.12$–0.14, $P = 0.32$–0.38). Consequently, C:P and N:P declined with elevation ($r = -0.32$ to -0.51, $P < 0.001$ to $P = 0.02$) and increased with MAT ($r = 0.34$–0.52, $P < 0.001$ to $P = 0.02$). Similarly, P_{mass} and P_{area} correlated negatively with MAP ($r = -0.36$ to -0.37, $P = 0.007$), while N_{mass} and N_{area} were independent ($r = -0.03$ to -0.07, $P = 0.64$–0.81), leading to an increase of C:P and N:P with MAP ($r = 0.39$–0.48, $P < 0.001$ to $P = 0.006$). Notably, CMA and C:N were independent of elevation ($r = 0.17$–0.20, $P = 0.15$–0.22) and, although CMA correlated weakly with MAT ($r = -0.28$, $P = 0.045$), C:N was independent

($r = -0.05$, $P = 0.13$), and both CMA and C:N were independent of MAP ($r = -0.05$ to -0.07, $P = 0.63$–0.73). Tissue composition and stoichiometry showed variable relationships with VPD. Across the matrix, N_{mass} correlated positively with VPD ($r = 0.32$, $P = 0.02$), and consequently C:N related negatively with VPD ($r = -0.34$, $P = 0.01$). By contrast, N_{area}, P_{mass}, P_{area}, N:P, C:P and CMA were independent of VPD ($|r| = 0.02$–0.16, $P = 0.26$–0.89).

Site OC was a stronger determinant of bryophyte morphology than climate (Fig. 3). CMA and CD correlated negatively with OC, whereas CH correlated positively with OC ($|r| = 0.46$–0.66, $P < 0.001$). N_{area} and P_{area} declined with OC ($r = -0.35$ to -0.37, $P = 0.005$–0.03), consistent with their relationships with canopy morphology described above, while N_{mass}, P_{mass}, N:P, C:N and C:P were independent of OC ($|r| < 0.01$–0.18, $P = 0.19$–0.97). Because N_{area} and P_{area} correlated with CMA, we calculated partial correlations. The correlations of N_{area} and P_{area} with OC, controlling for CMA, were non-significant ($r_{partial} = 0.08$–0.11, $P > 0.10$) but their correlations with CMA controlling out OC remained significant ($r_{partial} = 0.84$–0.89; $P < 0.001$; Table S4, row 3), indicating the morphological basis for tissue nutrient responses to OC.

Of the 20 correlations between traits and elevation, climate or OC, 5 and 12 were significant for old or young soil sites individually ($|r| = 0.33$–0.73, $P < 0.001$–0.05); three of the relationships, the correlations of CH, CD and CMA with OC, were significant for both soil ages with similar slopes and intercepts ($P = 0.25$–0.85, $P = 0.07$–0.75; Table S3). For 16 of the 20 correlations, there were significant relationships for individual taxa, and for two there were significant relationships for two taxa. The CMA negatively correlated with OC for *Acropodium* and *Campylopus* ($r = -0.77$ to -0.82, $P = 0.04$), with similar slopes and intercepts ($P = 0.11$ and $P = 0.08$, respectively). N:P and MAP were positively correlated for *Macromitrium* and *Racomitrium* ($r = 0.76$–0.85, $P = 0.02$–0.03); the slopes were similar ($P = 0.58$), and *Macromitrium* had a lower intercept ($P = 0.02$).

We resolved underlying relationships between traits and elevation, climate and OC using partial correlations. Across the whole data set, the five correlations with OC remained significant when partialing out the climate variables (Table S4). However, none of the nine correlations of traits with MAT or MAP had significant partial correlations after partialing out the other climate variable ($|r_{partial}| = 0.01$–0.25, $P = 0.07$–0.94; Table S4); thus the effects of MAT and MAP could not be separated for the Mauna Loa matrix. Similarly, several relationships on individual soil ages remained after partialing out other factors. For bryophytes on young or old soil considered individually, the relationships of CH and CD with OC remained after controlling for MAT and MAP ($|r_{partial}| = 0.40$–0.69, $P < 0.001$–0.03; Table S5). Only N:P versus MAP of the 16 trait-climate correlations found for individual taxa remained significant after partialing out another factor (MAT; $r_{partial} = 0.89$, $P < 0.05$; Table S6).
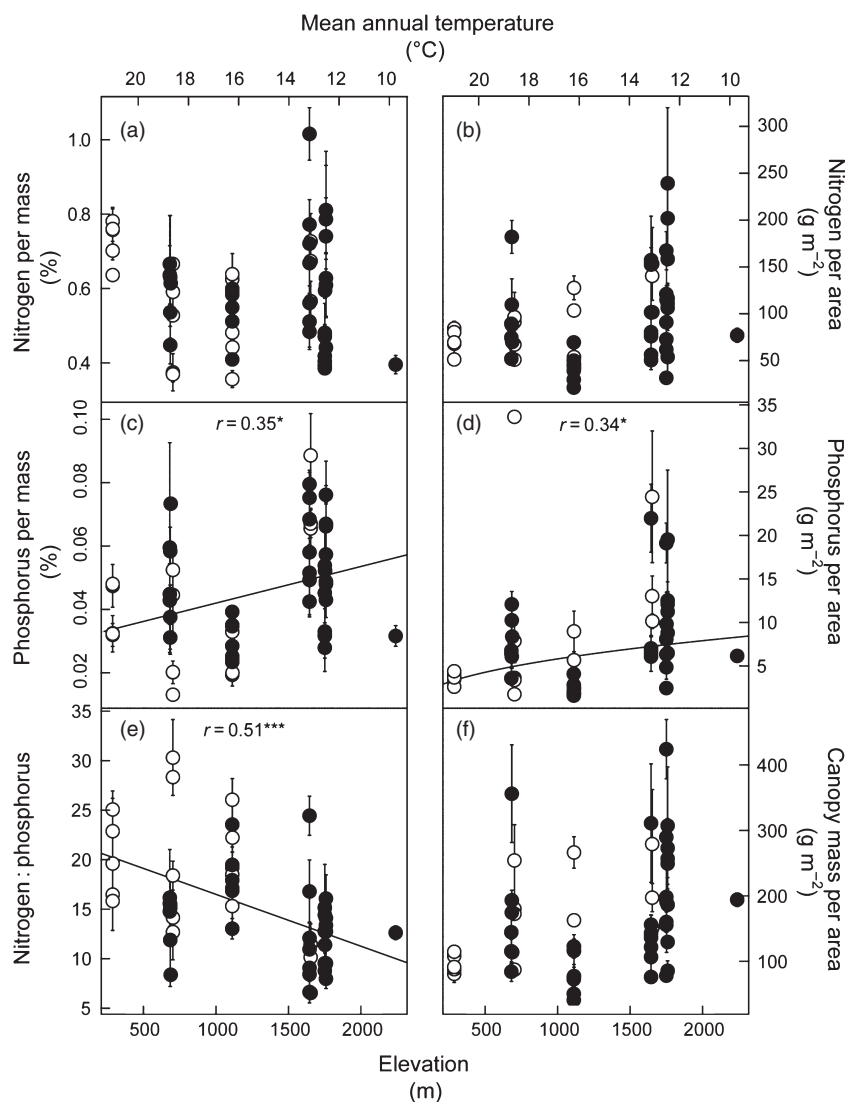
Fig. 2. Relationships of bryophyte nutrient concentrations and stoichiometric ratios with elevation and mean annual temperature: (a) nitrogen per dry mass (N_{mass}), (b) nitrogen per area (N_{area}), (c) phosphorus per dry mass (P_{mass}), (d) phosphorus per area (P_{area}), (e) nitrogen:phosphorus dry mass ratio (N:P), (f) canopy mass per area (CMA). Open and closed circles, respectively, indicate species averages from young and old lava flow sites. Fitted lines were determined by fitting ordinary least squares regression to untransformed data ($y = b + a \times elevation$) for C and E, and to log-transformed data [$\log y = \log b + a \times \log(elevation)$ for D; C: $a = 0.0301$(95% CI: 0.0182–0.0419), $b = 1.18 \times 10^{-5}$; D: $a = -0.655$ (−1.71 to 0.40), $b = 0.472$; E: $a = 21.8$ (18.4–25.1), $b = -0.00525$. Error bars = 1 SE. Significance levels: *$P < 0.05$; **$0.01 \geq P > 0.001$; ***$P \leq 0.001$. Note that similar relationships held for mean annual precipitation (Figure S2).

Discussion

Bryophyte nutrient composition and canopy morphology varied strongly with environmental variables across the Mauna Loa matrix. We found support for many of the stoichiometric principles established for tracheophytes, importantly extending the synthesis to a separate lineage, and indicating that trends previously established for global data sets hold across the ecosystem matrix of a single volcano. We also identified several important differences arising from the distinctive structure and function of bryophytes.

DEPENDENCE OF TISSUE NUTRIENT CONCENTRATION ON ENVIRONMENTAL AVAILABILITY: PLANTS REFLECT THE SOIL

Tissue concentrations per mass were related to nutrient availability. Indeed, in parallel with the greater general nutrient availability (Raich, Russell & Vitousek 1997), bryophyte N_{mass} and P_{mass} were higher on older soil when sites were matched by elevation. That pattern is consistent with

global trends for tracheophytes, and also with the local trend for the dominant tree, *M. polymorpha*, in which leaf nutrient concentrations per mass increased with soil age (Vitousek *et al.* 1992; Cordell *et al.* 1998). Our test was for a general effect on the bryophyte community of soil nutrient availability related to soil age and elevation. The bryophytes could be indirectly influenced by the soil nutrient availability through nutrients in leachates from the surrounding tracheophyte vegetation (reviewed in Glime 2007). Additionally, the bryophytes would have been influenced by the nutrient concentrations of the substrates immediately under the colonies, especially as bryophytes are ecosystem engineers that can affect soil conditions via effects including accumulation of organic matter, acidification and phenolic leakages (Turetsky 2003; Hagemann *et al.* 2010; Soudzilovskaia *et al.* 2010). We did not test for a relationship with the specific nutrient sources beneath each moss; such a detailed sampling for these diverse mosses was outside the scope of our study, and future research should elucidate specific inputs that might contribute to the landscape-scale patterns found.
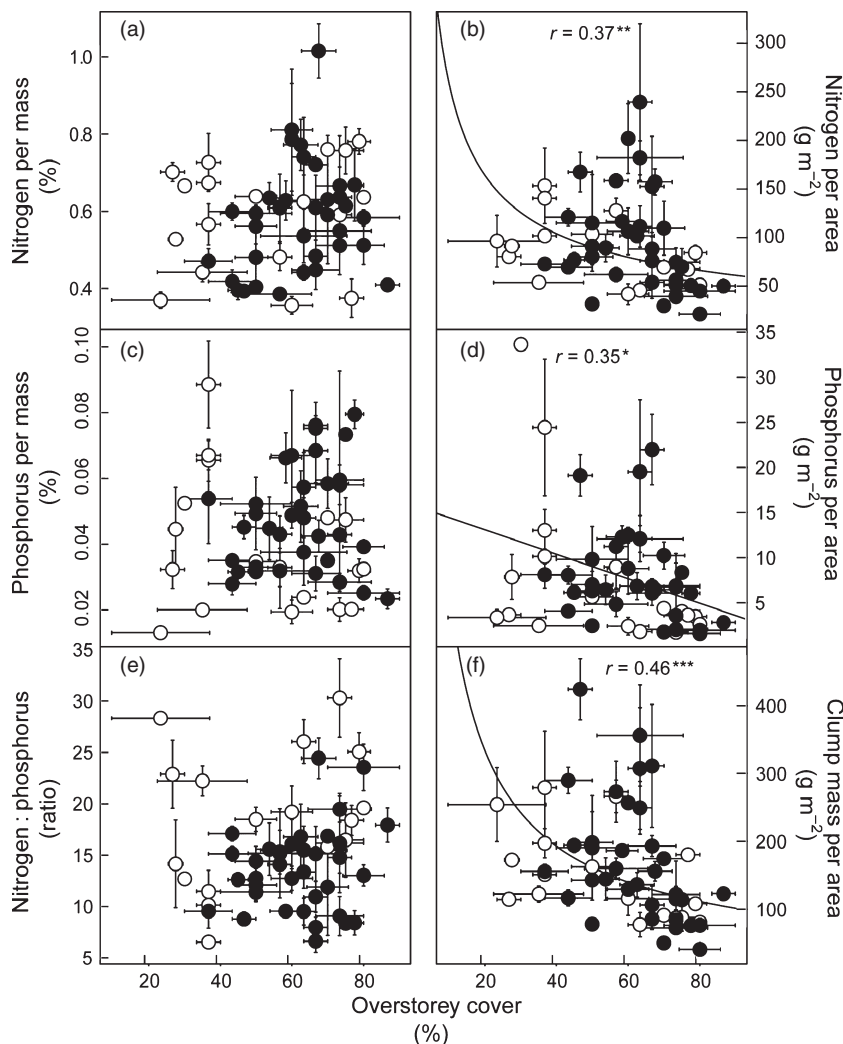
Fig. 3. Relationships of bryophyte nutrient concentrations and stoichiometric ratios with overstorey cover: (a) nitrogen per dry mass (N_{mass}), (b) nitrogen per area (N_{area}), (c) phosphorus per dry mass (P_{mass}), (d) phosphorus per area (P_{area}), (e) nitrogen:phosphorus dry mass ratio (N:P), (f) canopy mass per area (CMA). Symbols as in Figure 2. Lines are ordinary least squares regressions fitted to untransformed data ($y = b + a \times OC$) for D, and to log-transformed data [$\log y = \log b + a \times \log(OC)$] for B and F; B: $a = 3.07$ (95% CI: 2.27–3.87), $b = -0.656$; D: $a = 15.8$ (9.5–22.1), $b = -0.136$; F: $a = 3.55$ (2.80–4.31), $b = -1.22$. Error bars = 1 SE. Significance levels: * $P < 0.05$; **$0.01 \geq P > 0.001$; ***$P \leq 0.001$.

Despite the association of bryophyte tissue nutrient concentration with soil nutrient availability driven by soil age, tissue concentrations were not related to the decline in soil nutrient availability at higher elevations. Indeed the bryophytes on Mauna Loa showed an increase in P_{mass} and decline in N:P at higher elevations, and no elevational trend in N_{mass}. We note that tracheophytes generally show these same trends as found here for bryophytes with increasing elevation and cooler temperatures, i.e. not reflecting the soil nutrient availability along elevational gradients (Korner 2003; Reich & Oleksyn 2004). One notable exception is *M. polymorpha*, the dominant tree on Mauna Loa; for this species P_{mass} does decrease and N:P increases at higher elevation and lower MAT, corresponding to declining soil P availability coupled with a lack of directional trend in soil N availability (Vitousek *et al.* 1992; Martin & Asner 2009). This distinctive coupling of tree and soil nutrient availability for *M. polymorpha* may reflect the very low availability of soil nutrients to trees on lava flows (Cordell *et al.* 2001). By contrast, soil nutrient availability was apparently unimportant for explaining elevational differences in bryophyte nutrient concentration, perhaps due to their lower nutrient requirements and ability to meet their requirements from throughfall and accumulation of microsite organic

matter, and a primarily endogenous control of tissue P concentration potentially relating to cold tolerance (see following section).

DEPENDENCE OF TISSUE NUTRIENT CONCENTRATIONS ON MORPHOLOGY: LEAF STRUCTURE INFLUENCES COMPOSITION

As predicted from the 'Leaf structure influences composition' hypothesis, bryophyte tissue nutrient concentrations were linked with morphology. In tracheophytes, both mass and area-based nutrient concentrations are frequently related to LMA, which mediates their environmental associations. For the bryophytes in this study, CMA, and its determinants CH and CD, correlated positively with N_{area} and P_{area} (although only at $P < 0.1$ for CH with N_{area}) and not with N_{mass} and P_{mass}. The positive correlation of area-based nutrient concentrations with CMA for bryophyte canopies with greater numbers of leaf layers per canopy area (leaf area index) is analogous to the increased nutrient concentration in thicker and denser tracheophyte leaves. However, against expectations, we did not find a decrease of mass-based nutrient concentrations with increasing CMA as would have been found if

a higher CMA involved greater allocation to carbon rich structural components that 'diluted' the tissue nutrient concentration, as often found for tracheophyte leaves of higher LMA and longer leaf lifespan (Wright *et al*. 2004; Niinemets & Sack 2006). Notably, the independence of mass-based nutrient concentrations from CMA may have related to the particular range of bryophyte habitats sampled. While a previous study of 10 *Sphagnum* species with narrow variation in N_{mass} (0.64–0.90%) similarly found N_{mass} to be independent of CMA (Rice, Aclander & Hanson 2008), another study reported a negative correlation of N_{mass} and CMA for ten moss species along an irradiance gradient on Oahu Island, Hawaii (Waite & Sack 2010); that study included deep-shade adapted species of the forest floor possessing very low cell wall mass fraction and thus a wider range in N_{mass} than in this study (species ranged 0.24–1.51% by contrast with 0.36–1.02% in this study). The deep-shade species may not rely on thick cell walls for storing water as they experience a much lower degree of desiccation on the ever-moist forest floor, and thus can achieve higher N_{mass} (Dilks & Proctor 1979).

Also as predicted from the 'Leaf structure influences composition' hypothesis, the bryophytes exhibited higher C:N and C:P than are typical for tracheophytes. The average C:N mass ratio in this study, 76.8, was more than double the temperate and tropical tracheophyte community-level averages of 30.1 and 30.4 (McGroddy, Daufresne & Hedin 2004). The average C:P mass ratio in this study, 1164, was also higher than temperate and tropical tracheophyte community-level values of 357 and 952 (McGroddy, Daufresne & Hedin 2004). The higher C:P and C:N for bryophytes is consistent with greater allocation to cell wall relative to cell contents than in tracheophytes. We had also predicted higher N:P in bryophytes because concentrations of P are typically lower in cell wall than in cell membranes and metabolic components (Niinemets & Sack 2006). However, the N:P mass ratio of 14.8 for the Mauna Loa bryophytes was midway between tracheophyte temperate and tropical community-level values of 12.7 and 19.7 (McGroddy, Daufresne & Hedin 2004), and in the range reported for Hawaiian ferns and angiosperms, 10.8–16.5 (with one extreme value of 6.95; Amatangelo & Vitousek 2008). That the N:P for bryophytes is lower than expected suggests a higher P relative to cell wall material in bryophytes. One putative mechanism is a greater abundance of phospholipids for energy storage (Huang *et al*. 2009).

We found that area-based nutrient concentrations related to irradiance, an effect primarily mediated by CMA, which correlated positively with irradiance. This pattern is analogous to that in tracheophytes; LMA tends to increase for given species acclimating, or in the case of temperate deciduous species, adapting to increased light availability, developing thicker leaves with additional mesophyll layers and greater nutrient concentrations per leaf area (Givnish 1988; Sack, Grubb & Maranon 2003). Notably, considering CMA as analogous to LMA can point to similarities among tracheophytes and bryophytes despite their strong structural differences (Waite & Sack 2010). Bryophyte canopies differ fundamentally from tracheophyte leaves in many aspects, such as lacking stomata to control water loss from the photosynthetic tissues, capturing light with a complex system of displayed leaves on shoots, and retaining liquid water in cell walls and in the spaces among leaves. Thus, parallels between CMA and LMA are limited, though their analogy is beneficial to highlight similarities, such as the correlation with light availability, indicating convergent relationships of structure to ecology across these distinct lineages that evolved leaves independently.

By contrast, we found no relationship of CMA with elevation, MAP or VPD, as would have been expected by analogy with the LMA of tracheophytes, including for *M. polymorpha* at these sites (Cordell *et al*. 1998; Korner 2003; Poorter *et al*. 2009). One possibility is that such trends in LMA for tracheophytes are principally important for extending leaf lifespan under low resource conditions, whereas greater CMA in bryophytes may not typically confer greater longevity. An exception was that CMA was positively related to VPD on old soils, suggesting the possibility of climatic control of CMA under extreme conditions. Notably, CMA was negatively correlated with MAT, but here the control of nutrient concentrations per area by CMA was not strong; N_{area} was independent of MAT, and P_{area}, which was negatively correlated with MAT, remained so even after partialing out the effect of CMA.

We note that C:P and N:P did not show the trends with MAT, MAP, elevation, VPD or OC that would have been expected if cell wall thickness were associated with environmental factors as for tracheophytes, and if cell walls are low in P. In fact, N:P related to MAT in the opposite direction than expected from this hypothesis. As discussed below, a cold tolerance effect may have been the strongest determinant of climatic trends in P_{mass}, C:P and N:P.

BIOCHEMICAL CONSTRAINTS: STOICHIOMETRIC HOMEOSTASIS, AND THE 2/3 POWER LAW SCALING OF N AND P

We found that bryophytes adhered to the general rule that biological organisms require N and P in constrained proportions (Elser *et al*. 2010). We found that N_{mass} correlated positively with P_{mass}, and N_{area} with P_{area}. This finding, previously reported for tracheophytes (Thompson *et al*. 1997; Reich & Oleksyn 2004; Wright *et al*. 2004), indicates homeostasis for tissue-level stoichiometry. This study extended to bryophytes the allometric scaling of N_{mass} to P_{mass}; the scaling exponent 0.61 was consistent with the 2/3 value recently reported as general for angiosperms and gymnosperms (Reich *et al*. 2010).

DEPENDENCE OF PHOSPHORUS CONCENTRATION AND STOICHIOMETRY ON TEMPERATURE: COLD TOLERANCE

We found that bryophyte P_{mass} increased and C:P and N:P declined with lower MAT at higher elevations. The trend in N:P was consistent with the global trend of decreasing N:P with increasing latitude in tracheophytes, hypothesized to arise from a need for additional P in plants at lower temperature to mitigate slower chemical reactions (Reich & Oleksyn 2004;

Kerkhoff *et al.* 2005; Ordonez *et al.* 2009). We note that studies of tracheophytes have not resolved whether N:P was better related to soil P availability or climate because of their intercorrelation (Reich & Oleksyn 2004; Ordonez *et al.* 2009). Thus, the decline of N:P with latitude has also been ascribed to greater P availability in less leached temperate soils (McGroddy, Daufresne & Hedin 2004; Reich & Oleksyn 2004; Lovelock *et al.* 2007; Ordonez *et al.* 2009). That mechanism would not apply to the bryophytes in this study, because soil ages were relatively controlled. Further, the elevational trend in bryophyte N:P and C:P related to climate rather than to soil age; the N:P trend was found on the young soil sites considered individually (and on old soil sites, though non-significantly). Thus, the bryophytes appeared to incorporate more tissue P at higher elevation, despite reduced soil availability, suggesting greater physiological need for P at lower temperature, consistent with a cold tolerance mechanism and/or additional lipid energy storage.

GROWTH RATE UNRELATED TO VARIATION IN STOICHIOMETRY

Our results did not support the 'Growth rate – RNA' hypothesis in its simplest form, which predicts that slower growing plants would have relatively less P-rich RNA for protein synthesis and thus greater N:P (Elser *et al.* 2000). We found bryophyte N:P ratio to be similar to that for tracheophytes, despite the slower growth rate of bryophytes. Further, if bryophytes grow faster at higher moisture availability (Zotz & Rottenberger 2001; Wiklund & Rydin 2004), the increase of N:P with MAP and the lack of a trend with VPD did not support the growth rate hypothesis. A recent study of pine species found no increase in P_{mass} with increased growth rate, and suggested that P storage may mask any need for greater P supply (Matzek & Vitousek 2009). That may be also true for bryophytes, as P may be important in energy storage (Huang *et al.* 2009).

CONCLUSIONS AND FUTURE WORK

Bryophytes showed several strong similarities to tracheophytes in the relationships of composition and structure to climate. We found similar relationships of tissue N and P with soil availability, and of area-based nutrient concentrations with irradiance mediated by structure (with CMA partially analogous to tracheophyte LMA), and trends in P and N:P with elevation and temperature. Further, as for LMA in tracheophytes, bryophytes under higher irradiances had higher CMA. However, unlike for tracheophyte LMA, N_{mass} and P_{mass} were not related to bryophyte CMA, and these were independent of elevation.

We found that the bryophytes arrayed across striking local gradients in climate and elevation reflected trends in nutrient composition found globally for tracheophytes. Despite deep phylogenetic distance and fundamental morphological differences between bryophytes and tracheophytes, the bryophytes of this study supported the ubiquity of associations of nutrient concentrations and stoichiometry with environment among organisms. Future work should determine the extent to which these relationships arise due to evolution or to ecological sorting and assembly of traits across the matrix. Further, while this study focused on common bryophytes across a highly variable landscape of ecosystems, encompassing substantial morphological diversity, the interpretation is necessarily qualified by our narrow sampling of bryophyte diversity globally. This study included six families, with seven of the 12 sampled species within the family Dicranaceae. Future research is needed to extend the findings for stoichiometric trends to a broader range of bryophytes across resource gradients. Future work can also indicate the potential for predicting landscape nutrient concentrations across plant lineages and life forms based on general trends in nutrient concentrations and stoichiometry (Elser *et al.* 2010). This research holds exceptional promise. When nutrient concentrations and stoichiometry can be related to growth rates and carbon fluxes, this work may allow prediction, based on tissue nutrient concentrations, of bryophyte growth dynamics across environmental matrices, clarifying a major component of these ecosystems.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Hypotheses and principles tested from global stoichiometric theory developed principally for tracheophytes, recent citations, variables affected and specific predictions for tissue of bryophytes along the Mauna Loa matrix of soil ages and elevations.

Table S2a. Results of analyses of variance for canopy structure and composition traits for bryophytes across the Mauna Loa matrix, Island of Hawaii.

Table S2b. Results of analyses of variance for canopy structure and composition traits for bryophytes across the Mauna Loa matrix, Island of Hawaii, for samples from the three elevations for which there were both young and old lava flows.

Table S3. Slopes of relationships between variables significantly correlated for all data, or on young and/or old soils and/or within two or more taxa considered individually.

Table S4. Partial correlation analysis using all data.

Table S5. Partial correlation analysis using young soil and old soil data separately.

Table S6. Partial correlation analysis using individual taxon data.

Figure S1. Topographic map of the Hawaii Island with 300 m elevational contour lines and sampling locations with elevations.

Figure S2. Relationships of bryophyte nutrient concentrations and stoichiometric ratios with mean annual precipitation.

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