

# Shifts in bryophyte carbon isotope ratio across an elevation × soil age matrix on Mauna Loa, Hawaii: do bryophytes behave like vascular plants?

Mashuri Waite · Lawren Sack

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**Abstract** The carbon isotope ratio ( $\delta^{13}\text{C}$ ) of vascular plant leaf tissue is determined by isotope discrimination, primarily mediated by stomatal and mesophyll diffusion resistances and by photosynthetic rate. These effects lead to predictable trends in leaf  $\delta^{13}\text{C}$  across natural gradients of elevation, irradiance and nutrient supply. Less is known about shifts in  $\delta^{13}\text{C}$  for bryophytes at landscape scale, as bryophytes lack stomata in the dominant gametophyte phase, and thus lack active control over  $\text{CO}_2$  diffusion. Twelve bryophyte species were sampled across a matrix of elevation and soil ages on Mauna Loa, Hawaii Island. We tested hypotheses based on previous findings for vascular plants, which tend to have less negative  $\delta^{13}\text{C}$  at higher elevations or irradiances, and for leaves with higher leaf mass per area (LMA). Across the matrix, bryophytes spanned the range of  $\delta^{13}\text{C}$  values typical of  $\text{C}_3$  vascular plants. Bryophytes were remarkably similar to vascular plants in exhibiting less negative  $\delta^{13}\text{C}$  with increasing elevation, and with lower overstory cover; additionally  $\delta^{13}\text{C}$  was related to bryophyte canopy projected mass per area, a trait analogous to LMA in vascular plants, also correlated negatively with

overstory cover. The similarity of responses of  $\delta^{13}\text{C}$  in bryophytes and vascular plants to environmental factors, despite differing morphologies and diffusion pathways, points to a strong direct role of photosynthetic rate in determining  $\delta^{13}\text{C}$  variation at the landscape scale.

**Keywords** Altitudinal gradient · Elevation · Hawaii · Liverworts · Moss

## Introduction

Foliar carbon isotope ratio ( $\delta^{13}\text{C}$ ) can reflect ecology and physiology, especially because in vascular plants  $\delta^{13}\text{C}$  reflects changes in function along resource gradients. Less is known about bryophyte  $\delta^{13}\text{C}$  responses. We aimed to quantify the degree to which bryophytes resemble vascular plants (tracheophytes) in their landscape scale  $\delta^{13}\text{C}$  using the bryophyte flora distributed across Mauna Loa, Hawaii, a “natural experiment” for ecological research (Vitousek 2004).

Differences in the relative abundance of  $^{13}\text{C}$  and  $^{12}\text{C}$  in plant tissues relative to the atmosphere is caused by discrimination against the heavier  $^{13}\text{C}$  during photosynthesis by the carbon fixing enzyme RubisCO and during diffusion through air and liquid water (Farquhar et al. 1989). The  $\delta^{13}\text{C}$  is negatively related to discrimination;  $\delta^{13}\text{C}$  increases as the chloroplast  $\text{CO}_2$  concentration ( $c_c$ ) declines and carboxylation discriminates less against  $^{13}\text{C}$  (Farquhar and Sharkey 1982). The  $c_c$  can be affected by several factors which influence  $\text{CO}_2$  drawdown by photosynthesis relative to its rate of diffusion to the chloroplast. A high photosynthetic assimilation rate ( $A$ ) draws down  $c_c$  relative to the  $\text{CO}_2$  concentration of source air ( $c_a$ ) (Farquhar et al. 1989). Vascular plants control  $\text{CO}_2$  diffusion into the leaf with their stomata, which respond to numerous factors,

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especially opening with irradiance and high leaf water status, and closing under opposite conditions. A lower stomatal conductance ( $g_s$ ) or a lower mesophyll conductance should reduce  $c_c/c_a$  and lead to higher  $\delta^{13}\text{C}$  (Loreto et al. 1992; DeLucia et al. 2003; Seibt et al. 2008; Niinemets et al. 2009). Bryophytes differ from vascular plants fundamentally in lacking stomata in the dominant life stage (Schofield 1985), but span a wide range of  $\delta^{13}\text{C}$  from  $-21$  to  $-40$  ‰ (but primarily  $-22$  to  $-32$  ‰; Rundel et al. 1979; Proctor et al. 1992; Fletcher et al. 2004), very similar to  $\text{C}_3$  tracheophytes, which range from  $-24$  to  $-32$  ‰ (O’Leary 1988).

In vascular plants,  $\delta^{13}\text{C}$  tends to correlate with resource gradients for given species and across species sets. Typically,  $\delta^{13}\text{C}$  increases with elevation, associated with higher leaf mass per area (LMA) at lower temperatures (see below; Körner et al. 1988, 1991, 2003; Vitousek et al. 1990; Cordell et al. 1999). The  $\delta^{13}\text{C}$  also tends to correlate negatively with water availability, due to stomatal closure at low water availability (Farquhar et al. 1989). Further, vascular plant canopies exhibit increasing foliar  $\delta^{13}\text{C}$  with height (Medina and Minchin 1980; Garten and Taylor 1992; Ometto et al. 2006) due to effects linked with three resource gradients. According to the “water stress- $g_s$  effect”, higher irradiance with increasing canopy height (CH) may be associated with lower leaf water status, and lower  $g_s$  and  $c_c$  (Niinemets et al. 2004). According to the “irradiance-A effect”, high irradiance may drive higher  $A$  relative to  $g_s$ , decreasing  $c_c$  (Brooks et al. 1997; Le Roux et al. 2001; Duursma and Marshall 2006). Finally, according to the “source air effect”,  $\delta^{13}\text{C}$  may vary across natural irradiances due to a vertical gradient in air  $\delta^{13}\text{C}$  caused by soil respiration (Francey et al. 1985; Schleser and Jayasekera 1985; Ehleringer et al. 1986; Farquhar et al. 1989; Medina et al. 1991; Buchmann et al. 1997b; Sternberg et al. 1989, 1997; Schedlbauer and Kavanagh 2008); this effect is dampened by air mixing and typically accounts for only ca. 10–30% of the difference in foliar  $\delta^{13}\text{C}$  from understory to top-of-canopy (Broadmeadow et al. 1992; Brooks et al. 1997; Buchmann et al. 1997a, 2002; Sternberg et al. 1997).

Bryophytes may follow similar trends in  $\delta^{13}\text{C}$  with gradients as tracheophytes. The  $\delta^{13}\text{C}$  may correlate positively with elevation, as reported for *Sphagnum* (Menot-Combes et al. 2004; Skrzypek et al. 2007), potentially arising because of a “temperature effect” if lower temperature at high elevation reduces respiration relative to  $A$ , leading to reduced loss of the heavier isotope (cf. Gillon and Griffiths 1997; Barbour and Hanson 2009), thus reducing discrimination (Hamerlynck et al. 2002; Skrzypek et al. 2007), and/or because of lower atmospheric pressure leading to lower  $c_c$  (Menot and Burns 2001). A positive correlation of bryophyte  $\delta^{13}\text{C}$  with water availability has

also been shown due to “liquid film effect” that reduces  $\text{CO}_2$  diffusion (Proctor et al. 1992; Rice and Giles 1996; Williams and Flanagan 1996; Meyer et al. 2008). Bryophytes may also show increased  $\delta^{13}\text{C}$  with higher irradiance as found for the moss *Tortula ruralis* (Hamerlynck et al. 2002) and for three liverworts (Fletcher et al. 2006). This pattern could arise from the “irradiance-A effect” and/or from the “source air effect”, if forest floor bryophytes experience elevated  $\text{CO}_2$  originating from soil respiration (DeLucia et al. 2003).

The  $\delta^{13}\text{C}$  may also relate to leaf structure and composition. Across species of tracheophytes,  $\delta^{13}\text{C}$  correlates positively with LMA and nitrogen concentration per area ( $N_{\text{area}}$ ), themselves related (Hultine and Marshall 2000; Takahashi and Miyajima 2008; Li et al. 2009). A similar pattern was found for the Hawaiian tree *Metrosideros polymorpha* across elevations:  $\delta^{13}\text{C}$  positively correlated with LMA, apparently due to higher carboxylation rate related to the higher  $N_{\text{area}}$  (Vitousek et al. 1990; Cordell et al. 1998, 1999). In bryophytes, canopy mass per ground area (CMA) is analogous to tracheophyte LMA (Waite and Sack 2010) and may be related to higher  $N$  per ground area, and hence bryophyte  $\delta^{13}\text{C}$  might be expected to correlate with CMA.

Previous work has highlighted trends for a few bryophyte species along specific gradients. In a previous study, we found strong variation in bryophyte N and P stoichiometry on Mauna Loa (Waite and Sack 2011). Here, we conducted a landscape-scale analysis of variation in  $\delta^{13}\text{C}$  within and across species, and its correlation with climate (temperature and precipitation), forest and soil development, and overstory cover (OC). We also determined the correlation of  $\delta^{13}\text{C}$  with other functional traits and nutrient composition. Based on patterns within and across tracheophyte species, we hypothesized bryophyte  $\delta^{13}\text{C}$  would increase at (1) higher elevation, (2) higher irradiance, (3) lower precipitation and (4) higher CMA.

## Materials and methods

### Study site, species and sampling

Sampling took place across an elevational gradient on the windward slope of Mauna Loa, Hawaii Island, a model system for studies of ecosystem development (Vitousek et al. 1995; Raich et al. 1997). Mean annual temperature (MAT) declines with elevation on this slope at a lapse rate of  $6.4^\circ\text{C}/\text{km}$  (Juvik and Nullet 1994). Bryophytes were sampled at six elevations (287–2,239 m) on young lava flows (126–152 years old) and old lava flows ( $\geq 3,400$  years old; Lockwood et al. 1988) at ten sites (Table 1; Fig. 1). Bryophytes occupied seven primary

substrates (bark, humus, leaf litter, rock, soil, rotted wood, and tree fern trunk), and accounted for 10–80% of surface cover.

Eleven moss and one liverwort species were collected at ten sites (Table 1). Species were chosen that were sufficiently common to allow collection of replicates at given sites. The species varied in elevational range; the extreme cases were *Campylopus incarvatus*, found only at the 700 m old flow site, and *Racomitrium lanuginosum*, which, like the dominant tree species *M. polymorpha*, occurred at all sampled elevations. We collected samples of 40–150 cm<sup>2</sup> in projected area from three colonies per species, where possible, at each site (in 6 of 53 cases only a single colony was found for a given species). The projected area of each sample was traced on clear plastic and scanned (300 dots per inch; Epson 3170 Photo Scanner; Seiko Epson, Nagano, Japan) for area determination (using ImageJ 1.42q software; US National Institutes of Health, Bethesda, MD, USA). The substrate and the estimated height above ground for epiphytic colonies were recorded to the nearest 10 cm; non-epiphytic colonies were collected from rocks, fallen logs, and the ground surface. Samples were brought to the laboratory in plastic bags (Ziploc; SC Johnson, Racine, WI, USA) on ice and refrigerated until processing.

#### Climate data and overstory cover

We estimated the climate at each sampling site using a Geographic Information System model based on climate station data (Cao et al. 2007) and Geographic Positioning System coordinates (GPSmap 60CSx; Garmin, Olathe, KS, USA). We estimated mean annual temperature (MAT), precipitation (MAP), relative humidity (MARH) and vapor pressure deficit (VPD), calculated from MAT and MARH (Campbell and Norman 1998). VPD is a measure of atmospheric drought, i.e., the driving force for evaporation, and can be calculated as an absolute pressure difference (in kPa of vapor) or after normalizing by atmospheric pressure (mole fraction; i.e., unitless); the mole fraction VPD accounts for the effects of atmospheric pressure and temperature on diffusibility and thus more clearly indicates differences in driving force across elevations. Across sites the mole fraction VPD correlated strongly with absolute VPD ( $r = 0.92$ ;  $P < 0.001$ ), and the two estimates correlated similarly with other measured traits; only relationships with absolute VPD are presented. Forest overstory cover (OC, %), the proportion of the sky obscured from view (equivalent to canopy closure in Jennings et al. 1999), was visually assessed, to the nearest 10%. Visual canopy cover estimates involve a level of uncertainty, but correlate with measurements using a densiometer or hemispherical photography (Schott and Pieper 1985; Vora 1988; Bellow

and Nair 2003; Korhonen et al. 2006), especially given training as was undertaken previously to this study, to achieve a correspondence of  $\pm 5\%$  relative to hemispherical photos (Teti and Pike 2005; Korhonen et al. 2006; Paletto and Tosi 2009).

#### Measurements of structure, nutrient concentrations and $\delta^{13}\text{C}$

CH was determined with a ruler to the nearest mm of the green portion of the canopy at three random positions in each sample. The upper, green portions of the stems were cut from the older brown tissue using a razor blade, dried at 80°C for at least 48 h before determination of dry mass (AB204S/FACT Analytical Balance; Mettler Toledo, Columbus, OH, USA). Canopy mass per area (CMA) was determined by dividing dry mass by ground area. Dried samples were analyzed for  $\delta^{13}\text{C}$ , nitrogen per mass ( $N_{\text{mass}}$ ) and carbon per mass using high temperature combustion in an elemental analyzer (Costech ECS 4010; Valencia, CA, USA), with effluent passed into a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Delta V Advantage with a ConFlo III interface; ThermoFisher Scientific, Waltham, MA, USA; Fry et al. 1996). Samples were dry ashed in glass vials (Miller 1998), dissolved in 1 N HCL and analyzed for phosphorus per mass ( $P_{\text{mass}}$ ) using inductively coupled plasma-optical emission spectroscopy (Varian Vista MPX Instrument; Varian, Palo Alto, CA, USA; Porder et al. 2005). Concentrations of nitrogen and phosphorus per ground area ( $N_{\text{area}}$  and  $P_{\text{area}}$ ) were determined as, respectively,  $N_{\text{mass}}$  and  $P_{\text{mass}}$  multiplied by CMA. N:P was determined by dividing  $N_{\text{mass}}$  by  $P_{\text{mass}}$ ; C:N and C:P by dividing carbon per mass by, respectively,  $N_{\text{mass}}$  and  $P_{\text{mass}}$ .

#### Statistical analyses

Statistical procedures were applied using R 2.6.1 (R Development Core Team 2007). We tested for differences in  $\delta^{13}\text{C}$  across elevations, soil ages and species using three-way analysis of variance (ANOVA) and across substrate types using one-way ANOVA after log-transformation to improve normality and homoscedasticity (Zar 1999). Multiple pairwise comparisons of  $\delta^{13}\text{C}$  among substrate types was calculated with the Tukey honestly significant difference test (HSD; Zar 1999). Subsequent correlation analyses used species mean values for each site. Pearson correlations were determined for linear and power law relationships (i.e., linear relationships after log-transformation) for  $\delta^{13}\text{C}$  against environmental variables and bryophyte composition and structure variables. Relationships were tested for species mean values across all sites, and separately across only young and old soil ages

**Table 1** Bryophyte taxa and substrates sampled from young and/or old lava flows at seven elevations on Mauna Loa, Island of Hawaii, and the minimum, mean, and maximum values for average carbon isotope composition ( $\delta^{13}\text{C}$ ) across individuals at the sampled sites, and modeled climates for each site

Taxa sampled	Family	Substrate, average height above ground (m)	Elevations (m) and soil ages sampled							$\delta^{13}\text{C}$ (‰)
			300	700	1,100	1,650	1,750	1,760	2,200	
<i>Acroporium fusco-flavum</i>	Sematophyllaceae	Leaf litter–bark, 0.4	Y	Y, O	Y, O	O	O			–29.0, –30.4, –32.2
<i>Bazzania cf. trilobata</i>	Lepidoziaceae	Bark–humus, 0.4		Y, O	Y, O	O	O	O		–27.1, –29.5, –31.0
<i>Campylopus exasperatus</i>	Dicranaceae	Rock, 0.4		Y						–25.9
<i>Campylopus hawaiiicus</i>	Dicranaceae	Humus–bark, 0.6			O	O	O	O		–27.6, –29.1, –30.4
<i>Campylopus incurvatus</i>	Dicranaceae	Bark, 0.4		O						–31.4
<i>Campylopus schmidii</i>	Dicranaceae	Rock, 0.4					O			–27.5
<i>Dicranum speirophyllum</i>	Dicranaceae	Leaf litter–humus, 0.4			Y	Y, O	O	O	O	–28.7, –29.3, –29.9
<i>Leucobryum gracile</i>	Dicranaceae	Bark–humus, 0.6	Y	O	O	O		O		–30.2, –31.2, –32.4
<i>Leucobryum seemanii</i>	Dicranaceae	Humus, 0.4	Y	O						–32.5, –32.5, –32.6
<i>Macromitrium microstomum</i>	Orthotrichaceae	Bark, 1.8	Y	Y, O	Y, O	Y, O	O	O		–28.2, –29.4, –30.7
<i>Pyrrhobryum spiniforme</i>	Rhizogoniaceae	Bark–humus, 0.4		O	O	O		O		–28.2, –28.7, –29.5
<i>Racomitrium lanuginosum</i>	Grimmiaceae	Rock, 0.4	Y	Y	Y	Y	O	O		–24.7, –26.6, –28.9
Modeled climate										
Mean annual temperature (°C)			21.0	18.1	15.4	12.8	12.3	12.2	9.7	
Mean annual precipitation (mm)			4,514	5,840	4,320	2,840	2,420	2,360	1,510	
Mean annual vapor pressure deficit (kPa)			0.573	0.369	0.318	0.352	0.365	0.366	0.430	

Species nomenclature follows Staples et al. (2004); family nomenclature follows Tropicos, Missouri Botanical Garden [<http://www.tropicos.org/>]. Lava age is designated as ‘young’ (Y) for 1855–1881 flows and ‘old’ (O) for the 3,400-year-old lava flow (and the ca. 400-year-old lava flow at the 1,750 m site). *R. lanuginosum* was present but not sampled at the 2,200 m site because of difficulties distinguishing live from dead material

independently, and for each of the six taxa that were sampled at six or more sites (five species: *A. fuscoflacum*, *Bazzania cf. trilobata*, *D. speirophyllum*, *M. microstomum*, *R. lanuginosum*; and one genus, *Campylopus*). The  $\delta^{13}\text{C}$  relationship with OC was tested across substrate types using raw data rather than species means because a single species could be found on several substrates within a single site.

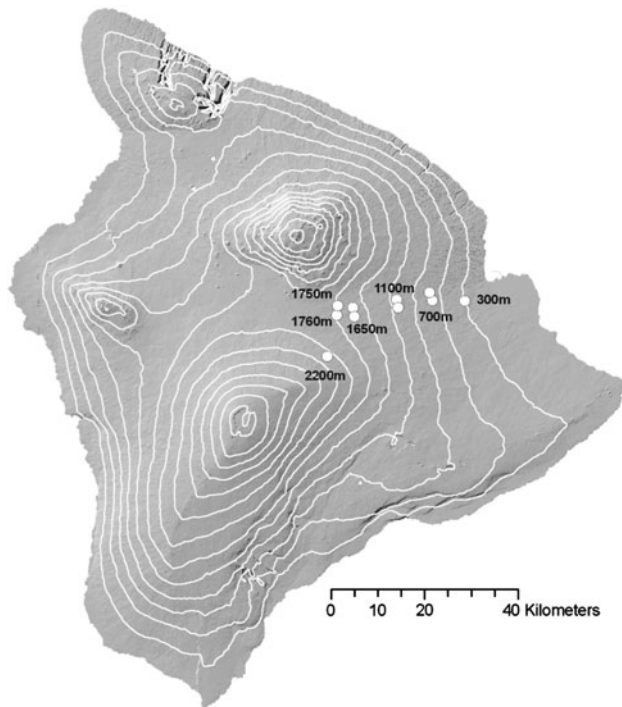
For relationships that were significant for all species across all sites and also for individual soil ages and/or for more than one individual taxon, we tested for shifts in the relationship. We tested for similarity of the slope and intercept between the soil ages or among taxa, using untransformed or log-transformed data according to which fitted best the relationship for all species across all sites (using SMATR; <http://www.bio.mq.edu.au/ecology/SMATR/>; Warton et al. 2006). Ordinary least squares regression was used for testing relationships between  $\delta^{13}\text{C}$  and climate variables or OC, and standard major axes were used in testing relationships

between  $\delta^{13}\text{C}$  and structure and composition traits, as appropriate given co-dependence of variables (Sokal and Rohlf 1995).

When bryophyte traits were correlated with climate variables that were themselves inter-correlated, we calculated partial correlations to resolve underlying relationships (using the *corpcor* package in R; Schaefer et al. 2007).

## Results

Species varied strongly in  $\delta^{13}\text{C}$ ; average values for species collected from at least four sites ranged from  $-26.3\text{‰} \pm 0.4$  for *R. lanuginosum* to  $-31.2\text{‰} \pm 0.4$  for *L. gracile*. Additionally, individual taxa varied strongly in  $\delta^{13}\text{C}$  across sites; for *R. lanuginosum*,  $\delta^{13}\text{C}$  differed by 4.2‰ from 300 to 1,650 m elevation on young soil, and within the genus *Campylopus*,  $\delta^{13}\text{C}$  differed by 5.5‰ from *C. exasperatus* at 700 m to *C. hawaiiicus* at 1,100 m (Table 1). Across all



**Fig. 1** Topographic map of Hawaii Island with 300-m elevational contour lines and sampling locations with elevations

sampled colonies, the  $\delta^{13}\text{C}$  showed significant differences across taxa, elevations and soil ages (three-way ANOVA;  $P < 0.001$  for each factor), with less negative values at higher elevation and on younger soil (Appendix Table A); on average the bryophytes on young and old soil had  $\delta^{13}\text{C}$  of  $-28.7\% \pm 0.5$  and  $-29.7\% \pm 0.2$ , respectively. Notably, all the climate variables correlated with elevation (for MAT,  $r = -0.999$ ; for MAP,  $r = -0.89$ ; for VPD,  $r = -0.68$ ;  $P < 0.001$ ), and MAT correlated with MAP ( $r = 0.92$ ,  $P < 0.001$ ) and VPD ( $r = 0.53$ ,  $P < 0.001$ ), though MAP was independent of VPD ( $r = 0.11$ ,  $P = 0.45$ ; Table 2). Across all the species and sites,  $\delta^{13}\text{C}$  correlated positively with elevation and negatively with MAT ( $|r| = 0.31$ – $0.32$ ,  $P < 0.05$ ; Fig. 2a) but not significantly with MAP or VPD ( $r = -0.23$  to  $-0.25$ ;  $P = 0.07$ – $0.10$ ; Fig. 2b). Across all sampled colonies, the  $\delta^{13}\text{C}$  showed significant differences across substrate type (one-way ANOVA;  $P < 0.001$ ) with colonies on the rock substrate showing  $\delta^{13}\text{C}$  values significantly higher than those on bark, humus and tree fern trunk (Tukey HSD;  $P < 0.001$ ) but no significant differences among other substrates (Tukey HSD;  $P = 0.08$ – $0.99$ ; Appendix Table B).

Across sites,  $\delta^{13}\text{C}$  correlated negatively with OC ( $r = -0.63$ ,  $P < 0.001$ ; Fig. 3a). In turn, OC was independent of elevation, MAT, MAP, and VPD ( $r = 0.09$ – $0.22$ ,  $P = 0.12$ – $0.52$ ). Part of the effect may have related to soil age, as OC differed between young and old soil sites from 52 to 62% on average (three-way ANOVA; elevation,

$P = 0.008$ ; substrate age,  $P < 0.001$ ; taxon,  $P < 0.001$ ), and, as described above,  $\delta^{13}\text{C}$  was higher on younger soil. However, the linkage of  $\delta^{13}\text{C}$  with OC also held on soils of given age (see below). The linkage of  $\delta^{13}\text{C}$  with OC also held on most individual substrates (bark, leaf litter, and rock) for which there were more than four samples ( $r = 0.45$ – $0.68$ ,  $P < 0.001$ ; Appendix Table B); the exception was on humus ( $r = 0.29$ ,  $P = 0.14$ ). The  $\delta^{13}\text{C}$  did not correlate with sampling height ( $r = -0.08$ ,  $P = 0.58$ ; a typical value of 0.4 m was used for non-epiphytic species on ground, rocks or logs).

Across all species and sites,  $\delta^{13}\text{C}$  correlated with CMA and  $N_{\text{area}}$  ( $r = 0.32$ – $0.37$ ,  $P = 0.01$ – $0.02$ ; Fig. 3b; Table 2). However,  $\delta^{13}\text{C}$  was independent of bryophyte CH, canopy density, and other nutrient traits ( $N_{\text{mass}}$ ,  $P_{\text{area}}$ ,  $P_{\text{mass}}$ , N:P, C:N, and C:P;  $|r| = 0.03$ – $0.26$ ,  $P = 0.06$ – $0.81$ ).

The relationships of  $\delta^{13}\text{C}$  with elevation, climate, morphology and composition that held across all sites were similar when calculated only for sites on young or old substrates. Thus, on both young and old soil ages considered independently,  $\delta^{13}\text{C}$  correlated with elevation ( $r = -0.53$  to  $-0.54$ ,  $P < 0.001$ – $0.02$ ), MAT ( $r = 0.55$ – $0.54$ ,  $P < 0.001$ – $0.02$ ), and OC ( $r = 0.42$ – $0.75$ ,  $P < 0.001$ – $0.01$ ), but not with bryophyte CH, density, or nutrient composition ( $N_{\text{area}}$ ,  $N_{\text{mass}}$ ,  $P_{\text{area}}$ ,  $P_{\text{mass}}$ , N:P, C:P, CH, and CD ( $|r| < 0.01$ – $0.32$ ,  $P = 0.06$ – $0.81$ ). The slopes of  $\delta^{13}\text{C}$  against elevation and MAT were similar for both soil ages ( $P = 0.65$ – $0.69$ ) with higher intercepts for the young soil ( $P < 0.001$ ). For  $\delta^{13}\text{C}$  against OC, the two soil ages showed the same relationship (i.e., similar slopes and intercepts;  $P = 0.28$ – $0.36$ ). However, in contrast with the data for all species and sites, on the young but not the old soil sites,  $\delta^{13}\text{C}$  correlated negatively with VPD and CMA ( $r = -0.50$  to  $-0.55$ ,  $P = 0.02$ – $0.03$ ), and on old but not on young soil,  $\delta^{13}\text{C}$  correlated positively with C:N and negatively with MAP ( $|r| = 0.35$ – $0.55$ ,  $P < 0.001$ – $0.04$ ).

When the taxa that occurred at six or more sites were analyzed separately, due apparently to the much lower variation in  $\delta^{13}\text{C}$  (Table 1), the only significant correlation of  $\delta^{13}\text{C}$  and an environmental or compositional trait was with OC for *Campylopus* and *R. lanuginosum* ( $r = -0.84$ ,  $P < 0.05$ ); the two taxa had similar slopes ( $P = 0.25$ ) with the higher intercept for *R. lanuginosum* ( $P < 0.001$ ). All other species showed non-significant empirical trends for this relationship ( $r = -0.43$  to  $-0.73$ ;  $P > 0.05$ ).

Partial correlation analyses were used to resolve the relationships of  $\delta^{13}\text{C}$  with OC, MAT, and MAP, keeping other variables constant. The correlations of  $\delta^{13}\text{C}$  with OC and MAT could be resolved as independent effects; the correlation with OC remained significant after partialing out the effects of MAT and MAP ( $r_{\text{partial}} = -0.61$ ;  $P < 0.001$ ; Table 3), and the correlation with MAT remained significant after partialing out the effect of OC

**Table 2** Correlations of  $\delta^{13}\text{C}$  with other measured traits from all sites, from young sites, old sites, and for given taxa

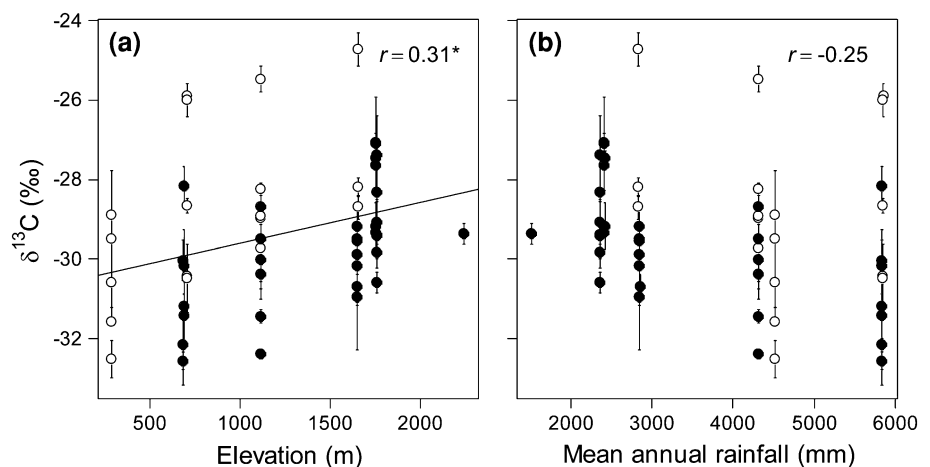
	All species/all sites ( $n = 53$ )	All species/young soil ( $n = 17$ )	All species/old soil ( $n = 33$ )	Within-species	
				CAMP ( $n = 6$ )	RALA ( $n = 6$ )
Sampling height above ground	-0.08 ( $n = 51$ )	-0.03	-0.11 ( $n = 31$ )		
Climate variables					
Elevation	<b>0.31*</b>	<b>0.56*</b>	<b>0.54***</b>	0.07	0.40
Mean annual temperature	<b>-0.32*</b>	<b>-0.55*</b>	<b>-0.54***</b>	-0.08	-0.44
Mean annual precipitation	-0.25	-0.14	<b>-0.55***</b>	-0.04	0.01
Vapor pressure deficit (absolute)	-0.23	<b>-0.55*</b>	0.17	0.21	-0.81
Overstory cover	<b>-0.63***</b>	<b>-0.75***</b>	<b>-0.42*</b>	<b>-0.89*</b>	<b>-0.84*</b>
Canopy morphology and composition					
Canopy height	0.15 ( $n = 49$ )	-0.09 ( $n = 13$ )	0.28	-0.82 ( $n = 5$ )	-0.52 ( $n = 4$ )
Canopy mass per area	<b>0.37**</b>	<b>0.50*</b>	0.27	0.52	0.01
Canopy density	0.13 ( $n = 49$ )	0.32 ( $n = 13$ )	0.00	0.65 ( $n = 5$ )	0.27 ( $n = 4$ )
Nitrogen per mass	-0.20	-0.23	-0.22	0.09	-0.39
Nitrogen per area	<b>0.32*</b>	0.40	0.21	0.61	-0.13
Phosphorus per mass	-0.03	0.16	-0.09	0.75	-0.02
Phosphorus per area	0.27	0.38	0.17	0.67	0.03
Nitrogen:phosphorus ratio	-0.05	-0.19	-0.17	-0.47	0.11
Carbon:nitrogen ratio	0.26	0.19	<b>0.35*</b>	-0.19	0.34
Carbon:phosphorus ratio	0.14	0.06	0.05	-0.77	0.27

Pearson correlation coefficients, with italicized values derived from log-transformed data, providing better fit and stronger significance. Replication is provided in parentheses in the first row, with exceptions denoted in the table

CAMP *Campylopus* spp., RALA *Racomitrium lanuginosum*

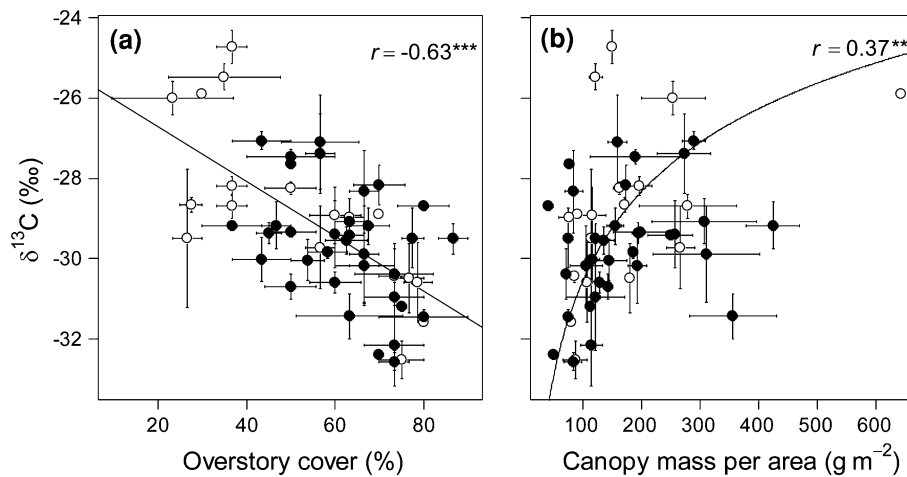
Significant correlations in bold: \*  $P < 0.05$ ; \*\*  $0.01 \geq P > 0.001$ ; \*\*\*  $P \leq 0.001$ . No within-species correlations were found for *Acroporium fuscoflavum* ( $n = 7$ ) *Bazzania* cf. *trilobata* ( $n = 7$ ) *Dicranum speirophyllum* ( $n = 6$ ) or *Macromitrium microstomum* ( $n = 9$ )

**Fig. 2** Relationship between bryophyte  $\delta^{13}\text{C}$  and **a** elevation and **b** mean annual precipitation (MAP) on Mauna Loa, Hawaii. Open and closed symbols represent colonies on young and old soil, respectively. Linear regression fitted to data in (a)  $\delta^{13}\text{C} = -30.6 + 0.00104 \times \text{elevation}$ . \* $P < 0.05$



when log-transformed data were analyzed ( $r_{\text{partial}} = -0.28$ ;  $P < 0.05$ ; Table 3). Notably, the relationship of  $\delta^{13}\text{C}$  with MAT lost significance after partialing out MAP, as did that with MAP after partialing out MAT ( $r_{\text{partial}} = -0.15$  to  $0.03$ ;  $P = 0.29$ – $0.83$ ; Table 3); thus, elevation, MAT and MAP could not be independently resolved as drivers of  $\delta^{13}\text{C}$ , given their inter-correlation across the Mauna Loa matrix.

Partial correlation analyses were also used to resolve the relationships of  $\delta^{13}\text{C}$  with OC, CMA, and  $N_{\text{area}}$ , because  $N_{\text{area}}$  and CMA were correlated ( $r = 0.91$ ;  $P < 0.001$ ), and both were negatively correlated with OC ( $r = -0.46$  to  $-0.37$ ;  $P < 0.001$ – $0.006$ ). The relationship of  $\delta^{13}\text{C}$  with OC remained significant after partialing out CMA and  $N_{\text{area}}$  ( $r_{\text{partial}} = -0.57$ ;  $P < 0.001$ ; Table 3). However, the relationship of  $\delta^{13}\text{C}$  with CMA or  $N_{\text{area}}$  lost significance after



**Fig. 3** Relationship between bryophyte  $\delta^{13}\text{C}$  and **a** elevation overstory cover (OC), and **b** bryophyte canopy mass per area (CMA). Open and closed symbols represent colonies on young and soil, respectively. Linear regression fitted to data in **(a)**  $\delta^{13}\text{C} = -25.3 - 0.068 \times \text{OC}$ . Standard major axis power law fitted to data in **(b)**  $\log(-1 \times \delta^{13}\text{C}) = 1.70 - 0.110 \times \log \text{CMA}$ . The correlation in

**(b)** remained significant when the outlier at the right was removed ( $r = 0.30, P = 0.03$ ).  $**0.01 \geq P > 0.001$ ;  $***P \leq 0.001$ . Notably, the relationship between  $\delta^{13}\text{C}$  and CMA appeared due to both variables being associated with OC; see partial correlation analysis in Table 3, and text

**Table 3** Partial correlation analyses of  $\delta^{13}\text{C}$  and environmental variables

	OC	Elevation	MAT	MAP	CMA	$N_{\text{area}}$
1	<b>-0.63***</b>	<b>0.31*</b>				
2	<b>-0.62***</b>		<b>-0.28*</b>			
3	<b>-0.61***</b>		-0.15	0.03		
4			-0.21	0.06		
5	<b>-0.57***</b>				0.03	0.03
6	<b>-0.57***</b>				0.12	
7	<b>-0.59***</b>					0.12

Each row represents a separate partial correlation analysis of  $\delta^{13}\text{C}$  with the factors in the columns, partialling out the effect of all the other factor(s) in that row. For example, the first value in row (1) presents the partial correlation of  $\delta^{13}\text{C}$  and OC, controlling for the effect of elevation

Values in italics were derived from log-transformed data (see Materials and methods)

Bold type indicates correlations that remained significant after partialling out the other factor(s)

CMA, canopy mass per area; MAP, mean annual precipitation; MAT, mean annual temperature;  $N_{\text{area}}$ , nitrogen per area; OC, overstory cover

Significance: \*  $P < 0.05$ , \*\*  $0.01 \geq P > 0.001$ , \*\*\*  $P \leq 0.001$ . The correlations with elevation and MAT remained significant after partialling out the effect of OC for log-transformed data but not untransformed data. Correlations shown without significance were non-significant using log-transformed or untransformed data

the other was partialled out, or after OC was partialled out ( $r_{\text{partial}} = 0.03\text{--}0.12$ ;  $P = 0.40\text{--}0.83$ ; Table 3).

**Discussion**

Across the Mauna Loa matrix landscape, the bryophytes varied greatly in  $\delta^{13}\text{C}$  due to species differences and their

being located across sites ranging markedly in elevation, climate and soil age. Across the landscape, the bryophytes exhibited similar correlations of  $\delta^{13}\text{C}$  with elevation and habitat irradiance as previously reported for tracheophytes. This finding indicates a potential for parallel underlying processes, despite the lack of stomata in the dominant bryophyte gametophyte life stage, and provides insights into functional relationships. These trends in bryophytes have previously been reported within a few individual species, primarily in the genus *Sphagnum* (Menot and Burns 2001; Fletcher et al. 2006; Skrzypek et al. 2007). Our study extends these findings across 12 species within eight genera, across natural gradients at a landscape scale. Notably, the bryophytes on Mauna Loa showed only a weak, non-significant negative correlation of  $\delta^{13}\text{C}$  with rainfall (MAP), despite its nearly three-fold variation, thus suggesting no important “liquid water film effect” integrated over long-term growth across sites. This finding contrasts with positive trends of  $\delta^{13}\text{C}$  with water availability previously reported in studies of *Sphagnum* species across varying water table heights and moisture contents (Proctor et al. 1992; Rice and Giles 1996; Williams and Flanagan 1996; Loisel et al. 2009) and precipitation (Menot and Burns 2001; Zhu et al. 2009). Notably, *Sphagnum* species are unusual among mosses in having photosynthetic cells that are surrounded by dead, hollow cells that hold water and would contribute to greater  $\text{CO}_2$  diffusion resistance. Without such modified water-holding cells, the leaves of typical bryophyte species may quickly drain to an optimal water content less restrictive to  $\text{CO}_2$  diffusion (Dilks and Proctor 1979). Of the study taxa, only *Leucobryum* has a cellular morphology approaching that of *Sphagnum*.

### Potential sources of the $\delta^{13}\text{C}$ –elevation correlation

The landscape-scale increase of  $\delta^{13}\text{C}$  with elevation in bryophytes on Mauna Loa was similar to that of *M. polymorpha* trees at these sites (Vitousek et al. 1990; Cordell et al. 1998, 1999) and that observed generally for vascular plants along elevational gradients worldwide (Körner et al. 1988, 1991, 2003; Morecroft et al. 1992; Hikosaka et al. 2002). This finding extends the elevational trends reported for *Sphagnum* (Menot and Burns 2001; Skrzypek et al. 2007) to a broader set of genera. In vascular plants, the trend was attributed to reduced  $\text{CO}_2$  partial pressure, associated with the lower atmospheric pressure at higher elevations, which would reduce  $c_c$  and increase  $\delta^{13}\text{C}$  (Körner et al. 1991), but later work concluded that such an effect would be too small to explain observed variation (Terashima et al. 1995; Körner 2003, 2007). The trend in vascular plants has also been attributed to increased leaf mass per area (LMA) with elevation (Körner 1989, 2003; Vitousek et al. 1990; Cordell et al. 1998; Hikosaka et al. 2002; Takahashi and Miyajima 2008; Li et al. 2009). A higher LMA in vascular plants may lead to lower mesophyll conductance, lower ratio of chloroplast to atmospheric  $\text{CO}_2$  partial pressure ( $c_c/c_a$ ) and higher  $\delta^{13}\text{C}$  (Terashima et al. 1995) because a greater leaf thickness increases the diffusion path length from the stomata to the mesophyll cell walls (Parkhurst 1994), and/or because thicker mesophyll cell walls reduce  $\text{CO}_2$  diffusion from intercellular air space to the chloroplasts (Niinemets 1999; Niinemets et al. 2009). We found that the  $\delta^{13}\text{C}$  decreased with decreasing bryophyte canopy mass per area (CMA), analogously to the decrease of  $\delta^{13}\text{C}$  with decreasing LMA in *M. polymorpha* (Vitousek et al. 1988), but whereas in *M. polymorpha* and in many other vascular plants LMA increases with elevation (Vitousek et al. 1988; Cordell et al. 1998; Hikosaka et al. 2002; Körner 2003), bryophyte CMA did not correlate with elevation but rather with OC (as discussed further below). These results indicate that the altitudinal trend in  $\delta^{13}\text{C}$  was likely due to lower  $\text{CO}_2$  pressure and/or the “temperature effect” as shown for three *Sphagnum* species (Menot and Burns 2001; Skrzypek et al. 2007). Such an effect contrasts with tracheophytes, for which there is no evidence for a general direct effect of temperature on  $\delta^{13}\text{C}$  (Troughton and Card 1975; Christeller et al. 1976). There is also the possibility that the declining cloud cover and thus increased irradiance, lower MAP and higher VPD at higher elevation might have contributed to the increase of  $\delta^{13}\text{C}$ . However, we found no correlation of  $\delta^{13}\text{C}$  with MAP or VPD, and thus such an effect was apparently not important at the landscape scale.

### Potential sources of the $\delta^{13}\text{C}$ –soil age and $\delta^{13}\text{C}$ –OC correlations

We observed a tendency for lower bryophyte  $\delta^{13}\text{C}$  on older soils on Mauna Loa. This finding is similar to that reported for leaves of *M. polymorpha* which have lower LMA and lower  $\delta^{13}\text{C}$  on the young flows (Vitousek et al. 1990). However, for the bryophytes, the effect was weak and apparently linked with the lower OC on young flows.

The strong correlation of bryophyte  $\delta^{13}\text{C}$  with OC across the landscape would likely have arisen from several factors. A first candidate is the “source air effect” if incomplete mixing resulted in air enriched in  $^{12}\text{C}$  due to soil-respired  $\text{CO}_2$  at bryophyte-level at sites with higher OC; such an effect would be stronger when canopies are dense, hindering airflow (Schleser and Jayasekera 1985; Broadmeadow et al. 1992). However, the bryophytes in this study were typically collected <0.5 m above ground, and up to only 5.5 m above soil, and tissue  $\delta^{13}\text{C}$  did not correlate with vertical height as expected if soil respiration were a major driver of the  $\delta^{13}\text{C}$ –OC relationship. The  $\delta^{13}\text{C}$  was significantly higher in colonies growing on rock than on organic substrates, as would be expected if substrate respiration had an important effect. However, colonies growing on humus, where substrate decomposition presumably leads to high  $\text{CO}_2$  production, did not differ significantly from those growing on bark substrates, and, most importantly, the  $\delta^{13}\text{C}$ –OC relationship held on individual substrates, including on rock. Thus, a vertical gradient in source air composition would likely contribute as a subtle effect, rather than as key driver of the relationship. The strong gradient in irradiance was likely the primary driver of the relationship, as reported for leaves of tracheophytes within forests (Buchmann et al. 1997b; Sternberg et al. 1997) and within the canopies of single trees (Le Roux et al. 2001). The  $\delta^{13}\text{C}$ –OC relationship may arise from the “irradiance-A effect” effect, i.e. a higher  $\delta^{13}\text{C}$  due to increased photosynthetic rate. The relationship might also arise from an indirect effect of water stress in more exposed, drier microhabitats leading to the development of thicker cell walls with greater water storage capacity, which during periods of high water availability would reduce  $\text{CO}_2$  diffusion (Dilks and Proctor 1979; Waite and Sack 2010). Such an effect may hold for individual species, but the lack of a significant relationship of  $\delta^{13}\text{C}$  with MAP or with VPD despite their varying by two- to three-fold across sites did not support the importance of such an effect at the landscape scale. We note that variation across taxa in cell size might also be expected to play a role; across three species of microalgae, larger cell size was related to reduced  $\text{CO}_2$  conductance and higher  $\delta^{13}\text{C}$  (Popp et al. 1998). That scenario is unlikely to be important across bryophyte species, in which larger cells tend to be



associated with deeper shade (Waite and Sack 2010) while higher  $\delta^{13}\text{C}$  was related to lower OC and hence greater irradiance.

Thus, the results best support a direct effect of irradiance on photosynthetic rate as a key driver of the bryophyte  $\delta^{13}\text{C}$ –OC relationship. Such an effect was found experimentally for two liverwort species grown at four irradiance levels;  $\delta^{13}\text{C}$  correlated positively with growth irradiance (Fletcher et al. 2006). Our findings extend this pattern across multiple species and show its potential importance at the landscape scale, across sites varying in forest successional stages. The effect is quantitatively strong; when the range of OC was divided into five bins, the average  $\delta^{13}\text{C}$  shifted from  $-27.1$  to  $-30.6\%$ . The effect was also strong on bark, leaf litter and rock substrates individually; the lack of the correlation on humus may be due to the lower range of OC values compared to the other substrate types. Notably, the correlation of OC and  $\delta^{13}\text{C}$  observed across all species and sites was also found within two of the six tested taxa, i.e., for *Campylopus* and in *R. lanuginosum*, which had the greatest range in OC values. Whereas these within-species relationships emerged from their wide distribution across OC levels, the across-species relationship reflected the assembly of diverse species along a light gradient (cf. Ackerly and Cornwell 2007).

We found that bryophyte  $\delta^{13}\text{C}$  correlated with the morphological variable CMA, analogously to LMA for tracheophyte leaves. This finding points to integrated adjustment of canopy form and physiology with irradiance for bryophytes across forest successional stages. However, by contrast with tracheophytes, in which the correlation of  $\delta^{13}\text{C}$  and LMA may arise in part from their relationship with mesophyll conductance, our partial correlation analysis indicated that in bryophytes, the relationship of  $\delta^{13}\text{C}$  with CMA arose indirectly due to relationships with OC. The high scatter in the correlation of  $\delta^{13}\text{C}$  with CMA, and their joint relationship with OC indicates that additional data would be needed to support a direct control of  $\delta^{13}\text{C}$  by CMA for any given species.

#### Implications and future work

The bryophytes of this study exhibited great similarities with vascular plants in landscape-scale patterns, showing independent and strong trends for lower carbon isotope fractionation with younger soil age, greater canopy openness and increasing elevation. The similarity with tracheophytes is especially remarkable given the centrality of stomata in explaining carbon isotope fractionation in tracheophytes and the lack of these structures in bryophytes. Our findings point to direct environmental impacts of light

and temperature on gas exchange rates as primary mechanisms for these landscape-level trends. We inferred the importance of given potential effects from correlation analyses at landscape scale; individual species may differ in the factors that determine  $\delta^{13}\text{C}$ . These findings highlight a necessity for additional mechanistic studies to clarify the underlying basis for these landscape-level trends. Indeed, for bryophytes, we lack a physiological predictor of  $\delta^{13}\text{C}$  signals, in the way that stomatal water use efficiency can be used for tracheophytes (reviewed in Seibt et al. 2008). This study, showing strong  $\delta^{13}\text{C}$  signals across the landscape scale, points to the importance of elucidating the determinants of carbon isotope discrimination in bryophyte structure and biochemistry (see Meyer et al. 2008). Such studies will need to determine isotope fractionation in real time for given species under ranges of controlled conditions to deepen the understanding of the landscape scale patterns demonstrated here.

We found the variation in  $\delta^{13}\text{C}$  in bryophytes at the landscape scale—including diverse taxa across diverse systems—to be information-rich, reflecting the signal of multiple environmental factors. This finding indicates the possibility of extending the usefulness of preserved bryophyte tissue to estimate past environmental conditions. Previous work emphasized that the lack of stomata can link bryophyte  $\delta^{13}\text{C}$  more closely with atmospheric  $\text{CO}_2$  concentration than for vascular plants (White et al. 1994; Fletcher et al. 2005, 2006, 2008), and pointed to the  $\delta^{13}\text{C}$  of fossilized *Sphagnum* as a proxy for moisture conditions, given that the “liquid film effect” would lead to higher  $\delta^{13}\text{C}$  in wetter climates (Loisel et al. 2009; Zhu et al. 2009). However, the lack of a relationship in our study between  $\delta^{13}\text{C}$  and MAP in the Mauna Loa bryophytes suggests that not all species would be good proxies for moisture conditions, though they may be proxies for other environmental factors. An avenue for future work is to determine how to model the combined effects of elevation, irradiance, soil age and temperature on given bryophyte floras. Alternatively, it may be possible to tease apart specific environmental signals, perhaps by using additional isotopes to carbon. Thus, bryophytes, by occupying, and responding to, a very wide range of environments could provide in their tissues recoverable information not only of physiological responses across gradients but also information of their climates and microhabitats.

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