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Interpreting bryophyte stable carbon isotope composition: Plants as temporal and spatial climate recorders

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Key Points:
- C isotopes provide an assimilation-weighted summary of bryophyte physiology
- Moss isotope values show most C gain that occurs close to optimal water content
- Thalloid liverworts are less sensitive to environmental variation than mosses

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Abstract Bryophytes are unable to control tissue water content although physiological adaptations allow growth in a wide range of habitats. Carbon isotope signals in two mosses (*Syntrichia ruralis* and *Chorisodontium aciphyllum*) and two liverworts (*Conocephalum conicum* and *Marchantia polymorpha*), whether instantaneous (real time, $\Delta^{13}C$), or organic matter (as $\delta^{13}C_{OM}$), provide an assimilation-weighted summary of bryophyte environmental adaptations. In mosses, $\delta^{13}C_{OM}$ is within the measured range of $\Delta^{13}C$ values, which suggests that other proxies, such as compound-specific organic signals, will be representative of historical photosynthetic and growth conditions. The liverworts were photosynthetically active over a wider range of relative water contents (RWC) than the mosses. There was a consistent 5‰ offset between $\Delta^{13}C$ values in *C. conicum* and *M. polymorpha*, suggestive of greater diffusion limitation in the latter. Analysis of a *C. aciphyllum* moss-peat core showed the isotopic composition over the past 200 years reflects recent anthropogenic CO_2 emissions. Once corrected for source-CO_2 inputs, the seasonally integrated $\Delta^{13}C_{OM}$ between 1350 and 2000 A.D. varied by 1.5‰ compared with potential range of the 12‰ measured experimentally, demonstrating the relatively narrow range of conditions under which the majority of net assimilation takes place. Carbon isotope discrimination also varies spatially, with a 4‰ shift in epiphytic bryophyte organic matter found between lowland Amazonia and upper montane tropical cloud forest in the Peruvian Andes, associated with increased diffusion limitation.

1. Introduction

Bryophytes represent the earliest group of land plants, including mosses (phylum Bryophyta), liverworts (phylum Marchantiophyta), and hornworts (phylum Anthocerotophyta), and demonstrate diverse life forms growing across varied habitats on all continents. It might be thought that "primitive" characteristics of bryophytes, including the dominance of haploid gametophytes, the requirement for liquid water to facilitate sexual reproduction during the alternation of generation life cycle, and the absence of stomata in all but a few moss and hornwort sporophyte tissues would limit bryophyte distribution and productivity. The inability to control water status (poikilohydry) requires that individual species are either tolerant of desiccation or restricted to areas where moisture inputs routinely exceed evaporation. However, these traits have facilitated the exploitation by some bryophytes of "extreme" environments in terms of temperature, exposure, and water availability in which most vascular plants fail to establish [*Longton*, 1988; *Proctor and Tuba*, 2002].

Bryophytes have made a significant contribution to both global carbon storage and biodiversity. Global peatlands, largely derived from bryophytes, currently contain approximately 600 Pg of stored carbon [*Yu et al.*, 2010] and represent a potential source of green house gas emissions through warming of permafrost in polar regions [*Koven et al.*, 2011]. There is extensive species diversity of bryophytes, particularly for mosses in temperate and boreal bogs and fens and leafy liverworts in montane tropical cloud forests [e.g., *Geffert et al.*, 2013; *Romanski et al.*, 2011]. Cryptogams (bryophytes, lichens, and algae) are estimated to contribute 7% of terrestrial net primary productivity [*Elbert et al.*, 2012].

Analysis of the stable isotope composition of bryophyte organic matter can be made from stratigraphic accumulations of peat and associated ancient and modern species diversity. Such isotopic markers offer insights into environmental conditions across either latitudinal and altitudinal gradients [e.g., *Ménot and Burns*, 2001; *Nichols et al.*, 2010; *Royles et al.*, 2012; *Sternberg and Ellsworth*, 2011; *Waite and Sack*, 2010]. The carbon isotope composition of bryophytes is subject to different controls to those seen in higher plants [*Farquhar et al.*, 1989; *Meyer et al.*, 2008; *Rice and Giles*, 1996; *Royles et al.*, 2013; *Williams and Flanagan*, 1996], although both to some degree are dependent on tissue water status. To maintain the positive water

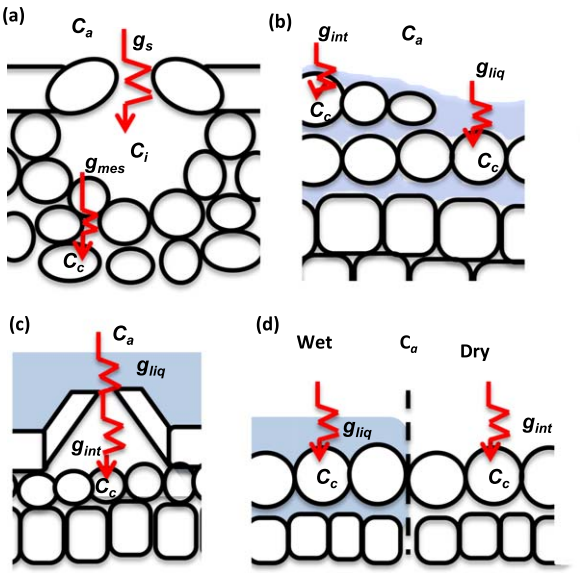

Figure 1. Diagrammatic cross sections (not to scale) through typical photosynthetic tissues where blue shading represents the liquid phase, C_a, C_i, and C_c are the atmospheric, intercellular, and chloroplastic CO_2 mole fractions, respectively, g_s, g_{mes}, g_{int}, and g_{liq} are stomatal, mesophyll, internal, and liquid phase conductances, respectively, and jagged arrows representing points of diffusion resistance. (a) Vascular leaf with guard cells around stomatal pore; (b) leafy liverwort with overlapping leaves, predominant in the Peruvian study (Figures 4 and 6); (c) a ventilated thalloid liverwort such as *M. polymorpha* or *C. conicum*; (d) a typical moss in wet/dry state, such as *S. ruralis* used in the lab measurements. Unlike leafy liverworts, most mosses have a midrib (costa/nerve) of enlarged/specialized cells. In all instances, C_c depends on the concentration difference with the atmosphere (C_a) and the extent of diffusion resistance to CO_2 between the atmosphere and the chloroplast. In vascular plants, the diffusion pathway is actively controlled via stomatal aperture (g_s), whilst in poikilohydric nonvascular plants, g_{liq} is uncontrolled and dependent on environmental conditions, but never the less represents a critical determinant of C_c.

balance required for photosynthesis and growth, bryophyte tissue directly absorbs precipitation, dewfall, or, under conditions of high humidity, atmospheric water vapor [*Helliker and Griffiths*, 2007]. An external layer of capillary water is held outside bryophyte tissue to delay desiccation under drying conditions [*Proctor and Tuba*, 2002; *Proctor et al*., 2007]. Bryophyte photosynthetic laminae vary from dense, largely undifferentiated thalli in some liverworts to finely divided leaflets, usually one layer of cells thick, in mosses and leafy liverworts (Figure 1). All bryophytes are dependent on diffusive supply of CO_2 across a variable outer liquid boundary layer, saturated tissues without air spaces (except in ventilated liverworts (Figure 1c), and finally through cell walls and internal membranes. Consequently, under environmental conditions in which a high tissue-to-air vapor pressure deficit is established, surface water first evaporates and the diffusive supply of CO_2 (and photosynthetic rate) initially increases, before tissues eventually become desiccated and metabolically inactive [*Rice and Giles*, 1996; *Royles et al*., 2013; *Williams and Flanagan*, 1996].

The capillary water around bryophyte tissue affects assimilation rate through the diffusive limitations to CO_2 supply (Figure 1), with minor fractionations occurring against $^{13}CO_2$ during delivery to the chloroplast attributed to the greater bidirection diffusion resistance to $^{13}CO_2$ molecules than the smaller, lighter and faster $^{12}CO_2$ molecules [*O'Leary*, 1988]. These fractionations amount to some 4.4‰ during diffusion in air adjacent to tissues, and 1.8‰ during dissolution [*Mook et al*., 1974] and diffusion [*O'Leary*, 1984] through liquid water, cell walls, and membranes [*Farquhar et al*., 1989]. Furthermore, $^{13}CO_2$ is slower to react and consequently the Rubisco can express a maximum biochemical discrimination against $^{13}CO_2$ of up to 29‰ [*O'Leary*, 1988]. Surface water extent and tissue density determine the relative dominance and magnitude of diffusion over metabolic fractionations. It is important to note that measurements normally assume a constant, bulk atmospheric CO_2 source composition (currently −8‰ [*White and Vaughn*, 2011]), with a progressive offset needing to be applied over the past 200 years due to anthropogenic emissions [*Francey et al*., 1999]. Variations in "source" CO_2 composition can also arise from more ^{13}C depleted biological respiratory inputs (in dense canopies or greenhouses), or the "tank" CO_2 used in some growth facilities or gas exchange equipment, which will alter the instantaneous compound-specific isotopic compositions of organic matter. Finally, the isotopic signal in source carbon species available to fully aquatic bryophytes (such as *Fontinalis antipyretica*) is also dependent upon alkalinity, *p*H, and temperature [*Osmond et al*., 1981].

In bryophytes, maximum $\Delta^{13}C$ is found simultaneously with both maximum carbon assimilation and light interception and conversion, measured as electron transport rates [*Meyer et al*., 2008; *Rice*, 2000; *Royles et al*., 2013], so $\Delta^{13}C$ provides an indication of the optimality of the photosynthetic conditions. When integrated over time in the cellulose and bulk organic matter (OM) of plant tissue, $\Delta^{13}C$ gives a relative

indication of growing season conditions [*Royles et al*., 2012]. The integrated $\Delta^{13}C_{OM}$ value has applications in environmental analysis over both space and time. In dendrochronological analysis $\Delta^{13}C_{OM}$ is largely dependent upon canopy stomatal conductance, and thus air temperature and tree water status [*Helliker and Richter*, 2008; *McCarroll and Loader*, 2004]. In Antarctic moss peat banks, the integrated $\Delta^{13}C$ signature of cellulose can be used to determine the relative assimilation rate at the time the moss was photosynthesizing over past millennia [*Royles et al*., 2012]. Similarly, the effect of environmental factors on the isotopic composition of plant tissue is apparent across gradients in elevation, where variations in source CO_2, temperature, precipitation, relative humidity, vapor pressure deficit, and light intensity all affect the extent of discrimination and consequently $\Delta^{13}C_{OM}$ [*Körner et al*., 1991; *Ménot and Burns*, 2001; *Waite and Sack*, 2011].

In this paper, the relative timing and dynamics of instantaneous carbon assimilation and the relationship between $\Delta^{13}C$ values measured instantaneously or integrated within organic matter will be compared for contrasting living bryophyte tissues. We will examine two moss species, with single-celled photosynthetic lamina in which there are no air spaces (Figure 1d), and two thalloid liverworts, which have dense, flat tissue structures with surface pores and air pockets saturated with water vapor (Figure 1c). The aim is to define the limits for variations in bryophyte $\Delta^{13}C_{OM}$, in such contrasting life forms, and to aid the interpretation of photosynthetic limitation across spatial and temporal scales to facilitate environmental reconstructions from measurements of stable isotope composition.

2. Materials and Methods

2.1. Ecology of Experimental Species

Experimental work was carried out on two moss (Figure 1d) and two ventilated liverwort species (Figure 1c) from contrasting habitats. *Syntrichia ruralis* (Weber and Mohr; synonymous with *Tortula ruralis* (Hedwig)) is a globally widespread desiccation tolerant moss species growing on calcareous substrates and forming turfs 10–20 mm deep [*Atherton et al*., 2010]. This moss is able to reactivate the photosynthetic apparatus rapidly when water is available, assimilate CO_2 during the brief intervals of appropriate hydration and irradiation, and then enter a reversible state of metabolic shutdown when tissue water becomes low [*Royles et al*., 2013]. In contrast, the moss *Chorisodontium aciphyllum* ((Hook. f. and Wilson) Broth) has a recorded distribution across the southern tip of South America and the maritime Antarctic [*Ochyra et al*., 2008]. *Chorisodontium aciphyllum* requires regular precipitation and forms large dome-shaped peat banks up to 2 m deep, which buffer changes in temperature and water availability and help to maintain a permanent water layer around the photosynthetic tissue [*Fenton*, 1980; *Gimingham and Smith*, 1971].

The thalloid liverworts *Conocephalum conicum* and *Marchantia polymorpha* both have widespread distributions and have large, dense, somewhat differentiated thalli, with some photosynthetic cells enclosed adjacent to air pockets in the thallus surface (Figure 1c). *Conocephalum conicum* (Underwood) has large, flat leathery, dichotomously branched thalli that form extensive, shallow mats in damp, shady habitats [*Atherton et al*., 2010]. In contrast, the most widely distributed hepatic in the world, *M. polymorpha* (Bischler and Boisselier), is more drought tolerant and tends to be found in disturbed habitats in full sun [*Atherton et al*., 2010].

2.2. Preparation of Experimental Bryophyte Material

Conocephalum conicum samples were collected from Cambridge University Botanic Garden "Life before Flowers" glasshouse, Cambridge, UK, where it was growing in full shade conditions (light level < 100 μmol m^{-2} s^{-1}; collected by A. Horwath (2009) and J. Royles (2012)). *Marchantia polymorpha* and *S. ruralis* samples were collected from populations growing in full sun around Cambridge, UK (light level: up to 2000 μmol m^{-2} s^{-1} in sun; A. Horwath (2009), H. Griffiths (2011), and J. Royles (2012)). Prior to each experiment, the bryophyte tissue was soaked for 24 h in deionized (DI) water to ensure full and consistent saturation with a water supply of constant and known isotopic composition and then maintained at 20°C in ambient external light conditions to fully activate the photosynthetic apparatus.

Samples of *C. aciphyllum* were collected during the 2009/2010 austral summer (Royles; Signy Island, Antarctica (Figure 4a)) where the moss was growing in full sun (Figure 4d) but light levels above 1000 μmol photons m^{-2} s^{-1} are unusual on Signy Island [*Davey and Rothery*, 1996]. Samples were transported to the UK at +4°C and then maintained in transparent plastic boxes at +4°C with a constant light source and air

humidified with DI as required. Under these conditions the top, green photosynthetic shoots continued to grow in a manner externally similar to that of *in situ* Signy Island moss.

Immediately prior to experimentation, green tissue of each species covering a basal area of 10 cm^2 was immersed in DI water and gently blotted to remove excess moisture but retain the external water directly associated with the thalli or leaflets. The tissue was then weighed to determine fresh mass (FM_0). This fresh mass includes some capillary water and is taken to represent the conditions following a rain event. Note that due to the anatomical and vascular differences between bryophytes and tracheophytes the measured fresh mass (FM_0) is not directly comparable between both groups.

2.3. Experimental Setup

Photosynthetic measurements and "online" gas collections were carried out using the LI6400-XT open gas exchange system (LiCor, Lincoln, NE, USA). Tissue samples were placed in an open-ended cylindrical dish of area 10 cm^2 and depth 10 mm, which was sealed within the Whole Plant Chamber (LI6400-17; LiCor, Lincoln, NE, USA) and illuminated using the RGB Light Source (LI6400-18; LiCor, Lincoln, NE, USA). All statistical and graphical analysis was completed using the R Software package (v. 2.10.1) [*R Core Development Team*, 2013].

2.4. Light and CO_2 Response Curves

To generate light-response curves, the instantaneous photosynthetic assimilation rate (*A*) of bryophyte tissue in the experimental cuvette was measured following 120 s exposure of the tissue to a range of photosynthetic photon flux densities from 0 up to a species-specific saturating photon flux density (maximum of 2000 μmol photons m^{-2} s^{-1}), whilst the inflowing CO_2 concentration was maintained at 400 ppm. Each curve was repeated for five replicate tissue samples.

For CO_2 response curves, the instantaneous assimilation rates of fresh bryophyte tissue samples were measured following exposure of the tissue within the gas exchange cuvette for 180 s to a range of external CO_2 concentrations (50–1500 ppm) whilst photon flux density was maintained at a saturating (but not photoinhibiting) intensity that was determined from the light-response curves (1000 μmol photons m^{-2} s^{-1} for *S. ruralis* and *M. polymorpha*; 500 μmol photons m^{-2} s^{-1} for *C. aciphyllum;* and 300 μmol photons m^{-2} s^{-1} for *C. conicum*). Each curve was repeated with five replicate tissue samples. The astomatous nature of bryophytes allows photosynthesis to be expressed as a function of external or atmospheric CO_2 concentrations (C_a).

Following completion of measurements, each bryophyte tissue sample was dried to a constant mass, and assimilation values expressed as CO_2 fixed per gram dry weight per hour.

2.5. Online Carbon Discrimination ($\Delta^{13}C$)

To determine the extent of carbon discrimination ($\Delta^{13}C$) during photosynthesis, air leaving the gas exchange cuvette was passed under positive pressure through a liquid nitrogen trap (−180°C). After 20 min, the CO_2 and condensed water vapor were successively purified from the liquid nitrogen trap into glass collection vials [*Harwood et al*., 1998]. Air collections were repeated over 20 min periods until the tissue ceased to be photosynthetically active. The mass of the tissue was measured between each gas collection to determine intermediate fresh weight values (FM_1–FM_7). At the end of the experiment, the moss and liverwort tissue was dried to a constant mass in an oven at 70°C and the dry mass determined (DM). Relative water content was calculated for each successive fresh mass (equation (1)).

$$RWC_{0-7} = ([FM_{0-7}] - DM)/DM * 100 \quad (1)$$

The isotopic composition of CO_2 ($\delta^{13}C_{VPDB}$) was measured directly using an Isotope Ratio Mass Spectrometer (IRMS; SIRA, VG Isotech, modified by Pro-Vac Services Ltd., Crewe, UK). The IRMS $\delta^{13}C$ values are expressed in per mill (‰) offset against a Vienna Pee Dee Belemnite standard (V-PDB). $\Delta^{13}C$ was then calculated based on the $^{13}C/^{12}C$ ratios of CO_2 in air entering (R_e) and exiting (R_o) the chamber, following *Evans et al*. [1986] (equation (2)).

$$\Delta^{13}C = \frac{\xi(\delta_o - \delta_e)}{1000 + \delta_o - \xi(\delta_o - \delta_e)} \quad (2)$$

where $\xi = p_e/(p_e - p_o)$ and δ_e, p_e, δ_o, and p_o are the isotopic composition of CO_2 and CO_2 partial pressures in air entering (e) and exiting (o) the chamber, respectively.

2.6. Determination of Isotopic ($\delta^{13}C_{OM}$) and Elemental (C:N) Composition of Organic Matter

The dried experimental bryophyte tissue was ground into a homogeneous powder. A precisely determined 1 mg subsample of powder was transferred into a 3 mm × 5 mm tin cup (Experimental Microanalysis Ltd., Okehampton, UK). The $\delta^{13}C_{OM}$ and $C{:}N_{OM}$ analyses were then completed at the Godwin Laboratory, University of Cambridge using a Costech Elemental Analyzer attached to a Thermo DELTA V mass spectrometer in continuous flow mode (Thermo Fisher Scientific, Waltham, MA, USA). Gases are separated and transported sequentially through a Thermo Conflo IV interface and "open split" into the mass spectrometer for analysis. The mass spectrometer software measures the $^{15}N/^{14}N$ and the $^{13}C/^{12}C$ ratio using two-point linear normalization [*Paul et al*., 2007] and two reference materials: IAEA-600 (Caffeine: $\delta^{13}C = -27.5‰$, when n = 4, measured $\delta^{13}C = -27.52‰$, sd = 0.04‰) and USGS 40 (L-glutamic acid: $\delta^{13}C = -26.2‰$, when n = 4, measured $\delta^{13}C = -27.20‰$, sd = 0.03‰).

2.7. Determination of Carbon Isotope Discrimination Over Time ($\delta^{13}C_{OM}$ and $\Delta^{13}C_{OM}$)

During November 2009, a frozen peat core was extracted on Signy Island, South Orkney Islands, Antarctica (60°34′S, 045°36′W; Figures 4a and 4d) from a *C. aciphyllum* dominated moss peat bank using an adapted ice corer (based on *Nørnberg et al*. [2004]). The core extended from the green growing surface to the underlying rocky substrata. The core was transported frozen to the UK for analysis where it was sliced longitudinally, with one half then divided into 5–7 mm wide transverse sections. Radiocarbon analysis was used to date successive points in the core and an age-depth model developed, as detailed in *Royles et al*. [2012]. Subsamples from the transverse sections of the core were dried, homogenized, and underwent $\delta^{13}C_{OM}$ analysis as described above (section 2.6).

Measured $\delta^{13}C_{OM}$ values were corrected for the variation in atmospheric CO_2 composition using the established isotopic composition of atmospheric CO_2 ($\delta^{13}C_a$) for each year of growth [*Francey et al*., 1999; *McCarroll and Loader*, 2004] as determined from the age-depth model (Site A) [*Royles et al*., 2012] using equation (3):

$$\Delta = \frac{\delta^{13}C_a - \delta^{13}C_{OM}}{1 + \delta^{13}C_{OM}} \quad (3)$$

where Δ represents the discrimination by the plant [*Farquhar et al*., 1982].

2.8. Determination of Spatial Variation in Carbon Isotope Discrimination ($\delta^{13}C_{OM}$)

Epiphytic bryophytes were collected between May 2008 and August 2008 from trees at eight locations along an altitudinal gradient in south-eastern Peru (Figures 4a–4c). Between 2 and 12 epiphytes (mosses and leafy liverworts), we collected at each location, which were classified, dried, and homogenized before undergoing $\delta^{13}C_{OM}$ analysis as described above (section 2.6).

3. Results

In order to interpret carbon isotope composition values preserved in bryophyte tissue, which have the potential to serve as a biochemical record, it is critical to understand the conditions under which carbon assimilation, via photosynthesis, occurred. First, it is important to define the physiological basis to the likely minimum and maximum rates of carbon gain and associated isotope discrimination for a given tissue type and associated water content. Second, these data need to be considered across a daily and seasonal basis to determine whether the isotopic composition values that integrate the extent of tissue hydration reflect the timing for the majority of carbon gain and contribution to growth, bulk organic residues, or cellulose signals. Our approach was to use gas exchange to determine optimal conditions for photosynthesis under laboratory conditions, in order to define light saturation and CO_2 use and to highlight the responsiveness of

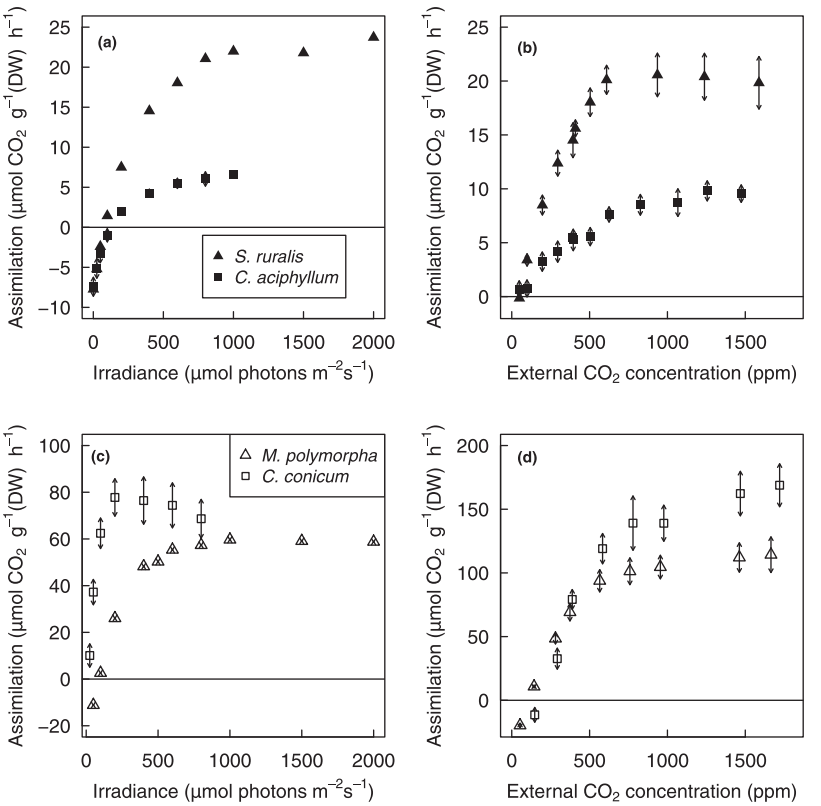

Figure 2. Instantaneous assimilation rate of CO_2, expressed per unit dry mass per hour, as a function of (a and c) irradiance and (b and d) external CO_2 concentration for the mosses *Syntrichia ruralis* (filled triangles) and *Chorisodontium aciphyllum* (filled squares) and the liverworts *Marchantia polymorpha* (open triangles) and *Conocephalum conicum* (open squares). Error bars represent one standard error, n = 5 samples per species under each treatment condition. Note different *y* axis scales.

the plants to variation representative of natural conditions. Real-time carbon isotope measurements were then used, to provide a virtually instantaneous isotopic discrimination value as a function of tissue water content, for comparison with the signature of organic material, which integrates carbon gain across an entire growing season.

3.1. Photosynthetic Characteristics of Experimental Bryophyte Species

The light-response curves reflected the growth environments of the bryophytes, with *M. polymorpha* and *S. ruralis*, both growing in fully exposed habitats, showing initial linear increases in photosynthesis under limiting irradiance, and light saturation at approximately 1000 μmol photons m^{-2} s^{-1}, compared to 500 μmol photons m^{-2} s^{-1} in *C. aciphyllum* and 300 μmol photons m^{-2} s^{-1} in the shade growing *C. conicum* (Figures 2a and 2c). The transition from limiting CO_2 to saturation (normally interpreted as limitation by other photosynthetic factors, such as light harvesting and RuBP regeneration) occurs around 600 ppm, well above current ambient CO_2 concentrations (400 ppm), suggesting that assimilation in all tissues is somewhat limited by diffusion in a current low CO_2 world (Figures 2b and 2d) at the tissue water contents maintained during measurements.

Combining the assimilation rates measured under both varying light and CO_2, *S. ruralis* consistently achieved significantly higher assimilation rates than *C. aciphyllum* (Figures 2a and 2b; Wilcoxon paired test, $V = 204$, $p < 0.0001$). Within the liverworts, assimilation by *C. conicum* was significantly higher than by *M. polymorpha* (Figures 2c and 2d; paired *t* test, $t = -3.40$, $p < 0.004$); however, this was restricted by the light levels at which *C. conicum* was photosynthetically active. On a unit tissue mass basis, the highest absolute assimilation rate was recorded in the liverwort *C. conicum*, at over 160 μmol CO_2 g^{-1} (DW) h^{-1}, which was 50% higher than the measured A_{max} in the second liverwort species, *M. polymorpha* and up to 20 times higher than the A_{max} measured in the moss *C. aciphyllum* (Figures 2b and 2d).

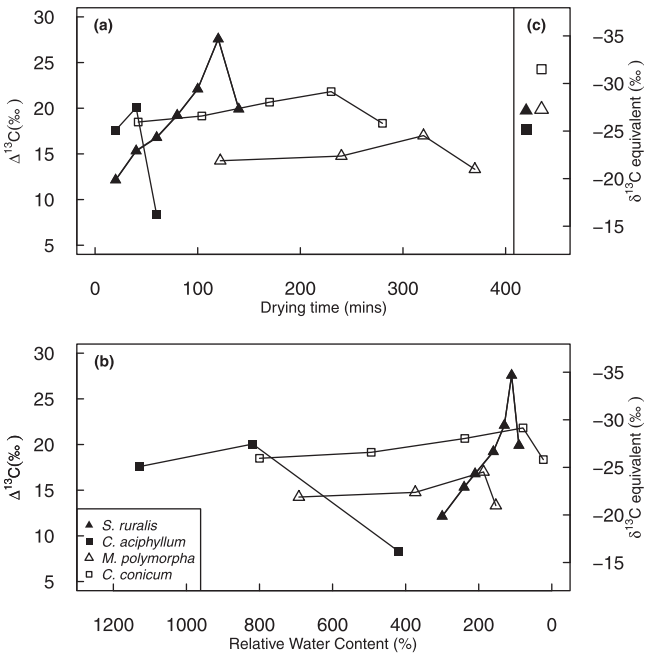

Figure 3. Online, real-time measurements of photosynthetic discrimination against $^{13}CO_2$ ($\Delta^{13}C$; equation (2)) for the mosses *Syntrichia ruralis* (filled triangles) and *Chorisodontium aciphyllum* (filled squares) and the liverworts *Marchantia polymorpha* (open triangles) and *Conocephalum conicum* (open squares) as a function of (a) time following saturation; (b) relative water content (equation (1); n = 2 per species); (c) mean carbon isotopic composition of organic matter for each species (n = 3; standard error bars covered by markers; see Table 1). The values on the two *y* axes are equivalent: $\Delta^{13}C_{equivalent}$ was calculated assuming that $\delta^{13}C_{source}$ was −8‰ (atmospheric) to give values comparable to those measured in organic matter ($\delta^{13}C_{equivalent} = \delta^{13}C_{source} - \Delta^{13}C/(1 + \Delta^{13}C/1000)$) [*Farquhar et al.*, 1989].

The *x* axis intercept, representing the transition from net respiration to net photosynthesis in such response curves, is also physiologically relevant. The light compensation points of *C. aciphyllum*, *S. ruralis*, and *M. polymorpha* were all very similar at 90 μmol photons m^{-2} s^{-1}. In comparison, the shade adapted *C. conicum* thalli had the lowest light compensation point (4 μmol photons m^{-2} s^{-1}) but also supported highest maximum rate of photosynthesis.

3.2. Instantaneous Isotopic Discrimination ($\Delta^{13}C$) and Organic Matter Composition

Previously, we have shown that for mosses, carbon isotope discrimination is a proxy for assimilation rate, since both are constrained by the diffusive supply of CO_2 into tissues by liquid water (boundary layer conductance) and internal, mesophyll conductance (Figure 1) [*Royles et al.*, 2012; *Royles et al.*, 2013]. Tissue dried out rapidly in the Antarctic moss *C. aciphyllum*, and net assimilation was maintained for 1 h, as compared to over 2 h in the temperate, desiccation tolerant *S. ruralis* (Figure 3a). The two liverwort species remained photosynthetically active for a substantially longer period of time than the mosses, up to 5 h in the case of *C. conicum* and 4 h in *M. polymorpha*. A narrower range in discrimination values were measured in the liverworts ($\Delta^{13}C$ range of 3‰ in *C. conicum*, 4‰ in *M. polymorpha*) than the mosses ($\Delta^{13}C$ range of 15‰ in *S. ruralis*, 14‰ in *C. aciphyllum*). When considered with respect to tissue relative water content (RWC, expressed relative to dry weight, equation (1)), the difference in the operating ranges of the moss species becomes apparent (Figure 3b), with *C. aciphyllum* active at RWC values of between 1100% and 400%, with tissues allowing a high rate of water loss relative to *S. ruralis*, which maintained RWC across a lower range of values (300–50%). In terms of response to RWC, both liverwort species had a similar active RWC range, intermediate between *S. ruralis* and *C. aciphyllum*, with *M. polymorpha* operating between 700% and 100%, and *C. conicum* at RWC values below 800%. The typical bimodal response in $\Delta^{13}C$, increasing as surface water is initially lost, followed by a rapid decline as tissues desiccate, is apparent for all species, but across the range of operating RWC values. Maximal $\Delta^{13}C$ values ranked in terms of *S. ruralis* > *C. conicum* > *C. aciphyllum* > *M. polymorpha*, with the highest value for *S. ruralis* (27.6‰) close to the maximum biochemical discrimination against $^{13}CO_2$.

Having defined the likely range of carbon isotope signals contributing photosynthate to growth across a range of conditions, we now compare the actual organic material composition ($\delta^{13}C_{OM}$) for each species. The most negative $\delta^{13}C_{OM}$ values were measured in *C. conicum*, at −31.5‰, compared with −27‰ in *M. polymorpha* and *S. ruralis* and −25‰ in *C. aciphyllum* (Table 1). When the source CO_2 effect is taken into consideration, and $\delta^{13}C_{source}$ was assumed to be atmospheric CO_2 with an isotopic composition of −8‰, the temporally integrated $\delta^{13}C_{OM}$ values can be directly compared to instantaneous $\Delta^{13}C$ (equation (3); Figure 3c), and the equivalence of the values highlights the link between the short term, leaf level measurements and the seasonal isotope discrimination values. The $\delta^{13}C_{OM}$ values of the two mosses (Figure 3c) fell

Table 1. Composition of bryophyte organic matter

Species	$\delta^{13}C_{OM}$ (‰)	Equivalent $\Delta^{13}C$ (‰)	N content (%)	C content (%)	C:N
Syntrichia ruralis	-27.15 ± 0.11^{a}	19.7	2.70 ± 0.08	41.9 ± 2.1	15.5 ± 0.4^{a}
Chorisodontium aciphyllum	-25.19 ± 0.05^{b}	17.6	1.53 ± 0.06	47.8 ± 1.7	31.3 ± 1.5^{b}
Marchantia polymorpha	-27.28 ± 0.15^{a}	19.8	1.47 ± 0.02	41.2 ± 2.2	28.1 ± 1.4^{b}
Conocephalum conicum	-31.50 ± 0.18^{c}	24.3	2.07 ± 0.37	44.0 ± 3.5	23.1 ± 5.0^{ab}

Mean ± standard error of the carbon isotopic composition ($\delta^{13}C_{OM}$: ANOVA $F(3,8)=414$, $p>0.0001$, superscripts indicate post-hoc Tukey's HSD tests significant at $p<0.0001$, equivalent source independent carbon isotope composition ($\Delta^{13}C$; $\delta^{13}C_{SOURCE} = 8‰$ [*White and Vaughn*, 2011] , and $\Delta^{13}C = (\delta^{13}C_{SOURCE} - \delta^{13}C_{OM})/(1+(\delta^{13}C_{OM}/1000)$ [*Farquhar et al.*, 1989]), nitrogen and carbon contents and carbon to nitrogen ratio (C:N) of organic matter samples from each of the four experimental species (ANOVA, $F(3,8) = 6.4$, $p=0.016$, superscripts indicate post-hoc Tukey's HSD tests significant at $p<0.05$) ($n=3$ for each measurement).

within the range of the online, instantaneous $\Delta^{13}C$ values (Figure 3a). In contrast, the $\delta^{13}C_{OM}$ values of the two liverworts were offset by approximately 3‰ from the $\Delta^{13}C$ values. Along with the least negative $\delta^{13}C_{OM}$ value *C. aciphyllum* was associated with the highest C:N ratio of 31.3, approximately double that of the *S. ruralis* tissue (15.5), due to both a higher carbon content and a lower nitrogen content in *C. aciphyllum* as compared with *S. ruralis* (Table 1). The liverwort C:N ratios were intermediate between the mosses.

3.3. Temporal Variation in $\delta^{13}C_{OM}$ and $\Delta^{13}C_{OM}$ Measured in Signy Island Peat Cores

The $\delta^{13}C_{OM}$ values of *C. aciphyllum* accumulated over time in the Signy Island moss-peat core covered a 3‰ range over the period from 1350–2000 A.D. (Figure 4a, 4c, 5a). Particularly striking was the progressive depletion from around −21‰ to −23.5‰ from 1700 A.D. onward, at a rate of approximately 0.7‰ per century, whereas prior to that time the $\delta^{13}C_{OM}$ values had fluctuated around -21 ± 0.5‰ for 400 years. Corrections for atmospheric source CO_2 isotope composition, which has changed due to anthropogenic CO_2

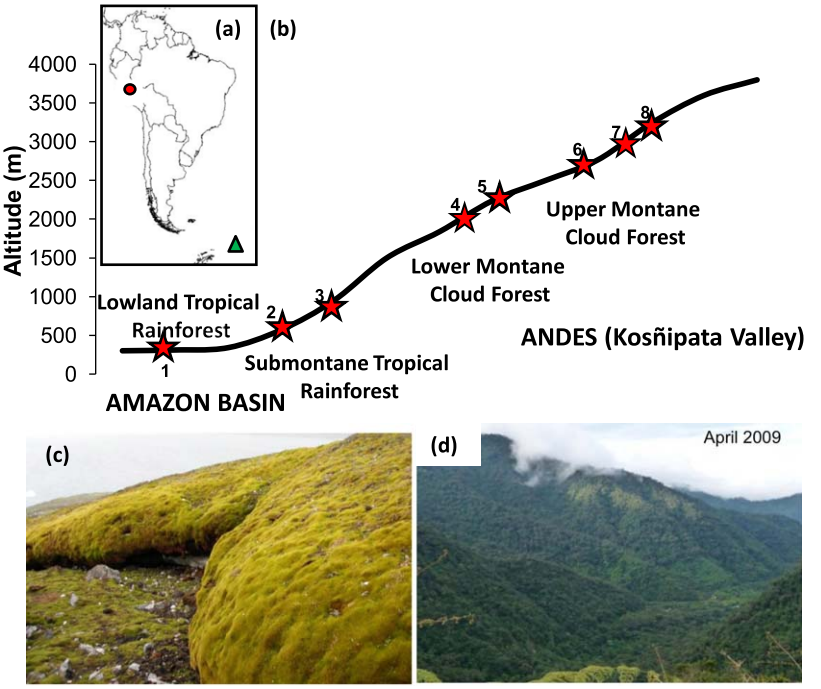

Figure 4. (a) Outline map of South America showing the location of the elevational gradient of epiphytic bryophytes in south-eastern Peru (circle) and Signy Island (triangle) from where the moss-peat core was extracted. (b) Schematic of altitudinal transect through the Kosñipata Valley, Peru. Sampling locations marked by stars. Key to numbers (geographical location and elevation above sea level): Madre de Dios district—(1) Cocha Cashu, 330 m, (2) Pantiacolla, 570 m; Cusco district—(3) Tono, 950 m; (4) Trocha Union VII, 2000 m; (5) Trocha Union VI, 2250 m; (6) Trocha Union IV, 2750 m; (7) Wayqecha IX, 3000 m; (8) Trocha Union II, 3200 m. (c) Photograph of section in Kosñipata Valley, Peru transect (photo: A. Horwath). (d) Photograph of typical Signy Island moss peat bank, approximately 1 m deep at edge (photo: J. Royles).

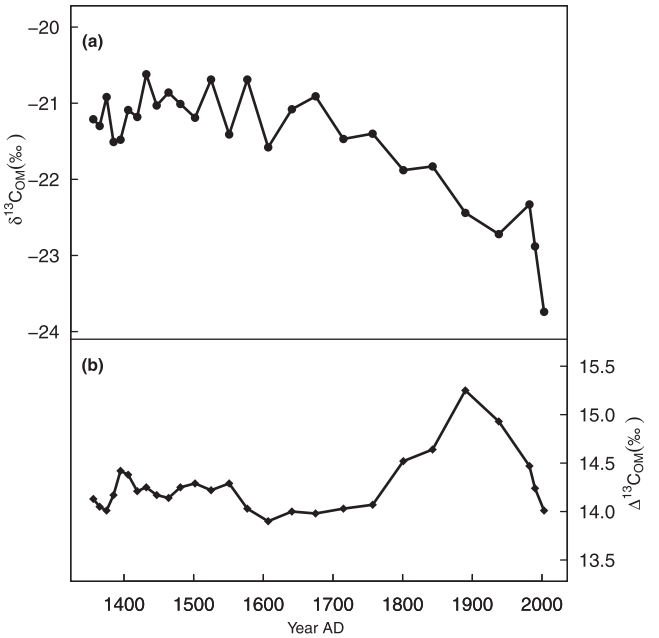

Figure 5. (a) Measured carbon isotope composition of organic matter ($\delta^{13}C_{OM}$) and (b) source-independent discrimination ($\Delta^{13}C_{OM}$; equation (3)) over time for a *Chorisodontium aciphyllum* peat core extracted from Signy Island (60°34′S, 045°36′W). See *Royles et al.* [2012] for age-depth model.

emissions [*Francey et al.*, 1999], allow source-independent $\Delta^{13}C_{OM}$ values to be determined (equation (3); Figure 5b). The most substantial fluctuation occurred between 1770 and 1900 A.D., with an increase of 1.3‰ followed by a decline in $\Delta^{13}C_{OM}$ over the past century from the peak of 15.3‰ to 14.5‰.

3.4. Spatial Variation in $\delta^{13}C_{OM}$ Measured in Peruvian Epiphytic Bryophytes

There was no significant difference in $\delta^{13}C_{OM}$ between the moss and leafy liverwort samples collected from the Peruvian transect (Figure 6a; Wilcoxon test, W = 273.5, n = 43, $p = 0.11$); however, across the 3000 m elevational gradient from the Amazon Basin to the high Andes in south-eastern Peru (Figure 4), the mean measured $\delta^{13}C_{OM}$ values covered a range of over 4‰ (Figure 6) and altitude was a highly significant driver of the measured variation (analysis of variance (ANOVA), n = 43, F = 31, $p < 0.0001$). The epiphytic bryophyte samples with the most negative $\delta^{13}C_{OM}$ values, of −31 to −32‰, were collected from the lowlands: below 1000 m asl. At three locations between 2200 and 3000 m asl, $\delta^{13}C_{OM}$ was around −27‰, and these less negative $\delta^{13}C_{OM}$ values coincided with the elevations in the upper montane tropical cloud forest at which most persistent cloud immersion at the vegetation level occurred. The sites with less frequent cloudiness (transitional zones) were characterized by intermediate values, −30‰ (2000 m asl) and −28.5‰ (3200 m asl) and corresponded to the lower and upper limits of the observed cloud bank.

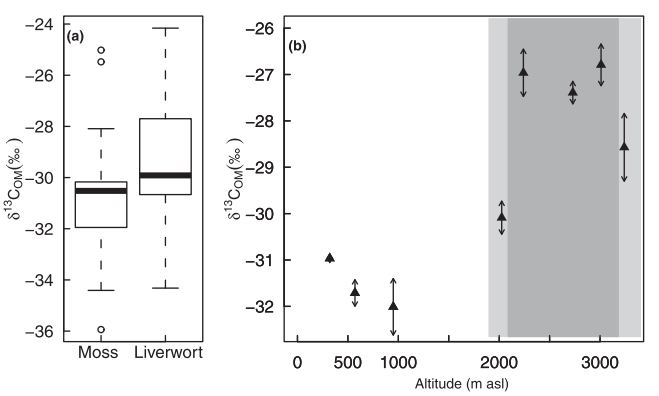

Figure 6. Measured carbon isotope composition of organic matter ($\delta^{13}C_{OM}$) from Peruvian epiphytic bryophytes (Figure 4): as a function of (a) life form and (b) elevation. Dark shading marks altitude of the observed cloud immersion zone; light gray corresponds to the transitional zones; unshaded areas represent lowlands. Error bars represent SE of the mean (n = 2–12). Plant material was collected during the dry season (May-August) of 2008 and identified as moss (n = 15) or leafy liverwort (n = 28) tissue. No significant difference between $\delta^{13}C_{OM}$ measured in mosses and leafy liverworts (Wilcoxon test, W = 273.5, n = 43, $p = 0.11$); however, $\delta^{13}C_{OM}$ varies significantly with altitude (ANOVA, n = 43, F = 31, $p < 0.0001$).

4. Discussion

The findings presented in this paper provide insights into limitations imposed on carbon gain as a function of hydration status for contrasting bryophyte life forms, showing that the isotopic composition of bulk organic material and cellulose provide an assimilation-weighted measurement reflecting typical growth conditions, which is important for the interpretation of stable isotope data from plant-based climate archives. The good overall agreement between real-time, instantaneous carbon isotope discrimination during photosynthesis and organic material

signals in mosses also suggests that compound-specific signals will reflect metabolic partitioning across a defined tissue water content operating range. As a general rule, for the finely divided leaflets of mosses, maximum carbon gain is tightly correlated with $\delta^{13}C$ and a narrow window of tissue water contents related to normal growth conditions. In the denser, planar thalli of liverworts, optimal water content is maintained for longer across a wider range of tissue water content, but tissue $\delta^{13}C$ is somewhat offset from the real-time, instantaneous discrimination values.

4.1. Photosynthetic Characteristics of Experimental Species

The photosynthetic gas exchange characteristics of the species examined highlighted both differences in physiology amongst bryophytes (Figures 2 and 3) and similarities which unite bryophytes (Figure 6a) when contrasted with vascular plants (Figure 1). When expressed on a dry weight basis, maximum CO_2 assimilation rates varied between bryophytes (Figure 2), but even the "high" liverwort assimilation values were approximately an order of magnitude lower than those of woody plants, when measured on an equivalent dry weight basis: at ambient CO_2 concentration, mean assimilation for a data compilation of woody species was 430 $\pm$ 200 μmol g^{-1} h^{-1} [*Niinemets*, 1999], compared to 50 μmol g^{-1} h^{-1} measured in the liverworts, and 5–10 μmol g^{-1} h^{-1} in the mosses. However, depending on growing season length and conditions (e.g., polar summer, temperate winter), even such low photosynthetic rates can translate into considerable total biomass accumulation over vast areas by peatland bryophytes, with growth often equivalent to around 10 mm yr^{-1} [*Loisel et al*., 2012].

When accounting for the contribution made to isotopic signals, it is also important to recognize the difference between long-term adaptation, and short-term acclimation, to contrasting growth conditions. Thus *S. ruralis* and *M. polymorpha*, a moss and a liverwort representative, are both genetically adapted for growth in exposed locations, as shown by the capacity to maintain high assimilation rates in light intensities equivalent to full sunlight (2000 μmol photons m^{-2} s^{-1}; Figures 2a and 2c). In comparison, the *C. aciphyllum* material was gathered on Signy Island (60°S) where light levels above 1000 μmol photons m^{-2} s^{-1} are unusual [*Davey and Rothery*, 1996], and due to the high levels of summer cloud only 40% of incident radiation received reaches the ground [*Schroeter et al*., 2010; *Walton*, 1982]. Consequently, *C. aciphyllum* light saturation was measured at a relatively low value, approximately 500 μmol photons m^{-2} s^{-1}, consistent with the long-term adaptation to low light. The maximum assimilation rate of *C. aciphyllum* measured in this study (8 μmol CO_2 g^{-1} DW h^{-1} or 92 μg CO_2 g^{-1} DW h^{-1}) was very similar to field measurements made on Signy Island of approximately 100 μg CO_2 g^{-1} ash free DW h^{-1} [*Davey and Rothery*, 1997]. It should also be recognized that the instantaneous rates of photosynthesis under natural conditions, encompassed by the light and CO_2 response measurements, endure for markedly different temporal periods because of the water retention characteristics of moss and liverwort surfaces, tissue, and colonies. Moss leaves, with a high surface area exposed to the atmosphere, low volume and minimal diffusion resistance are tightly coupled to the environment, with the colonial structure (e.g., carpet, cushion, or hummock) important for any prolonged maintenance of functional water contents. In contrast, the thalloid liverworts lie parallel to the ground and both *M. polymorpha* and *C. conicum* have thick, partially decay resistant epidermal tissues [*Graham et al*., 2004] that may limit both the rate of water loss and the maximum CO_2 diffusion rate, buffering environmental changes and tempering extreme physiological responses.

4.2. Instantaneous Isotopic Discrimination ($\Delta^{13}C$) and Organic Matter Composition

Across bryophytes, the extent of instantaneous carbon discrimination during photosynthesis over a drying curve can be divided into three phases: first, diffusion limitation (as surface water evaporates); second, optimal hydration that is associated with maximum assimilation; and third, desiccation-related metabolic compromise [*Meyer et al*., 2008; *Rice and Giles*, 1996; *Williams and Flanagan*, 1996]. These phases are apparent in all four of the species analyzed, although their range and extent varied substantially reflecting the different physiological and environmental sensitivities and constraints (Figures 3a and 3b). For ease of comparison, the extent of fractionation was also derived as an "equivalent" $\delta^{13}C$, to indicate the offset which would occur relative to the PDB standard (Figure 3c).

Under these conditions of relatively high ambient temperature and evaporative demand within the gas exchange cuvette, *C. aciphyllum* was photosynthetically active for the shortest period of time while maintaining the highest RWC range of 400–1100%, with maximum discrimination (more negative effective $\delta^{13}C$) measured when RWC was 800%. This may be attributable to the species natural colonial habit, as the moss

bank structure facilitates the maintenance of a consistent, high RWC throughout the growing season [*Gimingham and Smith*, 1971; *Ochyra et al*., 2008] which would be maintained for much longer periods in situ.

In contrast, desiccation resistant *S. ruralis* was photosynthetically active for longer than *C. aciphyllum* at lower range of RWC values (100–300%). The xerophytic morphology and ecology of *S. ruralis* is directly adapted to survival in exposed habitats; even after long periods of dormancy in response to drought, photosynthesis is rapidly resumed whenever sufficient water and light are available [*Hamerlynck et al*., 2002; *Oliver et al*., 2000]. The highest experimental discrimination value measured in *S. ruralis* was 27‰, approaching the maximum possible in a C_3 plant [*O'Leary*, 1988], which suggested that there was negligible diffusion limitation following the evaporation of the capillary water within the single-celled photosynthetic lamina (Figure 1d), and that the majority of the fractionation was accounted for by Rubisco carboxylation. The close agreement between real-time discrimination and organic $\delta^{13}C$ for the mosses suggests that both are well coupled to ambient atmospheric CO_2 supply, an important generalization for interpreting compound-specific variations.

For the liverworts, the extended periods of carbon gain and relatively constant, high instantaneous $\Delta^{13}C$ showed that both species maintain photosynthesis over a wide range of tissue RWC. Relative to mosses, the liverworts have a greater tissue volume to buffer changes in water content, as shown by the long curvilinear regions between the points of full turgor and turgor loss on the pressure-volume curves [*Proctor et al*., 1998]. The offset in instantaneous $\Delta^{13}C$ values between *M. polymorpha* and *C. conicum*, respectively, 17‰ and 22‰, suggests higher overall diffusion limitation in the former. There was also an equivalent offset in $\delta^{13}C_{OM}$ between *M. polymorpha* and *C. conicum* (Figure 3c; Table 1), but the more negative organic signals (relative to real-time signals) are possibly the consequence of two factors: either an increased contribution from isotopically depleted respired CO_2 in source air ($\delta^{13}C_a$) or secondary fractionations associated with respiration and metabolism [*Cernusak et al*., 2009]. *C. aciphyllum*, with the least negative $\delta^{13}C_{OM}$, would be predicted to have the lowest $\Delta^{13}C$ value, which was consistent with that measured experimentally. It would also be possible to use ^{18}O signals in tissue water to explore the extent of tissue water storage and turnover for comparison between mosses and liverworts [*Cernusak et al*., 2008; *Royles et al*., 2013].

The higher absolute N% and lower C:N ratios in *S. ruralis* and *C. conicum* (Table 1) suggest a higher investment in photosynthetic proteins, including Rubisco, which is consistent with the substantially higher assimilation rates measured in these species compared to *C. aciphyllum* and *M. polymorpha*. The high investment into photosynthetic apparatus provides an adaptive advantage for *S. ruralis* and *C. conicum* in the different habitats they colonize, by facilitating short, intense bursts of CO_2 assimilation and metabolic activity during brief periods of optimal environmental conditions.

4.3. Temporal Variation in $\delta^{13}C_{OM}$ and $\Delta^{13}C_{OM}$ Measured in Signy Island Peat Cores

The relevance of these physiological controls are further highlighted when, rather than using instantaneous measurements (online $\Delta^{13}C$ or assimilation) or integrated signals (C:N or $\delta^{13}C_{OM}$), as species-specific indicators, the variation in discrimination is considered over time or space. The moss-peat core from Signy Island showed temporal variation in *C. aciphyllum* $\delta^{13}C_{OM}$ (Figure 5a). The dependence of the composition of the organic matter on the isotopic composition of atmospheric CO_2 is clearly evident in the measured isotopic depletion in $\delta^{13}C_{OM}$ since the increased burning of fossil fuels has altered source $\delta^{13}C_a$ [*Francey et al*., 1999]. This demonstrates the importance of taking into account the isotopic composition of the source CO_2 inputs (see discussion above), as this temporal variation in $\delta^{13}C_{OM}$ due to variation in $\delta^{13}C_a$ has also been measured in lichens [*Máguas and Brugnoli*, 1996], another group of nonvascular plants, and is clearly evident during isotopic analysis of tree rings [*McCarroll and Loader*, 2004]. As Signy Island has cold year-round temperatures that suppress respiration rates from the minimal microfauna and flora present and is generally windy with no overgrowing canopy to prevent atmospheric mixing, it is reasonable to assume that the isotopic composition of source CO_2 has been equal to atmospheric CO_2 over the period of moss growth and peat-bank development. So, in this instance, using the atmospheric CO_2 values derived from tree rings [*McCarroll and Loader*, 2004] and ice-cores [*Francey et al*., 1999] was applicable as a correction factor between measured $\delta^{13}C_{OM}$ and source-independent discrimination.

Over time, $\Delta^{13}C_{OM}$ varied between 14.0‰ and 15.5‰ (Figure 5b): less than the maximum $\Delta^{13}C_{OM}$ measured experimentally in *C. aciphyllum* of 19‰, but within the range of both the diffusion limited and metabolically

limited values (Figure 3). The integrated $\Delta^{13}C_{OM}$ value over several seasons is expected to be less than the maximum value measured instantaneously under optimum conditions, as the measured $\Delta^{13}C_{OM}$ incorporates carbon assimilated at times in the season when the conditions are suboptimal, along with that fixed under optimal conditions [*Royles et al*., 2012]. The range of $\Delta^{13}C_{OM}$ values measured over time (1.5‰) was substantially lower than that measured instantaneously (12‰) partly due to the seasonal integration effect and the minimal contribution that net assimilation makes to $\Delta^{13}C_{OM}$ values at the extreme RWC values, and partly due to the colonial buffering effect of the large, extensive moss-peat banks [*Gimingham and Smith*, 1971; *Ochyra et al*., 2008].

4.4. Spatial Variation in $\delta^{13}C_{OM}$ Measured in Peruvian Epiphytic Bryophytes

In addition to temporal variation, the isotopic shift of over 4‰ in $\delta^{13}C_{OM}$ measured in epiphytic mosses and leafy liverworts across the 3000 m elevation Amazon–Andes gradient in south-eastern Peru (Figure 6) demonstrated the importance of considering contemporary spatial and topographical variation during the interpretation of $\delta^{13}C_{OM}$. Despite the taxonomic differences, the morphological similarity between most mosses and leafy liverworts (Figures 1b and 1d), with single rows of photosynthetic cells in contact with the external water and no structural restrictions to evaporative losses, largely determines $\delta^{13}C_{OM}$ within microclimates, hence the nonsignificant difference in values when the two groups were compared (Figure 6a). With a high abundance of mosses and liverworts in montane tropical cloud forests [*Romanski et al*., 2011] and close coupling to the environment these epiphytes can potentially provide important environmental markers. The most depleted $\delta^{13}C_{OM}$ values, around −31‰, were measured in samples from the lowlands, where higher temperatures and evaporative demand minimizes the extent of diffusion limitation. Within the observed cloud immersion zone, the persistently present external water layer on the bryophyte surfaces increased diffusion limitation and CO_2 uptake and lowered discrimination against $^{13}CO_2$. As a consequence, the most enriched $\delta^{13}C_{OM}$ values were measured within the montane cloud forest at 2000–3200 m asl. The trend in $\delta^{13}C_{OM}$ reflects those of temperature, relative humidity, and tissue-air vapor pressure difference [*Ménot and Burns*, 2001; *Waite and Sack*, 2011], all drivers of evaporation, which was reduced at higher altitude and particularly within the cloud immersion zone.

5. Conclusions and Implications

Bryophytes make a significant contribution to global carbon sequestration and storage, whether as living tissues in tropical forest epiphytes or temperate and polar mires, or as peat-bank reserves. The goal of this study was to demonstrate the underlying physiological constraints which determine carbon isotope signals in contrasting life forms, with a view to explaining the potential variation that could be found within compound-specific signals. For mosses (and probably also leafy liverworts), with finely divided photosynthetic lamina, the rapid evaporation from surfaces allows rapid shifts from diffusion-limited, low carbon isotope discrimination conditions to optimal photosynthesis with high $\Delta^{13}C$ (effectively more negative $\delta^{13}C$), prior to desiccation and biochemical limitation. The close agreement between real-time $\Delta^{13}C$ and $\delta^{13}C_{OM}$ in mosses from temperate and polar habitats suggest that the majority of carbon gain is undertaken at optimal tissue water contents, and that temporal signals associated with paleohistorical peat deposits or spatial variations along climatic gradients faithfully reflect the extent of surface liquid water limitation. In contrast, liverworts with pronounced thalloid gametophytes maintain $\Delta^{13}C$ across a much wider range of environmental conditions and tissue water contents, and while shifts between species are consistent with diffusion limitation in natural habitats, it is likely that an additional offset in $\delta^{13}C_{OM}$ implies that secondary fractionations and respiratory source inputs might complicate the interpretation of compound-specific signals in these denser tissues.

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