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Bryophyte stable isotope composition, diversity and biomass define tropical montane cloud forest extent

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Liverworts and mosses are a major component of the epiphyte flora of tropical montane forest ecosystems. Canopy access was used to analyse the distribution and vertical stratification of bryophyte epiphytes within tree crowns at nine forest sites across a 3400 m elevational gradient in Peru, from the Amazonian basin to the high Andes. The stable isotope compositions of bryophyte organic material ($^{13}C/^{12}C$ and $^{18}O/^{16}O$) are associated with surface water diffusive limitations and, along with C/N content, provide a generic index for the extent of cloud immersion. From lowland to cloud forest $\delta^{13}C$ increased from −33‰ to −27‰, while $\delta^{18}O$ increased from 16.3‰ to 18.0‰. Epiphytic bryophyte and associated canopy soil biomass in the cloud immersion zone was estimated at up to 45 t dry mass ha^{-1}, and overall water holding capacity was equivalent to a 20 mm precipitation event. The study emphasizes the importance of diverse bryophyte communities in sequestering carbon in threatened habitats, with stable isotope analysis allowing future elevational shifts in the cloud base associated with changes in climate to be tracked.

1. Introduction

Rapid rates of environmental change and population growth are threatening the ecological equilibrium of tropical ecosystems [1], particularly for tropical montane cloud forests (TMCF) [2,3]. Lowland deforestation and increasing temperatures are expected to cause an up-slope shift in the cloud base and thereby disrupt the unique climatic conditions of TMCF formations [4,5]; however, the exact location of the cloud base is difficult to identify. TMCFs are biodiversity hotspots, with a high level of endemism and genetic diversity [4,6–8], but only comprise a small proportion of tropical montane forest area (6.6%) [9]. The specialized TMCF epiphyte flora is dominated by bryophytes (primarily liverworts and mosses): basal land plant groups that cannot control vegetative thallus water use via stomata, and require frequent re-wetting through cloud immersion, precipitation and throughfall. TMCF habitats are characterized by cool temperatures and constant high relative humidity [10,11]: interception and deposition from fog can contribute over 75% of total precipitation [9]. TMCF areas provide important ecosystem services, including protection against soil erosion, stabilization of streamflow and

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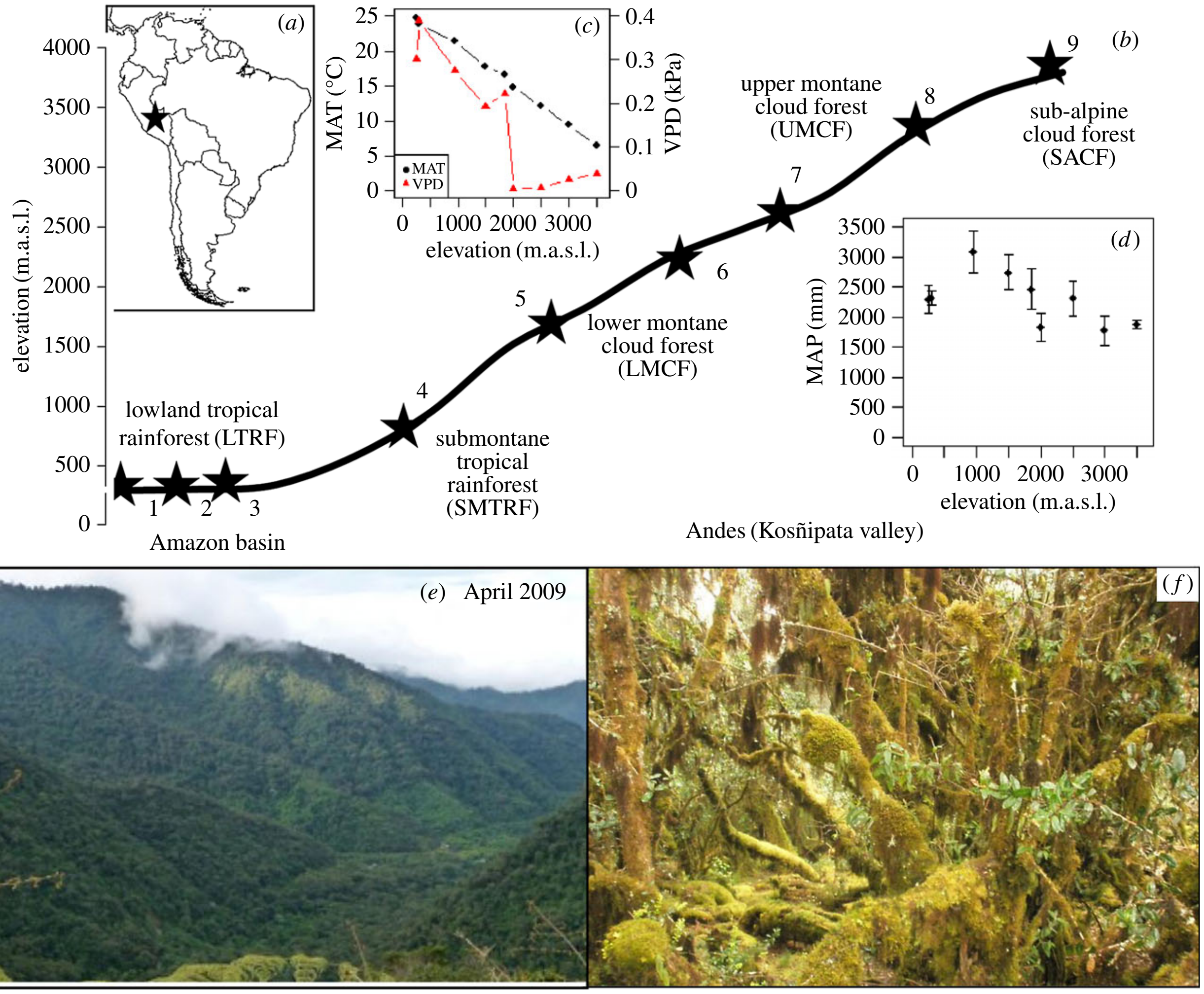

Figure 1. Description of fieldwork sites in Peru. (*a*) Location of fieldwork sites in Peru. (*b*) Elevational positions of sampling sites within their forest types (electronic supplementary material, table S1). (*c*) Mean annual temperature, mean annual vapour pressure deficit and (*d*) mean annual precipitation at nearest meteorological station to each site (electronic supplementary material, table S3). (*e*) View from 1600 m.a.s.l. towards the 1500 m.a.s.l. plots, showing the cloud base/transition zone in April 2008. (*f*) Interior of the sub-alpine cloud forest at 3600 m.a.s.l. (Online version in colour.)

provision of high-quality water for downstream populations, as well as tourism [1,9,12]; however, conservation efforts generally focus on more charismatic taxa in accessible areas.

Due to the difficulties of access and identification, few detailed investigations have been completed on the epiphytic bryophytes of cloud forests. We compiled a detailed inventory of epiphytic bryophytes from an elevational transect in Peru, and exploited the physiological capabilities of bryophytes to establish the current status of these largely unknown, challenging habitats and to assess the future resilience of the forests to potential upshifts in the cloud base. We hypothesize that the stable isotope composition of bryophytes will provide a taxon-independent index for the current lower limit of cloud immersion, and that within the cloud immersion zone (CIZ) epiphytic biomass and associated canopy organic matter forms a significant component of canopy biomass, relative to the low lands. Consequently, epiphytic biomass will be a significant store of carbon and water within the cloud immersion zone, which helps maintain the community against extreme precipitation and disturbance.

2. Material and methods

(a) Field sites

In research allied with the Andes Biodiversity and Ecosystem Research Group (ABERG) [13], nine forest plot sites were sampled three times in 2009 along a roughly 240 km transect from 200 metres above sea level (m.a.s.l.) in the Amazon lowland tropical rainforest (LTRF) to the tree line at 3600 m.a.s.l. on the east slope of the Peruvian Andes (Kosñipata Valley; figure 1; electronic supplementary material, table S1). Meteorological records show a decline in mean annual temperatures (MAT) with elevation (figure 1*c*). Vapour pressure deficit (VPD) declines rapidly to almost 0 kPa at approximately 2000 m.a.s.l. Mean annual precipitation (MAP) varies between 1750 and 3000 mm (figure 1*d*).

(b) Host tree selection and canopy access

During each collecting trip one tree per elevation was selected for canopy access using a 'random-walk' procedure where one set of random numbers (1–360) determined the direction and a second (0–90) the steps walked in the defined direction away from a central point. The host tree was the nearest tree to the endpoint that complied with safety guidelines for canopy access using the double rope technique. Sample collecting was completed at four strata: trunk at head height (HH), lower (LC), mid (MC), and upper crown (UC).

(c) Isotope analysis

During each trip three replicates of each of two most abundant epiphytic moss and liverwort species were collected at each of the four strata in the 27 sampled trees (i.e. 24 samples per accessed tree). Furthermore, two replicate sets of voucher specimens were collected for taxonomic determination. These samples were air dried, catalogued and stored in paper packets for analysis at the Smithsonian Tropical Research Institute (Panama). Isotope analysis was carried out at the Godwin Laboratory (Department of Earth Sciences, University of Cambridge). 0.5–0.75 mg aliquots of dried and milled plant material (188 samples) sealed in silver capsules were used for $^{18}\text{O}/^{16}\text{O}$ analyses by pyrolysis. The analysis was undertaken by EA-IRMS at using a Thermo Finnigan TC/EA attached to a Thermo Delta V mass spectrometer via a ConFlo 3. The precision of analyses is better than 0.4‰. 0.5–0.75 mg aliquots of the plant material (188 samples) sealed in tin capsules were used for $^{13}\text{C}/^{12}\text{C}$ and C : N analysis by combustion using a Costech Elemental Analyser attached to a Thermo MAT 253 mass spectrometer. Precision of analyses is better than 0.1‰ for $^{12}\text{C}/^{13}\text{C}$.

(d) $\delta^{13}\text{C}$ values in bryophytes

A fractionation of −4.4‰ is associated with diffusion of CO_2 in air, while fractionation associated with liquid phase diffusion is around −1.1‰. Consequently, if CO_2 supply was maximally constrained by diffusion, the measured $\delta^{13}\text{C}$ would shift from the atmospheric source CO_2 value (−8‰) to −13.5‰. Conversely, with unlimited CO_2 supply the maximum theoretical fractionation associated with Rubisco, −27‰, could be expressed so the offset including source CO_2 would find tissues of −35‰. The typical range of $\delta^{13}\text{C}$ values for C3 plants is −22 to −30‰. In bryophytes, the external water layer plays a significant role in determining the extent of fractionation against $^{13}\text{CO}_2$ as it limits the rate of diffusion from the atmosphere to chloroplast. Those more limited by diffusion (more closed stomata for C3 vascular plants, more surface liquid in bryophytes) tend to be at the less negative end of the $\delta^{13}\text{C}$ range; those where diffusion is less limiting (more open stomata for C3 vascular plants, less surface liquid in bryophytes) tend to have more negative $\delta^{13}\text{C}$ values.

(e) Epiphytic cover and surface organic matter collections

Bryophyte epiphytic diversity and biomass were quantified at the four canopy strata using a fixed-effort approach in which one hour was spent collecting all the visible species/morphotypes and all epiphytic material was removed from two 15 × 15 cm quadrats, giving a total of 24 quadrats at each elevation. Relevant botanical, ecological and biometric details (circumference of trunk at breast height (cbh), tree height (ht_{tot}), height to first branch (ht_1), number of principal branches (n) and lengths of principal branches (ht_{br})) about the host tree were recorded as well as percentage cover of each component. Plot census data of all trees were provided by ABERG for the Andean plots (950–3600 m), and from ACCA (Asociación para la Conservación de la Cuenca Amazónica), Dr Swamy (Duke University) and RAINFOR (University of Leeds) for the lowland plots (200–300 m). Tree surface areas were calculated from biometric measurements and allometric equations (electronic supplementary material, table S2 and figure S1). Epiphyte specimens were separated into life-form assemblages and morphotypes, for subsequent naming to family or, where possible to genus and species, using macro- and micro-morphometric analyses and keys [14,15] and comparisons to herbarium reference collections.

The organic matter in each sample quadrat was removed, bagged, weighed ('field weight'), and while fresh, divided into its components. In total 24 organic matter samples were collected at each elevation and 216 along the entire transect. Prior to drying, each organic matter component was used for laboratory-based measurements of maximum water content (MWC), defined as

$$\text{MWC} = 100 \times \frac{\text{sw} - \text{dw}}{\text{dw}}, \tag{2.1}$$

where sw is saturated weight of biomass, and dw is dry weight of biomass.

Saturated weight (sw) was obtained by submerging the organic matter in water for 30 min, leaving it to drip for 30 min and subsequently weighing. Dry weight (dw) of each sample was determined after drying in an oven at 70°C for 48–72 h.

(f) Tree allometry and calculation of tree surface area

Mean values of total tree height (ht_{tot}), dbh and the calculated A_{host} of three host trees at each elevation are listed in electronic supplementary material, table S2. To simplify the calculations of surface area of each host tree (A_{host}) we assumed that trees had a modular structure, composed of a cylinder (trunk to first branch) with other principal branches represented as a series of cones. Using the measured host tree parameters, the surface area of each host tree was calculated as

$$\begin{aligned} A_{\text{host}} &= \text{ht}_1 \times \text{cbh} \times \sqrt{(\text{ht}_{\text{tot}} - \text{ht}_1)^2 + \left(\frac{\text{cbh}}{2\pi}\right)^2} \\ &\quad + n \times \frac{c_{\text{br}}}{2} \times \sqrt{(l_{\text{br}})^2 + \left(\frac{\text{cbh}}{2\pi}\right)^2}, \end{aligned} \tag{2.2}$$

where ht_{tot} is total tree height, ht_1 is height from ground level to first principal branch, l_{br} is length of principal branches, cbh is circumference at breast height, c_{br} is average circumference (measured at base) of principal branches and n is the number of principal branches.

By correlating the calculated surface areas (A_{host}) with dbh (derived from measured cbh) of all host trees a 'tree A-dbh model' was derived from the best-fit regression equation (equation (2.3), electronic supplementary material, figure S1). Subsequently, in conjunction with the available plot census data ($\text{dbh}_{\text{tree}(i)}$) our model was used to compute the tree surface area ($A_{\text{tree}(i)}$) of each tree (i) with dbh > 10 cm in each forest plot along the Amazon–Andes transect:

$$A_{\text{tree}(i)} = -5.16 + 6.68^{0.006\times\text{dbh}_{\text{tree}(i)}} + 6.63^{0.03+\,\text{dbh}_{\text{tree}(i)}}, \tag{2.3}$$

where $\text{dbh}_{\text{tree}(i)}$ is the diameter of trunk at breast height of each tree (i) with dbh > 10 cm in each plot.

Finally, total tree surface area ($A_{\text{plot}(N)}$) for each 1 ha plot was the sum of the modelled tree surface areas of each tree ($A_{\text{tree}(i)}$) (electronic supplementary material, table S2),

$$A_{\text{plot}(N)} = \sum_{i=1}^{N} A_{\text{tree}(i)}. \tag{2.4}$$

(g) Upscaling epiphytic organic matter

In order to upscale the epiphytic organic matter measured in each sample quadrat (bmQ) to total surface organic matter per tree (bmT) it was necessary account for the percentage cover of the individual components on the entire host tree (cT) (figure 4*a*). By relating the recorded epiphytic cover in each quadrat (cQ) to the visually estimated percentage cover of each organic matter component on the entire host tree (cT) a factor cf was derived for each host tree:

$$\text{cf}_j = \frac{\text{cT}}{\text{cQ}_j}, \tag{2.5}$$

where cf_j is cover factor, cT is percentage epiphytic cover on entire host tree, cQ is percentage epiphytic cover in 15 × 15 m^2 quadrat.

Subsequently, the cf was used to compute the mass of organic matter on each host tree (bmT):

$$\text{bmT} = \text{bmQ} \times \text{cf}, \tag{2.6}$$

where bmT is epiphytic biomass of entire host tree and bmQ is epiphytic biomass in 15 × 15 m^2 quadrat. Using the cover factor

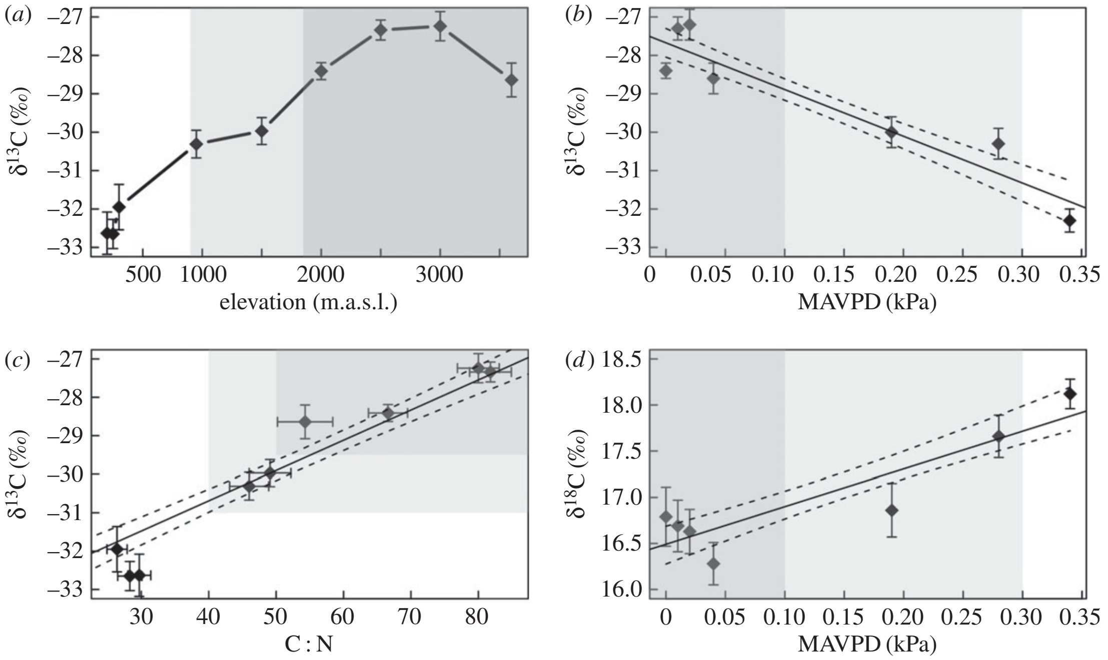

Figure 2. Stable isotope composition of bryophyte organic matter across elevational transect. (*a*) Mean δ^{13}C discrimination measured in epiphytic bryophyte organic matter as a function of elevation ($n = 188$). (*b*) Mean δ^{13}C as a function of mean annual vapour pressure deficit (MAVPD). (*c*) Mean δ^{13}C as a function of C/N ratio of bulk epiphytic bryophytes along elevational transect. (*d*) Mean δ^{18}O as a function of MAVPD. Shading represents the *k*-means data clusters: white background corresponds to locations where all data falls into cluster 1, dark grey corresponds to elevations with all data falling in cluster 2, and the light grey represents locations with a mixture of clusters: clusters and shading also represent extent of persistent cloud immersion from none in white to transitional in light grey and permanent in dark grey.

(cf) prevented any under or over represented biomass components in the individual sample quadrats giving rise to erroneous tree-level estimates (bmT), which were subsequently used for up scaling of total epiphytic surface organic matter at the stand-level. By using the 'Tree A-dbh model' (equation (2.3)) (electronic supplementary material, figure S1) and thus knowing the total tree surface area per 1 ha of land (A_{plot}) at each elevation (electronic supplementary material, table S2) we were able to express the tree-level biomass estimates (bmT) at the stand level (i.e. as tonnes of dry epiphytic organic matter per ha of forest; figure 5*b*).

3. Results

(a) Using stable isotope composition as a climate index

Independent of species, bulk plant δ^{13}C became less negative from the lowlands (−33‰) to 3000 m.a.s.l. (−27‰), before becoming more depleted at the highest elevation site (3600 m.a.s.l., −29‰; figure 2*a*). Less depleted δ^{13}C values were associated with lower mean annual vapour pressure deficits (MAVPD; figure 2*b*; Cor $= -0.66$, $r^2 = 0.43$, $p < 0.0001$, d.f. $= 160$), and δ^{13}C was inversely correlated with nitrogen content, shown as C/N ratio (figure 2*c*; Cor $= 0.64$, $r^2 = 0.40$, $p < 0.0001$, d.f. $= 185$). Organic δ^{18}O was also correlated with MAVPD (figure 2*d*; Cor $= 0.53$, $r^2 = 0.28$, $p < 0.0001$, d.f. $=$ 187) with the humid, high elevation sites experiencing lower evaporative enrichment than the Amazon basin (approx. 17‰, compared with greater than 18‰).

In order to establish any significant divisions in the bryophyte characteristics, *k*-means cluster analysis was completed to divide the stable isotope and CN data into two clusters, which was determined to be optimal from 30 indices. Consequently, all bryophyte samples collected at elevations lower than 940 m fell into group 1, and all those collected at elevations higher than 2000 m.a.s.l. in to group 2. For the site at 950 m.a.s.l., 21 of the 24 samples fell into group 1, whereas at 1500 m.a.s.l. 10 of the 24 samples fell into group 2. This clustering, which is taxon-independent, corresponds to the extent of diffusion limitation experienced by the epiphytic bryophytes, and thus acts as an index of the extent to which they experience cloud immersion. The division between the two clusters coincides with the significant drop in VPD measured at 2000 m.a.s.l. (figure 1*c*). At low elevation, there is high discrimination against $^{13}CO_2$ (more negative δ^{13}C values), and more evaporative enrichment of ^{18}O, indicative of rapid diffusion and a leaf surface with a minimal or absent external water layer: at elevations less than 1000 m the bryophytes do not experience cloud immersion. Above 2000 m the isotope values indicate less discrimination against $^{13}CO_2$ (less negative δ^{13}C values), and less evaporative enrichment of ^{18}O in leaf organic matter, corresponding to diffusion limitation due to the constant presence of an external water layer through cloud immersion. With 87.5% samples at 1000 m.a.s.l. in cluster 1, the elevation is likely to be largely cloud free, but the 1500 m.a.s.l. site with 42% values in cluster 1 and 58% in cluster 2, corresponds to transitional zone of cloud immersion that is periodically inundated but does not experience the persistent cloud immersion of the higher elevation sites. Thus the stable isotope compositions of epiphytic bryophytes provide a quantitative, taxon independent definition for the extent of the cloud immersion zone, the transitional cloud zones and regions which never experience cloud immersion, which is relevant to the interpretation of epiphyte biodiversity patterns.

(b) Bryophyte biodiversity within tropical forest canopies

Overall, the taxonomic analysis revealed 221 liverwort spp. (from 75 genera and 25 families) and 84 moss spp. (from

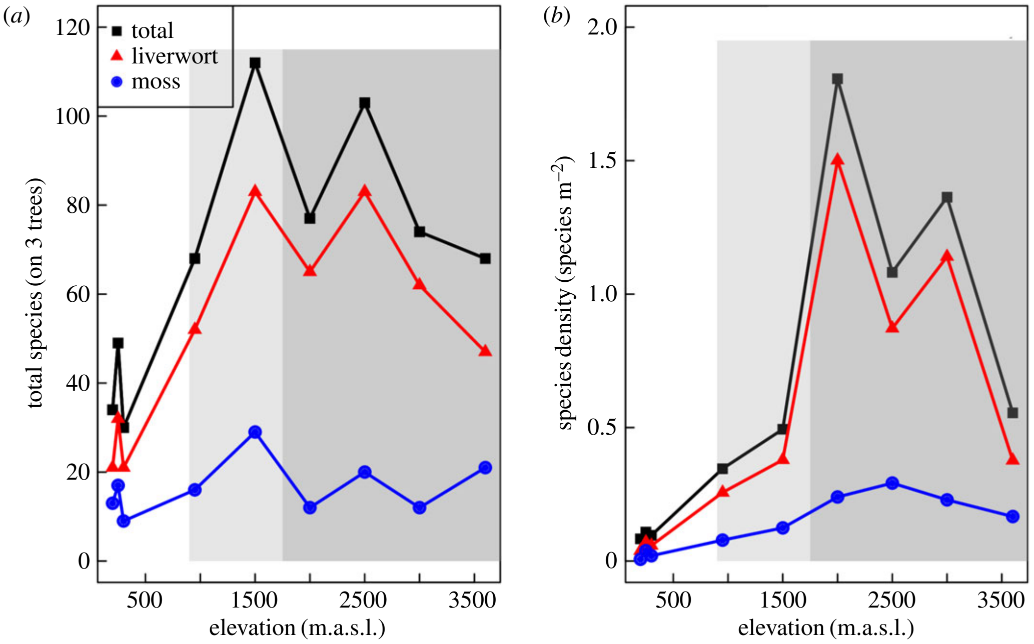

Figure 3. Diversity of epiphytic bryophytes in relation to elevation and tree size. (*a*) Species total collected from three trees at each sampling location. (*b*) Species total from three trees at each elevation normalized per m^2 bark surface area. Liverworts (triangles), mosses (circles) and total (squares). Shading represents the clusters of isotope data (figure 2), which relates to the extent of persistent cloud immersion from none in white to permanent in dark grey.

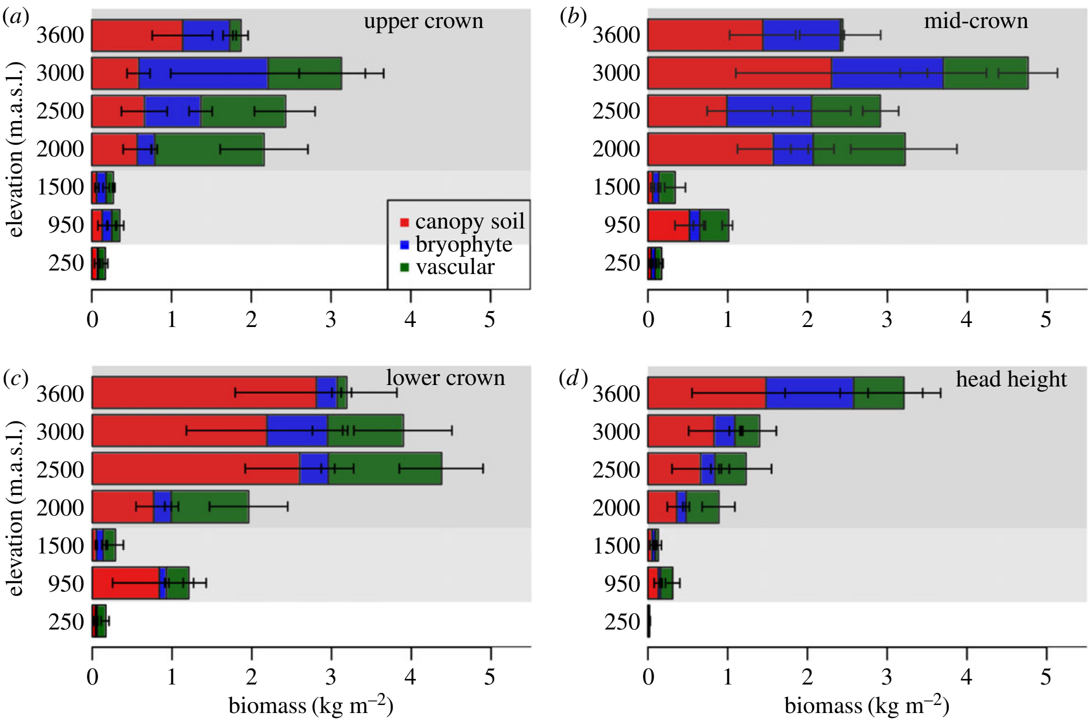

Figure 4. Distribution of epiphytic biomass components, separated between tree strata, across the elevational gradient: (*a*) upper crown (UC), (*b*) mid-crown (MC), (*c*) lower crown (LC) and (*d*) head height (HH). Shading represents the clusters of isotope data (figure 2), which relates to the extent of persistent cloud immersion from none in white to permanent in dark grey.

60 genera and 21 families; electronic supplementary material, table S4). This exceeds the 171 spp. from 16 families recently reported in the Eastern Andes of Central Peru [16]: this was without canopy access, showing the importance of collecting at the tops of the trees, despite the logistical difficulties it entails. Together, these two studies highlight the very high diversity of bryophytes, particularly in cloud-forest formations.

When species richness was expressed independent of tree size, the highest diversity of epiphytic bryophytes across the three sampled trees was identified at 1500 m.a.s.l. (114 spp., Lower Montane Cloud Forest (LMCF)): the climatically sensitive transition into the full cloud immersion zone (figure 3*a*). When normalized by tree surface area, three sites (2000 m.a.s.l., 2500 m.a.s.l., 3000 m.a.s.l.) with persistent cloud immersion had the highest epiphytic bryophyte species density (figure 3*b*), over twice that of the sites in the transitional cloud zones (1500 m). The highest-elevation site (3600 m) has lower species density: despite having isotopic signals compatible with full immersion, at this high elevation the cooler climate is likely to limit the extent of bryophyte colonization.

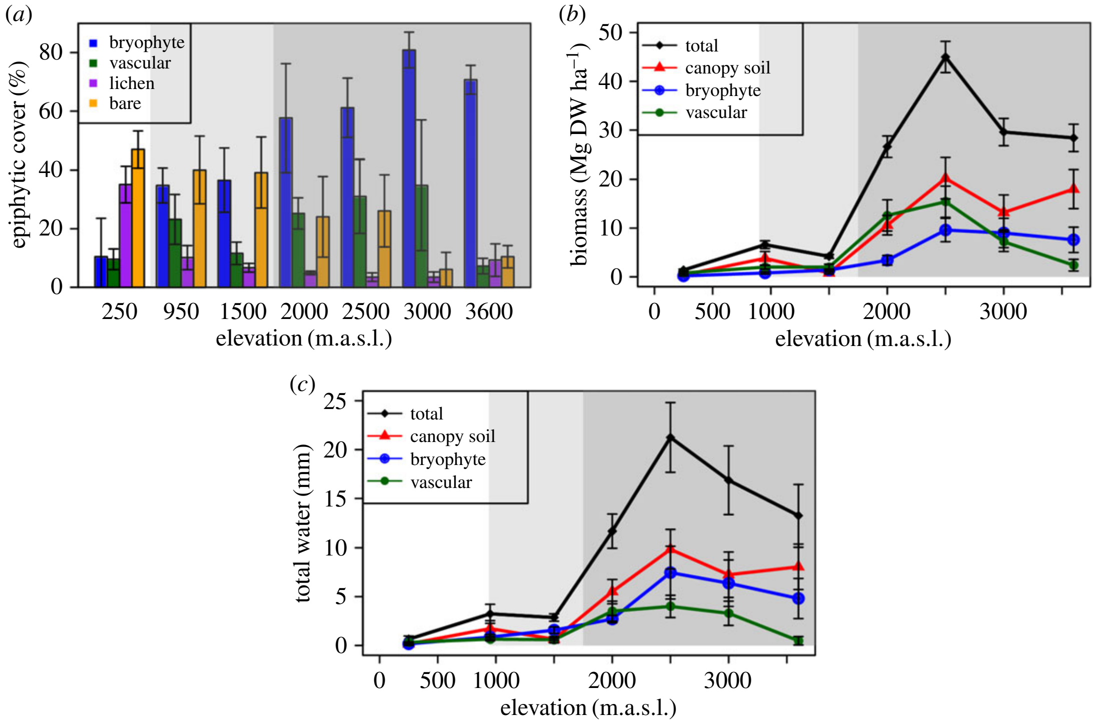

Figure 5. Contribution of epiphytic bryophytes to carbon and water storage. (*a*) Observed epiphytic cover of tree surfaces along the elevational gradient separated by component; $n = 3$ trees per elevation; total epiphytic cover is greater than 100% when biomass components overlap. (*b*) Estimated epiphytic biomass (dry weight) per hectare of forest. (*c*) Potential water storage capacity of epiphytic biomass calculated from saturated water holding capacity. The forest formations at each elevation are defined in the figure 1, which relates to the extent of persistent cloud immersion from none in white to permanent in dark grey. Error bars represent s.e. of mean.

The sites below 1000 m did not experience cloud immersion, and bryophyte species diversity decreased gradually with elevation from the sub-montane forest to the Amazon lowlands (figure 3*b*). The lowest species diversity was recorded in the lowland sites, with only 29 spp. in the terrace forest (300 m.a.s.l.) and 49 spp. in the flood plain forest (250 m.a.s.l.; figure 3*a*). These values are comparable to the 46 and 86 liverwort species recorded from four trees in the Peruvian lower and upper montane forest, respectively [17].

Liverwort taxa were dominant throughout the transect, contributing over 80% of species recorded in the upper montane cloud forest (electronic supplementary material, table S5). In the lowlands, over 95% of the liverworts were from a single family: the Lejeuneaceae (electronic supplementary material, table S6). The Dicranaceae dominated the moss flora of the high elevation forests, but were absent from the lowland Amazonian sites, where the Sematophyllaceae, Calymperaceae, and Neckeraceae were the most diverse families.

(c) Epiphyte organic matter and water retention capacity

The dominance of bryophyte and canopy soil biomass in the MC and LC profile sections within forest with persistent cloud immersion was striking (figure 4*b*,*c*), relative to lower elevation forest. Vascular epiphytic biomass was highest at all tree strata within the cloud immersion zone. The MC and LC epiphytic biomass reached a maximum of roughly 4.5 kg m^{-2} at 3000 m.a.s.l. (figure 4). At the highest elevation site (3600 m), samples at head height (HH) held the highest epiphytic biomass (approx. 3 kg m^{-2}) across the tree profile, elsewhere the epiphyte biomass at HH on tree trunks was much less than the higher canopy strata (figure 4*d*), this may be due to the reduced stature of the canopy at 3600 m.a.s.l., or that the HH strata provides a more sheltered habitat as compared with lower elevations.

In the lowlands, approximately 50% of tree bark remained bare, with highly dispersed and localized epiphytic distributions covering 10–20% of the tree surface (figure 5*a*). Generally increasing with elevation, the highest epiphytic cover of bryophytes (60–80%) and vascular plants (30–40%) coincided within the central cloud immersion zone (CIZ) at 2500–3000 m.a.s.l. At the stand level, maximum epiphyte and canopy soil dry biomass was found at the sites with persistent cloud immersion, equivalent to a total of *ca* 45 Mg DW ha^{-1} at 3000 m.a.s.l. (figure 5*b*). Approximately 50% of the total epiphytic load was composed of canopy soil, which represented the dominant biomass constituent on trees, particularly in the cloud forest. Within the central CIZ, bryophytes and vascular epiphytes each contributed 10–15 Mg DW ha^{-1}; however, outside the CIZ vascular plants constituted less than 5 Mg ha^{-1}. The lowest values were recorded in the lowland tropical rainforest (LTRF), where the development of vascular plants and associated canopy soil was restricted to around 1 Mg ha^{-1}, and bryophytes less than 0.5 Mg ha^{-1}.

Potential water retention capacity (figure 5*c*) was calculated by combining the stand-level estimates of epiphytic biomass (figure 5*b*) with the saturated water capacity of each component, as measured following harvest. The highest water holding capacity by epiphytic biomass was identified at 2500 m.a.s.l. and was equivalent to a 25 mm rain event, with 80% water retained by canopy soil and the living bryophyte mat. Vascular epiphytes (excluding bromeliad tanks) played a minor role in total canopy water storage (less than 5 mm). Potential water storage capacity showed a marked drop below 2000 m.a.s.l. The substantial and diverse epiphytic biomass of the cloud immersion zone can potentially hold 15–25 times more water than the epiphytes and associated organic matter in the Amazon lowlands. The epiphyte colonization as percentage cover (figure 5*a*), biomass combined with canopy soil per unit area (figure 5*b*) and water holding capacity (figure 5*c*) probably reflect the changing gradients of light availability, temperature, evaporative demand and water replenishment within each tree canopy along the elevational gradient.

4. Discussion

Environmental forcing, through increasing atmospheric CO_2, changing temperatures, precipitation frequency and intensity, as well as the occurrence of fire and land use change, is having significant and complex effects on Amazonian ecosystems [18], but these effects are difficult to accurately quantify without a complete understanding of the current status of the entire biome. Tropical montane cloud forests are biodiversity hotspots [4,6–8], yet the bryoflora is poorly characterized [16]. Thus, this detailed assessment identifying over 300 bryophyte species along a 3400 m elevational transect makes an important contribution towards providing a more detailed forest inventory, in addition to quantifying the extent of epiphytic biomass and revealing the potential for use of a stable isotope index to track the cloud base.

(a) Stable isotope composition of bryophytes defines the cloud immersion zone

The more enriched $\delta^{13}C$ values (−26.8 to −27.6‰) at 2500–3000 m.a.s.l. in the Andes were consistent with CO_2 uptake limited by diffusion due to surface liquid water [19], and carboxylation sink strength limited by low N content [20]. For liverwort and moss epiphytes from lowland Amazonia, higher mean annual temperatures lead to increased evaporative demand, and a high MAVPD. Such life forms are likely to cycle quickly between dry and rehydrated states, and lose surface water rapidly. In the lowland forests, bryophytes experience less liquid phase diffusion limitation and maximize photosynthetic carbon uptake (and have higher carboxylation capacity as N content) leading to higher carbon isotope fractionation and more negative $\delta^{13}C$ values [19]. This interpretation is supported by epiphyte $\delta^{18}O$ signals, with higher evaporative enrichment in tissue water leading to photosynthate incorporated into organic material during growth being relatively enriched in ^{18}O in the lowland epiphytes. One further climatic factor contributing to the differential in $\delta^{18}O$ signals between the high and lowlands is the possible extent of water vapour exchange under high humidity and low MAVPD conditions in the CIZ zone which shifts tissue water, and newly synthesized organic material, towards that of the source precipitation input signal [21]. The partial pressure of CO_2 declines with altitude, and is associated with less negative $\delta^{13}C$ values at altitude in vascular plants (approx. 1.1‰ per 1000 m [22]) and bryophytes (1.4–1.8‰ per 1000 m [23]). While changes in partial pressure contribute to the observed 6‰ shift in measured $\delta^{13}C$, it is insufficient to account for all the variation, nor explain the nonlinear response of $\delta^{13}C$ to altitude.

The separation of stable isotope and CN values into clusters that are physiologically explicable by the absence, intermittent presence or permanent presence of a cloud layer, and comparable with the decline in VPD, upholds our first hypothesis. As the isotope signals are niche- rather than taxon-specific, as the cloud base moves, the isotope composition of new bryophyte growth will reflect that of the new cloud-free conditions. A change in stable isotope composition therefore has the potential to reveal a shift in the cloud base even before any change in diversity associated with the new environmental conditions.

(b) Epiphytic bryophytes: the hidden diversity of cloud forests

Being hidden in the canopy [24] and consequently often only accessible through tree falls [16], epiphytes are rarely included in forest inventories [25] and therefore much bryophyte diversity remains unidentified and unquantified. Following extensive tree climbing and detailed sampling the identification of 130 species of bryophyte within the cloud immersion zone, almost half of the total along the whole transect and comparable to the diversity of Colombian cloud forests (135 spp. [26]), while exceeding that of Costa Rica (96 spp. [27]), emphasizes the critical role of cloud forests as biodiversity hotspots. The northern Andes are a liverwort hotspot, with high endemism, hence liverworts dominate moss species particularly at high altitudes with continuous water availability and high humidity [28]. Liverworts are generally more susceptible to moisture fluctuations than mosses, which are able to thrive in more disturbed, open and varied forests [28,29].The elevational trends of species richness, taxon diversity and life-form dominance along the Amazon–Andes gradient show that distinct bryophyte assemblages were found in the zones in which cloud cover was absent, persistent and transitory, as defined by the stable isotope composition.

(c) Epiphytic bryophytes are significant biomass components in high elevation cloud forests

Ecosystem biomass calculations usually focus on trees [30,31], and the contribution of epiphytes to canopy structure and function is difficult to quantify via remote sensing [32]. Across the Peruvian transect, tree biomass declines above 1500 m.a.s.l. [13,33]; however, vascular epiphytes have a high abundance in the wetter, cloudier conditions of the mid-elevation forests, as was previously observed in Mexico [34], Costa Rica [35], China [36] and across the Andes [37]. In contrast, this study shows that biomass of epiphytic bryophytes and arboreal canopy soil peaked at cooler, higher elevations. These differences in biomass accumulation highlight the distinct optimal climatic conditions required for maximum productivity of bryophytes and vascular plants [38,39]. Within trees, the highest accumulation of epiphytic biomass was found in the lower canopy as the large branches and open branch forks facilitate the accumulation of canopy soil and subsequent establishment and growth of plants [40]. Smaller epiphytes with less associated canopy soil were typical of the higher canopy strata, due to the reduced area available for colonization.

Previous estimates of above ground biomass in the same Peruvian cloud forest of 51–89 Mg C ha^{-1} above 2000 m.a.s.l. [41] and a mean for 3000–3630 m.a.s.l. of 63.4 Mg C ha^{-1} [25] do not include epiphytic biomass. Assuming that the measured epiphytic biomass is 50% carbon, the 30–50 Mg C ha^{-1} estimated above 2000 m.a.s.l. is a significant, and unaccounted for component of above-ground biomass. Carbon budgets for the cloud forests remain incomplete without inclusion of epiphytic biomass, and counter to some extent the declining net productivity of trees between sub-montane and montane forests [41].

(d) Water holding capacity of bryophytes

In addition to a role in the cloud forest carbon cycle, the water holding capacity of epiphytic bryophytes is significant. In the cloud forests of La Réunion Island (Mascarenes), the water holding capacity of the epiphytic bryophytes was calculated to be equivalent to a 5 (±2) mm rain event [42], similar to the potential values estimated in this study. Moisture derived from fog precipitation and stored by bryophytes has been estimated to exceed total annual rainfall by 50–90% [43–46], with the storage function of epiphytic biomass more important in seasonally drier areas, such as the SACF (3600 m.a.s.l.). Thus, it can be expected that the interception and subsequent slow release of moisture during the drier periods can make an important contribution to the hydrological conditions of this forest, where it is unlikely that the groundwater reservoir can sustain the observed dry-season river flow [47], and changes to the hydrological regime can increase the risk of landslides.

(e) Ecosystems at risk: disturbance of cloud forests

TMCF ecosystems are dynamic, with landslides removing around $6\ \mathrm{tC\ km^{-2}\ yr^{-1}}$ of vegetation from the Kosñispata Valley [48]. In 2010 a single storm triggered 185 landslides [48], most of which were below 1800 m.a.s.l., coinciding with the change in stable isotope composition (figure 2), a significant decrease in epiphytic biomass (figure 3) and a notable change in forest architecture. Deforestation of the lowland forests is predicted to lead to a reduction in cloud generation and consequently an irreversible loss of the adjacent cloud forest [5], which would have negative effects on canopy biota [49]. The current study predicts that an upslope migration of the cloudbank in response to environmental change would lead to a significant decrease in epiphytic biomass, reflecting the decline in epiphytic biomass that coincides with the change in stable isotope composition associated with the transition from permanently cloudy conditions, fitting with transplant experiments in which, among a complex set of responses due to the highly heterogeneous nature of the ecosystem, vascular epiphytes moved downslope to warmer, drier environments, were small and had reduced recruitment [50]. Over time, this would have critical knock-on effects on the accumulation of crown canopy soil and establishment of vascular epiphytes, as well as nutrient cycles [51]. By regulating the seasonal release of precipitation in the rainy season, and providing flood and erosion control, epiphyte mats can play a vital role as ‘capacitors’ in montane forest [3,49,52].

Following disturbance it takes approximately 30 years for 100% vegetation cover to return [48], and at least 200 years for the forest to reach maturity [53], though epiphyte recovery can be even slower [54]. Stable isotope analysis of bryophyte organic matter provides a clear index for cloud forest zones and the δ^{13}C-C/N-δ^{18}O relationship can serve as a climatic proxy for monitoring of cloud forest limits over time, especially as the cloud base is predicted to move upslope in response to increasing global warming and anthropogenic activities. The lower photosynthetic rates of cloud forest bryophytes as indicated by the reduced rates of isotope discrimination correspond to slow growth rates and emphasize the particular sensitivity of these species to environmental changes.

Data accessibility. This article has no additional data.

Authors’ contributions. Study was conceived by A.B.H. and H.G. Fieldsite network was set up by Y.M. and M.R.S. Fieldwork and sample processing were carried out by A.B.H. and R.T. Species identification was completed by A.B.H., J.A.G. and N.S.A. Plot census data were provided by W.F.-R., V.S. and J.P.L.F. ABERG meteorological data were compiled by J.M.R. Data analysis was completed by A.B.H. and J.R. Paper was drafted by J.R., A.B.H. and H.G.

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