Bryospheres in oligotrophic headwater streams provide nutrient-dense habitats and dominate stream nutrient cycling

Jessee J. B. Steele[1,4], Audrey N. Thellman[1,5], Olivia K. Vought[2,6], Emma J. Rosi[3,7], Tammy Wooster[3,8], Christopher T. Solomon[3,9], Emily S. Bernhardt[1,10]

[1]Department of Biology, Duke University, Durham, North Carolina USA

[2]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan USA

[3]Cary Institute of Ecosystem Studies, Millbrook, New York USA

E-mail addresses: [4]steele.jessee@gmail.com; [5]Present affiliation: Department of Earth, Marine, and Environmental Sciences, University of North Carolina at Chapel Hill, North Carolina USA, athellma@unc.edu; [6]ovought@umich.edu; [7]rosie@caryinstitute.org; [8]woostert@caryinstitute.org; [9]solomonc@caryinstitute.org; [10]To whom correspondence should be addressed, emily.bernhardt@duke.edu;

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Abstract: Stream bryophytes (mosses and liverworts) are widely recognized as important macroinvertebrate habitats, but their overall role in the stream ecosystem, particularly in nutrient cycling, remains understudied. Hubbard Brook Experimental Forest in New Hampshire, USA, contains some of the most extensively researched streams in the world, yet few studies mention their bryophytes. Perhaps this is because early estimates place bryophyte coverage in these streams at an insignificant 2%. However, data from 2019 show that contemporary coverage ranges from 4 to 40% among streams. To investigate how stream bryophyte cover may be changing over time and influencing stream nutrient stocks, we conducted field surveys, measured the mass of organic and inorganic bryophyte contents, and quantified nutrient uptake with bottle incubations of bryophyte mats. This study marks a novel attempt to map stream bryophyte coverage with estimates of C, P, and N stocks and fluxes. From our 2022 field surveys, we found that median bryophyte coverage varied across streams in the same catchment (0–41.4%) and shifted from just 3 y prior. We estimate that these bryophyte mats stored between 14 and 414 g of organic matter per m^2 of stream in the form of live biomass and captured particulates. Within 12 h of light incubation, 35 out of 36 bryophyte clump samples sorbed peak historical water-column concentrations of PO_4^{3-} as measured in the Hubbard Brook stream chemistry record. In Bear Brook, our scaled estimate of bryophyte mat NO_3^- uptake (2.3 g N/y) constitutes a substantial portion of previously estimated whole-stream NO_3^- uptake (12 g N/y). Cumulatively, our data demonstrate that bryophytes and their associated mineral substrates and biota—known as the bryosphere—are crucial in facilitating headwater stream nutrient cycling. These bryospheres may contribute significantly to interannual variability in stream nutrient concentrations within nutrient-poor streams, especially in climate-sensitive regions.

Key words: bryophyte, aquatic moss, Hubbard Brook, biomass, disturbance, anchor ice, nitrate, phosphate, detritus

Bryophytes (mosses and liverworts) are ubiquitous across many headwater streams, yet they have received little attention from stream ecosystem scientists. Bryophytes are widely recognized for their importance as macroinvertebrate habitat (Englund 1991, Suren 1991, Parker et al. 2007, Alvarez and Peckarsky 2013, Wood et al. 2016, Wulf and Pearson 2017), but there has been very little study of their impact on ecosystem energetics or nutrient cycling. The few studies that do exist found that despite their variable rates of photosynthesis (Fisher and Likens 1973, Ylla et al. 2007), stream bryophytes have greater area-specific uptake rates of P than periphyton (Steinman and Boston 1993) and similar physical sorption rates of P as sediments (Meyer 1979). Mulholland et al. (2000) and Peterson et al. (2001) used an isotopically enriched tracer experiment, ^{15}N-labeled NH_4^+, and found that bryophytes in Walker Branch, Tennessee, USA, had the highest area-specific NH_4^+ uptake rate of any organic substrate and retained 34% of this assimilated N^{15} tracer 75 d after the enrichment. Despite the implications of these early studies, there are few contemporary studies that directly consider bryophyte nutrient stocks and their role in nutrient cycling (Bowden et al. 1994, Finlay and Bowden 1994, Arscott et al. 1998).

This gap in the literature is somewhat surprising given the tremendous volume of research on the impact of bryophytes on global C and N cycling (Turetsky 2003), especially in peatlands (Gorham 1991, Yu 2012, IPCC 2014). An abundance of literature has demonstrated that bryophytes facilitate N cycling in nutrient-poor terrestrial environments like alpine forests, the tundra, and deserts (Chapin et al. 1987, Stark and Whittemore 2000, Gundale et al. 2011, Rousk and Michelsen 2017). Terrestrial bryophytes are critical early-successional species because they drive N fixation by hosting cyanobacteria (Rai et al. 2000, DeLuca et al. 2002, Arróniz-Crespo et al. 2014). These symbionts allow terrestrial bryophytes to be extremely efficient at assimilating atmospheric N_2 products into amino acids (Kahl et al. 1997, Zhu et al. 2018) compared with many vascular plants (Kotanen 2002). This N is stored as biomass for 3 to 10 y (Eckstein 2000)

74 because bryophytes are able to recapture N from their own senescing tissue (Liu et al. 2020). If
75 the N is not reabsorbed, it is often stored in terrestrial bryophyte litter that resists decomposition
76 because of high phenolic concentrations (Verhoeven and Toth 1995, Britton et al. 2018).
77 Many of the chemical properties that define the biogeochemistry of terrestrial bryophytes
78 appear in stream bryophytes as well. Like terrestrial bryophytes, aquatic bryophyte tissues and
79 litter are thought to be resistant to both decomposition (Stream Bryophyte Group 1999) and
80 herbivory (Glime 2006, Parker et al. 2007). Stream bryophytes also have a high cation exchange
81 capacity, allowing them to trap positively charged ions on their leaves (Brown and Bates 1990).
82 Because bryophytes lack effective cuticles, solutions and gasses can easily move across the cell
83 surface (Turetsky 2003). Combining these properties, engineers have demonstrated that aquatic
84 bryophytes can capture and store contaminants (Cenci 2001, Carrieri et al. 2021) and heavy
85 metals (Yoshimura et al. 2000, Vincent et al. 2001, Samecka-Cymerman et al. 2002). Their
86 ability to display visible symptoms of pollution makes bryophytes useful as indicator species for
87 water quality (Bleuel et al. 2005, Ecke 2018, Martin et al. 2024). While these properties have
88 been well explored within pollution chemistry, little is known about how they may influence
89 stream ecosystem nutrient cycling.
90 The structure of aquatic bryophytes also points to their potential ability to participate in
91 active nutrient exchange with the streamwater column (Fig. 1). Bryophytes can alter water flow
92 regimes (Suren 1991, Bowden et al. 2017), which allows them to capture and store sediments and
93 detritus (Suren 1991, Finlay and Bowden 1994, Muotka and Laasonen 2002, Turunen et al.
94 2018). By slowing water flow, stream bryophytes also provide refuge to a range of microbial
95 autotrophs and heterotrophs: cyanobacteria, protists, rotifers, diatoms, and other algae (Arscott et
96 al. 1998, Stream Bryophyte Group 1999, Alvarez and Peckarsky 2013, Bowden et al. 2017).

Even if stream bryophytes themselves do not substantially alter C, N, and P regimes, they host microbes that may be important for stream-wide nutrient dynamics.

It is impossible to characterize how relevant bryophyte nutrient cycling is without knowing how abundant they are within headwater streams and how their populations change over time. Many studies have shown that bryophytes are abundant in headwater streams (Suren 1991, Bowden et al. 1994, Mulholland et al. 2000, Virtanen et al. 2001, Ashkenas et al. 2004, Parker and Huryn 2006, Mulholland 2015), but it is poorly understood how variable they are across time. For example, early studies at Hubbard Brook Experimental Forest found that only 2% of Bear Brook was covered by bryophytes (Fisher and Likens 1973), whereas recent surveys show that median coverage of the same stream is ~36% (Vought et al. 2019). This increase is fascinating because early studies of bryophytes in Bear Brook found they were effective at sorbing P but discounted their role in ecosystem nutrient cycling because of their low abundance (Meyer 1979).

This study sought to characterize the role of aquatic bryophytes in nutrient cycling in a nutrient-poor stream ecosystem. We asked the following: 1) How does aquatic bryophyte abundance vary across headwater streams and across multiple sampling timepoints? 2) What are the C, N, and P stocks in bryophytes, and are these elements stored as tissue or in captured particulates? and 3) What capacity do bryophytes have for assimilating dissolved NO_3^- and PO_4^{3-}?

METHODS

Site description and study design

Our study took place in Hubbard Brook Experimental Forest (HBEF), located in New Hampshire, USA (latitude 43.947°N, longitude 71.724°W; Fig. 2). Since the 1960s, researchers

121 at HBEF have measured weekly water chemistry at watershed outflows using gauging weirs, and
122 since 2018, they have measured weekly algal biomass, insect emergence, and light availability
123 during the snow-free seasons (HBWatER 2024a, b, c). HBEF has been the subject of many
124 watershed-scale biogeochemical and ecological experiments (Table S1). Like many terrestrial
125 bryophyte-dominated habitats, the headwater streams in HBEF are inhospitable for most
126 organisms because they are steep and flashy, resulting in highly variable flow rates (Hall et al.
127 2001). With $^{1}/_{3}$ of the region's precipitation is delivered as snow (Hall et al. 2001), streamflow is
128 largely seasonal; around 47% of annual streamflow occurs just in Spring (Likens 2013).
129 Furthermore, a legacy of acid rain in the region has acidified the streams, making them extremely
130 nutrient poor (Bayer et al. 2021, Likens 2021).
131 To address our research questions, we conducted a field study to map bryophyte
132 abundance in headwater streams in the HBEF. We surveyed 9 stream reaches between May 31,
133 2022 and June 22, 2022 and recorded bryophyte abundance as well as reach characteristics.
134 During these surveys, we collected bryophyte samples from 2 stream sites for lab incubations to
135 calculate nutrient uptake rates and from 9 stream sites to calculate bryophyte mass and nutrient
136 stocks. We compared our survey data with data collected between June 25, 2019 and July 17,
137 2019 by Vought et al. (2019) using a Mann–Whitney U test to analyze differences in bryophyte
138 abundance between the 2 time periods. We also used ANOVA and linear regression to assess
139 relationships among nutrient uptake rates, bryophyte mass, and habitat and substrate types.
140 Finally, we used Pearson's correlation tests to assess relationships between bryophyte abundance
141 and stream characteristics. All data are available at the Environmental Data Initiative Data Portal
142 at the following link: https://doi.org/10.6073/pasta/2286895cbf1e5291af339d52002c502e (Steele
143 et al. 2024).
144

145 **Field surveys and bryophyte abundance**

146 To collect data on stream reach characteristics and bryophyte abundance, we conducted
147 field surveys at 9 stream reaches. We surveyed 50-m stream reaches beginning above the gauging
148 weir for numbered watersheds (W1–W6, W9) and quasi-random 200-m stream reaches in Bear
149 Brook and Paradise Brook. In each stream reach, we conducted longitudinal surveys (along the
150 stream) and lateral surveys (across the stream). For the longitudinal surveys, walking upstream,
151 we recorded stream characteristics at every meter. At the center of flow, we recorded the depth
152 (cm), dominant habitat type (pool, riffle, slide, or cascade), substrate type (boulder, bedrock,
153 cobble, pebble, sand, or wood), and the presence of organic material (e.g., stick, algae, leaf litter,
154 bryophyte). For lateral surveys in the 50-m stream reaches (W1–W6, W9), we randomly selected
155 10 longitudinal meter markers to conduct transects. For lateral surveys in the 200-m Bear Brook
156 and Paradise Brook reaches, we divided the 200-m reaches into four 50-m segments and
157 randomly selected 10 longitudinal meter markers to conduct transects in each of the segments. At
158 the lateral transect markers, following the methods of Vought et al. (2019), we recorded the
159 width, bryophyte coverage, and leaf litter coverage (cm) for both wetted and active channel cross
160 sections. We defined the active channel margins by breaks in slope. We avoided seeps and
161 additional nutrient inputs in the reaches.

162 For each stream that we surveyed, we performed a Mann–Whitney U test in R (version
163 1.2.1335; R Project for Statistical Computing, Vienna, Austria) to determine if the stream's %
164 bryophyte coverage changed between 2019 and 2022. This test was appropriate because %
165 bryophyte coverage of transects is not normally distributed in streams. We calculated Pearson's
166 correlation coefficients and resulting *p*-values between bryophyte coverage and ambient stream
167 conditions (stream slope [from Likens and Bormann 2013], pH, and streamwater temperature

[from HBWatER 2024a]; data collected 2017–2021), habitat type, and substrates (Tables S2, S3, S4, respectively).

Nitrate and phosphate uptake

To measure nutrient uptake, we collected bryophyte samples from each of the 200-m reaches in Bear Brook and Paradise Brook. All samples were collected with a 6.8-cm-diameter circular cutter during lateral transect surveys. To ensure that we collected replicate samples from across the distribution of habitat types (cascade, pool, slide), we collected 6 samples/habitat from a stratified random selection of sampling sites where we observed bryophytes in each of the 2 streams (18 samples/stream for a total of 36 samples).

Next, we prepared our bryophyte samples for nutrient incubation. We drained excess water from bryophyte samples on a sieve and placed them right-side-up in open mason jars (0.47-L capacity). We filled the jars with 400 mL of water from Paradise Brook and removed air bubbles caught in the bryophyte samples. We placed the jars into a tray and used randomly generated numbers to assign location in the tray and water sampling order. The tray was pre-filled with enough water to submerge the bottom half of the jars. We covered the jars with foil and placed them in a refrigerator in the dark at 12°C (ambient summer water temperature) for 24 h to allow suspended sediments to settle.

After the settling period, we mixed the overlying solutions by withdrawing water near the top of the bryophyte clump with a syringe and reintroducing that water near the top of the jar. We repeated this process 3×. After mixing we removed 120 mL of stream water and measured initial NO_3^- and PO_4^{3-} concentrations for these pre-incubation samples as described below. We then replaced the volume of water removed with 120 mL of NO_3^- and PO_4^{3-} stock (1.06 mg/L NO_3-N and 0.012 mg/L PO_4-P, respectively, made from KNO_3 and KH_2PO_4). We incubated the samples

for 12 h under a growlight and 12 h in the dark at 12°C. We collected 60 mL of water after light and dark incubations, filtering the samples through a Whatman GF/F glass-fiber filter (0.7-µm pore size). We froze the filtered water samples until they could be analyzed on an ion chromatograph (Dionex ICS-2000 with an AS-18 analytical column; Thermo Fisher Scientific, Waltham, Massachusetts) for their NO_3^- and PO_4^{3-} concentrations.

We applied a repeated measures analysis of variance (RM-ANOVA) to our NO_3^- uptake data to assess whether bryophyte clumps assimilated more NO_3^- in light or dark conditions. After removing 1 outlier, our data satisfied normality and sphericity conditions requisite for this test. We performed linear regression analysis in R on log-transformed NO_3^- uptake and ash-free dry mass data from Bear Brook and Paradise Brook samples to test whether bryosphere mass explained variation in NO_3^- uptake. Models were fit separately for NO_3^- uptake under light and dark conditions. As with the repeated measures analysis of variance described above, the data met normality assumptions after the removal of 1 outlier. The same analyses could not be applied to PO_4^{3-} sorption because there was no remaining PO_4^{3-} in the water column for 35 out of 36 samples after light incubation.

Nutrient stocks

In addition to the 36 bryophyte clumps collected from Bear Brook and Paradise Brook, we collected 1 sample/transect from 5–8 lateral transects in each upper watershed (W1–W6, W9) during lateral transect surveys, for a total of 84 bryophyte samples for nutrient stock analysis. To prepare each sample for desiccation, we rinsed it in a tub with 2 L of deionized water or a specimen cup with 100 mL of deionized water, depending on the size of the sample. We then poured the sample through stacked sieves (1.19 mm and 125 µm) into a pre-weighed container and recorded the mass of the filtrate (mg). Coarse materials caught in the top sieve (>1.19 mm)

were placed in a paper bag, and fine materials caught in the bottom sieve (between 125 µm and 1.19 mm) were placed in pre-weighed aluminum tins. All samples were air dried for 3 months and then weighed, which may have introduced variability in mass measurements because of water retention. Samples were then heated to 550°C for 2 h in a muffle furnace to combust all organic material in accordance with methods described by Benfield et al. (2017). We then weighed the resulting ash-free dry mass. We performed ANOVA analyses to assess whether bryospheres accumulated higher proportions of detritus or sediments between local habitats or substrates.

We used the ash-free dry mass to calculate C, N, and P content of the bryophyte samples. Assuming the common ratio of C in organic matter, 50% of the mass lost between dry and ash-free mass can be estimated as C. There are no published C:N:P ratios for the species found in the study site (*Fontinalis antipyretica* and *Scapania undulata*). Thus, we estimated the amount of organic N and P contained in our bryophyte tissue by using low-end (145:10:1) and high-end (103:8:1) molar C:N:P ratios for a similar woodland lotic bryophyte species, *Porella pinnata* (Steinman 1994). The low-end ratio is similar to what Fernandez-Martinez et al. (2021) identified as the median molar C:N:P ratio of 35 different aquatic and semi-aquatic bryophyte species (142:8:1). Because bryophyte C:N:P ratios can vary widely based on the species and the setting (Martinez et al. 2024), this approach is not ideal and points to a need for better characterization of aquatic bryophyte nutrient stocks.

Scaling to stream-wide and watershed-scale estimates

We estimated the amount of C, N, and P stored in bryophyte mats across the full length of our studied streams based on data collected from our surveyed reaches (Fig. 2). Because data from our measures of bryophyte mass were skewed towards low values, we used the median

values of bryophyte cover and dry mass to estimate reach-scale bryophyte C and nutrient stocks per unit bryophyte mass. We estimated stream reach area and the bryophyte coverage per stream reach by applying midpoint Riemann sums on our transect data. We used the following equation to estimate C content per square meter of stream (g C/m^2) for each reach:

$$\text{C content per m}^2\text{ of stream} = \frac{C}{a}\left(\frac{s_m}{s_w}\right), \quad \text{Eq. 1}$$

where C is the median C content (g) of our bryophyte samples collected from a given stream reach, a is the area of each bryophyte sample we collected in that reach (0.0036 m^2), s_m is the total area of the reach covered by bryophytes (m^2), and s_w is the total area of the stream reach (m^2). We then used stoichiometric element ratios from the literature, as noted above, to estimate the range of standing stocks of bryophyte N and P in each stream.

We approximated watershed-scale stream bryophyte mass by multiplying our reach-scale estimates by the total stream area in each watershed. We determined stream area for each watershed by multiplying satellite-derived stream lengths (USDA Forest Service, https://doi.org/10.6073/pasta/c62e92e0eada569e8580f5541b064dac, accessed 17 January 2022) by the mean transect widths measured during our surveys. We chose this method because Vought et al. (2019) documented that stream widths in the study system are fairly consistent from 1st-running water to the sampling reaches at the watershed outflow. Using the same approach and the C:N:P ratios described above, we also estimated watershed-scale bryosphere standing stocks of C, N, and P for all study watersheds. These watershed-scale estimates of bryosphere C, N, and P content allowed us to compare standing stocks of bryosphere nutrients with the export of elements from the watershed on an annual basis. If we assume that the bryosphere is a primary reservoir for stream nutrients, we can compare these standing stocks with annual watershed

exports of dissolved inorganic N (DIN = NO_3-N and NH_4-N) and PO_4-P from each study watershed (HBWatER 2024a).

RESULTS

Bryophyte abundance

We found that bryophyte coverage was highly variable both within and among streams. Data from our lateral transects demonstrated that a stream that was completely carpeted by bryophytes in one section can be devoid of bryophytes in another section (Fig. 3). Furthermore, median % cover across stream reaches in 2022 ranged from as little as 0% to as high as 41.4%. This variability in coverage among streams was not related to differences in the streams' mean slope (a proxy for the frequency of bed-moving flows), nutrient availability, pH, or temperature (Table S2). However, we observed that bryophyte cover differed with channel geomorphology, with bryophytes appearing to be more abundant in points of flow constriction and in shallower water. Likewise, bryophytes were present more often on immobile substrates, like boulders and bedrock, than pebbles or cobbles (Table S5). However, differences in the distribution of habitat types (i.e., slide, cascade, riffle, pool) and substrates (i.e., bedrock, boulder, cobble, pebble, etc.) among streams were not related to differences in steam-level % bryophyte coverage (Tables S3, S4). In addition, we also observed that the thickness and complexity of bryophyte communities varied spatially. Bryophyte mats accumulated in some parts of the stream to depths of >15 cm and consisted of both live and dead tissue (Fig. 4A), whereas other locations were dominated by thin layers (<2 cm) of exclusively live bryophyte tissue (Fig. 4B).

Bryophyte coverage was also variable over time, although changes in bryophyte coverage across years was not consistent among streams. W1 had <5% median coverage in both 2019 and 2022, but bryophytes in W9 increased in median coverage from 10 to 41% between years

(Mann–Whitney U, $W = 34.5$, p-value = 0.009). Conversely, median bryophyte coverage in Bear Brook declined from 36 to 24% between 2019 and 2022 (Mann–Whitney U, $W = 406$, p-value = 0.05). Bryophyte coverage did not notably shift in any other stream (Table S6).

Bryophyte nutrient stocks

There were large differences in bryophyte mat nutrient storage capacity between collected samples. Our bryophyte sample masses ranged widely from the sparsest sample, which contained only 0.0005 g organic matter (OM)/cm^2 of bryophyte, to the thickest bryophyte mat we sampled, which contained 0.959 g of OM/cm^2 of bryophyte. Median bryophyte-associated OM across streams ranged from 0.007 to 0.20 g OM/cm^2 of bryophyte ($n = 84$). It is interesting to note that the stream with the largest increase in bryophyte % coverage had the 2nd-lowest median organic mass content (W9 OM = 0.007 g/cm^2, $n = 8$).

For most samples, most OM was contained within living and dead bryophyte tissue, though each mat had a reservoir of captured organic materials that were rinsed out (particle diameter = 125 µm–1.19 mm), ranging from 0.00002 to 0.18 g/cm^2 ($n = 84$). These captured organic particulates constituted between 0.5 and 74.2% of the total organic mass in bryophyte clumps, averaging 9.7 ± 10% of the total OM. We found no differences in the OM ratio of bryophytes based on their local habitat (ANOVA, $F_{3,332} = 0.9$, $p = 0.4$) or substrate (ANOVA, $F_{5,498} = 0.8$, $p = 0.6$).

Based on our abundance and OM data, we estimated stream-scale bryophyte-associated OM to range between 14 and 414 g/m^2 of stream, depending on the stream (Fig. 5C). Using published C:N:P ratio ranges for a bryophyte species similar to those found in HBEF (Steinman 1994), we estimated the standing stock of N in bryophyte tissue to range from 0.6 to 0.7 g N/m^2 of stream in our most barren stream and upwards of 18.5 to 20.7 g N/m^2 of stream in our mossiest

stream. Likewise, we estimated the organic P stock from bryophyte tissue to range from 0.1 to 0.2 g P/m^2 of stream in our most barren stream and 3.7 to 5.2 g P/m^2 of stream in our mossiest stream.

In addition to trapping organic material, bryophyte mats also accumulated large quantities of mineral sediments (Fig. 6). Inorganic material ranged from 0.3 to 2250 mg/cm^2 of bryophyte across all of our samples (n = 84). The highest mineral content was in thick mats, where it constituted up to 95% of the total mass of a bryophyte clump. We found no relationship between bryophyte mat inorganic content and local habitat type (ANOVA, $F_{3,332} = 0.4$, $p = 0.7$) or substrate type (ANOVA, $F_{5,498} = 0.3$, $p = 0.9$). All bryophytes contained small particles of magnetite (diameter < 125 µm) that clung to a magnetic stir rod.

Bryophyte nutrient uptake

We found that bryophyte clumps had high NO_3^- assimilation and PO_4^{3-} sorption rates. When incubated with water-column concentrations of NO_3^- (320 µg NO_3-N/L) and PO_4^{3-} (20 µg PO_4-P/L) that represent the highest measured concentrations in the Hubbard Brook stream chemistry record, 35 out of 36 bryophyte clumps sorbed all available PO_4^{3-} within our 12-h light incubations. Because we performed light and dark incubations consecutively, PO_4^{3-} concentrations were too low after light incubation to measure sorption in dark conditions in all but 1 sample, which sorbed the little PO_4^{3-} remaining in the water column. Our bryophyte samples also completely removed available NO_3^- from the water column within a 24-h period. One bryophyte clump from Bear Brook released NO_3^- into the column during light incubation. Although net NO_3^- uptake was also notably higher in light than dark conditions for samples from both Paradise (RM-ANOVA $F_{1,34} = 18.4$, $p = 0.0006$) and Bear Brook (RM-ANOVA $F_{1,32} = 10.9$, $p = 0.004$; Table S7), all bryophyte clumps also assimilated NO_3^- during the dark

incubations (Fig. 7A–D). In fact, 7 out of 36 samples assimilated NO_3^- at a marginally higher rate in the dark incubations than the light incubations.

Total bryophyte mat mass only weakly explained differences in NO_3^- uptake rates (Table S8). Under light conditions mass explained ~16% of variability in NO_3^- uptake rates between samples in both Paradise Brook and Bear Brook. Under dark conditions mass explained a greater portion of variability (36%) in NO_3^- uptake rates in Bear Brook but did not explain any variability in NO_3^- uptake rates in Paradise Brook (Table S8). By scaling 24-h NO_3^- assimilation rates from our jar incubations and using our estimates of total stream bryophyte mass, we estimated whole-stream N uptake rates due to bryophyte-associated NO_3^- assimilation to be 2.3 g N m^{-2} y^{-1} for Bear Brook and 1.4 g N m^{-2} y^{-1} for Paradise Brook.

Bryophyte species composition differed between Bear Brook and Paradise Brook, as did NO_3^- uptake rates within species. All Paradise Brook samples contained *S. undulata*, with 1 out of 18 samples also including a small fraction of *F. antipyretica* (Fig. 7B, D). Conversely, Bear Brook samples were more mixed: *S. undulata* was found in 10 out of 18 samples, and *F. antipyretica* was dominant in 16 out of 18 samples (Fig. 7A, C). In Bear Brook, samples containing *S. undulata* had higher mass-specific NO_3^- uptake rates than pure *F. antipyretica* samples (Fig. 7C). In contrast, the mass-specific uptake rates in *S. undulata* from Paradise Brook spanned the entire range of mass-specific uptake rates seen in Bear Brook for both taxa (Fig. 7D). Out of 36 bryophyte clumps prepared for nutrient incubation, 7 contained live Dipteran larvae. Each of these 7 samples were collected from bedrock slides or cascades.

DISCUSSION

Our study represents a novel attempt to 1) characterize change in stream bryophyte coverage over time and 2) quantitatively measure their nutrient storage and uptake capacity in

streams of the Hubbard Brook Experimental Forest. Understanding the variability of stream bryophyte coverage over time—and where nutrients are stored and transformed within bryophyte mats—is critical to assess their importance to stream nutrient cycling. Our data suggest that in streams with expansive bryophyte mats, the bryosphere is a significant and dynamic site of nutrient cycling.

Bryophyte abundance

Within streams, bryophytes were most commonly found in points of flow constriction and on stable substrates like bedrock and boulders (Table S5), consistent with previous observations of headwater streams (Steinman and Boston 1993, Suren 1996, Vought et al. 2019). Similar to Suren (1996), we found no difference in stream stability or substrate between streams covered in moss or liverworts. Steinman and Boston (1993) found that bryophytes accumulate more mass in riffles and on boulders, but we did not observe strong differences in bryophyte mat mass between habitat and substrate type. This difference in our results is likely because the stream in their study, Walker Branch, is proportionally dominated by flat and slow-flowing runs with larger stretches of pebbles and sand. In comparison, the HBEF streams we studied are steeper, faster flowing, and have a higher proportion of stable substrate with a step–pool morphology.

Among streams, median bryophyte coverage ranged from 0 to 41.4%. Though the streams we surveyed had different proportions of ideal bryophyte habitat (i.e., bedrock or boulder slides), we found no consistent pattern in the distribution of different substrates and habitat types between the most and least bryophyte-dominated streams (Tables S3, S4). Therefore, although substrate and habitat type partly explained differences in bryophyte distribution within streams, it did not explain differences in coverage and mass among streams. Regardless, our results align with previous studies that found that small substrates, like pebbles and sand, are incapable of

accruing large amounts of moss because of bed disturbance flows (Steinman and Boston 1993, Suren 1996, Scarlett and O'Hare 2006). Other likely drivers of bryophyte abundance, such as stream slope, pH, temperature, and nutrient availability, did not explain differences in bryophyte coverage across streams (Table S2). Collectively, none of our commonly measured variables were sufficient to explain the high spatial and temporal variability of bryophyte coverage that we observed across HBEF headwaters.

In addition to high spatial variability, we found that change in bryophyte coverage between 2019 and 2022 was greater than we expected. Two streams, Bear Brook and W9, had large shifts in bryophyte coverage over time—with one experiencing a 31% increase and the other a 12% decrease in coverage. However, most streams (7 out of 9) did not appear to have notable changes in bryophyte coverage. For Bear Brook, we can compare our recent surveys of bryophyte coverage (24–36%) with a much earlier survey conducted by Fisher and Likens in 1973 (2%). There was a major winter storm in 1968, the year before Fisher and Likens surveyed Bear Brook (S. Fisher, Arizona State University, Tempe, AZ, personal communication), which may have led to a decrease in bryophyte coverage at that time. Past studies demonstrate that winter storms decrease stream moss abundance (Steinman and Boston 1993) and that stream areas with abundant anchor ice are devoid of moss (Lind et al. 2014), which suggests that weather disturbance events may drive bryophyte distribution in HBEF streams. If this is the case, sensitivity to extreme weather events may be a more important driver of change than rising temperatures in stream bryospheres. As extreme weather events become more common (Rahmstorf and Coumou 2011), scour from storms and winter freezing may become more frequent. We have very limited information about the ability of bryophyte mats to recover from scouring. Steinman and Boston (1993) observed that after a winter storm disturbance, the bryophyte % coverage recovered within a couple of months, whereas total mass had not fully

406 recovered after 6 mo. In that study the relative ratios of species also changed after the storm,
407 which—as our data suggest—could influence bryosphere nutrient uptake.

408 It is evident that the bryophyte cover and mass in any stream can vary greatly over longer
409 time periods, but with only 3 y of data we cannot confidently conclude whether bryophytes are
410 becoming more or less abundant in these headwater streams. Because of the high spatial
411 variability of bryophyte coverage in these streams, it is also possible that the differences we
412 observed between 2019 and 2022 were due to spatial variability in the stream bed (i.e.,
413 happenstance sampling of less- or more-dense bryophyte sections). It is likewise important to
414 note that error may be introduced because of differences in practices between sampling teams.
415 Nonetheless, our data raise the question of what drives interannual differences in stream
416 bryophyte populations and distribution. Our data suggest that stream bryophyte populations can
417 be quite dynamic, and annual static quadrat survey data is necessary to determine whether valley-
418 wide abundance of bryophytes is increasing or decreasing in HBEF.

419 Understanding how bryophyte populations change over time helps us understand to what
420 extent they contribute to stream nutrient cycling. For example, although Meyer (1979) identified
421 that bryophytes efficiently sorb P in Bear Brook, she discounted their overall contribution to P
422 capture because Fisher and Likens (1973) previously reported bryophyte coverage to be a mere
423 2%. As years passed, studies of Bear Brook began reporting anecdotal increases in bryophyte
424 abundance (Findlay et al. 1997), but the lack of formal surveys until 2001 (TW, unpublished
425 data) and 2019 has proliferated the misconception that contemporary HBEF streams are
426 characteristically devoid of bryophytes.

427

428 **Bryophyte nutrient stocks**

We estimate that in the summer of 2022, bryophytes stored between 14 and 414 g OM/m^2 of stream. Our data suggest that bryophyte organic matter stocks in some HBEF streams are among the highest of those reported in the literature and that variation across HBEF streams spans nearly the full range of variation reported for streams in the literature (Fig. 8). The range of organic matter that we estimated for HBEF streams is most similar to the range identified by Virtanen et al. (2001) in the Kuusamo streams of Finland (150–650 g OM/m^2). In the stream with the least bryophyte abundance (W1), the N stock within the bryosphere is ~$^1/_{50}$ of annual N watershed exports, and the P stock is roughly equivalent to P watershed exports (Fig. 9). In contrast, in the stream with the most bryophyte abundance (W2), the bryosphere stored >8× more N and P in the stream channel than is exported from the watershed each year.

In these otherwise nutrient-poor streams, the bryosphere provides an important standing stock of inorganic nutrients that may limit or fuel the production of algal, microbial, and insect biota. Knowing where nutrients are stored within these bryophyte mats is important to understand their role in nutrient cycling. Although it is possible that bryophytes could decrease nutrient availability for other stream organisms by outcompeting them for nutrients and storing nutrients in inedible tissues (Glime 2006, Parker et al. 2007), it is more likely that bryophytes increase a stream's overall ability to capture and store nutrients by providing physical structure to capture detritus and host epiphytic algae, microbes, and insects. Aquatic bryophytes harbor algae and macroinvertebrates in fast-flowing waters in which they otherwise would not be able to subsist (Arscott et al. 1998, Stream Bryophyte Group 1999, Alvarez and Peckarsky 2013, Bowden et al. 2017). The physical structure of bryophytes also allows them to capture potentially bioavailable particulate matter that may otherwise wash downstream. From our ash-free dry mass samples, we found a mean of 90.3 ± 10% of OM was stored as bryophyte tissue, whereas most inorganic

452 matter was captured as sediment. However, captured particulates constituted upwards of 74% of
453 organic matter in some bryophyte mats.
454 It is important to note that the organic matter stocks in bryophyte mats are relatively small
455 compared with coarse woody debris. In Bear Brook, for example, large woody debris contributes
456 622 to 1120 g C/m^2 of stream (Findlay et al. 1997), whereas bryophyte mats contain an estimated
457 21 g C/m^2 of stream. Despite woody debris contributing ~30 to 50× more C than contributed by
458 bryophyte mats, they both act as physical obstructions that alter streamflow, such that they
459 increase a stream's overall nutrient retention and cycling capability. Large woody debris can act
460 as a dam, trapping dense accumulations of leaf litter that can harbor macroinvertebrates and
461 microbes (Tank et al. 2010), similar to bryophytes. Unlike woody debris, however, bryophytes
462 have living tissue with nutrient demands in addition to those of the biota they harbor.
463
464 **Bryophyte nutrient uptake**
465 Previous work at HBEF provided conclusive evidence that stream bryophytes are highly
466 effective at sorbing and assimilating P (Meyer 1979). Our results suggest that bryophyte mats
467 may also drive instream NO_3^- assimilation. By scaling the mean 1-d NO_3^- uptake from our Bear
468 Brook bryophyte incubations with the stream-level bryophyte cover, we estimated that
469 bryophyte-associated NO_3^- uptake (2.3 g N m^{-2} y^{-1}) constitutes a substantial portion of
470 previously estimated whole-stream NO_3^- uptake rates in this stream (12 g N m^{-2} y^{-1}) (Bernhardt
471 et al. 2003). It is important to note that our methods allowed us to measure net nutrient uptake
472 rates. It is possible that gross nutrient turnover rates in the bryosphere are considerably higher.
473 Our results further demonstrate that NO_3^- uptake in bryophyte mats is not attributable to
474 bryophytes alone but may be due in large part to the organisms they host. NO_3^- uptake was
475 generally greater in light than dark conditions, but all bryophyte clumps assimilated NO_3^- during

476 the dark incubation period as well. Because bryophytes are photosynthetic, this result suggests
477 that heterotrophs make important contributions to NO_3^- uptake in the bryosphere. This
478 conclusion is further supported by our finding that mass only explained ~16% of variation in
479 NO_3^- uptake in light conditions among samples from both Bear Brook and Paradise Brook, but
480 upwards of 36% of variation in samples from Bear Brook under dark conditions. The amount of
481 photosynthetic tissue contained in bryophytes does not scale linearly with mass. Instead, larger
482 bryophyte mats tend to consist of large accumulations of dead tissue beneath living tissue. Thus,
483 we propose that mass explained more variation in our Bear Brook samples under dark conditions
484 because the effect was driven by increased heterotrophic uptake due to increased surface area for
485 colonization, and the effect was not drowned out by the uptake attributable to photosynthesis. It
486 is possible that mass did not explain any variation in uptake in Paradise Brook under dark
487 conditions because those samples contained different taxa than those from Bear Brook.

488 We measured different NO_3^- uptake rates between samples that were dominated by *F.*
489 *antipyretica* vs those dominated by *S. undulata* in Bear Brook where these 2 species co-occur.
490 This difference might lead one to believe that *S. undulata* characteristically uptakes less NO_3^-
491 than *F. antipyretica* uptakes. Yet, we observed that the range in NO_3^- uptake across samples from
492 Paradise Brook—where we almost exclusively found *S. undulata*—was similar to the full range
493 of NO_3^- uptake rates observed from samples in Bear Brook, regardless of the sample's bryophyte
494 taxon composition. Although it is possible that there is a negative interaction effect between the 2
495 species, our study did not contain enough mixed bryophyte samples from Paradise Brook for
496 comparison, meaning this effect could equally be due to ambient differences between Bear Brook
497 and Paradise Brook. It is interesting to speculate and well worth further exploration to discover
498 whether these 2 bryophyte taxa support distinct communities of epifauna and microbes that may
499 influence their role in nutrient uptake.

Almost all (35 out of 36) bryophyte clumps sorbed annual peak stream concentrations of PO_4^{3-} within 12 h of light incubation, consistent with previous studies that found stream bryophytes sorb P (Meyer 1979, Steinman and Boston 1993). Because there were no detectable concentrations of PO_4^{3-} remaining in the water column after light incubation, we could not measure PO_4^{3-} sorption in the dark. Therefore, we could not distinguish whether the consumption of PO_4^{3-} we observed in the light incubation was due to physical sorption or biological uptake. For this reason, we are using the term sorption to represent all removal of PO_4^{3-} from the water column. Future studies should compare PO_4^{3-} uptake in light and dark conditions to better understand whether bryosphere removal of PO_4^{3-} in headwater streams is driven by biological uptake or sorption. However, the rapid sorption rates we measured may help elucidate why stream organisms are able to survive in such oligotrophic water columns. If sorbed P is held in bioavailable fractions, then a bryophyte mat's constituent organisms may not need to source their P from the nutrient-poor water column. Water-column nutrient content alone may be insufficient to characterize the nutrient profile of headwater streams with developed bryophyte mats.

Cumulatively, our results suggest that HBEF streams contain dynamically changing, complex, and extensive bryospheres with high nutrient uptake and storage capacity. We found that the spatial and temporal distributions of the bryosphere in HBEF streams are not explainable solely by ambient stream conditions and resource availability. Our results raise interesting questions around the extent to which disturbance events influence the distribution, composition, and recovery of the bryosphere, which is important for stream ecosystem function.

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726 **FIGURE CAPTIONS**

727 Fig. 1. Stream bryophyte structure in Hubbard Brook Experimental Forest in New Hampshire,
728 USA. A.—Streams at Hubbard Brook are largely dominated by aquatic and semi-aquatic
729 bryophytes. B.—These bryophytes are most abundant in bottlenecks with laminar flow,
730 often trapping debris. The left of the boulder shows what may be scour—a patch of moss
731 ripped off and yet to grow back. C.—Although the streams look verdant, bryophyte mats
732 often contain several centimeters of dead tissue, providing structure to retain detritus and
733 biota. Paintings by Emma Rosi. Paintings are based on photos and were all painted with
734 the same pigments to be more color, light, and saturation balanced than source photos.

735

736 Fig. 2. Map of the study site in Hubbard Brook Experimental Forest, New Hampshire, USA,
737 which included 7 headwater streams in upper watersheds (W1–W6, W9) and 2 additional
738 stream reaches (Bear Brook [BB] and Paradise Brook [PB]). The inset map shows W9,
739 which is southwest of the other stream reaches. We surveyed bryophyte coverage and
740 collected bryophyte samples along stream stretches denoted in black. Our stream-level
741 estimates of bryophyte-associated stocks and NO_3^- uptake are based on how much of the
742 grey-outlined extrapolated streams are covered by bryophytes. We measured flux data at
743 the weirs.

744

745 Fig. 3. Comparison of bryophyte abundance within streams between years in Hubbard Brook
746 Experimental Forest, New Hampshire, USA. Some streams had similar coverage over
747 time (i.e., W1, W2, and W6), and others varied (i.e., BB and W9). W9's % bryophyte
748 coverage increased between years from 10 to 41% (Mann–Whitney U, p-value = 0.009),
749 whereas BB's coverage declined from 36 to 24% (p-value = 0.05). No other streams had a

consistent change in coverage between years. These results demonstrate that there is no clear trend in bryophyte coverage change over time, but coverage can change over relatively short time spans. Survey data for 2019 was collected by Vought et al. (2019). For numbered watersheds, n = 7–10. For Bear Brook (BB) and Paradise Brook (PB), n = 40. Boxes encompass the minimum, interquartile range, median, and maximum; outliers are depicted as points.

Fig. 4. Comparison of types of bryophyte mats in streams in Hubbard Brook Experimental Forest, New Hampshire, USA. A.—A thick (>15 cm), developed bryophyte sample from a bedrock slide in W2. Most bryophyte tissue is dead and nonphotosynthetic, and water flows through the whole profile. B.—A thin (<2 cm) bryophyte sample from a cobble in W9, consisting exclusively of live tissues attached directly to bare rock.

Fig. 5. A—.Bryophyte cover at the stream scale in Hubbard Brook Experimental Forest, New Hampshire, USA. B—.Organic matter (OM) stocks for each stream at the mat scale. C—.OM stocks for each stream extrapolated to the stream scale. Bryophyte coverage varied within streams and between streams, likely, in part, because of variable stream geomorphology. Bryophytes generally shared similar profile depths—and thus organic matter stocks—between streams, except those in W2, which were more massive. Taken together, despite being located in the same larger catchment, these streams varied widely in bryophyte abundance and OM density.

Fig. 6. Bryophyte mats in Hubbard Brook Experimental Forest, New Hampshire, USA, accumulate large quantities of mineral sediments, especially within thick clumps. Each

774 bar represents 1 bryophyte clump, with its organic matter content above the horizontal
775 line and its inorganic mineral content below the line. By mass, 45.1% of sampled
776 bryophyte mats were proportionally dominated (>50%) by mineral material (n = 84).
777
778 Fig. 7. Aerial (A, B) and mass-specific (C, D) NO_3^- uptake rates of bryophyte clumps in light and
779 dark conditions in Bear Brook (A, C) and Paradise Brook (B, D) in Hubbard Brook
780 Experimental Forest, New Hampshire, USA. Uptake rates are expressed in terms of NO_3^-.
781 Paired points represent 1 bryophyte clump that was incubated under both light and dark
782 conditions, and size displays the sample's total mass, ranging from 0.02 to 25.93 g (n =
783 36). We found that bryophyte clumps assimilated more NO_3^- under light conditions than
784 dark conditions (Table S7), suggesting that photosynthetic uptake contributes to NO_3^-
785 uptake. In Bear Brook, bryophyte mats containing *Scapania undulata* had greater mass-
786 specific NO_3^- uptake rates than pure *Fontinalis antipyretica* samples. Although more-
787 massive samples generally assimilated more NO_3^-, mass only weakly explained
788 differences in NO_3^- uptake between samples (Table S8).
789
790 Fig. 8. Comparison of organic matter stocks in bryophyte mats across surveyed streams from this
791 study and other studies. Data labeled with open squares are from this study. Data labeled
792 with closed circles are from (Suren 1991, Bowden et al. 1994, Mulholland et al. 2000,
793 Virtanen et al. 2001, Ashkenas et al. 2004, Parker and Huryn 2006, Mulholland 2015).
794 Variability in bryosphere organic matter between streams in Hubbard Brook Experimental
795 Forest is comparable to variability seen at the continental scale. Furthermore, some of the
796 streams at Hubbard Brook are among the most dominated by bryophyte organic matter.

797 Methods used to find comparison streams and detailed coordinates for each referenced
798 stream are in Table S9.
799
800 Fig. 9. Comparison of bryosphere N and P stocks to watershed N and P exports in streams in
801 Hubbard Brook Experimental Forest, New Hampshire, USA. Bars represent the estimated
802 standing stocks of bryosphere N and P in the 2 watersheds with the lowest (W1) and
803 highest (W2) bryophyte cover. Numbers above the arrows represent watershed N and P
804 flux estimates and are the mean annual fluxes reported for each of these watersheds from
805 water years 2017 to 2021 (HBWatER 2024a).