Quantifying moss-associated nitrogen fixation in New England peatlands

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Kirsten K. Coe
Assistant Professor, Department of Biology
Middlebury College, Middlebury, VT

Abstract:
Nitrogen (N) limits productivity across terrestrial ecosystems, and the primary way in which N enters the biosphere is through fixation via diazotrophic organisms (those that convert N_2 into useable forms like NH_4^+), such as cyanobacteria. In some ecosystems, mosses can form symbiotic associations with N-fixing cyanobacteria, and moss-associated N fixation can represent a critical N source to communities. While such associations have been demonstrated in arctic and boreal ecosystems, considerably less attention has been paid to temperate systems. This is despite the ecological importance of mosses in systems such as temperate peatlands. The goal of this project was to examine the magnitude and drivers of moss-associated N fixation in New England peatlands. We conducted *Sphagnum* (peat moss) field incubations in three bogs spanning a latitudinal gradient from Western MA to Northern VT, using $^{15}N_2$ tracers coupled with modeling of biotic and abiotic predictor variables for N fixation. We also applied UV fluorescence microscopy in paired field-collected specimens to concomitantly examine cyanobacterial abundance as a spatial and temporal predictor of N fixation. Two undergraduate students under the mentorship of principal investigator Coe conducted field incubations, microscopy, species identification, and analyses of stable isotope data, as well as analyses using NOAA-derived remote sensing predictors of N fixation. Overall, we document widespread evidence of moss-associated N fixation across our sites, where N fixation rates were, on average, greater than what has been reported in boreal and arctic regions. We also identified six *Sphagnum* species that associated with N-fixing cyanobacteria and discovered that cyanobacterial symbionts may migrate within individual shoots throughout the growing season. Environmental variables captured across sites, such as recent temperatures and longer-term precipitation, were strong predictors of N fixation rates.

Nitrogen (N) availability is the principal limiting factor on primary productivity across terrestrial systems (Vitousek & Howarth, 1991). The primary input of N to the biosphere involves conversion of gaseous N_2 into the biological available form, NH_4^+, via N fixation, a process accomplished by a diversity of diazotrophic bacteria including cyanobacteria. Mosses can form symbiotic associations with N-fixing cyanobacteria (Adams & Duggan 2008), and moss-associated N fixation can represent a critical N source to plant and soil communities (Gundale et al. 2011, Rousk et al. 2013). While such associations have been demonstrated in arctic and boreal ecosystems (e.g., forests and peatlands; DeLuca et al. 2002, Berg et al. 2013), less attention has been paid to temperate systems where mosses are ecologically important. Bogs are a type of *Sphagnum* moss-dominated peatland commonly found throughout New England for which we possess scant information on the extent and importance of moss-associated N fixation. To assess the magnitude and drivers of moss-associated N fixation in New England bogs, we examined three bogs spanning a latitudinal gradient: northernmost (Carmi Bog, VT), mid-latitude (Leicester Bog, VT), and southernmost (Hawley Bog, MA). We conducted *Sphagnum* field incubations in the three bogs using $^{15}N_2$ tracers coupled with modeling of biotic and abiotic predictor variables for N fixation.

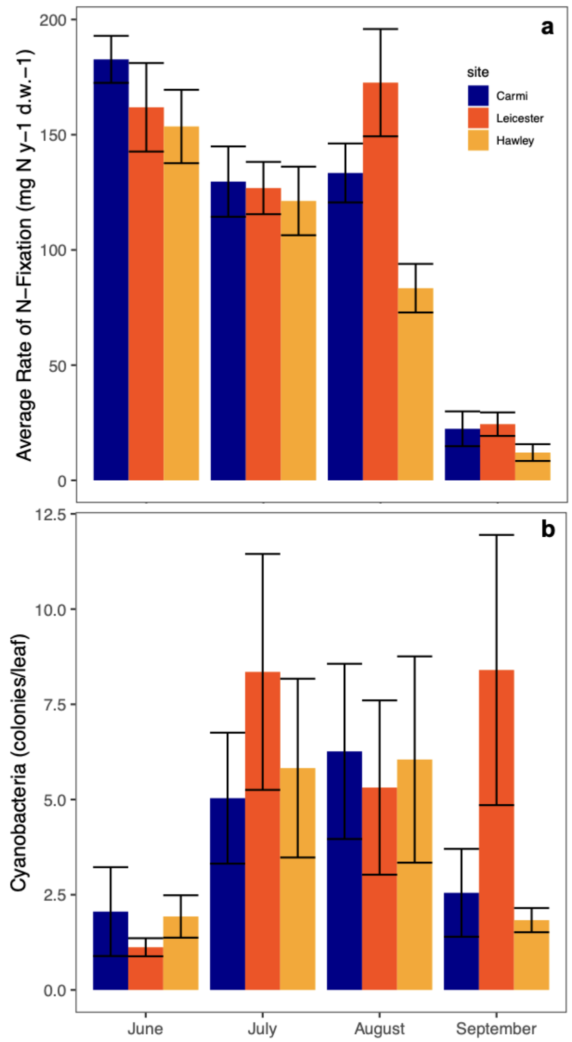

Figure 1: **a)** Mean *Sphagnum*-associated N fixation (mg N y^{-1} dw^{-1} ± SE) and **b)** cyanobacterial colonization (colonies per leaf) measured seasonally across three bogs (Carmi, Leicester, Hawley) spanning a N-S latitudinal gradient in New England.

We found widespread evidence of *Sphagnum*-associated N fixation across all sites over the course of the growing season (**Fig. 1a**). Overall, N fixation rates were highest in June and declined over time (P<0.001) and exhibited a positive relationship with latitude (P<0.05; 2-way ANOVA). Mosses incubated in our Northernmost VT site (Carmi) displayed highest N fixation rates overall. However, our mid-latitude VT site (Leicester) had the highest rates of fixation in August (P<0.05). N fixation rates in these New England bogs were, on average, 0.5-3 times greater than what has been reported in boreal and arctic regions.

Cyanobacterial colonization detected by UV-fluorescence microscopy also varied across sites as well as over the growing season. While these two factors were not significant predictors of colonization overall, we identified several trends in colonization over time across 2/3 of the bogs: a bell-shaped distribution peaking mid-growing season. In our mid-latitude site, Leicester Bog, we observed very high colonization late in the season, comparable to July (**Fig. 1b**).

We identified six *Sphagnum* species across the sampled bogs that associated with N-fixing cyanobacteria to different degrees (up to 38 colonies per leaf; **Fig. 2**). While we did not detect a significant effect of species on N fixation, there was a significant effect of species on cyanobacterial colonization (P<0.05), and *S. magellenicum* exhibited the highest average rate of colonization (mean 11.4 colonies per leaf). Overall, N fixation was only weakly predicted by cyanobacterial colonization (P>0.05 across all months), likely because of limits on the ability to detect cyanobacterial presence consistently in the variable and diverse microclimate that is a *Sphagnum* shoot. Within the species that formed associations, cyanobacterial symbionts were present in lower regions of moss shoots early in the growing season and moved into the capitulum (shoot tops) to peak in abundance in August. In terms of *Sphagnum* diversity across the bogs, two species (*S. rubellum* and *S. magellanicum*) were found in all three bogs, while *S. teres* was only found in Leicester bog.

Strong predictors of moss-associated N fixation, in addition to latitude and time of year, included a suite of environmental variables. We analyzed NOAA-based remote-sensing records of 115 environmental variables for the locations of each of our measurement sites, including maximum, minimum, and average temperature, relative humidity, cloud cover, and precipitation at different time intervals prior to field incubations. Our preliminary analysis revealed that mean and minimum temperatures four hours prior to measurement were the strongest positive predictors of N fixation, while precipitation occurring up to two weeks prior to measurement had a strong negative effect on N fixation (**Table 1**). Perhaps surprising for a poikilohydric semi-aquatic plant (i.e., where tissue hydration is dependent on environmental water availability) and its symbionts, warmer and drier conditions may result in higher fixation rates, at least in these bogs.

Funding received from the New England Botanical Society in 2021 helped make this field and laboratory research possible. The data presented in this report were collected by two undergraduate students at Middlebury College under the mentorship of principal investigator Coe and are being incorporated into a senior honors thesis for one student. The results of this work reveal that moss-associated N fixation is indeed occurring across New England bogs across a diversity of *Sphagnum* species, which will assist in future estimates of N inputs and nutrient cycling throughout the region.

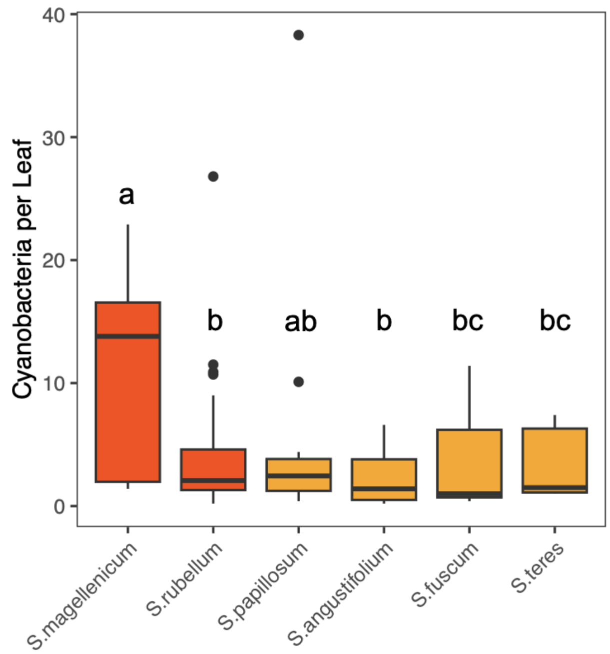

Figure 2: Variation in cyanobacterial colonization rates across *Sphagnum* species collected from three New England bogs. ***S. magellenicum*** and ***S. rubellum*** (shown in red) were found in all sites, while the others (orange) were found in two or fewer sites. Dark bars within boxplots represent median colonization for each species and dots above plots represent statistical outliers. Significant differences in colonization frequency across species are denoted by different lowercase letters (P<0.05).

Predictor variable	**Effect on N fixation**	***P***	**r^2**
4-hour T_{mean}, °C	positive	<0.01	0.196
4-hour T_{min}, °C	positive	<0.01	0.213
72-hour precipitation, mm	negative	<0.0001	0.114
14-day precipitation, mm	negative	<0.0001	0.150

Table 1: Best individual environmental predictors of *Sphagnum* associated N fixation based on preliminary analyses of 115 NOAA-extracted potential predictors.

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