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Biological N2 fixation mainly controlled by Sphagnum tissue N:P ratio in ombrotrophic bogs

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Most of the 18 Pg nitrogen (N) accumulated in northern nutrient-poor and *Sphagnum*-dominated peatlands (bogs and fens) can be attributed to N_2-fixation by diazotrophs either associated with the live *Sphagnum* or non-symbiotically in the deeper peat such as through methane consumption close to the water table. Where atmospheric N deposition is low (< 0.2 g m^{-2} y^{-1}), ombrotrophic bogs rely on N_2-fixation as the primary source of N that sustains primary production. Due to high energetic requirements, N_2-fixation depends on the available phosphorus (P). Anthropogenic impacts in the last 200 years increased atmospheric N deposition, resulting in a switch from N to P limitation in *Sphagnum*, suggested by the increase in tissue N:P to $>$16. It is unclear how *Sphagnum*-hosted diazotrophic activity may be affected by N deposition and thus changes in N:P ratio.

First, we investigated the effects of long-term addition of different sources of nitrogen (0, 1.6, 3.2 and 6.4 g N m^{-2} y^{-1}as NH_4Cl and $NaNO_3$), and phosphorus (5 g P m^{-2} y^{-1}as KH_2PO_4) on *Sphagnum* nutrient status (N, P and N:P ratio), net primary productivity (NPP) and *Sphagnum*-associated N_2fixation at Mer Bleue, a temperate ombrotrophic bog. We show that N concentration in *Sphagnum* tissue increased with larger rates of N addition, with a stronger effect on *Sphagnum* from NH_4 than NO_3. The addition of P created a 3.5 fold increase in *Sphagnum* P content compared to controls. *Sphagnum* NPP decreased linearly with the rise in N:P ratio, while linear growth declined exponentially with increase in *Sphagnum* N content. Rates of N_2-fixation determined in the laboratory significantly decreased in response to even the smallest addition of both N species. In contrast, the addition of P increased N_2 fixation by up to 100 times compared to N treatments and up to 5-30 times compared to controls. The change in N_2-fixation was best modeled by the N:P ratio, across all experimental treatments.

Secondly, to test the role of N:P ratio on N_2-fixation across a range of bogs, eight study sites along the latitudinal gradient from temperate, boreal to subarctic zone in eastern Canada were selected. From each bog, two predominant microptopographies, hummocks and hollows, were tested for both N_2-fixation activity in the laboratory and *Sphagnum* tissue concentrations of N, P and N:P ratio. We found that 65% of the variance in the increase of N_2-fixation activity was explained by the decrease in N:P ratio in hollows (n = 73) but only about 20% in hummocks (n = 78). Changes in neither N or P concentration alone explained the increase in N_2-fixation better than N:P ratio. We interpret that the difference between hollows and hummocks results from the availability of moisture that further limits N_2-fixation. When moisture is not a limiting factor, i.e. in hollows, N:P ratio will be the best predictor of N_2-fixation in bogs.

Both studies suggest that the relative P availability to *Sphagnum*-associated diazotrophs, measured as a tissue N:P ratio, best describes N_2-fixation activity in bogs, especially ones exposed to larger N deposition.