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## High potential of nitrogen fixation in pristine, ombrotrophic bogs in Southern Patagonia

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Nitrogen (N) input in pristine peatlands occurs via natural input of inorganic N through atmospheric deposition or biological dinitrogen ( $N_2$ ) fixation. However,  $N_2$  fixation is to date mostly attributed to bacteria and algae associated to Sphagnum and its contribution to plant productivity and peat buildup has been often underestimated in previous studies. Based on net N storage, exceptionally low N deposition, and high abundance of vascular plants at pristine peatlands in Southern Patagonia, we hypothesized that there must be a high potential of non-symbiotic  $N_2$  fixation not limited to the occurrence of Sphagnum. To this end, we chose two ombrotrophic bogs with spots that are dominated either by Sphagnum or by vascular, cushion-forming plants and sampled peat from different depths for incubation with  $^{15}N_2$  to determine  $N_2$  fixation potentials. Moreover, we analyzed  $^{15}N_2$  fixation by a nodule-forming, endemic conifer inhabiting the peatlands. Results from  $^{15}N_2$  uptake were compared to the conventional approach to study  $N_2$  fixation by the acetylene reduction assay (ARA).

Using  $^{15}N_2$  as a tracer, high non-symbiotic  $N_2$  fixation rates of 0.3-1.4  $\mu$ mol N g $^{-1}$  d $^{-1}$  were found down to 50 cm under micro-oxic conditions (2 vol.%) in samples from both plots either covered by Sphagnum magellanicum or by vascular cushion plants. Peat N concentrations suggested a higher potential of non-symbiotic  $N_2$  fixation under cushion plants, likely because of the availability of easily decomposable organic compounds as substrates and oxic conditions in the rhizosphere. In the Sphagnum plots, high  $N_2$  fixation below 10 cm depth would rather reflect a potential fixation that may switch on during periods of low water levels when oxygen penetrates deeper into the peat.  $^{15}N$  natural abundance of live Sphagnum from 0-10 cm pointed to N uptake solely from atmospheric deposition and non-symbiotic  $N_2$  fixation.  $^{15}N$  signatures of peat from the cushion plant plots indicated additional N supply from N mineralization.

Nitrogen fixation by the conifer *Lepidothamnus fonkii* was exceptionally high, reaching 3.1  $\mu$ mol N g<sup>-1</sup> d.w. d<sup>-1</sup> detected in roots, stems, and green biomass. For *L. fonkii*, we could identify a specific association with *Beijerinckiaceae* as N<sub>2</sub> fixing bacteria in the root nodules, whereas the rhizosphere peat was dominated by other diazotrophs.

The ARA considerably underestimated N<sub>2</sub> fixation and can thus not be recommended for peatland studies.

Our findings suggest that non-symbiotic or associative  $N_2$  fixation overcomes N deficiency in different vegetation communities and has great significance for N cycling and peat accumulation in pristine peatlands.