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Rates of biological nitrogen fixation in Central European wetlands: $^{15}N_2$ experiments along a regional air-pollution gradient and in vertical peat profiles

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Microbial N_2-fixation helps to sustain carbon accumulation in pristine peatlands and to remove CO_2 from the atmosphere. As breaking the triple bond of atmospheric N_2 is energy-intensive, it is reasonable to assume that increasing inputs of reactive N (N_r; NH_4^+-N and NO_3^--N) *via* anthropogenic pollution will lead to downregulation of N_2-fixation. However, recent studies indicated adaptation of diazotrophs to changing N_r inputs. To verify this assumption, we studied four *Sphagnum*-dominated peat bogs in the Czech Republic, differing in N_r deposition by a factor of two (~16 and ~8 kg N_r ha^{-1} yr^{-1} in the north and south, respectively). We hypothesized that the more N_r-polluted northern sites, Velke jerabi jezero (VJ) and Cerny potok (CP), would exhibit lower biological N fixation rates (BNF) than the less polluted southern sites, Cervene blato (CB) and Zdarecka slat (ZS). Wet *Sphagnum* was collected in spring 2017/2024. At the end of laboratory incubations in a $^{15}N_2$ atmosphere, *Sphagnum* exhibited an increase in $d^{15}N$ values from about -3 ‰, typical of all sites, to between 27 and 266 ‰ at VJ, CP and ZS. Over the 7-day experiment, no change in $d^{15}N$ values was recorded at CB. The calculated BNF rates increased in the order: VJ < ZS < CP (0.8, 9.6, and 49 ug N per gram of dry *Sphagnum* per day, respectively). Replicated vertical peat core profiles from CP (north) and ZS (south) were subjected to a similar $^{15}N_2$ experiment. The investigated depths were 0, 10, 20, and 30 cm. In spring, ^{15}N-tracer incubations provided the following BNF downcore gradients: from 49 to 85 ug N at CP, and from 10 to 0.2 ug N at ZS. The late summer BNF rates were around 30 ug N throughout the profile at CP, and from 47 to 97 ug N downcore at ZS (all values are for $gram^{-1}$ day^{-1}). A large seasonal/between-site variability in BNF was observed. Our data indicate that atmospheric N_r inputs were not the main control of BNF in the studied Central European peat bogs. Other site characteristics, *e.g.,* phosphorus availability, NH_4^+/NO_3^- ratios, and moisture conditions, served as important BNF drivers.