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# Effects of long-term elevated $CO_2$, warming, and prolonged drought on *Pleurozium*-associated diazotrophic activity and abundance

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Nitrogen ($N_2$) fixation is the primary natural influx of N to terrestrial ecosystems, and changes in $N_2$ fixation may have consequences for primary productivity and thus ecosystem function. We studied the activity and abundance of diazotrophs associated with the feather moss *Pleurozium schreberi* in a temperate heathland, after seven years of global change manipulations, including elevated atmospheric $CO_2$ (510 ppm), increased temperature (0.5-1.5 $^{\circ}$C), and prolonged pre-summer droughts (4-6 weeks /year). Acetylene reduction assay was carried out monthly to monitor $N_2$ fixation rates throughout one year, while *nif*H copy abundance, serving as a diazotroph abundance estimate, was measured by quantitative polymerase chain reaction (q-PCR).

Prolonged summer droughts significantly increased both $N_2$ fixation and *nif*H copy abundance, contrasting previous studies that demonstrate a direct negative correlation between $N_2$ fixation and water availability. A shift in the relative abundance of $N_2$-fixing bacteria from the green, upper parts of the moss stem to the lower, brown parts was observed. This shift could make diazotrophs less sensitive to desiccation, enabling $N_2$ fixation to be upheld for longer during drought and thus causing higher abundance. Increased temperature likewise had a positive effect on the diazotroph abundance, although this did not translate into increased activity. Possibly, warming protects diazotrophs during extreme cold events, while actual $N_2$ fixation is limited by water, disregarding a rise in potential $N_2$ fixation caused by higher abundance. Increased $CO_2$ caused no significant diazotroph response.

Our study showed that long-term increase in temperature and recurrent drought events cause higher diazotroph abundance in *Pleurozium schreberi* and thus enhance the potential $N_2$ fixations rate. Furthermore, our results indicate that diazotrophs may alter colonization patterns and thereby actively remain in the moss fraction less likely affected by desiccation. In consequence, *Pleurozium*-associated $N_2$ fixation may become an even more important contributor of N for terrestrial ecosystems in a predicted future climate.