



An ecophysiological study of the *Azolla filiculoides*- *Anabaena azollae* association

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The long term effects of salinity stress on the growth, nutrient content and amino acid composition of the *Azolla filiculoides* – *Anabaena azollae* association was studied in a laboratory experiment. It was demonstrated that the symbiosis could tolerate salt stress up to 90 mM NaCl, even after a 100 day period of preconditioning at salt concentrations that were 30 mM NaCl lower. In the 120 mM NaCl treatment the *Azolla filiculoides* survived, but hardly any new biomass was produced. It was shown that during the experiment, *A. filiculoides* became increasingly efficient in excluding salt ions from the plant tissue and was thus able to increase its salt tolerance. The amino acid analysis revealed that the naturally occurring high glutamine concentration in the plants was strongly reduced at salt concentrations of 120 mM NaCl and higher. This was the result of the reduced nitrogenase activity at these salt concentrations, as was demonstrated in an acetylene reduction assay. We suggest that the high glutamine concentration in the plants might play a role in the osmoregulatory response against salt stress, enabling growth of the *A. filiculoides* -*Anabaena azollae* association up to 90 mM NaCl. In a mesocosm experiment it furthermore was demonstrated that *Azolla* might manipulate its own microenvironment when grown at elevated salt concentration (up to \sim 50 mmol•L⁻¹) by promoting salinity stratification, especially when it has formed a dense cover at the water surface.

Beside salt stress, we also studied the growth of *Azolla filiculoides* in response to elevated atmospheric carbon dioxide concentration, in combination with different light intensities and different pH of the nutrient solution. The results demonstrated that as compared to the control (ambient pCO₂ concentrations), *Azolla filiculoides* was able to produce twice as much biomass at carbon dioxide concentrations that were five times as high as the ambient pCO₂ concentration. However, it was also shown that this response was much less pronounced at light intensities that were too high or too low, or when the nutrient solution had a higher pH. At higher light intensities and higher pH the growth response to elevated atmospheric CO₂ was probably overruled by an increased competition with algae, while at low light intensities, light became limiting in the photosynthetic processes. In order to get a better understanding of the carbon metabolism of the *Azolla* -*Anabena* association we are currently performing experiments with labeled bicarbonate and/or carbon dioxide. Analysis of the samples is still in progress but should be ready at the time of the congress.

The fore mentioned results might help to better understand the occurrence of ancestral *Azolla* species in the central Arctic Ocean some 50 mya, as was found by Brinkhuis et al. (2006). The results obtained in the experiment with elevated atmospheric pCO₂ concentrations for example were useful in estimating the impact of the Arctic *Azolla* bloom on carbon drawdown. Speelman et al. (2009) estimated that storing $0.9 \cdot 10^{18}$ to $3.5 \cdot 10^{18}$ g carbon would result in a 55 to 470 ppm drawdown of pCO₂ under Eocene conditions, indicating that the Arctic *Azolla* bloom might have had a significant effect on global atmospheric pCO₂ levels through enhanced burial of organic matter.