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No observed effect of ocean acidification on nitrogen biogeochemistry in a summer Baltic Sea plankton community

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SUPPLEMENTARY MATERIALS

Enrichment of mesocosms with ^{15}N-N_2 gas

Four of six mesocosms spanning the range of fCO_2 treatments were enriched with the isotopically labelled ^{15}N-N_2 gas to investigate the fate of newly fixed N in this plankton community under future ocean acidification conditions. A similar approach to Mohr et al. (2010), as described for the N_2-fixation incubations (see Section 2.2), was employed on a larger scale. A total of approximately 1500 L of unfiltered seawater was collected from the Baltic at ca. 10 m depth and pumped into the laboratory building at Tvärminne Zoological Station. Mesocosm enrichment occurred in two pulses on *t22* and *t26*. We added this in two steps because of the limited number of bags available for preparing the ^{15}N-N_2 enriched seawater. For the first step, seawater was filtered and collected as for the N_2-fixation incubations in bags (thermoplastic polyurethane, ~30 L capacity) with a tap and a crimp sealed septum (N20 grey butyl rubber plugs, Macherey and Nagel) on opposite ends of the bag. The large physical effort required to dissolve the gas by 'bag-slapping', as commonly done for small volumes using the method described by Mohr et al. (2010), led to a modification of the enrichment method for the second enrichment step. Water was collected and degassed as previously described through the degassing membrane. Instead of collecting the water directly after this step, the water then passed through a second membrane that was flooded with ^{15}N-N_2 gas and was connected to an overflow system which allowed monitoring of gas dissolution (Fig. A). The high surface area in the membrane enhanced the labelled gas dissolution. This enriched water was then pumped directly into the empty collection bags using a peristaltic pump without contact with the atmosphere. One complete cartridge of gas (500 mL, nitrogen - ^{15}N-N_2, 98 atom % ^{15}N, Sigma Aldrich, Lot no.: SZ1670V, SZ1423V, CX0937) was added per bag through the septum. A total of 150 L of enriched seawater prepared was added to four mesocosms (M3, M5, M6, M8), and 100 L unenriched filtered seawater was added to the other two mesocosms (M1, M7) as isotope label controls on *t22* and *t26*.

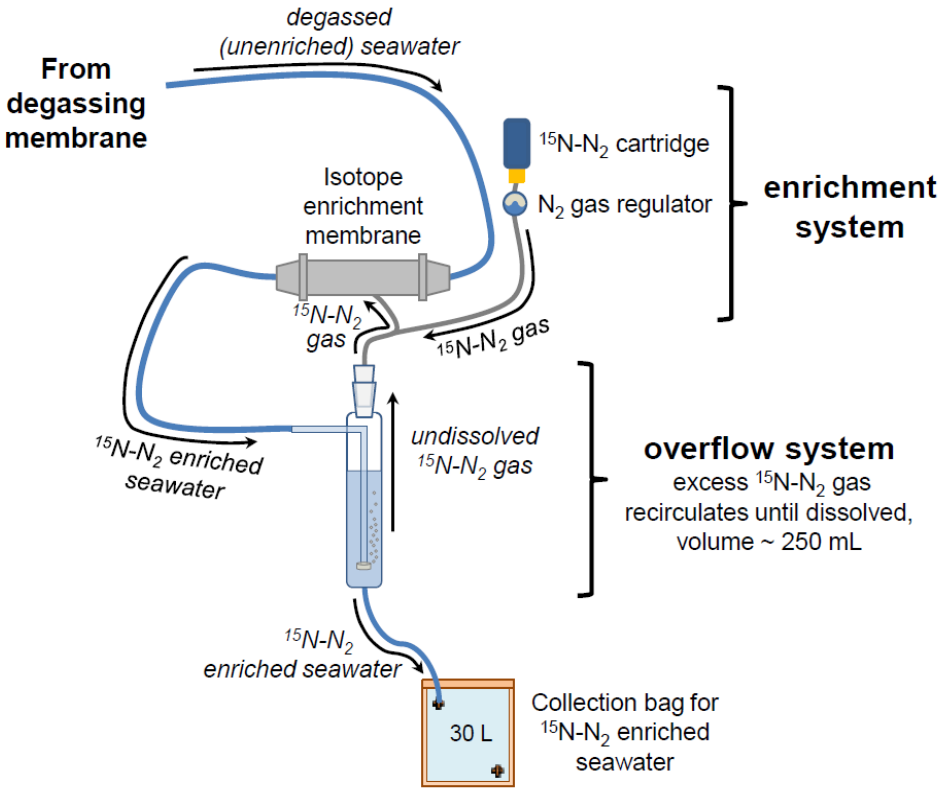

Figure S1. Diagram of set-up used for large-scale preparation of ^{15}N-N$_2$ enriched seawater which was added to selected mesocosms.

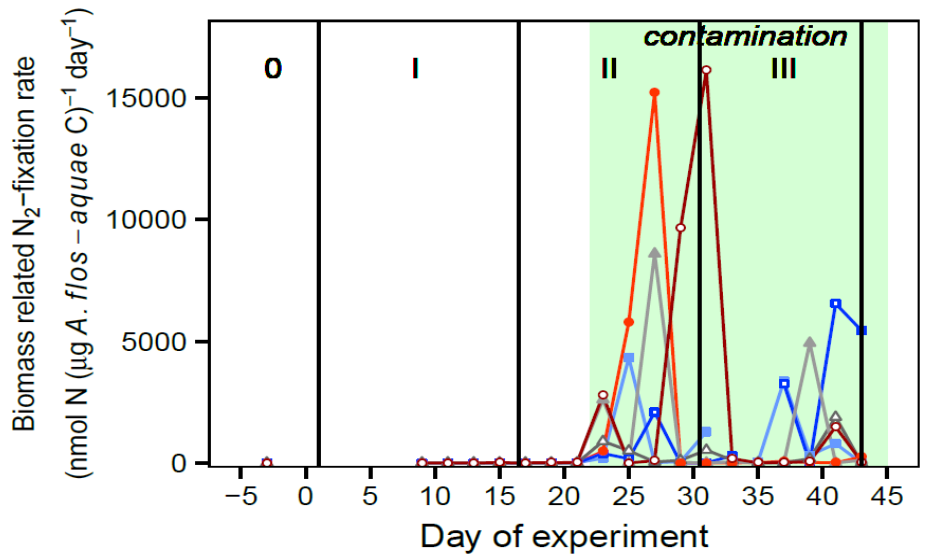

Figure S2. *A. flos-aquae* carbon-normalised N_2-fixation rates over the study period. Where data points are missing before *t9*, rates were either below detection limit (0.15 nmol N L^{-1} d^{-1}) or did not coincide with sampling for phytoplankton abundance counts. Green shaded area between *t23* and *t43* indicates when contaminated ^{15}N-N_2 gas was used in incubations (see Dabundo et al. 2014) and added to mesocosms.