



## Does long-term soil warming affect microbial element limitation? A test by short-term assays of microbial growth responses to labile C, N and P additions

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Human activities have caused global warming by 0.95 °C since the industrial revolution, and average temperatures in Austria have risen by almost 2 °C since 1880. Increased global mean temperatures have been reported to accelerate carbon (C) cycling, but also to promote nitrogen (N) and phosphorus (P) dynamics in terrestrial ecosystems. However, the extent of warming-induced increases in soil C, N and P processes can differ, causing an eventual uncoupling of biogeochemical C, N and P cycles, and leading to altered elemental imbalances between available plant and soil resources and soil microbial communities. The altered dynamics in soil C and nutrient availability caused by increased soil temperature could shift the growth-limiting element for soil microorganisms, with strong repercussions on the decomposition, mineralization and sequestration of organic C and nutrients. The latter relates to the conservative cycling of limiting elements while elements in excess are mineralized and released at greater rates by microbial communities.

Despite the many laboratory and in situ studies investigating factors that limit soil microbial activity, most of them explored nutrient addition effects on soil respiration or soil enzyme activities. A critical assessment, however, clearly indicated the inappropriateness of these measures to deduce growth-limiting nutrients for soil microbes. Similar to studies of plant nutrient limitation, unequivocal assessment of soil microbial element limitation can only be derived from the response of microbial growth to element amendments. To our knowledge this has not been performed on soils undergoing long-term soil warming.

In this study, we therefore investigated the effect of long-term soil warming on microbial nutrient limitation based on microbial growth measurements in a temperate calcareous forest soil. Soil samples were taken from two soil depths (0-10, 10-20 cm) in both control and heated plots in the Achenkirch soil warming project (>15 yrs soil warming by + 4 °C). Soil samples were pre-incubated at their corresponding field temperature after sieving and removal of visible roots. The soils were

amended with different combinations of glucose-C, inorganic/organic N and inorganic/organic P in a full factorial design, the nutrients being dissolved in  $^{18}\text{O}$ -water. After 24 hours of incubation, microbial growth was measured based on the  $^{18}\text{O}$  incorporation into genomic DNA. Nutrient (co)limitation was determined by comparing microbial growth responses upon C and nutrient additions relative to unamended controls. Basal respiration was also measured based on the increase in headspace  $\text{CO}_2$ , allowing to estimate microbial C use efficiency (CUE). The fate of C and nutrient amendments was finally traced by measurements of inorganic and organic extractable and microbial biomass C, N and P. This study will thereby provide key insights into potential shifts in limiting nutrients for microbial growth under long-term soil warming, and into concomitant effects on soil C and nutrient cycles.