



## **Stoichiometric controls of soil carbon and nitrogen cycling after long-term nitrogen and phosphorus addition in a mesic grassland in South Africa**

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Terrestrial ecosystems have experienced rising nitrogen (N) inputs with consequences for belowground carbon (C) and N dynamics. This study investigates how long-term N and phosphorus (P) additions affect microbial community composition, and to what extent microbial homeostasis explains changes in different processes involved in soil C and N cycling in response to element addition. We studied a 66-year-old nutrient addition experiment in a mesic grassland in South Africa, consisting of four different levels of N addition (0, 7, 14, and 21 g N m<sup>-2</sup>yr<sup>-1</sup>) with and without P addition (0, and 9 g P m<sup>-2</sup>yr<sup>-1</sup>).

Despite strong changes in the microbial community, the biomass C:N ratio did not change with N addition suggesting that microorganisms adjust rates of C and N cycling to their stoichiometric demands. Gene abundances of enzymes involved in degradation of labile C compounds (e.g. cellulose, hemicellulose, and chitin) as well as  $\beta$ -glucosidase and N-acetylglucosaminidase activities increased with elevated N availability and pointed to a more intensive investment into microbial C acquisition. In contrast, N addition decreased microbial N acquisition as indicated by reduced leucine-aminopeptidase activity and lower non-symbiotic N<sub>2</sub> fixation rates. Further, intensified N addition induced microbes to release excessive N into their environment, and accordingly net N mineralization was 4.0 - 6.7 times higher in the N fertilized treatments. Nitrogen addition and associated soil acidification decreased the microbial biomass and respiration and altered the community composition with prokaryotes being more affected than fungi. Nitrogen addition increased the relative abundance of gram-positive over gram-negative bacteria and favored taxa with low genome-size. While N addition strongly affected processes of the C and N cycle, P addition did not change soil element cycling.

Taken together, our findings support the concept that element cycling processes can be explained by the property of the soil microbial biomass to maintain its biomass stoichiometry and by its reaction to soil acidification. This highlights that predicted elevated N inputs might largely shape soil C and N cycling through microbial stoichiometric adaptations with positive feedbacks on the CO<sub>2</sub> sink function.