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The effect of long-term nutrient deficiency on the abundance and community composition of arbuscular mycorrhizal fungi in a mountainous grassland

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Arbuscular mycorrhiza (AM) fungi are associated with almost all land plants and provide soil nutrients and other benefits to their plant hosts in exchange for photosynthetic products. While fertilization regimes in managed grasslands or agricultural systems are tailored for increasing plant biomass, their potential effects on AM fungi are rarely taken into account. Nutrient-driven changes in abundance and community composition of AM fungi, however, may feedback on ecosystem performance in the long term. Therefore, it is necessary to get a better understanding on how AM fungal communities respond to changes and imbalances in soil nutrient availabilities.

Here, we evaluated how long-term nutrient deficiency of phosphorus (P), nitrogen (N) and potassium (K) affects the abundance and community composition of AM fungi in a mountainous grassland. In addition, we investigated how the responses of AM fungi to those deficiencies were modulated by liming and the type of fertilizer addition (inorganic versus organic).

Our study was carried out on a long-term nutrient deficiency experimental grassland site in Admont (Styria, Austria), established in 1946. Different fertilization treatments were applied for more than 70 years in a randomized block design, including numerous combinations of inorganic (P, N, K with/without lime) and organic (solid manure and liquid slurry) fertilizers. The hay meadow at the site is cut three times per year and biomass is not returned to the system. Therefore, biomass and nutrients have been continuously removed for decades, leading to different types of soil nutrient deficiency. In this study, we collected both root and soil samples in July 2019 and quantified AM fungi and other microbial groups by measuring neutral fatty acid (NLFA) and phospholipid fatty acid (PLFA) biomarkers, respectively. Additionally, we applied DNA and RNA-based amplicon sequencing of the 18S rRNA gene to identify AM fungal community composition.

Our data shows that deficiencies of one or more elements had a major impact on both AM fungal biomass and community composition. AM fungal biomass was higher in plots that received no

fertilizers compared to inorganically fertilized plots, but lower in plots which were deficient only in certain single or multiple elements, specifically in plots fertilized with inorganic N only (i.e., deficient in P and K). Conversely, liming and organic fertilizer amendments increased AM fungal biomass compared to plots containing inorganic fertilizers without lime. Across all treatments, AM fungal biomass was positively correlated with pH and soil water content, and negatively with dissolved N compounds, indicating indirect effects via responses of other soil parameters to nutrient deficiency. Long-term nutrient deficiency also altered plant community composition, which may also have indirectly affected AM fungal communities.

We conclude that long-term nutrient deficiency, and in particular the stoichiometry of available nutrients, strongly affects the abundance and community composition of AM fungi in grassland soil. This response may be linked to changes in plant community composition or soil chemistry both as a result and as a cause, emphasizing the complexity of feedbacks determining the response of grassland ecosystems to changing nutrient conditions.