

Exploring Potential Interactions of Bacteria and Arbuscular Mycorrhizal Fungi Using Network Analyses of an Annual Grassland Soils

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Project Goals: Soil surrounding plant roots is home to diverse biological activity and carbon (C) cycling. A substantial quantity of C moves from roots to the surrounding soil via mycorrhizal hyphae. A primary goal of our project is to understand and quantify how multi-trophic interactions mediate and control soil C dynamics; we focus here on one of the key components of belowground food webs, the interaction of arbuscular mycorrhizal fungi (AMF), bacteria, and roots. We applied co-occurrence network analyses to the bacterial and fungal communities sampled from a California annual grassland soil growing *Avena fatua* in a greenhouse experiment. Our goal was to identify covarying AMF and bacteria taxa adjacent to and distant from roots at different growth stages of the plant. We used patterns of covariation and coexclusion to identify potential AMF-bacterial interactions as a first step in elucidating interactions and their mechanisms.

AMF form associations with 80% of land plant species, facilitating the transfer of plant-derived C into surrounding soils, and increasing the volume of soil from which plants can access nutrients. The presence of AMF is known to correlate with changes in soil bacteria, and the interactions between plant, AMF, and bacteria likely play a significant role in mediating soil C dynamics. Yet less is known about the identities of associated AMF and bacterial species, the timing and location of interactions, and the mechanisms involved. Here we explore the co-occurrence patterns between soil fungi and bacteria using Random Matrix network analyses, expecting network analyses to provide novel information towards understanding multi-trophic interactions in soil food webs. In a greenhouse experiment with a highly replicated design, we studied both rhizosphere and bulk soils associated with different growth stages of the annual grass *Avena fatua*, under conditions mimicking two growing seasons in the relevant Mediterranean climate with a summer dry down between growing seasons. Sequences of ITS and 16S rRNA genes were used to investigate fungal, including AMF, and bacterial communities over the stages of plant growth.

Roots impacted both bacterial and fungal communities in the rhizosphere. Overall, rhizosphere communities had lower diversities and compositions distinct from those in bulk soils. As plants grew through both seasons, rhizosphere bacterial and fungal communities underwent dramatic succession and a decrease in diversity, while communities remained much less changed in bulk soil. Dry down (simulating summer) caused significant shifts in both rhizosphere and bulk community compositions, with a stronger impact on the diversity of bacteria than fungi. AMF detected in these soils also differed in composition between season and sampling locations. Interestingly, when compared to rhizosphere soil, the diversity of detected AMF was higher in bulk soil, where diversity increased across the two seasons, despite the dry down period.

Co-occurrence networks were constructed for both rhizosphere and bulk soils at different plant growth stages, including all pairs of fungi and bacteria whose abundances covaried. Using a random matrix theory-based detection method, levels of covariation significance were determined for each network. Twenty-two AMF OTUs from five genera, including *Glomus*, *Funneliformis*, *Claroideoglomus*, *Acaulospora*, and *Paraglomus*, were captured by the network. These AMF and their covarying bacteria formed larger networks that increased in size over time in bulk soil but not rhizosphere soil, and in the second season compared with the first. The increase in covarying AMF-bacteria pairs over time in bulk soil was consistent with the increase of AMF diversity in these soils.

We found more covarying AMF-bacteria pairs in bulk soil than rhizosphere soil. In the same system, covarying bacteria-bacteria and bacteria-fungi pairs were generally more frequent in rhizosphere soil where root exudates provided a source of labile C. There are several possible, complementary, explanations for the pattern of increased co-occurrence of AMF-bacteria pairs in bulk soil. As plants take up nutrients from the rhizosphere, a depletion zone is generally established directly around the root, leading AMF to extend hyphae beyond that zone to reach required nutrients. In fact, both AMF mycelium and spores may be more abundant in bulk soil than rhizosphere soil, paving the way for more interactions with bacteria in bulk soil. We found AMF taxa co-occurring with bacteria of the orders Myxococcales, Spinnigobacterales, and Xanthomonadales, including groups known to degrade biopolymers. It has been proposed that AMF exudates provide C to bacteria in bulk soil, possibly promoting decomposition by bacteria. AMF may then take up nutrients freed up by the decomposition. AMF spores have been reported to contain intracellular bacterial microbiomes which include bacteria from the order Burkholderiales; this bacterial order was found associated with AMF in our networks.

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