

Carbon Transported by Arbuscular Mycorrhizal Fungi to Soil Alters the Characteristics of Soil Organic Carbon as well as the Soil Microbial Community

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Project Goals: Our project asks how cross-kingdom and within-kingdom interactions (involving viruses, bacteria, archaea, fungi, protists, microfauna, and plant roots) provide a functional framework for nutrient cycling in grassland soils. We are using stable isotope probing, NanoSIMS, metagenomic and metatranscriptomic sequencing, exometabolomics, network analysis, and ecosystem modeling to unravel how biotic interactions shape nutrient availability and loss pathways and how these interactions and pathways differ among soil compartments (rhizosphere, detritosphere, hyphosphere, and bulk soil). In the work presented here, our primary goals are to: (1) illuminate how arbuscular mycorrhizal fungi (AMF) move carbon (C) beyond the plant rhizosphere into bulk soil and affect the early stages of carbon stabilization, and (2) investigate the influence of AMF on soil bacterial communities and bacterial facilitation of nutrient availability to plants.

Arbuscular mycorrhizal fungi (AMF) consume up to 20% of plant photosynthetic carbon (C) and grow extensive hyphal networks into the soil; AMF thus have the potential to influence soil C dynamics. Plant root litter, exudates, and microbial residues are thought to be the primary sources of organic C in soil, and they are subsequently transformed into soil organic matter (SOM) by a series of chemical and microbial processes. However, the mechanisms responsible for the persistence of SOM are complex and the roles of specific microbial groups are not well characterized. AMF represent an important pathway for the flow of C from plants into the soil and may thereby alter surrounding soil bacterial communities. These bacteria can colonize hyphae, consume hyphal exudates, and help AMF mobilize nutrients in soil.

We used ¹³C stable isotope tracing to measure the transfer of C from the host plant *Avena barbata*, a widespread annual grass, into the soil via the AMF, *Rhizophagus intraradices*. In a greenhouse experiment, we used a two-chamber microcosm design to distinguish the fluxes of C from AMF from those of roots. To illuminate how AMF affect the early stages of C accumulation, we tracked ¹³CO₂ as it was fixed by host plants and transferred to AMF over the course of six weeks during the exponential phase of plant growth. We assessed changes in C chemistry with solid state ¹³C nuclear magnetic resonance (NMR) spectroscopy in soil accessible to AMF only. We characterized the form of AMF-contributed C by density gradient fractionation into three pools: a heavy fraction (likely mineral associated), a free light fraction (likely still as free hyphae), and an occluded light fraction (likely hyphae protected in aggregate structures).

Organic C in these soil fractions has distinct rates of biochemical and microbial degradation and these fractions are widely thought to represent ecologically relevant soil subunits.

Using nanoscale secondary ion mass spectrometry (NanoSIMS), we determined that hyphae and roots had a similar level and distribution of ^{13}C enrichment. By isotope ratio mass spectrometry (IRMS), we found that after six weeks of labeling, 26.7 mg of AMF-transported ^{13}C remained in the soil, which accounted for 1.1% of the total soil C pool. Of the ^{13}C that remained in the soil, 17.8 mg or 67% of the in the free light fraction, 2.2 mg or 8% of the total soil C in the occluded fraction, and 6.7 mg or 25% in the heavy fraction. Thus, after six weeks, 33% of the C transported by AMF was in a potentially protected form (mineral associated or aggregate-occluded). ^{13}C -NMR spectra showed a larger carbohydrate peak in the spectra for soil with AMF compared to soil without AMF. This suggests that AMF produced organic compounds in the form of hyphae and/or released metabolites that contained a large proportion of carbohydrates.

We also investigated the influence of AMF hyphae on soil bacterial communities beyond the direct influence of roots, finding that AMF significantly modified the soil bacterial community composition but not diversity. Out of a total of 3019 amplicon sequence variants (ASVs), nineteen ASVs significantly increased and seventeen ASVs significantly decreased in relative abundance in the presence of AMF by DESeq analysis. Over half of the ASVs (and sequences) that responded to the presence of AMF, either positively or negatively, were *Proteobacteria*. A number of the ASVs that increased in relative abundance in the presence of AMF in our study match bacterial taxa that are often found in the rhizosphere, including *Arthrobacter crystallopoietes*, *Caulobacter sp.*, *Rhizobium sp.*, *Dongia sp.*, and two *Verrucomicrobia* taxa.

In summary, AMF moved a substantial amount of C from plants beyond the rhizosphere compartment into the bulk soil. About a third of that C occurred as mineral-associated and aggregate-occluded forms, which may comprise C forms with some initial stability. NMR spectroscopy indicated an increased presence of organic C compounds (such as chitin) that can be associated with AMF hyphae. AMF C inputs modified the bacterial community, potentially enhancing AMF access to N and P.

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