

Microbial and Viral Niche-Differentiation in Time-Resolved Metatranscriptomes from Rhizosphere and Detritosphere Soil

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Project goals: Our primary research goals are to identify and quantify the importance of key interactions including predation, competition, and cooperation as controllers of (i) N and C mineralization/immobilization, (ii) depolymerization of macromolecular organic N compounds, (iii) nitrification and denitrification, and (iv) N and C transfers by fungi and fauna that enhance or constrain N-cycling processes. Our primary hypotheses are: (1) Viral infection of bacteria, fungi, and fauna initiates cell lysis and plays a significant role in macromolecular depolymerization, and N-mineralization and culls specific communities mediating N-transformations. (2) Faunal predation mobilizes biomass nutrients present in microbiota, differentially affects specific prey populations, and potentially transports viruses, bacteria and fungi across soil habitats. (3) Fungi mediate C transfer, transport bacteria, shape bacterial N-transformation, and themselves are key to depolymerization, mineralization, immobilization, and denitrification. (4) Dominant interactions differ by soil habitats, affecting N process rates.

The rhizosphere is a hotspot for microbial activity and biomass and the entry point for organic carbon. Plants deposit a significant proportion of their photosynthates into soil as root biomass or exudates, and plant-derived polymeric carbohydrates such as cellulose and hemicellulose are the most abundant polysaccharides in soil. Additionally, breakdown and utilization of macromolecular nitrogen may be mediated in soil near roots. Rhizodeposits create a high resource, high activity environment, and stimulate microbial succession as roots grow and senesce, potentially selecting for organisms that benefit mineral nutrition and plant health. This bloom of microbial growth may trigger a succession of viral populations in response. Rhizodeposits may also stimulate depolymerization by cellulases, chitinases, proteases, and carbohydrate degradation genes. Using *Avena fatua*, a common annual grass, we analyzed time-resolved metatranscriptomes to compare microbial functional dynamics related to C and N processing in rhizosphere, detritosphere, and combined rhizosphere-detritosphere habitats. We also used this dataset to delve into the largely unknown realm of soil RNA viruses and explore their diversity and spatiotemporal dynamics.

During three weeks of root growth, microbial community composition shifted only slightly, whereas gene expression profiles changed significantly, indicating that mRNA is more sensitive to changes in environmental conditions and can reflect community shifts before they are detectable by diversity markers. With carbohydrate active enzymes (CAZy) identified in the metatranscriptomes, we used hierarchical clustering and mapping to a site-specific metagenome to identify functional guilds. These guilds revealed taxa specializing in rhizosphere carbohydrate degradation vs. taxa that specialize in detritus degradation and, surprisingly, a guild that specialized in the breakdown of aging roots. Rhizosphere and detritosphere guilds expressed enzymes for cellulose and xylan degradation and their byproducts. This indicates that complex

cross-feedings networks could promote coexistence within highly interconnected rhizosphere communities (Nuccio et al., 2020).

Nitrogen cycling gene expression varied over time and in the presence/absence of detritus, with distinct rhizosphere, detritosphere and aging root functional guilds. The taxonomy of these guilds often mirrored those previously identified using CAZymes. Expression of extracellular proteases was significantly lower in the rhizosphere compared to bulk soil. Ammonia oxidation (AO) transcripts were dominantly archaeal and more highly expressed in bulk soil, which may reflect competition for ammonium with plant roots. While archaeal ammonia oxidation genes were several-fold more abundant than their AO bacterial counterparts, expression of archaeal ammonia monooxygenase was higher by orders of magnitude, implying that the overexpression of AO genes cannot be attributed simply to a higher abundance of ammonia oxidizing archaea.

Our large metatranscriptomics dataset also allowed us to identify RNA viruses—which are understudied in most environments and have been largely ignored in soil (with the exception of plant pathogens). There is almost no knowledge of the diversity and host range of these viruses, which could have a significant impact on microbial community structure and soil carbon cycling. We targeted the RNA-dependent RNA-polymerase (RdRp) gene, which is universal to RNA viruses. We improved the set of hidden Markov models (HMMs) used to identify this gene and significantly increased the known diversity of RNA viruses in existing databases. Most of these viruses likely infect abundant taxa such as fungi and Proteobacteria, indicating that they may significantly affect microbial dynamics in soil. The temporal dynamics of RNA-viruses we identified also indicate that they are replicating, and therefore infecting their hosts, potentially leading to a release of OC into soil. The diversity of RNA viruses as well as of potential hosts was structured by the presence or absence of detritus (Starr et al., 2019).

Our analyses are a step towards understanding multitrophic interactions in soil and emphasize the need to think beyond community structure. Gene expression studies may be valuable in elucidating the complex to-and-fro between plants and their associated microbial and viral communities, as well as identifying the guilds of organisms that orchestrate C and N processing in soil.

References

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