

Next-generation sequencing reveals patterns of co-occurrence in the soils of Great Smoky Mountains National Park

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ABSTRACT

Nematodes and bacteria are highly important in the maintenance and ecology of soil communities. Bacteria are responsible for the breakdown and subsequent transformation of recalcitrant organic matter into biomass that is accessible to other groups of organisms. Nematodes exert top-down influence on bacterial populations through predation and grazing of biofilms. It has been shown that nematodes display a preference for certain bacterial taxa as food items. Many of these taxa are capable of nitrogen fixation and dissimilatory metal metabolism, implying that nematode food preference may affect the chemical and trophic state of the soils they inhabit. Interactions between soil nematodes and bacteria will be investigated via next generation sequencing and data analysis using QIIME 2. Co-occurrence networks will be used to establish possible ecological roles in the soils of Great Smoky Mountains National Park.

INTRODUCTION

Nematodes and bacteria are among the most abundant and significant groups of soil organisms. One estimate suggests that there may be as many as 4.4×10^{20} nematodes living within forest soils globally, with a total biomass of 300 million tons. Nematodes may be predatory or parasitic, feeding on a domain spanning variety of organisms, including bacteria, archaea, fungi, plants, and other nematodes. The extreme metabolic versatility of bacteria allows them to play various roles in soil ecology and health. This includes the capacity to degrade recalcitrant organic matter, such as cellulose, lignin, keratin. These organic carbon species are unavailable to most other soil inhabitants. Conversion of these recalcitrant organics into bacterial biomass allows carbon, that might otherwise be unreachable, to re-enter circulation throughout the soil ecosystem. Aside from acting as effective decomposers, bacteria are largely responsible for nitrogen fixation; the process in which molecular nitrogen is converted into an assimilable form such as ammonia. Among all nematode feeding types, bacterivores, nematodes that consume bacteria, are the most abundant in soil, representing over half of all free-living nematodes. Bacterivorous nematodes have been demonstrated to display foraging preferences. Given the crucial role bacteria play in soil function and nutrient cycling, differential grazing can be expected to affect soil heath and succession, as well as above-ground diversity and biomass. Quantifying bacterial, fungal and nematode assemblages with next-generation sequencing and qPCR allows co-occurrence networks to be constructed. These in silico representations of ecological communities offer a powerful tool for hypothesis generation.

METHODS

Soil samples were collected at 19 sites across Great Smoky Mountain National Park. From these samples fungal ITS and bacterial 16S rRNA gene copies were quantified via next-generation sequencing. Nematode 28S rRNA genes were quantified via qPCR. Sequence data generated by next-generation sequencing were then denoised, subjected to de novo clustering, and classified in QIIME 2. α -diversity indices were also calculated from this data. Data were then imported into CoNet, an ecological network inference tool. Co-occurrence networks were visualized in Cytoscape 3 and networks were modularized using ModuLand2.0.

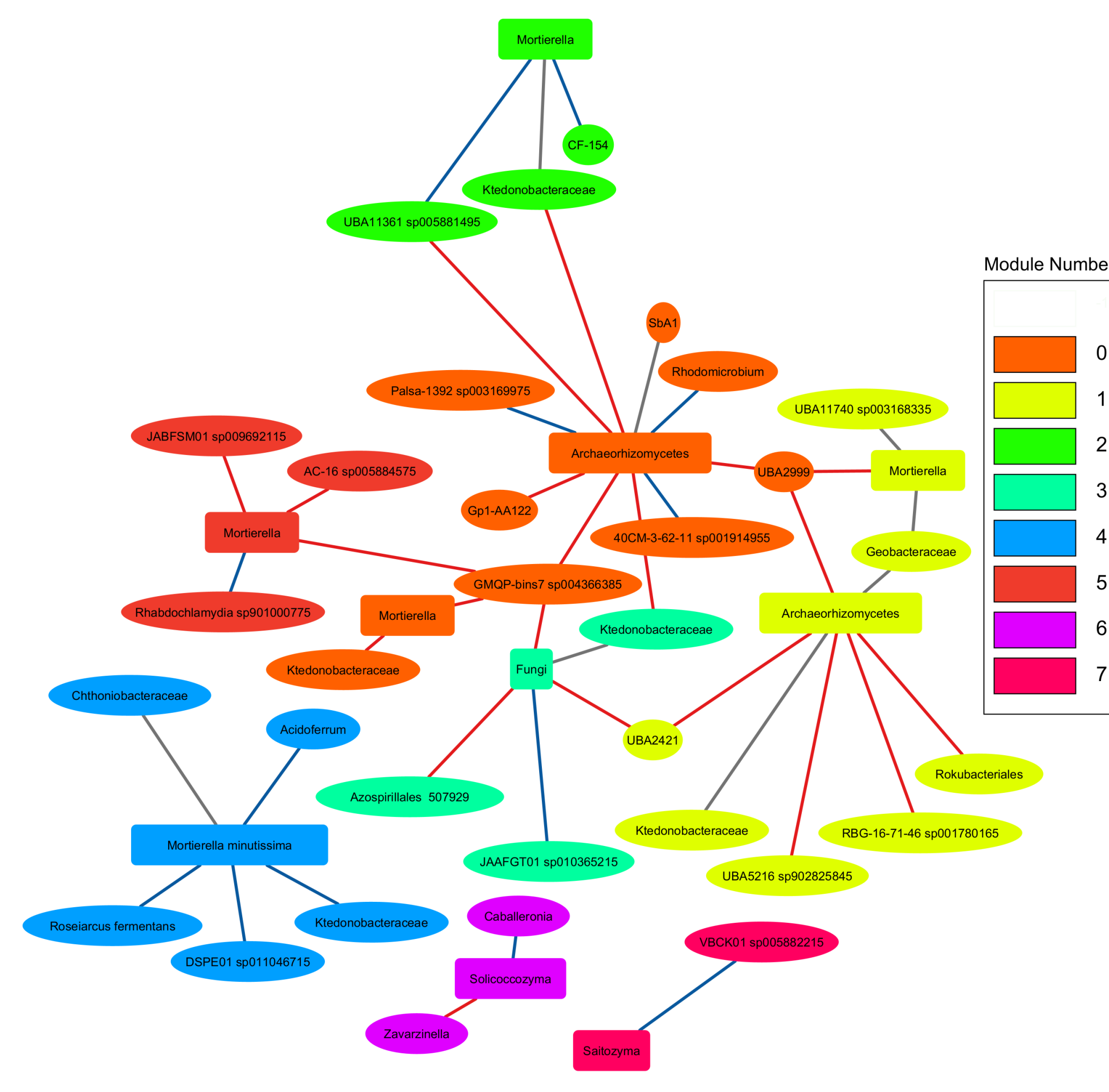


Figure 1: Bipartite co-occurrence network generated from next-generation sequencing data characterizing the identity and abundance of bacteria and fungi at 19 sites across GSMNP.

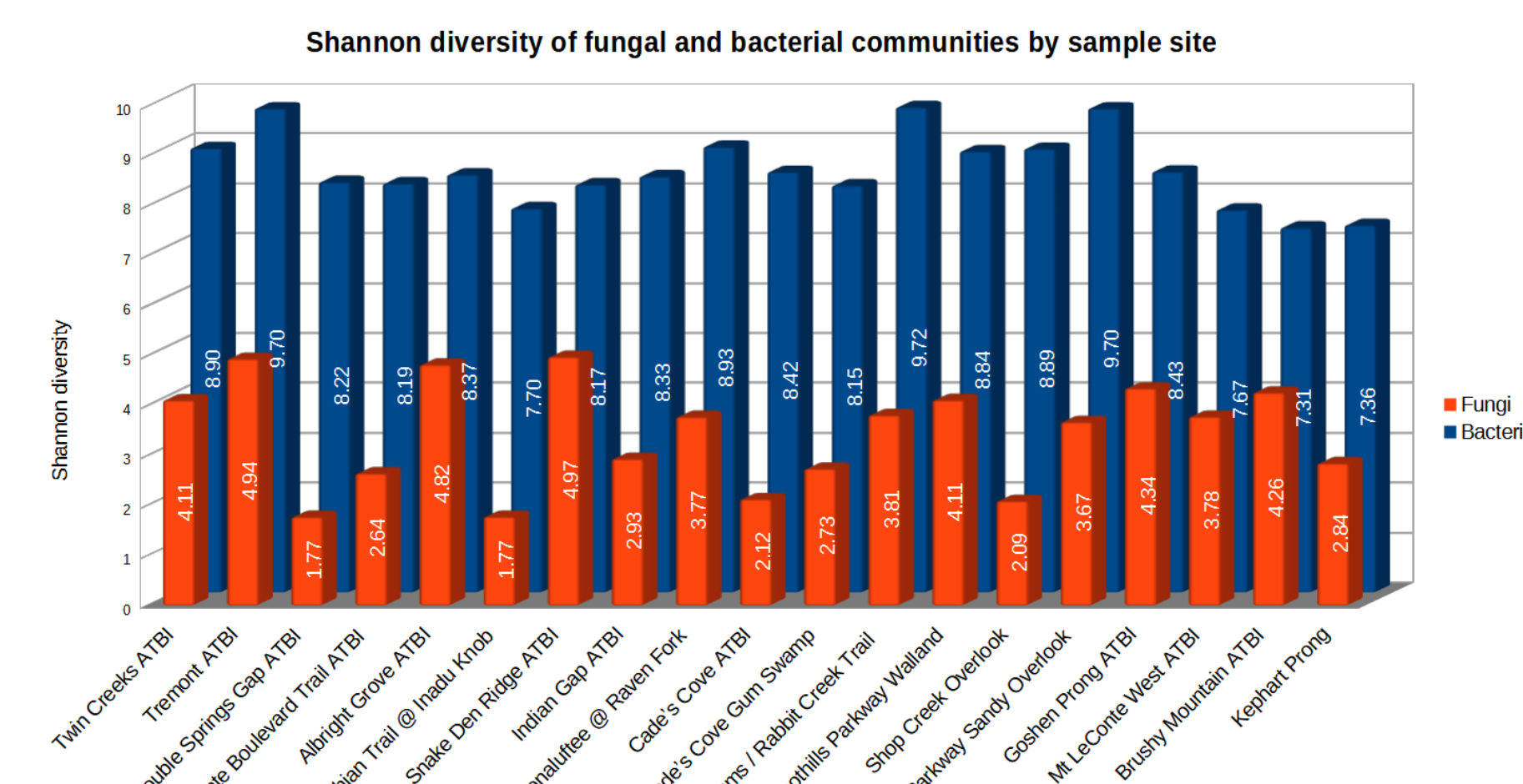


Figure 2: Shannon diversity of sampled fungal and bacterial assemblages.

RESULTS

Only very weak correlation between bacterial diversity and abundance of bacterivorous nematode taxa ($R^2 = 0.131$). Relationships were however found by co-occurrence network inference ($p < 0.05$). The most abundant bacterial phyla were Acidobacteria, Proteobacteria, Planctomycetota and Chloroflexota. The most abundant fungal phyla were Basidiomycota, Ascomycota and Mortierellomycota. The most abundant nematode genus was the omnivorous worm *Labronema*.

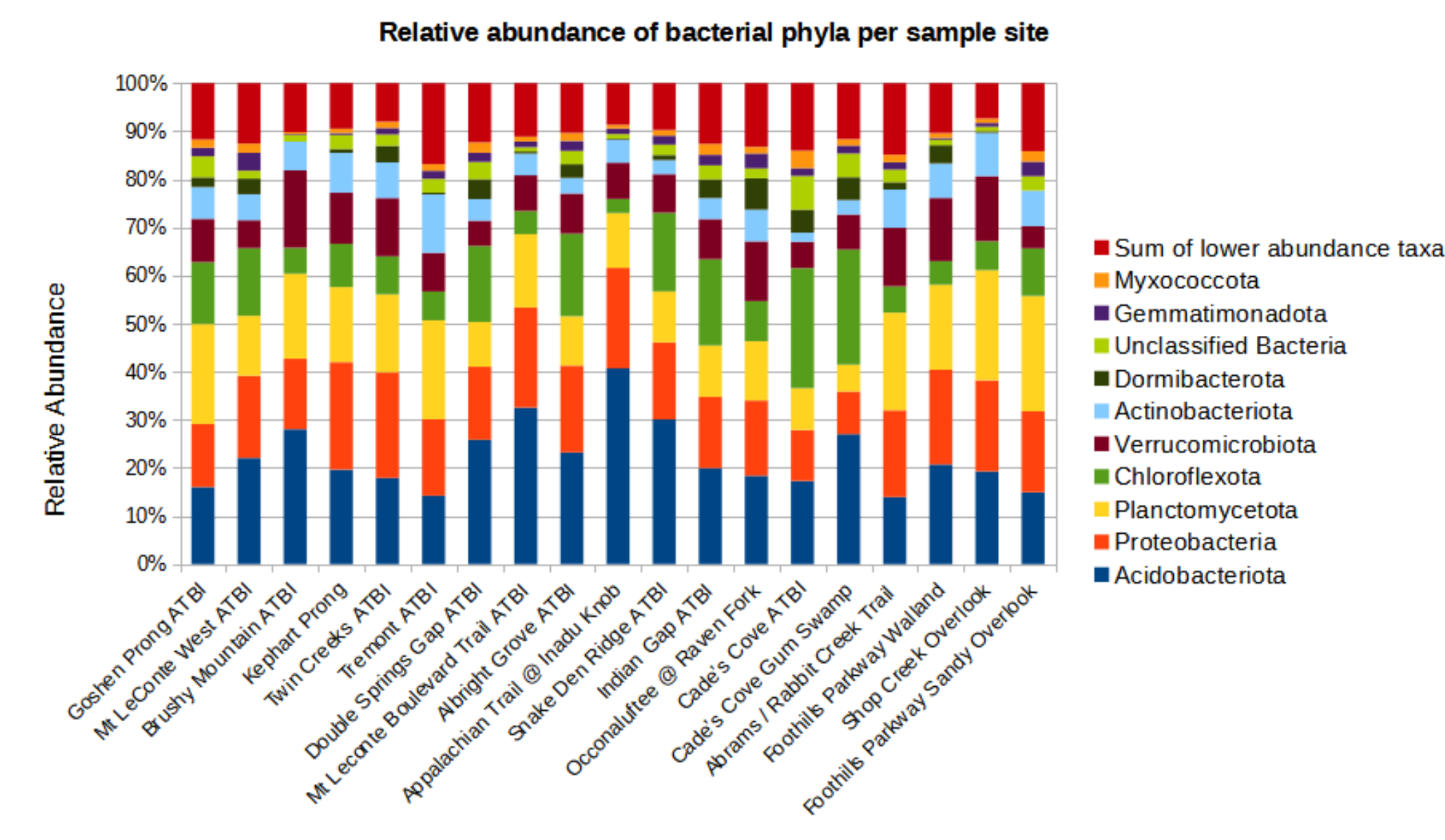


Figure 3: Relative abundance of bacterial phyla

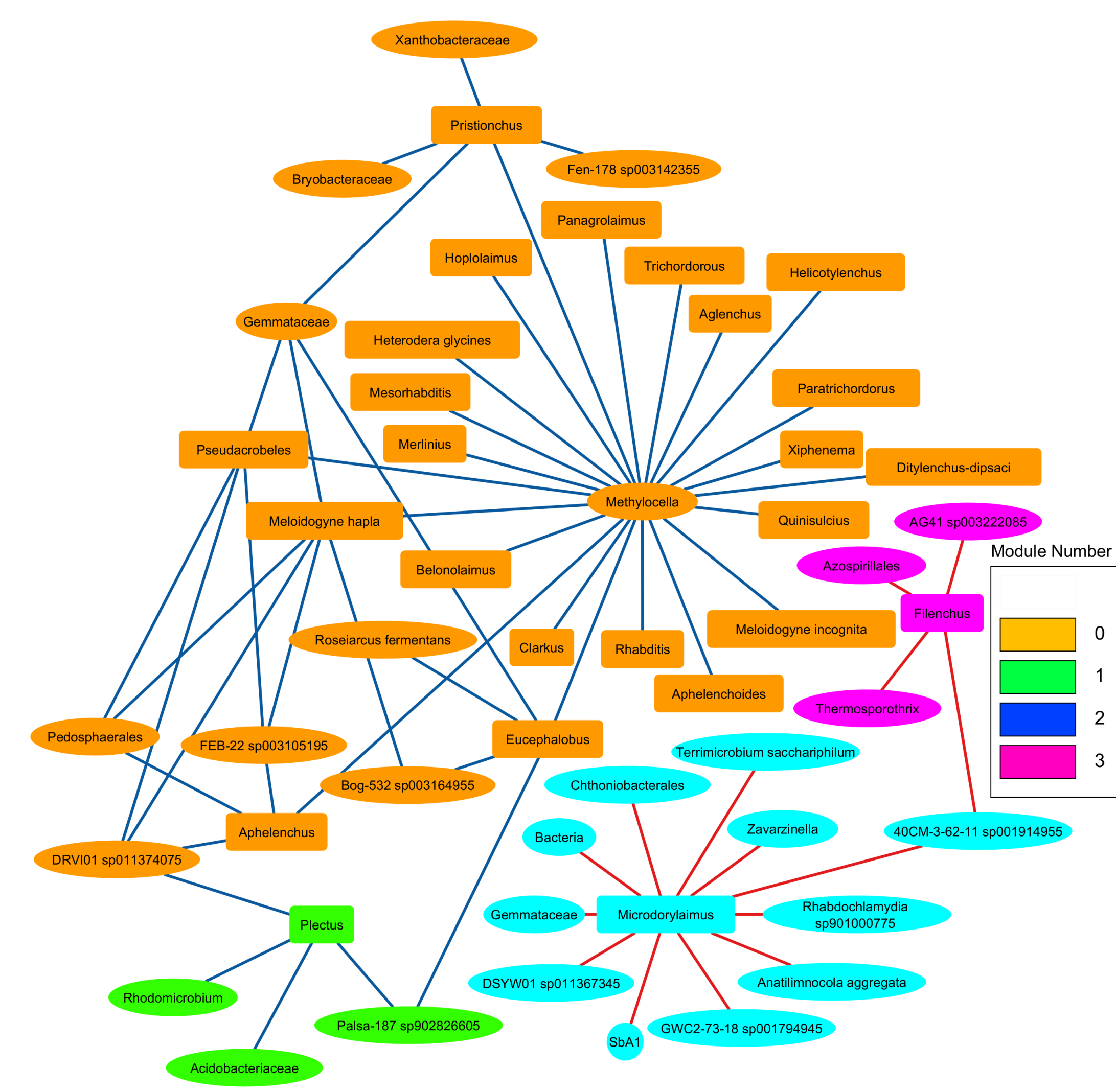


Figure 4: Bipartite co-occurrence network generated from next-generation sequencing data and qPCR data characterizing the identity and abundance of bacteria and nematodes at 19 sites across GSMNP.

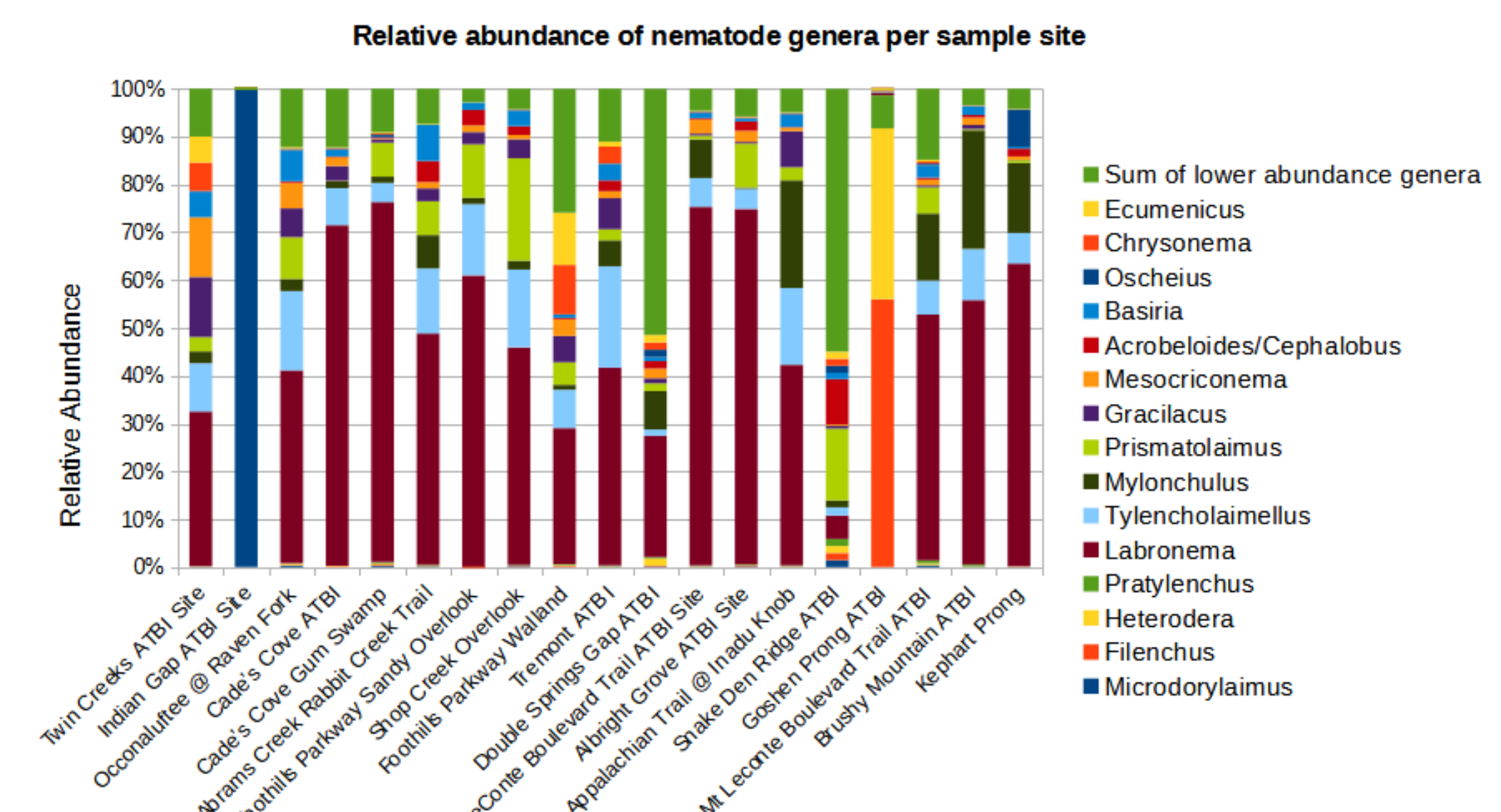


Figure 5: Relative abundance of nematode genera

CONCLUSIONS

Both mutual exclusion and co-occurrence were found in inferred networks. While some of these may be direct trophic relationships, such as predation or parasitism, most are likely to be indirect. It is necessary to be skeptical when interpreting co-occurrence networks. The relative abundances of bacterial and fungal phyla were within the expected range and displayed sampling biases introduced by the sampling protocol and extraction protocol followed. Shannon indices calculated for fungal assemblages were in all cases lower than Shannon indices calculated for bacterial communities. This is not unexpected as bacteria are more abundant in soil and are more diverse.

- Collecting replicate samples would improve the reliability of future studies and is considered "good practice," and may mitigate the following.
- Ribosomal gene copy number varies from taxon to taxon and can prevent direct inference of organism abundance from quantified genes.
- Multicellular and poly-nucleate organisms have a disproportionately large impact on abundance estimates.

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