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Comparative fungal community analyses using metatranscriptomics and ITS-amplicon sequencing from Norway spruce

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Microbial communities are major players in carbon and nitrogen cycling globally and are of particular importance for plant communities in the nutrient poor soils of boreal forests. Especially relevant are the fungal communities in the soil that interact with the plants in multiple ways, indirectly through their pivotal role in the breakdown of organic matter and, more directly, through mycorrhizal symbiosis with plant roots. Large-scale disturbances of these complex microbial communities can lead to shifts in soil carbon storage with unknown and global-scale long-term consequences. To understand the dynamics of these communities and their relationship to associated plants in response to climate change and anthropogenic influence, we need a better understanding of how modern “omics” methods can help us to understand compositional and functional shifts of these microbiomes. Microbial gene expression and functional activity can be assayed with RNA sequencing (RNA-Seq) data from environmental samples. In contrast, currently phylogenetic marker gene amplicon sequencing data is generally used to assess taxonomic composition and community structure of the microbiome. Few studies have considered how much of this structural and taxonomic information is included in RNA-Seq transcriptomic data from matched samples. Here we describe fungal communities using both RNA-Seq and fungal ITS1 DNA amplicon sequencing to compare the outcomes between the methods. We used a panel of root and needle samples from mature stands of the coniferous tree species *Picea abies* (Norway spruce) growing in untreated (nutrient deficient) and nutrient enriched plots at the Flakaliden forest research site in boreal northern Sweden. We created an assembly-based, reproducible and hardware agnostic workflow to taxonomically and functionally annotate fungal RNA-Seq data obtained from Norway spruce roots, which we compared to matching ITS amplicon sequencing data. We show that the community structure indicated by the fungal transcriptome is in agreement with that generated by the ITS data, while also identifying limitations imposed by current database coverage. Furthermore, we show examples to demonstrate how metatranscriptomics data additionally provides biologically informative functional insight at the community and individual species level. These findings highlight the

potential of metatranscriptomics to advance our understanding of interaction, response and effect both between host plants and their associated microbial communities, and among the members of microbial communities in environmental samples in general.