Structure of orchid mycorrhizal fungal communities in soil does not affect endo-mycorrhizal fungal communities within orchid roots

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Introduction

Orchidaceae is considered as one of the largest and most diverse plant families with an estimated 20,000-35,000 species (Dressler, 1993). Orchid mycorrhizal associations are different from other types of mycorrhizae in that mycorrhizal symbiosis is considered necessary for seed germination, and early plant development. Orchids show considerable variation in mycorrhizal interactions ranging from partial mycoheterotrophy to obligate mycoheterotrophy, and from generalist to specialist fungal partner requirements. While seed germination in orchids is known to be influenced by both the abundance of mycorrhizal fungi in soil (McCormick et al, 2016), none to few studies have focused on correlating diversity and abundance of orchid mycorrhizal fungal (OMF) species in soil on the structure of mycorrhizal fungal communities within the roots of adult orchid plants.

Hypothesis

Hypothesis: The structure of mycorrhizal fungal communities in orchid roots is driven by the diversity and abundance of orchid mycorrhizal fungi in the rhizosphere. Fungal communities in soil where orchids do not occur are structured differently in comparison to soil at sites that host orchid plants.

Materials and Methods

Study species: *Platanthera praeclara* (Fig. 1a) was selected as a model species due to its wide geographic distribution within the Tallgrass Prairie of North America. High specificity toward its mycobionts has been reported (Tovar, 2015) in this rare species across time and space despite its wide geographic range.

Sample collection – soil was sampled at sites where orchids occur and at sites where the orchid species is absent across four states (Iowa, Minnesota, Nebraska, and North Dakota) within the United States, and Manitoba in Canada (Fig. 1b)

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DNA extraction from 0.5g of each soil samples

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Sequencing - ITS2 + partial LSU region of fungal rDNA

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Bioinformatics - PEAR, QIIME, VSEARCH

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Biostatical analyses in R version 3.2.4.

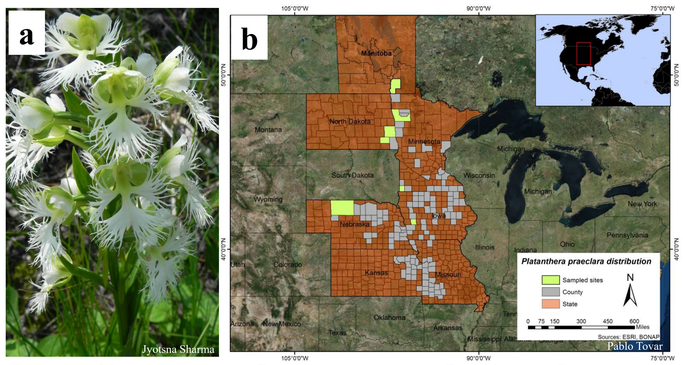

Figure 1: a) Model orchid species *Platanthera praeclara*, b) Distribution of *P. praeclara*

Results

- **Analysis of variance (ANOVA) did not detect differences in OTU diversity of OMF species in soil sampled across 12 sites (p = 0.50). Further, soil representing rhizosphere of orchids and soil where orchid plants were absent hosted similar OMF communities (p = 0.09).**
- **The relative abundances of generally known OMF families showed Tulasnellaceae as the most abundant (Fig. 2a) at sampled sites, whereas Ceratobasidiaceae was the most abundant OMF family within the roots of *P. praeclara* (Fig. 2b).**

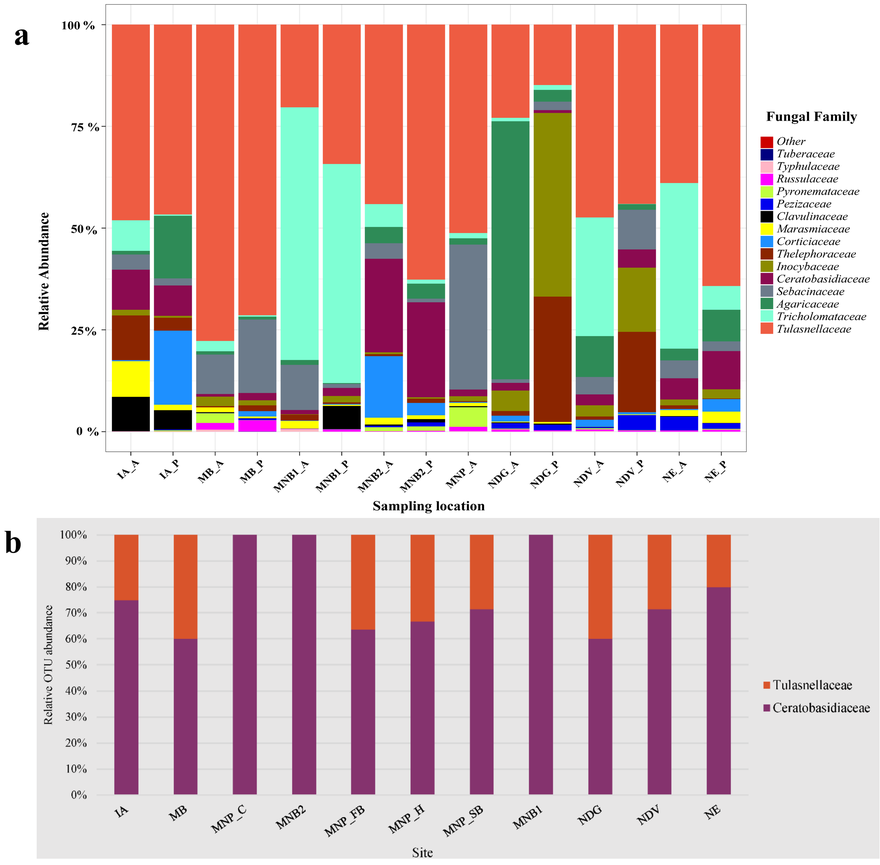

Figure 2: Relative abundances of general known OMF families in **a)** soil collected from Iowa (IA), Minnesota (MNP, MNB1, MNB2), Nebraska (NE), and North Dakota (NDG, NDV) in United States, and Manitoba (MB) in Canada; **b)** roots *of P. praeclara* plants. MNP site is represented by three sites, MNP_FB, MNP_H and MNP_SB.

- **Within Ceratobasidiaceae, *P. praeclara* specific OTUs exhibited 5-75% abundance in soil where orchid plants were present, and 0-20% abundance where orchid plants were absent (Fig. 3). *Platanthera praeclara* specific Tulasnellaceae OTUs were observed only at MNP_P and NDV_P sampling locations with <1% abundance.**

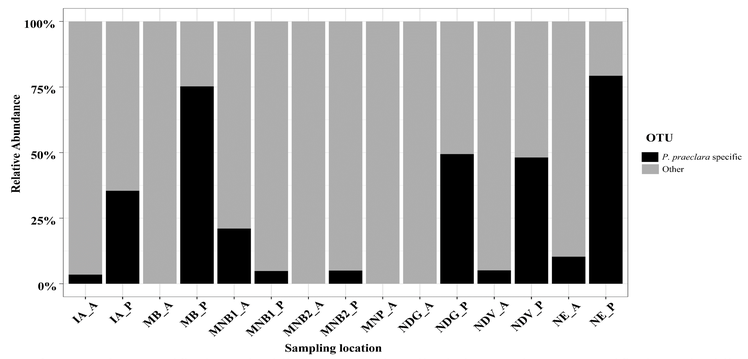

Figure 3: Abundances of *P. praeclara* specific Ceratobasidiaceae OTUs in soil relative to other non-specific Ceratobasidiaceae OTUs

- **Permutational multivariate analysis of variance (PERMANOVA) based beta diversity comparisons with both unweighted and weighted Bray-Curtis dissimilarity index revealed differences in richness and abundance of *P. praeclara* specific OTUs in soil across sampling locations (p <0.05).**
- ***P. praeclara* specific OTU-based hierarchical clustering analyses clustered orchid-present (population names followed by 'P') and orchid-absent (population names followed by 'A') sites separately (Fig. 4).**

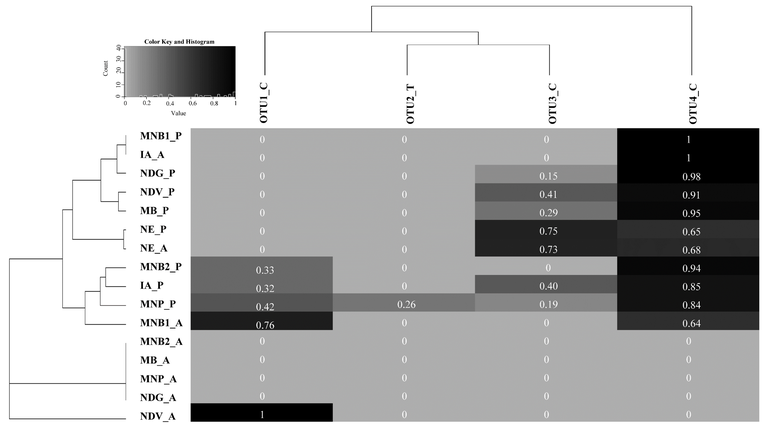

Figure 4. Principal Coordinate Analysis with weighted Bray-Curtis dissimilarity values

Conclusions

- The specificity of *P. praeclara* toward a few members of Ceratobasidiaceae is not driven by the richness and abundance of OMF families in soil given the higher abundance of Tulasnellaceae fungal family in soil. This specificity is likely a result of long evolutionary history and high specificity between OMF species and its host plant.
- Within Ceratobasidiaceae, *P. praeclara* roots exhibited high specificity toward its mycobionts independent of their abundance in soil.
- The absence of *P. praeclara* specific Ceratobasidiaceae and Tulasnellaceae OTUs at sampling locations not occupied by *P. praeclara* is likely the reason for the orchid's restricted and fragmented populations.

Acknowledgements

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