Distribution of Mycorrhizal Fungi in Different Soil Zone with Pyrosequencing Approach

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Abstract

We collected 83 soil samples distributed in all five soil types of Saskatchewan, and amplified 18s rDNA as our target DNA segment with primer pairs NS1/NS4 and AMV4.5NF/AMDGR. Sequencing results show that there is rich diversity of AM and other soil fungi in summer season but fungal composition vary among different soil zones and culture management.

Introduction

Most plants live in mutualistic association with soil fungi. In the temperate grassland biome, arbuscular mycorrhizal (AM) fungi, which have a very ancient origin dating back to the Early Devonian, over 400 million years ago, when the early AM fungi helped plants' ancestors to colonize the continents of Earth (Strullu-Derrien and Strullu 2007). Today AM fungi collectively form the order Glomeromycota. In soil, AM fungi form dynamic hyphal networks, which are shared by the members of plant communities. AM hyphal networks can extend well the ability of multiple nutrient uptake of plant, especially soil P nutrient. On the other hand, plant can also provide carbon back for development of AM fungi. This good relationship is very important support for plant-AM symbiosis.

Although mycorrhizal fungi give the sign of benefits to plants, we didn't known well about how it works and how we can effectively use their relationship. So the essential to understand is to discovery the diversity or distribution of mycorrhizal fungi naturally exit in our cropping system. Our objective is to discover AM fungal resources in cultivated soils of the Canadian Prairie using a novel, very powerfull, cultureless, DNA-based method to study biodiversity. Pyrosequencing is very high sensitive and deep sequencing that were popular applied to research the richness, diversity and community composition of microecosystem, which can offer high-throughput DNA analysis of large number of soil micro-organism. This powerful and sensitive sequencing method we used to detect AMF diversity in our soil sampling sites.

Material and method

Sampling

July 2009 summer, we sampled 48 commercial wheat fields in organic or conventional management and 34 wheat plots in research stations.

DNA extraction, PCR and pyrosequencing

Soil raw DNA was extracted from soil samples with UltraClean® Soil DNA Isolation kit. Nest-PCR amplified 18s rDNA as target region of mycorrhizal fungi with primer pairs NS1/NS4 and AMV4.5NF and AMDGR. PCR products were pyrosequenced under contract at Génome Québec (Montréal, Québec, Canada).

AM fungi DNA sequencing data analysis

Bioinformatics: verication and preparation of the sequences for data processing using Mothur v.1.15.0. Sequences were clustered into OTUs, and Chao1 and ACE sample richness estimators were calculated by Mothur. Taxonomic assignment was performed by BLAST representative sequence from each OTU against GenBank nr/nt nucleotide database.

Results

Table 1. Total Number of Fungal Sequences Obtained from the Soil Samples, by Soil Type

Soil type	No. of sample	No. of $OTUs_{0.03}$	Chao1	ACE	No. of *Glomeromycota* sequences
Black	23	2357	3870	4937	3754
Brown	25	1989	3067	3639	1966
Dark Brown	20	2036	3371	4150	1964
Dark Gray	7	651	1157	1410	515
Gray	2	219	282	305	81

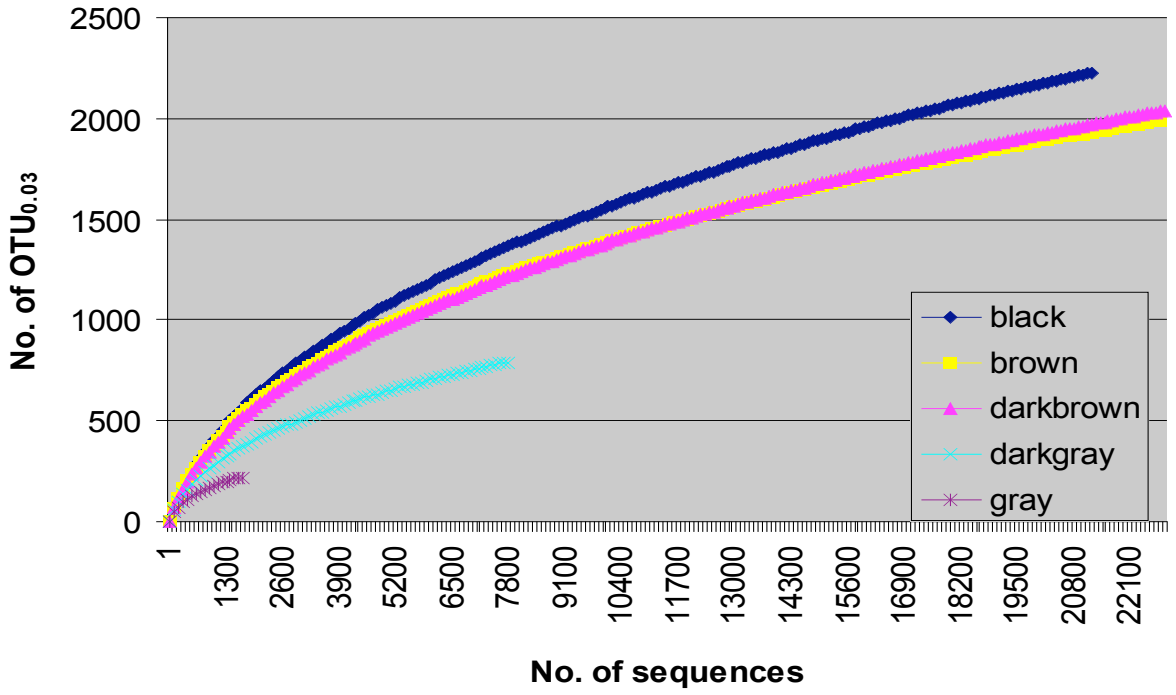

Fig. 1 Rarefaction Curves for Fungal $OTUs_{0.03}$ Identified from the Five Soil Zones

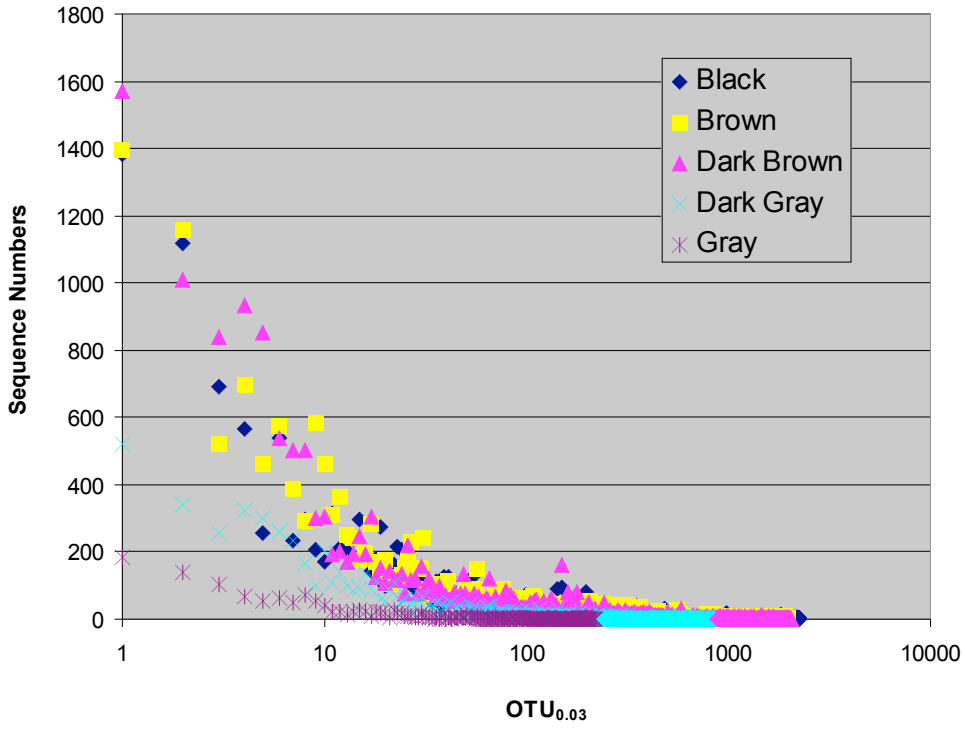

Fig. 2 Rank-abundance of Fungal $OTUs_{0.03}$ in the Five Soil Types

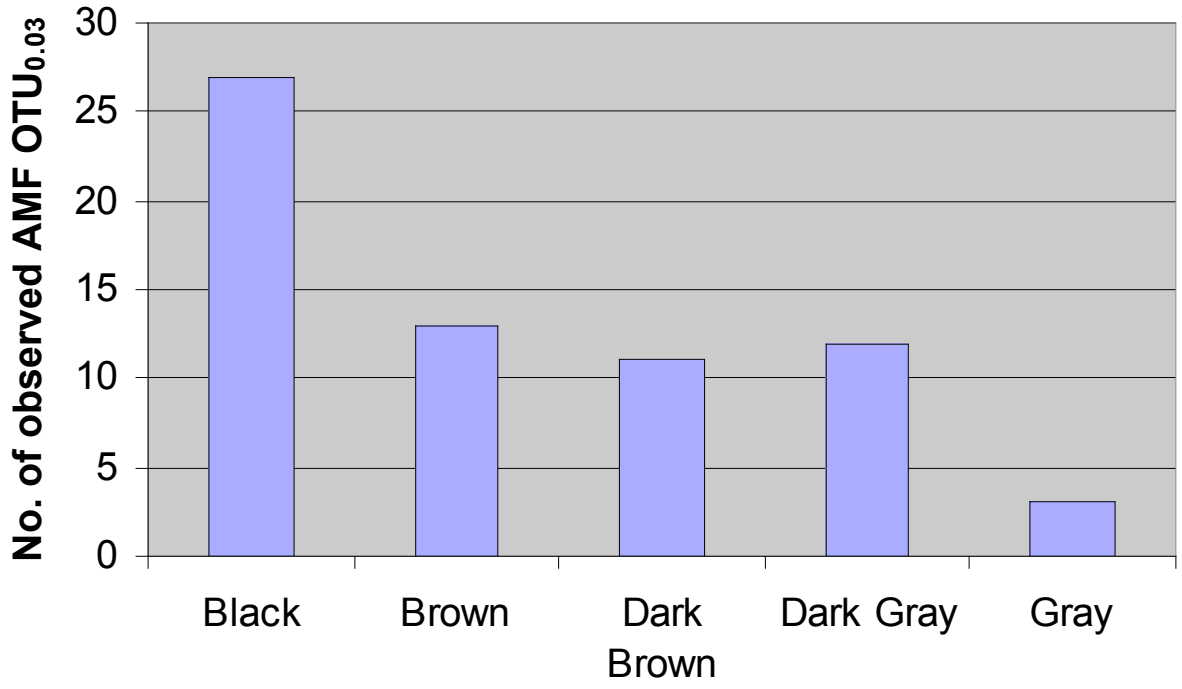

Fig. 3 AM Fungi Diversity in the Five Soil Zones

Conclusion

There are more AM fungi in cultivated soil than previously thought based on traditional methods. The richness of the AM fungi population of cultivated Prairie soil varies with soil zones. AM fungi taxonomic richness and abundance is higher in the Black Soil zones. AM fungi diversity is probably underestimated in the Gray Soil zone due to insufficient sampling.