

# Estimating the AM Fungal Resources of Wheat Fields

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**Key Words:** Arbuscular mycorrhizal fungi, soil phosphorus supply, soil bioresources, biogeography, wheat phosphorus uptake, wheat phosphorus nutrition

## Abstract

The arbuscular mycorrhizal (AM) fungi are a taxonomic group of microscopic soil dwellers generally promoting the growth of most plant species, including wheat, by mobilizing soil minerals, in particular soil P. Despite the importance of the AM fungi for plant nutrition, no technology exists to identify the fields in which AM fungi are doing a good job with feeding P to crops and the fields where agronomic interventions are required. We set out to fill this gap and examined 225 commercial fields in 2007, 2009 and 2010. We first found that P yields were limited by P supply rates below  $3.5 \mu\text{g P } 10 \text{ cm}^{-2} \text{ day}^{-1}$ . Fifty seven percent of the organic fields surveyed were below this threshold. A trend for higher P use efficiency in organic than conventional wheat crops at low soil P fertility levels was concurrent with higher levels of AM root colonization. The AM fungal communities of soils were simpler than expected. Some 122 phylotypes were found, but only ten of these accounted for over half of the AM fungal DNA sequences encountered. The main drivers of AM fungal diversity varied with the species of AM fungi. Among all the variables considered, soil organic matter level and soil texture were most often significantly correlated with the relative abundance of the different AM fungi. The research results suggest the possibility of using simple regression models to estimate the quality of soil AM fungal communities invisible to the naked eye. It appears that national databases on soils and climate, and soil analyses using PRS<sup>TM</sup>-probe can be used to develop cost effective tools allowing the management of nutrient efficient wheat production systems based on ecological principles.

## Introduction

The management of arbuscular mycorrhizal (AM) fungi to improve fertilizer use efficiency in crops could mitigate the impact of increase mineral fertilizer prices, which are rising due to declining natural reserves of phosphate and fossil fuel involved in the production of P and N fertilizers (Fixen 2009). It would also reduce the loss of nutrients from cultivated fields through leaching and denitrification, which negatively impacts environmental quality and contributes to global warming (Buczko and Kuchenbuch 2007; Snyder et al. 2009).

The AM fungi form dense hyphal networks in the top layer of soil where they can account for 25% of total soil microbial biomass. One gram of top soil can contain 100 m of AM hyphae (Leake et al. 2004). The main benefit plants gain from their association with AM fungi comes from the ability of their hyphal networks to mobilize and transport soil nutrients (Liu et al. 2007). The hyphae of AM fungi explore intensively the soil matrix beyond the nutrient depletion zone that develops around the roots as plant uptake of nutrients is faster than replenishment from the

bulk of soil. Nutrients are loaded in AM fungal hyphae, rapidly transported within AM fungal networks, and transferred to the plant symplast via a symbiotic interface formed at the level of the plasma membrane of root cells (Govindarajulu et al. 2005; Parniske 2008). This vehicle for rapid transport is particularly important for nutrients that do not move toward the roots with mass flow as plants are drawing water. This is the case of phosphorus, a nutrient required in large amount by plants, and the micronutrients Cu and Zn (Liu et al. 2000a; Liu et al. 2007). The AM fungi associated to roots can also stimulate decomposers and improve their host plant productivity by linking mineralization to plant nutrient demand (Atul-Nayyar et al. 2008). This is particularly important for plant N nutrition from organic sources (Hodge and Fitter 2010).

Despite the importance of AM fungi in plant nutrition this soil bioresource has largely remained unexploited. The lack of a simple and inexpensive method to assess the ‘health’ of AM fungal community in cultivated soils has been a main barrier to the management of AM fungal resources in agroecosystems. The AM fungi do not grow in pure culture; they are microscopic in size and hidden in the soil matrix. We investigated the possibility of using simple indicators to assess the abundance and structure of the AM fungal community living in cultivated soils.

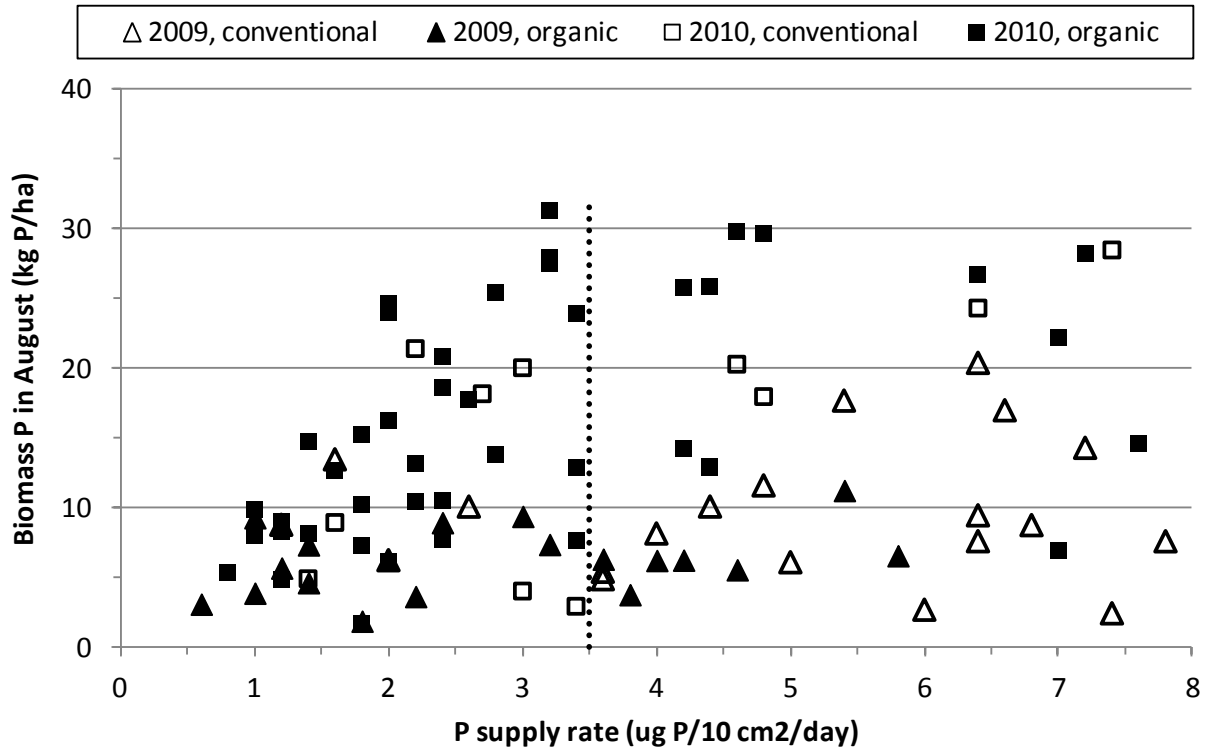
## Methods

We documented the diversity of AM fungi in wheat-growing fields, and sought practical indicators of the relative abundance and taxonomic assemblages of AM fungi through a survey of 225 field sites under organic and conventional management, which was conducted in 2007, 2009, and 2010 in all Prairie soil zones and on Podzolic soils of eastern Canada. We described the AM fungal communities living in these soils using cutting-edge metagenomic DNA analysis techniques, measured soil nutrient supply rates with PRS<sup>TM</sup>-probe, and gathered soil physical property data from the National Soil Database and weather data from Environment Canada ([www.climate.weatheroffice.gc.ca](http://www.climate.weatheroffice.gc.ca)). The AM fungal DNA sequences found in soils were grouped, based on similarity, into ‘operational taxonomic units’ (OTUs), which is a proxy for species. The taxonomic and environmental data were subjected to multiple regression analysis using PROC REG in SAS to model the distribution of these OTUs in the landscape.

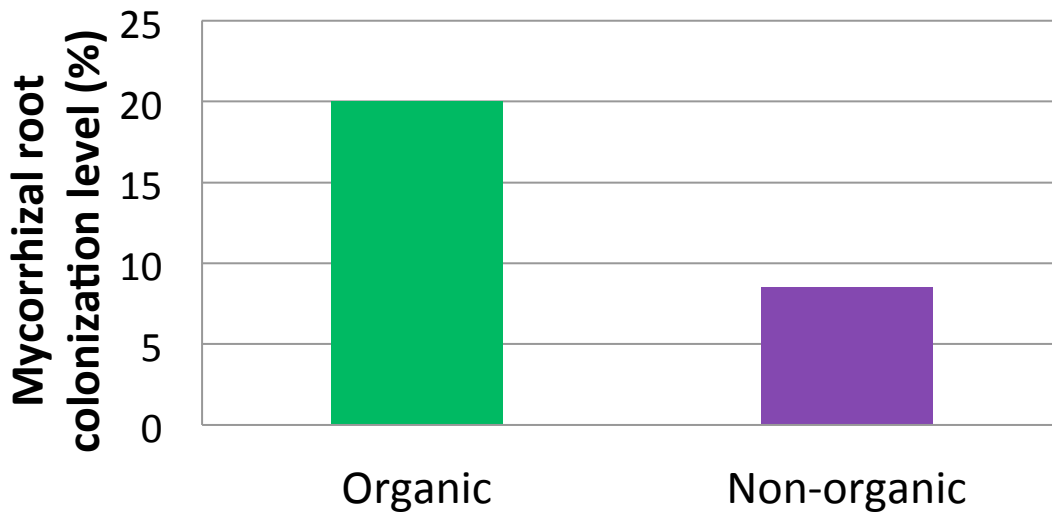
We also examined the relationship between plant P uptake and soil P supply capacity in a subset of 113 Prairie soils growing wheat from our 2009-2010 dataset to document the extent of P fertilization needs in Prairie soils. Segmented regression analysis was used to define the P fertility threshold below which a wheat crop becomes P limited.

## Results

Soil P supply rate spanned between 0.6 and 62  $\mu\text{g P } 10 \text{ cm}^2 \text{ day}^{-1}$ . No relationship was found between soil P supply rate and wheat biomass P across the whole range of soil P fertility encountered, suggesting that soil P fertility was not the major factor limiting wheat P uptake in most Prairie fields. Threshold analysis revealed that P uptake in wheat growing in soils with a P supply rate below 3.5  $\mu\text{g P } 10 \text{ cm}^2 \text{ day}^{-1}$  was likely limited by soil P fertility (Fig. 1).



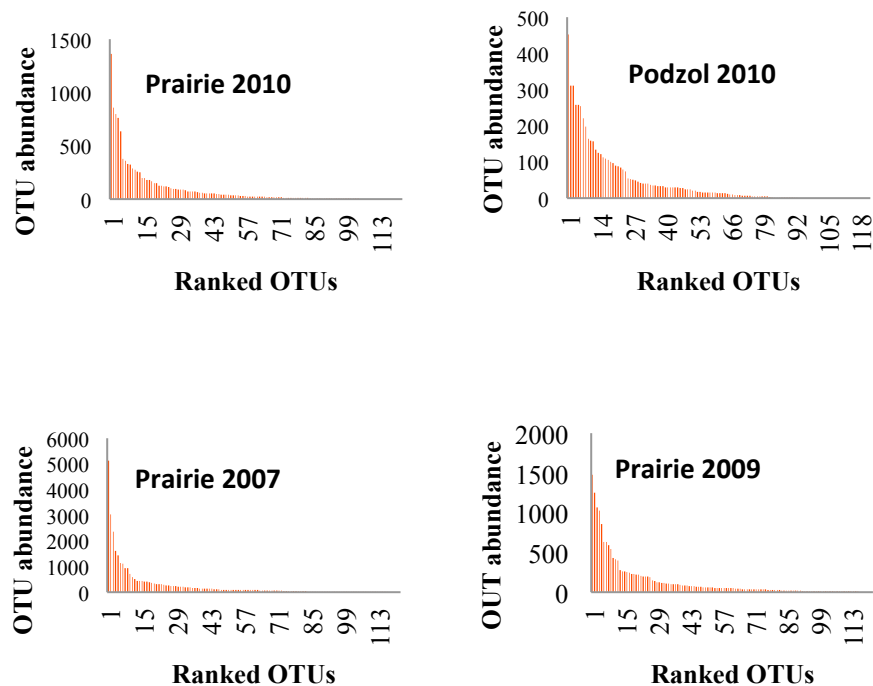
**Figure 1.** Relationship between August P yield and P supply rate for sites with supply rates < 8  $\mu\text{g P } 10 \text{ cm}^2 \text{ day}^{-1}$ .



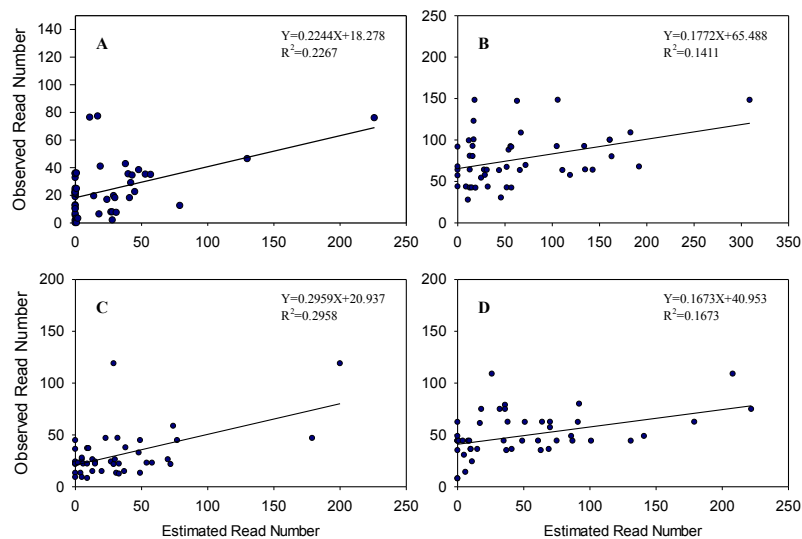
**Figure 2.** Level of wheat root colonization by AM fungi in organic and conventionally managed farms, with standard errors.  $N= 172$  Prairie fields visited in 2009 or 2010;  $P < 0.0001$ , according to ANOVA.

Fifty seven percent of the organic fields surveyed in the Prairie had a soil P supply rate below the  $3.5 \mu\text{g P } 10 \text{ cm}^2 \text{ day}^{-1}$  threshold suggesting that soil P fertility is an important limiting factor in organic wheat production. A trend for superior P uptake efficiency in organic than conventional wheat crops, at the low end of soil P fertility range (Fig. 1), was concurrent with higher levels of wheat root colonization by AM fungi under organic production (Fig. 2). This observation may reflect the fact that organic fields have lower P fertility levels than conventionally managed fields. The development of the AM symbiosis is enhanced and the contribution of AM fungi to plant P nutrition is increased at low soil P fertility levels (Liu et al. 2007).

Some 122 AM fungal OTUs were found in cultivated soils of the Prairie and of eastern Canada (Fig. 3). Most OTUs represented rare species and a few were very dominant. In 2007, 2009 and 2010, as much as 60%, 52% and 55% of all the AM fungal sequences found in Prairie soils belong to one of the ten most abundant OTUs, in these years. In the Podzols (only in 2010), 48% of all the AM fungal sequences belong to the “top ten” OTUs. Seven of these “top ten” OTUs



**Figure 3.** Ranked abundance of AM fungal OTUs in the Prairie and eastern Canada Podzolic soils, measured in the different years of the study.



**Figure 4.** Examples of relationship found between the number of reads of AM fungal OTUs observed in soil samples and estimated by best multiple regression models. Here, OTUs A, B, C, and D.

were among the ‘top ten’ most abundant OTUs found in all four years/regions, suggesting that anywhere in Canadian cultivated soils the same few dominant generalist AM fungi co-existing with numerous rare specialized AM fungal species. OTUs belonging to the genera *Claroideoglossum*, *Funnelliformis*, and *Rhizophagus* were the most abundant; a few OTUs belonging to *Diversispora* and *Entrophosphora* were also found.

The distribution of the different OTUs of AM fungi among the different fields surveyed was driven by different soil physico-chemical and climatic variables. A model significantly ( $P \leq 0.05$ ) explaining the distribution of AM fungal sequences was found for 30 of the 47 seven most abundant AM fungal OTUs. The  $R^2$  of these models ranged from 0.08 to 0.46. The relative abundance of five AM fungal OTUs was not related to any of the environmental variables measured.

### Highlights and Practical Use of the Research

We conclude that estimating the abundance of the different AM fungal species living in Canadian cultivated fields might be possible using regression models. This opens the possibility to develop cost effective tools allowing the management of the AM fungal resource. The data required to feed the models developed are soil fertility measures generated with the PRS<sup>TM</sup>-probe technology, at Western Ag Innovations Laboratory, and data freely available in the National Soil Database and at Environment Canada. Next steps toward the development of this tool include the identification of AM fungal OTUs that are related to efficient P uptake by wheat. Plants are to some degree selective in their association with AM fungi and only a subset of AM fungal species is expected to contribute to nutrient uptake in plant (Montesinos-Navarro et al. 2012). The final

management tool should combine models estimating the abundance of a subset of AM fungal species.

Phosphorus limitation is an important problem in organic wheat production in the Prairie, since 57% of the organic wheat fields surveyed had a soil P supply rate under the threshold of  $3.5 \mu\text{g P } 10 \text{ cm}^{-2} \text{ day}^{-1}$ , below which wheat P uptake is restricted by soil P fertility. However, improving nutrient use efficiency will become increasingly desirable in all types of production systems with increasing prices of fertilizers and the recognition of environmental impacts related to inefficient use of nutrients in agriculture. Effective decision-making tools to assess the quality of indigenous AM fungal communities in cultivated fields will help manage nutrient efficient wheat production systems based on ecological principles.

### **Acknowledgement**

Thank you to Western Ag Innovations, the Canadian Wheat Board, Growing Forward, and Agriculture and Agri-Food Canada for their financial support within the framework of the Organic Science Cluster.

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