Microbial phosphorus pools in a long-term organic farming system in Scott, Saskatchewan

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Abstract

Organic farmers across Saskatchewan face widespread phosphorus (P) shortages. Due to the lack of inputs in organic systems, organic farmers must rely on mechanisms like crop rotation and naturally-occurring arbuscular mycorrhizal fungi (AMF) for plant P supply. Crops that are not colonized by AMF (non-mycorrhizal) can decrease colonization of a following crop. An experiment was carried out to look at varying P pools in four cropping sequences under organic management, and also to determine if mustard (non-mycorrhizal) was delaying the colonization of wheat following it. Soils from the four cropping sequences were measured for inorganic P (P_i), AMF spore density (SD), phospholipid fatty acid analysis (PLFA, for AMF biomarker counts), and alkaline phosphatase activity (ALPase, related to AMF metabolic activity). Plants were measured for AMF colonization and P content and uptake of above-ground biomass. A lack of significant difference in AMF activity indicated that mustard was not depressing colonization. The combination of low P_i levels, no inputs, and crop rotation were most likely creating optimal conditions for AMF colonization.

Introduction

Many organic farms across Saskatchewan (SK) face phosphorus (P) shortages. Phosphorus is often tied up with calcium compounds in the soil, making it largely inaccessible to plants (Buckman and Brady, 1969). It is also immobile within the soil profile (Brady, 1990). The principles of organic farming do not allow the use of fertilizers, so it becomes necessary for organic farmers to rely on natural inputs for soil P fertility. Crop rotation becomes very important in these systems. Cereal crops like wheat, barley, and mustard are heavy nutrient users that do not replenish the soil system, while legumes like pea, lentil, and alfalfa fix nitrogen (N_2) in the soil. The benefits of legume crops will often carry over, and produce higher yields of cereals following them (Beckie and Brandt, 1997). When legumes are plowed under as green manure, farmers may see many benefits including increased nutrient cycling, decreased nutrient

losses, decreased erosion, and weed suppression (Phatak et al, 1987; Dapaah and Vyn, 1998; Delgado et al, 2001). Proper crop rotation means maintaining a balance between nutrient users and nutrient fixers.

Arbuscular mycorrhizal fungi (AMF) are also important for P in organic systems. They form a symbiotic relationship with 80% of terrestrial plants (Zhu et al, 2007). In exchange for carbon, AMF colonize and extend the root surface area of plants, allowing them to explore a larger area of the soil and increase P uptake. A non-mycorrhizal (uncolonized) crop in rotation has been seen to depress colonization of a following crop (Bendini et al, 2007; Karasawa et al, 2002). The degree of AMF colonization is also affected by its environment. Colonization is often decreased in high P environments (Allen et al, 1981; Douds and Schenk, 1990), indicating it will only perform in P-deficient systems.

For this experiment, AMF infection and other microbial P parameters were measured under differing cropping sequences in a long-term (18-year) organic system. Soil and plants were sampled from the Agriculture and Agri-Food Canada research station in Scott, SK. They were taken from the Alternative Cropping Study (ACS), which is an existing experiment looking at different cropping sequences with three diversity levels and six phases (Table 1). The diversity levels are named for the crops appearing in them (LOW=low diversity, DAG=diverse annual grain, DAP=diverse annual perennial), and each phase is present every year. The experiment consists of a high-input, reduced-input, and organic system, but only the organic system was sampled for this experiment. Samples were taken to represent each diversity level and to examine AMF colonization following a non-mycorrhizal crop (mustard). The cropping sequences were wheat-pea (WP), lentil green manure-wheat (LGrMW), mustard-wheat (MW), and wheat-barley (WB).

Table 3-1. Sampled Plots in Scott, Saskatchewan

Input Level	**Diversity Level**	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**	**Phase 5**	**Phase 6**
Organic	LOW	Lentil green manure	***Wheat†***	Wheat	Lentil green manure	Mustard	Wheat
	DAG	Lentil green manure	Wheat	***Pea***	Barley	Lentil green manure	Mustard
	DAP	Mustard	***Wheat***	***Barley***	Alfalfa	Alfalfa	Alfalfa

†Sampled plots are italicized.

The study objectives were (i) to evaluate microbial P in diverse cropping sequences under long-term organic management and (ii) to identify when AMF colonization initiates after a non-mycorrhizal crop (mustard).

Materials and Methods

Soils were taken at pre-seeding to measure inorganic soil P (P_i) and AMF spore density (SD, number of AMF spores 100 g $soil^{-1}$), at pre-seeding and flowering for phospholipid fatty acid analysis (PLFA), and at flowering for alkaline phosphatase (ALPase) activity. Plant samples were taken at flowering for %AMF colonization and plant P concentration and uptake. Spore density and PLFA measurements (AMF biomarker 16:1ω5c, Olsson, 1999) were taken to assess potential AMF activity and AMF colonization and ALPase activity represented actual activity. Alkaline phosphatase is often related to AMF metabolic activity (Gianinazzi-Pearson and Gianinazzi, 1978; Guillemin et al, 1995; Jajabi-Hare et al, 1990).

Inorganic soil P was measured via modified Kelowna extraction (Qian et al, 1992) and AMF SD via the wet-sieving technique of Dandan and Zhiwei (2007). PLFA was determined via the method of White et al (1979), and ALPase via Tabatabai and Bremner (1969). Plant AMF colonization was performed following the ink and vinegar staining of Vierheilig et al (1998) and counted according to the gridline-intersection method of Giavonnetti and Mosse (1980). Plant P concentration was measured through total acid P digestion (Thomas et al, 1967).

Results and Discussion

Soil inorganic P levels did not vary significantly between cropping sequences, but P_i in WB was lowest (Table 2). Spore density of AMF was also not significantly different between sequences (Figure 1). The AMF biomarker 16:1ω5c was detected in greater amounts at flowering than pre-seeding, indicating its abundance increased in the presence of growing crops, but no significant differences were detected (Figure 1). Plant AMF colonization and ALPase activity were also not significantly different at flowering (Figure 1). Plant P concentrations of the above-ground biomass were significantly lower in MW, and plant P uptake of the above-ground biomass was significantly lower in WB (Table 2). Factors other than P are affecting yield and P-uptake of WB, due to its high P concentrations and low P-uptake. However, this is beyond the scope of this project.

The non-significance of SD, AMF biomarker counts, AMF colonization, and ALPase activity between cropping sequences indicates continuous AMF activity throughout the growing season. Despite previous studies to the contrary, mustard is not depressing the colonization of the wheat following it (Bendini et al, 2007; Karasawa et al, 2002). Furthermore, plant P concentrations are average, despite low Pi levels (0.2%, Tisdale and Nelson, 1975). It is possible that AMF is

responding to the low P_i environment, and colonizing each plant equally. This has been seen previously (Koide, 1991). In fact, decreased AMF colonization is often seen in systems where high amounts of P are added in the form of fertilizer (Asimi et al, 1980; Hinsinger, 2001). The combination of low soil P_i and the lack of P-input in this system may be promoting AMF colonization equally on all crops, and trumping any effects that non-mycorrhizal crops would have in a conventionally managed system. These conditions preserve spore counts and the living AMF DNA, allowing AMF to draw up its resources every growing season.

Crop rotation may also play a role for AMF activity and colonization. Although variations were not significant, the barley from WB had the lowest overall SD, AMF biomarker counts, and AMF colonization, WP had the highest SD and AMF biomarker counts, and surprisingly, MW had the greatest colonization at flowering.

Table 2. Soil P_i and Plant P Concentration and Uptake at Flowering

Cropping Sequence	Soil PO_4 (lbs $acre^{-1}$)	Plant P concentration (mg kg^{-1})	Plant P uptake (kg ha^{-1})
WP	15.1	4.1a†	11.2a
LGrMW	16.4	3.5b	18.8b
MW	15.0	3.1b	16.5b
WB	12.6	3.6b	7.0a
$LSD_{0.05}$	**ns‡**	**0.7**	**4.7**

‡ns indicates non-significance

†Means followed by the same letter are not significantly different according to $LSD_{0.05}$. Means are grouped by date and cropping sequence.

To further understand these patterns, it is necessary to refer back to the crop rotation plan (Table 1). In Phase 1, or 2010 for the sampled plot of barley, mustard was planted, followed by wheat in 2011 and barley in 2012. All three crops in this succession are heavy nutrient users. It is therefore unsurprising that by 2012, barley would grow in a relatively nutrient-depleted plot.

The higher SD and AMF biomarker counts in WP can also be attributed to crop rotation. Peas increase soil N levels through N_2 fixation. Despite the wheat that was planted in this plot in 2011, which would have depleted the soil somewhat, LGrM was grown there in 2010. The addition of organic matter and nitrogen by LGrM and pea outweigh the nutrient depletion caused by wheat, and promote conditions for AMF SD and biomarker counts. The elevated AMF colonization in the MW rotation is less clear, but may be related to a carryover effect of alfalfa (N_2-fixer) from 2010. The lack of inputs in this organic system meant that LGrM, pea, and alfalfa are the only inputs available, and are more reliable determinants of plant AMF colonization than mycorrhizal or non-mycorrhizal crops. Crop rotation is the only factor that changes between rotations.

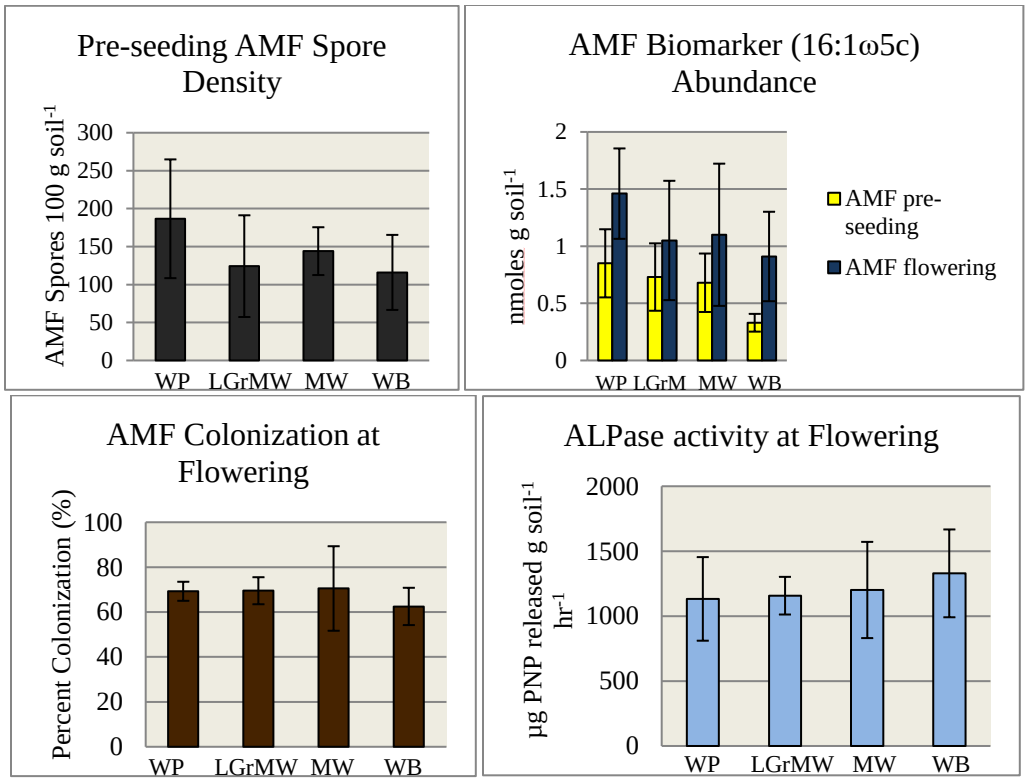

Figure 1. AMF activity at pre-seeding and flowering.
The graphs in this figure show potential AMF activity (spore density, PLFA) and actual AMF activity (colonization, ALPase). No significant differences were detected between cropping sequences. WP=wheat-pea, LGrMW=lentil green manure-wheat, MW=mustard-wheat, and WB=wheat-barley.

Conclusions

In this organic system, low P_i and crop rotation are more important for AMF colonization than mycorrhizal and non-mycorrhizal cropping sequences. More specifically, the presence of mustard is not depressing the colonization of wheat following it, and it may be due to these conditions.

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