ORGANIC CROP MANAGEMENT CAN DECREASE LABILE SOIL P AND PROMOTE MYCORRHIZAL ASSOCIATION OF CROPS

BY

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Submitted to the Faculty of Graduate Studies of
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ABSTRACT

Welsh, Catherine M. M. Sc. The University of Manitoba, December 2006. Organic management can decrease labile soil P and promote mycorrhizal association of crops. Major Professor: Dr. Mario Tenuta.

A principle concern with organic farming is for the potential depletion of phosphorus reserves in soil. In the Canadian Prairies, many organic farming systems have been shown to be deficient in plant available phosphorus. One of the main objectives of this study was to determine which organic management systems are in danger of depleting phosphorus reserves and if large reserves still exist in these systems in unavailable forms. Another was to determine if the colonization of arbuscular mycorrhizal fungi (AMF) could assist crops in low phosphorus organic systems in taking up phosphorus from the soil. The final objective of this study was to establish whether different management or rotation systems select for specific communities of AMF allowing for the future identification of efficient AMF species for inoculation in the field.

The research was carried out at a 14 year-old study at Glenlea, in Southern Manitoba. The site has 3 different 4-year rotations under organic and conventional management: Wheat – Alfalfa – Alfalfa – Flax (forage-grain) with and without manure-compost, Wheat – Pea – Oat – Flax (grain-only), and a restored tall grass prairie planting (prairie). The conventional treatments received synthetic fertilizers as well as pesticides whereas the organic treatments received no inputs other than manure-compost.

The modified Hedley sequential extraction procedure was used to extract different phosphorus pools from readily plant available, to moderately available, to unavailable phosphorus. The organic systems had lower concentrations of readily plant available phosphorus fractions than conventional but recalcitrant fractions were not significantly different ($P < 0.05$). The cumulative phosphorus balance of organic and conventional systems demonstrated that organic grain-only systems reduce labile fractions at a low rate due to low yields. However in high yielding systems such as forage-grain, labile phosphorus fractions considerably decreased over 14 years of organic management. Nitrogen limitations considerably affected yields in organic grain-only rotations more than phosphorus limitations in high yielding forage-grain systems. This study demonstrates that only high yield, high phosphorus export organic systems are a concern for developing plant available phosphorus deficiency in the long-term. Labile phosphorus concentrations were generally high in this soil being on average 35 mg kg^{-1} for conventional and 18 mg kg^{-1} for organic systems (Modified Kelowna). It is expected that where concentrations are within the agronomic response to phosphorus, depletion of available phosphorus will impart nutrient deficiency in the short-term. According to published values for agronomic response thresholds, the phosphorus concentrations in the organic forage-grain rotations are below this threshold and are considered phosphorus limited whereas the organic grain-only rotation will likely not become limited by phosphorus for another six years.

Colonization and spore populations of AMF were analyzed in order to determine the effect of crop treatment on AMF activity. Colonization levels as percent arbuscules were higher ($P < 0.05$) in organic than conventional systems; crop rotations did not significantly differ in colonization. The same was true for mycorrhizal spore populations in the forage-grain rotation ($P < 0.05$). The organic systems had lower labile soil phosphorus than conventional and the forage-grain rotation had lower phosphorus concentrations than the grain-only rotations ($P < 0.05$); however plant tissue phosphorus concentration was only effected by rotation indicating the increase in AMF colonization in organic systems may have been an important contributor to increased phosphorus uptake in these systems. Correspondence analysis indicated a shift in AMF community structure likely occurred between the prairie and the rest of the treatments and between the organic and conventional grain-only rotations; however, this shift may have been more related to selection of AMF species by crops than phosphorus limitation.

In conclusion, the Glenlea Long-term Rotation study has shown that in a soil with initially high plant available phosphorus there is not necessarily an immediate crisis in long-term organic farming due to phosphorus depletion as the reduced yield of organic systems imparts reduced phosphorus export. For the time scale of 10s of years, in high yielding forage export systems, a challenge will be to utilize the large pool of phosphorus in recalcitrant forms to help provide proper phosphorus nutrition. The colonization of AMF may allow crops to survive in low phosphorus conditions while recalcitrant phosphorus reserves are cycled into labile pools. From this study it appears that AMF communities were altered more by crop rotation than by management and labile phosphorus concentration; however, more work needs to be done in order to determine the AMF species composition with the use of molecular techniques.

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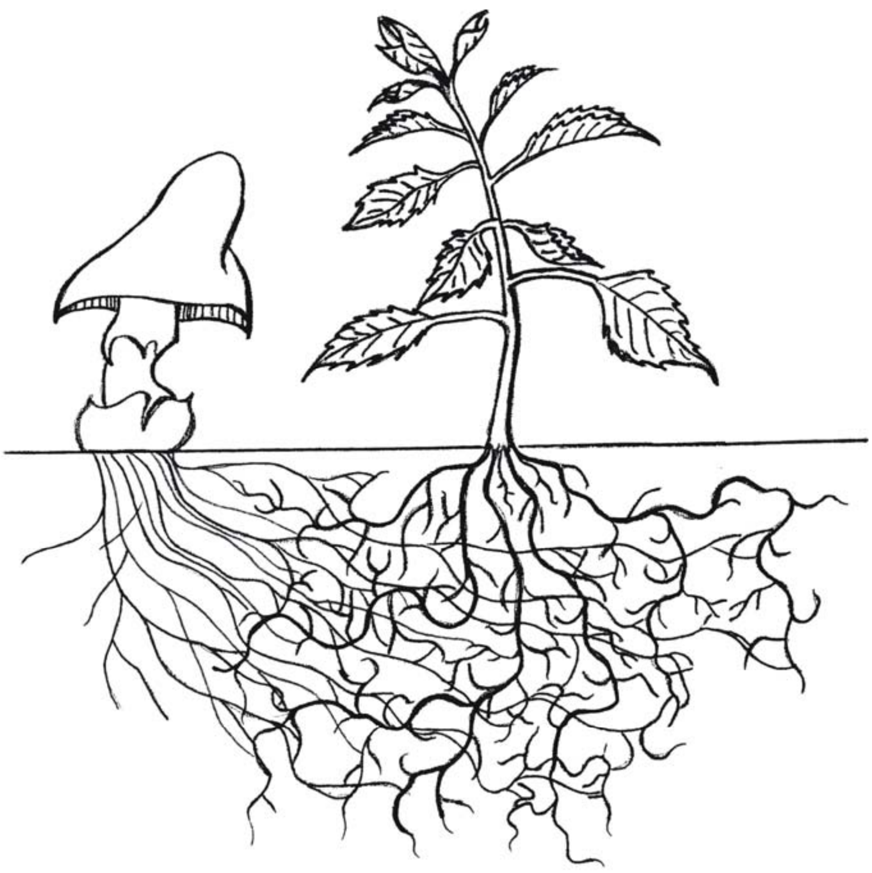
DEDICATION

The following thesis is dedicated to all those hard working scientists that want to make a difference in this world.

Cheers.



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1. GENERAL INTRODUCTION

1.1 Organic Farming

A major difference between organic and conventional farming, in addition to limiting the use of synthetic pesticides and fertilizers, is the commitment of organic producers to farming as natural systems to achieve sustainability. This commitment leads to increased diversity of crops, increased diversity and activity of soil organisms that help maintain soil fertility and structure (Hansen et al. 2001), and often a decreased cost of crop production by relying less on external inputs. The reliance of organic systems on biological nutrient cycling as opposed to fertilizer application can lower the risk of nutrient leaching caused by application of highly soluble fertilizers, with the exception of some organic systems that use green manure plowdowns and high rates of manure applications (Hansen et al. 2001). Organic systems also lower energy costs by discontinuing synthetic fertilizer application. For example, Hoeppner et al. (2005) found energy use was decreased in organic systems by 50%. Much of the returns on crop yields in conventional farming are spent on expensive fertilizer inputs which are applied annually. Switching to organic farming while maintaining a balanced phosphorus budget could be part of a solution to reducing production costs, decreasing nutrient leaching and release from dependence on pesticides.

The soil microbial community plays a major part in nutrient cycling and soil fertility and so are very important in organic farming systems. This community can be influenced by cropping practices such as rotation, type of fertilizer and pesticide application. The management of organic farming systems tends to favour a more active soil microbial community as compared to conventional systems (Hansen et al. 2001). One of the most important functions of the soil microbial community is in its ability to enhance nutrient uptake by plants. Many soil organisms form symbiotic associations with plants to provide them with valuable nutrients such as nitrogen (e.g. bacteria of the Rhizobiaceae with plants of the Fabaceae) and phosphorus (mycorrhizal fungi with a variety of plants). These soil organisms are essential in organic systems as synthetic fertilizers are not used to supply nitrogen and phosphorus.

1.2 Low Phosphorus Organic Systems

Due to the lack of synthetic fertilizer addition, organic systems are often nutrient limited and phosphorus is particularly lacking in the Canadian prairies. In a study of 14 organic farms across the Canadian prairies and North Dakota farms were generally sufficient in nitrogen, potassium and sulphur, but often deficient in phosphorus (Entz et al. 2001). Many long-term studies have demonstrated the decline in available phosphorus and also crop yields without the addition of synthetic fertilizer (Gosling and Shepherd 2005; Oehl et al. 2002; Campbell et al. 1993). Campbell et al. (1993) confirmed that when fertilizer was withheld for 34 years, crop yields steadily declined even in crop rotations that included nitrogen-fixing crops. In a 15-year study comparing management systems, organic systems had much lower plant available phosphorus levels than conventional systems (Gosling and Shepherd 2005). In a separate study, after 21 years of organically managed soils, which received organic manure, had a negative annual phosphorus balance; whereas the annual budget in conventionally managed soils, which received synthetic fertilizers, was positive

(Oehl et al. 2002). These studies imply that management of many organic systems is not sustainable with regards to phosphorus fertility.

In a study by Oberson et al. (1993), a sequential phosphorus extraction procedure was done on soils collected from conventional, bio-dynamic and bio-organic cropping systems. The soils from the bio-dynamic and bio-organic systems had lower available phosphorus concentrations than soils from the conventional system even with the addition of phosphorus in the form of manure (28 kg P ha^{-1} $year^{-1}$ for bio-dynamic) and manure plus mineral fertilizer (31 kg P ha^{-1} $year^{-1}$ for bio-organic). However, in the unavailable phosphorus pools, there was no significant difference with management system indicating a large reserve of phosphorus still exists in organic systems.

The possibility of a large phosphorus reserve in organic systems raises the question of whether there is a crisis in organic systems in regards to soil phosphorus concentration. It is obvious that more than just available phosphorus needs to be measured to determine the level of fertility in an organic management system. Unavailable phosphorus reserves will eventually be cycled into available pools by the action of plant roots and microbial activity (Hedley et al. 1982). The ability of crops to grow on soils low in available phosphorus while unavailable reserves are made available may determine the sustainability of organic systems.

1.3 Arbuscular Mycorrhizal Fungi in Organic Systems

Arbuscular mycorrhizal fungi (AMF) are the most important soil organisms in regards to increasing phosphorus uptake by plants. These fungi form symbiotic relationships with many plants by trading most of the phosphorus they absorb to their hosts in exchange for plant sugars. Numerous crops (such as flax) rely heavily on AMF to obtain sufficient phosphorus for growth in systems low in phosphorus such as many organic and low-input systems (Mäder et al. 2000; Thingstrup et al. 1998). The AMF can increase phosphorus absorption mainly through the increase in surface area for nutrient absorption provided by fungal hyphae emanating from colonized roots (referred to as extraradical hyphae) (Smith and Read 1997). Low phosphorus organic systems therefore have a large potential to benefit from the association of AMF to offset the lack of available phosphorus that occurs without the use of conventional fertilizers. This lack of available phosphorus leads to an increased colonization of AMF in organic systems (Entz et al. 2004; Oehl et al. 2002; Mäder 2000).

Densely branched clusters of hyphae, known as arbuscules, grow between the cell wall and cell membrane of plant cells and are proposed to be the primary exchange location of nutrients between plant and fungus. An increase in arbuscular colonization therefore represents an increase in nutrient exchange between plant and fungus (Smith and Read 1997). Decrease in tillage (McGonigle and Miller 1993) and decrease in soil phosphorus concentration (Dekkers and van der Werff 2001) can both lead to increases in arbuscular colonization.

In addition to colonization, AMF diversity is also altered by management systems. High input monoculture cropping systems as well as intensive tillage can decrease the species diversity of AMF in soil to those providing less efficient transfer of phosphorus to crops (Kucey and Paul 1983; Oehl et al. 2003). Contrarily, pasture systems as well as cropping systems with multi-year crop rotations can increase mycorrhizal diversity due to the increase in host diversity (Oehl et al. 2003).

In order to determine whether there is an increase in nutrient exchange between organic and conventional systems, it is necessary to determine arbuscular colonization rather than colonization in general. In addition, it will also be useful to determine whether an increase in arbuscular colonization is accompanied by a shift in diversity to more efficient AMF communities in organic systems.

1.4 Glenlea Long-term Rotation Study

Reported here is a field study that examines whether the reduction of phosphorus in organic systems is a general depletion or only a reduction in plant available fractions, and whether AMF are able to increase phosphorus uptake in crops growing in organic systems. For this research a comparison was done of organic and conventional management systems under three different four-year crop rotations. The study was implemented in 1992 and is Canada's oldest organic/conventional comparison study. Direct comparison between organic and conventional systems is difficult because these systems vary widely in management practice and crop rotations. In many previous comparison studies the crop rotations were not identical between management systems, but to simplify analysis of results, the three four-year crop rotations in this study were identical in conventional and organic systems. These rotations included a grain-only rotation, a forage-grain rotation, and a forage-grain rotation receiving a one time application of manure-compost. The different crop rotations were chosen to show to what extent rotation affects phosphorus and AMF activity in addition to management system. The only difference in management was that none of the synthetic fertilizers and pesticides that were used in the conventional systems were added to the organic systems. A native tall grass prairie was planted at implementation of the study and remained as an undisturbed section of land (save for a burning every four years) with no additions or removals for the duration of the field study. The prairie could therefore serve as a benchmark for phosphorus concentrations in the soil and a reflection of the AMF activity in a more natural system in Glenlea.

Both AMF activity and phosphorus were analyzed in the Glenlea study. The soil for the phosphorus extraction study was sampled in 2004 and the phosphorus pools extracted were water-soluble, sodium bicarbonate-soluble, sodium hydroxide-soluble and hydrochloric acid-soluble. A cumulative phosphorus balance was conducted to explain the differences in phosphorus pools with rotations in relation to differing removal rates. The roots for AMF analysis were sampled in 2005 and colonization of hyphae and arbuscules was measured to determine whether potential for AMF-mediated nutrient exchange was increased in organic systems. The AMF spore analyses included spore enumeration to measure AMF activity in organic relative to conventional systems, and spore diversity to detect shifts in AMF diversity with management.

1.5 Objectives

The thesis follows with two research study chapters. The objectives of these studies were to determine:

Chapter 2

- Which organic systems will encounter phosphorus limitation problems and approximately how long until limitation will occur.
- Whether organic farming leads to a general depletion of reserves or rather only a decline in plant available fractions.

Chapter 3

- If AMF are able to assist crops in absorbing phosphorus in low phosphorus organic systems through increase in nutrient exchange.
- Whether different management or rotation systems select for species of AMF based on phosphorus transfer efficiency.

A general discussion of findings follows in Chapter 4.

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2. HIGH YIELDING ORGANIC CROP MANAGEMENT DECREASES PLANT AVAILABLE SOIL PHOSPHORUS[1]

2.1 Abstract

Phosphorus is a non-renewable resource, raising concerns that agricultural practices may deplete soil phosphorus reserves. Organic farming with low phosphorus inputs has been shown to result in deficient levels of plant available phosphorus in the Canadian Prairies. The purpose of this study was to determine if common organic management systems are depleting phosphorus reserves or if large reserves still exist in unavailable forms. The research was carried out at a 14 year-old study at Glenlea, in Southern Manitoba. The site has 3 different 4-year rotations under organic and conventional management: Wheat – Alfalfa – Alfalfa – Flax (forage-grain) with and without manure-compost, Wheat – Pea – Oat – Flax (grain), and a restored tall grass prairie planting (prairie). The conventional treatments received synthetic fertilizers as well as pesticides whereas the organic treatments received no inputs other than manure-compost. The modified Hedley sequential phosphorus extraction procedure revealed organic rotations to have lower concentrations of readily plant available phosphorus fractions than conventional but recalcitrant fractions were not significantly different ($P < 0.05$). The prairie had phosphorus concentrations similar to conventional in all fractions. The cumulative phosphorus balance of organic and conventional systems demonstrated that organic grain-only systems reduce labile fractions at a low rate due to low

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Cynthia Grant: Agriculture and Agri-Food Canada, Brandon Research Centre.
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yields. However, in high yielding systems such as forage-grain, labile phosphorus fractions decreased substantially over 14 years of organic management to below the agronomic response threshold. Nitrogen limited yields in organic grain-only rotations more than phosphorus in high yielding hay export systems. This study illustrates that only high yield, high phosphorus export organic systems are a concern for developing phosphorus deficiency depending on initial reserves and the length of time without additional inputs.

2.2 Introduction

Organic farming systems are increasingly being adopted by producers both in Canada and the United States. In North America alone the organic market is growing at a rate of about 20% per year and this rate is even higher in the United Kingdom (Griffiths et al. 2001). In Canada, the total acreage of farms under organic certification was estimated in 2003 to be 966,482 acres and estimates for the market value of organic production ranges from $2.64 million in Atlantic Canada to $20 million in British Columbia (Macey 2004). Producers may switch to organic farming in belief it is more sustainable and profitable than conventional methods, which depend on synthetic fertilizers and pesticides. Organic farming relies less on fertilizer and pesticide input and may be a means of limiting excessive nutrients and build-up of pesticides in the environment. There is a general trend to decrease phosphorus levels in soils as an approach to limit eutrophication. It is important to balance the amount of phosphorus removed from the soil by crop uptake with fertilizer additions to prevent over-accumulation of labile phosphorus (Sharpley et al. 1994). Organic cropping tends to rely on nutrient cycling and the action of microorganisms and less on external inputs; therefore over-accumulation is not often a problem (Hansen et al. 2001). Furthermore, much of the returns on crop yields in conventional farming are spent on expensive fertilizer inputs which are applied annually. Switching to organic farming while maintaining a balanced phosphorus budget could be part of a solution to reducing production costs, decreasing nutrient loading to the environment and release from dependence on pesticides.

One uncertainty limiting adoption of organic farming is the depletion of soil nutrients over time. In a study of 14 organic farms across the Canadian Prairie provinces and North Dakota, farms were generally sufficient in nitrogen, potassium and sulphur, but often deficient in phosphorus (Entz et al. 2001). The crops grown on the farms surveyed included forages, cereals, pulses and oilseeds with 42% of the land seeded to soil building crops such as sweet clover and alfalfa. These soil building crops are able to add nitrogen to the soil, but not phosphorus. The problem with nutrient management in low input and organic systems is that phosphorus cannot be fixed from the atmosphere as nitrogen can. In these systems, legumes are often included in the rotation to provide nitrogen to crops; however, supplementation of phosphorus from external sources is required to add phosphorus to the system if crop production and export of phosphorus is to be sustainable. For example, Campbell et al. (1993) demonstrated that when fertilizer was withheld for 34 years, crop yields steadily declined even in crop rotations that included nitrogen-fixing crops, phosphorus being the nutrient limiting yields.

There is increasing evidence that organic farming systems are reducing phosphorus reserves to sustain crop yields. A long-term study in the United Kingdom comparing soils managed organically and conventionally for at least 15 years demonstrated that organic systems had much lower plant available phosphorus levels than the conventional systems (Gosling and Shepherd 2005). The reduction of available phosphorus was attributed to a loss in the organic systems rather than a gain in conventional systems because fertilizer guidelines in the United Kingdom limit a buildup of phosphorus in conventional systems.

Further evidence for the reduction of phosphorus reserves in organic systems was revealed in a 21 year phosphorus budget calculated for organic and conventional soils (Oehl et al. 2002). The budget revealed that the organically managed soils, which received organic manure, had a negative annual phosphorus budget; whereas the annual budget in conventionally managed soils, which received synthetic fertilizers, was positive. The crops in these organic farm systems likely obtained their phosphorus partially at the expense of soil reserves.

Many organic farming practices may reduce labile phosphorus, but we do not have much information on which organic systems will encounter phosphorus limitation and when, or which forms of phosphorus specifically are being reduced in these systems. Therefore, it is necessary to compare the levels of phosphorus in organic and conventional soils under different rotations, and to examine specifically the reduction of different fractions of phosphorus, particularly plant available pools. Other pools are also important as they can provide phosphorus to the plant available pool over time.

Reported here is a field study that examines whether the reduction of phosphorus in organic systems is a general depletion or only a reduction in plant available fractions. For this research a comparison was done of phosphorus fractions between organic and conventional management under three different crop rotations. The phosphorus pools extracted were from labile pools (water-soluble and sodium bicarbonate-extractable forms) to moderately labile (sodium hydroxide-extractable) to non-labile (hydrochloric acid-extractable). A cumulative phosphorus balance was done to explain the differences in phosphorus pools with rotations in relation to differing removal rates. The crop rotations included a grain-only rotation, a forage-grain rotation without composted cattle manure and a forage-grain rotation with manure-compost to replace nutrients taken up by the crops. A native tall grass prairie was planted at the beginning of the rotation study and has remained as an undisturbed section of land (save for a burning every four years) with no additions or removals for the duration of the field study. The prairie could therefore serve as a benchmark for phosphorus levels in the soil before beginning the long-term crop rotation study. In order for an equal comparison between the organic and conventional systems, it was necessary to grow the same crops under the same rotations. The only difference in management was that none of the synthetic fertilizers and pesticides that were used in the conventional systems were added to the organic systems.

2.3 Materials and Methods

2.3.1 Site Description and Treatments

The research was conducted at the Glenlea Crop Rotation Study, which began in 1992 and is Canada's oldest field crop organic system study. Glenlea is located 20 km south of Winnipeg, Manitoba (N 49,39,0 / W 97,7,0). Soil was sampled in 2004, being the 13th year of the study. Each management system had three types of four-year crop rotations: Wheat – Alfalfa – Alfalfa – Flax (forage-grain) with and without composted cattle manure, and Wheat – Pea – Oat – Flax (grain-only). In the forage-grain rotation a 4 x 26 m section from each replicate was treated with manure-compost in the fall of 2002. A native tall grass prairie planting (including *Andropogon gerardii* Vitman var., *Sorghastrum nutans* (L.), *Panicum virgatum* (L.), *Agropyron smithii* (Rydb.), *Elymus lanceolatus* (Scribn. and Smith) Gould. and *Elymus trachycaulus* (Link) Gould ex Shinners) established at the beginning of the rotations study was also sampled to provide a benchmark for initial total phosphorus. The experimental design was a split plot randomized complete block, with three replicate blocks. The soil was mapped as predominantly Rego Black Chernozem soil of Red River or Scantenbury series (Michalyna 1970). The texture of the soil is 9% sand, 26% silt and 66% clay with a pH_{H_2O} of 7.4 and an organic matter content of 7.7%.

The conventional treatments received synthetic fertilizer based on soil test recommendations. The soil samples were collected in late fall or early spring each year from all three replicates, and then combined across replicates before analysis for nitrogen, phosphorus, potassium and sulphur. Phosphorus fertilizer was applied in the seed row during seeding and nitrogen was applied with the seed (with respect to limits for each crop). The rest of the nitrogen was broadcast as ammonium nitrate, or ammonium sulphate when sulphate was needed, directly after planting. Herbicides (including glyphosate and Imazamox) were applied in-crop to conventional plots at recommended rates based on economic thresholds. For the forage-grain with manure-compost rotation, composted conventional cattle manure of medium nutrient concentration obtained from Dr. Katherine Buckley of the Agriculture Canada Research station at Brandon, Manitoba was added as a one time application in 2002 at 10 tons ha^{-1} fresh weight to both conventional and organic plots. The application rate of total nitrogen and phosphorus in the cattle manure was 11 kg ha^{-1} and 24 kg ha^{-1} respectively.

No synthetic fertilizer was added to the prairie or the organic rotations. The prairie grass was burned in the fall of 1998 and 2002. The burning of the prairie grass is part of a natural cycling process in prairie systems as it allows nutrients that are taken up in plant matter to be returned to the soil. Without burning, decaying plant matter is built up over time and nutrients are released very slowly from this plant material thereby decreasing productivity of the living plants in the system.

2.3.2 Soil Analysis and Sequential Extraction of Phosphorus

A macronutrient analysis as well as a sequential extraction of phosphorus provided detailed and current information about phosphorus concentrations and other properties of the soil. The top 15 cm of soil was collected on October 15, 2004 using a Dutch auger (5 cm) from the three crop rotations under organic and conventional management. Samples were also taken from the restored tall grass prairie planting. Fifteen random samples were taken from each plot and mixed together as one composite sample per plot. The soil samples were then air-dried for one week and ground prior to analysis.

A portion of the air-dried soil was analyzed for concentration of macronutrients and determination of chemical properties. Nitrate was extracted by dilute calcium chloride dihydrate solution (0.15% w/v) and then passed through a Technicon AutoAnalyzer II instrument where it underwent cadmium reduction before being analyzed for nitrite by colorimetry. Sulphate was extracted with dilute calcium chloride dihydrate solution (0.15% w/v) and analyzed by inductively coupled plasma spectrometry (ICP). Potassium and phosphorus were analyzed using the modified Kelowna methods and the Technicon AutoAnalyzer II was used for automated colorimetry analysis. A flame photometer was attached to the Technicon for potassium analysis. Electrical conductivity and pH were determined by mixing the soil and water in a ratio of 1:2 (soil:water) and organic matter was measured using the wet oxidation and titration (Walkely Black Method) (Carter 1993).

The other portion of the soil was used for the sequential extraction of increasingly recalcitrant plant available phosphorus pools by means of the modified Hedley procedure

(Hedley et al. 1982). The air-dried soil samples were ground to pass a 2 mm mesh screen and subsamples of 0.5 g taken for sequential extraction. For each extraction step there was a shaking period of 16 h at 20°C followed by centrifugation at 10,000 x g for 10 min at 0°C. The supernatant including phosphorus extracted with solution was poured onto a Buchner funnel and was vacuum filtered through a 45 µm MicronSep, Cellulosic filter paper before analysis. Total phosphorus in each fraction was analyzed by ICP. The first extraction determined labile phosphorus from the plant available pool in the water-soluble form using 30 mL of deionized water as the extractant. Residual soil was then extracted with 30 mL of 0.5 M sodium bicarbonate ($NaHCO_3$) to determine labile phosphorus from the plant available pool as inorganic phosphorus (P_i) weakly associated with Fe and Al, as well as organic phosphorus (P_o) associated with soil organic surfaces and humic and fulvic acids. Next, the soil was extracted with 30 mL of 0.1 M sodium hydroxide (NaOH) to determine the pool of intermediate phosphorus lability with P_i strongly associated with Fe and Al and P_o strongly associated with humic and fulvic acids. Apatite-type P_i and some occluded P_i was extracted in the last phosphorus pool using 30 mL of 1 M hydrochloric acid (HCl).

2.3.3 Cumulative Phosphorus Balance

A cumulative phosphorus balance was determined to compare the rates of phosphorus removal of the rotations throughout the study which could explain current differences in phosphorus pools observed among rotations. The balance was calculated using data for total dry matter yield, estimated phosphorus content of harvested crops and fertilizer added. From 1992 to 2001, the yield was calculated by harvesting the crops in 10 m strips with a Hege (Hege Maschinen GmbH, Walkdenburg, Germany) plot combine. From 2002 to 2004, the grain was harvested in 26 m strips with a Massey Ferguson 8-XP small plot combine

(Kincaid Equipment Manufacturing, Haven, KS). After harvesting, the grain was cleaned, weighed and dried at 65°C for 72 hours. Alfalfa harvest yields were determined by collecting above ground biomass in three 1 m^2 quadrats before mechanical haying of the whole plot. Total dry matter production was then determined after visual separation of weeds, and drying of the biomass for 72 hours at 55°C prior to weighing.

Estimated rates of phosphorus removal were determined using standard reference values for phosphorus concentration of harvested crop components published in the Manitoba Soil Fertility Guide (2004) and the mass per hectare of harvested component. The straw was removed from all plots at grain harvest from 1993 to 1999 and was returned to plots starting in 2000; therefore, straw phosphorus was included in phosphorus removal calculations until 2000.

The cumulative phosphorus balance for each year was determined by subtracting phosphorus removed by the crops from the phosphorus added. The annual balances were then summed together to estimate the surplus or loss of phosphorus from the soil due to management and rotation over the years 1992 to 2004.

Flax grain samples from 2003 were analyzed for actual grain phosphorus and nitrogen content in order to measure the impact of treatments on crop phosphorus status and to compare with values from the Manitoba Soil Fertility Guide. Flax grain from 2003 was used rather than wheat kernels from the 2004 harvest because flax has more difficulty absorbing phosphorus from the soil than wheat when phosphorus is limiting. The phosphorus was determined by dry ashing which involved digesting the grain samples in 2 Normal HCl at 550°C for 5 hours and then analyzing the samples by ICP. The nitrogen content was determined by colorimetric analysis after filtering grain samples mixed with water to remove large particles.

2.3.4 Statistical Analyses

Extracted phosphorus components and yield were compared using a split plot design and the Statistical Analysis Software program (SAS Institute 2001). Prior to the analysis, the data were tested for homogeneity of variance (Levene's) and for normality (Shapiro-Wilks). Three different ANOVAs were performed on the results: The first ANOVA used a split plot design with management (organic and conventional) and rotation as main effects which excluded the prairie and forage-grain with compost-manure rotations. A second ANOVA was conducted on both forage-grain rotations using a split plot design with management and manure-compost addition as the main effects. A third ANOVA was conducted to compare agriculture systems with natural systems and used the organic rotations and the prairie treatment in the analysis. All analysis were performed at the $\alpha = 0.05$ significance level and are presented in tables in the following results section.

2.4 Results

2.4.1 Yield

Both management and rotation had an effect on crop yields in the Glenlea Long-term Study. Yields were greater with conventional management than with organic management in most years of the study ($P < 0.05$) (Table 2.1). The exceptions were the second and third years of the study (1993 and 1994) in which management had no effect on yields (Appendix I, Table 5.1). There was also an interaction between management and rotation in every year except 2000 and 2004 (Table 2.1). The interaction was likely due to the very low yields in the organic grain-only rotation, which would bring down the average yield for the grain-only rotation and may have lead to an erroneous significant effect of rotation on yield. Yields were greater in the organic forage grain rotation than the organic grain-only in every year of either flax or wheat. The manure-compost did not significantly affect the wheat yields in the forage-grain rotation in 2004 (Table 2.1).

2.4.2 Soil Macronutrient and Chemical Analysis

Management system and crop rotation affected concentrations of various macronutrients and also soil properties including plant available phosphorus, nitrate, organic matter and pH (Table 2.2). Available phosphorus concentrations (modified Kelowna) were lower in organic management systems than conventional systems ($P < 0.0001$) and phosphorus levels in forage-grain without manure-compost rotations were lower than grain-only ($P < 0.0005$). However, there was an interaction between management and rotation in the ANOVA comparing the soil test phosphorus concentrations in the grain-only and the forage grain system. This interaction was due to a high concentration of phosphorus in the conventional forage-grain rotations compared with the other treatments. Compost addition increased soil test phosphorus concentration in the forage-grain rotation but did not effect nitrate concentration. Phosphorus concentrations were higher in the prairie treatment than the organic rotations, but nitrate concentration was much lower in the prairie than organic rotations. Management system did not significantly affect nitrate levels; however, grain-only rotations had lower nitrate levels than forage-grain rotations ($P < 0.005$). The organic matter concentration was higher in forage-grain rotations than grain-only but addition of manure-compost had no significant effect on soil organic matter ($P < 0.05$). In contrast to nitrate and organic matter levels, pH was not affected by rotation but was lower in the conventional systems than organic (Table 2.2).

2.4.3 Extractable Phosphorus Components

Management system had a large influence on concentration of phosphorus in plant available and moderately available extractable phosphorus forms (water, sodium bicarbonate and sodium hydroxide extractable phosphorus) but no effect on recalcitrant forms (hydrochloric acid extractable phosphorus). Organic systems had lower phosphorus concentrations than conventional systems in all forms except the recalcitrant (Table 2.3), indicating the organic systems had a greater proportion of phosphorus in the recalcitrant fraction than conventional systems. Manure-compost addition had no effect on phosphorus concentration in any of the extractable phosphorus forms (Table 2.3).

The relative decline of phosphorus in the organic rotations was measured using the native prairie planting as a benchmark. The organic forage-grain rotations had greater declines in phosphorus than organic grain-only rotations in all fractions except recalcitrant phosphorus (Table 2.3). Contrary to the organic forage-grain rotation, the organic grain-only had similar levels of phosphorus to the prairie in all fractions; however, when looking at the total extractable phosphorus, the organic grain-only did show a significant decline in phosphorus from the prairie.

2.4.4 Phosphorus Balance

The estimated cumulative phosphorus balance was dependent on management system as well as rotation in this study. The conventional systems had a surplus of phosphorus addition for the study period, whereas the organic rotations reduced the reserve of phosphorus. The alfalfa crop was expected to remove the most phosphorus per year of any crop; therefore the loss of phosphorus was greatest after the two year stand of alfalfa when there was no replacement of phosphorus either by fertilizer or manure-compost. The difference between the organic and conventional cumulative phosphorus balance was about 80 kg/ha for the grain-only rotation, and 190 kg/ha in the forage-grain rotation (Table 2.4). The addition of manure-compost raised the overall phosphorus balance in the organic forage-grain rotation by approximately 20 kg/ha and the conventional by approximately 25 kg/ha (Appendix A for annual running cumulative balance).

2.4.5 Phosphorus and Nitrogen Concentration of Flax Grain

The expected concentration of phosphorus in flax grain used to calculate cumulative phosphorus balance was taken from the Manitoba Soil Fertility Guide (2004) and so it was important to determine if these values were representative for this particular study. The results from the analysis revealed that the organic grain-only rotation had a very high concentration of phosphorus in the flax grain samples and this high phosphorus concentration was the reason for the significant interaction between rotation and management (Table 2.5). Because of this interaction, there was likely no significant effect of management on flax grain phosphorus concentration even though it was significant at $\alpha = 0.05$. The flax grain of most treatments had phosphorus concentrations that were higher than the values listed in the Soil Fertility Guide (2004) (Table 2.5).

In order to determine whether nitrogen was limiting in the soil, flax grain nitrogen concentrations were also analyzed. Unlike the phosphorus levels, the grain-only rotations had lower grain nitrogen concentrations than the Manitoba Soil Fertility Guide (2004) values and were also lower than the nitrogen in the grain of forage-grain rotations. The forage-grain rotations had higher concentrations of nitrogen in flax grain than the grain-only rotations

(Table 2.5). Management system, however, did not have a significant effect on flax grain nitrogen concentrations. There was no significant effect of compost addition on either nitrogen or phosphorus concentrations in the flax grain.

Table 2.1 Flax and wheat grain yield for the Glenlea long-term study for years where common crops were grown for all management treatments.

	Rotation	Management	1995 (Flax)	1996 (Wheat)	1999 (Flax)	2000 (Wheat)	2003 (Flax)	2004 (Wheat)
					kg ha^{-1} dry weight			
	Grain-only	Conventional	1877 (247)[1]	3276 (313)	1378 (600)	2402 (517)	1693 (84)	1282 (87)
	Grain-only	Organic	961 (123)	1122 (277)	606 (280)	441 (253)	231 (23)	553 (80)
	Forage-grain	Conventional	1712 (59)	3514 (126)	1454 (148)	2854 (114)	1328 (209)	1872 (272)
	Forage-grain	Organic	1373 (112)	2022 (259)	1379 (117)	1372 (174)	482 (264)	680 (153)
	Forage-grain compost	Conventional	-[2]	-	-	-	-	1988 (169)
	Forage-grain compost	Organic	-	-	-	-	-	897 (240)
ANOVA Tests	Management and Rotation	Management	**	***	*	***	***	***
		Rotation	n.s.	***	*	**	n.s.	**
		Interaction	*	**	*	n.s.	**	n.s.
	Management and Compost Addition	Management	-	-	-	-	-	***
		Compost	-	-	-	-	-	n.s.
		Interaction	-	-	-	-	-	n.s.

n.s. (not significant at $P < 0.05$), * ($P < 0.05$) ** ($P < 0.01$), *** ($P < 0.001$)

[1] Values shown are the average of three replicate values with ± 1 standard deviation of the average given in parenthesis.

[2] Dash indicates treatment not present and test unable to be performed.

Figure 2.1 A picture of wheat in the grain-only rotation under organic management in 2004 before harvest. F^- (no fertilizer), P^- (no pesticides).

Table 2.2 Soil parameters determined in fall 2004 following a wheat crop at the Glenlea Long-term Rotation Study.

	Rotation	Management	Nitrate-N	Modified Kelowna Soil Phosphorus	pH_{H_2O}	EC ($dS\ m^{-1}$)	Organic Matter (%)[2]
			mg kg^{-1} dry soil				
	Grain-only	Conventional	8.33 (0.58)[1]	37.67 (7.09)	6.63 (0.23)	0.57 (0.11)	7.18 (0.20)
	Grain-only	Organic	7.67 (2.08)	30.33 (7.51)	7.03 (0.35)	0.74 (0.31)	6.67 (0.49)
	Forage-grain	Conventional	13.67 (0.58)	30.67 (7.23)	6.57 (0.25)	0.74 (0.26)	7.89 (0.46)
	Forage-grain	Organic	13.00 (2.65)	8.67 (4.04)	7.00 (0.36)	0.87 (0.17)	7.93 (0.84)
	Forage-grain compost	Conventional	14.00 (1.00)	35.33 (8.08)	6.57 (0.23)	0.65 (0.11)	8.09 (0.10)
	Forage-grain compost	Organic	13.33 (1.53)	14.00 (4.58)	7.07 (0.35)	0.95 (0.14)	8.12 (0.44)
	Prairie	Prairie	2.00 (0.00)	34.67 (9.45)	6.73 (0.06)	0.73 (0.09)	8.03 (0.86)
ANOVA Tests	Management and Rotation	Management	n.s.	**	**	n.s.	n.s.
		Rotation	**	**	n.s.	n.s.	*
		Interaction	n.s.	*	n.s.	n.s.	n.s.
	Management and Compost Addition	Management	n.s.	***	**	*	n.s.
		Compost	n.s.	*	n.s.	n.s.	n.s.
		Interaction	n.s.	n.s.	n.s.	n.s.	n.s.
	Prairie and Organic	Rotation	***	*	n.s.	n.s.	n.s.

n.s. (not significant at $P < 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$)

1 Values given are the average of three replicate values with ± 1 standard deviation of the average given in parenthesis.

2 Percent organic matter was converted from percent carbon using the conversion value of 1.724 (Wilson and Staker, 1932). Analysis for percent carbon was conducted by Manitoba Agriculture, Food and Rural Initiatives (MAFRI). All other analyses were conducted by Norwest Labs, Winnipeg MB.

Table 2.3 Phosphorus concentration in sequential soil extractions of treatments from the Long-term Crop Rotation Study in 2004.

	Rotation	Management	Water	Sodium Bicarbonate	Sodium Hydroxide	Hydrochloric Acid	Total Extractable P[2]
					mg P kg^{-1} dry soil		
	Grain-only	Conventional	12.49 (0.83)[1]	65.31 (12.98)	145.86 (7.50)	121.04 (10.66)	344.69 (31.37)
	Grain-only	Organic	8.31 (7.28)	49.35 (11.83)	110.44 (12.33)	148.64 (29.92)	316.73 (11.43)
	Forage-grain	Conventional	10.65 (1.90)	52.75 (5.19)	132.47 (15.86)	123.65 (9.52)	319.52 (32.18)
	Forage-grain	Organic	7.12 (0.60)	27.32 (8.22)	94.26 (25.60)	130.74 (29.17)	259.44 (8.10)
	Forage-grain compost	Conventional	14.69 (4.37)	60.25 (9.55)	140.34 (17.37)	128.66 (12.00)	343.94 (42.54)
	Forage-grain compost	Organic	7.57 (6.82)	32.82 (10.76)	100.52 (18.21)	145.80 (24.58)	286.71 (12.08)
	Prairie	Prairie	12.46 (1.90)	70.70 (8.03)	140.78 (5.97)	129.30 (12.57)	353.24 (26.23)
ANOVA Tests	Management and Rotation	Management	n.s.	***	**	n.s.	*
		Rotation	n.s.	**	n.s.	n.s.	*
		Interaction	n.s.	n.s.	n.s.	n.s.	n.s.
	Management and Compost Addition	Management	*	***	**	n.s.	**
		Compost	n.s.	n.s.	n.s.	n.s.	n.s.
		Interaction	n.s.	n.s.	n.s.	n.s.	n.s.
	Prairie and Organic	Rotation	n.s.	*	n.s.	n.s.	**

n.s. (not significant at $P < 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$)

1 Values given are the average of three replicate values with ± 1 standard deviation of the average given in parenthesis.

2 Total extractable phosphorus is the sum of water, sodium bicarbonate, sodium hydroxide and hydrochloric acid-extractable phosphorus fractions.

Table 2.4 Estimated cumulative (1992-2004) phosphorus balance for crop rotations and organic and conventional management systems at the Glenlea long-term study.

Rotation	Management	Cumulative P removal	Cumulative P nutrient addition	Cumulative P Balance
			$kg\ P\ ha^{-1}$	
Grain-only	Conventional	107.26[1]	139.88	32.61
Grain-only	Organic	52.05	0.00	-52.05
Forage-grain	Conventional	160.07	230.56	70.49
Forage-grain	Organic	117.92	0.00	-117.92
Forage-grain compost	Conventional	160.56	255.02	94.46
Forage-grain compost	Organic	118.83	24.46	-94.37

[1] The cumulative phosphorus removal was calculated using values for phosphorus concentration in harvested crop from the Soil Fertility Guide (2004) and crop yield.

Table 2.5 Phosphorus and nitrogen concentration in flax grain samples harvested in 2003 from the Glenlea long-term study[1].

Rotation	Management	Nitrogen	Phosphorus
		g kg^{-1} dry weight of grain	
Grain-only	Conventional	35.52 (1.12)[2]	5.23 (0.45)
Grain-only	Organic	32.48 (1.27)	8.07 (0.59)
Forage-grain	Conventional	41.76 (0.70)	5.23 (0.59)
Forage-grain	Organic	40.64 (3.00)	4.80 (1.56)
Forage-grain compost	Conventional	40.21 (1.18)	4.80 (0.36)
Forage-grain compost	Organic	38.24 (2.12)	5.87 (1.36)
ANOVA Tests – Management and Rotation	Management	n.s.	*
	Rotation	**	**
	Interaction	n.s.	**
ANOVA Tests – Management and Compost Addition	Management	n.s.	n.s.
	Compost	n.s.	n.s.
	Interaction	n.s.	n.s.

n.s. (not significant at $P < 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$)

[1] The grain samples were analyzed by Norwest Labs, Winnipeg MB. Typical nutrient concentrations in flax grain are 37.9 g kg^{-1} dry weight for nitrogen and 4.88 g kg^{-1} dry weight for phosphorus according to the Manitoba Soil Fertility Guide, 2004.

[2] Values given are the average of three replicate values with ± 1 standard deviation of the average given in parenthesis.

2.5 Discussion

A major concern for the sustainability of organic systems is that phosphorus reserves can be rapidly depleted and yields reduced without the replacement of phosphorus removed by the crop. The purpose of this study was to determine whether the depletion of phosphorus in organic systems is an immediate concern or if organic crop yields can be sustained for long periods of time by soil phosphorus reserves. By combining the sequential phosphorus extraction data with the expected cumulative phosphorus balance from the Glenlea long-term study, it was possible to examine whether the reduction of phosphorus in organic systems is a general depletion or a reduction only in plant available fractions.

2.5.1 Reduction in Plant Available Phosphorus in Organic Systems

Fourteen years of organic management at Glenlea led to a substantial reduction in plant available phosphorus concentrations in both the grain-only and forage-grain rotations. Concentrations of available soil phosphorus are often lower in organic systems than in conventional systems (Gosling and Shepherd 2005; Entz et al. 2001). The phosphorus status in organic systems will largely depend on whether or not manure-compost or some other external source of phosphorus is applied in these systems, how often it is applied and the phosphorus content of the amendment.

In addition to management system, the type of crop rotation also has an impact on the reduction of plant available phosphorus. There was a reduction of available phosphorus in the forage-grain rotation due to the high removal of plant matter compared with the grain-only rotation and this removal will be further discussed in section 2.5.3. The restored tall grass prairie had available phosphorus levels similar to those of conventional systems because phosphorus was recycled in the prairie system.

The chemical and physical properties of the soil can also determine the level of plant available phosphorus. The heavy clay soil texture such as that found at Glenlea causes retention of phosphorus mostly due to large amounts of labile calcium and magnesium (Ige et al. 2005). These ions have been known to retain phosphorus in unavailable forms, thereby decreasing plant available phosphorus levels in both organic and conventional systems. Clay content has been correlated to increases in phosphorus in stable, plant-unavailable forms (McKenzie and Bremer 2003). The properties of the soil can also determine the agronomic response of crop yields to added fertilizer (Read et al. 1973). Heavy clay soils have a high soil fixing capacity and so large additions of phosphorus may be required for a response in yield (Morel et al. 1989).

Phosphorus can also be retained by immobilization in organic matter and biological organisms (Flaten et al. 2003). Long term organic management systems tend to have increased levels of organic matter and microbial activity (Entz et al. 2004; Clark et al. 1998; Drinkwater et al. 1995) which may lead to a decrease in plant available phosphorus. It is likely that the retention of phosphorus in organic matter also played a role in the reduction of plant available phosphorus in the organic management systems at Glenlea, especially in the forage-grain rotations which had the highest levels of organic matter. This increase in organic matter may also have implications in carbon sequestration and therefore the organic matter in the forage-grain rotation deserves further scrutiny.

2.5.2 Fractionation and Total Extractable Phosphorus

The plant available phosphorus concentrations have both agronomic and environmental significance, but this is only a portion of the total phosphorus found in the soil. The amount of phosphorus in recalcitrant fractions is also important because this component may eventually re-supply phosphorus to plant available pools. Sequential phosphorus extraction allows for measurement of different pools of phosphorus present in the soil at the time of soil analysis.

The differences in labile and moderately labile extractable components between organic and conventional systems were similar to those found in the plant-available phosphorus levels measured by the modified Kelowna technique. The water, sodium bicarbonate, and sodium hydroxide extractable phosphorus levels were all lower in organic than conventional systems. These results are not surprising in that added phosphorus fertilizer as well as cropping practices tend to affect labile fractions more than unavailable fractions (Zheng et al. 2003a). There was not a large difference between organic and conventional management in the sodium bicarbonate extractable phosphorus pool of the grain-only rotation as compared with these differences in the forage-grain rotation. The similarity is possibly due to low rates of removal and mobilization of the sodium hydroxide pool into the more plant available fraction. The conversion of the moderately available sodium hydroxide pool into plant available sodium bicarbonate-extractable phosphorus has been observed in other studies when soil phosphorus is low (Zheng et al. 2003b; Guo et al. 2000; Zhang and MacKenzie 1997). In these studies, phosphorus also tended to accumulate in the sodium hydroxide pool when fertilizer was applied in excess of plant uptake and was therefore acting as both a sink for excess phosphorus and a source when phosphorus was low. In the Glenlea Long-term Rotation study, the sodium hydroxide pool was likely a phosphorus source in the organic rotations and a sink for the conventional rotations which could account for the greater difference in phosphorus concentration between management systems in the grain-only rotation in this pool compared to other pools.

As with the modified Kelowna phosphorus results, the conventional systems had levels of phosphorus comparable to the restored native tall-grass prairie planting in all plant available pools in the Glenlea Long-term Rotation study. It is fair to say that the total amount of phosphorus should not have changed in the prairie system from what it was prior to prairie establishment as no phosphorus was deliberately added or removed from the prairie. This indicates that the export of phosphorus in the crops of the conventional system was balanced with fertilizer addition in this study. As expected, the organic systems had lower plant available phosphorus than the prairie system because no fertilizer was added to replace the phosphorus taken up in crop harvests.

Contrary to the available phosphorus pools, the recalcitrant phosphorus pool showed no differences in either management or rotation. The hydrochloric acid-extractable phosphorus pool was the largest out of all the phosphorus pools in this study because of the large amounts of labile calcium and magnesium in the soil at Glenlea. It is possible that crops in the organic rotations were only tapping into the labile phosphorus fractions, leaving the recalcitrant fractions untouched due to inaccessibility. The resilience of the recalcitrant phosphorus pool has been confirmed in other studies where neither crop rotation nor fertilizer addition had any effect on changing this pool (Zheng et al. 2003a; 2003b). However, these phosphorus pools are known to be sparingly soluble and can slowly become available through cycling and the action of roots when labile phosphorus pools are low (Guo et al. 2000; Hedley et al. 1982). This suggests that in organic systems there still exists a large pool of phosphorus that is unavailable to crops in its current form, but may eventually become available through nutrient cycling in the soil.

The reduction of total extractable phosphorus in organic systems was calculated by combining the recalcitrant and available phosphorus totals. The differences between organic and conventional management systems were less extreme than what was observed in the plant available phosphorus pools. Even though no external nutrients were supplied to the organic grain-only rotation, this rotation had total phosphorus levels that were very similar to its conventional counterpart. To make sense of these results, it is necessary to take into account the phosphorus added and removed from the system in the different crop rotations and incorporate these into an estimated cumulative phosphorus balance.

2.5.3 Phosphorus Balance

The sequential phosphorus extraction and nitrate analyses indicated that organic systems are so far being sustained by declining pools of plant available phosphorus and initial nitrate concentrations. Yields began to fall in organic systems after the first two years of the study, indicating it took two years for organic management to decrease the soil phosphorus and nitrate concentrations and increase the population of weeds. This drain in available phosphorus with organic systems has also been observed in the 21 year study by Oehl et al. (2002) and the 34 year study by Campbell et al. (1993). However, considering how long these studies have been in rotation, it is interesting to note that phosphorus levels are still adequate to support a crop, even when fertilizer is withheld completely. An expected cumulative phosphorus balance makes it possible to estimate how much phosphorus is removed over the years of cropping and consequently gives an indication of the sustainability of the system.

According to phosphorus balance calculations, the organic forage-grain rotation had a phosphorus deficit that was much larger than the organic grain-only rotation. When considering the phosphorus removed from organic grain-only and organic forage-grain rotations, it is not surprising that the organic forage-grain had such a large deficit. One reason for the decline of phosphorus in forage-grain rotations is that alfalfa harvests removed large amounts of phosphorus in their plant matter and unlike grain, the straw is not replaced. The benefit of legumes in a crop rotation is the additional nitrogen provided to the soil; however, legumes are unable to add phosphorus. A long term study of the soil fertility of crop rotations without fertilizer in Indian Head, Saskatchewan by Campbell et al. (1996) compared the nitrogen and phosphorus concentrations in soil for a continuous wheat and wheat with fallow rotation with a wheat rotation including a three year stand of hay. The wheat rotation including hay had lower available phosphorus levels than either wheat rotation, indicating the removal rate of phosphorus in the hay stand was highest. An annual phosphorus budget was calculated for typical organic farms in the United Kingdom with legumes and with or without fertilizer (Berry et al. 2003). This budget revealed that organic legume farms without fertilizer had a much larger phosphorus deficit than the organic farms with fertilizer. The results of these studies emphasize that legumes in a rotation can drain available phosphorus faster than grain-only crop rotations.

In addition to the large amounts of nutrients removed in the plant matter during alfalfa harvests, another factor which contributed to the large deficit of phosphorus in the forage-grain rotations at Glenlea was the higher grain yields. When comparing the grain yields of the organic grain-only and forage-grain rotations in years when flax and wheat was common to both rotations, the organic forage-grain yields were consistently higher than the grain-only. It is obvious that a higher yielding cropping system, which includes no fertilizer, will remove nutrients at a higher rate than a low yielding system.

An important point to note is that the large deficit of total phosphorus in the forage-grain rotations is not supported by a large reduction in the total extractable phosphorus measured by sequential extraction, nor is it supported by an increase in phosphorus export in the cumulative phosphorus balance calculations. Because the removal rates used in the calculation of the phosphorus balance were based on typical concentrations phosphorus in crops from the Soil Fertility Guide (2004), these estimated deficits were not entirely accurate. The inaccuracy of these removal rates became apparent after examining the grain phosphorus concentration of the flax seed harvested in 2003. The concentration of phosphorus in the grain of the organic grain-only rotations was about 1.6 x that of the values from the guide and was also higher than the organic forage-grain and all conventional rotations. Therefore, the phosphorus balance estimated for this study probably underestimated the amount of phosphorus removed in the organic grain-only rotation.

One reason for the higher phosphorus concentration in the organic flax seed of the grain-only rotation could be due to an inverse relationship between high grain yield and low nutrient content. This type of inverse relationship has been observed in many cases with wheat grain nitrogen content (Dick et al. 1985; Pleijel et al. 1999), but is not well documented in the case of phosphorus concentration in flax seed. The inverse effect commonly seen in wheat grain nitrogen results from the dilution of nitrogen in the kernel that occurs when the wheat yields are increased and there is less nitrogen available to be shared amongst the kernels. The yields for the organic grain-only rotation at Glenlea were very low and therefore might explain why more phosphorus was contained in the flax seed. The conventional crops were also much taller than the organic crops during the growing season and therefore the nutrients were spread over a smaller biomass in organic systems than conventional.

The higher colonization of endomycorrhizal fungi, a beneficial soil fungus, in the organic systems could be another explanation for the high phosphorus concentration in organic flax grain of the grain-only rotation. Working at Glenlea, Entz et al. (2004) observed a higher colonization of endomycorrhizae in flax roots in the organic than conventional rotations in 2003. Endomycorrhizal fungi are able to increase the absorption of phosphorus in colonized plants when phosphorus concentrations in the soil are low due to the increase in surface area for nutrient absorption provided by the endomycorrhizal hyphae (Smith and Read 1997). The increase in phosphorus absorption could lead to an increase in translocation of phosphorus into the grain.

Even though the phosphorus deficit calculated for the organic systems at Glenlea is an expected one, the fact remains that in these systems phosphorus is being removed without being replaced. Many organic systems will eventually become phosphorus limited and will be unable to sustain crop yields.

2.5.4 Phosphorus Limitation in Organic Systems

The difficulty of replacing phosphorus is one of the major concerns in organic systems. Organic production cannot continue indefinitely without added phosphorus from external sources because phosphorus reserves will eventually be depleted. However, the rate at which phosphorus will be depleted in these organic systems depends on different factors.

One factor which may slow down phosphorus limitation in organic crops is how quickly unavailable phosphorus reserves can be cycled into plant available forms, and how much phosphorus is contained in these reserves. In this study, the plant available phosphorus concentrations were low in the organic systems, but the recalcitrant levels were not significantly different from the conventional rotations. Some crops, such as alfalfa, may have the ability to transform stable, residual phosphorus fractions into moderately labile phosphorus pools (Daroub et al. 2001). Another crop that has been known to mobilize recalcitrant phosphorus reserves is buckwheat. The hypothesis behind this ability of buckwheat is that this crop can decrease the rhizosphere pH by secreting H^+ which, in turn, mobilizes phosphorus that is bound to calcium ions in unavailable forms (Zhu et al. 2002; Hedley et al. 1982). The mobilization of recalcitrant phosphorus reserves is one way that the rate of phosphorus limitation in organic systems can be slowed down.

The colonization of endomycorrhizal fungi can also assist in delaying the rate of phosphorus limitation, since endomycorrhizae can help plants absorb phosphorus from the soil (Smith and Read 1997). There is some evidence that endomycorrhizal fungi are also able to increase phosphorus availability by mobilizing unavailable reserves (Cabala-Rosand and Wild 1982; Kucey et al. 1989; Grant et al. 2005). On the other hand, in several experiments involving ^{32}P-labeled phosphorus reviewed by Bolan (1991), the specific activities of phosphorus in mycorrhizal and nonmycorrhizal plants were the same. Since these beneficial fungi cannot increase available phosphorus in the soil, their benefit would be more in their ability to allow the sustenance of crop yields when phosphorus is low.

The mobilization of phosphorus reserves by some crops and the increase in phosphorus absorption by endomycorrhizal fungi will help slow down phosphorus limitation in organic systems, but replacement of nutrients taken up by crops is the only way to make these systems sustainable. While the one time application of manure-compost did increase the available phosphorus in the forage-grain rotations at Glenlea, this increase was not significant. Applying the manure-compost more frequently would help replace the phosphorus removed by the crops. This presumption is supported by a survey involving four different organic horticulture farms in the United Kingdom which all had imported animal manure (Watson et al. 2002b). A positive annual phosphorus budget was calculated for each of these systems ranging from 1.7 to 89 kg ha^{-1} yr^{-1}. The large range was due to different management strategies and crop rotations on each farm. In another study involving a comparison between organic, low-input and conventional farming systems, the organic system had the highest annual surplus of phosphorus due directly to the high phosphorus content in the animal manure applied (Clark et al. 1998). The ability of composted animal manure inputs to sustain organic agriculture systems therefore depends on a careful balance of the inputs and exports of the system to avoid large phosphorus deficits and surpluses.

In order to determine whether phosphorus is really limiting in the organic systems at Glenlea it is necessary to compare phosphorus levels in the plant available pools with the agronomic response threshold. According to the Manitoba Soil Fertility Guide (2004), the concentration of phosphorus above which flax crops do not respond to added fertilizer is 15-18 mg P kg^{-1} dry soil (Olsen phosphorus). The amount of available phosphorus extracted by modified Kelowna methods is greater than that of Olsen phosphorus (Kleinman et al. 2001); therefore, in order to assess whether or not the organic soils at Glenlea are below the agronomic response threshold it is necessary to convert modified Kelowna to Olsen P. The conversion was done using a regression equation from the relationship between Olsen and modified Kelowna phosphorus developed by Kumaragamage et al. (2007) for soils of $pH > 7$. After conversion, the Olsen phosphorus concentrations in the organic systems at Glenlea would be roughly 23 mg P kg^{-1} for the grain-only, 6 mg P kg^{-1} for forage-grain and 10 mg P kg^{-1} for forage-grain with manure compost. According to these calculations both of the organic forage-grain rotations are below the agronomic response threshold and are given a rating of low for soil phosphorus availability by the Manitoba Soil Fertility Guide (2004). The phosphorus concentration is the organic grain-only rotation is well above the agronomic threshold and is rated as very high for phosphorus availability. Considering the low removal rates of phosphorus in the organic grain-only rotation, it will likely be another six years before this rotation becomes phosphorus limited.

2.5.5 N Limited Systems

Though phosphorus limitation is a major problem in many organic systems, nitrogen is actually considered the main limiting nutrient in organic systems (Watson et al. 2002a). The present study indicated the soil at Glenlea is probably more limited by nitrogen than phosphorus. The low yields in the grain-only rotation were suspected to be due to nitrogen limitation rather than phosphorus because the organic forage-grain rotation had concentrations of available phosphorus that were much lower than the organic grain-only system, yet the forage-grain rotation yields were generally similar for organic and conventional systems. The two consecutive years of alfalfa would have been enough to build up nitrogen concentrations in the forage-grain rotation (Kelner et al. 1997), whereas the one year of fababeans in the grain-only system was not sufficient to supply nitrogen to the soil. In their review of the role of nitrogen in organic farming, Berry et al. (2002) stated that pea residues can supply only 20-25 kg N ha^{-1} to the following crop; conversely, pasture crops such as clover can supply in excess of 100 kg N ha^{-1} yr^{-1}. The nitrogen benefit of the two-year alfalfa period in the organic forage-grain rotation can be seen when comparing the difference in nitrogen content of the forage-grain soil with that of the organic grain-only rotation. The latter system appears to be nitrogen limited whereas the former system is sufficient in nitrogen.

Unlike phosphorus concentrations, the nitrogen concentrations in the flax grain more closely reflected the nitrogen limitations in the soil. The organic grain-only rotation had the lowest nitrogen in both the soil and the flax grain. This suggests that whereas in low phosphorus soils plants may be able to mobilize phosphorus in the rhizosphere or increase phosphorus uptake possibly through the association with endomycorrhizal fungi, it may have been more difficult for plants to increase nitrogen uptake when the nitrogen levels were low in the soil.

2.5.6 Conclusions

Organic systems may reduce plant available phosphorus where exported phosphorus is not replaced, especially high yielding hay export systems. The rate of conversion of non-labile to labile phosphorus in the heavy clay soil was not fast enough to replace phosphorus lost from the plant available pool by plant uptake, keeping this pool very low. The system was high in phosphorus prior to implementation of the study and therefore crop yields were sustained by this initial phosphorus reserve for the duration of the study. After fourteen years of organic management, the soil in organic forage-grain rotations has become phosphorus limited whereas the organic grain-only rotation will likely not be limited by phosphorus for another six years. The soil at the study site was overall more nitrogen limited than phosphorus. Nitrogen limitation was apparent because the yield of flax in the forage-grain rotation was similar under both organic and conventional management after the two year stand of alfalfa, whereas in the grain-only rotation the organic flax yields were much lower than conventional. The flax grain in the organic grain-only rotation also showed signs of nitrogen deficiency. Therefore, removal of nutrients from organic crops may deviate from published values due to nutrient deficiencies in the soil. Addition of manure-compost to organic systems every four years, depending on application rate and crop removal, should be sufficient to overcome phosphorus limitation. The inclusion of phosphorus-solubilizing crops such as buckwheat or inoculants such as *Penicillium bilaiae* may help to mobilize large reserves of recalcitrant phosphorus in organic systems. Mycorrhizal fungi are also important in low-input and organic systems because they allow greater exploration of the soil for phosphorus when phosphorus is limiting. The results of the Glenlea Long-term Rotation study revealed that nitrogen limitation is a more immediate concern in organic rotations that do not include nitrogen fixing crops than phosphorus limitation in high yielding hay export systems.

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3. ORGANIC CROP MANAGEMENT PROMOTES MYCORRHIZAL COLONIZATION AND DIVERSITY[1]

3.1 Abstract

Arbuscular mycorrhizal fungi (AMF) are one of the most important soil organisms for assisting in plant nutrient uptake from soil, particularly phosphorus (P). With interest in lowering soil P levels for enhanced environmental sustainability, promoting the activity of mycorrhizal fungi may be critical to maintaining crop yields. This study compares organic and conventional systems and different crop rotations to determine the extent of their impact on AMF colonization and community structure. The research was carried out at the 14 year study at Glenlea, in Southern Manitoba. This site consists of 3 different 4-year rotations under organic and conventional management: Flax – Alfalfa – Alfalfa – Wheat (forage-grain) with and without composted cattle manure, Flax – Oat – Fababean – Wheat (grain-only), and a restored tall-grass prairie (prairie). Percentage AMF colonization as arbuscules was higher ($P < 0.05$) in organic than conventional systems; crop rotations did not significantly differ in colonization. The same trend in treatments was observed for mycorrhizal spore populations ($P < 0.05$) as for colonization. The organic rotations had lower labile P than conventional and the prairie had P levels similar to conventional systems. The grain-only rotation had higher labile P than the forage-grain rotations ($P < 0.05$); however, tissue P concentration did not differ among any treatments indicating AMF may have contributed to increasing P uptake in

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organic systems. Correspondence analysis indicated a shift in AMF community structure likely occurred between the prairie and the rest of the treatments and between the organic and conventional grain-only rotations; however, this shift may have been more related to selection of AMF species by crops than P limitation.

3.2 Introduction

Mycorrhizal fungi are one of the most important soil organisms for assisting in nutrient uptake by plants. These fungi form symbiotic relationships with many plants by trading most of the phosphorus they absorb to their hosts in exchange for plant sugars. Various plants rely heavily on arbuscular mycorrhizal fungi (AMF) to obtain sufficient phosphorus for growth in systems low in phosphorus such as many organic and low-input systems (Mäder et al. 2000). Phosphorus absorption can be increased by AMF colonization mainly through the increase in surface area for nutrient absorption provided by extraradical fungal hyphae emanating from colonized roots (Smith and Read 1997).

Promoting the beneficial role of AMF in agriculture systems relies upon understanding the effects of management practices upon mycorrhizal colonization of crops. Colonization is inhibited by high concentrations of phosphorus in the soil (Mäder et al. 2000), extensive tillage which breaks up the network of extraradical hyphae (McGonigle and Miller 1993; Evans and Miller 1988), and applications of some pesticides at certain rates (Gosling et al. 2006). Mycorrhizal colonization can be increased by a crop rotation system that ensures a host plant is always present in the field (Alvey et al. 2001).

In addition to colonization, AMF diversity is also altered by management systems. High input monoculture cropping systems as well as intensive tillage can decrease the species diversity of AMF in soil to those providing less efficient transfer of phosphorus to crops (Kucey and Paul 1983; Oehl et al. 2003). In contrast, pasture systems as well as cropping systems with multi-year crop rotations can increase mycorrhizal diversity due to the increase in host diversity (Oehl et al. 2003).

Crops in organic systems have a large potential to receive benefits from AMF association. These systems tend to increase AMF colonization and diversity by the very principles that define organic agriculture. For example, synthetic pesticides are not used in organic production, soils are managed by addition of organic materials, and crop diversity is encouraged to reflect ecological balances in nature (IFOAM 2005). In following these principles, organic agricultural systems are consequently dependent on an active microbial community for processes such as nutrient cycling. AMF therefore play a critical role in these ecosystems in supplying phosphorus to crops to offset the lack of available phosphorus that occurs without the use of synthetic fertilizers. The low plant phosphorus concentration due to the lack of available soil phosphorus leads to an increased colonization of plants by AMF in organic systems (Entz et al. 2004; Oehl et al. 2002; Mäder 2000). However, when organic systems are high in phosphorus, AMF colonization would not be beneficial because plants already have access to sufficient phosphorus. In this case, plants limit colonization to conserve their sugars for growth and reproduction (Gosling et al. 2006).

We know that crops in most organic systems are more highly colonized by AMF than conventional systems, but little is known about how colonization structurally varies between organic and conventional systems. It is the arbuscules that specifically lead to an increase in nutrient exchange and seemingly are a better indication of phosphorus acquisition of plants via AMF colonization (Marx et al. 1982).

The experiment reported here used the Glenlea Long-term Rotation Study to examine whether arbuscular colonization is increased in organic systems leading to an increase of phosphorus absorption in plant tissue under low phosphorus conditions. Arbuscular and hyphal colonization concentrations were compared between organic and conventional management under three different crop rotations. A benefit-to-cost ratio of AMF colonization was calculated to determine where and if colonization is helping or hindering crop yields (Dekkers and van der Werff 2001). A restored tall-grass prairie treatment served as a comparison of agriculturally managed systems to a system which has been untouched. In order for an equal comparison between the organic and conventional systems, it was necessary to grow the same crops under the same rotations. The only difference in management was that none of the synthetic fertilizers and pesticides that were used in the conventional systems were added to the organic systems.

It was also important to consider AMF spore abundance and diversity in this study to further test for an effect of management or rotation on AMF. In addition, detection of a shift in AMF diversity with different management systems and crop rotations using multivariate analysis may indicate that an AMF species composition is selected for by crop management that enhances the plant-fungus symbiotic relationship.

3.3 Materials and Methods

3.3.1 Site Description and Treatments

The research was conducted at the Glenlea Crop Rotation Study, which began in 1992 and is Canada's oldest field crop organic system. Glenlea is located 20 km south of Winnipeg, Manitoba (N 49,39,0 / W 97,7,0). This long-term rotation was soil sampled in 2005, in the 14th year of the study. Each management had three different four-year crop rotations: Flax – Alfalfa – Alfalfa – Wheat (forage-grain) with and without composted cattle manure, and Flax – Oat – Fababean – Wheat (grain-only). The treatments (such as manure, fertilizer and pesticides) were the same as detailed previously in chapter 2 (section 2.3.1). In the forage-grain rotation, a 4 x 26 m section from each replicate was treated with manure-compost in the fall of 2002. A native tall grass prairie planting (including *Andropogon gerardii* Vitman var., *Sorghastrum nutans* (L.), *Panicum virgatum* (L.), *Agropyron smithii* (Rydb.), *Elymus lanceolatus* (Scribn. and Smith) Gould. and *Elymus trachycaulus* (Link) Gould ex Shinners) established at the beginning of the rotation study was also sampled to provide a benchmark for initial total phosphorus and also to monitor natural AMF populations in the soil. The experimental design was a split plot randomized complete block, with three replicate blocks. The soil was mapped as predominantly Rego Black Chernozem soil of Red River or Scantenbury series (Michalyna 1970). The texture of the soil is 9% sand, 26% silt and 66% clay with a pH_{H_2O} of 7.4 and an organic matter content of 7.7%.

3.3.2 Yield Measurement

From 2002 to 2005, the grain was harvested in 26 m strips with a Massey Ferguson 8-XP small plot combine (Kincaid Equipment Manufacturing, Haven, KS). After harvesting, the grain was cleaned, weighed and dried at 65°C for 72 hours. Alfalfa harvest rates were determined by collecting three 1 m^2 quadrats of dry matter before mechanical haying of the whole plot. Total dry matter production for other crops was determined in the same way as alfalfa harvest rates, except after separating the weeds, the crop was dried for 72 hours at 55°C and then weighed.

3.3.3 Soil and Plant Nutrient Analysis

The flax samples were collected from the Glenlea study to be analyzed for concentration of macronutrients. The above-ground flax biomass samples were collected on July 22nd, 2005 from the three different crop rotations under organic and conventional management when flax plants were about four weeks old. Four plant cuttings in 20 cm long sections were taken at the base of the plants from each plot, with two cuttings taken 1 m away from both the front and back of the plots on either side. The samples were then dried in a drying room for two weeks in paper bags. After drying, the samples were weighed prior to analysis.

Flax grain samples from 2005 collected during harvest were analyzed for phosphorus and nitrogen concentration. The forage-grain with manure-compost rotation was left out of the analysis because the third replicate had insufficient yields and was not harvested. The phosphorus in both grain and tissue samples was determined by dry ashing which involved digesting the samples in 2 Normal hydrochloric acid (HCl) at 550°C for 5 hours and then analyzing them by inductively coupled plasma spectrometry (ICP). The nitrogen concentration of the digest was determined by automated colorimetric analysis after filtering samples mixed with water to remove large particles.

Soil samples to be analyzed for macronutrients and spore isolation were also taken from the plots. The top 15 cm of soil was sampled on July 29th, 2005 using a Dutch auger (5 cm width). Nine random samples were taken and mixed together for a plot and approximately half of each of the samples were broken up and dried for two weeks in a drying room prior to analysis. The other half was kept at 5°C to be used later for spore extraction. Nitrate was extracted by dilute calcium chloride dihydrate solution (0.15% w/v) and then analyzed using a Technicon AutoAnalyzer II instrument where it underwent cadmium reduction before being analyzed for nitrite by colorimetry. Sulphate concentration of the same extraction solution was determined by ICP. Potassium and phosphorus were analyzed using the modified Kelowna methods and the Technicon AutoAnalyzer was used for automated colorimetry analysis. A flame photometer was attached to the Technicon for potassium analysis. The EC and pH were determined by mixing the soil and water in a ratio of 1:2 (soil:water) and organic matter was measured using the wet oxidation and titration method (Walkely Black Method) (Carter 1993). Plant tissue phosphorus was determined by HCl digestion and then analysis by ICP and nitrogen was determined as ammonia by colorimetric analysis using a Technicon system after filtering samples mixed with water to remove large particles.

3.3.4 Root Sampling

The root samples were collected for analysis of root length density and mycorrhizal colonization. Four root samples were collected from each plot on July 22nd, 2005. Two samples were taken 1 m away from both the front and back of the plots 1 row in from either side. A Tulip Bulb Planter (7 cm diameter) was placed over top of a single plant and pressed down in the soil to a depth of 10 cm. In this way, the soil extracted with the root samples was of equal, calculable volumes and could be used to determine soil moisture and bulk density. Bulk density for each plot was determined by taking a 15 g subsample of soil from the root samples and drying it for 24 hours at 105°C. The root samples were washed using a Root Washer (Gillison's Variety Fabrication Inc., GVF Root Washer) and then stored in 70% ethanol until needed.

3.3.5 Root Density Determination

The root density in each core sampled was estimated quantitatively using the method outlined by Tennant (1975). After root washing, roots were placed in a rectangular tray (24.5 x 17.5 x 4.5 cm) which had a grid (3.5 cm x 3.5 cm squares) on the bottom. The roots were separated into roots attached to flax plants and free roots in the soil, which included weed roots, cut into 2 cm long fragments and submerged in water before counting. Starting with the top horizontal line of the grid and scanning right to left, top to bottom, all roots which bisected a line were counted and the number recorded. The roots bisecting the vertical lines were counted next scanning top to bottom and moving right to left. By summing up the total intersections of both horizontal and vertical lines, a total number of bisecting roots was calculated and entered into the formula for root length, R.

$$R = 11/14LN \qquad [1]$$

Where L is the length of one side of a square on the grid, and N is the total number of bisecting roots counted. The average root density beneath each plant was calculated by dividing the root length by the bulk volume of soil removed by the Tulip Bulb Planter. See Appendix II for a more detailed description of methods.

3.3.6 Mycorrhizal Colonization

After calculating root density, only the roots attached to a flax plant were used in the clearing and staining procedure for mycorrhizal colonization determination (Brundrett et al. 1996). The flax roots were first placed in FAA (formalin-acetic acid-alcohol fixative) for at least one week to fix the roots so the fungal morphological characteristics remained intact for viewing. A subsample of the root sections was then rinsed with deionized water three times by soaking in Petri dishes, the water was poured off, and 10% potassium hydroxide (KOH) solution added to the dishes. The roots in KOH were then poured into beakers and autoclaved for 10 minutes to clear them. After clearing, the root segments were rinsed as before. When rinsed, Chlorazol Black E (0.03% w/v in 1:1:1 lactic acid, glycerol and water) solution was added which stains root and hyphal cell walls a very dark green. The roots were left in the stain and placed in an oven at 90°C for 90 minutes. The roots were then rinsed again and glycerol was added to destain the roots overnight.

The stained roots were then ready to be viewed under the microscope for colonization scoring following the methods of McGonigle et al. (1990). The root segments were mounted on slides for viewing under the light microscope at 250x magnification. The slide was moved up, down and across and when a root, hypha, or arbuscule was bisected with the vertical cross-hair in the ocular lens it was counted as one. However, when both hyphae and arbuscules were intersected on the same root, only the arbuscules were scored. Counting was stopped when the amount of root sections evaluated reached 100. A percentage of hyphae and arbuscules per root sections counted could then be determined. The ratio of arbuscular colonization to total colonization (percent arbuscules plus hyphae alone) was calculated to estimate the benefit to cost ratio of mycorrhizal colonization in the different treatments (Dekkers and van der Werff 2001). The percent hyphae alone and arbuscules were also multiplied by the flax root length density to determine the mycorrhizal benefit-to-crop production (Brundrett et al. 1996). If the colonization density appears small compared with percent colonization, the plant biomass is likely small and therefore crop production may not benefit from the association. See Appendix II for a more detailed description of methods.

3.3.7 Spore Abundance and Diversity Determination

Spores were isolated from the soil samples so their populations could be used to further evaluate the effect of treatment on AMF activity and also to determine if shifts in

AMF diversity occurred with treatment. The method used for spore isolation is based on that of Brundrett et al. (1996). Briefly, a random subsample of the soil in each plot (100 g) was soaked in deionized water overnight before being passed through a stack of mesh screens. After soaking, the soil was manually broken up until most clay particles were suspended in the water. The soil and water mixture was then slowly poured through five stacked mesh screens (500, 212, 150, 53, and 45 μm diameter openings) and water run through the screens until clear. The debris (including isolated spores) remaining on the 212, 150, 53, and 45 μm screens were then collected by rinsing with deionized water into a round-bottom centrifuge tube for each screen. The debris/water mixture was then mixed to submerge the debris before centrifuging at 2,000 *g* for 5 minutes. The supernatant that included low density organic material was poured off and the pellet re-suspended in a 50% (w/v) sucrose solution. This mixture was then centrifuged at 2,000 *g* for 1 minute. The supernatant (including spores) was then poured through a small 45 μm screen and rinsed with deionized water to wash the spores, the pellet was discarded. The rinsed spores were collected into a centrifuge tube with water and poured through a Buchner funnel followed by vacuum filtration through a 45 μm pore-size MicronSep, Cellulosic filter paper.

The spores mounted on the filter paper could then be easily seen and counted under the dissecting microscope at 20x magnification. There were too many spores on the filter paper to count; therefore, five random locations on each paper were selected and photographed using QCapture Pro digital photography (Media Cybernetics 2003). The spores in each picture could then be measured and counted at the same time using Image-Pro Discovery software (Media Cybernetics 2004) by dragging a line across the longest diameter of each spore. The number of spores counted was then converted to total spores per 100 g soil using area of the photographed sections and the area of the filter paper. The spore measurement was converted from pixels to µm using a stage micrometer and measuring the length in pixels with the Discovery software.

For spore diversity determination, the spore types were categorized into different groups for diversity determination. The spore populations were broken down into two different size fractions (< 110 µm and > 110 µm) and three different colour groups (clear, brown, and black). The resulting six different spore categories (otherwise known as operational taxonomic units - OTUs) were used to determine spore diversity in each rotation. The amount of each different OTU in each rotation was determined and this data analyzed by multivariate statistical analysis (Canonical Discriminant Analysis) to observe groupings of OTU's according to treatment. Though our method did not access the number of genetic taxonomic units it should indicate if treatments altered the AMF community in soil.

3.3.8 Statistical Analysis

Hyphae, arbuscules and spore populations were compared using a split plot design and the Statistical Analysis Software program (SAS Institute 2001). Prior to the analysis, the data were tested for homogeneity of variance (Levene's) and for normality (Shapiro-Wilks). The protected Least Significant Difference (LSD) test was used to determine significant differences ($P < 0.05$) among rotations and between organic and conventional management. Three different ANOVAs were performed on the results: The first ANOVA used a split plot design with management (organic and conventional) and rotation as main effects which excluded the prairie and forage-grain with compost-manure rotations. A second ANOVA was conducted on both forage-grain rotations using a split plot design with management and manure-compost addition as the main effects. A third ANOVA was conducted to compare agriculture systems with natural systems and used the organic rotations and the prairie treatment in the analysis. All analysis were performed at the $\alpha = 0.05$ significance level and are presented in tables in the following results section. A regression analysis was done for total colonization and spore populations to determine if there existed a correlation between these two variables.

Differences in spore community types were analyzed and depicted using correspondence analysis (CA) and the CANOCO Software Program (Biometris, Plant Research International 2004). A simple ordination biplot was created with scaling focused on interspecies differences. With CA, samples assemble along a major gradient (Axis 1) which encompasses most of the variance in the abundance of different spore types. Axis 2, which is perpendicular to Axis 1, is the secondary gradient and accounts for further variance in the spore data but less than Axis 1.

3.4 Results

3.4.1 Yield

In 2005, the crop yields were not significantly affected by management system but were affected by rotation (Table 3.1). The crop yields in the grain-only rotation were lower than the forage-grain. However, the results for historical crop yields reported in section 2.4.1 show conventional management yields were greater than organic management with the exception of the first two years of the study (1993 and 1994) in which management had no effect on yields (Appendix I, Table 5.1). The organic and conventional flax yields in 2005 were very similar in the forage-grain rotation, which was why management had no effect on yield. Climate likely had an effect on crop yield in this case, particularly the large amount of precipitation in July (155 mm) – Environment Canada.

3.4.2 Hyphal and Arbuscular Colonization

The percent of the root colonized by hyphae did not differ among any of the treatments; however, percent arbuscular and total colonization were altered. The prairie grass had a very high percent colonization of hyphae alone but none of the commercial crop rotations differed significantly in the colonization of hyphae alone (Table 3.2). However, arbuscular colonization and total colonization (hyphal and arbuscular) were higher in organic treatments than in conventional (Table 3.2). The colonization of arbuscules in the prairie grass was not significantly higher than the arbuscular colonization in the organic systems ($P < 0.05$), but was higher than both of the conventional forage-grain rotations. The type of AMF colonization was also visually different in the prairie than in the flax roots (Figure 3.1a and b). The ratio of arbuscular colonization to total colonization (benefit to cost) was not significantly different between organic and conventional treatments; however, the organic forage-grain rotation had a higher benefit to cost ratio than the tall-grass prairie indicating the prairie may not have been benefiting from AMF colonization to the same extent as the forage-grain rotation (Table 3.2). Neither rotation nor manure-compost addition had any effect on root colonization by AMF.

The differences in arbuscular colonization between management systems were not as pronounced when multiplied by the density of flax roots in the plots, but the hyphal density showed the same trend as hyphal colonization (Table 3.2). As with percent colonization of hyphae alone, the density of hyphae alone was not significantly altered by either management or rotation. Contrary to the findings for percent arbuscular colonization, the arbuscular density was only significantly higher in organic systems when looking at the effect of manure-compost addition. The prairie was the only treatment which differed in total colonization density (Table 3.2). The average root density did not differ in any of the treatments but the percent of the root density composed of flax plants was slightly altered (data not shown). The flax root density was lower in the organic rotations (21.1 cm mL^{-1}) than in the conventional (29.9 cm mL^{-1}), which would compensate for differences in arbuscular density that were calculated for arbuscular colonization.

3.4.3 Soil and Plant Nutrient Analysis

Organic systems had lower concentrations of plant available soil phosphorus than conventional systems and the forage-grain rotation had lower phosphorus than the grain-only. The concentration of available phosphorus in the prairie system (24 mg kg^{-1} dry soil) was similar to the conventional systems (Table 3.1). The plant tissue phosphorus concentrations, however, did not differ among management systems but were still higher in grain-only than forage-grain rotations (Table 3.1). Neither management nor rotation had any effect on flax grain phosphorus concentration.

There were no significant differences in soil nitrate nitrogen levels in 2005 but there were some differences in flax tissue and grain nitrogen concentration. The tissue nitrogen concentration was lower in organic systems than in conventional, but no significant differences were found with rotation or compost addition (Table 3.1). However, the rotation effect was significant for nitrogen in the grain with the grain-only being less than the forage-grain. The nitrogen concentration in the flax grain of the grain-only rotation was also lower than the average values for nitrogen concentration in flax grain stated in the Manitoba Soil Fertility Guide, 2004 (37.9 g kg^{-1} dry weight). No differences were observed with management system in regards to flax grain nitrogen concentration (Table 3.1).

3.4.4 Spore Abundance and Diversity Determination

Both spore populations and spore diversity were affected by treatments in this study. The spore populations per g of dry soil, as with the arbuscular colonization, were higher in the organic systems compared with the conventional; whereas the populations were not significantly affected by rotation (Figure 3.2 and 3.3). The prairie had the highest number of spores but the number was not significantly higher than the spores counted in the organic systems. Spore populations were also correlated to total mycorrhizal colonization ($P < 0.05$, $r^2 = 0.72$).

Different groups of spores were apparent in various quantities among the treatments (Table 3.3 and Figure 3.4). The correspondence analysis biplot showed that some plots were grouped together by treatment, and these treatments were found in different quadrats (Figure 3.5). The distribution of plots was determined mostly by the first axis (Axis 1), which accounted for 53 percent of the variation of the species data and to a lesser extent by Axis 2 which accounted for 27 percent of the variation. The small brown spore group was highly weighted on axis 1 (Figure 3.5) suggesting that this spore type was highly variable among different plots. The abundance of the large black spore group appeared to be inversely related to the abundance of small brown spores. The prairie plots were found strictly in the upper left quadrat suggesting that large black spores were abundant in these plots and that small brown spores were for the most part absent. The organic grain-only plots occurred in the upper right quadrat suggesting their distribution was determined mostly by the presence of small brown spores. The rest of the spores did not have as large an impact on the distribution of plots in this biplot. There was no clear pattern of distribution of plots in the organic or conventional forage-grain treatments.

Table 3.1 Flax grain yield, soil test P and nitrate-N, total P and N concentration in flax tissue (sampled July 22, 2005) and flax grain P and N concentration (at harvest) for the different treatments at the Glenlea Long-Term Crop Rotation study.

Rotation	Management	Flax yield ($kg\ ha^{-1}$)	Soil test P ($mg\ kg^{-1}$ dry soil)	Soil Nitrate-N ($mg\ kg^{-1}$ dry soil)	Total P concentration: Tissue ($g\ kg^{-1}$ dry matter)	Total P concentration: Grain ($g\ kg^{-1}$ dry matter)	Total N concentration: Tissue ($g\ kg^{-1}$ dry matter)	Total N concentration: Grain ($g\ kg^{-1}$ dry matter)
Grain-only	Conventional	845.0 (94.3)[1]	32.3 (2.52)	9.00 (2.00)	3.90 (0.265)	7.27 (0.058)	4.34 (0.21)	3.30 (0.10)
Grain-only	Organic	351.4 (48.3)	22.0 (2.00)	5.67 (2.08)	4.10 (0.794)	7.87 (0.252)	2.89 (0.25)	3.11 (0.10)
Forage-grain	Conventional	1243.9 (396.9)	15.3 (5.51)	9.00 (3.61)	3.03 (0.577)	6.97 (0.666)	4.00 (0.93)	3.70 (0.31)
Forage-grain	Organic	1139.3 (720.6)	8.33 (3.21)	7.33 (1.53)	3.43 (0.850)	6.20 (1.47)	3.45 (0.26)	3.61 (0.20)
Forage-grain compost	Conventional	1098.5 (360.6)[2]	21.0 (8.19)	10.67 (3.79)	3.73 (0.503)	7.05 (0.21)[2]	4.17 (0.10)	3.40 (0.12)[2]
Forage-grain compost	Organic	457.6 (81.67)[2]	8.00 (2.65)	8.67 (1.15)	3.50 (0.529)	7.20 (0.28)[2]	3.43 (0.42)	3.30 (0.00)[2]
ANOVA Tests: Management and Rotation	Management	n.s.	**	n.s.	n.s.	n.s.	*	n.s.
	Rotation	*	**	n.s.	*	n.s.	n.s.	**
	Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ANOVA Tests: Management and Compost Addition	Management	-[3]	*	n.s.	n.s.	-	n.s.	-
	Compost	-	n.s.	n.s.	n.s.	-	n.s.	-
	Interaction	-	n.s.	n.s.	n.s.	-	n.s.	-

n.s. (not significant at $P < 0.05$), * ($P < 0.05$), ** ($P < 0.01$)

1 Values given are the average of three replicate values with ± 1 standard deviation of the average in parenthesis.

2 The yield and grain phosphorus averages for the forage-grain manure-compost rotations are an average of two replicates due to inadequate harvest of the third replicate.

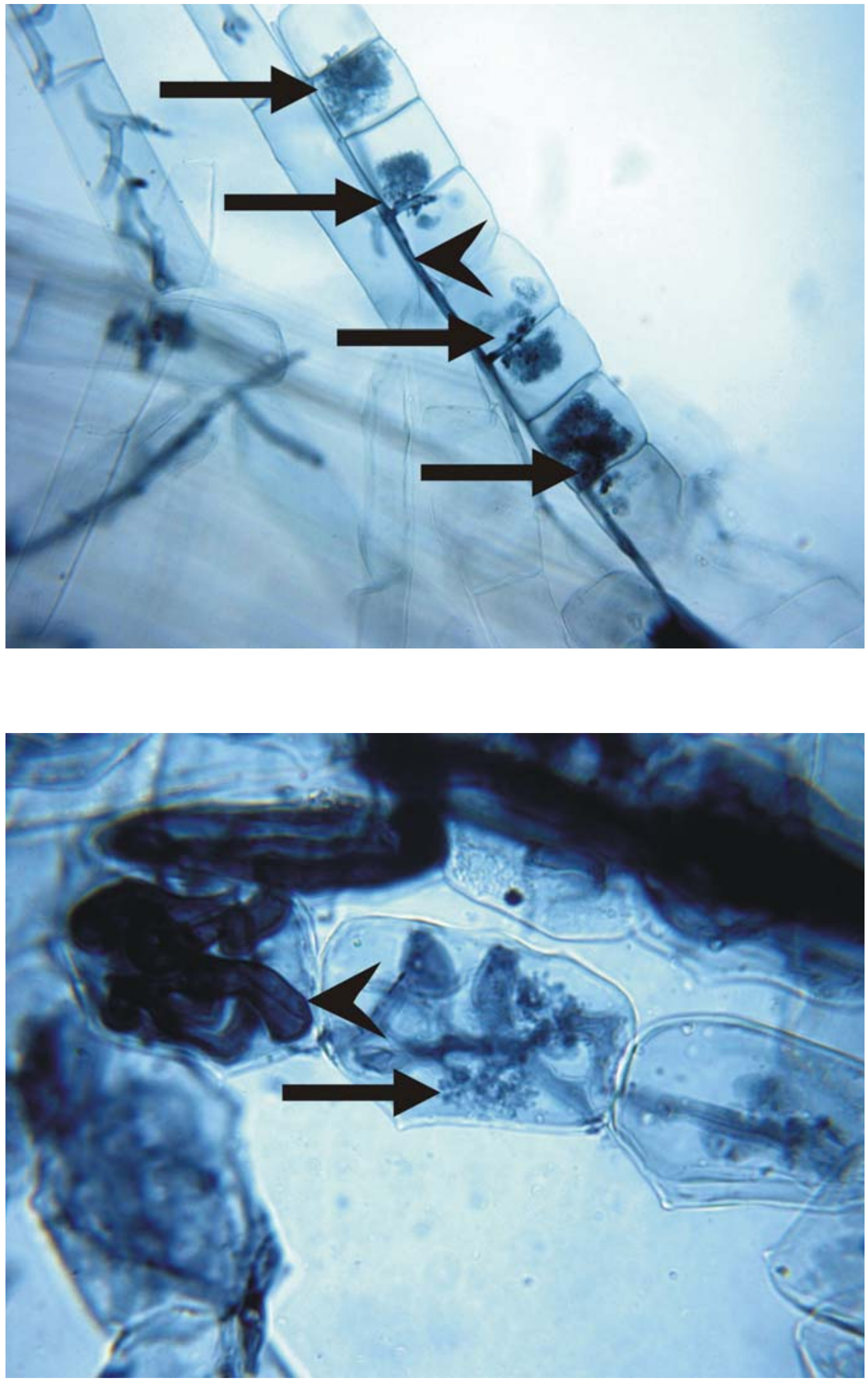
3 No statistics done due to lack of third replicate.

Table 3.2 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization and the benefit to cost ratio (AC:TC) of colonization as well as hyphal (HCD), arbuscular (ACD) and total (TCD) colonization density in flax roots at the Glenlea Long-Term Crop Rotation study.

Rotation	Management	HC (%)	AC (%)	TC (%)	HCD (cm mL^{-1})	ACD (cm mL^{-1})	TCD (cm mL^{-1})	AC:TC
Grain-only	Conventional	9.7 (4.04)[1]	22.7 (11.2)	32.3 (14.2)	0.68 (0.35)	1.48 (0.58)	2.16 (0.88)	0.686 (0.079)
Grain-only	Organic	15.0 (2.65)	34.7 (3.51)	49.7 (4.16)	1.36 (0.71)	3.11 (1.47)	4.47 (2.16)	0.698 (0.043)
Forage-grain	Conventional	12.0 (8.72)	16.0 (6.24)	28.0 (14.7)	1.02 (0.65)	1.48 (0.35)	2.49 (0.94)	0.629 (0.168)
Forage-grain	Organic	10.3 (5.13)	34.7 (8.33)	45.0 (7.00)	1.00 (0.33)	3.82 (2.24)	4.81 (0.219)	0.768 (0.107)
Forage-grain compost	Conventional	15.7 (6.35)	14.0 (4.00)	29.7 (7.51)	1.09 (0.26)	1.07 (0.59)	2.17 (0.72)	0.478 (0.113)
Forage-grain compost	Organic	11.0 (5.20)	24.7 (8.39)	35.7 (7.64)	1.20 (0.65)	2.55 (0.56)	3.75 (0.42)	0.684 (0.168)
Prairie	Prairie	47.7 (10.07)	29.3 (10.26)	77.0 (3.46)	11.79 (2.58)	7.31 (2.91)	19.10 (1.66)	0.381 (0.134)
ANOVA Tests: Management and Rotation	Management	n.s.	**	*	n.s.	n.s.	n.s.	n.s.
	Rotation	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ANOVA Tests: Management and Compost Addition	Management	n.s.	**	n.s.	n.s.	*	n.s.	n.s.
	Compost	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ANOVA Tests: Prairie and Organic	Rotation	***	n.s.	***	***	n.s.	***	*

n.s. (not significant at $P < 0.05$), * ($P < 0.05$) ** ($P < 0.01$), *** ($P < 0.001$)

[1] Values shown are the average of three replicate values with ± 1 standard deviation of the average given in parenthesis.



a

b

Figure 3.1 Arbuscules (arrows) and hyphae (arrow heads) of arbuscular mycorrhizal fungi in a) flax roots under 250x magnification, and b), *Andropogon gerardii* (Vitman var.) roots of the prairie rotation under 400x magnification. Roots had been cleared with 10% KOH and then stained with Chlorazol Black E.

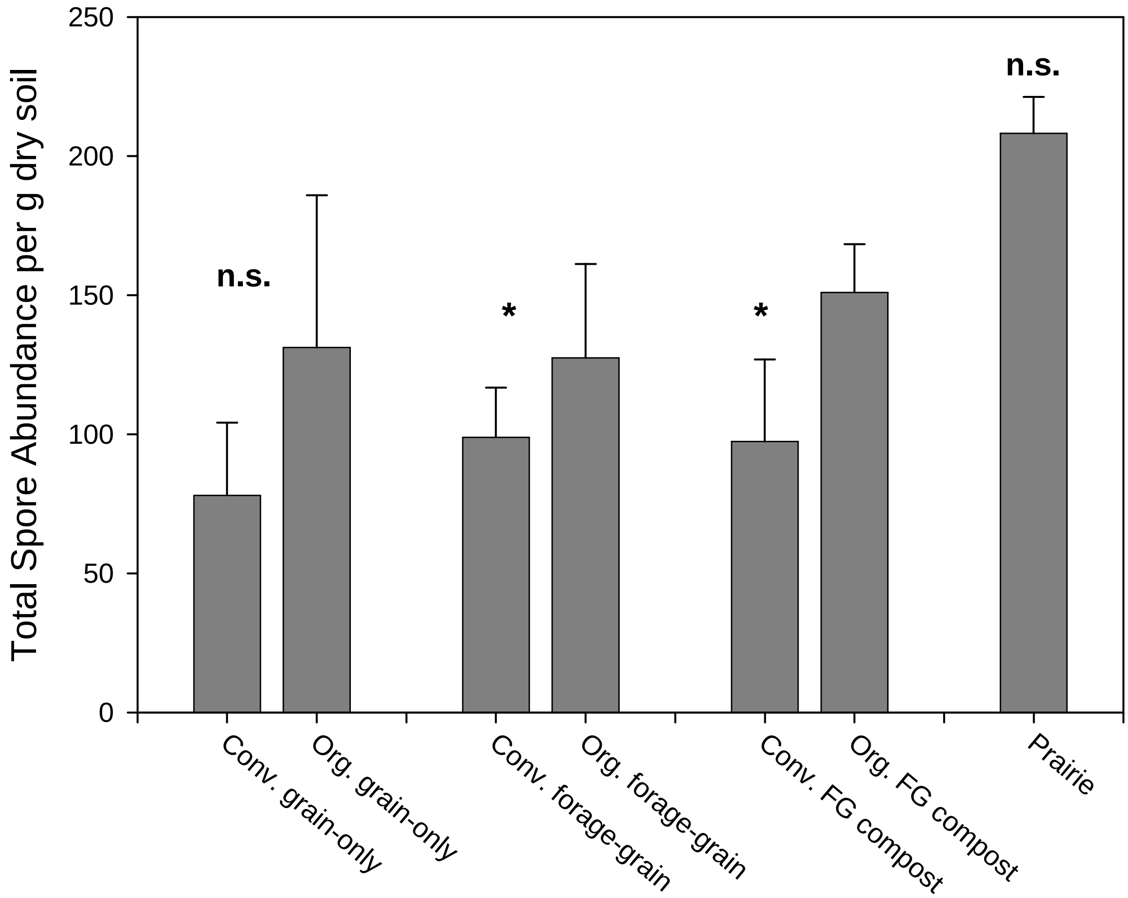


Figure 3.2 Total spore abundance per g of dry soil in different management and rotation systems at the Glenlea Long-term Rotation study. The soil was sampled on July 29 2005, and spores were isolated using the sucrose centrifugation method (Brundrett et al. 1996). Organic systems were higher in spore totals than conventional in both forage grain rotations (*) but management had no effect on spore totals (n.s.) in the grain-only rotation ($P < 0.05$). Rotation had no effect on spore totals and the prairie was not significantly higher than the organic systems ($P < 0.05$). The error bars represent +1 standard deviation of three replicate values.

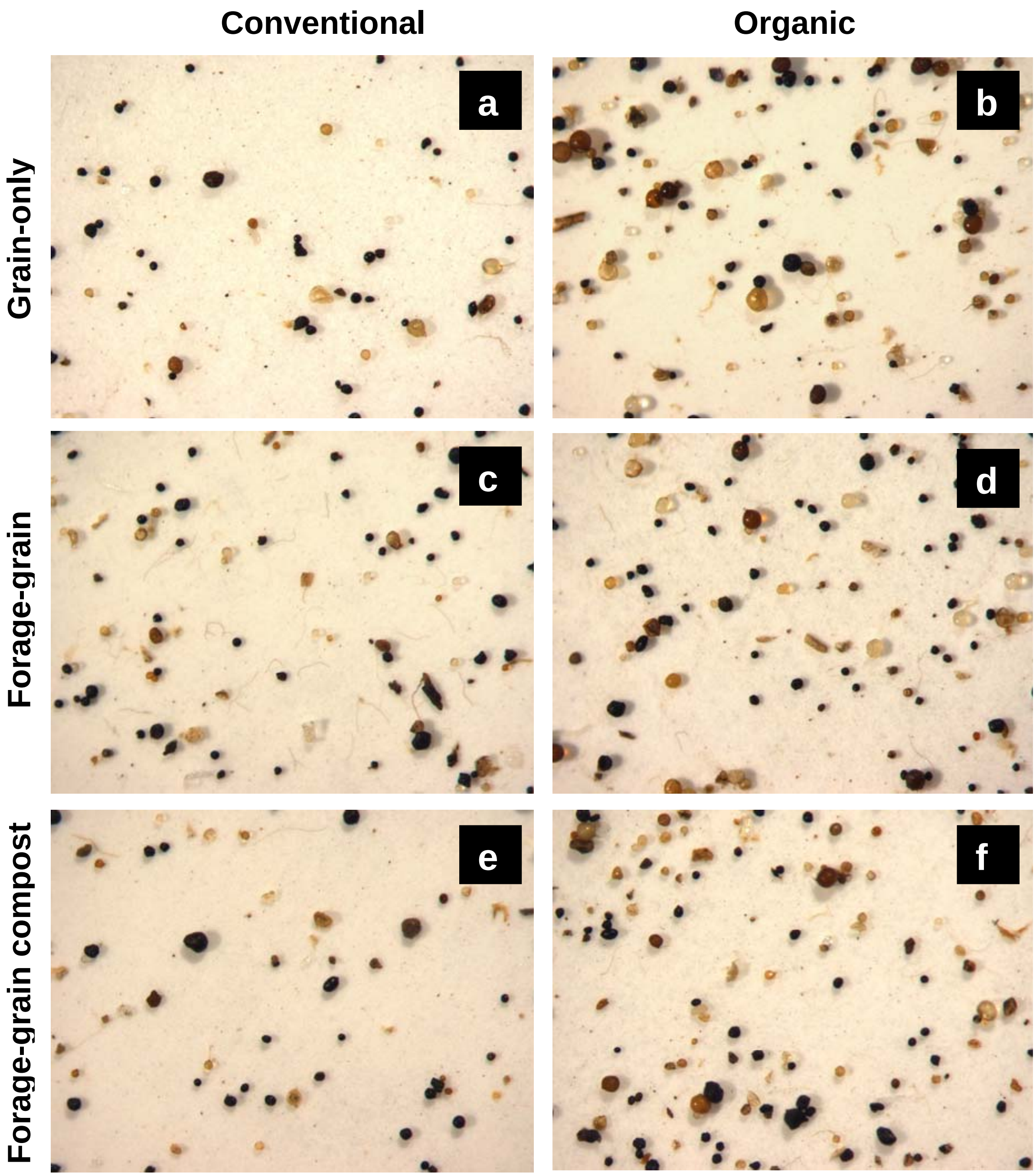


Figure 3.3 Pictures of spores on a section of filter paper isolated from 100 g of soil from each of the different treatments: a) conventional grain-only, b) organic grain-only, c) conventional forage-grain, d) organic forage-grain, e) conventional forage-grain with manure-compost, f) organic forage-grain with manure-compost. The digital camera was attached to a dissecting microscope and the pictures taken at 20x magnification. The area photographed for each picture was approximately 9.3 mm^2.

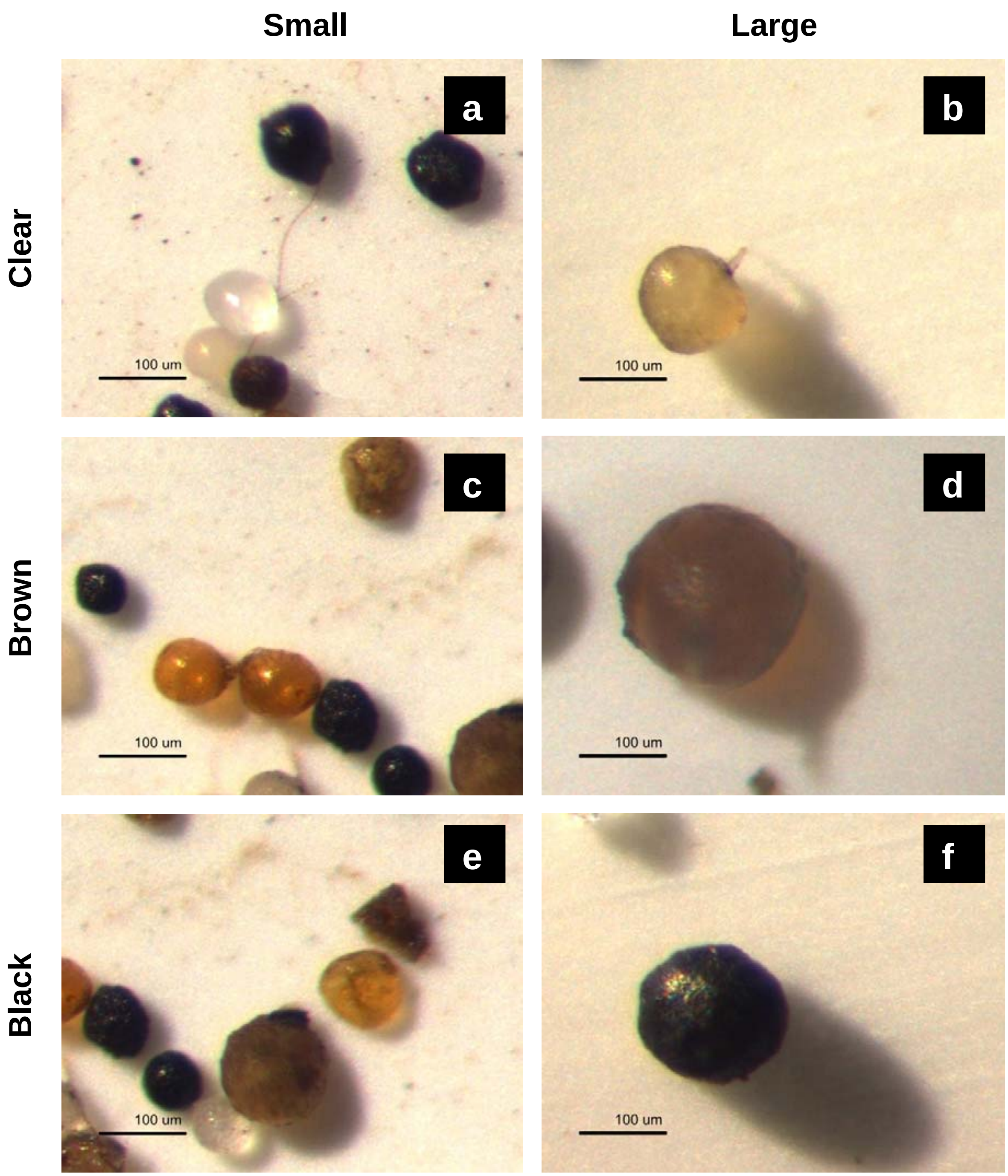


Figure 3.4 Different spore types used in diversity estimation on filter paper isolated from 100 g of soil from each of the different treatments: a) small clear, b) large clear, c) small brown, d) large brown, e) small black, f) large black. The digital camera was attached to a dissecting microscope and the pictures taken at 40x magnification. The spore of interest is the spore directly above the scale bar. The bars indicate scale being 100 µm in length.

Table 3.3 Abundance of spores in the six different operational taxonomic unit (OTU) categories (clear, brown and black spores greater or less than 110 µm) per 100 g of dry soil from the Glenlea Long-Term Crop Rotation study.

		Clear spores		Brown spores		Black spores	
Rotation	**Management**	**< 110 µm**	**>110 µm**	**< 110 µm**	**>110 µm**	**< 110 µm**	**>110 µm**
				Spores 100 g^{-1} dry soil			
Grain-only	Conventional	692 (187)[1]	35 (14)	511 (133)	3 (1)	2892 (927)	318 (135)
Grain-only	Organic	1839 (646)	56 (85)	841 (85)	81 (65)	2514 (325)	405 (215)
Forage-grain	Conventional	1195 (139)	35 (30)	1139 (735)	63 (50)	3586 (488)	439 (112)
Forage-grain	Organic	1030 (321)	75 (84)	502 (195)	112 (37)	4667 (1208)	587 (322)
Forage-grain compost	Conventional	913 (589)	32 (50)	786 (506)	77 (19)	3809 (1254)	359 (102)
Forage-grain compost	Organic	794 (272)	26 (26)	1244 (693)	76 (76)	4632 (1312)	546 (352)
Prairie	Prairie	1465 (303)	15 (13)	517 (177)	72 (15)	4408 (902)	1493 (523)

[1] Values given are the average of three replicate values with ± 1 standard deviation of the average in parenthesis.

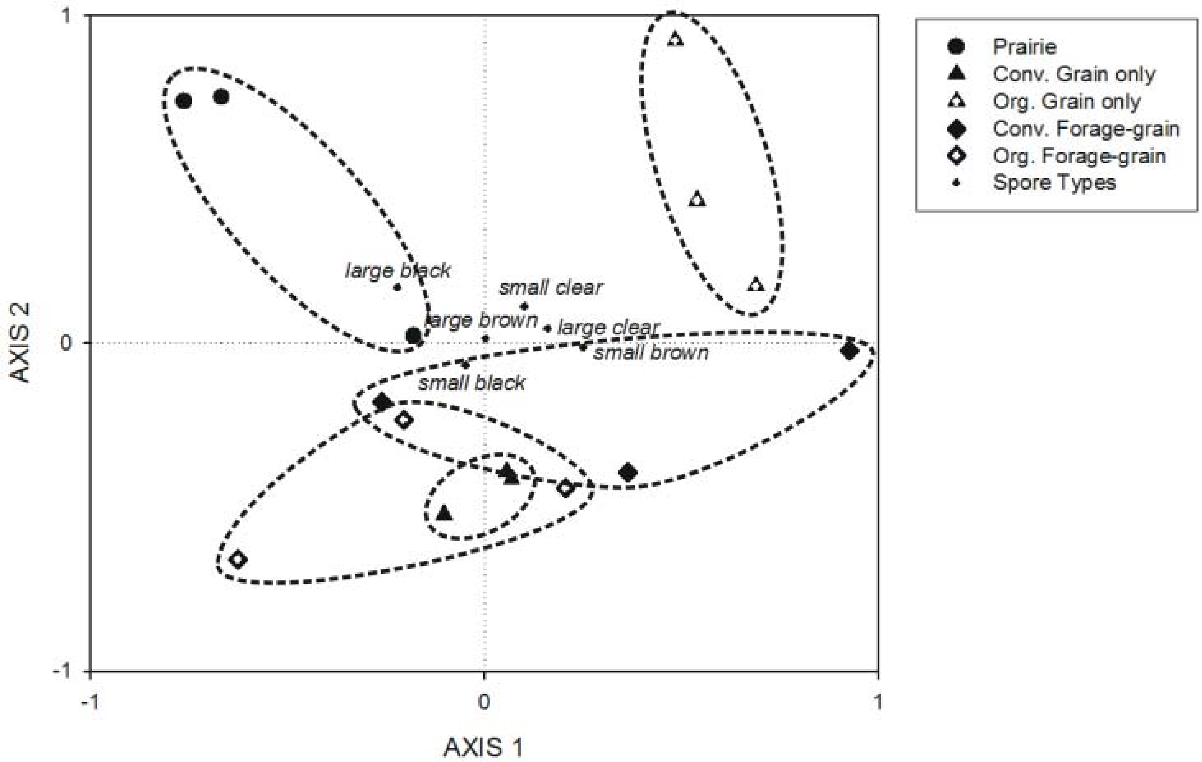


Figure 3.5 Ordination biplot results of correspondence analysis of spore abundances in the different treatments. Analysis was done using CANOCO Software Program (Biometris, Plant Research International 2004). Symbols correspond to the different treatments and each treatment has three replicates. The distance of the spore type from the origin represents how great an influence the spore has on the orientation of the points on the two dimensional plot.

3.5 Discussion

The purpose of this study was to determine whether arbuscular colonization is increased in low phosphorus organic systems leading to an increase of phosphorus absorption in plant tissue. The spore abundance data were used to support the hypothesis that organic management has a vital influence on AMF types in soil. In addition, AMF diversity was analyzed to determine the influence of crop management on AMF community structure. By combining these results with phosphorus uptake, it was possible to assess the importance of AMF communities in increasing phosphorus absorption of crops in low phosphorus organic systems at the Glenlea Long-term Rotation study.

3.5.1 Arbuscular and Hyphal Colonization

Organic management increased arbuscular colonization whereas hyphal colonization was not altered. Hyphae are only the means by which nutrients and water are moved from the soil throughout different parts of the fungi; arbuscules transfer these nutrients (in particular phosphorus) directly to the plant cells (Marx et al. 1982). The importance of arbuscules for exchange of nutrients between AMF and plants is based on evidence of high ATP-ase activity in the arbuscules indicating active transport mechanisms, in addition to their large surface area available for exchange of nutrients. The colonization levels for both hyphal and arbuscular colonization in the Glenlea Long-term Rotation Study are lower than other published data (Dekkers and van der Werff 2001) but are similar to levels found in maize by McGonigle and Miller (1993) that were sampled at approximately the same period after planting as in our flax study. The difference between colonization levels in organic and conventional systems may be slightly underestimated given the slower morphological development of flax crops in the organic systems. The flax in the conventional systems would have been colonized earlier in this case and had more time to develop, yet colonization was still higher in organic systems.

It is important to also look at colonization density of arbuscules and hyphae as this measurement is more related to the cost to benefit ratio of colonization (Brundrett 1996). Interestingly, when percent colonization is converted to colonization density the differences in colonization caused by treatment are no longer apparent. A similar observation was also made by Dekkers and van der Werff (2001). These colonization differences vanish on a land area basis when accounting for flax root length density because the positive effect of phosphorus on increasing root length may be larger than the effect of reduced phosphorus levels on colonization. Amijee et al. (1989) observed this effect at low additions of phosphorus when root length increased in response to the phosphorus but the added phosphorus was not enough to inhibit colonization. The larger variance in root length density in organic systems may also decrease the differences in colonization density between management systems.

For crops that are highly dependent on colonization of AMF for phosphorus uptake, such as flax, the colonization of AMF increases the amount of phosphorus absorbed compensating for a reduced root system and can be of great benefit to the plant when phosphorus is at low levels in the soil. Thingstrup et al. (1998) found flax to be highly dependent on AMF colonization when soil phosphorus levels were below 40 mg kg^{-1} (Olsen phosphorus). The sodium bicarbonate ($NaHCO_3$) extractable phosphorus in soil of the organic forage-grain rotation at Glenlea was around 40 mg kg^{-1} in 2004 (Table 2.3) and $NaHCO_3$-phosphorus from sequential extractions tends to estimate available phosphorus concentrations that are 2.5 times that of Olsen phosphorus (Kleinman et al. 2001). This low plant available phosphorus concentration indicates that organic flax crops were relying heavily on AMF for uptake of phosphorus. The sodium bicarbonate extractable phosphorus in the grain-only soil was around 60 mg kg^{-1} in 2004 (Table 2.3) indicating these flax crops were not benefiting from the mycorrhizal colonization to the same extent as the forage-grain crops. This high phosphorus in the soil combined with high colonization of AMF in the grain-only rotation suggests that the benefit of colonization may be out weighted by the cost. It is possible that the AMF are actually parasitizing the flax rather than benefiting them, although the benefit to cost ratio does not suggest that parasitism existed in this case. The arbuscules are the benefit in this relationship because they provide the location for nutrient exchange, and the AMF biomass or total colonization represents the drain on carbon from the plant to support fungal growth (Dekkers and van der Werff 2001). Because AMF may also assist in nitrogen uptake in plants (Smith and Read 1997), it is possible that the AMF colonization was increased in the grain-only rotation in the Glenlea study to assist in nitrogen acquisition. The benefit of AMF colonization in soils that are more nitrogen limited than phosphorus has also been observed by Monz et al. (1994) who found AMF colonization to be negatively correlated to nitrogen concentration of the shoot.

The increase in colonization observed in the organic grain-only rotation may also have been a result of an abundance of mycorrhizal weeds. The colonization of crops can be influenced by the presence of both mycorrhizal and nonmycorrhizal weeds (Gosling et al. 2006). Entz et al. (2004) found an abundance of green foxtail (*Setaria viridis*), a mycorrhizal weed, in the grain-only rotations and wild mustard (*Brassica kaber*), a nonmycorrhizal weed, in the forage-grain rotations in 2002. The presence of nonmycorrhizal weeds in a rotation may have a similar effect to that of nonmycorrhizal plants and summer-fallow in reducing

AMF colonization of crops in that rotation. In contrast, mycorrhizal weeds may increase colonization when grown in the presence of a host crop (Harrier and Watson 2003). The abundance of AMF inoculum in the soil due to the increase in host plants with the presence of the green foxtail could lead to an increase in colonization of flax crops in the grain-only rotation compared to the forage-grain rotation.

3.5.2 Nutrients in Soil and Flax Plant Material

The 14 years of organic management at Glenlea reduced plant available phosphorus concentrations in both the grain-only and forage-grain rotations. It is common to see lower levels of available phosphorus in organic systems than conventional systems (Gosling and Shepherd 2005; Entz et al. 2001). In addition to management system, certain crop rotations can also reduce available phosphorus. There was a reduction of available phosphorus in the forage-grain rotation due to the high removal of plant matter as forage compared with the grain-only rotation as demonstrated in section 2.4.4. The restored tall grass prairie had available phosphorus levels similar to those of conventional systems due to conservation of initial phosphorus levels through nutrient cycling.

Both the yield and phosphorus concentration of flax in the grain-only rotation did not reflect available phosphorus levels in the soil in 2005. The yields in the grain-only rotation were much lower than the forage-grain yields even though the available phosphorus was higher in the grain-only rotation. Furthermore, the phosphorus concentration in organic flax grain and tissue of the grain-only rotation was higher, though not significantly higher than the conventional flax grain and tissue. It appears that nitrogen, not phosphorus and weeds, was not the limiting nutrient in the grain-only rotations that lead to the decline in yields. Even though soil nitrate concentrations were not significantly different, nitrogen concentration was lower in the flax tissue at four weeks after emergence in the grain-only rotation possibly due to a difficulty in absorbing this nutrient from the soil. Vitousek (1982) stated that when annual crops are grown in nutrient limiting soils they favour slow growth and increased uptake of available nutrients. It is possible that the increase in phosphorus concentration was a compensation for the loss of nitrogen.

The uptake of phosphorus in excess of current plant needs as occurred in the grain-only rotation indicates a decrease in phosphorus use efficiency, calculated as yield per unit of phosphorus uptake. The 'luxury consumption' of phosphorus seen in the plant material may, however, be beneficial to serve as storage when phosphorus reserves are low (Koide 1991). The phosphorus concentrations in the soil solution are highly variable throughout the growing season, in particular organic phosphorus which fluctuates with changes in microbial biomass (Stewart and Tiessen 1987). It is therefore important to view levels of phosphorus in the soil solution as a snapshot of plant available phosphorus and not the absolute concentration throughout the year.

The increase in phosphorus concentration in the organic grain-only rotations was not explained by an increase in root growth because surface flax-root length density was actually lower in the organic systems than the conventional four weeks after emergence. The flax plants in organic systems were smaller at the time of sampling and therefore the root biomass was lower. Dekkers and van der Werff (2001) also found that phosphorus level had a positive effect on root length density. The total root-length density, which included weeds sampled, was not significantly different between organic and conventional systems. This lack of difference suggests that more weeds were found in the organic than the conventional systems and is consistent with the findings of Entz et al. (2004) for the Glenlea long-term crop rotation.

3.5.3 Composted cattle manure

The addition of manure-compost to the organic forage-grain rotation did not significantly reduce arbuscular colonization (*P* < 0.05) from the organic forage-grain without manure-compost rotation. The addition of phosphorus fertilizer in the form of manure-compost, depending on the rate, does not normally reduce mycorrhizal colonization as much as conventional fertilizers likely due to the lower level and slower release of phosphorus in plant available forms (Joner 2000; Harinikumar and Bagyaraj 1989). The manure-compost addition may also have a beneficial effect on mycorrhizal colonization indirectly due to the increase in organic matter which improves various aspects of soil structure (Harinikumar and Bagyaraj 1989). Gryndler et al. (2005) discovered humic substances found in organic matter stimulated growth of mycelium and mycorrhiza formation in the AMF species *Glomus claroideum* BEG 23, possibly because of improvement in nutrition in the soil provided by the humic substances.

The effects of manure-compost on AMF colonization are mainly dependent on available phosphorus concentration provided by the manure-compost. When manures that are high in available phosphorus, such as chicken manure, are added to the soil these often have inhibitory effects on mycorrhizal colonization (Gosling et al. 2006). The amount of available phosphorus (modified Kelowna) was not increased in the soil with the addition of manure-compost compared with the organic forage-grain without manure-compost rotation in the Glenlea study; therefore it is likely that the amount of available phosphorus in the manure-compost was low. The flax in the organic forage-grain with manure-compost rotation was likely still benefiting from the association of AMF and this was also indicated by the benefit to cost ratio which was similar to that of the grain-only rotation. The conventional forage-grain with manure-compost rotation received both conventional phosphorus fertilizers and manure-compost and was therefore high in phosphorus and low in arbuscular colonization and benefit-to-cost ratio.

3.5.4 Spore Populations and Diversity

The increases in spore populations were correlated to increases in total mycorrhizal colonization. This type of correlation is often the case with annual plants during the growing season because spores are the propagules of AMF and will increase when the mycorrhizal community is healthy and productive (Smith and Read 1997). This relationship has also been observed in reduced tillage systems due to the greater opportunity for the establishment of hyphal networks (Galvez et al. 2001). The lower available phosphorus in organic systems was likely the greatest contributor to the larger density of spores. In a greenhouse experiment, Douds and Schenck (1990) observed decreased sporulation of different AMF species in the presence of high soil phosphorus. In the field, AMF spore populations of different species were found to be lower under conventional than low input farming but this may have been due to higher plant diversity in addition to lower phosphorus levels in organic systems (Douds et al. 1993).

The average population of the spores per gram of soil in the Glenlea study was relatively high compared to other studies (Smith and Read 1997). However, the spores were collected five weeks after planting rather than at the end of the season when spore populations normally reach a maximum. It is possible that many of the spores counted were produced the previous fall (2004). During the growing season, the AMF propagules are likely mycelia rather than spores; and during fall senescence of crops, spores become the AMF propagules (An et al. 1993). The life cycle of mycorrhizae are highly related to the life cycle of their host plants and therefore the maximum spore production will depend on the growth stage of the plants (An et al. 1993; Douds and Chaney 1982).

Groupings of plots into treatments were observed with the spore data in the correspondence analysis biplot suggesting that a shift in AMF community structure occurred with different management systems. However, this grouping was only observed for the prairie, organic grain-only and conventional grain-only treatments and not the forage-grain treatments. A separation on the biplot of the native prairie from all other management systems was expected since the management of the prairie was profoundly different from the other systems. A shift in diversity between conventional and organic systems was also expected, which was observed in the grain-only rotation but not in the forage-grain rotation. However, differences in plant available phosphorus levels in the soil were more extreme between organic and conventional management in forage-grain rotations than grain-only, indicating the shift in diversity between management systems in the grain-only rotation may have been due to selection of AMF species by crop plants rather than a shift in diversity caused by phosphorus limitation. Crop rotation therefore may have had more of an impact on AMF diversity than management system.

The changes in AMF spore diversity in the Glenlea study are interesting, but a more thorough look into spore identification is required to determine how an optimum AMF community structure can be selected by a well designed crop management system. Many other studies have shown different cropping systems affect AMF community structure and spore diversity. Typically, high input agriculture systems, like the conventionally managed systems at Glenlea, have a lower AMF diversity than more natural systems (Oehl et al. 2004, 2003; Douds and Millner 1999; Helgason et al. 1998; Kucey and Paul 1983). Organic systems tend to have higher AMF diversity due to lower phosphorus in the soil, higher weed populations, and characteristically long crop rotations that increase host diversity and consequently AMF diversity (Oehl et al. 2004). A more diverse AMF community is important because mycorrhizal species differ in benefits to their host plants such as plant growth stimulation and phosphorus uptake efficiency (Van der Heijden et al. 1998). Not only do intense, high input systems reduce AMF diversity, they have also been known to increase the dominance of certain, less beneficial AMF species (Oehl et al. 2004; Kucey and Paul 1983). High input monoculture systems tend to select for fast growing, inefficient mycorrhizal symbionts that have little benefit to their host plants and will likely become parasitic leading to poorer host growth (Johnson 1993). In the Glenlea study, the correspondence analysis biplot indicated a shift in AMF diversity was driven more by rotation system than by phosphorus limitations and management system. More investigations are needed to determine what factors lead to changes in AMF community structure at the Glenlea Long-term Rotation study.

3.5.5 Conclusions

AMF were found to be responsive to lower phosphorus in organic systems in this study. It is difficult to determine the extent of AMF contribution to increased phosphorus uptake in crops without a closer examination of phosphorus movement (i.e. tracing radioactive labelled phosphorus from fungus to plant). However, arbuscular colonization was higher in organic compared with conventional systems and even though organic systems were lower in available phosphorus, phosphorus concentration in the plant tissue and grain tissue was not lower in organic systems. The dilution of nutrients in the conventional systems due to larger biomass in addition to colonization of AMF in organic systems was likely the reason for the high phosphorus in organic flax tissue and grain.

The benefit to cost ratio of mycorrhizal colonization suggested colonization was a beneficial association in most of the systems. Because nitrogen rather than phosphorus was limited in the grain-only rotation, the high AMF colonization could be a means to increase nitrogen absorption in the crops through the extraradical hyphae and extension of the plant root system. Spore populations were higher in organic systems suggesting that the AMF community was thriving more in organic than the conventional systems. The groupings of plots into treatments and the separation of these treatments in the correspondence analysis biplot indicated a shift in AMF community structure likely occurred between the prairie and the rest of the treatments and between the organic and conventional grain-only rotations; however, this shift may have been more related to selection by crops than phosphorus limitation.

3.6 Acknowledgements

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4. GENERAL DISCUSSION AND CONCLUSIONS

The Glenlea Long-term Rotation study was designed as an attempt to answer some questions pertaining to potential problems with organic farming. First, there is the problem of supplying adequate phosphorus to organic systems, which raises the question to what rate are phosphorus reserves being depleted? Another important question being does organic farming lead to a general depletion of reserves or rather a decline in only plant available fractions? This leads into another question and that is if the decline of phosphorus in organic systems is in only plant available fractions, are AMF able to assist crops in absorbing phosphorus in soils low in labile phosphorus while unavailable reserves are slowly made available? Finally, do different management or rotation systems select for certain species of AMF making it possible to design crop rotations to select for AMF communities that are highly efficient in transferring phosphorus to crops? These questions will be discussed further in light of the results of the Glenlea Long-term Rotation Study.

4.1 Phosphorus Fractionation

The decline in plant available phosphorus in organic systems was confirmed in the Glenlea study with both the results from the modified Kelowna phosphorus extraction and the sequential phosphorus extraction procedure (modified Hedley). The pools that were reduced according to the sequential extraction procedure were the labile phosphorus (water and sodium bicarbonate-extractable) as well as the moderately labile phosphorus pool (sodium hydroxide-extractable). The moderately labile phosphorus pool has been known to act as both a sink and a source when phosphorus is either high or low, respectively (Zheng et al. 2003b; Guo et al. 2000; Zhang and MacKenzie 1997). The ability of this pool to be used by plants or act as a sink may be the reason why the conventional system had much higher phosphorus than the organic system in the sodium hydroxide-extractable phosphorus pool.

The low concentration of labile phosphorus in the organic systems (particularly the forage-grain rotation) can be a problem for agriculture but is a benefit to the environment. Agricultural runoff is a contributor to eutrophication of rivers and lakes, and phosphorus in particular leads to rapid growth in blue-green algae (Sharpley et al. 1994). Applying fertilizer to increase plant available phosphorus is difficult in soils with high clay content, such as the soil in Glenlea, as phosphorus tends to be adsorbed quickly on clay surfaces or precipitated as dicalcium phosphate and is then in unavailable forms (Flaten et al. 2003). Increasing the application rate of fertilizer increases plant available phosphorus but also leads to a greater risk of runoff losses and therefore eutrophication. Organic systems such as the organic forage-grain system at Glenlea, when managed properly, may avoid problems of conventional systems by utilizing AMF association to increase phosphorus absorption in low phosphorus environments.

Unlike the labile phosphorus pools, the recalcitrant phosphorus did not differ between organic and conventional systems in the Glenlea study. The resistance of the recalcitrant phosphorus pool has been confirmed in other studies where neither crop rotation nor fertilizer addition had any effect on changing this pool (Zheng et al. 2003a; 2003b). However, these phosphorus pools are known to be sparingly soluble and can be made available through cycling and the action of roots when labile phosphorus pools are low (Guo et al. 2000; Hedley et al. 1982). Another way to mobilize the recalcitrant phosphorus pool is to include phosphorus mobilizing crops such as buckwheat, with the ability to decrease the rhizosphere pH by secreting H^+ ions which release phosphorus bound to calcium ions (Zhu et al. 2002; Hedley et al. 1982). This suggests that in organic systems there still exists a large pool of phosphorus that is unavailable to crops in its current form, but may eventually become available through nutrient cycling in the soil.

What was missing from the sequential fractionation procedure in the Glenlea study was a separation of the pools into organic and inorganic phosphorus. The differences in the organic phosphorus fraction between management systems in particular would have been interesting to measure due to speculation that organic systems have high phosphorus in organic fractions. Long-term conventional management tends to deplete organic phosphorus fractions as compared to native sites (Daroub et al. 2001; Hedley et al. 1982). However, there is little evidence that the organic phosphorus can be increased by adopting different management systems (Zheng et al. 2003b; Daroub et al. 2001; Oberson et al. 1993). Zheng et al. (2003b) found organic phosphorus was more related to soil texture; specifically, heavy clay soils that had high levels of sodium hydroxide (NaOH) extractable organic phosphorus because this pool contains phosphorus bound to humic and fulvic acids which tend to be adsorbed on clay surfaces. Daroub et al. (2001) determined that organic phosphorus was not increased with adoption of an organic management system even after seven years as compared with conventional practices. In their 13-year study comparing biodynamic, bio-organic and conventional treatments, Oberson et al. (1993) did not find an increase in organic phosphorus, however they did observe temporal fluctuations in sodium bicarbonate extractable organic phosphorus likely resulting from mineralization processes in the microbial biomass. Therefore, had the organic phosphorus pool been measured in the Glenlea study, the sodium hydroxide and hydrochloric acid organic phosphorus fractions may have been high in both the organic rotations and conventional rotations at Glenlea. There may also have been higher microbially-bound phosphorus in the organic rotations, which can provide available phosphorus sporadically throughout the growing season through mineralization processes.

The extraction of phosphorus by chemical means is a very useful method for determining the amounts of phosphorus in labile and recalcitrant fractions, but may not reflect the phytoavailability of phosphorus. There are many factors which can affect the availability of phosphorus to plants that must be taken into account when analyzing phosphorus pools determined by sequential extraction. These factors include the interaction of phosphorus with organic and inorganic soil parts, rates of transfer between labile phosphorus into stable inorganic phosphorus, mobilization capabilities of certain crops, and climatic variation (McKenzie and Bremer 2003). Colonization by AMF can also increase the ability of plants to absorb phosphorus and must therefore also be taken into consideration when assessing phosphorus availability (Grant et al. 2005). A more accurate estimation of the phytoavailability of phosphorus would therefore include these factors to correlate with plant uptake of phosphorus.

4.2 Cumulative Phosphorus Balance

According to the calculated cumulative phosphorus balance, the phosphorus additions in conventional rotations were in excess of plant uptake producing a surplus of phosphorus in the system, while in the organic systems a phosphorus deficit occurred because the phosphorus removed in crop harvests was not replaced in the organic system. The forage-grain rotation had a larger deficit than the grain-only rotation which was consistent with the lower levels of phosphorus in labile pools and also with the higher removal of nutrients in the two year alfalfa stand. The large deficit in the organic forage grain system would indicate that this system is not sustainable; however, crop yields are still being maintained in these systems even when no phosphorus has been replaced. In an economic analysis of the Glenlea study, Entz et al. (2004) discovered that the organic forage-grain rotation had the highest net return and the lowest input cost of crop production. This study indicated that even though yields were smaller in the organic forage-grain rotation, the net income was actually higher than that of conventional due to reduction of inputs. It is therefore important to look at more than just yield when comparing the performances of organic and conventional systems.

It is possible that if the soil in Glenlea been low in phosphorus before implementation of the study, the depletion of phosphorus reserves in the organic systems may have been accelerated and a large reduction in crop yields would have been observed in this study. Nevertheless, the phosphorus fractionation procedure indicated that a large pool of phosphorus in recalcitrant forms existed in the organic systems, seemingly unaffected by cropping system. This pool can be made available through cycling and the action of roots when available phosphorus is low (Guo et al. 2000; Hedley et al. 1982) and may have been able to sustain the organic cropping systems even in a soil that was initially low in phosphorus. However, a reduction in the recalcitrant pool was not observed in organic systems of the Glenlea study. A follow-up will have to be done for the Glenlea Long-term study to determine how long the organic systems can be sustained on current phosphorus concentrations.

By using published values for phosphorus concentration in harvested crop material for cumulative phosphorus balance calculations, some information about the removal of phosphorus from crop harvests may have been missed. This deduction is based on the large variation in measured phosphorus concentrations of flax grain among treatments and the inconsistency in soil phosphorus concentrations. It still remains unclear why the phosphorus concentration in the organic flax grain of the grain-only rotation was much larger than the phosphorus in the conventional flax grain. The available phosphorus in the soil was not different between management systems in this rotation; therefore the large variation in phosphorus uptake must be due to other factors. The explanation must lie with either the activity of roots increasing uptake of phosphorus in the plant or interaction with the microbial population including the association of mycorrhizal fungi in the organic system. Nitrogen limitation may also have been a factor in the increased uptake of phosphorus. Vitousek (1982) stated that when annual crops are grown in nutrient limiting soils they favour slow growth and increased uptake of available nutrients. It is possible that the increase in phosphorus concentration observed in the Glenlea study was a compensation for the loss of nitrogen.

The main reason why the phosphorus was highest in the organic grain-only rotation was likely due to the dilution effect that occurs with larger yields. The conventional yields were larger than organic and therefore nutrients were spread over a greater biomass. The organic grain-only rotation had yields consistently lower than any other rotation, therefore nutrients could be concentrated in the smaller biomass. More research is needed to understand phosphorus uptake by plants, why it is increased in certain situations and whether organic and conventional crops differ significantly in the uptake of nutrients. It would be prudent to do a study on phosphorus concentration in harvested plant material under different yields and soil nutrient concentrations to determine how much phosphorus concentration may vary under these conditions.

4.3 AMF Colonization and Diversity

The increased colonization of arbuscules in the flax roots of organic systems at Glenlea as opposed to conventional is an indication of higher rate of nutrient exchange between plant and fungus. As mentioned above, this could be a reason for the discrepancy of plant concentration of phosphorus and concentration of phosphorus in the soil. There was no sign of phosphorus deficiency in the flax grain of the forage-grain rotation yet the available phosphorus in the soil was quite significantly reduced from the conventional systems. Again, this supports the claim that with the colonization of AMF organic cropping systems can be sustained on low phosphorus soils while recalcitrant phosphorus reserves are slowly being cycled into available pools.

The benefit to cost ratio calculated for AMF colonization was higher in all treatments than published values (Dekkers and van der Werff 2001) indicating in all situations the association likely had some benefit to the crop. The lowest ratio occurred in the conventional forage-grain rotation with added manure-compost, even though the highest phosphorus occurred in the conventional grain-only rotation. The benefit to cost ratio was expected to be lower in crops grown on high phosphorus soils because plants do not benefit from the increase in phosphorus provided by exchange through arbuscules. In this case, the high arbuscular colonization in the grain-only rotations may have been a means for the plant to increase the uptake of nitrogen from the soil (Monz et al. 1994). It may be necessary to further investigate the benefits that AMF colonization has on increasing uptake of nitrogen in low nitrogen systems.

AMF spore abundance was increased in organic systems at the Glenlea Long-term study. This increase along with the increase in arbuscular colonization in the organic systems supports that AMF activity was higher in organic systems than conventional. It is interesting to note that even though conventional crops were larger at the time of spore collection, which would also mean AMF had more time to colonize the root and form arbuscules, there was still a higher amount of spores in the organic systems. It is likely that the differences in spore abundance may have been underestimated between organic and conventional systems. Many of the spores isolated from the soil may have actually been the spores produced from the fall of 2004 since the spores were collected in the middle of the growing season rather than at the end when spores are more abundant (Douds and Millner 1999).

The correspondence analysis (CA) biplot revealed a shift in AMF community structure occurred with different management systems. However, this grouping was observed for only the prairie, organic grain-only and conventional grain-only treatments and not the forage-grain treatments. This result is interesting because the differences in plant available phosphorus levels in the soil were more extreme between organic and conventional management in forage-grain rotations than grain-only. If bioavailable phosphorus concentration in the soil determined the AMF species composition then the shift in diversity would have occurred between management systems in the forage-grain rotation rather than the grain-only rotation. In this case, the difference in AMF community structure may have been due to selection of AMF species by crop plants rather than a shift in diversity caused by phosphorus limitation.

The use of molecular techniques for AMF species identification would be a more accurate method to define AMF communities in the Glenlea study. Molecular techniques allow the identification of the active AMF community colonizing the roots at a given time. One of the first studies to use molecular methods to show that land use intensity decreased AMF species diversity as compared to native lands was Helgason et al. (1998), who used differences in restriction-fragment length polymorphism (RFLP) to determine species composition. However, many species were missed in this study using RFLPs because the authors did not include some of the more atypical fungi in the order Glomales (Redecker et al. 2000). In other studies polymerase chain reaction (PCR) primers have been used to detect species of AMF in five major phylogenetic subgroups and seven genera of Glomeromycota (Hijri et al. 2006; Redecker 2000). Hijri et al. (2006) concluded that low input management systems with good crop rotations can preserve AMF diversity, whereas high input agriculture leads to selection of few inefficient AMF species. They attributed the low diversity in the high input systems mainly to the lack of crop rotation, but phosphorus concentration may have also played a role.

The next logical step for the Glenlea Long-term Rotation study is to identify AMF communities to species level using PCR primers. This tool will make it possible to determine how species composition changes with management systems and with different rotations. Ultimately, the goal will be to identify the efficient species of AMF in certain crop rotations to allow application in the field. When converting to organic systems from conventional intensive crop production, population of indigenous mycorrhizae are low and not optimal for symbiosis. These organic systems have a high potential to benefit from the inoculation of efficient AMF communities especially during the first phases in conversion.

4.4 Conclusions and Recommendations

Does organic farming lead to a general depletion of reserves or rather only a decline in plant available fractions?

- Using Glenlea as a model, organic systems reduce plant available phosphorus where exported phosphorus is not replaced especially in high yielding hay export systems.
- There is no immediate crisis in long-term organic farming when phosphorus is initially high in the soil since a large pool of phosphorus is still present in recalcitrant forms.
- After 14 years of organic management, the soil in organic forage-grain rotations has become phosphorus limited whereas organic grain-only rotations will likely not develop phosphorus limitation for another 6 years.
- Nitrogen rather than phosphorus limitation was the likely reason for lower yields in the organic grain-only rotation compared to conventional.

Recommendations:

- Add sufficient rates and/or frequencies of manure-compost to replace phosphorus removed by crops.
- Use phosphorus solubilizing crops such as buckwheat in organic crop rotations and inoculations of phosphorus solubilizing bacteria to mobilize recalcitrant pools.
- Include nitrogen fixing crops in rotations to maintain nitrate concentration in the soil.

Are AMF able to assist crops in absorbing phosphorus in soils low in labile phosphorus while unavailable reserves are slowly cycled into available pools?

- The increased colonization of arbuscules in the flax roots of organic systems compared with conventional at Glenlea is an indication of a higher rate of nutrient exchange between plant and fungus.

- The benefit-to-cost ratios of AMF colonization were high in all treatments indicating the AMF association was benefiting the crops.
- There was no sign of phosphorus deficiency in the flax grain of the forage-grain rotation yet the available phosphorus in the soil was quite significantly reduced from the conventional systems

Recommendations:

- Organic systems should be managed to maintain AMF populations to allow increased uptake of phosphorus when available levels are low.
  - Limit inclusion of non-mycorrhizal crops in rotation
  - Reduce tillage
  - Do not use summer-fallow, instead use green fallow and cover-crops

Do different management or rotation systems select for certain species of AMF making it possible to design crop rotations to select for AMF communities that are highly efficient in transferring phosphorus to crops?

- The correspondence analysis (CA) biplot revealed a shift in AMF community structure occurred with prairie, organic grain-only and conventional grain-only treatments.
- The shift in AMF community more likely related to selection of species by crops and not phosphorus limitation.

Recommendations:

- Molecular techniques such as the use of PCR primers and sequencing would be a more accurate method of identifying AMF communities to species level.

- Ultimate goal of the AMF community identification is to isolate efficient AMF for inoculation in organic rotations, particularly when converting from conventional systems

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5. APPENDICES

Appendix I – Cumulative Phosphorus Budget

Table 5.1 Calculated cumulative (1992-2004) phosphorus balance for crop rotations and organic and conventional management systems at the Glenlea long-term study.

Year	Crop	Rotation	Management	Average yield[1]	P concentration in harvested crop[2]	Annual P removal	Annual P fertilizer addition	Cumulative P Balance
				kg ha^{-1}	g kg^{-1} dry weight		kg ha^{-1}	
1992	Wheat	Grain-only	Conventional	0.00	0.00	0.00	11.00	11.00
	Wheat	Grain-only	Organic	0.00	0.00	0.00	0.00	0.00
	Wheat	Forage-grain	Conventional	0.00	0.00	0.00	11.00	11.00
	Wheat	Forage-grain	Organic	0.00	0.00	0.00	0.00	0.00
1993	Pea	Grain-only	Conventional	409.45	6.26	2.56	11.00	19.44
	Pea	Grain-only	Organic	575.45	6.26	3.60	0.00	-3.60
	Alfalfa	Forage-grain	Conventional	3031.53	2.73	8.26	18.30	21.04
	Alfalfa	Forage-grain	Organic	3215.10	2.73	8.76	0.00	-8.76

Table 5.1 Calculated cumulative (1992-2004) phosphorus balance for crop rotations and organic and conventional management systems at the Glenlea long-term study (continued).

Year	Crop	Rotation	Management	Average yield[1]	P concentration in harvested crop[2]	Annual P removal	Annual P fertilizer addition	Cumulative annual P Balance
				kg ha^{-1}	g kg^{-1} dry weight		kg ha^{-1}	
1994	Oat	Grain-only	Conventional	2664.43	5.60	14.92	11.00	15.52
	Oat	Grain-only	Organic	2893.30	5.60	16.20	0.00	-19.80
	Alfalfa	Forage-grain	Conventional	6060.68	2.73	16.52	17.60	22.12
	Alfalfa	Forage-grain	Organic	6062.47	2.73	16.52	0.00	-25.29
1995	Flax	Grain-only	Conventional	1876.78	5.85	10.98	13.29	17.82
	Flax	Grain-only	Organic	960.90	5.85	5.62	0.00	-25.43
	Flax	Forage-grain	Conventional	1712.26	5.85	10.02	13.29	25.39
	Flax	Forage-grain	Organic	1373.26	5.85	8.04	0.00	-33.32
1996	Wheat	Grain-only	Conventional	3275.92	5.83	19.09	10.52	9.25
	Wheat	Grain-only	Organic	1122.11	5.83	6.54	0.00	-31.97
	Wheat	Forage-grain	Conventional	3513.64	5.83	20.47	10.52	15.43
	Wheat	Forage-grain	Organic	2022.07	5.83	11.78	0.00	-45.11

Table 5.1 Calculated cumulative (1992-2004) phosphorus balance for crop rotations and organic and conventional management systems at the Glenlea long-term study (continued).

Year	Crop	Rotation	Management	Average yield[1]	P concentration in harvested crop[2]	Annual P removal	Annual P fertilizer addition	Cumulative annual P Balance
				$kg\ ha^{-1}$	$g\ kg^{-1}$ dry weight		$kg\ ha^{-1}$	
1997	Pea	Grain-only	Conventional	1130.53	6.26	7.08	9.59	11.76
	Pea	Grain-only	Organic	777.17	6.26	4.87	0.00	-36.84
	Alfalfa	Forage-grain	Conventional	7889.77	2.73	21.50	17.16	11.09
	Alfalfa	Forage-grain	Organic	7052.60	2.73	19.22	0.00	-64.33
1998	Oat	Grain-only	Conventional	2105.87	5.60	11.79	25.17	25.13
	Oat	Grain-only	Organic	475.97	5.60	2.66	0.00	-39.50
	Alfalfa	Forage-grain	Conventional	5045.67	2.73	13.75	13.29	10.62
	Alfalfa	Forage-grain	Organic	3732.33	2.73	10.17	0.00	-74.50
1999	Flax	Grain-only	Conventional	1378.27	5.85	8.07	8.23	25.30
	Flax	Grain-only	Organic	605.67	5.85	3.54	0.00	-43.05
	Flax	Forage-grain	Conventional	1453.93	5.85	8.51	4.14	6.25
	Flax	Forage-grain	Organic	1378.97	5.85	8.07	0.00	-82.57

Table 5.1 Calculated cumulative (1992-2004) phosphorus balance for crop rotations and organic and conventional management systems at the Glenlea long-term study (continued).

Year	Crop	Rotation	Management	Average yield[1]	P concentration in harvested crop[2]	Annual P removal	Annual P fertilizer addition	Cumulative annual P Balance
				kg ha^{-1}	g kg^{-1} dry weight		kg ha^{-1}	
2000	Wheat	Grain-only	Conventional	2401.82	4.19	10.06	10.08	25.31
	Wheat	Grain-only	Organic	440.63	4.19	1.85	0.00	-44.89
	Wheat	Forage-grain	Conventional	2854.44	4.19	11.95	10.08	4.37
	Wheat	Forage-grain	Organic	1371.75	4.19	5.74	0.00	-88.32
2001	Pea	Grain-only	Conventional	0.00	0.00	0.00	0.00	25.31
	Pea	Grain-only	Organic	0.00	0.00	0.00	0.00	-44.89
	Alfalfa	Forage-grain	Conventional	6836.87	2.73	18.63	0.00	-14.26
	Alfalfa	Forage-grain	Organic	5155.60	2.73	14.05	0.00	-102.37
2002	Oat	Grain-only	Conventional	1918.75	4.73	9.08	11.00	27.23
	Oat	Grain-only	Organic	783.98	4.73	3.71	0.00	-48.60
	Alfalfa	Forage-grain	Conventional	5915.11	2.73	16.12	96.18	65.80
	Alfalfa	Forage-grain	Organic	3799.00	2.73	10.35	0.00	-112.72

Table 5.1 Calculated cumulative (1992-2004) phosphorus balance for crop rotations and organic and conventional management systems at the Glenlea long-term study (continued).

Year	Crop	Rotation	Management	Average yield[1]	P concentration in harvested crop[2]	Annual P removal	Annual P fertilizer addition	Cumulative annual P Balance
				kg ha^{-1}	g kg^{-1} dry weight		kg ha^{-1}	
2003	Flax	Grain-only	Conventional	1693.03	4.88	8.26	13.29	32.26
	Flax	Grain-only	Organic	231.17	4.88	1.13	0.00	-49.73
	Flax	Forage-grain	Conventional	1328.47	4.88	6.48	13.29	72.61
	Flax	Forage-grain	Organic	481.87	4.88	2.35	0.00	-115.07
2004	Wheat	Grain-only	Conventional	1281.67	4.19	5.37	5.72	32.61
	Wheat	Grain-only	Organic	553.33	4.19	2.32	0.00	-52.05
	Wheat	Forage-grain	Conventional	1871.67	4.19	7.84	5.72	70.49
	Wheat	Forage-grain	Organic	680.00	4.19	2.85	0.00	-117.92
	Wheat	FG manure	Conventional	1988.33	4.19	8.33	5.72	94.46
	Wheat	FG manure	Organic	896.67	4.19	3.76	0.00	-94.37

1 Values shown are the average of three replicates.

2 Values for phosphorus concentration in harvested crop material from the Manitoba Soil Fertility Guide, 2004.

Appendix II – Standard Operational Procedure for Working with AMF

2.1 Root Length Density

2.1.1 Materials – Refer to MSDS for more information

Root Washer (Gillison's Variety Fabrication Inc., GVF Root Washer)	
125ml Nalgene polypropylene wide mouth bottle - Autoclavable	(Fisher Cat. # 02-893A)
Fine forceps	(Fisher Cat. # 08-953C)
Fine dissecting scissors	(Fisher Cat. # 08-951-20)
Formaldehyde	(Fisher Cat. # F79-500)
Acetic Acid	(Fisher Cat. # A491-212)
Ethanol	

2.1.2 Chemical Formulae

Glacial Acetic Acid Ethanol Formaldehyde: 18:1:1

450ml (95%) Ethanol: 25ml (37%) Formaldehyde: 25ml Acetic acid

2.1.3 Root Sampling

Take four root samples per plot: two samples 1 m away from both the front and back of the plots on either side. Place a Tulip Bulb Planter (7 cm diameter) over top of a single plant and press down in the soil to a depth of 10 cm. In this way the soil extracted with the root samples will be of equal, calculable volumes and could be used to determine soil moisture and bulk density. Wash the root samples using a Root Washer. Place a handful of roots with soil in each washing cylinder at a time and allow at least 5 min per sample depending on soil texture (clays can take longer). Let wash until water coming out of the collecting sieve is clear and collect roots from sieve using forceps. Store roots in 70% ethanol in 125 mL Nalgene wide-mouthed bottles until needed.

2.1.4 Root Length Determination

The methods for root density estimation follow those outlined by Tennant (1975). Prepare a grid with square measuring 3.5cm x 3.5cm (you can alternatively use 1cm x 1cm, 2cm x 2cm etc.). Laminate the grid to prevent water damage. Place the grid under a clear lasagna dish (24.5 x 17.5 x 4.5 cm) (laminated grids can also be placed in aluminum lasagna trays of the proper dimensions). Add about 2-3cm of water to the dish. Cut the roots into 2 cm fragments and divide the sample of roots mixed with debris into as many batches as necessary to facilitate counting. Starting with the top horizontal line of the grid and scanning right to left, top to bottom, count all roots which bisect a line and record the number. Count the roots bisecting the vertical lines next by scanning top to bottom and moving right to left. By summing up the total intersections of both horizontal and vertical lines, a total number of bisecting roots can be calculated and entered into the formula for root length, *R*.

$$R = 11/14NL \qquad [1]$$

Where *L* is the length of one side of a square on the grid, and *N* is the total number of bisecting roots counted. Calculate the average root density beneath each plant by dividing the root length by the bulk volume of soil removed by the Tulip Bulb planter.

EXAMPLE

Sample 1 from Glenlea - June 1st , 2005

Batch 1

VERTICAL	HORIZONTAL
1 ---- 12	1 ---- 9

2 ---- 20	2 ---- 19
3 ---- 30	3 ---- 53
4 ---- 22	4 ---- 22
5 ---- 17	5 ---- 22
6 ---- 26	6 ---- 31
7 ---- 16	
8 ----0	
TOTAL = 143	TOTAL = 156

Batch 2

TOTAL = 150	TOTAL = 136

SUM TOTAL = 585

R = 11/14 (585) (3.5)

= 1608.75 cm

Root Density = R/soil volume

= $1608.75/(3.5)^2(10)$

= 13.13 cm mL^{-1}

After counting roots to determine root density, collect only the fine roots attached to a flax plant and place in a 125 mL Nalgene wide-mouthed bottle. Add FAA (formalin-acetic acid-alcohol fixative) just enough to cover the roots and leave for at least one week before calculating mycorrhizal colonization. This solution fixes the roots so the fungal morphological characteristics remain intact for viewing.

2.2 Mycorrhizal Colonization

2.2.1 Materials – Refer to MSDS for more information

Pyrex* Vista* Glass Culture Dishes	(Fisher Cat. # 70160-60)
100 mL Pyrex* Griffin Beakers	(Fisher Cat. # 02-540H)
BD Plastic Disosable Transfer Pipet	(Fisher Cat. # 13-669-12)
Fine forceps	(Fisher Cat. # 08-953C)
Glycerol	(Fisher Cat. # G37-4)
Potassium Hydroxide	(Fisher Cat. # P251-3)
Chlorazol Black E.	(Fisher #AC40440-0250)
Autoclave	
Light Microscope	

2.2.2 Chemical Formulae

Chlorazol Black E: (0.03% w/v in 1:1:1 lactic acid, glycerol and water)

0.45g Chlorazol Black E: 300ml Lactic acid: 300ml Glycerine: 300ml Water

Note: Chlorazol Black E. is a strong carcinogen, avoid all contact, use gloves.

10% Potassium Hydroxide:

10 g Solid Potassium Hydroxide: 100ml Water

2.2.3 Colonization Determination

The staining of AMF follows the methods of Brundrett et al. (Brundrett et al. 1996). Take a random subsample of roots from FAA for staining and rinse with deionized water three times by soaking in glass Culture Dish. Refer to MSDS for proper disposal procedure for FAA. Remove water by aspiration with disposable Transfer Pipet and add 10% Potassium Hydroxide (KOH) solution to the dishes. Pour the roots in KOH into 100 mL beakers and autoclave for 10 minutes to clear them. After clearing, rinse the root segments as before in glass Culture Dishes, then add Chlorazol Black E (0.03% w/v in 1:1:1 lactic acid, glycerol and water) solution just to cover the roots. Place the glass Culture Dishes in the oven at 90°C for 90 minutes. Aspirate Chlorazol Black E stain with disposable Transfer Pipets and dispose in properly labelled container according to MSDS regulations. Rinse the roots three times again and add glycerol to destain overnight.

The scoring of mycorrhizal colonization follows the methods of McGonigle et al. (1990). Using fine forceps, arrange roots horizontally on a slide (about 13 per slide) and squash with a cover slip. Carefully soak up excess glycerol from the area around the outside of the cover slip so as not to get residue in the viewing areas. Mount the slide under the microscope at 250x magnification and move the slide up, down and across. Using the vertical line of the cross-hair of the microscope, count when a root, hyphae or arbuscule is bisected. When both hyphae and arbuscule are intersected on the same root, only count the arbuscule. Occasionally a higher magnification may be needed to verify arbuscules and hyphae if they are unclear. Stop counting when the amount of root sections evaluated reaches 100. The percentage hyphal and arbuscular colonization per root sections counted can now be determined using the numbers of hyphae and arbuscules bisected.

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