THE EFFECT OF PULSE CROPS ON ARBUSCULAR MYCORRHIZAL FUNGI IN A DURUM-BASED CROPPING SYSTEM

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ABSTRACT

Pulses are an important component in crop rotations in the semiarid Brown soil zone of southern Saskatchewan, Canada. Besides their capability to fix nitrogen, pulse crops establish a strong symbiotic relationship with arbuscular mycorrhizal fungi (AMF), which have been shown to increase nutrient and water uptake through hyphal extensions in the soil. Incorporating strongly mycorrhizal crops in a rotation may increase inoculum levels in the soil and benefit the growth of a subsequent crop. The objective of this study was to determine if AMF potential and colonization of a durum crop is significantly affected by cropping history and to assess the impact of pulses in crop rotations on the abundance and diversity of AMF communities in the soil. In 2004 and 2005, soil, plant, and root samples were taken on *Triticum turgidum* L. (durum) with preceding crops of *Pisum sativum* L. (pea), *Lens culinaris* Medik (lentil), *Cicer arietinum* L. (chickpea), *Brassica napus* L. (canola) or *Triticum turgidum* L. (durum). Although there were few differences in soil N and P levels, previous crop had a significant effect ($p<0.05$) on durum yields in both years. A previous crop of pea was associated with the highest yields, while the durum monocultures were lowest. Arbuscular mycorrhizal potential and colonization were significantly affected ($p<0.05$) by cropping history, but not consistently as a result of inclusion of a pulse crop. Phospholipid and neutralipid fatty acids (PLFA/NLFA) were completed to analyse the relative abundance of AMF (C16:1ω5), saprophytic fungi (C18:2ω6), and bacteria in the soil. The effect of treatment on the abundance of AMF, saprotrophic fungi and bacteria were not significant ($p<0.05$), but the changes over time were. These results demonstrate that although previous crop may play a role in microbial community structure, it is not the only influencing factor.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

Pulses are an important component in crop rotations in southern Saskatchewan. Conservation tillage in the brown soil zone of Saskatchewan typically includes a combination of cereals, pulses, and oilseeds in the rotation cycle. The benefits of pulse crops in a rotation are not completely understood. Known benefits of pulse crops are mostly attributed to their ability to fix N, but the non-N benefits are not well understood . Besides their capability to fix nitrogen, pulse crops establish a symbiotic relationship with arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi help the plants take up nutrients and water, while the plant supplies the fungi with carbon. Arbuscular mycorrhizal fungi can increase uptake through hyphal extensions in the soil. The fungi work by colonizing the root and then extending hyphae out into the soil. Hyphae have the ability to increase soil-root contact, increase exploration in micropores, extract water, and improve water holding capacity . The extraradical hyphae provide contact between the AMF and the host, thereby contributing to nutrient uptake in the soil . The fungal hyphae have the ability to penetrate soil pores that are inaccessible to non-mycorrhizal roots . Since water, N and P are often limiting factors for plant growth on the Brown Chernozemic soils in the semiarid region of Saskatchewan, increased levels of AMF in the soil may decrease fertilization and disease management costs. Increased water infiltration, decreased soil erosion, and better soil aeration stemming from improved soil aggregation are other AMF-derived benefits.

Based on previous studies, it was hypothesized that pulse crops would increase the biodiversity and abundance of AMF communities under a subsequent durum crop. A corollary to this hypothesis was that there will be an increase in N and P uptake by the plant. The objectives of the study were to analyse the impact that pulse crops have on the nutrient pools of N and P under a subsequent durum crop, as well as to determine changes in the microbial communities.

2. LITERATURE REVIEW

2.1 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with four out of five terrestrial plants. The AMF extend hyphae into the soil, which increase nutrient and water uptake through soil exploration. The benefits associated with AMF are widely reported, but because the fungi have a low level of host specificity it has been difficult to identify and evaluate their function in different ecosystems . Mycorrhizal colonization commences with signals between the host and the fungi. Spore germination and activation of hyphal growth are influenced by chemical signals conducted in the root zone.

2.1.1 Life cycle

Spores, infected root fragments, and hyphae are the main sources of AMF propagules in the soil. The hyphal network, along with root fragments, is the primary source of root colonization, while spores are responsible for long-term survival . When conditions are adequate, spores are able to germinate in the absence of a host . Although spontaneous germination is achievable, AMF typically cannot complete its life cycle if the host plant is absent. The exception to this was reported by Hildebrant et al. (2006), who found that AMF *Glomus intraradices* growth was stimulated by isolates of *Paenibacillus validus.* The plant root was successfully substituted by bacteria.

The AMF life cycle is completed in five main developmental stages. The spore remains inactive in the soil until germination is initiated and pre-symbiotic mycelial growth occurs . Once there is recognition by the host, colonization, arbuscular growth, and extraradicle hyphal extension are commenced. The final step in the AMF lifecycle is spore production.

Although the fundamental steps of the AMF life cycle have been identified, the molecular signals resulting in the breaking of dormancy and activating germination are unclear. Various potential effects have been studied including pH, temperature, moisture, mineral and organic nutrients, plant species (host/non-host) and microorganisms . The effect of pH and temperature on germination is often dependent on the situation that they are adapted to . Moisture has been found to produce variable effects. Some isolate spores are independent of soil moisture (i.e. *Gigaspora margarita*) whereas others are inhibited by low water content (i.e. *G. intraradices, G. mosseae* and *A. longula*). Other AMF show tolerance within an acceptable range of moisture content (*G. macrocarpum, G. clarum* and *G. etunicatum*). Also, isolates in an area often have an ecological adaptation to the moisture conditions in which they are found . Mineral and organic nutrients have an inconsistent effect on germination, but it may be stimulated by microorganisms in the rhizosphere. Host/non-host relationships do not have an effect on germination since spores can germinate in the absence of the host; however, the plant has an effect on the growth of fungi following germination .

2.1.2 Water and nutrient uptake

Arbuscular mycorrhizal fungi can increase uptake through hyphal extensions in the soil. The fungi work by colonizing the root and extending hyphae out into the soil. Hyphae have the ability to increase soil-root contact, increase exploration in micropores, extract water, and improve soil water holding capacity . The extraradicle hyphae provide contact between the AMF and the host, thereby contributing to nutrient uptake in the soil . The fungal hyphae (2-5 μm diameter) are smaller than root hairs (10-20 μm diameter) therefore have the ability to penetrate soil pores that are inaccessible to non-mycorrhizal roots .

Along with nutrients, water is also transported to the host plant. Marulanda et al. evaluated the ability of AMF to uptake water for host plants under drought stress. In their study, six different isolates were used to inoculate plants. Colonization occurred in four out of the six isolates and resulted in greater water depletion than in the uninoculated control plants. The other two isolates did not result in significant

differences . Mycorrhizal effects on plant water uptake are not as significant and consistent as the impact of increased P uptake on the host plant .

Augé et al. also observed that as the water is infiltrated out of large soil pores, it remains only in micropores and is less available to roots. Since hyphae have the ability to enter smaller pores, the hyphal growth had a large effect on water uptake. They also found that AMF in semi-arid climate may have evolved to be better at reducing drought stresses than those isolates existing in a moist climate .

2.1.3 Aggregate stability

Soil structure plays a critical role in soil water dynamics, vegetative growth and dynamics, and providing an appropriate habitat for microorganisms. According to Tisdall and Oades (1982), aggregation is based on three classes of binding agents: transient, temporary, and persistent. The transient binding agents consist of microbial and plant derived polysaccharides and mucigels, which can be rapidly decomposed. The temporary binding agents normally persist over the growing season or longer and include fibrous roots and hyphae. The persistent binding agents consist of aromatic humic materials and are responsible mostly for the stability of microaggregates . Arbuscular mycorrhizal fungi are most important as temporary binding agents .

Arbuscular mycorrhiza contribute to soil aggregate stability as a result of hyphae extending into the soil . They also produce a glycoprotein, glomalin, which acts as a relatively stable hydrophobic glue . Different isolates of AMF produce various levels of the glycoprotein, which can be measured using an enzyme-linked immunosorbent assay (ELISA) . Increased levels of glomalin in the soil aid in the stabilization of soil aggregates even during wetting and drying cycles.

2.2 Factors affecting AMF biodiversity and abundance

The function and abundance of AMF are influenced by a combination of environmental and plant factors. The environmental factors include the soil type, temperature, pH, moisture, and dissolved nutrients. The plant factors include the species, age, and biomass of the particular plant . Requirements for dormancy, germination, and sporulation must also be met. The soil conditions are constantly

changing and in addition, fluctuations in temperature and plant growth result in changes in the microbial community. Seasonality also plays a large role in determining the microbial community composition.

2.2.1 Seasonality

Seasonal variations in soil properties result from seasonal changes in ambient temperature and the growth of annual crops. Gavito et al. found that temperature (10 and 15°C) had a highly significant influence on total plant mass, mycorrhizal colonization, total P, and P-use efficiency. The intraradical colonization measured by Gavito et al. (2003) was 40% at 10°C and 71 % at 15°C. They concluded that soil temperature has a significant effect on mycorrhizal development and function . Liu et al. (2004) also found that increases in root zone temperature positively influenced colonization and root length after 15 weeks (Table 2.1). On the contrary, Baon et al. found that the root zone temperature did not have an effect on AMF colonization of barley roots for indigenous Canadian Prairie species. The ability of AMF strains to survive in temperate climates is important in Saskatchewan since they must have the potential to over winter . Suboptimum root temperature can reduce potential growth for the plant roots, and the extraradical hyphae.

2.2.2 Management

To ensure that there is a positive influence of mycorrhizal fungi on crop production (1) the crop can be inoculated with an effective strain or (2) use management

Table 2.1 Effect of root zone temperature on root colonization, root length, and shoot height of sorghum 15 weeks after seeding (Liu et al., 2004).

Temperature (°C)	Root Colonization (%)	Root Length (cm)	Shoot Height (cm)
10	10.0[c]†	519[b]	79.5[b]
15	17.4[b]	623[b]	133[a]
23	59.2[a]	816[a]	130[a]

†Values given are treatment means (n=5) with same letters within columns representing no significant difference at p=0.05 as determined by LSD tests.

practices to increase activity of effective indigenous strains . Agronomic practices influence the physical and chemical properties of the soil, which in turn affect microbial populations. Cereal grains such as durum are well adapted to a semi-arid climate and short growing seasons . Commonly, producers have also included fallow in their crop rotations in an attempt to conserve moisture in these areas . Traditional cereal monocultures and frequent mechanical tillage lead to substantial soil erosion by wind and water.

Many producers in the semi-arid brown soil zone have incorporated no till or minimum tillage into their cropping rotations. The trend to increase no till practices is largely an attempt to minimize soil erosion. This change in management is advantageous to AMF since tillage is known to disrupt hyphal networks throughout the soil. The largest effect is early in the season when the plants benefit by early uptake, until the network becomes reestablished . Since tillage has become less common in an attempt to decrease soil erosion, other methods must be utilized to conserve nutrients and water. This has resulted in a substantial increase in the number of acres seeded to pulse crops in southern Saskatchewan. Conservation tillage in the brown soil zone of Saskatchewan typically includes a combination of cereals, pulses, and oilseeds in the cycle. For these systems to be sustainable they must be agronomically feasible and economically viable .

2.2.2.1 Host crops

Mozafar et al. examined root colonization (nonmycorrhizal fungi and mycorrhizal fungi) and shoot nutrient concentrations over three years with rotations of maize, winter wheat, and canola (non-host) under three tillage treatments. The study concluded that changes in the nutrient concentrations of maize and wheat leaves were not a result of physical or chemical soil properties, but probably a result of root colonization by a combination of mycorrhizal and nonmycorrhizal fungi.

Crop rotation encourages diversity of the AMF community . Crops that are highly mycorrhizal dependent (i.e. pulses) have shown substantial differences in dry weight of roots and shoots, root length and overall colonization, and P uptake per plot in mycorrhizal inoculated plants compared to non-inoculated plants .

Shibata and Yano (2003) conducted a study comparing P uptake using cropping rotations including pigeon pea (Cajanus cajan [L.] Millsp), peanut (Arachis hypogaea L.), and soybean (Glycine max [L.] Merrill). These plants were chosen because they effectively utilize non-labile P sources. Three different P sources were used. Since sterilized soil was not used, there was some colonization in the uninoculated plants. These indigenous fungi were obviously not as effective as the *Gigaspora margarita* that was used to inoculate the plants. Increases in P acquisition enhanced by mycorrhizal inoculation were reported as 10 fold in pigeon pea, 6 in peanuts, and 3 in soybean.

Pulse crops are important when considering symbiotic plant-microbial interactions because roots are colonized by arbuscular mycorrhizal fungi and nitrogen-fixing rhizobia, both important soil microorganisms . There is currently an incomplete understanding of how including pulses in a cropping rotation affects the microbial community. In order to manage AMF in agriculture, an understanding of the interactions existing between crops, AMF, and environmental factors (including soil factors) must exist.

The relationship between a pulse crop, AMF, and *Rhizobium* species is a tripartite association . Different interactions occur depending on the strains of species for an association with a particular plant. A complementary *Rhizobium* and AMF species combination will result in optimal benefits to the plants. This interaction is of optimum importance when P is limiting in soils, since nitrogen fixation will be impaired.

Incorporating pulses into a crop rotation results in both nitrogen and non-nitrogen benefits to the subsequent crop. The non-nitrogen benefits include increased P, K, and S availability, improved soil structure, and a decrease in disease and weed populations . Controversial results have been published with regard to the nitrogen and non-nitrogen benefits resulting from incorporating pulses into cropping rotations. Stevenson and van Kessel found during a pea rotational study in the black soil zone the N-benefits only accounted for 8% of increased yields in the subsequent wheat crop. Contrary findings from Campbell et al. , involved a twelve year wheat-lentil rotational system, where they examined the N supplied by the soil and the N economy of this system. Results indicated that lentil preceding wheat did not increase the yield

exceeding that expected from N_2 fixation. It appears from these studies that abiotic factors may regulate the N benefits of legumes. They reported that a combination of greater N uptake in the wheat-lentil system and dry conditions from continuous cropping resulted in minimum NO_3 leaching out of system . Over time, decreases in NO_3 leaching will potentially enhance the sustainability of the agricultural system.

Incorporating host plants, such as pulses, in a cropping rotation with no till cropping systems will promote AMF diversity and increase soil inoculum levels. Non-host plants such as *Brassicas* spp. have been found to reduce or delay colonization in the subsequent crop

2.2.2.2 Non-host crops

Canola is a non-host plant and delays mycorrhizal colonization of subsequent crops in a rotation (Gavito, 1998). This is presumably a result of the reduction of mycorrhizal propagules in the soil and the canola roots excreting toxic compounds. Gavito (1998) measured the effects of treatments by measuring colonization potential at the beginning and end of cropping cycles in pot assays and in the field. Previous crops of canola resulted in significant delays in colonization in both field and pot studies. Including canola as a pre-crop had a much greater effect on subsequent crop colonization compared to tillage and P fertilization.

2.2.2.3 Fertilization

There have been contradictory studies reporting the effect of fertilization on AMF colonization and nutrient uptake. High levels of P fertilization may lead to dramatic decreases in root colonization and spore production , resulting in the overall conclusion that AMF are not useful in highly fertilized systems. However, the level of colonization does not depend solely on fertilization, but also on the dependency of the plant species . Balser et al. found that AMF levels, measured by FAME analysis, were much higher in the fertile treatments, compared to the N-limited and the P-limited treatments. Johnson found after comparing fertilized and unfertilized soil that in general, N addition increased colonization whereas P addition decreased colonization. It is also possible that root colonization is predominately independent of dry matter

yield . In theory, optimum levels of soil nutrients should stimulate AMF and result in maximal yields .

3. SOIL AND PLANT FACTORS ARE INFLUENCED BY INCLUSION OF PULSES IN A DURUM-BASED CROPPING SYSTEM

3.1 Introduction

Agroecosystem 12 of the northern Great Plains is characterized by long, cold winters and short, warm summers and is usually drier than surrounding regions . Water, N and P are the most limiting factors to crop production in this area and frequent fallowing has traditionally been used in an attempt to conserve water and nutrients for subsequent crops. Conservation tillage in this brown soil zone of Saskatchewan typically includes a combination of cereals, pulses, and oilseeds in the cycle (Table 3.1). For these systems to be sustainable they must be agronomically feasible and economically viable . Including pulse crops in a rotation has been widely recognized as beneficial by both producers and researchers, as they may increase the yield and/or quality of a subsequent durum crop and contribute to a more stable farm income . The production of the pulse crops is limited by soil characteristics and climate . For example, some pulse crops are well suited to semiarid regions since they may not endure extreme moisture conditions . The use of fallow has been on a steady decline (Figure 3.1a), as crop diversifications including pulses (particularly pea and lentil) is on the rise (Figure 3.1b).

Research in the semi-arid region of Saskatchewan has produced inconsistent results concerning the effect of pulse crops on durum yield (Miller et al. 2003). Besides forming a relationship with arbuscular mycorrhizal fungi (AMF), legumes also form a symbiotic relationship with Rhizobia. Pulse crops may have both N and non-N benefits to subsequent crops. The non-N benefits may include increased water-use efficiency (WUE), decreased disease incidence, and H_2 fertilization. These bacteria lead to the formation of nodules on the roots which can fix atmospheric N into a plant available

Table 3.2 Cropping rotations in southern Saskatchewan in 2003 included cereals, pulses, oilseeds and fallow. Values are presented in thousands of hectares .

Pea	Lentil	Chickpea	Wheat†	Canola	Summerfallow
988	547	53	3779	2309	2612

† Wheat includes winter wheat, spring wheat and durum.

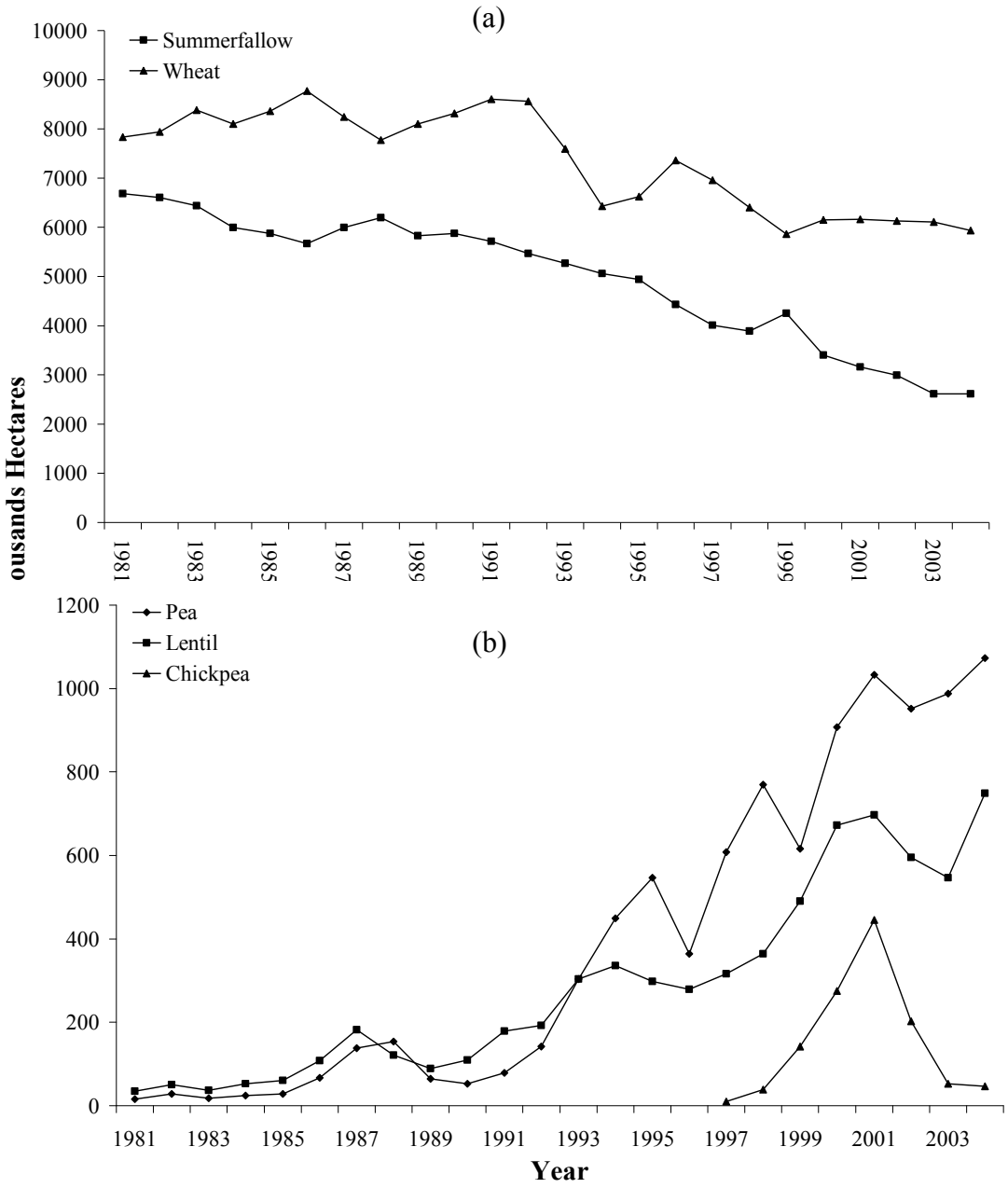

Figure 3.1 Saskatchewan hectares allocated to summer fallow and wheat and (a) pulse crops (b) between 1981 and 2003. Wheat includes winter wheat, spring wheat and durum .

form. A byproduct of this process is a H_2 gas, an energy source for chemoautotrophic bacteria with plant growth promotion activity (Dong et al. 2003).

A two year study near Swift Current, Saskatchewan compared the effect of previous crop on soil and plant factors. There were three repetitions of durum following chickpea, lentil, pea, durum, and canola. Plant and soil samples were taken at emergence, five-leaf, flag leaf, anthesis and physiological maturity. Since nutrients and water are often limiting factors for plant growth in Southern Saskatchewan, soil nutrient pools, plant nutrient uptake, and WUE were analysed to determine by which mechanism chickpea, lentil, pea, durum or canola affect the yield of subsequent durum crop.

3.2 Objective and hypothesis

I hypothesized that there would be an increase in N and P plant uptake by the plants. The objective of this study was to assess the impact of pulses in crop rotation on plant N and P uptake and the available soil N and P levels in a durum crop. This hypothesis was tested using a combination of laboratory and field experiments.

3.3 Materials and methods

3.3.1 Site description

The experiment was a 2-yr field plot study (2004 and 2005), conducted at the South Farm of the Semiarid Prairie Agricultural Research Centre (SPARC), in Swift Current, Saskatchewan, Canada (latitude, 50º18'N; longitude: 107º 41'W). It was located in the Brown soil zone on a Swinton Silt Loam (Orthic Brown Chernozem), in Agroecosystem 12 of the northern Great Plains . Three replicates of five treatments were compared i.e., the inclusion of *Pisum sativum* L. (pea), *Lens culinaris* Medik (lentil), *Cicer arietinum* L. (chickpea), *Brassica napus* L. (canola) or *Triticum turgidum* L. (durum) at stage II of 3-year rotations with fallow at stage I and durum at stage III (Table 3.2), in 5 x 24 m plots[1]. Conventional tillage at a depth of 7.5 cm was used to maintain fallow stage.

[1] Durum following durum was grown in 15 x 24 m plots

Table 3.3 Crop varieties used in the experiment were common varieties grown in the region.

Rotation†	Variety	Inoculant
Fallow-**lentil**-durum	CDC Sovereign	Nitragin C
Fallow-**pea**-durum	CDC Handel	Nitragin C
Fallow-**chickpea**-durum	Myles	Nitragin GC
Fallow-**durum**-durum	AC Avonlea	n/a
Fallow-**Argentine canola**-durum	Liberty Link Invigor 2573/2733‡	n/a

† Crops in bold were preceding crops of interest and correspond to the indicated variety. All sampling was completed in the durum phase of the rotation.
‡ The canola variety used in 2004 was Liberty Link Invigor 2573 and in 2005 was Liberty Link Invigor 2733.

Since the durum monoculture once represented the best management practice under conventional till, it was included in the study as a check rotation. The impacts of these crops on soil quality and growth of durum were evaluated. Crops were fertilized each year to equalize soil fertility among treatments, as determined by soil tests. For durum plots the total N level was equalized to 73 kg ha^{-1} with urea (46-0-0). This was calculated as 73 minus the amount of N in top 60 cm as determined by fall soil sampling. Ammonium phosphate (11-51-0) was routinely applied with seeds at approximately 45 kg ha^{-1}. All fertilizer was side banded 2.5 cm to the side and below the seed. Durum (AC Avonlea) was seeded using a Flexicoil 5000 air drill equipped with Stealth Double Shoot Knives with 22.5 cm spacing (Appendix A). Seeding rate was 100 kg ha^{-1} and seeding depth 5 cm. Agronomic details are provided in Appendix A.

Precipitation and temperature were recorded at an Environment Canada weather station (Latitude 50° 16' N; Longitude 107° 43' W). Total precipitation was 407 mm in 2004 and 366 mm in 2005. Mean climatic data (54-years) indicates that the site receives an average of 361 mm of annual precipitation and a yearly mean temperature of 3.6ºC, with a minimum of -13.2ºC in January and a maximum temperature of 18.6ºC in June. Mean daily temperatures and daily precipitation for the 2004 and 2005 growing seasons are displayed in Figure 3.2.

3.3.2 Soil and plant sampling and analysis

Soil and plant samples were taken at five main physiological stages (Table 3.3). Four soil samples, 2 between row and 2 in row, along a diagonal transect per plot were taken at emergence, five-leaf, flag-leaf, anthesis, and physiological maturity using a hand core sampler (r=2.5 cm) to a depth of 7.5 cm. The samples were bulked into one composite sample and put through 2 mm sieves. Two plants were taken at four locations per plot using a trowel. Pre-seeding soil samples were taken (2 per plot) to a depth of 120 cm and analysed for gravimetric soil moisture, NO_3, NH_4 and PO_4. Plant shoots were dried, weighed and ground before N and P analysis. Tissue digestion

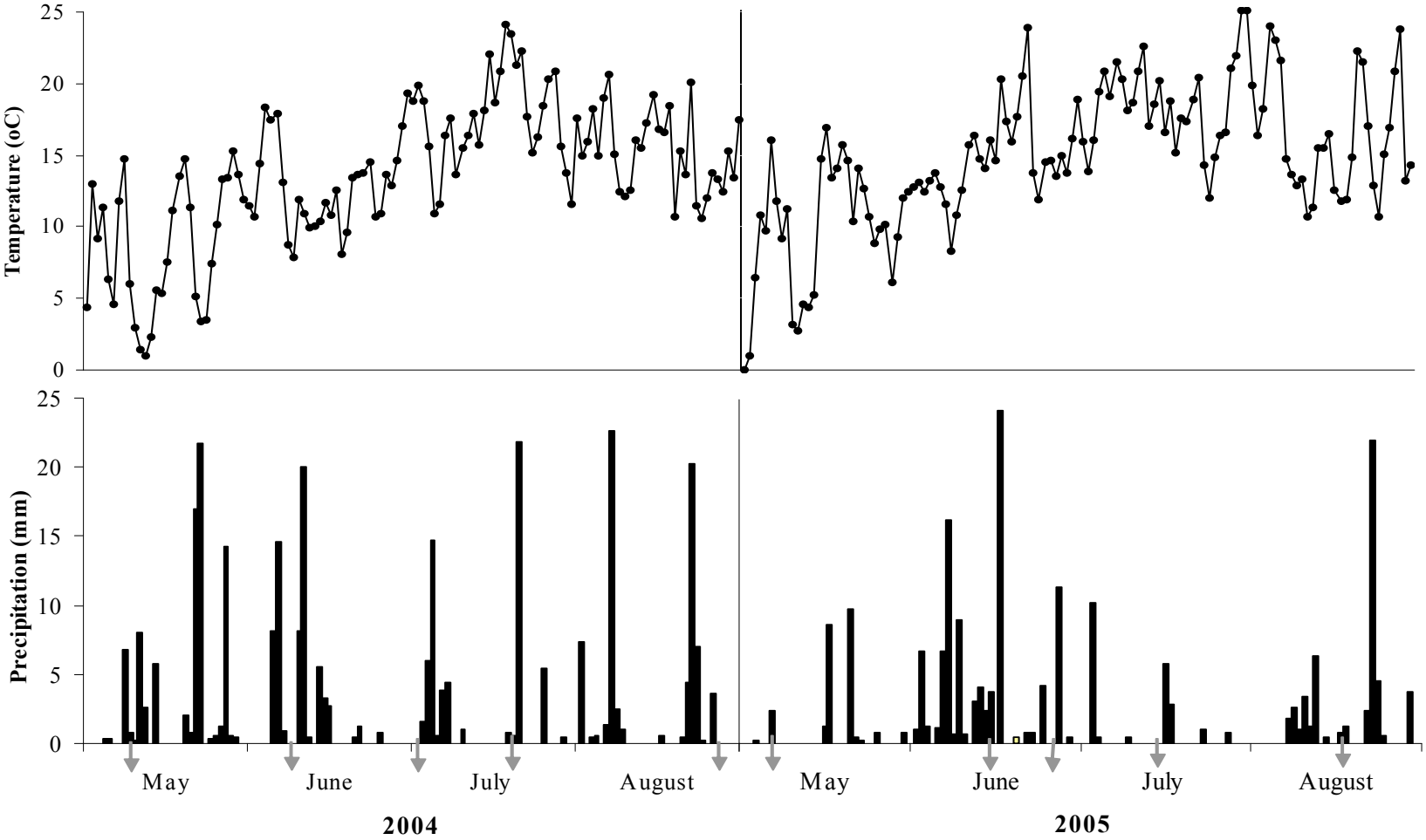

Figure 3.2 Precipitation and mean daily temperature throughout the 2004 and 2005 growing season. Data was recorded at the Environment Canada Swift Current CDA weather station. Arrows represent sampling times (emergence, five leaf, flag leaf, anthesis and maturity).

Table 3.4 Soil and plant samples for AC Avonlea were taken in 2004 and 2005 at five main growth stages.

Growth stage	Sampling date	
	2004	2005
Pre-seeding	Apr-19	Apr-20
Emergence	May-10	May-06
Five-leaf	Jun-09	Jun-14
Flag leaf	Jul-01	Jun-27
Anthesis	Jul-20	Jul-15
Physiological maturity	Aug-30	Aug-18

was completed and N and P concentrations were measured on the autoanalyzer. Soil extractible N and P samples were analysed for KCl extractable NH_4/NO_3 and Resin P . The soil N and P concentrations were then determined using a Technicon segmented flow AutoAnalyzer at the SPARC Chemistry Laboratory.

Soil N and P fluxes were measured using anion exchange membranes (AEMs). The membranes used in this study were 2.25 cm x 6.5 cm pieces cut from 0.457 m by 1.143 m resin-coated membrane sheets (Ionics # 5200253). A 0.3 cm hole was punched in one end to attach coated wire to a flag for ease of retrieval. Beginning at emergence, two membranes were placed vertically 1 cm below the soil surface at four positions per plot in between rows. At five leaf, flag-leaf and anthesis the AEMs were replaced, and collected at physiological maturity. The membranes were washed and extracted as described by Ziadi et al. . Gravimetric soil moisture (Equation 3.1) was calculated for each sampling time and water use efficiency of the grain and straw was determined at the end of the growing season.

$$\theta_d = \frac{(\text{wt of wet soil}) - (\text{wt of dry soil})}{(\text{wt of dry soil})} \qquad [3.1]$$

Water use efficiency is a measure of how much plant biomass is produced with one unit of water. The average bulk densities from the test site (Table 3.4) were used to convert gravimetric to volumetric moisture. The amount of water used by plants is calculated as the difference between the spring and fall values of moisture in the soil profile plus all rainfall during this time period, which is then used to calculate durum water use efficiency (biomass/total water). Runoff is not considered significant in this study since the field site is quite level (Miller et al., 2003).

3.3.3 Statistical analysis

Field plots were replicated three times using a randomized plot design where precrop species form the main plots and sampling time as subplots . Variations in soil conditions and plant tissue were measured throughout the growing seasons in 2004 and 2005. When normality could be reached within a sampling

Table 3.5 Bulk density values (0-120 cm) calculated from soil samples at the Phase II field site near Swift Current, Saskatchewan.

Soil Depth (cm)	Bulk Density (g cm^{-3})
0-15	1.22
15-30	1.25
30-60	1.36
60-90	1.39
90-120	1.55

time, the effects of treatments were analysed using analysis of variance (ANOVA). Tukey's test was used for comparison of means at p=0.05 using SYSTAT v.10 (Point Richmond, USA). Shapiro-Wilks' test was used for normality assessments and non-normal data was transformed when allowable. Normality could not be achieved for AEM NO_3, NH_4, gravimetric soil moisture, plant biomass, plant P, and plant N (2004 only). Kruskal-Wallis nonparametric tests were used to determine treatment effects and mean differences (SYSTAT v.10) on these factors. All sampling and statistical analysis was completed in 2004 and 2005.

3.4 Results and discussion

3.4.1 Plant dry mass and tissue analysis

The previous crop had a significant effect (p<0.05) on the yield of the following durum crop in both 2004 and 2005. A previous crop of pea was associated with the highest yields of durum (Table 3.5) in both 2004 and 2005. The durum monoculture resulted in the lowest yields in both years, similar to finding by Miller et al. in southwest Saskatchewan. Miller et al. reported that the effect of broadleaf crops on subsequent wheat yield is influenced by soil type, as increases in Agroecosystem 12 were 35% in clay and only 14% in the loam at Swift Current. At a study site in close proximity, Gan et al. reported that durum following pulse or canola increased grain yield by 7% and 5% respectively, when compared to a previous crop of spring wheat. This study also reported that effects of crop rotation on durum yield was higher in years when precipitation was above the long-term average . In 2005, durum grain yield was significantly higher following pea, lentil, and canola than chickpea and durum (Table 3.5). This is unusual considering that plant density was much lower on durum following pea and lentil than all other crops (Table 3.5), but may be a result of the ability of durum to fill in open space through tillering. A preceding crop of canola resulted in the second highest yield and the highest biomass (Figure 3.3) in the subsequent durum crop.

Table 3.6 Durum plant densities, grain yield, plant N and P as influenced by previous crops in 2004 and 2005.

Previous Crop	Plant Density (Plant m^{-2})		Yield (t ha^{-1})		N (mg kg^{-1})		P (mg kg^{-1})	
	2004	2005	2004	2005	2004	2005	2004	2005
Chickpea	194b †	120b	3.18bc	2.01b	0.285ab	0.227a	0.037a	0.039a
Lentil	219ab	85c	3.68ab	2.43a	0.290a	0.263a	0.035b	0.036a
Pea	193c	90c	4.03a	2.64a	0.294a	0.267a	0.034c	0.036a
Durum	223ab	174a	2.68c	2.01b	0.290a	0.223a	0.033d	0.035a
Canola	236a	172a	3.22bc	2.60a	0.276b	0.238a	0.030e	0.035a

† Values given are treatment means (n=3) with same letters within columns representing no significant difference at $p<0.05$ as determined by Tukey's test.

Canola grows well in the short, cool season typical of the Canadian Prairies, and can also handle high temperatures and periods of drought .
Kruskal-Wallis nonparametric tests revealed that in 2004 and 2005 durum biomass (Figure 3.3) was significantly different between sampling times (p<0.001). In 2004, canola followed by durum resulted in little accumulation of biomass between anthesis and maturity, but after pea 35% of biomass was accumulated during this time period (Figure 3.3). The treatment effect on durum biomass was insignificant for all sampling times except at maturity for both sampling years. In 2004, the preceding lentil crop was significantly higher than the durum monoculture, while in 2005 canola was higher than durum and chickpea (p<0.05) (Figure 3.3). The lower durum monoculture biomass results correspond with the data of durum monoculture yields (Table 3.5).

In 2004 and 2005, durum tissue P concentration was higher after chickpea, lentil and pea and lower after canola and durum, although in 2005 the trend was not significant (p<0.05) (Table 3.5). During the 2004 growing season, canola was lower than all other preceding crops but differences (p<0.05) were greatest at the flag leaf stage and before maturity. In 2005, there were no significant differences detected (p<0.05).

Previous crops significantly affected the N content of durum only in 2004 (p<0.05), where higher yields were also obtained (Table 3.5). The time when the N is released from pulse stubble was found to affect the growth response of the subsequent crop . Nitrogen that is released early in the season affects tillering and therefore overall yield, while N released later has a greater effect on size and protein of grain kernels . It was also reported by Campbell et el. that yield responses to N increases with water availability.

Different plant species included in cropping rotations may influence the availability of water and nutrients to the subsequent crop to a depth below that of surface sampling (7.5 cm). Gravimetric soil moisture, NO_3, NH_4, and PO_4 were

Biomass (kg ha^{-1})

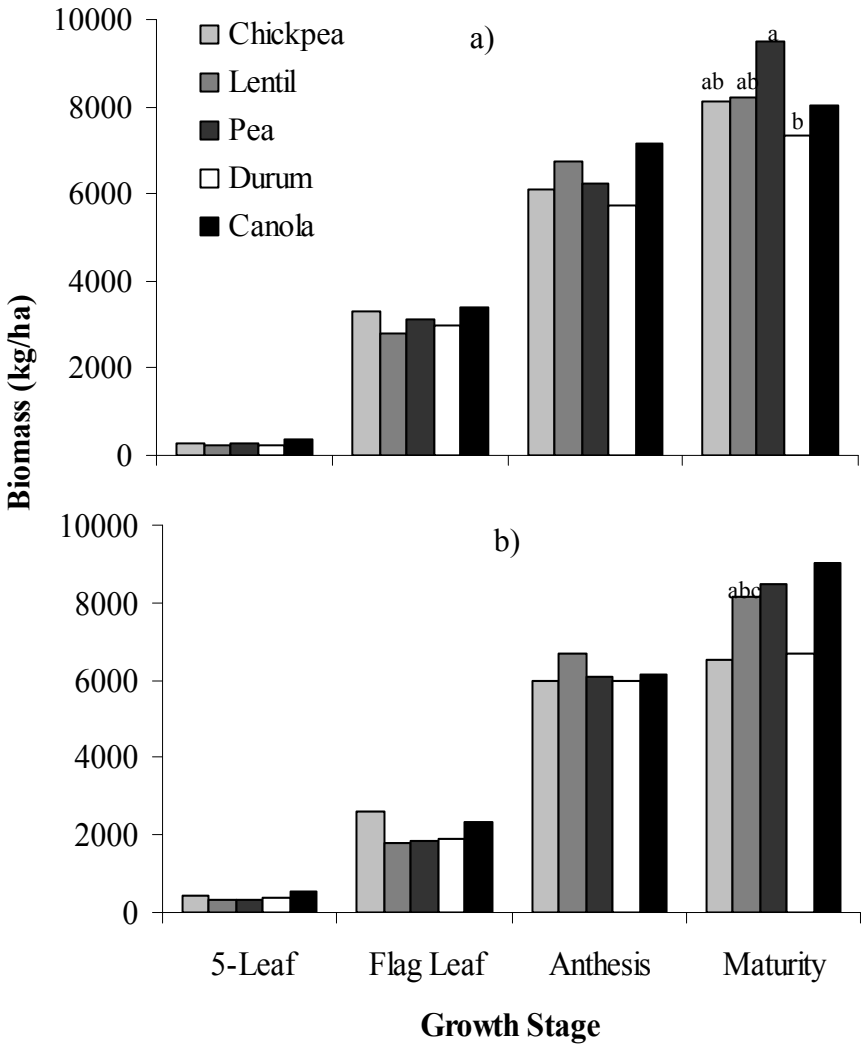

Figure 3.3 Durum biomass accumulation in (a) 2004 and (b) 2005, as influenced by previous crop at each sampling stage. Letters represent significant differences between treatments during the sampling time. An absence of letters indicates no significant difference (p<0.05).

analysed before seeding to determine residual effects of the previous crop throughout the soil profile. Gravimetric soil moisture values were not significantly different (p<0.05) below 30 cm. From 0-15 cm in 2004 and 0-30 cm in 2005, soils planted to durum the previous year consistently had significantly higher soil moisture (%), since the durum stubble functions as a snow trap (Table 3.6, 3.7).

Soil nutrient analysis revealed more varied results, especially over the two sampling years. In 2004, soil NO_3 revealed differences (p<0.05) from 0-15 cm with the pulse crops having higher values than durum and canola (Table 3.6). In 2005, the differences observed were at the lower soil depths (30-120 cm), with lentil and pea being highest (Table 3.7). Pre-seeding in 2004, soil sampled after a durum crop contained the highest amount of NH_4 for the first two sampling depths (Table 3.6). The opposite occurred from 30-120 cm, with durum values being significantly lower than all other crops. The 7.5 – 15 cm depth for durum had an unusually high value. Soil PO_4 measurements were different between treatments only at the surface. There was a substantial decrease in PO_4 as sampling went down the soil profile (Table 3.6, 3.7).

3.4.2 Soil extractible N and P

The previous crop effect on durum yield may be attributed to durum N tissue concentration but not to their effect on soil nutrient availability. Soil available PO_4 showed no significant effect of previous crop at any of the sampling times. The analysis of available NO_3 and NH_4 (mg kg^{-1}) were rarely significantly different (p<0.05) between treatments (Table 3.8, 3.9). The high level of NH_4 at emergence is a result of N fertilization applied with the durum seed. The previous crop was significant for available NO_3 at flag-leaf (2004, Table 3.8) and maturity (2005, Table 3.9) and available NH_4 was significant at flag-leaf (2004), where the durum monoculture was consistently lowest. The large elimination of the N effect was probably accomplished by the adjustment of N fertilization rates as determined by soil tests. A three year study in southwestern Saskatchewan comparing the effect of pea, lentil, and chickpea stubbles on wheat (yield and quality) also found that the N

Table 3.7 Gravimetric soil moisture, nitrate, ammonium, and phosphate pre-seeding the 2004 growing seasons to a depth of 120 cm.

Soil depth (cm)	Previous crop				
	Chickpea	Pea	Lentil	Durum	Canola
	Gravimetric soil moisture (%)				
0-7.5	10.94±0.9b†	12.40±0.9b	11.93±0.3b	17.13±0.2a	12.45±1.6b
7.5-15	13.07±0.7b	14.76±0.4b	14.06±0.6b	19.66±0.5a	15.25±0.5b
15-30	15.46±1.4	17.35±0.3	16.71±0.6	18.20±1.0	17.79±0.4
30-60	9.54±1.3	13.38±2.4	12.78±0.8	11.10±2.4	14.30±0.6
60-90	9.33±1.1	11.03±2.5	10.74±0.7	8.75±1.0	10.34±0.7
90-120	9.82±0.9	11.21±1.5	9.72±0.6	10.60±0.7	9.80±1.3
	NO_3 (mg kg^{-1})				
0-7.5	6.14±0.8a	4.02±0.2ab	3.70±0.4ab	2.44±0.4b	4.35±0.9b
7.5-15	5.43±0.5a	3.68±0.1b	2.96±0.5b	2.78±0.4b	2.22±0.4b
15-30	4.20±1.2	3.53±0.7	2.83±1.1	2.76±0.3	1.76±0.3
30-60	1.58±1.1	2.18±0.9	1.40±0.1	3.12±0.6	1.32±0.1
60-90	3.61±1.0	2.74±1.1	1.94±0.1	4.22±0.7	1.14±0.1
90-120	4.19±0.7	4.61±1.8	3.98±1.4	4.60±0.9	1.76±0.2
	NH_4 (mg kg^{-1})				
0-7.5	4.00±0.8b	3.57±1.0b	2.87±0.3b	29.68±6.4a	3.33±0.5b
7.5-15	3.00±0.1b	2.77±0.0b	3.22±0.4b	8.28±2.3a	2.55±0.1b
15-30	2.90±0.1	3.53±0.8	2.85±0.1	2.64±0.5	3.25±0.6
30-60	3.61±0.4a	3.35±0.2a	3.35±0.1a	0.48±0.2b	2.93±0.1a
60-90	5.68±1.1a	4.86±0.0a	5.10±0.3a	0.05±0.0b	4.34±0.3a
90-120	5.52±0.0a	5.35±0.3a	5.43±0.0a	0.29±0.3b	4.75±0.2a
	PO_4 (mg kg^{-1})				
0-7.5	24.46±2.4a	17.73±3.6a	21.28±1.1a	2.75±0.8b	24.91±3.5a
7.5-15	14.95±1.6a	12.78±3.4ab	14.03±0.8ab	2.68±1.7b	23.71±3.9a
15-30	4.78±0.3	7.35±3.9	4.39±0.7	3.90±1.7	7.88±1.3
30-60	1.49±0.3	1.88±0.5	1.71±0.2	1.12±0.3	2.94±0.8
60-90	0.55±0.3	1.16±0.4	0.82±0.2	1.60±0.8	1.48±0.1
90-120	0.63±0.2	1.25±0.5	0.76±0.3	2.72±0.7	0.92±0.2

† Values followed by a different letter across rows are significantly different according to Tukey's test ($p<0.05$) ± the standard error of the mean. Values with no letters are not significantly different.

Table 3.8 Gravimetric soil moisture, nitrate, ammonium, and phosphate pre-seeding the 2005 growing seasons to a depth of 120 cm.

Soil depth (cm)	Previous crop				
	Chickpea	Pea	Lentil	Durum	Canola
	Gravimetric soil moisture (%)				
0-7.5	13.54±0.6b†	14.37±0.4b	14.23±0.7b	18.99±0.5a	14.58±1.2b
7.5-15	16.42±0.8b	16.34±0.7b	16.61±0.5b	19.56±0.7a	16.51±0.3b
15-30	16.69±0.8b	17.87±0.6b	17.33±0.1b	20.48±0.4a	17.29±0.3b
30-60	10.34±1.5	9.87±0.5	11.47±0.4	15.03±2.4	12.72±1.5
60-90	8.47±0.3	9.74±0.7	8.96±0.1	9.48±1.9	7.70±0.6
90-120	8.67±0.2	9.01±0.9	9.82±0.4	9.02±0.5	7.96±0.6
	NO_3 (mg kg^{-1})				
0-7.5	2.92±0.9	3.37±0.4	2.45±0.5	2.10±0.3	1.99±0.9
7.5-15	2.83±1.1	5.07±1.1	2.75±0.6	1.53±0.2	2.05±1.3
15-30	2.17±0.4	4.41±0.6	3.86±0.6	1.85±0.3	2.90±1.0
30-60	0.71±0.2b	2.73±0.4a	2.55±0.7a	1.12±0.1ab	1.72±0.2ab
60-90	0.64±0.2b	2.53±0.7a	1.72±0.1a	0.54±0.1b	0.65±0.1b
90-120	0.91±0.1a	1.51±0.4a	1.52±0.3a	0.37±0.0b	0.92±0.2a
	NH_4 (mg kg^{-1})				
0-7.5	3.49±0.7	3.87±0.4	3.35±0.5	3.85±0.4	2.62±0.3
7.5-15	4.22±1.1	4.17±1.2	3.44±0.2	4.12±0.2	2.54±0.2
15-30	3.54±0.3	3.81±0.4	3.50±0.2	4.11±0.3	3.74±0.2
30-60	3.84±0.4	4.57±0.2	4.31±0.6	3.50±0.7	3.68±0.1
60-90	6.05±0.3	5.71±0.5	6.21±0.7	4.84±0.7	5.16±0.5
90-120	6.35±0.3	4.94±1.2	6.42±0.2	6.52±0.3	6.13±0.3
	PO_4 (mg kg^{-1})				
0-7.5	43.38±3.6a	31.37±2.4ab	33.11±1.8ab	28.52±3.0b	37.90±2.9ab
7.5-15	16.14±3.9	13.34±1.7	12.33±1.2	17.08±2.6	14.19±4.8
15-30	5.80±0.8	4.13±0.5	4.81±0.8	4.48±0.3	4.79±1.1
30-60	3.31±0.6	2.59±0.7	2.57±0.8	2.01±0.2	2.98±0.2
60-90	1.76±0.0	1.72±0.3	1.42±0.3	0.79±0.2	1.68±0.2
90-120	1.90±0.6	1.56±0.6	1.60±0.4	0.92±0.1	1.67±0.2

† Values followed by a different letter across rows are significantly different according to Tukey's test ($p<0.05$) ± the standard error of the mean. Values with no letters are not significantly different.

Table 3.9 Soil moisture and nutrients for the sampling times over the 2004 growing season.

	Previous crop				
Time†	Chickpea	Pea	Lentil	Durum	Canola
			Gravimetric soil moisture (%)		
1	14.33±0.3‡	15.70±0.2	16.07±0.5	15.01±0.4	16.22±0.3
2	17.70±0.3	18.33±0.6	18.47±0.4	17.91±1.0	20.18±0.7
3	6.58±0.2	7.39±0.1	7.04±0.3	7.53±0.3	7.39±0.5
4	6.88±0.4	7.39±0.6	7.27±0.4	6.63±0.4	6.85±0.8
5	20.31±1.0	20.46±0.9	21.12±0.9	22.34±0.6	22.47±0.2
			NO_3 (mg kg^{-1})		
1	11.47±1.6	10.03±1.6	11.70±0.4	10.03±0.5	9.50±0.7
2	4.93±0.8	7.53±0.1	7.10±1.8	7.30±3.8	5.13±1.5
3	5.83±0.7a	3.37±0.4ab	5.63±1.4a	2.03±0.1b	3.40±0.3ab
4	3.27±1.5	3.07±0.0	2.53±0.6	1.73±0.6	2.57±0.5
5	4.73±0.3	4.97±0.3	3.83±0.5	3.10±0.7	3.90±1.7
			NH_4 (mg kg^{-1})		
1	60.83±12.0	52.23±13.7	45.97±11.0	60.75±9.8	21.97±13.5
2	0.43±0.1	1.27±0.4	1.30±0.3	5.40±5.1	0.87±0.8
3	1.17±0.3	0.90±0.1	1.13±0.4	0.53±0.2	0.87±0.1
4	3.87±2.3	1.43±0.1	1.13±0.2	0.97±0.2	0.93±0.1
5	2.90±0.1	2.83±0.4	2.10±0.4	1.87±0.4	1.50±0.2
			PO_4 (mg kg^{-1})		
1	0.76±0.1	0.71±0.1	0.72±0.1	0.64±0.0	0.71±0.2
2	0.58±0.1	0.52±0.1	0.57±0.1	0.53±0.0	0.52±0.1
3	0.63±0.1	0.51±0.1	0.46±0.1	0.53±0.0	0.55±0.1
4	0.85±0.1	0.83±0.1	0.96±0.1	0.73±0.0	0.81±0.1
5	0.92±0.2	0.69±0.1	0.80±0.1	0.74±0.1	0.71±0.1
			Soil PO_4 flux (µg cm^{-2} d^{-1})		
2§	0.08±0.0	0.06±0.0	1.64±0.0	0.07±0.0	0.05±0.0
3	0.09±0.0	0.07±0.0	0.07±0.0	0.07±0.0	0.09±0.0
4	0.06±0.0	0.10±0.1	0.06±0.0	0.06±0.0	0.07±0.0
5	0.05±0.1	0.03±0.0	0.05±0.0	0.07±0.0	0.07±0.0
			Soil NO_3 flux (µg cm^{-2} d^{-1})		
2§	1.60±0.1	1.47±0.5	1.67±0.1	1.27±0.3	2.40±0.5
3	0.71±0.1	0.53±0.1	0.51±0.0	0.47±0.1	0.59±0.0
4	0.57±0.1	0.37±0.1	0.57±0.1	0.32±0.1	0.43±0.0
5	0.49±0.1	0.36±0.1	0.49±0.1	0.54±0.1	0.48±0.1

† Sampling times refer to (1) emergence, (2) five-leaf, (3) flag-leaf, (4) anthesis, and (5) physiological maturity.

‡ Values followed by different letters across rows are significantly different according to Tukey's test (p<0.05) ± the standard error of the mean.

§ Soil nutrient flux is measured using anion exchange membranes that are placed in the soil at emergence, removed at the next sampling date, and replaced with a new membrane.

Table 3.10 Soil moisture and nutrients for the sampling times over the 2005 growing season.

	Previous crop				
Time†	Chickpea	Pea	Lentil	Durum	Canola
	Gravimetric soil moisture (%)				
1	16.15± 1.0‡	16.84±0.3	15.75±0.4	17.56±0.4	17.38±0.9
2	20.18±0.3	20.81±0.3	20.14±0.2	21.30±0.5	21.20±0.3
3	17.34±0.4	16.74±0.2	17.88±0.4	18.53±1.3	18.51±0.4
4	6.77±0.3	8.37±1.1	8.00±0.6	9.01±0.4	8.18±0.3
5	10.47±0.7	11.12±0.6	10.96±0.3	11.08±0.1	10.59±0.2
	NO_3 (mg kg^{-1})				
1	10.53±1.3	11.30±0.4	11.23±1.0	9.50±1.3	10.20±0.2
2	4.07±0.5	6.47±0.4	5.83±0.8	4.57±1.2	4.43±0.3
3	6.10±1.2	8.10±3.0	8.07±2.8	3.60±1.2	4.47±0.7
4	2.45±0.8	1.83±0.9	2.33±0.9	0.63±0.4	1.33±0.2
5	7.93±0.3a	6.63±1.3ab	7.37±0.2a	4.37±0.1b	5.30±0.4ab
	NH_4 (mg kg^{-1})				
1	35.57±16.5	23.60±14.4	17.80±5.9	18.27±13.6	12.87±7.9
2	2.03±1.0	2.63±0.5	2.93±1.9	2.07±0.3	1.83±0.4
3	2.20±0.1a	1.60±0.3ab	1.70±0.2ab	0.93±0.0b	1.67±0.2ab
4	3.30±1.9	1.77±0.7	2.13±1.2	2.80±1.6	1.43±0.5
5	12.50±1.6	9.93±0.3	11.07±0.2	13.53±2.7	10.40±0.8
	PO_4 (mg kg^{-1})				
1	1.05±0.0	0.77±0.1	0.91±0.2	0.73±0.1	0.74±0.1
2	0.68±0.1	0.53±0.1	0.55±0.1	0.33±0.1	0.40±0.0
3	0.83±0.1	0.73±0.0	0.74±0.1	0.47±0.0	0.62±0.1
4	0.72±0.1	0.71±0.1	0.68±0.0	0.50±0.0	0.68±0.0
5	0.77±0.0	0.76±0.1	0.83±0.1	0.59±0.0	0.81±0.1
	Soil PO_4 Flux (μg cm^{-2} d^{-1})				
2§	0.03±0.0	0.02±0.0	0.02±0.01	0.04±0.0	0.06±0.0
3	0.07±0.0	0.05±0.0	0.04±0.0	0.06±0.0	0.09±0.0
4	0.01±0.0	0.10±0.0	0.17±0.1	0.09±0.0	0.17±0.0
5	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
	Soil NO_3 Flux (μg cm^{-2} d^{-1})				
2§	2.27±0.1	3.10±0.1	3.27±0.4	1.91±1.0	2.28±0.2
3	2.06±0.7	1.48±0.1	1.08±0.0	1.62±0.7	1.11±0.4
4	0.66±0.1	0.73±0.2	0.81±0.1	0.61±0.2	0.61±0.1
5	0.23±0.1	0.20±0.0	0.17±0.0	0.18±0.0	0.14±0.0

† Sampling times refer to (1) emergence, (2) five-leaf, (3) flag-leaf, (4) anthesis, and (5) physiological maturity.

‡ Values followed by different letters across rows are significantly different according to Tukey's test ($p<0.05$) ± the standard error of the mean.

§ Soil nutrient flux is measured using anion exchange membranes that are placed in the soil at emergence, removed at the next sampling date, and replaced with a new membrane.

effect from a previous pulse crop was neutralized by adjusting the fertilizer rates . They no significant difference in the overall soil N pools, however, differences did occur with the amount and time of N uptake by the plants . In general, the levels of NO_3 and NH_4 decreased over time, most likely a result of residual N and initial release of N fertilizers applied at seeding. Without adjusting fertilizer rates, Miller et al. (1998) reported that the N benefits of pulses in rotation may be potentially large in the semi-arid region of southern Saskatchewan.

Controversial results have been published with regard to the N and non-N benefits resulting from incorporating pulses into crop rotations. Stevenson and van Kessel found during a pea rotational study in the black soil zone that the N-benefits only accounted for 8% of increased yields in the subsequent wheat crop. Contrary findings from Campbell et al. involved a twelve year wheat-lentil rotational system where they examined the N supplied by the soil and the N economy of this system. Results indicated that lentil preceding wheat did not increase the yield exceeding that expected from N_2 fixation

Soil NO_3 and PO_4 fluxes measured using AEMs varied significantly through time but not as a result of durum following a previous crop (Table 3.8, 3.9). Although the use of AEM membranes provide valuable information about changes in nutrient availability over the growing season, it is possible that results may be affected by reduced contact with the soil surface or interference by plant roots. It is also possible that periods with no moisture may affect the results obtained. Mamo et al. reported that a wet-dry cycle of one day had a significant effect ($p<0.05$) on ion exchange resin desorption of PO_4-P but not on NO_3-N or NH_4-N. However, when 30 wet-dry cycles were applied there was a significant effect on desorption of PO_4-P, NO_3-N and NH_4-N, which may potentially underestimate levels in the field. This is similar to results from Kjonaas who found reduced adsorption of NO_3 by AEMs caused by drying.

3.4.3 Water use efficiency

Since water is often a limiting factor in Southern Saskatchewan, WUE is considered important for durum yield. Durum following durum had the lowest WUE in both 2004 and 2005, while pea had the highest in 2004 and canola in 2005 (Table 3.10). In 2004, both WUE and yield values were higher than in 2005, most likely a result of more precipitation over the growing season (238 mm in 2004 compared to 192 mm in 2005). Durum had the greatest WUE after pea, increasing both N concentration and yield, while WUE was lowest after durum. This agrees with the results of Miller et al. (1998) that the shallow rooting patterns of pea and lentil result in less water needed for recharge for the subsequent durum crop. These crops also mature 2-3 weeks earlier than durum resulting in a partial fallow effect. Although WUE was lower after a durum monoculture than wheat grown after broadleaf crops, the trend was not significant (p=0.11) .

Inoculation of pulse crops can lead to increased N availability due to N fixation, of which the benefits can carry over into subsequent years. Even though there were yield differences between the treatments, these cannot be explained by N benefits since there were no significant differences in the soil nutrient pools. However, it is not possible to rule out the possibility that the yield increases of durum following pulses are not a result of soil N benefits. There are many factors that may have affected the results. For example, the two soil samples taken in between the rows may have diluted the in row samples when combined for the composite sample. The analysis was also not representative of the entire root zone, considering that durum roots may advance below the 7.5 cm

A 3-yr study by Miller et al. (2003), conducted both on clay and a silt loam site, showed wheat yield increases of 35% and 14%, respectively, following a broadleaf crop. The differences between sites could neither be explained by WUE or the soil N pool, since fertilizer N had been adjusted to eliminate this effect. It has also been reported that yield variability following pulse crops could be caused by a lack of N from ineffective nodulation or that the effects of N may be limited due to

Table 3.11 Water use efficiency (WUE) of durum plants following durum, chickpea, lentil, pea and canola.

Previous Crop	2004		2005	
	---------- kg ha^{-1} mm^{-1} ----------			
	WUE Straw	WUE Grain	WUE Straw	WUE Grain
Chickpea	19.53ab†	14.34a	21.03a	11.17ab
Lentil	19.13ab	15.13a	17.94a	12.54ab
Pea	22.54b	16.90a	20.05a	13.37b
Durum	17.71a	12.86a	17.55a	10.26a
Canola	19.83ab	13.69a	24.29a	13.46b

† Values for treatment means (n=3) with the same letter within columns are not significantly difference at p=0.05 as determined by Tukey's test.

unpredictable climate . A long-term study by Campbell et al. reported that potentially mineralizable N from a lentil in a crop rotation was small in the initial years but increased with time, suggesting a cumulative effect. Other non-N benefits such as increased microbial activity , disease reduction, or hydrogen fertilization activity may have played a significant role.

The increased nitrogen fixation activity, and subsequent yield increase resulting from inclusion of pulses in rotation may be directly related to the inoculant strain used. Pea and lentil were inoculated with Nitragin C (*Rhizobium leguminosarum*). This may partially explain the higher yields of durum after pea and lentil, but not after the chickpea that was inoculated with Nitragin GC (*Mesorhizobium ciceri*). Dong et al. (2003) revealed that there were significant plant growth responses in soils pretreated with H_2, applied to simulate hydrogen release from nodules lacking Hup capability, in both a legume crop (soybean) and non-legume crops (barley, canola and wheat). Plant biomass of canola, cereals, and un-inoculated legumes revealed increases as high as 30%. The increased plant growth using H_2 fertilization systems may explain some of the increased soil fertility and plant growth as a non-N benefit of legumes in rotation.

3.5 Conclusion

By the end of the growing season, the crop preceding durum had an effect on durum production but not on soil nutrient pools. Previous crops of pea and lentil triggered high yields despite reduced durum emergence in 2005. Since the N fertilizer adjustment did not neutralize the pulse crop effect on durum yield, it is assumed that non-N benefits play a substantial role in this effect. The yield increase following pea and lentil may be partially attributable to increased WUE, differences in microbial activity, or possibly a factor such as hydrogen uptake activity. Diversifying a cropping rotation to suit the conditions of a region can improve nutrient uptake, soil quality, and microbial activity . In general, pulse crops appear to have a positive effect on a subsequent cereal crop in the northern Great Plains .

1. RESPONSE OF ARBUSCULAR MYCORRHIZAL FUNGI AND CHANGES IN MICROBIAL COMMUNITY COMPOSITION TO PULSE CROPS IN A DURUM BASED CROPPING SYSTEM

1.1 Introduction

Microbial community composition in soil is influenced by a variety of factors. Bossio et al. ranked the importance of different environmental variables on changes in microbial community composition in decreasing order of importance as: soil type, sampling time, specific farming operation (i.e., fertilization), management system, and spatial variation in the field.

To adequately exploit the benefits of AMF in agricultural production, we must understand their dynamics to have a predictable and consistent outcome. Changes in AMF population dynamics in response to controlled variables may be measured through various microbial techniques. Relative difference in inoculum levels may be determined at the beginning of the growing season to be used as a baseline.

Various methods have been identified for determining the inoculum potential of soil . The traditional method for estimating AMF in soil involves wet sieving the soil to separate out spores and hyphae, followed by quantification through microscopic counts. The most probable number method (MPN) may be used when it is difficult to measure the number of organisms in soil . Since spore counts will not include other infective propagules in the soil, the MPN method is a more accurate indicator of viable AMF in the soil . The MPN method, which was developed as a method in bacteriology, involves serial dilutions of the soil, growth of a trap plant, and analysis of the colonization of their roots. The MPN of infective propagules is

significantly correlated to the number of spores found in the soil . Since the mathematical calculations are time-consuming, statistical tables have been developed for estimating the MPN and more recently, computer programs to calculate for more possible combinations .

A convenient method for determining mycorrhizal potential is an infectivity assay. This method is appropriate in comparing the mycorrhizal potential in soils of differing treatments. To conduct this assay, trap plants are grown in the soil to be assayed. After a few weeks, when colonization has started but before it reaches its full potential, the plants are examined for colonization . The timing of analysis is important because at the point of maximum colonization, plants of different treatments may become uniformly colonized and thus no differences observed.

A new method to examine the size, nature, and physiological state of the soil microbial community by extracting, purifying, and analyzing fatty acid methyl esters (FAME) from soil now exist . Phospholipid fatty acids (PLFA) are valuable indicators of the active soil microbial community because they are structurally diverse, highly biologically specific, and are able to characterize living biomass as well as changes in the microbial community . Phospholipids are rapidly released after cell death and quickly metabolized, resulting in the sole measurement of living or active biomass. Phospholipid fatty acids measurements can be used to characterize the living or active biomass while PLFA profiles represent soil microbial community structure. The marker commonly used as fungal biomass indicator is C18:2ω6, while C16:1ω5 is the AMF indicator. Even though PLFA C16:1ω5 can also be found in the cell membranes of some bacteria, the NLFA is specific to AMF (Olsson, 1999). This method does not allow for fine scale analysis of diversity.

1.2 Objectives and hypothesis

I hypothesized that pulse crops would increase the inoculum potential of soil and this includes colonization in a subsequent durum crop, resulting in a higher abundance of AMF and a change microbial community composition. The objective of this study was to: (1) determine if AMF inoculum potential and colonization in a durum crop was significantly affected by cropping history and (2) assess the impact of pulses in crop rotations on microbial community abundance and dynamics. This hypothesis was tested using a combination of laboratory, growth chamber and field experiments.

1.3 Materials and Methods

1.3.1 Soil and root sampling

Since the microbial population is dynamic and changes over the growing season, sampling was completed at four times. Soil samples and root samples were taken at intermediate growth stages between emergence, five-leaf, flag-leaf, anthesis, and physiological maturity (Table 4.1) as predicted using the degree-day (Equation 4.1) model of AC Avonlea crop development (Hong Wang unpublished). The growing degree day is calculated each day beginning the day after plant emergence, using a base temperature of zero.

$$\text{Growing Degree Day} = \sum_{n}^{1} \left[\frac{T_{max} + T_{min}}{2} \right] - \text{Base Temperature} \qquad [4.1]$$

Two between rows and two within row soil samples were taken to a depth of 7.5 cm along a diagonal transect with a hand operated soil sampler (5 cm diameter). The samples were bulked into one composite sample, put through 2 mm sieves, and stored at -12^{o}C until fatty acid methyl ester (FAME) analysis. Two plant roots were removed using a trowel at the four locations along the transect for mycorrhizal root colonization determinations. Soil sampled at emergence was used for the AMF infectivity assay in the growth chamber. Soil samples for the MPN assay were taken

between five-leaf and flag-leaf in 2005 only. One FAME analysis from chickpea, pea, lentil, durum and canola was completed using 4 g of soil on a dry mass equivalent basis. Soil temperature and moisture were monitored continuously over the growing season using time-domain reflectrometry in 2 replicates only (Figure 4.1).

1.3.2 Root colonization

Roots were washed thoroughly after sampling to remove any adhering soil, while placed over 2 mm sieves to minimize fine root loss. The durum roots were then cut into 1 cm fragments and 2 replicates from each plot were placed in plastic cassettes. The roots were then cleared and stained using an ink vinegar solution as described by Vierheilig et al. . The percent root colonization for each plot was determined using the gridline-intersect method from Giovanetti and Mosse ..

1.3.3 Inoculum potential of arbuscular mycorrhizal fungi in soil

Pre-germinated leek seeds were planted in 100 g of soil of appropriate dilution. Leek plants were used as the test plant because they have a high mycorrhizal dependency. Dilution soils were disinfected by autoclaving for 1 h periods on two consecutive days. Soils were diluted using autoclaved soil at 1, ¼, 1/64, 1/256, and 1/1024 by shaking for 10 min using a twin shelf dry blender (The Patterson Kelly Co., Pennyslvania). Each dilution was repeated 5 times for each treatment of pea, lentil, chickpea, durum and canola. The plants were watered to saturation daily and harvested after 46 days. The roots were prepared as described in Section 4.3.2 and rated as positive in the presence of colonization and negative for lack of colonization. Most probable number estimations for mycorrhiza were made using a computer MPN calculator .

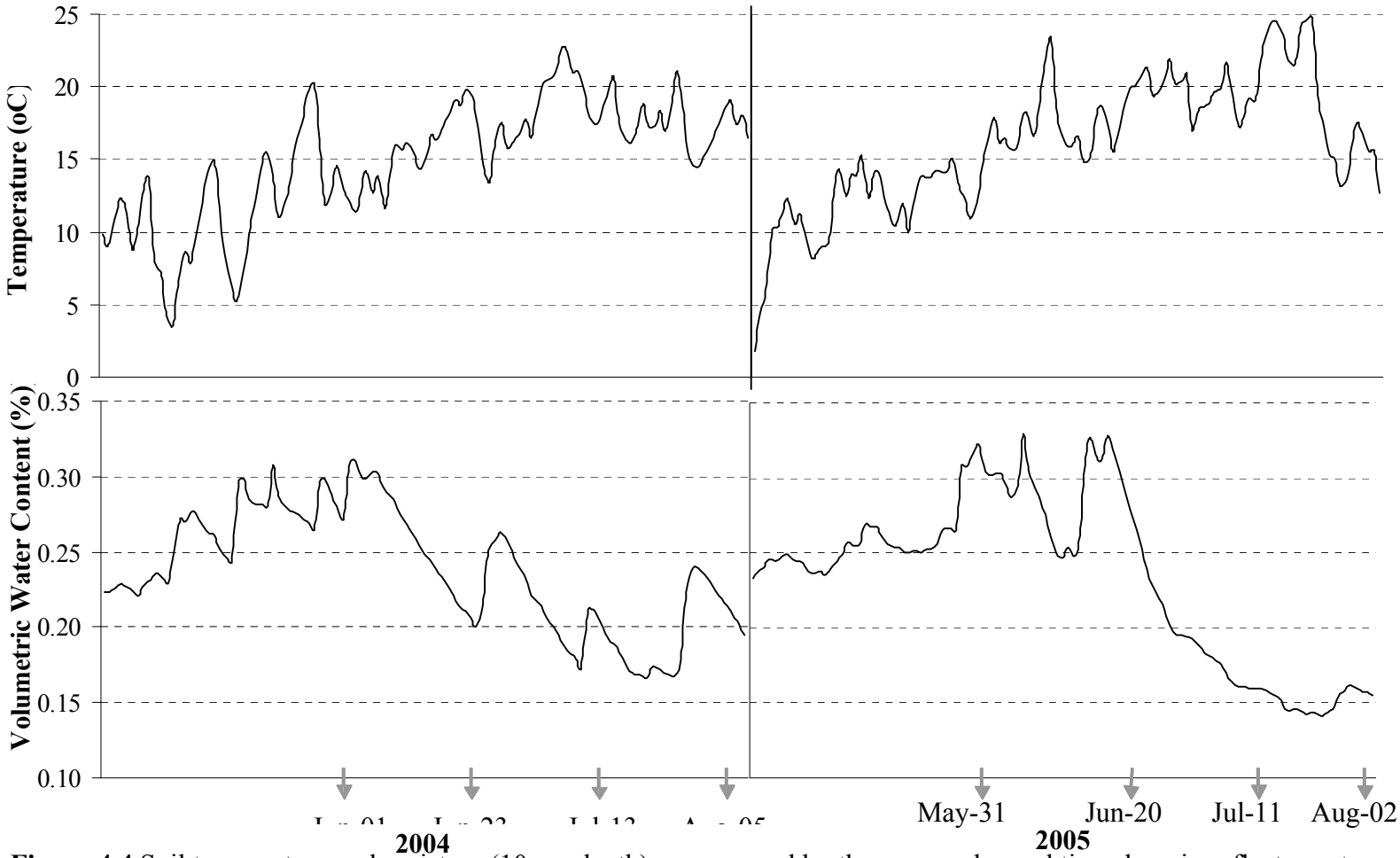

Figure 4.4 Soil temperature and moisture (10 cm depth) as measured by thermocouples and time domain reflectrometry probes. Data is the average collected at 2 replicates only over the growing season. Arrows represent sampling times during the growing season.

Table 4.12 Sampling dates in 2004 and 2005 as determined by growing degree days for AC Avonlea.

Growth stage/code	Target degree day †	Actual sampling degree day		Sampling date	
		2004	2005	2004	2005
Emergence	0				
T1.5	179	189.85	211.65	Jun-01	May-30
Five-leaf	357				
T2.5	485	466.35	512.45	Jun-23	Jun-20
Flag leaf	613				
T3.5	773	781.85	874.45	Jul-13	Jul-11
Anthesis	933				
T4.5	1241	1205.10	1245.20	Aug-05	Aug-02
Physiological Maturity	1550				

† Target degree days were determined using a model for AC Avonlea durum developed by Wong, H. (unpublished).

1.3.4 Infectivity potential of arbuscular mycorrhizal fungi in soil

Pre-germinated durum seeds were planted in 20 g of soil taken from the field study at the beginning of the growing season. Four plants per plot were grown for a total of 12 per treatment in the growth chamber with 23ºC/18ºC day/night temperatures and a photoperiod of 16 hours. Plants were watered daily and four control plants were established to monitor colonization levels. When control plants reached colonization levels of 20-30% (after 25 days in 2004 and 35 days in 2005) the plants were harvested and percent mycorrhizal colonization was determined using the procedure described in Section 4.3.2.

1.3.5 Fatty acid methyl esters (FAME) analysis

The soil microbial structure and fungi physiological status were determined through analyses of PFLA/NLFA. Lipid extraction and preparation for analysis was done using a procedure modified from Peterson and Klug and previously described in detail by . Briefly, lipids were extracted from the soil and separated into fractions using silica gel filled columns. The fatty acids of the phospholipids and neutral lipid fractions were transmethylated and analyzed by gas chromatography on a VARIAN 3900 GC with a flame ionization detector, a CP-8400 auto sampler and Star Chromatography software.

Peaks were identified through comparison to standards using Supelco Bacterial Acid Methyl Esters (#47080-U), MJS Biolynx #MT1208 for 16:1ω5, linoleic acid, 18:2ω6c, as indicator for saprotrophic fungi , and C19:0 as an internal standard. Results from chosen bacterial indicators (Table 4.2) were the summed means for the bacterial index.

1.3.6 Statistical analysis

Field plots were replicated three times using a randomized complete block design where precrop species was the main treatment. Variations in the soil microbial community were measured throughout the growing seasons in 2004 and

Table 4.13 Systematic names of fatty acids chosen to represent bacteria for fatty acid methyl ester (FAME) analysis.

Fatty acids	Systematic name†
C12:0	Methyl dodecanoate
C13:0	Methyl tridecanoate
2-OH C12:0	Methyl 2-hydroxydodecanoate
3-OH C12:0	Methyl 3-hydroxydodecanoate
iC15:0	Methyl 13-methyltetradecanoate
2-OH C14:0	Methyl 2-hydroxytetradecanoate
3-OH C14:0	Methyl 3-hydroxytetradecanoate
iC16:0	Methyl 14-methylpentadecanoate
iC17:0	Methyl 15-methylhexadecanoate
C17:0	Methyl heptadecanoate
2-OH C16:0	Methyl 2-hydroxyhexadeanoate
C18:1cis	Methyl cis-11-octadecanoate
C18:0	Methyl octadecanoate

†Source: Stenerson, 2007

2005. Shapiro-Wilks' test was used for normality assessments and non-normal data was transformed when allowable . Normality could be reached across all sampling times for mycorrhizal fungi PLFA (2004 and 2005), mycorrhizal fungi NLFA (2004 only), saprotrophic fungi NLFA (2004 only), and bacteria (2005 only). The effects of treatment and time were measured using repeated-measures analysis of variance (MANOVA) using JMP v.3.2.6 (Cary, NC, USA) for these variables. When MANOVA was not possible, time periods were split and analysed using ANOVA. Tukey's test was used for comparison of means at p=0.05 using SYSTAT v.10 (Point Richmond, USA). Normality could not be achieved for Shannon-Weaver biodiversity indices and several fatty acids. Kruskal-Wallis nonparametric tests were used to determine the significance of treatment effects on these variables (SYSTAT v.10). The Shannon Index of diversity (Equation 4.2) was calculated for each plot where p_i = peak area of the peak over the area of all peaks (Spellerberg and Fedor, 2003). A higher index reflects higher biodiversity of the microbial community.

$$H = -\sum_{i=1}^{n} pi \ln pi \qquad [4.2]$$

Regression analysis revealed relationships between the most probable number of AMF propagules, infectivity potential, and AMF root colonization (JMP v.3.2.6). Spearman's correlation analysis was used to display associations between soil microorganisms, soil nutrients, and durum grain yield (SYSTAT v.10).). Discriminant analysis was used to assess the changes in the soil microbial communities over time, as defined by FAME fingerprints using Systat v.10. All sampling and statistical analysis was completed in 2004 and 2005 unless otherwise specified.

1.4 Results and discussion

1.4.1 Potential of arbuscular mycorrhizal fungi in soil

The results from the infectivity assay of arbuscular mycorrhizal fungi in durum roots with previous crops of chickpea, pea, lentil, durum and canola was inconsistent between years (Table 4.3). In 2004, there was significantly lower

infectivity potential when Argentine canola preceded durum (4.7%) than all other rotations. The soil from plots with chickpea preceding durum resulted in the highest average colonization of 15.7%, followed by pea (12.1%). In 2005, durum after durum resulted in the highest colonization levels (24.9%). Soil from durum following pea, lentil, and canola resulted in significantly lower colonization than the durum monoculture.

The MPN assay, completed only in 2005, revealed similar results to the infectivity assay (Table 4.4). The durum treatment had the most viable propagules per gram of soil (39), followed by chickpea (21), pea (15), and lentil (10). Canola preceding durum resulted in the lowest number of infected propagules at 7 per gram of soil (Table 4.5). The lower inoculum potential of durum following canola was expected because of the typical delayed colonization after non-host crops (Hamel, 1996). The consistency in results for all treatments with no positive evaluations at any lower dilution than a negative appears (Appendix B), indicating that the experiment met the basic assumption of MPN that the organism of interest was able to produce positive results . The last dilution for all treatments resulted in negatives. However, AMF numbers may be underestimated if the fungi are specific to the host plant being investigated .

Even though the infective mycorrhizal potential is a more accurate alternative to this method, it is extremely time consuming. The mycorrhizal soil infectivity (MSI_{50}) is measured using plant populations grown in a dilution series of soil. An MSI_{50} unit is then determined by calculating the amount of soil required for colonization of 50% of the plants . The estimates may also be affected by the duration of the test and the temperature (Liu, 1994). The MPN estimates were tested using regression analysis with the actual infectivity potential at the beginning of the growing season.

The number of infective mycorrhizal propagules at the beginning of the growing season may influence root colonization. A regression analysis between the infectivity potential assay and the MPN assay (Figure 4.2b) revealed a positive

Table 4.14 Infectivity potential for arbuscular mycorrhizal fungi, using field soil at the beginning of the growing season in 2004 and 2005 as influenced by previous crop in a growth chamber experiment.

Previous Crop	Mycorrhizal root colonization (%)	
	2004	2005
Chickpea	15.7 a†	13.2 ab
Pea	12.1 a	9.9 b
Lentil	9.8 a	9.6 b
Durum	10.7 a	24.9 a
Argentine Canola	4.7 b	10.0 b

† Values for treatment means (n=3) with the same letter within columns are not significantly difference at p=0.05 as determined by Tukey's test.

Table 4.15 Estimates of infective propagules of mycorrhizal fungi per gram of soil and upper and lower 95% confidence intervals (CI) as determined by most probable number determinations using field soil at the beginning of the 2005 growing season.

Previous Crop	# of infective propagules	Upper 95% CI	Lower 95% CI
Chickpea	21	54	8
Pea	15	35	6
Lentil	10	23	4
Durum	39	89	17
Canola	7	17	3

relationship (r^2=0.93, p=0.01). This suggests that higher numbers of AMF propagules in soil results in greater infectivity in durum roots, when tested in a greenhouse setting. A similar relationship existed (Figure 4.2a) between the estimated number of AMF propagules for the different treatments and the mean durum root colonization for all sampling times in 2005 (r^2=0.83, p=0.03). Past research has shown that the MPN of viable AMF propagules shows a significant positive correlation with spore counts (p<0.0001) . The number of propagules calculated in the soil was affected by cropping history, with corn being higher than soybean.

1.4.2 Root colonization

Mycorrhizal colonization after the pulse crops was high, especially in lentil-treated plots where durum exhibited the highest colonization between the five-leaf and flag-leaf stages of development (Table 4.5). The 2004 growing season showed significant differences (p<0.05) between treatments only at the third sampling date with lentil preceding durum resulting in significantly higher mycorrhizal root colonization (22.3%) when compared with canola (2.3%).

Using canola as a precrop resulted in no colonization for the first 2 sampling times, with a maximum colonization reaching only 5.7% between anthesis and maturity. Since canola is a non-mycorrhizal crop, it was expected that AMF development would be delayed in the subsequent durum crop, but the treatment effect did not taper off at the end of the growing season. The impact of canola precrop in this case, is compounded by the impact of fallow in the rotation stage I i.e., the year before canola cropping.

Over the 2005 growing season, the durum monoculture had significantly higher (p<0.05) mean root colonization (20.3%) than all other treatments. Splitting the sampling times revealed significant differences for the first three sampling times. On May 31st, percent mycorrhizal colonization of durum following lentil (10.6), durum (8.7), and chickpea (7.6) were significantly higher than canola (0.7). At the June 20th sampling, pea and durum were significant (p<0.05) with only durum

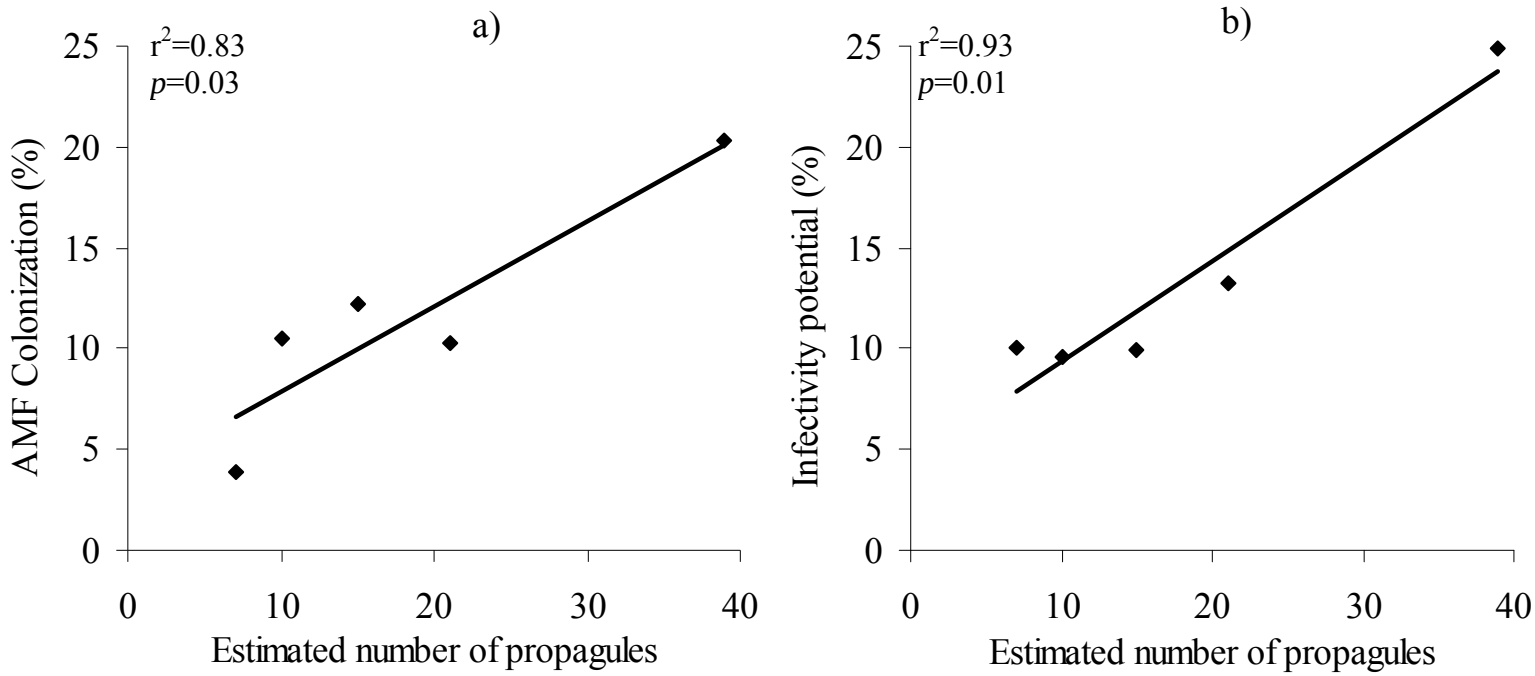

Figure 4.5 Regression analysis of the estimated number of propagules and a) arbuscular mycorrhizal fungi (AMF) colonization and b) infectivity potential (N=5) in spring 2005.

Table 4.16 Effect of previous crop on mycorrhizal colonization of durum wheat roots in 2004 and 2005.

Previous Crop	Mycorrhizal root colonization (%)							
	2004				2005			
	Jun-01	Jun-23	Jul-23	Aug-05	May-31	Jun-20	Jul-11	Aug-02
Chickpea	0.0†	7.3	16.6 ab	19.3	7.6 a	8.0 ab	12.7 ab	11.7
Pea	1.0	5.6	12.6 ab	23.7	4.0 ab	14.0 a	15.3 ab	14.3
Lentil	0.0	4.1	22.3 a	28.0	10.6 a	8.0 ab	11.7 ab	12.3
Durum	0.0	6.0	13.3 ab	14.7	8.7 a	26.3 a	23.0 a	20.3
Canola	0.0	0.0	2.3 b	5.7	0.7 b	3.0 b	6.0 b	11.3

† Values for treatment means (n=3) with a different letter within columns are not significantly difference at p=.05 as determined by Tukey's test. Absence of letters indicates no significant difference

showing significance at the following July 11th sampling. The August 2nd sampling indicates that the effect of treatment had tapered off near the end of the growing season, since there were no significant differences. However, the colonization of canola preceding durum at the final sampling date in 2005 was more than double that of 2004.

Previous studies have also found that there is a lag phase of AMF colonization, followed by an increase and then a leveling off. Sampling time has a strong influence on colonization determinations and in general, colonization increases with time over the growing season . The change in colonization levels may also differ between plant species. For example, after studyingAMF colonization of wheat plants, Talukdar and Germida reported colonization in wheat plants at the tillering stage, which then increased until panicle emergence, and then declined. Lentil, however, showed rapid increases until flowering and then leveled off . Gavito et al. reported that a previous crop of canola significantly affected mycorrhizal colonization until the 6 leaf stage, and never exceeded 10%. In comparison, pre-maize plants were colonized 45% when at the 3 leaf stage of plant development. Previous cropping with an AMF non-host reduced colonization in the subsequent host crop . In addition, canola has been reported to release toxic compounds from the roots affecting soil microorganisms .

The relationship between mycorrhizal infectivity potential of durum roots at emergence and mean AMF colonization (%) over the growing season was looked at using regression analysis. In 2004, a positive relationship existed but revealed that they were not highly related (r^2=0.35, p=0.02), indicating that the infectivity potential in spring had a slight effect on the level of mycorrhizal colonization in durum roots over the season (Figure 4.3a). The second year, revealed a similar but less significant dependency (r^2=0.22, p=0.10) of AMF colonization on the infectivity potential (Figure 4.3b).

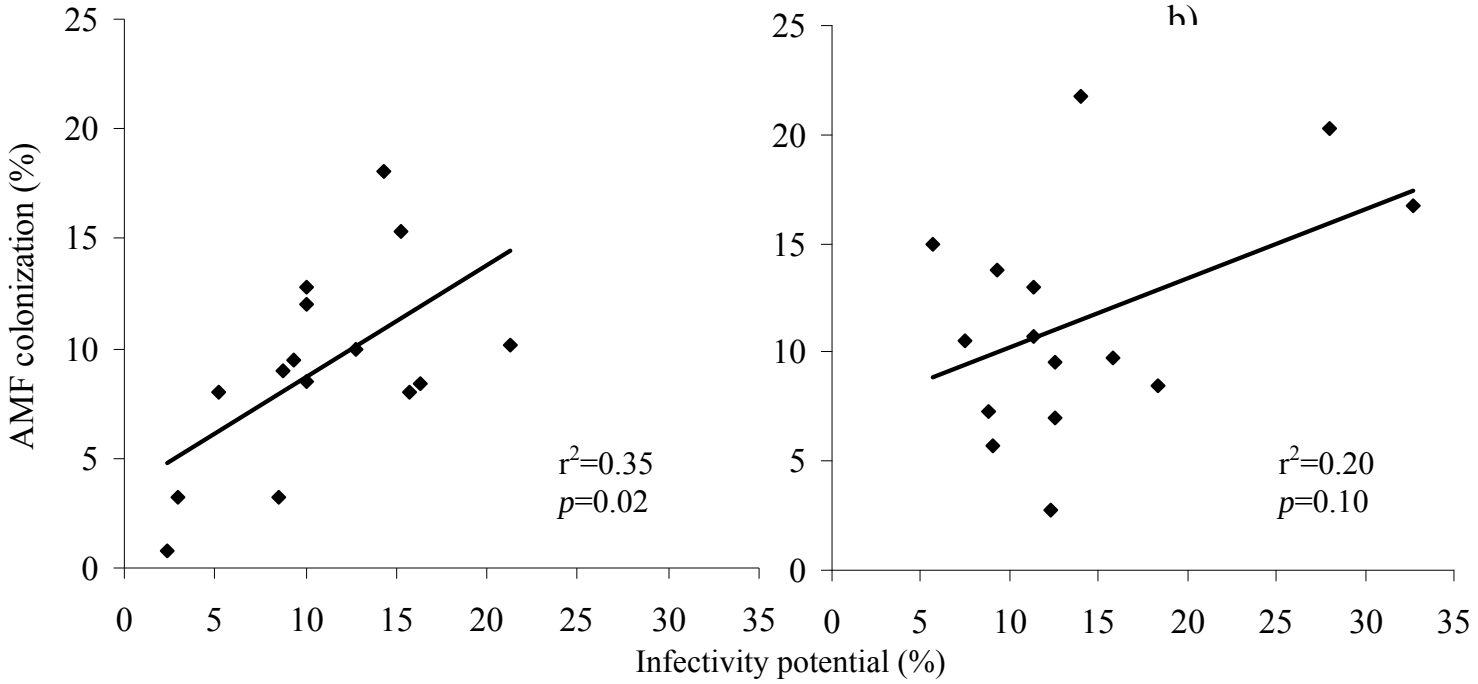

Figure 4.6 Regression analysis of the infectivity potential of mycorrhizal fungi in durum roots at the beginning of the growing season and actual mean mycorrhizal colonization in durum roots at four sampling times in the field during a) 2004 and b) 2005 (N=15).

1.4.3 Fatty acid methyl esters (FAME) analysis

The results for phospholipids and neutral lipid fatty acids in 2004 and 2005 are displayed in Table 4.6 as the means for AMF, saprotrophic fungi, and bacteria. Data could be normalized for mycorrhizal fungi PLFA (2004 and 2005), mycorrhizal fungi NLFA (2004 only), saprotrophic fungi NLFA (2004 only), and bacteria (2005 only). The effects of preceding crops on these variables were measured using MANOVA to determine the effect of treatment, time, and treatment x time. This analysis did not reveal any significant effects of treatment, time, or interaction of the variables measured ($p<0.05$).

The remaining fatty acids analysed with Kruskal-Wallis non-parametric tests revealed that there were also no significant differences between treatments ($p<0.05$) (Table 4.6). However, saprotrophic fungi (C18:2ω6c), in 2004, showed significant differences between treatments at $p=0.06$. Canola preceding durum resulted in the highest relative number of saprotrophic fungal NLFA at 0.033 µg per gram of soil, followed by lentil (0.031), chickpea (0.023), and pea (0.021). The durum treatment had the lowest number at 0.014 µg per gram of soil, which was also the case for all other fatty acids calculated except C18:2ω6c (NLFA) in 2004 and C16:1ω5 (PLFA and NLFA) in 2005. In general, the results from FAME analysis were inconsistent between years. In 2004, pulse crops (chickpea, pea, and lentil) preceding durum resulted in higher values for mycorrhizal fungi (NLFA and PLFA) and the bacteria group, than canola and durum. Since C16:1ω5 PLFA may also be used to characterize gram negative bacteria, the neutral lipid portion may be a better representative of AMF levels in the soil because it is only used to characterize storage compounds in fungi. Higher levels of AMF storage compounds in the soil ultimately should result in more mycorrhizal colonization of plant roots, if other factors remain favourable.

In 2005, there were no obvious trends but a few interesting observations. Canola preceding durum resulted in the highest relative values of mycorrhizal fungi PLFA and all bacteria PLFA. This was not predicted considering that canola has been reported to release toxic compounds such as isothiocyanates, which can have a

Table 4.17 Impact of previous crop on PLFA and NLFA (μg/g) of mycorrhizal fungi (C16:1ω5), saprotrophic fungi (C18:2ω6c) and all bacteria.

Previous Crop	C16:1ω5		C18:2 ω6c		All Bacteria
	PLFA	NLFA	PLFA	NLFA	PLFA
			2004		
Chickpea	0.341 *(0.10)*†	0.120 *(0.04)*	0.019 *(0.003)*	0.023 *(0.01)*	3.585 *(0.70)*
Pea	0.290 *(0.06)*	0.120 *(0.07)*	0.019 *(0.002)*	0.021 *(0.003)*	4.810 *(1.54)*
Lentil	0.593 *(0.30)*	0.401 *(0.30)*	0.022 *(0.003)*	0.031 *(0.004)*	3.674 *(0.42)*
Durum	0.198 *(0.03)*	0.052 *(0.01)*	0.023 *(0.004)*	0.014 *(0.004)*	2.789 *(0.29)*
Canola	0.271 *(0.04)*	0.063 *(0.02)*	0.029 *(0.004)*	0.033 *(0.01)*	3.419 *(0.28)*
			2005		
Chickpea	0.085 *(0.02)*	0.037 *(0.01)*	0.016 *(0.01)*	0.026 *(0.01)*	1.657 *(0.35)*
Pea	0.100 *(0.02)*	0.056 *(0.02)*	0.007 *(0.003)*	0.026 *(0.01)*	2.062 *(0.33)*
Lentil	0.070 *(0.01)*	0.039 *(0.01)*	0.008 *(0.01)*	0.096 *(0.08)*	1.780 *(0.19)*
Durum	0.085 *(0.02)*	0.184 *(0.11)*	0.005 *(0.003)*	0.012 *(0.01)*	1.480 *(0.23)*
Canola	0.165 *(0.04)*	0.064 *(0.03)*	0.012 *(0.01)*	0.064 *(0.05)*	2.216 *(0.26)*

† Values are treatment means (n=12) with the standard error of the mean displayed in parentheses.

strong detrimental effect on the active microbial community . Durum mycorrhizal fungal NLFA was almost three times higher (0.184 μg/g) than canola (0.064 μg/g), the next highest value and as with other factors, was not significant (p>0.05).

The soil microbial community is dynamic and can fluctuate over the growing season, and between years. For example, mycorrhizal fungal NLFA of the durum monoculture went from being the lowest in 2004, to the highest in 2005. It is well known that populations in the food web fluctuate in a non-stable equilibrium, as influenced by the abiotic environment conditions (Neutel et al., 2007; Hamel et al., 2006). At one point, one population is high and later another population is high.

In general, analysis of neutral and phospholipids fatty acids has been a valuable tool for microbial community analysis. Arbuscular mycorrhizal fungal biomass is adequately represented by C16:1ω5 PLFA . The 16:1ω5c and C18:2ω6c are useful for distinction between relative abundance of AMF and saprotrophic fungi in a field experiment . Bossio et al., reported that PLFA profiles were replicable when evaluating environmental factors of the soil microbial community under different cropping management. Even though FAME analysis did not appear to be affected by treatment, many of the fatty acids were affected by time. Mycorrhizal fungal PLFA and NLFA (Figure 4.4a, b) were significantly affected by sampling time in 2004 (p=0.05). The greatest change in relative values appears to occur between the July 13 and August 5 dates. In 2005, the relative values did not follow the same trends (p=0.06) and were lower at the final August 2nd sampling date than they were at the initial May 30th sampling.

Saprotrophic fungal PLFA in 2004 showed significant differences (p=0.05) and also had increased at the August 5 sampling date (Figure 4.4c). The 2005 FAME analysis resulted in more fluctuation over the growing season, and dropped off at the end of the season (p=0.08). The relative value of storage compounds for saprotrophic fungi (NLFA) was highly insignificant in 2005 (Figure 4.4d). Although there appears to be a sharp increase at the July 11 sampling time, the change is not significant (p<0.05) and shows a high degree of standard error. Fluctuations over

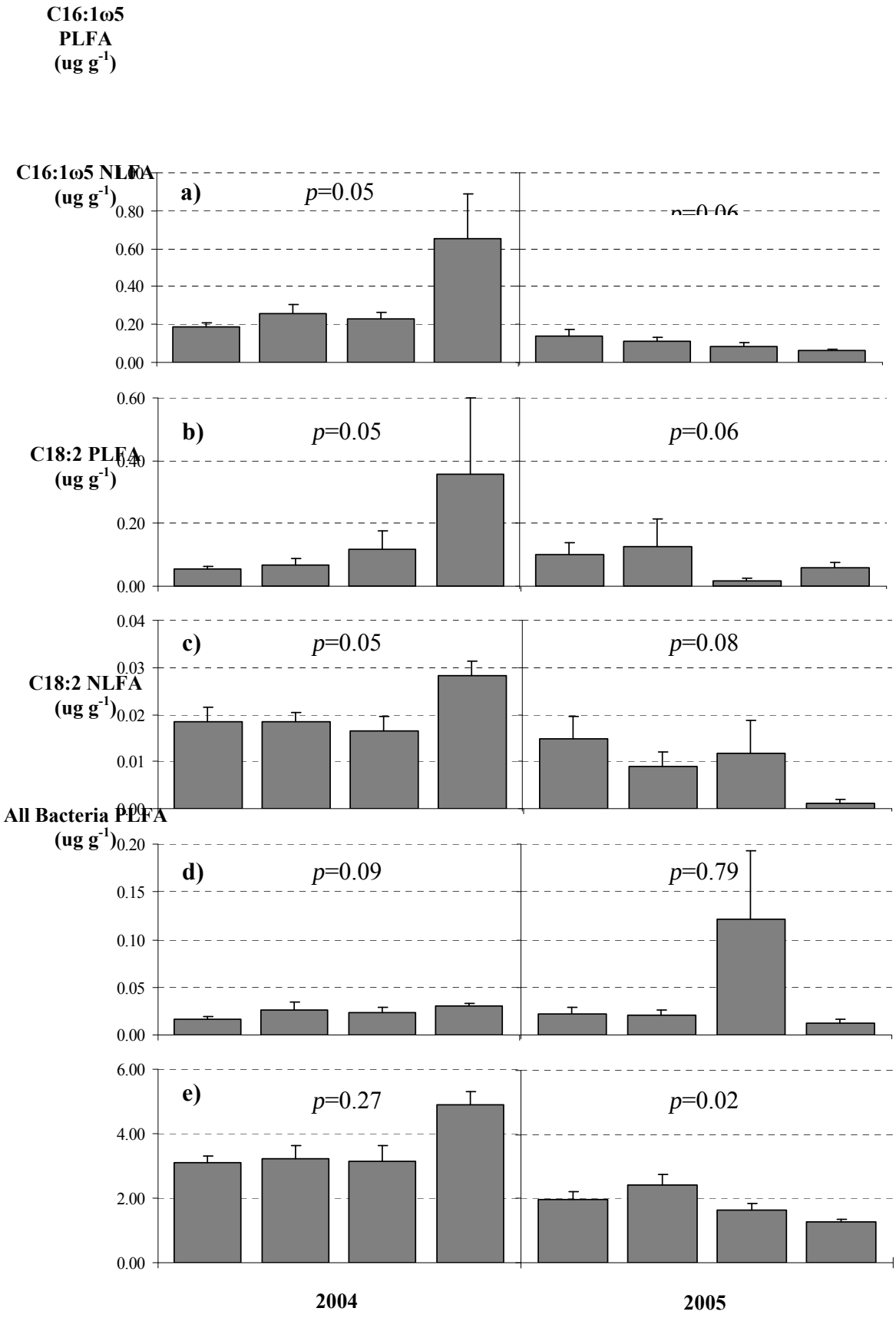
C16:1ω5 PLFA (ug g-1)
C16:1ω5 NLFA (ug g-1)
a)
p=0.05
p=0.06
0.00
0.20
0.40
0.60
0.80
C18:2 PLFA (ug g-1)
b)
p=0.05
p=0.06
0.00
0.20
0.40
0.60
C18:2 NLFA (ug g-1)
c)
p=0.05
p=0.08
0.00
0.01
0.02
0.03
0.04
All Bacteria PLFA (ug g-1)
d)
p=0.09
p=0.79
0.00
0.05
0.10
0.15
0.20
e)
p=0.27
p=0.02
0.00
2.00
4.00
6.00
2004
2005

Figure 4.7 Variation in the microbial abundance and community status as evaluated with fatty acid methyl ester (FAME) indicators showing relative abundance of (a) active arbuscular mycorrhizal fungi; (b) storage lipids in arbuscular mycorrhizal fungi; (c) active saprotrophic fungi; (d) storage lipids in saprotrophic fungi; and (e) active bacteria in 2004 and 2005. Bars represent standard errors of the means.

Considering that soil microbes are closely associated with their environment, it is understandable that the microbial community changes would reflect changes in environmental conditions more than other variables .

The Shannon index of diversity was calculated from PLFAs to determine diversity differences as influenced by previous crops (Table 4.7). Since the data could not be normalized, significant differences were tested using the Kruskal-Wallis nonparametric test. This revealed that neither treatment (p=0.95) nor time (p=0.07) were significantly different (p<0.05) in either year. Bossio et al. looked at PLFA profiles from organic, low-input, and conventional farming systems and found no difference in the Shannon's diversity index (based on PLFA relative abundance) for management system or time.

In 2004, the indices for durum following canola were highest followed by pea, durum, and chickpea (Table 4.8). Durum following lentil resulted in the lowest biodiversity index of 2.287, although the differences were not significant (p<0.05). The second year revealed slightly different results with previous crop of durum having the highest biodiversity index, followed by canola. This is similar to results published by Lupwayi et al. that the Shannon index for bacterial functional diversity of bulk soil indicated that the cereal rotation of barley-barley monoculture (2.33) was higher than the canola-canola (1.96) and inoculated pea-barley rotation (1.67). The same trend occurred when the functional diversity of the rhizosphere was measured, although the differences were non-significant (p>0.05). In comparison, Lupwayi reported that microbial diversity increased under wheat when preceded by pea or red clover, compared to wheat monoculture. In a similar study, Oehl et al. reported that crop rotations with lower input levels had a significantly higher diversity index (p<0.05) than sites of maize monocropping with high input levels.

The treatments were combined to reveal overall shifts in the microbial community over the growing season (Figure 4.5). A similar analysis from a long-

Table 4.18 Shannon index of diversity calculated from PLFAs to determine diversity differences as influenced by previous crops.

Previous Crop	PLFA	
	2004	2005
Chickpea	2.306 *(0.05)*†	2.277 *(0.03)*
Pea	2.327 *(0.02)*	2.188 *(0.07)*
Lentil	2.287 *(0.06)*	2.242 *(0.05)*
Durum	2.315 *(0.02)*	2.285 *(0.06)*
Canola	2.342 *(0.02)*	2.264 *(0.05)*

† Values for treatment means (n=12) with the standard error of the mean in parentheses.

term study in close proximity to our study site by Hamel et al. also used PLFA profile to reveal changes in microbial community structure with time ($p<0.0001$) and suggested that sudden events such as heavy rainfall on a dry soil may play a substantial role in changes of the active soil microbial population in this area. To detect possible changes over time, relationships were evaluated between soil microorganisms, soil N and P, and durum grain yield within each year. In 2004, AMF root colonization was positively correlated with all measured factors except soil NO_3 flux and point in time NO_3 (Table 4.8). Previous studies have found that colonization is higher if moderate to low levels of P fertilization are applied to field soils , but there is little if any correlation with NO_3-N or plant available P . The majority of the soil nutrient analysis showed negative correlations with the fatty acid groups. Positive correlations existed between AMF colonization and all of the fatty acids with all others, indicating that if a factor causes one to change, the entire microbial community shifts.

In 2005, grain yield showed little association with AMF colonization (-0.01) or C16:1ω5 (-0.02) (Table 4.9). However, C16:1ω5 NLFA and C18:2 NLFA revealed a higher, negative correlation (-0.48 and -0.33 respectively). Bacterial PLFA showed little correlation (0.09) with durum grain yield, which is similar to results published by Lupwayi indicating that there was no correlation between bacterial populations and wheat yield. Mycorrhizal colonization showed little association with C16:1ω5 PLFA (0.08) and NLFA (0.05) but revealed a negative relationship with the remaining fatty acid groups. Wamberg et al. reported with investigating the effect of mycorrhizal fungus on microbial activity in the rhizosphere of pea plants that there was no significant effect of AMF on bacteria levels ($p=0.24$). They also stated that there is no consistent evidence that mycorrhizal colonization has a positive effect on soil bacteria. Contrary to the results in 2004, colonization was negatively correlated with all measured soil nutrient factors except NO_3 flux. Fatty acid groups C16:1ω5 PLFA, C16:1ω5 NLFA and C18:2 PLFA produced a negative correlation with the majority of measured soil parameters (Table 4.9).

1.5 Conclusion

The mycorrhizal potential and colonization in a durum crop was significantly affected by cropping history, but not consistently as a result of inclusion of pulse crops. The abundance of AMF and changes in the microbial community were not consistent between the two years, for both time and treatment showed significant effects ($p<0.05$) only when analysed for differences between the four sampling times within each year. This variability in results created difficulty in drawing conclusions and reveals the extent of the dynamic microbial community and many factors influencing these changes. This is also similar to the supporting scientific evidence revealing conflicting results concerning microbial community dynamics and influencing factors.

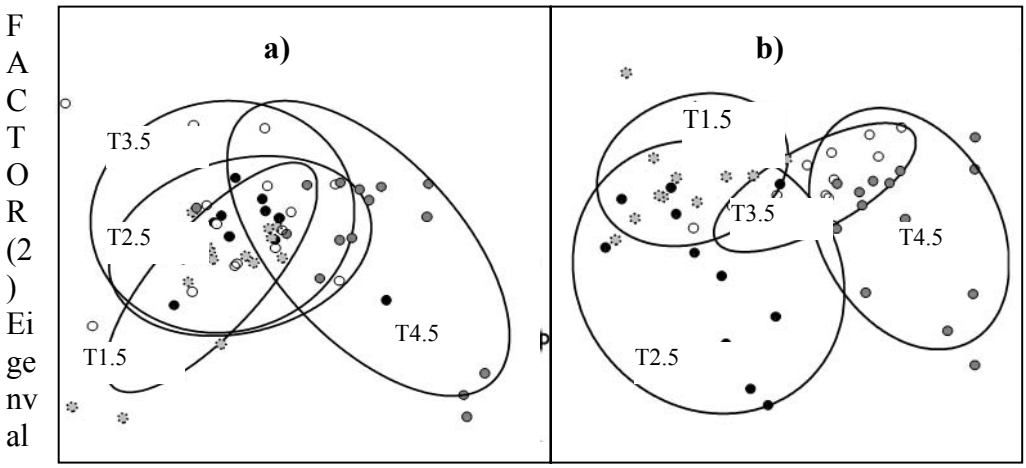

Figure 4.8 Discriminant analysis of the PLFA profiles over time in (a) 2004 and (b) 2005 ($p<0.001$, N=60). Sampling times refer to mid points between main physiological plant growth stages of (T1.5) emergence and five-leaf; (T2.5) five-leaf and flag leaf; (T3.5) flag leaf and anthesis; (T4.5) anthesis and physiological maturity.

Table 4.19 Spearman's correlations between soil microorganisms, soil nutrients, and durum grain yield in 2004.

	Grain yield	AMF Root†	C16:1ω5 PLFA	C16:1ω5 NLFA	C18:2 PLFA	C18:2 NLFA	Bacteria PLFA	Soil moisture	Soil NO_3 flux	Soil PO_4 flux	Soil PO_4	Soil NO_3	Soil NH_4
Grain yield	1.00												
AMF Root†	0.29	1.00											
C16:1ω5PLFA	0.06	0.40	1.00										
C16:1ω5NLFA	-0.14	0.44	0.80	1.00									
C18:2PLFA	-0.02	0.10	0.54	0.31	1.00								
C18:2NLFA	0.19	0.09	0.36	0.46	0.29	1.00							
BacteriaPLFA	0.37	0.27	0.40	0.16	0.50	0.27	1.00						
Soil moisture	0.20	0.12	-0.04	0.09	-0.36	0.25	-0.60	1.00					
Soil NO_3 flux	-0.04	-0.26	-0.21	-0.11	-0.29	-0.21	-0.26	-0.16	1.00				
Soil PO_4 flux	-0.07	0.06	-0.04	-0.17	-0.17	-0.03	0.41	-0.48	0.21	1.00			
Soil PO_4	0.13	0.22	-0.09	0.13	0.004	-0.21	-0.26	-0.16	0.06	-0.50	1.00		
Soil NO_3	0.39	-0.04	-0.18	-0.27	-0.57	-0.25	-0.15	0.01	0.58	0.19	-0.16	1.00	
Soil NH_4	0.35	0.24	0.24	0.13	-0.15	-0.15	0.06	-0.10	0.25	-0.16	-0.08	0.72	1.00

† AMF Root Colonization

Table 4.20 Spearman's correlations between soil microorganisms, soil nutrients, and durum grain yield in 2005.

	Grain yield	AMF Root†	C16:1ω5 PLFA	C16:1ω5 NLFA	C18:2 PLFA	C18:2 NLFA	Bacteria PLFA	Soil moisture	Soil NO_3 flux	Soil PO_4 flux	Soil PO_4	Soil NO_3	Soil NH_4
Grain yield	1.00												
AMF Root†	-0.01	1.00											
C16:1ω5PLFA	-0.02	0.08	1.00										
C16:1ω5NLFA	-0.48	0.05	0.39	1.00									
C18:2PLFA	0.25	-0.31	0.43	0.43	1.00								
C18:2NLFA	-0.33	-0.18	-0.20	0.39	-0.24	1.00							
BacteriaPLFA	0.09	-0.21	0.21	0.23	0.43	-0.14	1.00						
Soil moisture	0.42	0.10	-0.58	-0.33	-0.31	0.05	0.04	1.00					
Soil NO_3 flux	0.07	0.50	-0.02	-0.11	-0.19	0.01	-0.2	0.20	1.00				
Soil PO_4 flux	0.09	-0.43	0.08	-0.29	0.19	-0.15	0.07	-0.11	-0.43	1.00			
Soil PO_4	0.15	-0.63	-0.15	-0.31	-0.03	-0.04	0.31	0.05	-0.42	0.48	1.00		
Soil NO_3	-0.14	-0.48	-0.28	-0.08	-0.19	0.30	0.19	-0.12	0.09	0.01	0.56	1.00	
Soil NH_4	-0.54	-0.45	-0.15	0.10	-0.19	0.34	-0.14	-0.35	-0.66	0.10	0.17	0.14	1.00

†AMF Root Colonization

2. PULSE CROPS IN A DURUM-BASED CROPPING SYSTEM: IMPACT ON THE BIODIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI

2.1 Introduction

Biodiversity is important at all levels of an ecosystem, including below ground. Unknown numbers and species of microorganisms interact within the soil carrying out many different functions. Arbuscular mycorrhizal fungi (AMF) colonize the roots of most terrestrial plants and play an important role in soil ecology. In agricultural soils of the Brown Chernozem zone of Southern Saskatchewan it has been documented that AMF diversity is lower than in other soils of the province . Crop management can play an important role in increasing the biodiversity and abundance of AMF in a field. Management practices such as reduced tillage and inclusion of highly mycorrhizal host species are beneficial.

When measuring the importance of AMF in crop rotations, both the relative abundance and the species composition must be considered. In order to measure biodiversity of AMF, the species present must be identified. The traditional method of identification was to look at spore morphology. Besides being extremely tedious and time consuming, the use of spore morphology may lead to lower than actual estimates of diversity because of variability in expression of visual traits . Also, spores in the soil can not be directly linked to a host plant.

Polymerase chain reaction (PCR) may be used to identify deoxyribonucleic acid (DNA) that is extracted directly from the soil without the need for isolation . The components of PCR include heat stable DNA polymerase *Thermus aquaticus* (*Taq*), two oglionucleotide primers, reaction mix, and the required series of incubation conditions . The DNA must be fragmented before the amplification to minimize interference of DNA from non-target organisms . However, the determination of DNA from spores in the soil may not reflect the

AMF that is colonizing plant roots so the DNA extracted from the roots may also be analysed. The resulting DNA can then be purified and sequenced to determine the different species present. Greater differences in sequences are indicative of being phylogenetically further apart .

There are both advantages and disadvantages of using PCR for DNA analysis. For example, if the quantity of target DNA is very low, a simple PCR reaction may not adequately amplify DNA to be visualized on a gel . The amplification process must be optimised in order to obtain enough DNA for analysis. Also, when analysing community differences, rare species may go undetected because of competition for cloning with common ones . The DNA fragments obtained are also sensitive to mutations and have the potential to react with non-target strains . The advantages of using PCR for DNA analysis include that it has the potential to be very specific and once the equipment and knowledge for use are acquired they can be relatively inexpensive and easy to use . However, PCR does not provide any phylogenetic information without further denaturing or sequencing.

Capillary array electrophoresis-single-strand conformation polymorphism (CAE-SSCP) analysis can be done on the ABI PRISM ® 3130. SSCP analysis works under the assumption that non-denaturing conditions allow DNA molecules with different nucleotide sequences to display unique conformations . A PCR reaction is done using fluorescently labeled primers, followed by denaturation of the PCR product and rapid cooling to prevent the strands from re-annealing . The capillary electrophoresis has resolution to a single base pair and is ideal for resolving very minute polymorphisms. This method is ideal for detecting differences between samples before sequencing is completed. It can greatly reduce the costs associated with sequencing, since selection of samples is based on the electrophoresis results.

When preparing for sequencing, the DNA templates should be free of organic residues and high salts, which can be accomplished using PCR cleaning kits i.e. the QiaQuick PCR purification kit (Qiagen). Since too much DNA may result in poor readings, a fluorometer or spectrophotometer may be used to accurately measure DNA concentration. For example, for a 500 bp PCR product, 10-20 ng of template is used in

the sequencing reaction. The DNA sequences may be compared to known sequences of AMF to identify which species are present.

2.2 Objective and hypothesis

I hypothesized that pulse crops would increase the biodiversity of AMF communities under a subsequent durum crop. The objective of this study was to assess the possibility of using DNA analysis to determine the impact of pulses in crop rotations on the biodiversity of AMF communities. This hypothesis was tested using molecular techniques to extract and analyse DNA from soil and roots taken from the field.

2.3 Materials and methods

2.3.1 Site description

For a general site description refer to section 3.3.1.

2.3.2 Determining AMF in soil and roots

The possibility of using PCR to determine AMF biodiversity in soil, as influenced by cropping history, was examined on soil and root samples with adhering soil. Soil and root samples were taken from the field on July 11, 2005 and frozen (-20º) until DNA extraction could be completed. The flag leaf stage (July 11) was chosen as the sampling date because by this time there should be sufficient fungi present for detection and identification.

Total genomic root DNA was extracted from a random subsample of roots using the DNEASY Plant DNA extraction kit (MoBIO). A 100 mg sample of unwashed frozen root material (-20°C) was further chilled in liquid nitrogen and ground in a 2 ml plastic centrifuge tube using a pestle. Complete pulverization of the root material was avoided to minimize shearing of the DNA. Genomic soil DNA was extracted from 0.65 g of soil using UltraClean™ Soil DNA Kit (BIO/CAN Scientific). All DNA was purified using polyvinylpolypyrrolidone spin columns .

DNA was amplified using nested PCR protocol. Briefly, all DNA was first amplified using general fungal primers NS1 (5'GTAGTCATATGCTTGTCTC) and NS41 (5'CCCGTGTTGAGTCA AATTA) combined with the *Taq* PCR Master Mix

system (Qiagen: Hilden, Germany). The PCR cocktail for each reaction included 31.75 µL nanopure H_2O, 5 µL PCR buffer, 1 µL d NTPs, 0.25 µL *Taq* DNA, 5 µL of 5 mM primers, and 2 µL genomic DNA. The DNA was diluted 1:10 before amplification in a thermocycler (PTC-100, M. J. Research, Inc., MA) using the following settings: 94°C for 3 minutes; 35 cycles (94°C for 1 minute, 50ºC for 1 minute, 72ºC for 1 minute); and 72ºC for 10 minutes. The first PCR products were diluted to 1:100 (root) and 1:50 (soil) before the second stage PCR using primers NS1-GC (5'CGCCCG CCGCGCGC GGCGGGCGGGGCGGGGGCACGGTTGGAGGGCAAGTCTGGTGCC) and AM1 (5'GTT TCCCGTAAGGCGCCGAA) with 6-FAM fluorescent labeled primer (Sigma). The thermocycler was set at 94°C for 3 minutes; 35 cycles (94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute) and 72°C for 10 minute. A control reaction without template was included in each run to rule out the presence of contaminant DNA.

PCR products were visualized on 1.25% agarose gels stained using 1 µL/100 mL of ethidium bromide in TAE buffer. 5 µL of 1X loading dye was mixed with 5 µL PCR product. A 1 kb DNA ladder (Sigma) was run in a separate lane. Gel electrophoresis was run at 250 V for 105 minutes. Gels were illuminated with a DyNa Light Dual Intensity UV Transilluminator (Labnet) and images were photographed using a Kodak EDAS29 digital camera.

The PCR products were prepared for single-strand conformation polymorphism (SSCP) by capillary array electrophoresis (CAE) by adding 9.5 µl of cocktail (HiDi, ROX, H_20) to a loading plate with 0.5 µl of PCR. The wells were mixed with a pipette and centrifuged before denaturing for 5 minutes at 95ºC and snap-cooled on ice. The plate was covered with septa and placed on the ABI Prism ® 3130 Genetic Analyser (Applied Biosystems) for analysis.

PCR products were run on an agarose gel (1.25%) at 250 V for 1 hr and 45 min. Visible bands were selected for sequencing based on results of product size (bp) determined by CAE. Samples with different product sizes and with visible bands but no peak were extracted and purified using a QIAquick Gel Extraction Kit (Qiagen). Samples eluted in nuclease free water were diluted to 0.05 ug uL^{-1} before being sent to the National Research Center Plant Biotechnology Institute (Saskatoon, Saskatchewan) for sequencing.

2.4 Results and discussion

Analysis of soil and root nested PCR product by capillary electrophoresis revealed polymorphisms between treatments of chickpea, pea, lentil, durum and canola. A clear polymorphism is shown between the roots of canola preceding durum (561 bp) and pea preceding durum (556 bp) (Figure 5.1). The DNA samples extracted from the soil samples resulted in fewer identifiable peaks than root DNA.

Analysis of PCR products from roots yielded 25% more identifiable peaks than from soil (Table 5.1). Chickpea, pea, lentil and durum treatments all revealed peaks at 556 bp for the root samples. Chickpea, durum, and canola had common peaks at 558 bp. The canola treatment also resulted in a peak at 561 bp, which was not identified in any other sample for either soil or roots.

The samples extracted from soil resulted in only 6 out of 15 peaks being identified. Soils from canola preceding durum did not display peaks, and lentil and durum both had only one out of the three peaks. The most common product size was 556 bp, found with 42% of the samples. The fragment sizes 557 and 558 were in 8% and 12%, respectively, and the remaining 559, 660, and 561 bp were all found in only 4% of the root and soil samples.

Running the nested PCR products on agarose gels using electrophoresis yielded adequate bands around 560 bps. The soil product resulted in two samples that did not show any bands under UV illumination (Figure 5.2), while all of the durum roots resulted in bands (Figure 5.3). This is similar to results from the CAE and it is possibly a problem of a lower concentration of fungal DNA in the soil than in the roots. The 0.65 g of subsampled soil used for DNA extraction may not accurately represent fungal diversity in the soil.

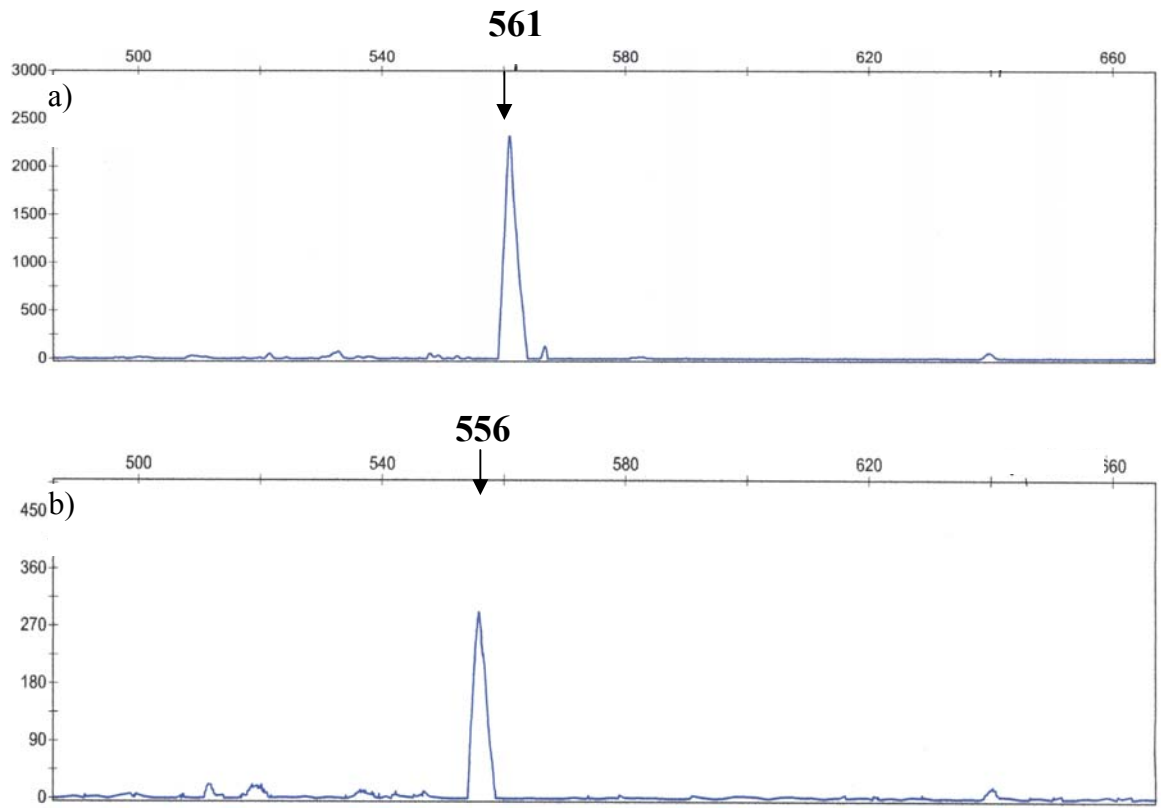

Figure 5.9 Identification of nucleotide polymorphism by fluorescence-based capillary electrophoresis indicates the size (bp) of root PCR fragments from (a) the canola treatment and (b) the pea treatment. Values on the vertical axis are relative fluorescent units.

Table 5.21 Fluorescence-based capillary array electrophoresis determines product size (bp) from durum soil and root PCR samples with preceding crops of chickpea, pea, lentil, durum, and canola.

		Product size (bp)				
Sample		Chickpea	Pea	Lentil	Durum	Canola
Soil						
	1	556	557	556	560	0
	2	556	559	0	0	0
	3	0†	0	0	0	0
Root						
	1	556	556	556	556	558
	2	558	556	556	558	561
	3	0	556	557	0	0

†Samples with 0 indicate that no peak was present or was too low to be identified by the software.

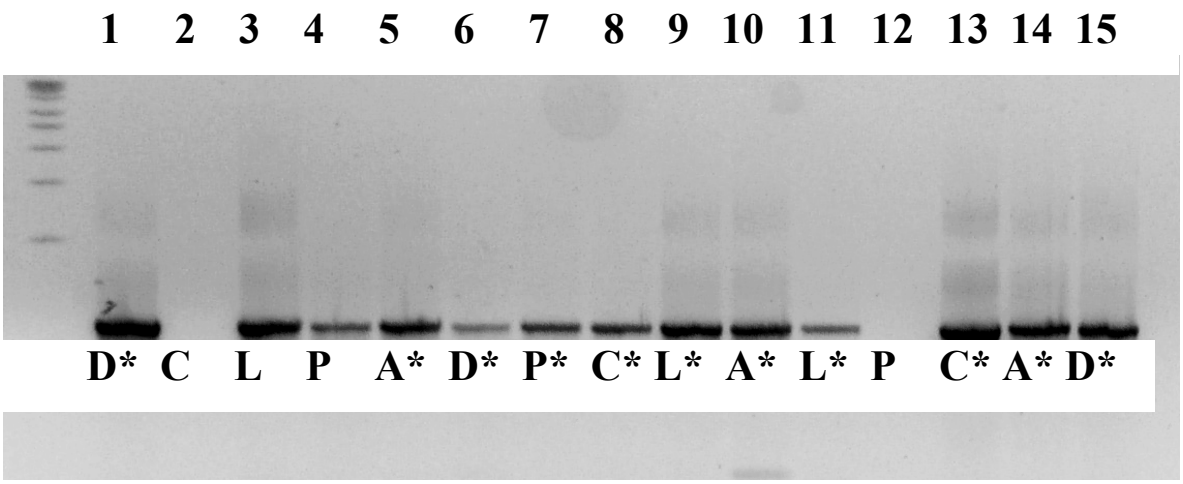

Figure 5.10 Amplified from the soil, the 18s rRNA gene fragment is illuminated at ~550 bp on agarose gel. Letters in the lanes represent treatments of chickpea (C), pea (P), lentil (L), durum (D), and argentine canola (A). The approximate sizes of the bands were determined using a 1 kb DNA ladder from Sigma. * Bands were chosen for extraction and purification for sequencing.

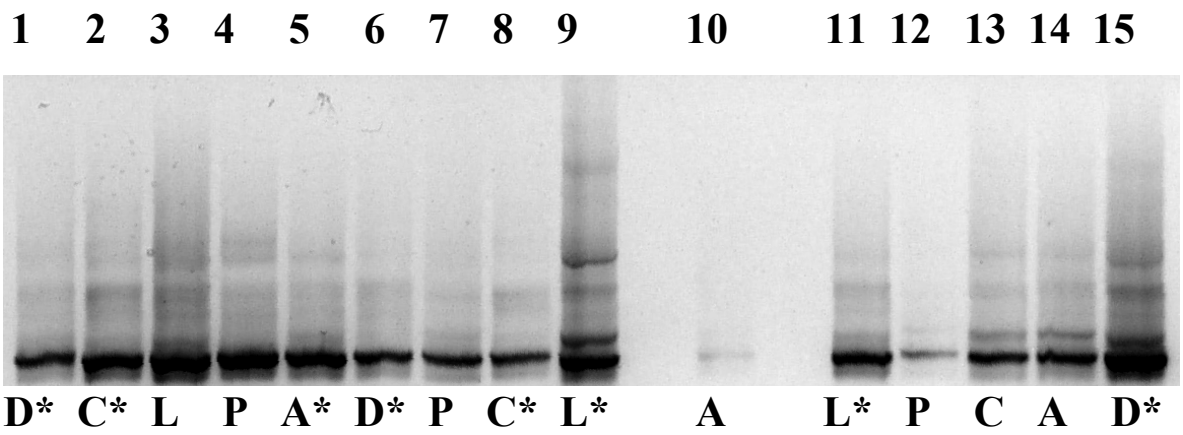

Figure 5.11 Amplified from durum roots, the 18s rRNA gene fragment is illuminated at ~550 bp on agarose gel. Letters in the lanes represent treatments of chickpea (C), pea (P), lentil (L), durum (D), and argentine canola (A). The approximate sizes of the bands were determined using a 1 kb DNA ladder from Sigma. * Bands were chosen for extraction and purification for sequencing.

Bands representing different CAE identified peaks, plus all samples resulting in no peaks, were sent for sequencing. Out of the 19 samples run, no usable DNA sequences were obtained. The cause of this has not been clearly identified, but could be a result of more than one peak inhibiting the sequencing. Ma (2004), analysed DNA from soil and roots in the Brown soil zone of Saskatchewan. Using denaturing gradient gel electrophoresis (DGGE) also did not get any usable sequences. Without sequences it is impossible to positively identify any AMF species in the soil or roots.

Some possible reasons why sequencing did not yield usable results have been identified. The most likely problem related to this experiment was the selection of primers. The primers NS1 and AM1 may amplify more than one species of AMF, or possibly other fungi. Although the CAE showed one distinctive peak in the samples, it is possible that the gel extraction technique used to isolate the DNA before sequencing did not isolate only one species. It is also possible that something occurred while cleaning the DNA and preparing it for analysis, including the something occurring during shipping of samples between cities. The final possible problem identified was that the sequencing equipment was not functioning properly. In future studies, a positive control should always be run when sequencing the samples to ensure the functionality of the equipment.

2.5 Conclusion

Current molecular techniques can be effective and efficient in identifying arbuscular mycorrhizal fungi in soil and roots. However, optimising the conditions for analysis may be difficult and time consuming. One problem in this study was that the primers used (NS1 and AM1) are not necessarily specific to AMF. Lack of primer specificity can result in inaccurate estimates with respect to fungal diversity . Although capillary array electrophoresis was able to show polymorphisms in product size from the different treatments, it can not be concluded without further evidence, that these are AMF species.

6. GENERAL CONCLUSIONS

In recent years, pulses have played an increasingly important role in agriculture in Agroecosystem 12 of southern Saskatchewan. Relationships between plants, soils, and microorganisms are complex systems important for agricultural production. Greenhouse studies, with controlled climatic conditions, are often not representative of field conditions. This study attempted to understand the interactions existing between crops, AMF, and environmental factors (including soil factors) in a field setting. These interactions are often complex and difficult to predict consistently since soil conditions are constantly changing and fluctuating resulting from seasonal changes in ambient temperature and the growth of annual crops.

Pulses appear to have a positive effect on a subsequent durum crop when incorporated into a durum-based cropping rotation. Agroecosystem 12 of the northern Great Plains is characterized by long, cold winters and short, warm summers and is usually drier than surrounding regions . Pulse crops are an important consideration because their roots are colonized by arbuscular mycorrhizal fungi and nitrogen-fixing bacteria, both important soil microorganisms (Perotto et al., 1994). Through N fixation and increased soil exploration by hyphal growth, they can contribute to a more stable farm income (Zentner et al., 2002). This study supports this theory since the previous crop had a significant effect ($P<0.05$) on the yield of the following durum crop in both 2004 and 2005, with pea being associated with the highest yields. This increase could not be explained by N benefits since there were no significant differences in the soil nutrient pools. Since the potential of mineralizable N resulting from pulses in a crop rotation may be small in the initial years but increase in time (Campbell et al., 1992), it would be interesting to measure changes in soil nutrient pools and microbial activity over a longer time period. Changes in the microbial community were analysed over the 2004 and 2005 growing seasons. The mycorrhizal potential and colonization in a durum crop were significantly affected by cropping history, but not consistently as a result of inclusion of pulse crops. There were also variations between years, making it difficult to present a concrete conclusion and thus revealing the dynamic nature of microbial communities.

Investigation of the impact of pulse crops on the soil microbial community produced varied results between the years, for both FAME analysis and AMF colonization determinations. Broughton and Gross identified some possible reasons as to why they did not see differences in the soil microbial community as: (1) no relationship existed between the microbial community and soil and plant factors; (2) the microbial community is mostly influenced by long-term factors, and is stable over the season; (3) inappropriate sampling time to reveal changes; or (4) sampling or analysis technique was not specific to detect actual changes in the microbial community. Considering the lack of treatment effect of pulse crops preceding durum ($P<0.05$) on variables representing the microbial community, it is possible that a limited relationship exists between the microbial community and soil and plant factors or that it is related to sampling since numbers may depend on the sampling location, which may change between bulk soil and the root interior . In comparison, Larkin et al. identified one of the most important factors of soil microbial change as the present plant species.

Although AMF root colonization measurements and fatty acid analysis give an adequate picture of microbial community structure, it does not give an indication of functional diversity . It would be interesting to investigate the effect of chickpea, lentil, and pea on a subsequent durum crop's microbial functional diversity in comparison to canola and the durum monoculture. For example, Lupwayi et al. found that the functional diversity in the rhizosphere of a nonlegume monoculture was greater than one following pea. Community level physiological profiling (i.e. using Biolog ® plates), combined with FAME analysis would give valuable information about both microbial composition and functionality. It would be informative to determine the functional diversity of the microbial community and identify the species present. Although this study attempted to identify the species of AMF present in soils and roots of different cropping rotations, more time is needed for problem solving and to

identify specific primers for DNA analysis.

More studies are needed to find optimum cropping cycles to increase the diversity and function of mycorrhizal fungi and optimise soil processes, while controlling plagues. Including pulses in these rotations can result in both N and non-N benefits for a subsequent crop. However, changing environmental conditions have made it difficult to expose trends using data from only 2 years and longer term tests are necessary to determine and predict consistent outcomes.

7. LITERATURE CITED

APPENDIX A

Agronomic details

Table A.1 Agronomic activities and sampling details over the growing season in 2004 and 2005.

Activity	Details	Date	
		2004	2005
Preseeding weed control	Roundup Transorb (1.25 L ha^{-1})	Apr-26	Apr-22
Seeding	AC Avonlea (100 kg ha^{-1})	Apr-29	Apr-26
Post emergent weed control	Butril M (1.0 L ha^{-1}); Horizon (2.38 L ha^{-1})*	Jun-03	Jun-16
Harvest	Hand sample	Aug-27	Aug-10
Harvest	Wintersteiger	Sep-01	Sep-01

* 2005 only

APPENDIX B

Most probable number of arbuscular mycorrhizal fungi

Table B.1 Most probable number assay using soil dilutions to determine the inoculum potential of arbuscular mycorrhizal fungi at the beginning of the growing season in 2005.

Previous Crop	Rep	Soil Dilutions†					
		Undiluted	1/4	1/16	1/64	1/256	1/1024
Chickpea							
	1		+	-	-		
	2		+	+		-	
	3	+	+	+	-	-	-
	4				+	-	-
	5	+	+	+		-	-
Pea							
	1	+	+	+	-	-	-
	2		+	-	+	+	-
	3	+	+	-			-
	4	+	+	-	-	-	-
	5	+		+		-	
Lentil							
	1	+	+			-	
	2	+	-	+	-	-	-
	3	+	-	-	-		
	4	+	+				-
	5	+	+	+	+		
Durum							
	1	+	+	+	-	-	-
	2	+	+	+	+	-	
	3	+	+	+	+	-	-
	4	+	+	+	-	-	-
	5	+	+	+	-		
Canola							
	1		+		-	-	
	2	+			-	-	-
	3	+	+	+	-		-
	4	+	+	-	-	-	
	5	+	-	-	+	-	-

† Root colonization determinations on leek plants indicate presence (+) or absence (-) of arbuscular mycorrhizal fungi. No symbol indicates that analysis could not be completed.