# PERSISTENCE OF AN INTRODUCED NON-INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGUS, *RHIZOPHAGUS IRREGULARIS* AND THE IMPACT ON INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES AND SUBSEQUENT GROWTH OF PULSE CROPS

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## ABSTRACT

The use of non-indigenous commercial arbuscular mycorrhizal fungi as bio-fertilizers is increasing worldwide without a clear understanding of the persistence and consequences on the indigenous AMF communities and crop productivity. To address this research gap, a three-year field incubation study using open-ended soil cores transplanted to four sites in Saskatchewan was initiated in 2011. A growth chamber study was also carried out in 2014 to examine the impact of AMF inoculants of different origins on the alteration of indigenous AMF communities and subsequent crop growth performance of lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.), and field pea (*Pisum sativum* L.).

Non-indigenous *Rhizophagus irregularis* inoculant was applied into soil cores in which field pea-wheat-field pea were subsequently grown in three consecutive cropping seasons (2011 to 2013). The 18S rRNA gene pyrosequencing data from trap roots of field pea revealed that a single application of the commercial inoculant persisted in roots competing with indigenous AMF over three crop seasons in two of the four sites and declined in the remaining two sites and was undetectable by the third cropping season. Inoculation resulted in a significant alteration of the resident AMF communities and suppression of some indigenous AMF taxa that were low in abundance (*Septoglomus*, *Archaeospora*, *Diversispora* and *Entrophospora*). Inoculation was one of the significant driving factors regulating the composition and diversity of indigenous AMF communities.

Phylogenetic analysis using pyrosequencing was efficient in detecting and quantifying the relative abundance of AMF and discriminated between introduced and indigenous AMF taxa in roots. Locally isolated Semiarid Prairie Agricultural Research Centre (SPARC) AMF inoculant strain, *F. mosseae* B04 significantly enhanced shoot N and P uptake and biomass in pulse crops with minimum disturbance to resident AMF communities in roots compared to commercial inoculant strain, *R. irregularis* 4514535. Inoculation with Glomeromycota In-vitro Collection (GINCO) inoculant strain, *F. mosseae* DAOM 221475 also enhanced N uptake in chickpea; however, uptake of P and biomass response were variable between crops. Strong positive correlations existed between the relative abundance of major indigenous AMF taxa (*Rhizophagus* and *Funneliformis*) and shoot N, P uptake and biomass production of lentil

chickpea and pea. Growth performances were mediated by the influence of indigenous AMF taxa as a consequence of inoculation by inoculant that was locally isolated.

Assessment of pyrosequencing data with pooled versus non-pooled replicated trap root samples (2011 and 2013 crop seasons) prior to DNA extraction showed that the relative abundance of major (highly abundant) indigenous AMF genera was similar in both sampling strategies. Abundance of minor (low abundant) AMF genera was significantly reduced and was undetectable in some root samples as a consequence of pooling replicates. Pooling replicates reduced the cost of analyses and reduced efforts significantly but it compromised estimates of AMF community composition and diversity.

These results raised several questions such as 1) does inoculant anastomose genetically with different individual strains, 2) how does genetic manipulation impact rhizosphere microbial communities and subsequent plant growth and productivity, 3) what are the important determinants for the survival of introduced inoculants, 4) does inoculation have direct or indirect impact on growth performance, etc. All these relevant questions regarding the mechanism and nature of competition between indigenous and non-indigenous AMF taxa in different crops, soils, climates and subsequent crop productivity over long-term warrant further investigation.

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## DEDICATION

I dedicate this dissertation to the departed soul of my mother, *Amena Rahman* who always dreamed of me obtaining a higher education for the benefit of mankind.

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| | |
|---|---|
| 18S rRNA | 18 Small Sub Unit Ribosomal RNA gene |
| AAFC | Agriculture and Agri-Food Canada |
| AMF | Arbuscular Mycorrhizal Fungus |
| ANOVA | Analysis of Variance |
| BLAST | Basic Local Alignment Search Tool |
| CSIDC | Canada-Saskatchewan Irrigation Diversification Centre |
| DGGE | Denaturing Gradient Gel Electrophoresis |
| DNA | Deoxyribonucleic Acid |
| GINCO | Glomeromycota In vitro Collection |
| ITS | Internal Transcribed Spacer |
| LSU | Large Sub Unit (of ribosomal DNA) |
| MF | Melfort |
| MRPP | Multi-Response Permutation Procedures |
| mt-DNA | Mitochondrial DNA |
| NGS | Next Generation Sequencing |
| NMDS | Non-Metric Multi-Dimensional Scaling |
| NS | Non-Significant |
| OL | Outlook |
| OTUs | Operational Taxonomic Units |
| PCR | Polymerase Chain Reaction |
| Per-MANOVA | Permutation based Multivariate Analysis of Variance |
| qPCR | Quantitative Polymerase Chain Reaction |
| RA | Relative Abundance |

| | |
|---|---|
| rDNA | Ribosomal DNA |
| RFLP | Restricted Fragments Length Polymorphism |
| RNA | Ribonucleic Acid |
| RT-PCR | Real-time Polymerase Chain Reaction |
| SC | Swift Current |
| SE | Standard Error |
| SPARC | Semi-Arid Prairie Agricultural Research Centre |
| SSU | Small Sub Unit |
| ST | Scott |

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Introduction

World food production must increase because according to United Nations estimates, the human population is expected to exceed 8 billion by 2024 (United Nations, 2014). To feed this growing world population without impairing the environment, more sustainable food production technologies are necessary (Fitter, 2012). The most promising and realistic approach is to manage soil nutrients in crop farms through enhancing the nutrient use efficiency of synthetic chemical fertilizers and exploring the use of soil microbes for altering nutrient availability. Manipulation of soil microbial communities offers the potential for improved crop productivity with reduced inputs (Verbruggen et al., 2012). In particular, nitrogen (N) and phosphorus (P) are the primary limiting factors for increasing crop productivity (Tilman et al., 2001). The potential two key groups of microorganisms naturally occurring in soils for improving N and P acquisition are N-fixing bacteria and arbuscular mycorrhizal fungi (AMF), respectively (Cakmak, 2002; Rodriguez and Sanders, 2015). Significant technological development has been achieved to applying N-fixing bacterial inoculants in cropping systems; however, significant research progress for efficient use of AMF has not been made and well adopted in field crop production systems, despite the enormous potential (Rodriguez and Sanders, 2015).

Arbuscular mycorrhizal fungi are classified in the phylum Glomeromycota and as a biotrophic symbiont, they live in plant roots and have the capability to form a mutualistic symbiotic relationship with the roots of more than 90% of terrestrial plants (Koide and Mosse, 2004). Arbuscular mycorrhizal fungi are not able to synthesize carbon; instead AMF receive carbon from host plants and, in return, AMF provides nutrients and water to the host plant.

Plant P uptake by AMF is well recognized in agricultural and horticultural crop production sectors (Sanders and Tinker, 1971; Hayman, 1983). However, numerous other benefits of AMF to host plants have been documented (Gosling et al., 2006) including increased

resistance to soil pathogens (Newsham et al., 1995), tolerance of salinity and heavy metals (Díaz et al., 1996; Mohammad et al., 2011), uptake of macronutrients other than P, including N, potassium (K) and magnesium (Mg) (Clark and Zeto, 2000; Smith, 2009), and uptake of some micronutrients (Gildon and Tinker, 1983; Azaizeh et al., 1995). In addition, AMF play an important role in improved drought resistance (Augé et al., 1994), water acquisition (Marschner and Dell, 1994; Augé et al., 2001) and soil aggregate stability (Wright and Upadhyaya, 1998). They also help phytoremediation (Turnau and Haselwandter, 2002) and enhance resistance to foliar-feeding insects (Gange and West, 1994).

Most soils already contain diverse AMF communities (Ceballos et al., 2013). Indigenous AMF communities are inherently beneficial for nutrient uptake, and enhancing biomass and crop yields (Abbott and Robson, 1982; Berman and Bledsoe, 1998; Richardson, 2001; Verbruggen et al., 2013). However, to stimulate root colonization, introduction of AMF inoculants has been used for decades to increase the density of local AMF populations (Koide and Mosse, 2004; Malusá et al., 2012). Commercial inoculant industries have been aiming to produce AMF inoculant for supporting plant production around the world. Agricultural inputs-based industry formulating AMF inoculant for multiple benefits and they are considered as plant health insurance (Gianinazzi and Gianinazzi-Pearson, 1988). However, the biofertilizer properties of AMF differ between isolates, depending on host-specific interactions and numerous ecological factors (Smith and Smith, 1996). Recent use of AMF inoculants as commercial bio-fertilizers has raised concerns, because the ecological consequences of inoculation are still unexplored. Questions remain regarding: 1) how these exotic/introduced commercial AMF isolates or strains interact with existing indigenous AMF populations; 2) the impact of mass-released non-indigenous AMF propagules on indigenous AMF communities; 3) whether introduced AMF will persist in crop soils; and 4) the ultimate effects on plant growth.

The influence of introduced and resident AMF on plant growth may be extremely complex in nature. The long term consequences of introduced AMF inoculants on indigenous AMF communities or even other rhizosphere communities and subsequent crop productivity are unknown. The conservation and preservation of local indigenous soil microbial communities may be extremely important for sustainable crop production and require investigation of the

possible consequences of the application of microbial inoculants in sustainable crop production systems.

The ecology and biology of AMF communities are fundamental aspects to be explored when assessing the mechanisms by which introduced AMF interact with the existing indigenous AMF communities. The impact of AMF inoculants on indigenous communities should be known prior to mass-release of AMF inoculant in soil. Little is known about the persistence and establishment of introduced AMF in crop roots in pre-established existing indigenous AMF communities and the consequences of indigenous AMF communities for long-term cropping systems. Moreover, several key factors are important to understand such as the adaptability of introduced AMF isolates/strains to new environmental conditions, genetic variations within AMF species which affect crop growth and productivity, the enhancement of crop growth and yield as a direct result of interactions between introduced AMF and local indigenous AMF or without interactions, and individual contribution to plant productivity (Verbruggen et al., 2013; Rodriguez and Sanders, 2015). Positive and negative contributions of introduced AMF inoculant taxa with different origin to nutrient uptake, biomass, yield and plant productivity responses have been investigated (Wilson and Hartnett, 1998; Dai et al., 2014; Koziol et al., 2015). Information on the estimation of the actual occurrence of introduced inoculant separated from the occurrence of existing indigenous AMF taxa within a colonized root is currently unavailable. In order to determine the relative contribution by the different AMF group assemblages in roots to plant productivity, separate relative abundance of indigenous and introduced AMF taxa in roots is necessary. It is generally assumed that AMF associations promote plant growth. However, several reports showed a negative correlation between the level of mycorrhizal root colonization and plant growth variables in field and greenhouse (Wilson and Hartnett, 1998; Veiga et al., 2011; Dai et al., 2014).

Plant growth performance can be attributed to differences in the ability of different species of AMF taxa (Van der Heijden et al., 1998; Rodriguez and Sanders, 2015). The evidence of mycorrhiza-induced suppression in the plant P uptake pathway via root hairs and the epidermis (Smith and Smith, 2011; Smith et al., 2011) has been documented. For example, complete suppression of the P uptake pathway in several plant species, including *Medicago truncatula* inoculated with different isolates of *R. intraradices* have been shown (Smith et al.,

2004; Grunwald et al., 2009). Plants inoculated with different AMF species respond differently (Klironomos and Hart, 2002). For example, two strains of an AMF species, *R. intraradices*, extracted from geographically different sources exhibited different root colonization rates and had variable correlations (positive to negative) with plant productivity variables (Rasouli-sadaghiani et al., 2010; Colombo et al., 2013). The inoculation with AMF isolates/strains coupled with interactions between indigenous and introduced AMF taxa in response to environmental variables including soil, climate, host, and their ultimate contribution to plant productivity, potentially influence sustainable crop production in future.

### 1.2 Research hypotheses and objectives

The following six hypotheses were tested:

1. Inoculation with commercial non-indigenous AMF inoculant strain, *R. irregularis* will alter indigenous AMF community composition and diversity in the trap roots of field pea.
2. The commercial non-indigenous AMF strain, *R. irregularis* will not persist in soils beyond a single cropping season and persistence will vary with soils and climates.
3. Variable root occupancy will be achieved by inoculant strains of different origin resulting in different contribution to nutrient uptake and biomass accumulation in lentil, chickpea and field pea.
4. The contribution of introduced AMF inoculant taxa to crop productivity (nutrient uptake and biomass accumulation) will be greater than that of indigenous AMF taxa.
5. The 18S rDNA pyrosequencing technology can discriminate between indigenous and introduced AMF strains and can be used to quantify the relative abundance of introduced AMF strains.
6. Pooling biological replications of trap root samples prior to DNA extraction reduces the richness, diversity and structural composition of 2011 and 2013 samples compared to non-pooling of replications using a high throughput pyrosequencing platform.

This dissertation research addresses the following three specific objectives:

1. To examine the persistence of an introduced commercial non-indigenous AMF inoculant strain, *R. irregularis* and its impact on the composition and diversity of the indigenous AMF communities in the field pea trap roots grown in core soils collected from four locations across the Saskatchewan Prairie.
2. To assess the impact of indigenous and non-indigenous AMF inoculants on the existing indigenous AMF communities in roots and the contribution to crop productivity (P and N uptake and biomass accumulation) whether mediated by the indigenous inoculant, non-indigenous inoculant or existing indigenous AMF communities.
3. To compare the impact of pooling and non-pooling replicated sampling strategy on the richness, diversity and compositional structure of AMF communities in field pea trap roots over two cropping seasons using a 454 pyrosequencing platform.

### 1.3 Organization of the dissertation

The research presented in this dissertation is organized in a manuscript format. A total of 6 chapters, of them Chapter 3, Chapter 4 and Chapter 5, are the original research manuscripts containing abstract, materials and methods, results, discussion and conclusions. Chapter 1 is the general introduction, including overall research hypotheses and objectives of this dissertation. Literature review in Chapter 2 provides an overview and background for the topics of this dissertation as a whole. A synthesis of the thesis works is provided in Chapter 6, along with conclusions.

For the research chapters, Chapter 3 presents a three-year field incubation study of how commercial non-indigenous AMF inoculant (*Rhizophagus irregularis*) influenced the diversity and composition of resident indigenous AMF communities in field pea trap roots and also monitored the persistence of this inoculant over three consecutive crop seasons. This field incubation study was established across the three Prairie soil zones at four locations in Saskatchewan Agriculture and Agri-Food Canada (AAFC) research farms. The second study (Chapter 4) aimed to assess the impact of three inoculants with different origins including the commercial *R. irregularis* inoculant on indigenous AMF communities and subsequent crop productivity. The main objective of this study was to examine whether the abundance of

introduced inoculant or the abundance of indigenous AMF community in roots as a consequence of inoculation was correlated with crop growth parameters including shoot N, P uptake and biomass accumulation.

In both field and growth chamber studies (Chapter 3 and 4), the impact of inoculation with non-indigenous and locally isolated AMF inoculants on the existing indigenous AMF taxa in roots of pulse crops was assessed based on the relative abundance of 18S rRNA gene using high-throughput pyrosequencing platform.

Chapter 5 presents comparisons between pooling and non-pooling four replicates prior to DNA extraction, through estimating the richness, diversity and composition of AMF taxa using the pyrosequencing platform. The replicated root samples from the 2011 and 2013 crop seasons used in Chapter 3 were reanalyzed by pooling four reps prior to DNA extraction. The objective was to demonstrate how the indigenous AMF community composition, diversity, and persistence of introduced AMF inoculant varied in pyrosequencing technology with and without replication, as the sample analyses using NGS tools involve heavy workload and costs. Some of the results from Chapter 3 such as Shannon diversity indices of replicated root samples of 2011 and 2013 were repeated in Chapter 5 to compare with the Shannon diversity indices for pooled (4 replicate sample combined into one composite) root samples from 2011 and 2013 samples.

The final chapter, Chapter 6 of this dissertation contains the synthesis and conclusions of three research chapters (3, 4 and 5) along with future research directions to address some unanswered questions.

The appendices, A, B and C provide the number of absolute and relative sequence reads of 18S rRNA gene of indigenous AMF and introduced inoculants taxa in each treated sample used in Chapter 3, 4 and 5, respectively.

# CHAPTER 2

# BACKGROUND AND REVIEW OF LITERATURE

## 2.1 Introduction

Arbuscular mycorrhizal fungi, phylum Glomeromycota, form one of the most common and oldest symbiotic associations with plants on the earth. Based on recent AMF molecular taxonomy (Oehl et al., 2011c), Glomeromycota are comprised of five orders (*Archaeosporales, Diversisporales, Gigasporales, Glomerales* and *Paraglomerales*), 14 families, 29 genera and over 230 species (Schüβler et al., 2001; Redecker, 2002; Walker and Schüßler, 2004; Palenzuela et al., 2008; Oehl et al., 2011a; b; Schüssler and Walker, 2011). This symbiont can form associations with roots in over 80% of plant species (Smith, 2009). Indigenous AMF communities can be inherently beneficial for nutrient uptake and enhancing crop productivity (Liu et al., 2012; Köhl et al., 2014). Arbuscular mycorrhizal fungi spread extra-radical mycelium through the soil and the zone of influence around the hyphae is known as the mycorrhizosphere (Linderman, 1988; Artursson et al., 2006).

The expanding commercial arbuscular mycorrhizal fungi (AMF) inoculant industry promotes inoculation as a tool to improve crop productivity and sustainability in agricultural ecosystems. However, research suggests that introducing non-indigenous commercial AMF inoculant strains affects the diversity, structure, and composition of beneficial resident indigenous AMF communities (Koch et al., 2011; Jin et al., 2013a; b). Studies also show exchange of genetic material between inoculants and indigenous AMF is possible (Börstler et al., 2010; Colard et al., 2011). Genetic alteration of existing indigenous AMF communities may alter their ability to enhance crop growth and yield (Koch et al., 2004, 2006; Angelard et al., 2010). Advanced molecular metagenomics reveals the composition of AMF communities in soils and roots to be highly dynamic (Dai et al., 2013; Bainard et al., 2014b). Questions remain regarding the establishment and persistence of mass-release field inoculants and their impact on indigenous AMF communities over multiple crop seasons. Monitoring viable AMF propagules of introduced inoculants in cropping systems is challenging and complicated by complex genetics.

Understanding the mechanisms of interaction among indigenous AMF, introduced non-indigenous AMF, and host plants requires appropriate molecular tools. Consequently, this review covers the general benefits of AMF in cropping systems, current knowledge about potential molecular methods for examining introduced AMF persistence, and the long-term impacts on existing AMF and subsequent crop productivity. Factors affecting successful inoculation including soils, environmental parameters, and host plants are also discussed. Highlighted are prospects, challenges, and limitations of molecular techniques used for assessing persistence of AMF inoculants in agricultural soils.

**2.2 Benefits of AMF in cropping systems**

Prior to colonization of plant roots, the developmental stages of AMF are comprised of three phases; 1) spore germination; 2) hyphal growth; and 3) host recognition and aspersorium formation (Douds and Nagahashi , 2000). Spores can germinate in the absence of host; however, the rate of spore germination can be enhanced by the root exudates of host plants (Douds and Nagahashi , 2000). Several studies showed that root colonization by AMF is stimulated in low nutrient soils and decreases with the application of phosphorus fertilizers (Hayman et al., 1975; Read et al., 1976; Vivekanandan and Fixen, 1991). The main benefit of AMF to plants is to increase uptake of macro and micronutrients, particularly increasing P uptake in AMF colonized plants ( Gildon and Tinker, 1983; Clark and Zeto, 2000; Smith, 2009). This typically is attributed to an increase in surface area of soil contacted by plant roots due to the mycorrhizosphere effect (Bolan, 1991). The relative dependency of a crop plant on AMF for nutrient uptake is determined by soil conditions and root factors such as surface area, abundance, growth and length of root hairs, and available root exudate (Aggarwal et al., 2011; Moebius-Clune et al., 2013).

The contribution by AMF-crop symbiosis to crop yields is well established. Lekberg and Koide (2005) conducted a meta-analysis of 290 published field and greenhouse studies related to the benefit from AMF-crop symbiosis. They concluded that AMF root colonization resulted in a 23% yield increase in a variety of crop plants. McGoniglea (2011) reported that increased AMF colonization resulted in an average yield increase of 37% in a survey of 78 published field trials. Uptake of macronutrients other than P including nitrogen (N), potassium (K) and magnesium

(Mg) (Clark and Zeto, 2000; Smith, 2009) and uptake of some micronutrients (Gildon and Tinker, 1983) have been reported.

In addition to the benefits of AMF for nutrient uptake, other benefits of AMF to host plants have been recognized (Gosling et al., 2006) including improved drought resistance (Abdelmoneim et al., 2014) water acquisition (Marschner and Dell, 1994; Augé et al., 2001), soil aggregate stability (Wright and Upadhyaya, 1998), increased resistance to soil pathogens (Newsham et al., 1995), tolerance of salinity and heavy metals (Díaz et al., 1996; Kaldorf et al., 1999; Mohammad et al., 2011), and improved phytoremediation (Turnau and Haselwandter, 2002).

### 2.3 Significance of AMF inoculant application and establishment in agricultural soils

The mechanisms regulating the structure and diversity of AMF communities are poorly understood, although several studies suggest their importance (Abbott and Robson, 1981; Alkan et al., 2006; Alguacil et al., 2015) and show that environmental factors such as annual rainfall, geographical location, and soil biological content significantly correlate with the distribution and assemblages of AMF communities (Ndoye et al., 2012; Torrecillas et al., 2013). Terrestrial ecosystems contain diverse AMF associations with co-existing plant communities. Abundance and diversity of AMF likely contribute to improved plant growth and yield and maintaining sustainable crop production systems. However, intensive agricultural production systems commonly have lower AMF diversity than natural ecosystems and the associated soil management practices are regarded as key constraints on AMF genetic diversity (Verbruggen and Kiers, 2010). Intensive agricultural practices cause broken and mismatched hyphal networks and are negatively associated with the abundance of AMF populations ( Mcgonigle and Miller, 1996; Schalamuk and Cabello, 2010). Repeated fallow periods and extensive tillage practices gradually reduce the absolute abundance of infective propagules (Karasawa and Takebe, 2012). Additionally, Maherali and Klironomos (2007) reported that AMF species from multiple lineages were replaced with the species from a single evolutionary lineage in certain AMF communities with a concomitant reduction in the species richness and plant productivity. Further, Mummey et al. (2009) suggested that colonization of roots by specific AMF species may influence subsequent colonization by other closely related species. An indigenous AMF community that is

disadvantaged in terms of absolute abundance or diversity may benefit from AMF inoculant application (Janoušková et al., 2013). There is, however, no clear understanding of the impact of inoculation on alteration of AMF community structure, diversity, and composition (Rodriguez and Sanders, 2015).

Use of commercial AMF inoculants to enhance agricultural productivity has been expanding, with manipulation of AMF communities being practiced at the field scale. This manipulation is achieved either through introduction of particular AMF inoculant strains, or by managing resident indigenous communities. The goals of manipulation through the application of AMF inoculants are to overcome limitations in adequacy (such as lack of diversity) or quality of resident indigenous AMF propagules and to address complex ecological consequences of plant-fungal interactions that are not functioning properly (Verbruggen et al., 2013).

Pellegrino et al. (2012) demonstrated that both indigenous and non-indigenous inoculants could be equally effective in increasing plant growth and yields. However, in one study, *Claroideoglomus etunicatum* applied as an inoculant in crop soil successfully colonized the root of sweet potato, but two other introduced inoculants were inefficient in colonizing roots (Farmer et al., 2007). They suggested that choosing an inoculant from an AMF taxon (family or genus) which is absent or low in abundance in a particular habitat may be an inoculation strategy with the best chance of success.

Host diversity affects the establishment of AMF associations in field soils, because the associations depend on recognition by host plants (Klironomos, 2003; Ehinger et al., 2009). Local indigenous AMF community composition also plays a role (Maherali and Klironomos, 2007). Establishment of a newly introduced AMF species or strain may be challenging if it is to compete with well-adapted existing indigenous communities. Antunes et al. (2009) suggested that the application dose, form of inoculants, and the genetic characteristics of target communities are the most important factors for successful establishment of an AMF inoculant. Low level additions of inoculants may decrease both initial establishment and subsequent impact on indigenous AMF (Janoušková et al., 2013). Other environmental factors such as local climate and soil properties also affect establishment of introduced inoculants (Maherali and Klironomos, 2007).

Mycorrhizal fungal reproduction and root colonization are influenced by seasonal dynamics, with abundance of AMF spores largely fluctuating over the growing season (Brundrett and Abbott, 1994). There are numerous reports that the impact of root colonization by AMF on plant growth varied from positive to negative in response to multiple environmental conditions, such as light intensity, temperature, rainfall events, and soil nutrient availability (Abiala et al., 2013). Inoculants must adapt to particular field conditions, such as the tillage environment (Schnoor et al., 2011), soil types, and pH (Oehl et al., 2010). The degree of infectivity of AMF varies with different soil types (Oehl et al., 2010). Díaz and Honrubia (1995) report that the introduction of *G. fasciculatum* enhanced plant biomass in sterilized soil, whereas introduction did not influence plant growth in unsterilized soil with indigenous AMF.

### 2.4 Factors affecting long-term persistence and effectiveness of indigenous and non-indigenous AMF in agricultural soils

A limited number of studies has assessed AMF inoculant persistence and efficacy over cropping seasons, post inoculation. The effectiveness and persistence of AMF inoculants in agricultural soils is important for sustainable crop production practices. Levels of spore persistence for introduced and indigenous AMF in field soils have a great impact on nutrient availability, particularly P, so AMF persistence could eventually minimize fertilizer costs for crop production (Hart and Trevors, 2005; Verbruggen et al., 2013; Rodriguez and Sanders, 2014). Host specificity is an important factor for persistence. Some AMF taxa are host specialists, whereas many others are generalists (Öpik and Moora, 2012). An inoculant strain that is a generalist is likely to persist longer (Verbruggen et al., 2012). Additionally, AMF spores can survive for several years without host plants. Research results indicate that the spores remained viable for several years in a low-Arctic meadow habitat (Pietikainen et al., 2007). One study tested the survival of spores under storage conditions and found that the half-life of *G. claroideum* spore is 2 yr at 4 ˚C and 3.5 yr at 24 ˚C (Wagner et al., 2001). Johnson et al. (2013) found that the absence of host plants for three years did not affect high levels of AMF diversity.

Local climate and resident AMF community composition and soils are important variables that must be compatible with introduced AMF inoculants for ultimate persistence and effectiveness (Oehl et al., 2010; Verbruggen and Toby Kiers, 2010; Verbruggen et al., 2012).

The alteration of soil microbial ecology in agricultural soils following the introduction of non-indigenous AMF inoculant has consequences for effectiveness and persistence (Mummey et al., 2009; Antunes et al., 2009; Koch et al., 2011), although the ecological impact of AMF inoculants on soil microbial communities and plant productivity is still largely unexplored. Antunes et al. (2009) reported that in disturbed soil, a non-indigenous inoculant, *G. irregulare,* significantly improved the P content of host plants in the presence of an indigenous mycorrhizal population. Mosse (1977) reported that growth response to inoculation in field soils was significantly higher when there were few resident endophytes. However, several studies have shown the ineffectiveness of AMF inoculants in unsterilized soils where indigenous microflora were present (Hetrick et al., 1991).

Although the application of AMF inoculants in agriculture is important for environmentally sustainable crop production systems, knowledge about the ecological consequences of inoculant interaction with other microbial populations and the effect on indigenous AMF communities is limited. There is no clear indication of how and which structure of the indigenous AMF community is likely to contribute effectively to crop production systems, nor a full understanding of what is actually contributing to crop yield: whether AMF inoculation directly affects yield potentials, or indigenous community alteration as a response to inoculation indirectly affects yields (Rodriguez and Sanders, 2014). The alteration of an indigenous AMF community may potentially alter crop yield without necessarily changing the rate of root colonization in response to inoculation in a particular cropping system (Rodriguez and Sanders et al., 2015).

It is not always obvious if indigenous AMF diversity is threatened due to the introduction of non-indigenous AMF strains, nor is it clear if long-term establishment and persistence should be viewed as a positive outcome, although this might lead to less frequent applications and reduced input costs. The type of AMF inoculant (species or strain) and the rate of application dose are important issues when considering the consequences (positive or negative) of inoculation on the resident indigenous AMF community composition (Antunes et al., 2009; Koch et al., 2011; Verbruggen et al., 2012). Little is known about how indigenous mycorrhizal communities respond to introduced non-indigenous AMF isolates under different soil management practices, climatic conditions, and crop production systems. Antunes et al. (2009)

indicated that the addition of AMF has no negative impact on resident AMF community composition, either under disturbed (cultivated) or undisturbed (uncultivated) soil conditions. According to their study, non-indigenous commercial AMF inoculants have a lower chance of becoming problematic if the indigenous AMF community already has integral hyphal networks and has colonized most of the plant roots in an undisturbed soil. Likewise, the study showed that the introduction of commercial non-indigenous AMF isolates at the recommended dose to maize producing farm soils located in Ontario, Canada, did not affect resident AMF community structure. The study did not demonstrate how indigenous AMF diversity responds to non-indigenous AMF inoculation. Mummey et al. (2009) reported that ribotype richness of indigenous AMF communities in colonized roots was largely decreased when two AMF strains (*Glomus* sp.) were added in a field soil. Koch et al. (2011) reported that introduction of the non-indigenous AMF isolate (*G. irregulare*) into a Canadian field soil resulted in a drastic decrease of detected terminal-restricted fragments (T-RFs) in plant roots, indicating that the addition of *G. irregulare* to field soil had a negative effect on indigenous AMF diversity. However, AMF occurring at low frequency may not have been detected, and some *G. irregulare* species were also present in the field soil. Their findings imply that introduced *G. irregulare* successfully established and became dominant over the resident AMF communities, although *G. irregulare* did not outcompete all indigenous AMF. Addition of AMF inoculants into a pre-established AMF soil system, resulting in an increase in the total AMF density of infective propagules, may enhance competition among root colonizing AMF and eventually decrease the effectiveness of an AMF taxa in promoting plant growth and productivity (Janoušková et al., 2013).

Work on monitoring field-released non-indigenous AMF strains implies a potential risk in that genetic exchange between introduced and resident indigenous AMF strains, may occur resulting in outcrossing with lower fitness (Börstler et al., 2008; Colard et al., 2009; Roger et al., 2013; Beaudet et al., 2014). Understanding the genetic exchange events and mechanisms among introduced and indigenous strains presents a challenge for future research in the area of AMF inoculants. Genetic interchange and manipulation among indigenous and non-indigenous AMF can influence the success of symbiotic association (Colard et al., 2011). Single AMF species have multiple biotypes that differ genetically from each other and each isolate from a single species can contribute differentially to plant growth (Koch et al., 2004). A single spore or hypha

of AMF can have numerous nuclei together, thus the multi-genomic structure of AMF (Kuhn et al., 2001) in nature leads to an unexpectedly higher genetic diversity of AMF (Gollotte et al., 2004). DNA polymorphism is detectable in a single spore and in an isolate of a single species (Pawlowska and Taylor, 2004; Colard et al., 2011). As a result, there is disagreement around genetic manipulation, variations among AMF populations, and their subsequent role in plant productivity. Therefore, deployment of suitable technology is needed to quantify changes in AMF communities in terms of richness, evenness, and diversity in response to inoculation with non-indigenous AMF.

### 2.5 Molecular tools to assess persistence of AMF field inoculants and diversity, structure, and composition of indigenous AMF communities

The detection and quantification of introduced AMF in plant roots over a period of time is necessary for understanding the ecological consequences of inoculation in field conditions. In recent years, advances in molecular detection of field inoculants have been promising, both for the quality control of commercial inoculants and for assessing the benefits of inoculation. Identifying the conditions under which the applied inoculant successfully establishes and persists in plant roots, and maximizes yield and nutrient uptake, particularly acquisition of P, is the ultimate target. It is important to determine the levels of establishment and persistence of introduced AMF inoculants over multiple seasons to know with what frequency inoculants should be applied for maximum economic and ecological advantage in sustainable cropping systems.

Recent advancements of high-throughput DNA sequencing technologies, such as 454 pyrosequencing and Illumina platforms (Mi-Seq and Hi-Seq), have been applied in studies that use large-scale sampling with a sufficient number of replications to profile AMF assemblages in agricultural systems (Dai et al., 2012; 2013; Lindahl et al., 2013; Bainard et al., 2014b). These metagenomic technologies could be an efficient means of detecting consequences of AMF inoculation by tracing potential alterations of resident communities over cropping seasons.

In recent years, the likelihood of detection and monitoring AMF inoculant strains has increased greatly through advances in molecular methods including mitochondrial ribosomal

DNA (rDNA) and terminal restriction fragment length polymorphism (T-RFLP), to target small sub-unit (SSU) and large sub-unit (LSU) regions of rDNA fragments (Alguacil et al., 2011; Krak et al., 2012; Pellegrino et al., 2012; Sykovora et al., 2012). Some well-developed molecular tools are already utilized for tracking introduced AMF strains in field situations and monitoring the persistence of AMF inoculants in order to understand whether inoculants should be applied every season or less frequently. Mitochondrial large sub-unit (mtLSU) ribosomal RNA gene sequences were used to identify unique haplotypes of AMF isolates existing in nature (Börstler et al., 2008, 2010; Croll et al., 2009; Sanders and Croll, 2010). Formey et al. (2012) reported sequencing mitochondrial genomes using NGS platforms such as 454 pyrosequencing, and Illumina platforms to characterize the intra- and inter-strain mitochondrial genome variability of *R. irregularis*. Two subclades of *R. irregularis* were identified based on the type of polymorphic (variability generating element/VGE) characteristics. Sykorova et al. (2012) are the first to have demonstrated how to detect an inoculated *R. irregularis* isolate BEG140 using mtLSU rDNA markers in the roots of *Phalaris arundinacea* grown in coal mine soils.

Real-time and quantitative polymerase chain reactions (qRT-PCR) are well-established techniques for the detection and quantification of AMF, but nuclear ribosomal DNA (nrDNA) is not an appropriate genomic region for amplifying closely related genotypes of two different strains, or even two different isolates of a species (Stockinger et al., 2010; Krak et al., 2012). Better resolution is obtained with the large sub-unit mitochondrial DNA (LSU-mtDNA) of AMF, which has recently been successfully examined (Krak et al., 2012; Formey et al., 2012). Krak et al. (2012) reported applying a RT-PCR assay targeting LSU-mtDNA to quantify the mtDNA gene copy number of two isolates of *G. irregulare* co-existing in the colonized roots of *Medicago sativa*. This newly-developed genetic approach could allow for discrimination between AMF strains mass-released as inoculants in the field soils and resident AMF strains belonging to the same species. A preliminary characterization using phylogenetic analysis of mtLSU sequences of different haplotypes of a single species (non-indigenous strain versus indigenous strain) is a prerequisite to quantifying the actual persistence of inoculant strains (Stockinger et al., 2009). Dai et al. (2014) extensively profiled AMF communities based on long reads (mean length: 751.7 bp) of the SSU rDNA region using high-throughput 454 GS-FLX+ pyrosequencing technology. In one of their studies (Dai et al., 2013), 122 operational taxonomic

units (OTUs) of major AMF taxonomic groups (representing 56 distinct species with 97% similarity to GenBank reference sequences) were detected using 454 pyrosequencing of 18S rRNA genes from an extensive soil survey of AMF diversity (337 soil samples of croplands, natural areas, and roadsides across the Canadian prairie and Atlantic maritime eco-zones). These results indicate that NGS technology reveals a greater diversity and population dynamics of AMF communities in the highly fertile Chernozemic agricultural soils than expected based on the previous studies using molecular methods other than NGS.

**2.6 Diversity and composition of AMF in Canadian Prairie soils**

Thirty-three dominant AMF operational taxonomic units (OTUs) were found in 76 wheat fields over the Chernozemic Great Groups of the Prairie region (Dai et al., 2013). The dominant members of the Glomeromycota were previously determined based on spore morphology (Talukdar and Germida, 1993) and 18S rRNA gene sequence (PCR-DGGE) analysis (Ma et al., 2005). Spores of *Rhizophagus fasciculatum*, *Claroideoglomus luteum* NT4, *C. etunicatum*, *Funneliformis mosseae*, *Glomus versiforme* (Talukdar and Germida, 1993; Ma et al., 2005), *G. aggregatum*, *G. pansihalos*, and *Entrophospora infrequens* (Boyetchko and Tewari, 1993) were found in cultivated Canadian Prairie soils. *Funneliformis mosseae* is the dominant and ubiquitous species in Prairie soils (Avio et al., 2009) and the presence of *F. constrictum*, *R. iranicus*, *C. viscosum* and *G. decipiens* was identified over different Chernozem Great Groups. Sequences of *Scutellospora calospora* in a Dark Gray Chernozem were reported by Ma et al. (2005). Spores of *Acaulospora denticulate* were found in soils of Chernozem Great Groups (Talukdar and Germida, 1993). Additionally, numerous unknown *Glomus* sequences found in Chernozem soils were reported by Ma et al. (2005), Yang et al. (2010), and Dai et al. (2012).

A soil survey was conducted to describe AMF diversity across the Prairie landscape (Hamel et al., 2013). They reported that the most common species were *G. irregulare*, *G. claroideum*, *G. monosporum. Diversispora spurca*, *G. clarum*, *G. cubense*, *G. eburneum*, *G. etunicatum*, *G. fasciculatum*, *G. geosporum*, *G. intraradices*, *G. luteum*, *G. microaggregatum*, *G. mosseae*, *G. viscosum*, and *Paraglomus occultum*. In recent years, several researchers (Hamel et al., 2013; Dai et al., 2013, 2014; Bainard et al., 2014a, 2014b) have explored a larger genetic

diversity of AMF communities using different NGS platforms compared to traditional and other molecular tools in Prairie agroecosystems.

### 2.7 Prospects, challenges, and limitations for studying AMF inoculant persistence and impact on the indigenous AMF communities in agricultural soils

A clear understanding of the ecological consequences of AMF inoculants on resident AMF communities and possible interactions with other microbial populations is limited. Several studies published from 2007 to 2014 described the use of massively parallel pyrosequencing technology profiling AMF community compositions and diversity from environmental field samples (Dai et al., 2013, 2014; Bainard et al., 2014a, 2014b). Specifically, the persistence of introduced AMF inoculants, their impact on the indigenous AMF communities, and subsequent plant growth performance both in field and greenhouse conditions were documented (Farmer et al., 2007; Antunes et al., 2009; Koch et al., 2011; Alguacil et al., 2011; Krak et al., 2012; Pellegrino et al. 2012; Sykorova et al., 2012; Jin et al., 2013a, 2013b). Jin et al. (2013b) examined the effect of co-application of seven fungicides and a commercial non-indigenous AMF inoculant, *G. irregulare* on the compositional structure of indigenous AMF communities using 454 pyrosequencing of 18S rRNA gene regions (target AMF primers: 550 bp) in a greenhouse study. They mention that pyrosequencing technology was capable of detecting a commercial AMF inoculant strain (*G. irregulare* as OTU10) from pea roots colonized by indigenous and non-indigenous AMF inoculant strains. A potential prospect is therefore indicated for high-throughput 454 pyrosequencing and other platforms (such as Illumina) in monitoring long-term persistence of commercial non-indigenous AMF inoculants and quantifying the abundance of indigenous and commercial AMF strains in field-grown colonized root samples.

Researchers have examined the persistence of non-indigenous AMF inoculants, assessed the impact of introduced inoculants on the indigenous AMF communities using different molecular tools with variable success. Most of the research was carried out under greenhouse and few were undertaken in field conditions. No molecular tools and methods for the detection and quantification of introduced AMF strains from the indigenous AMF were perfect, however, researchers explained their drawbacks and further research issues for better estimates of

indigenous and existing resident AMF communities. The highlights of the eight published studies, highly relevant to the current thesis research are presented in Table 2.1.

In conclusion, recently-developed molecular techniques like NGS allow the characterization of AMF communities in agricultural systems to be highly accurate. Many molecular techniques have been proven useful for answering a number of important questions about many aspects of inoculation, such as the nature of competition and interactions among AMF species in soils and roots, and the genetic variability within isolates of a single species. Additional studies would allow the potential of high-throughput metagenomics to be realized, enhancing chances of successful detection, quantification, and evaluation of mass-released AMF inoculant strains in field crop soils. Advanced molecular tools are able to explain the nature and mechanisms of interactions and the competition between indigenous and non-indigenous AMF. Inoculant tracking is essential for quantifying root colonization by inoculants alone and understanding the nature of interactions among local AMF, commercial AMF, and with plants. The NGS technology for DNA sequencing offers possibilities for very extensive studies with huge amounts of sequence data. This may aid the understanding of these dynamic interactions and help in exploring the mechanisms of genetic interchange among indigenous and introduced AMF strains. Current genomic techniques need to be validated with various AMF inoculants from different sources and genetics, multiple host crops, field soils, and seasonal variations to achieve consistent efficiency when applying inoculants as bio-fertilizers for sustainable agriculture. Exploring the ecological impact of inoculation on the genetic diversity of indigenous AMF and subsequent plant growth could help in design and formulation of efficient AMF biofertilizers for each crop, soil type, and climatic eco-zone across the globe. This could help lower the environmental impacts of intensive agriculture by reducing the use of synthetic fertilizers and pesticides in crop production systems.

**Table 2.1** Highlights of recently published articles on the detection and quantification of the persistence of mass-release AMF inoculants in the field and greenhouse trials and their subsequent impact on resident indigenous AMF communities.

| Main objective of the study | Time course | Molecular technique | Outcome/Result | Drawback of method | Reference |
|---|---|---|---|---|---|
| To monitor and evaluate the persistence of 3 AMF species in a field condition | -Sampled 6 weeks after planting and inoculation for 2 consecutive years | -DNA extracted from roots<br>-Targeted region LSU of rDNA<br>-PCR-Sanger sequencing | -*G. mosseae* and *G. etunicatum* were successfully detected but *G. intraradices* was common in Chinese trial field and it was difficult to determine the success of inoculation of *G. intraradices* | -Persistence of detected inoculated species was not quantified<br>-Did not distinguish between two co-existing isolates of same species | Farmer et al. (2007) |
| To assess the impact of a commercial AMF inoculant, *G. intraradices*, in an agricultural soil on the structure and functioning of indigenous AMF community | -Sampled 3, 6, and 9 weeks of post inoculation | -DNA extracted from roots<br>-Targeted region LSU-rDNA<br>-PCR-T-RFLP | -Introduced commercial AMF inoculant (*G. intraradices*) did not affect structure of resident AMF community at recommended dose | -PCR-T-RFLP was unable to differentiate inoculated strain from same group of indigenous strains<br>-quantified total indigenous AMF phylotypes or groups rather than individual indigenous AMF taxa | Antunes et al. (2009) |
| To examine the persistence and survival of mixed indigenous AMF inoculants in field soils | -Sampled 3 times, latest 14 months after inoculation | -DNA extracted from roots<br>-Targeted region SSU of rDNA<br>-PCR-cloning, Sanger sequencing | -*G. intraradices* was detected and estimated % sequences in plant roots | -No quantification of gene copies of *G. intraradices*<br>-Unable to discriminate coexisting isolate of same species | Alguacil et al. (2011) |

**Table 2.1** Continued

| Main objective of the study | Time course | Molecular technique | Outcome/Result | Drawback of method | Reference |
|---|---|---|---|---|---|
| To examine the impact of addition of *G. intraradices* inoculant on indigenous AMF community composition in a greenhouse condition | -10-months study<br>-Sampled roots at harvest | -DNA extracted from roots<br>-Targeted LSU-rDNA, PCR-T-RFLP | -Added *G. intraradices* to field soils, impacted negatively on the diversity of resident indigenous AMF community composition,<br>-Quantified total indigenous AMF phylotypes or groups rather than specific taxa of indigenous AMF | -Detected the presence of *G. intraradices* in field soils,<br>-Unable to distinguish inoculated strain from indigenous strains of G. *intraradices* | Koch et al. (2011) |
| To assess the suitability of the method of mtDNA amplification for monitoring and detection of introduced AMF isolates in roots | -Sampled roots 6, 12, 26 weeks post inoculation | -DNA extracted from roots<br>-Targeted region mtLSU and nrLSU of rDNA<br>-qPCR compared efficacy of mtLSU and nrLSU regions | -Quantified 2 isolates of a single species *G. intraradices* in colonized roots using mtDNA based qPCR assay<br>-Able to discriminate two closely related coexisting genotypes of *G. intraradices* | -Samples used from greenhouse trial require modification and validation of the methods with environmental field samples | Krak et al. (2012) |

**Table 2.1** Continued.

| Main objective of the study | Time course | Molecular technique | Outcome/Result | Drawback of method | Reference |
|---|---|---|---|---|---|
| To assess the effect of inoculation with non-indigenous AMF inoculant on the structure and functioning of indigenous AMF community in pot-cultured pea roots | -Sampled roots at 42 days post inoculation | -DNA extracted from roots<br>-Targeted SSU-ITS and LSU of rDNA<br>-PCR-cloning, RFLP-Sanger sequencing | -Inoculation with *G. irregulare* significantly reduced the diversity and composition of resident AMF assemblies in field pea roots.<br>-80 AMF phylotypes (OTUs) were detected<br>-Successfully amplified multiple priming sites and longer gene sequences | -Unable to distinguish inoculated AMF isolate of *G. irregulare* from isolates of same resident species<br>-Did not address the above issue<br>-Techniques require modification/adjustment for environmental field samples | Jin et al. (2013a) |
| To assess the impact of co-applied seed-fungicides and commercial AMF inoculant (*R. irregularis*) on three indigenous AMF communities in controlled conditions | -Sampled roots 8 weeks after inoculation | -DNA extracted from roots<br>-18S-SSU of rDNA<br>-454 pyro-sequencing | -The systemic fungicides reduced the abundance of indigenous AMF and the suppression is pronounced in the presence of commercial AMF inoculant strain<br>-The commercial inoculant strain, *R. irregularis* was successfully detected in roots in the presence of indigenous AMF communities<br>-Recovered 39 AMF OTUs from colonized roots | -Require adjustment of this technique with environmental field samples<br>-Fungicide alone and combined impact of fungicide and inoculant on indigenous AMF communities was determined<br>- The impact of inoculant on indigenous AMF community was not estimated | Jin et al. (2013b) |

**Table 2.1** Continued.

| Main objective of the study | Time course | Molecular technique | Outcome/Result | Drawback of method | Reference |
|---|---|---|---|---|---|
| To examine the persistence of an introduced AMF inoculant, *R. irregularis*, in roots in a contaminated field soil | -Sampled 3 times per year over 3 consecutive years | -DNA extracted from roots<br>-Targeted region mtLSU PCR-RLFP and Sanger sequencing | -Haplotype A of inoculated *R. irregularis* established and detected in roots 3-year post-inoculation even though several indigenous haplotypes of same species co-existed and established in the roots<br>-Unable to discriminate haplotypes of inoculant from indigenous ones | -Used indirect methods of quantification<br>- Determine the presence or absence of isolate in roots and estimated the percent of the persistence of inoculant haplotypes | Sýkorová et al. (2012) |
| To monitor the success of inoculation by two non-indigenous AMF isolates of *F. mosseae* in field maize roots | -Sampled yearly for 2 consecutive years | -DNA extracted from roots<br>-Targeted region LSU-SSU-ITS of rDNA<br>-PCR-RFLP Sanger sequencing | -Detected two introduced isolates of *F. mosseae* in field crop soils 2 years after inoculation<br>-Able to discriminate indigenous and non-indigenous *F. mosseae* strains<br>- Successfully detected inoculant strains in roots by PCR-RFLP based sequencing analysis | -Doubted the appropriateness of long nrDNA markers to discriminate phylogenetically similar taxa of AMF | Pellegrino et al. (2012) |

# CHAPTER 3

# PERSISTENCE OF AN INTRODUCED NON-INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGUS, *RHIZOPHAGUS IRREGULARIS* AND THE IMPACT ON INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES

## 3.1 Preface

This chapter assesses the persistence of an introduced commercial non-indigenous arbuscular mycorrhizal fungal strain, *Rhizophagus irregularis*, and its impact on the indigenous AMF diversity, structure and composition in field pea and wheat in Chernozemic soils of Saskatchewan, as assessed using high-throughput 454 pyrosequencing of the 18S rRNA gene. The AMF community analyses were performed using field pea trap roots grown in soil samples collected from the field cores at each harvest in 2011, 2012 and 2013. The study used soil cores collected from geographically unrelated sites which were subsequently transplanted to other sites, thereby facilitating an assessment of the impact of climate on AMF communities. This soil core transplantation study investigated the influence of climatic conditions on (1) the establishment and survival of non-indigenous AMF inoculants and (2) the indigenous AMF community in different soil types over three consecutive years (2011 to 2013). The study assessed the ecological consequences of introducing *R. irregularis* as a biological disturbance and the resulting effect on the local indigenous AMF communities.

## 3.2 Abstract

Inoculation of crop plants with non-indigenous AMF as a bio-fertilizer is increasing worldwide without clear evidence of the persistence and consequences of these inoculants on the existing indigenous AMF communities. To address this knowledge gap, a three-year field incubation study at four locations across Saskatchewan was initiated in 2011. At each of the sites, an AMF inoculant containing *Rhizophagus irregularis* was applied to open-ended soil cores in which a host plant was subsequently grown during three growing seasons. Additionally,

replicated soil cores from each site were relocated to each of the other three sites. The persistence of introduced non-indigenous AMF and the impact on the composition and diversity of indigenous AMF in the trap roots of the field pea were assessed using 18S rRNA gene pyrosequencing technology. The introduced inoculant strain was detected in Swift Current and Outlook soils after three growing seasons, 27 months after inoculation, whereas persistence in the remaining Scott Dark Brown and Melfort Black soils was limited. Inoculation resulted in significant suppression, displacement, and alteration of minor indigenous AMF taxa (*Rhizophagus, Septoglomus, Diversispora, and Archaeospora*). This occurred in all soils used in this study. When soils were transplanted to other locations, *Claroideoglomus* became predominant over the other two dominant genera (*Glomus* and *Funneliformis*) in response to inoculation. Inoculation was recognized as one of the significant driving factors regulating the composition of indigenous AMF communities. The impact of inoculation on AMF diversity was influenced by soil type ($P$=0.0002) according to Per-MANOVA analysis. This research provides insight into the effects and persistence of an introduced non-indigenous commercial AMF strain on the existing indigenous AMF community diversity, structure, and composition.

### 3.3 Introduction

Soil microbes are of increased commercial significance as more organisms are used as inoculants for bio-fertilisation and biological management of plant diseases in sustainable agriculture. Arbuscular mycorrhizal fungi (AMF) improve plant health by increasing the accessibility of nutrients, improving plant root growth, and bio-protection of plants from soil-borne pathogens (Harrier and Watson, 2004). In terms of enhancement of soil beneficial biological properties, an increase in the microbial community, activity and diversity are key (Tilman et al., 2001; Van Der Heijden et al., 2008).

Arbuscular mycorrhizal fungi are classified in the phylum *Glomeromycota.* They are considered bio-trophic symbionts as they live in the roots. They can influence plant biodiversity (Van der Heijden et al., 1998), and increase the uptake of phosphorus in agriculture and horticulture systems (Sanders et al., 1977; Hayman, 1983). They promote water acquisition, (Marschner and Dell, 1994; Augé et al., 2001) and plant fitness in polluted environments (Kaldorf et al., 1999). Arbuscular mycorrhizal fungi were developed as bio-fertilizers over the

last two decades; however, the symbiosis is not mutualistic in all circumstances and may be parasitic to the host plant (Smith and Smith, 1996). Bio-fertilizer properties of AMF differ between isolates, depending on host-specific interactions and numerous ecological factors (Verbruggen et al., 2013).

The ecological consequences of introducing commercial non-indigenous AMF inoculants into cropped soils on the indigenous AMF communities, which are inherently beneficial for crop production, remains relatively unexplored. The application of non-indigenous AMF inoculants has greater consequences in crop soils due to the potential changes in the soil microbial community ecology (Mummey et al., 2009; Antunes et al., 2009; Koch et al., 2011). Little is known about how indigenous mycorrhizal communities respond to non-indigenous AMF isolates under different soil management practices, climatic conditions, and crop production systems. There is no clear indication of how and which aspects of indigenous AMF communities (either richness, evenness or diversity) are likely to contribute effectively to crop production systems. Also, the alteration of indigenous AMF communities may potentially alter crop yields without necessarily increasing or decreasing the rate of colonization due to the introduction of non-indigenous AMF species in that particular cropping systems (Rodriguez and Sanders, 2015).

Several studies have suggested the importance of structure and diversity of AMF communities; however, the mechanisms by which AMF assemble in soils and roots are poorly understood (Abbott et al., 1984; Alkan et al., 2006). For example, Maherali and Klironomos (2007) reported that AMF communities can influence microbial community assembly in plant roots. Thus, colonization of roots by specific AMF species may influence subsequent colonization by other closely related species. Establishment of an AMF association depends on recognition by the host plant (Klironomos, 2003; Ehinger et al., 2009) and other environmental factors, such as local weather parameters, soil properties, or the indigenous AMF community composition (Maherali and Klironomos, 2007). Few studies have assessed the persistence, establishment and efficacy of inoculants over the cropping seasons post inoculation. In recent years, the ability for long-term monitoring of field AMF inoculant strains is now possible through use of molecular tools (Sýkorová et al., 2007; Alguacil et al., 2011; Krak et al., 2012; Pellegrino et al., 2012). Molecular tools such as 18S rRNA gene pyrosequencing, mtLSU-

cloning, 18S rRNA gene-RFLP are now used to monitor persistence of AMF inoculants in order to understand whether an inoculant should be applied every season or less frequently (Sýkorová et al., 2007; Pellegrino et al., 2012; Jin et al., 2013b; Rodriguez and Sanders, 2015). Recent advancement of high-throughput next generation sequencing (NGS) technology such as 454 pyrosequencing and Illumina (Mi-Seq or Hi-Seq) sequencing allows profiling of AMF assemblages in agricultural systems (Öpik and Moora, 2012; Lindahl et al., 2013; Dai et al., 2013; Bainard et al., 2014a, 2014b). Thus NGS technologies could be efficient tools to assess the consequences of AMF inoculation and potential alteration of the resident AMF community over cropping seasons.

The objectives of this three year-term field incubation study were: 1) to examine the influence of soil types and climates on the persistence of an introduced non-indigenous commercial AMF inoculant strain, *R. irregularis*, and 2) to assess the impact of an introduced inoculant strain on the composition, structure and diversity of the indigenous AMF communities. This present research approach is unique in the manner of manipulating soil cores by transplantation in different climatic soil zones over multiple seasons. It was hypothesised that the non-indigenous AMF strain, *R. irregularis* would not persist in trap roots longer than one cropping season and indigenous AMF community would be altered in response to inoculation.

## 3.4 Materials and Methods

### 3.4.1 Installation of soil cores, site descriptions, experimental treatments and layout

Four sites were established within three different soil zones in Saskatchewan. Sites were selected to assess the interaction effect of soil type and the respective climatic conditions (precipitation and temperature) on persistence of a non-indigenous AMF inoculant containing *R. irregularis*. The soils were also chosen to assess the impact of inoculation on resident AMF community composition, structure and diversity over a three-year period. The field incubation study was initiated in May 2011 at sites located at the Agriculture and Agri-Food Canada (AAFC) research farms located at Swift Current (latitude: 50°18'00.000" N, longitude: 107°44'00.000" W and elevation: 825.00 m), Scott (52°21'35.064" N, longitude: 108°50'05.004" W, elevation: 659.60 m), Melfort (latitude: 52°49'00.000" N, longitude: 104°36'00.000" W and elevation: 480.10 m), and Outlook Canada Saskatchewan Irrigation

Diversification Centre (CSIDC) (latitude: 51°29'00.000" N, longitude: 107°03'00.000" W and elevation: 541.00 m).

The experiment was conducted over three consecutive cropping seasons using a minimally disturbed aluminum soil core system exposed to ambient outdoor conditions. In the first year, 24 undisturbed open-ended soil cores (37 cm depth, 20 cm diameter) were collected from each of the four different field sites representing three different soil zones by inserting the cores into the soil using a truck mounted hydraulic press and subsequently extracting the cores manually. Eight replicated soil cores from each site were transported to each of the other locations where they were reinstalled to a depth of 37 cm with two rows distancing of 45 cm between two cores (see the image of the experimental layout, Fig. A.3.2). Thus, each site had 32 cores, with eight from each original site-reinstalled at each location. Commercially available AMF inoculant (MYKE® PRO GR containing active propagules of *R. irregularis*) was introduced to half of the cores (16 cores each site) following a completely randomized design. The remaining cores remained uninoculated and therefore represented indigenous AMF populations. The persistence of AMF inoculants was monitored in inoculated soil cores in response to soil types (soil physicochemical properties) and ambient climatic factors over three cropping seasons.

### 3.4.2 Inoculation, fertilization, and seeding in soil cores

Field pea (*Pisum sativum* L., CDC Meadow) was used as a host crop for the 2011 and 2013 cropping season. Wheat (*Triticum aestivum* L., CDC Go) was grown as a rotational crop in 2012 between the two field pea seasons. Soil cores were hand seeded (six pea and nine wheat seeds) and seedlings were thinned to three pea and five wheat seedlings per core. Seedlings were thinned two-weeks post seeding. Rhizobia inoculant (N-Prove® containing *Rhizobium leguminosarum* bv *viceae* 5.0 x $10^8$ viable cells per gram inoculant, Novozymes BioAg, Canada) was applied to pea seeds at seeding in 2011 and 2013 at the recommended rate (equivalent to 3 mL $kg^{-1}$ seed). Seed inoculation was performed 30 minutes prior to sowing into the cores. No inorganic chemical fertilizers and pesticides were used for the 2011 and 2013 cropping season, but urea was applied once during wheat seeding at the rate of 0.60 g $core^{-1}$ (0.28 g N $core^{-1}$). Weeds were controlled by hand three to four times during the growing season.

The AMF inoculant (MYKE® Pro GR, Premier Tech, Québec, Canada) with 110 viable spores-propagules of *Rhizophagus irregularis* $g^{-1}$ inoculant was applied at a rate of 2.4 g soil $core^{-1}$ (area of soil core 0.03 $m^2$). The actual application rate was equivalent to the recommended in-row application rate, assuming a 22 cm row spacing and a 2.5 cm row width (7.50 kg $ha^{-1}$). Farmers generally apply inoculant into the furrow along with seed. To apply inoculant, the top 5 cm of soil was removed from each core, the inoculant (2.4 g) was spread onto the surface of the soil, and then the surface soil was replaced into the surface of the cores. Rhizobia inoculated seeds were then placed at a depth of 4 cm. Seeding holes were filled, and 650 mL of water was applied to each of the cores. The AMF inoculation occurred only at the initiation of the study (2011) and was not repeated. No AMF inoculants were added into the soil cores during the 2nd and 3rd cropping seasons. The schedules of seeding, harvesting and trap culture for duration of experimental sites are listed in Table 3.1.

### 3.4.3 Initial soil sampling

Prior to seeding, composite soil samples were collected from each study site from the 0 to 15 cm depth in May 2011 using a JMC Backsaver N-2 (3.048 cm diameter) soil core (Clements Associates, Inc, IA 50208, USA). Samples were stored in plastic bags and maintained at -20 °C. Complete nutrient (macro and micro) profiles, organic matter content and necessary physico-chemical properties of soil were determined for each soil of the experimental sites, and are summarized in Table 3.2.

### 3.4.4 Plant nutrient uptake and measurement

At the end of each cropping season (September 2011, 2012 and 2013), field pea and wheat were harvested by hand. The above ground portions of the plants were cut off at ground level and bagged for determination of biomass dry weight. Shoot and grain samples were dried at 70 °C for 48 h, separated, weighed again, and ground to pass through a 0.5 mm pore size screen. Representative samples of shoot and seed were digested using sulfuric acid-peroxide and analyzed for nutrient concentration of P and N using a Technicon™ Auto Analyzer (Technicon Industrial Systems, Tarrytown, USA). The P and N contents of both shoot and grain were determined using the methods described by Thomas et al. (1967).

**Table 3.1** Schedules of seeding and harvesting the incubated soil cores at four field locations in Saskatchewan.

| Information of Cultivation | Years | Sites/Locations† | | | |
|---|---|---|---|---|---|
| | | **Swift Current** | **Outlook** | **Scott** | **Melfort** |
| Seeding date | 2011 | 9th June | 8th June | 6th June | 7th June |
| | 2012 | 30th May | 17th May | 26th May | 24th May |
| | 2013 | 21st May | 22nd May | 23rd May | 24th May |
| Harvest date | 2011 | 10th September | 7th September | 5th September | 9th September |
| | 2012 | 24th August | 22nd August | 28th August | 4th September |
| | 2013 | 20th August | 23rd August | 26th August | 1st September |

†Sites located at SPARC: Swift Current, CIDC: Outlook, AAFC research farm at Scott and Melfort, Saskatchewan, Canada.

NB: Soils (0-15 cm) at harvest dates were collected and used for field pea trap culture experiments between 25th October to 30th December, 2011, 3rd November to 4th January, 2012, and 24th October to 25th December, 2013.

**Table 3.2** Physical and chemical characteristics of initial soil, collected from the the experimental sites in 2011.

| Soil Properties and Depth (0-15 cm) | Soil Order, Great Groups and Experimental Sites† | | | | Methods |
|---|---|---|---|---|---|
| | Brown Chernozem (Swift Current) | Dark Brown Chernozem (Outlook) | Dark Brown Chernozem (Scott) | Black Chernozem (Melfort) | |
| Organic Carbon (g $kg^{-1}$) | 19.0 | 17.0 | 19.0 | 55.0 | Walkley Black method (Walkley and Black, 1934) |
| Organic Matter (g $kg^{-1}$) | 33.1 | 28.9 | 33.9 | 94.3 | Walkley Black acid digestion method |
| Total N (g $kg^{-1}$) | 1.6 | 1.7 | 1.5 | 5.0 | LECO-combustion method (Kowalenko et al., 2001) |
| Avail. P (mg $kg^{-1}$) | 41.1 | 66.8 | 59.1 | 34.4 | Calcium chloride solution (Comm. Soil Sci. Plant Anal. 25, 1994) |
| Avail. K (mg $kg^{-1}$) | 327 | 228 | 708 | 371 | |
| Avail. S (mg $kg^{-1}$) | 6.7 | 57.8 | 3.7 | 7.8 | ICP-AES method (Zhao et al., 1994) |
| Fe (mg $kg^{-1}$) | 49.9 | 8.4 | 16.3 | 134 | Metal-DTPA method (Roca and Pomares, 1991) |
| Cu (mg $kg^{-1}$) | 0.59 | 0.5 | 0.41 | 0.78 | |
| Mn (mg $kg^{-1}$) | 17.2 | 3.6 | 9.14 | 24.4 | |
| Zn (mg $kg^{-1}$) | 1.01 | 1.67 | 1.6 | 4.14 | |
| Soil pH | 6.6 | 7.01 | 5.9 | 7.9 | Soil: water extraction method (Sparks et al., 1996) |
| EC (dS $m^{-1}$) | 0.21 | 0.37 | 0.19 | 0.18 | |

†Sites located at SPARC: Swift Current, CIDC: Outlook, AAFC research farm at Scott and Melfort, Saskatchewan, Canada.
Soil Order and Great Groups: Soil classification working group, 1998. The Canadian System of Soil Classification. Agric. and Agri-Food Can. Publ. 1646 (Revised). 187 pp.

### 3.4.5 Soil sample collection for AMF trap culture

Soil samples were collected from 0 to 15 cm using JMC Backsaver N2 soil core (Clements Associates, Inc, IA, USA) (2 cores per treatment, approx. 150g soil per core) from inoculated and uninoculated aluminum soil cores at field pea and wheat harvest as described previously. The JMC Backsaver was washed with 70% ethanol (to avoid contamination) between cores. Soil samples were transported in a cooler and preserved at -20 °C for trap culture use.

The collected core soils were used for a trap culture conducted in a growth chamber (phytotron) with ambient day/night temperatures of 24 °C/18 °C with 16 h day lengths. The trap culture using field pea as a host plant was used to determine AMF species composition (Ferrol et al., 2004). Core soils were mixed mechanically and passed through a 4 mm sieve before use. The fine sand (Microcrystalline Silica CAS, Unimin Corp, USA) was sterilized by autoclaving three times on the liquid autoclave cycle (120 ◦C, 2 hr) and subsequently placed in sterile 750 mL plastic pots containing 400g soil/sand mix (1:1). The soils were thoroughly homogenized (1:1, w/w) with sterilized fine sand. Before sowing, field pea seeds were surface disinfected by immersing in a 0.5% sodium hypochlorite solution for 20 min and washing in sterile tap water (Saucer and Burrough, 1986). A total of one host x four soil types x two treatments x four sites = 32 x 4 replicated pots (128) each season were arranged for this trap culture. Control pots were maintained using respective autoclaved soil samples. Pots were irrigated as needed and the half-strength Hoagland (Hoagland and Arnon, 1950) solution (N: 211, S: 64, K: 236, Mg: 48, Ca: 200, B: 0.01, Cu: 0.01, Fe: 0.5, Mn: 0.1, Mo: 0.02, Zn: 0.01 µg $mL^{-1}$) without P was applied (100 mL per pot) onto soil: sand mix prior to seeding. The nutrient solution and all other materials used in this trap culture were sterilized to avoid any possible contamination. Field pea were allowed to grow for 8 weeks. Harvested trap roots were thoroughly rinsed in tap water free of soil then washed with deionized water to remove any residue soil particles and debris and blotted dry. The cleaned roots were immediately immersed in liquid N and preserved at -80 ◦C until molecular analysis. These methods were repeated for the 2012 and 2013 soils collected from field cores at harvest.

### 3.4.6 DNA extraction from AMF trap field pea roots

The DNA was extracted from each root sample using Qiagen Plant DNeasy kits (QIAGEN, Mississauga, ON) according to the manufacturer's recommended protocol. One hundred milligrams of root tissue were freeze dried and placed in 2-mL screw-top micro-centrifuge tubes with 5-mm ceramic beads, and pulverized to a powder using Precellys® 24 tissue homogenizer (Bertin Technologies, USA). A total of 24 samples at a time were processed at 4000 rpm for three cycles (30s per cycle) to homogenize the root tissue for further DNA extraction. The pure genomic DNA from roots (both plant and fungal) was eluted in Tris EDTA (TE) buffer for further use.

### 3.4.7 18S rRNA gene pyrosequencing methods

To analyze the AMF community associated with trap field pea roots, a nested PCR protocol was used to amplify 800 bp partial fragment of the AMF 18S rRNA gene for 454 pyrosequencing (Dumbrell et al., 2011). The universal eukaryotic primers NS1 and NS4 (White et al., 1990) were used in the first round of PCR followed by the AMF specific primer pair AML1 and AML2 (Lee et al., 2008) The forward primer (AML1) and reverse primer (AML2) also included tags CS1 and CS2 (Fluidigm Corp., San Francisco, CA) that were anchors in a third PCR reaction adding Titanium MIDs and Lib-L adaptors sequences. The sequences of primers, tags and adaptors are included in Table 3.3.

The first polymerase chain reaction (PCR) conditions were as follows: initial denaturing step at 95°C for 15 min; 30 cycles at 95°C for 30 sec; 50°C for 30 sec; 72°C for 1 min 30 sec; and a final extension step at 72°C for 3 min, with a 5 µL reaction volume including 1 µL of 1/10 diluted DNA template, 1 mM dNTPs, 0.4 µM of each primer (NS1 and NS4) and FastStart High Fidelity (Roche, 04 738 292 001). Five microliters of reaction mixture in the second round of PCR included FastStart High Fidelity (Roche), 1 µL of diluted nested PCR product, and 0.4 µM of each primer (AML1-CS1F and AML2-CS2R). The conditions for the second round of PCR were as follows: initial denaturing step at 95°C for 15 min; 33 cycles at 95°C for 30 sec; 60°C for 30 sec; 72°C for 1 min 30 sec; and final extension step at 72°C for 5 min.

The third PCR was performed to incorporate 10 nt-MIDs (Titanium Lib-L forward-MDs-CS1 and Titanium Lib-L reverse adaptor-CS2) and contained 0.5 µL of diluted PCR, 1 µL of 2 µM barcodes, 0.5 µL of DMSO, 0.1 µL FastStart High Fidelity (Roche) and 0.2 µL of 10 mM dNTP. The third PCR conditions were: initial denaturing step at 95°C for 10 min; 15 cycles at 95°C for 15 sec; 60°C for 30 sec; 72°C for 1 min; and a final extension step at 72°C for 3 min.

All final PCR products were run on 2% agarose gel and quantified using picogreen. Samples were combined into pools of 96 samples based on their MIDs. Each pool was purified with three AMPure XP (Agencourt/ Beckman Coulter) protocols (ratio 0.5) and quantified using Qubit 2.0 Fluorometer (Life Technologies, CA, USA). A total of 386 replicated DNA samples for three sampling seasons (128 per season x 3 = 386 samples for 2011, 2012 and 2013 sampling seasons) were analyzed and pyrosequenced in this study. The samples were run on an Agilent 2100 Bioanalyzer using a high sensitivity DNA kit to confirm the size and quality of amplicons. Finally, unidirectional sequencing was performed in half region runs for each pool on a GS-FLX+ system (454 Life Sciences/ Roche Applied Science) at McGill University and Génome Québec Innovation Centre (Montréal, QC, Canada) for pyrosequencing analysis.

### 3.4.8 DNA extraction and pyrosequencing of AMF inoculant strains

The commercial AMF inoculant fungal strain, *R. irregularis*, was subjected to pyrosequencing for its identity at the species level and to differentiate the introduced inoculant from the indigenous AMF communities. Sucrose density gradient centrifugation (Gerdemann and Nicolson, 1963) was used to extract spores from the commercial and non-indigenous *R. irregularis* inoculant. Spores were cleaned by re-suspending in a 40% (v/v) sucrose solution (Struble and Skipper, 1988) and centrifugation was carried out at 1400 rpm for 5 min. The supernatant was poured into the sieves (44 µm diam). The spores on the sieves were carefully rinsed with tap water and spores were picked by pipette using a dissecting microscope. Spores were cleaned by transferring into doubled distilled water and left for 24 h at 4 °C. Abnormal looking spores such as discolored or broken spores were identified using a dissecting microscope and discarded. Spores were surface disinfected with two washes of 2% chloramine T and rinsed in two washes of PCR-grade water (Helgason et al., 2002).

**Table 3.3** Primers, tags and 454 Lib-L adaptors used for PCR amplification of 18S rRNA gene for pyrosequencing analyses of the AMF community assemblages in trap field pea roots and AMF inoculant used in this study.

| †PCR Primers | Primers Sequence (5´ to 3´) | Reference |
|---|---|---|
| NS1 | GTAGTCATATGCTTGTCTC | (White et al., 1990) |
| NS4 | CTTCCGTCAATTCCTTTAAG | (White et al., 1990) |
| AML1 | ATCAACTTTCGATGGTAGGATAGA | (Lee et al., 2008) |
| AML2 | GAACCCAAACACTTTGGTTTCC | (Lee et al., 2008) |
| CS1 (Tag) | ACACTGACGACATGGTTCTACA | |
| CS2 (Tag) | TACGGTAGCAGAGACTTGGTCT | |
| Lib-L adaptor (Forward) | CCATCTCATCCCTGCGTGTCTCCGACTCAG | |
| Lib-L adaptor (Reverse) | CCTATCCCCTGTGTGCCTTGGCAGTCTCAG | |

†First PCR primers: NS1Forward/NS4Reverse, second PCR primers: AML1-CS1 Forward/AML2-CS2 Reverse and third PCR 454 Lib-L forward adaptor-10nt-MIDs-CS1/454 Lib-L reverse adaptor-CS2.

Extraction of DNA from clean AMF spores was performed as per a modified method described by Gamper et al. (2008). Briefly, instead of extracting genomic DNA by washing a single spore in PCR-grade water, approximately 100 surface disinfected spores were placed in a 1.5 mL Eppendorf tube containing 50 µL PCR-grade water and crushed with a sterilized micro-pestle. Sixty microliters of 100 mM Tris (pH 8.0) and 20 µL of 20% (w/v) Chelex-100 resin (Bio-Rad laboratories, Hercules, CA, USA) were added to the crushed spore suspension and gently vortexed and incubated at 95°C for 5 min and then cooled on ice. The suspension was then centrifuged (1400 rpm) for 1 h and the resulting pellet discarded. The supernatant contained the pure DNA although the quantity was low (<10 ng). This supernatant was directly used as a DNA template for PCR amplification. Pyrosequencing protocols including PCR conditions and library preparations were performed as described in Section 3.4.7.

### 3.4.9. Bioinformatics

A total of 37 405, 28 648, and 42 174 18S rRNA gene sequence reads were obtained from the AMF trap field pea roots harvested following the 2011, 2012 and 2013 cropping seasons, respectively. The raw pyrosequencing reads were processed using MOTHUR version 1.31 (Schloss et al., 2009) to clean the ambiguous nucleotides (average score of quality <30) (Huse et al., 2010). The excessively long homopolymers and short and low-quality sequence

reads were removed from the dataset using the command “trim.seqs”. The average 650 to 850 bp long 18S rRNA gene sequences were targeted for downstream analysis. The clean sequences were aligned against Silva eukaryotic references databases (http://www.mothur.org/w/index.php?title=Silva_reference_files&redirect=no ) using a k-nearest neighbour consensus and Bayesian approach using the command “align.seqs”. The commands “screen.seqs”, “filter.seqs” (vertical = T, trump =), “remove.seqs” were used to detect poorly aligned sequences, which were removed from the data set. At this stage, chimeric sequence reads were detected and removed using “chimera.uchime” and “remove.seqs”, respectively. The original Uchime reference sequence files were downloaded from the public Uchime domain (http://drive5.com/usearch/manual/uchime_algo.html) (Edgar et al., 2011). The commands “precluster.seqs”, “classify.seqs” were used to classify sequences which belong to AMF phyla Glomeromycota (non-Glomeromycota sequences were removed from the data set using the command “remove.seqs”). Constructing the distance matrix and cluster sequences was performed using a further neighbour algorithm and operational taxonomic units (OTUs) were determined based on 97% similarity using the command “dist.seqs” and “cluster.seqs”. Finally, the “get.oturep” command was used to identify representative sequences for each OTU. The singletons (one sequence) and doubletons (two sequences) that clustered into OTUs were removed from the data set using the command “remove.reqs”. At this stage, resulting fasta files (OTUs sequences file format) of each sample were merged together using the command “merge.seqs”. The combined file (fasta) was uploaded in CD-HIT Suite (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=cd-hit) to re-cluster the representative OTU sequences using sequence identity cut-off to 0.97. Non-Glomeromycota sequences that remained were manually deleted from the fasta file. Only the sequences considered representative OTUs produced a match with 97% similarity or above and above 90% query coverage in the blast search of the GenBank non-redundant representative sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) (Zhang et al., 2000; Kent, 2002). The number of sequence reads for each OTU identified as any of the AMF taxa (genus) in each individual sample was considered the absolute abundance of that AMF community using the command “classify.otu”. The list file generated after clustering OTUs for each sample was used to estimate Shannon diversity indices ($H'$) using the command “collect.single” based on the formula referred by Shannon (1948).

### 3.4.10 Phylogenetic tree analysis

The OTU sequences from field pea trap roots including the OTUs identified as introduced *R. irregularis* inoculant strain, and AMF reference sequences (closest match <97%) from GenBank were aligned using ClustalW. The unaligned sequences were removed and the aligned sequence file was saved as an aligned sequence file in Mega format. The neighbour-joining phylogenetic reconstruction (Saitou and Nei, 1987) was used to build a phylogenetic tree using MEGA v.6 (Tamura et al., 2013). Bootstrap replication method was set at a confidence level of 1000 with the Kimura 2 parameter model. The nomenclature of AMF taxa was used as the classification of Schüssler and Walker (2011).

### 3.4.11 Statistical analysis

For AMF community analysis, the sample was normalized by calculating the proportional reads (number of representative sequences) of an OTU in a sample divided by the total number of absolute sequence reads of all OTUs in that sample (Amend et al., 2010). The significance of the effect of different fixed factors (inoculation, soil type, climatic site and their interaction) on the relative abundance of the compositional AMF taxa was tested according to a three-way analysis of variance (ANOVA) (Anderson, 2005) using SAS (SAS Institute Inc., NC 27513-2414, USA). Prior to total community analysis, the proportional reads of the OTUs from inoculant strain, *R. irregularis* were separated (inoculant OTUs reads subtracted from total reads of an inoculated sample) from each inoculated sample, so that the actual effect of inoculation on the community alteration was determined.

Before statistical analyses, relative abundance of indigenous AMF and introduced inoculant was estimated from the absolute number of sequence reads of OTUs in each sample. To determine the persistence of the inoculant over three cropping seasons, the absolute abundance of *R. irregularis* sequence reads in each inoculated sample was divided by the total absolute indigenous AMF sequence reads of the same sample quantified as relative abundance of introduced inoculant. Similarly, to determine the relative abundance of individual indigenous AMF taxa (such as *Glomus, Funneliformis, Claroideoglomus*), the absolute abundance of inoculant was subtracted from the total abundance of AMF taxa, then the absolute abundance of

each indigenous AMF taxa sequence reads in each inoculated sample was divided by the total absolute AMF sequence reads (including inoculant) quantified as relative abundance of each indigenous AMF taxa. The absolute abundance of each AMF taxa in uninoculated control sample was also divided by the total absolute abundance of all AMF taxa present in control sample. The detailed calculation of the relative abundance of indigenous AMF taxa and inoculant is presented in appendix A. Before analyses, all non-parametric (percent relative abundance) data were subjected to a normality test. Skewness and kurtosis of percent relative abundance data of AMF communities were performed.

The three data sets (2011, 2012 and 2013) were run together to test the effect of inoculation, soil and site (climate) on the relative abundance of different AMF taxa, Shannon diversity index and the persistence of introduced inoculant (relative abundance of *R. irregularis*) using a three- way analysis of variance (ANOVA). Tukey's honestly significant difference ($P<0.05$) was determined using PROC MIXED in SAS v.2.0.4 to assess the significance of differences among the persistence level of inoculant, *R. irregularis* and Shannon diversity index for each sample.

## 3.5 Results

### 3.5.1 Plant growth performances and climatic conditions at the study sites

The crop biomass and nutrient uptake for three consecutive cropping seasons (2011 to 2013) were presented in the appendix D. The historical precipitation (mm) and temperature (°C) annual average over 30 years (1981–2010) at each experimental site are summarized in Fig. 3.1. The precipitation and temperature during the growing season (May to September) in 2011, 2012 and 2013 are presented in Fig. 3.2. The climate data (temperature and precipitation) were received from Environment Canada (http://climate.weather.gc.ca/climate_normals/index_e.html).

Both temperature and precipitation fluctuated during the cropping seasons of the three study years compared to 30 year cropping season average at both Scott and Melfort. The temperature and precipitation were consistent with 30 years normal during the study period at Swift Current and Outlook. According to the historical the 30-year average precipitation and temperature, the Scott and Melfort sites received relatively high amounts of precipitation and low temperatures resulting in those sites being relatively wet and cool. In contrast, Swift Current and Outlook were drier and warmer regions (Fig. 3.1).

### 3.5.2 AMF Community sequence analysis using GS-FLX+ pyrosequencing platform

A total of 37 405, 28 648 and 42 174 AMF sequences of 18S rRNA gene were obtained from field pea trap root samples, after cleaning and removal of short, ambiguous and chimera sequences for the 2011, 2012 and 2013 seasons, respectively. The number of absolute and relative sequence reads obtained from GS-FLX+ 454 pyrosequencing technology for three cropping seasons are reported in Appendix A (Tables A.3.1 to A.3.6).

The 18S rRNA sequence length ranged from 650 to 800 bp which was over 87% of the sequence length of 18S rRNA gene fragment amplified by the AML/AML2 primer pair. The nested PCR protocol with the NS1/NS4 and AML1/AML showed fairly good AMF specificity for all data over the three years of sampling with an average of less than 19% of the sequences from non-Glomeromycota microorganisms.

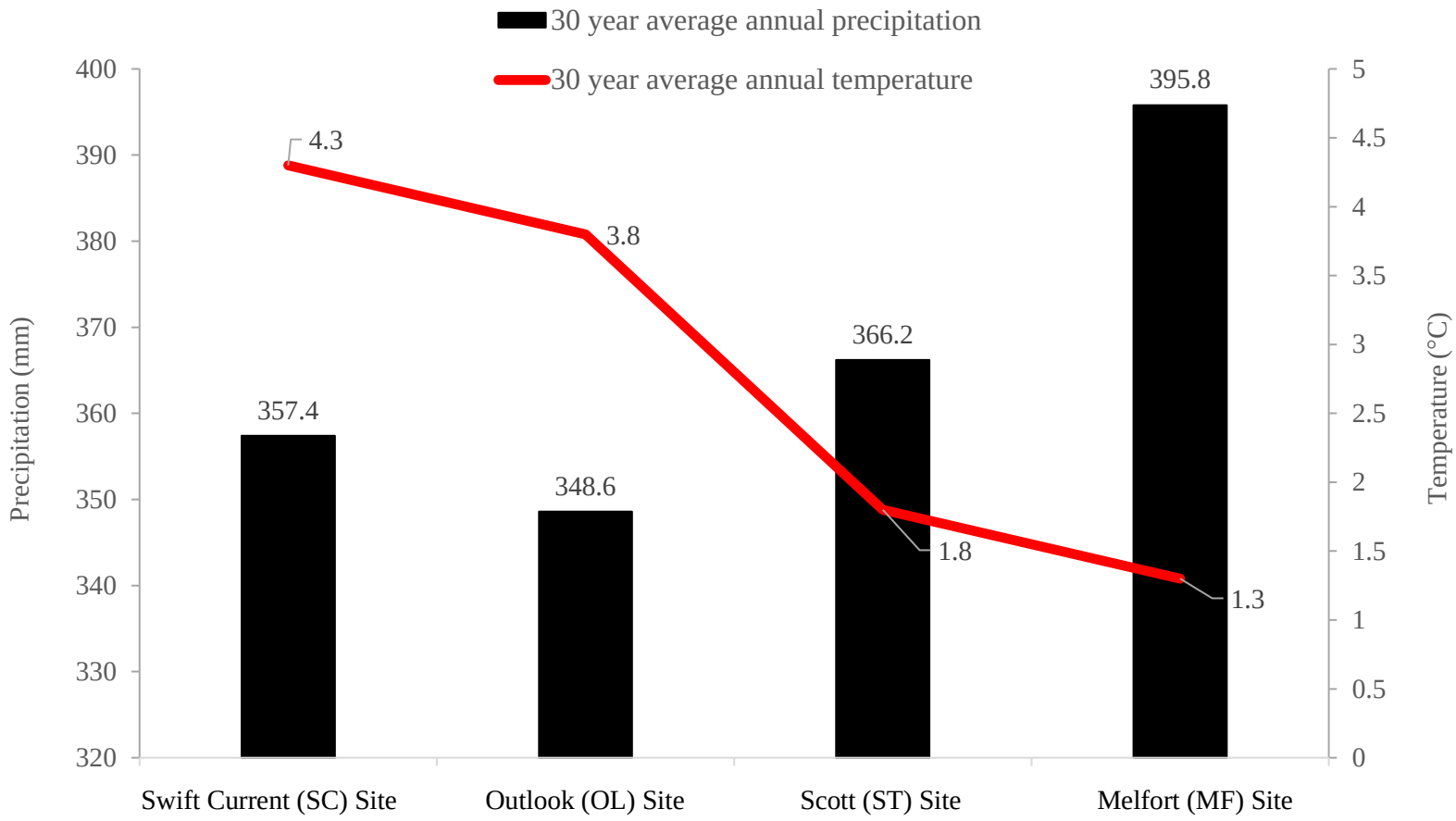


**Figure 3.1** 30-years average annual (1981-2010) precipitation (mm) and temperature (°C) at four study sites in Saskatchewan.

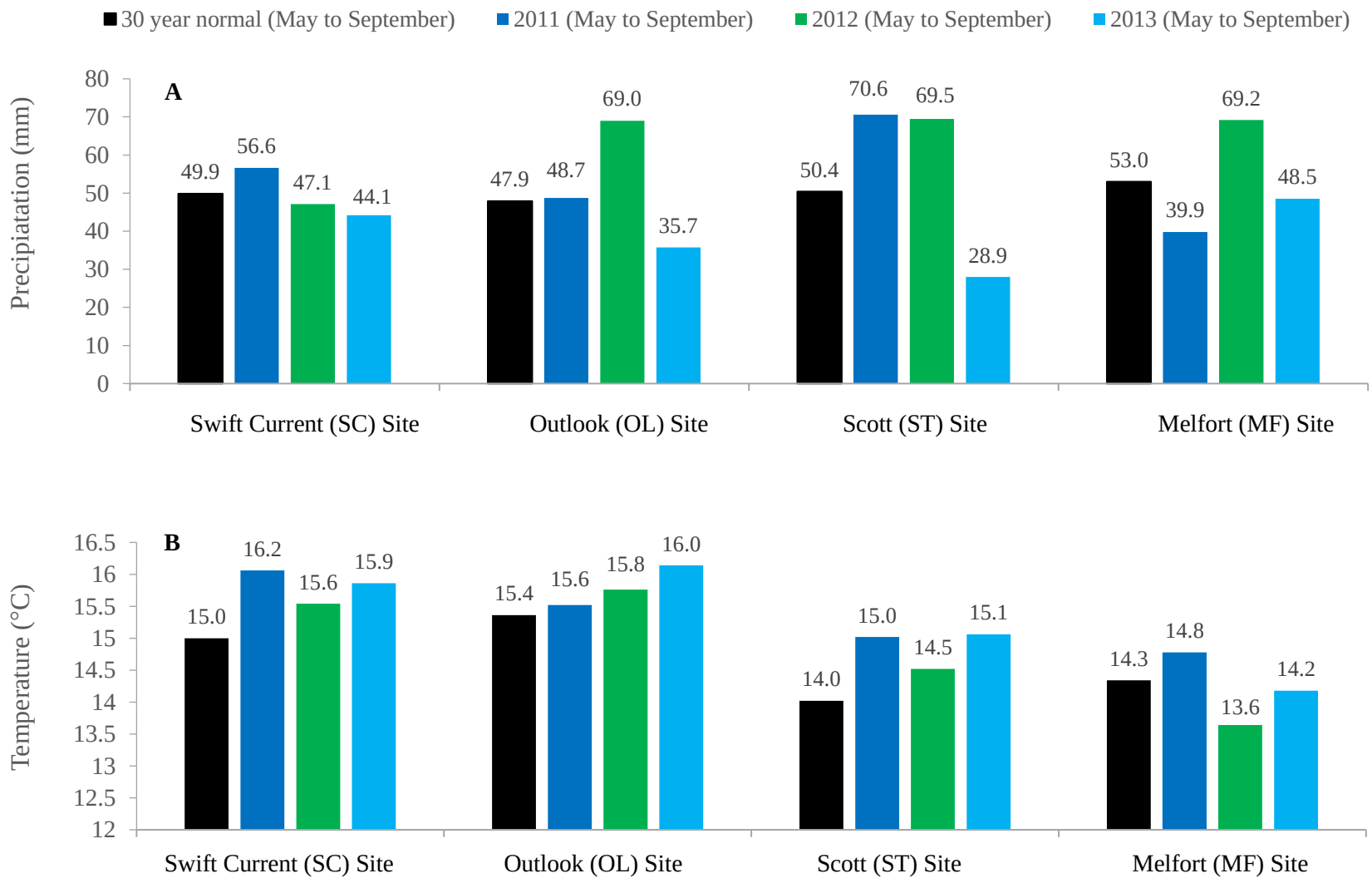


**Figure 3.2** 30-years (1981-2010), 2011, 2012 and 2013 average crop season (May to September) **A.** precipitation (mm) and **B.** temperature (°C) at four study sites in Saskatchewan.

The sequence reads were clustered based on 97% sequence similarity into 86, 30 and 72 OTUs for year 1, year 2 and year 3, respectively representing nine genera under six families. Glomeraceae (*Rhizophagus, Glomus and Funneliformis),* Claroideoglomeraceae *(Claroideoglomus),* Diversisporaceae *(Diversispora)* and Paraglomeraceae *(Paraglomus)* were commonly found in the trap roots from the three sampling years. In addition, Glomeraceae (*Septoglomus)* and Archaeosporaceae (*Archaeospora)* in 2011 and Acaulosporaceae (*Entrophospora)* in 2012 and 2013 root samples were detected (Tables 3.5 to 3.7).

The Glomeraceae represented the majority of the OTUs accounting for 28 out of 86 following pea in year 1 (indigenous *Rhizophagus*-9, *Funneliformis*-12, *Glomus*-17 *and Septoglomus*-6*)* including four OTUs from commercial non-indigenous *R. irregularis* inoculant followed by Paraglomeraceae (18 OTUs), Claroideoglomeraceae (12 OTUs) and Archaeosporaceae (6 OTUs), Diversisporaceae (2 OTUs) in year 1 (2011).

In year 2 after wheat (2012), 15 months after inoculation, sixteen OTUs belonged to Glomeraceae (indigenous *Rhizophagus*-1, *Funneliformis*-9, *and Glomus*-5) including one OTU detected from commercial non-indigenous *R. irregularis,* seven OTUs belonged to Claroideoglomeraceae, four OTUs belonging to Paraglomeraceae, two belonging to Diversisporaceae and one belonging to Acaulosporaceae (*Entrophospora*).

A total of 72 OTUs were detected in year 3 after pea, 27 months after inoculation. Of these, 47 OTUs were from Glomeraceae (indigenous *Rhizophagus*-8, *Funneliformis*-16, *and Glomus*-21) including two OTUs belonging to the commercial non-indigenous *R. irregularis,* 13 OTUs from Claroideoglomeraceae, six OTUs belonged to Diversisporaceae, three OTUs from Paraglomeraceae, and three OTUs from Acaulosporaceae (*Entrophospora*).

### 3.5.3 Distinguishing the introduced commercial non-indigenous *R. irregularis* inoculant strain from the indigenous *Rhizophagus* community

The representative sequences of 86 OTUs from the 2011 sampling season and 12 reference sequences from NCBI databases were used to construct a neighbor-joining phylogenetic tree (Saitou et al., 2007) for molecular identification of AMF community taxa designated as an operational taxonomic unit (OTU). The OTU generated from the commercial

AMF inoculant was clustered with the other 13 OTUs which had a higher level of similarity with known reference sequences according to the BLAST (Basic Local Alignment Search Tool) GenBank (Fig. 3.3). The OTU signature generated from the commercial *R. irregularis* inoculant spore had the closest match (99%) with *R. irregularis* (accession no. FR750222.1). The 13 OTUs namely, OTU1, OTU3, OTU9, OTU13, OTU18, OTU34, OTU37, OTU38, OTU55, OTU56, OTU60, OTU75 and OTU84 were clustered together with high levels of similarities varying from 97% to 99% with the reference sequences of AMF genus *Rhizophagus* from the NCBI GenBank BLAST search and showed relatively high bootstrap values in the phylogenetic analysis (Fig. 3.3).

The 13 OTUs of the *Rhizophagus* cluster were distributed throughout the sample set of 2011. Of them, four OTUs (OTU1, OTU3, OTU37 and OTU84) were detected in the trap roots from inoculated field soils, where AMF inoculant was introduced. Also these four OTUs were the closest match with the OTU signature generated from *R. irregularis* inoculant and the reference sequence from GenBank (accession no. FJ009618.1). Thus, these four OTUs were confirmed to be associated with the introduced commercial non-indigenous *R. irregularis* inoculant (Fig. 3.3 and Table A.3.4).

The other nine OTUs (OTU9, OTU13, OTU18, OTU34, OTU38, OTU55, OTU56, OTU60, and OTU75) within the *Rhizophagus* cluster were found both in the inoculated and the uninoculated control (representing only indigenous AMF) soil cores from Outlook and Melfort and were assumed to be indigenous *Rhizophagus* already present in the soils (Fig. 3.3 and Table A.3.1).

Similar techniques were applied to detect introduced non-indigenous *R. irregularis* inoculant from the data sets of the 2012 and 2013 samples. With regards to the 2012 sample set, 30 AMF OTUs were generated, with only two OTUs (OTU22 and OTU25) being clustered with the *Rhizophagus* OTU group. OTU25 showed the closest match (99%) with the reference sequence of *R. irregularis* from GenBank (accession no. FJ009618.1) and the OTU signature, generated from introduced *R. irregularis* inoculant, and thus OTU25 was confirmed to be the OTU signature from the introduced *R. irregularis* (Fig. 3.4 and Table. A.3.5). The OTU22 in the *Rhizophagus* cluster was found both in the inoculated and uninoculated control soil cores of

Outlook and Melfort and thus, it is likely that this represents an indigenous *Rhizophagus* already occurring in those soils.

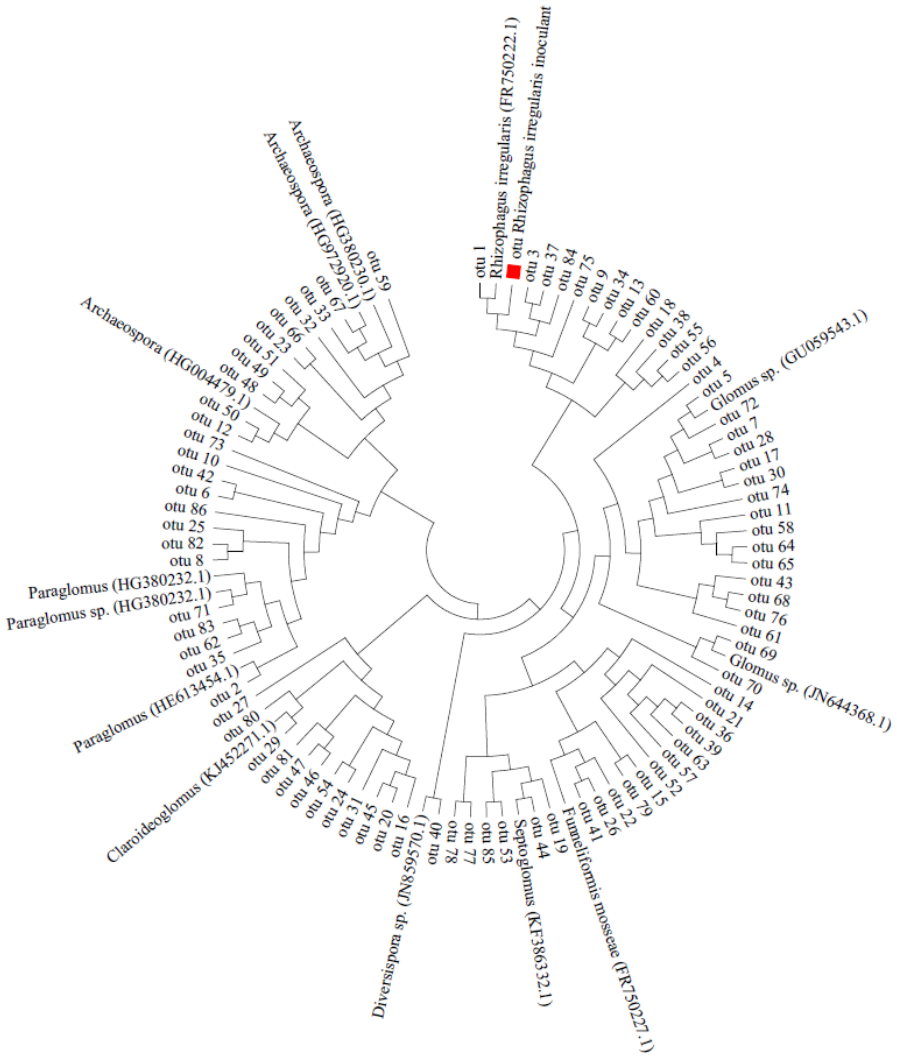


**Figure 3.3** Phylogenetic analysis of 86 AMF OTUs in the field pea trap roots, detected by pyrosequencing in year 1 (2011), 3 mo after inoculation at four sites. AMF OTUs are clustered as *Rhizophagus*, *Glomus*, *Funneliformis*, *Septoglomus*, *Claroideoglomus*, *Diversispora*, *Archaeospora* and *Paraglomus* groups. Phylogenetic relationships are obtained by neighbor-joining analysis of AMF 18S rRNA gene. GenBank reference sequences are indicated within a parenthesis. Sequence representing the commercial non-indigenous AMF inoculant strain, *R. irregularis* is marked with a red box.

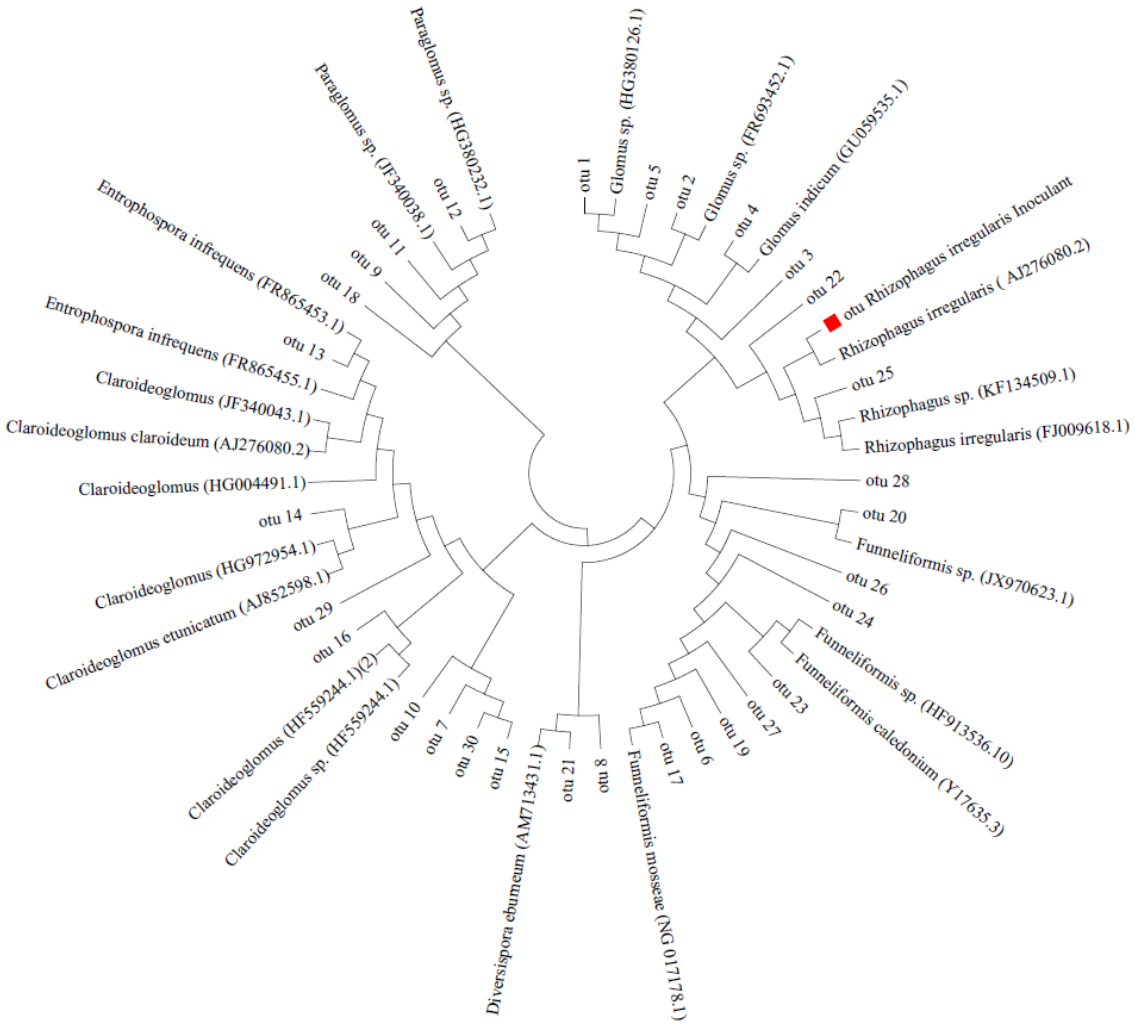


**Figure 3.4** Phylogenetic analysis of 30 AMF OTUs in the field pea trap roots, detected by pyrosequencing in year 2 (2012), 15 months after inoculation at four sites. AMF OTUs are clustered as *Rhizophagus*, *Glomus, Funneliformis, Septoglomus, Claroideoglomus, Diversispora*, *Archaeospora* and *Paraglomus* groups. Phylogenetic relationships are obtained by neighbor-joining analysis of AMF 18S rRNA gene. GenBank reference sequences are indicated within a parenthesis. Sequence representing the commercial non-indigenous AMF inoculant strain, *R. irregularis* is marked with a red box.

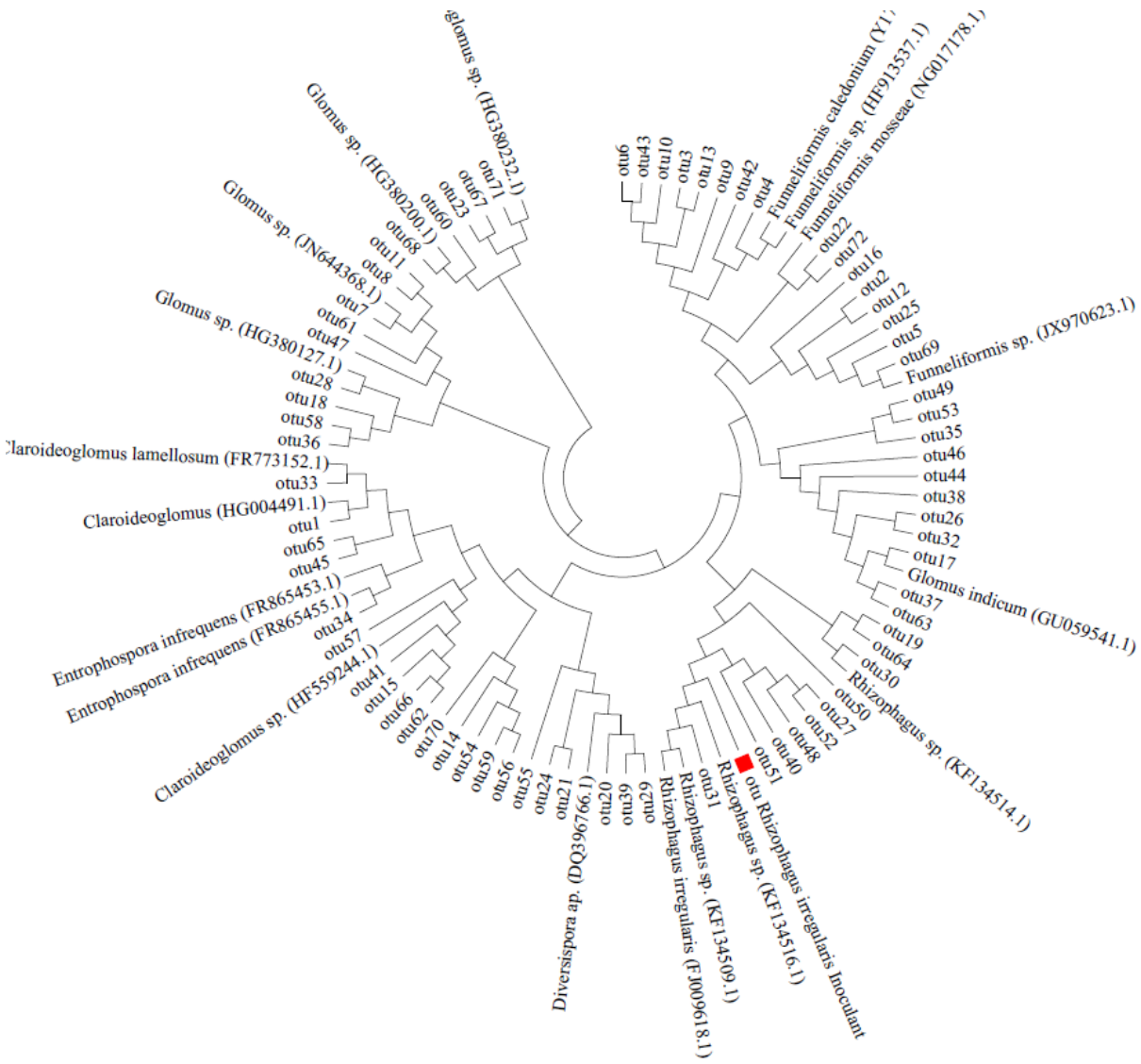


**Figure 3.5** Phylogenetic analysis of 72 AMF OTUs in the field pea trap roots, detected by pyrosequencing in year 3 (2013), 27 months after inoculation at four sites. AMF OTUs are clustered as *Rhizophagus, Glomus, Funneliformis, Septoglomus, Claroideoglomus, Diversispora, Archaeospora* and *Paraglomus* groups. Phylogenetic relationships are obtained by neighbor-joining analysis of AMF 18S rDNA. GenBank reference sequences are indicated within a parenthesis. Sequence representing the commercial non-indigenous AMF inoculant strain, *R. irregularis* is marked with a red box.

A total of 72 OTUs were generated from the 2013 sample set, 27 months post inoculation. Of these, 10 OTUs (namely, OTU19, OTU27, OTU30, OTU31, OTU40, OTU48, OTU50, OTU51, OTU52 and OTU64) were clustered with the OTU generated from *R. irregularis* inoculant and assigned to *Rhizophagus* taxonomic group with varying similarity ranging from 97% to 99% according to the known reference sequences obtained from a GenBank BLAST search. OTU31 and OTU50 were found in the inoculated soil cores and showed the closest match (99%) with the reference sequence of *R. irregularis* from GenBank (accession no. FJ009618.1) and the OTU signature generated from *R. irregularis* inoculant; thus, OTU31 and OTU50 were confirmed as the OTUs generated from introduced *R. irregularis* inoculant. The other eight OTUs were detected both in the inoculated and uninoculated control cores of Outlook and Melfort and are considered to represent indigenous *Rhizophagus*. (Fig. 3.5 and Table A.3.6).

The *Rhizophagus* taxonomic group was not detected in uninoculated Swift Current and Scott soils. The relative abundance of 18S rRNA gene of the introduced *R. irregularis* inoculant strain (OTU31 and OTU50) was only found in the trap roots from inoculated core soil samples of Scott and Swift Current in the 2011 data set (Table A.3.4).

### 3.5.4 Persistence of *R. irregularis* inoculant in some Saskatchewan Prairie soils

Persistence of inoculant was defined as the number of sequence reads in OTUs belonging to the *R. irregularis* inoculant divided by the total number of sequence reads in OTUs (from indigenous AMF taxa) found in each inoculated sample. This was then referred to as relative abundance of *R. irregularis* as presented in Fig. 3.6. The pyrosequencing data for the relative abundance of 18S rRNA gene of *R. irregularis* inoculant revealed that the inoculant established and persisted in the trap roots in all of the soils. The significant ($P<0.01$) influence of soil and site on the prolonged survival (persistence) of the introduced *R. irregularis* inoculant strain in soil cores incubated at different locations was determined using maulti-way analysis of variance (ANOVA). A significant interaction between the soil and site (climate) on the relative abundance of *R. irregularis* inoculant was detected (Table 3.4).

In year 1, at harvest, three months after inoculation (September 2011), significantly ($p \leq 0.05$) higher abundance of *R. irregularis* sequence reads were detected in the trap roots of Scott

(ST) soil (32%) followed by Swift Current (SC) (27%), Melfort (MF) (19%) and Outlook (OL) (18%) soils. In year 2, 15-months post inoculation, *R. irregularis* inoculant was not detected in ST soil; however, the *R. irregularis* sequences were detected in SC (15%) and OL (5%) and MF (4%). Only the SC (15%) and OL (4%) soils had *R. irregularis* sequences present 27-month post inoculation. The *R. irregularis* sequences were not detected in trap roots of ST in year 2 and year 3, and MF soils in year 3 (Fig. 3.6).

Moving soils from their original location affected the persistence of the inoculant (Fig. 3.6). Persistence was both enhanced and reduced, depending on soil. For example, when SC soil moved to OL site, the inoculant did not persist beyond year 2. Similarly, the level of persistence of inoculant was significantly lower when SC soil moved to OL site over three cropping seasons. The SC soil transplanted to the ST and MF sites showed no persistence at all at year 2 and year 3 but SC soil remained unchanged in the OL site in the year 1. An opposite trend was observed in the OL soil when it was transplanted to MF site, where consistently higher abundance of *R. irregularis* relative to OL soil at the OL site was found with 16%, 15% and 14% persistence in 2011, 2012 and 2013. The MF soil transplanted to all other sites was not found to be suitable for the survival and establishment of *R. irregularis* in trap roots in year 2 and year 3 (Fig. 3.6).

### 3.5.5 Effect of introduced inoculant, *R. irregulsris* on relative abundance of indigenous AMF taxa over three cropping seasons

The influence of inoculation on the relative abundance of different indigenous AMF taxa compared to uninoculated control over four soils and sites was estimated for three cropping seasons (Tables 3.5 to 3.7). The inoculation with commercial non-indigenous *R. irregularis* significantly ($p \leq 0.01$) altered all nine AMF taxa detected in pea trap roots over three cropping seasons. The magnitude of alteration of major indigenous AMF taxa (*Glomus, Funneliformis* and *Claroideoglomus)* in response to inoculation varied in soils and sites. The alteration of these taxa persisted over the seasons and was detectable even 27 months after inoculation. For example, *Glomus* was significantly reduced in transplanted soils but no changes in original soils were detected in year 1 over the four sites. However, the relative abundance of *Glomus* significantly declined in almost all soils in year 3, with the exception of a significant increase of relative abundance of *Glomus* in SC soil at SC site, OL and ST soils at MF site (Table 3.7).

*Funneliformis* was significantly increased in OL and MF soils but unaffected in SC and ST soils in response to inoculation in year 1 (Table 3.5). However, in year 3, *Funneliformis* was significantly reduced in OL and enhanced in MF soils. An inconsistent pattern of distribution of *Claroideoglomus* in response to inoculation was detected over the sites for three cropping seasons (Tables 3.5 to 3.7).

The minor (less abundance) AMF taxon, namely *Rhizophagus,* was significantly ($p \leq 0.01$) reduced by inoculation relative to uninoculated control over three cropping seasons. A decreasing trend of indigenous *Rhizophagus* abundance in pea trap roots as a consequence of inoculation with non-native *R. irregularis* was observed over three consecutive crop seasons (Tables 3.5 to 3.7). Similarly, inoculation resulted in a significant reduction in *Septoglomus* and *Archaeospora* in year 1 across the sites. However, they were not detected in year 2 and 3. *Entrophospora* was detected in the SC soil at the SC site for the first time in year 2 with a significant ($p \leq 0.01$) reduction in the the inoculated soil. The *Paraglomus* community was affected by inoculation over the cropping seasons with a significant decrease in ST soil at ST and OL sites (year 1 and 2) and OL soil at the OL site (year 3) (Tables 3.5 to 3.7). However, inoculation significantly enhanced the relative abundance of indigenous *Paraglomus* in MF soil at MF site over year 2 and 3. Most importantly, *Rhizophagus, Diversispora* and *Archaeospora* were suppressed in response to inoculation with non-indigenous *R. irregularis* over the soils and sites for three consecutive cropping seasons (Tables 3.5 to 3.7).

**Table 3.4** Three-way analysis of variance (ANOVA). The effect of inoculation, soil, site (climate) on field pea trap root associated AMF communities, Shannon diversity and persistence (relative abundance) of commercial AMF inoculant, detected by 18S rRNA gene pyrosequencing in 2011, 2012 and 2013 (1st, 2nd and 3rd cropping seasons).

| | Root occupancy (relative abundance) (%) of indigenous AMF taxa† | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sources | *Rhizophagus* | *Glomus* | *Funneliformis* | *Septoglomus* | *Claroideoglomus* | *Diversispora* | *Archaeospora* | *Paraglomus* | *Entrophospora* | Shannon diversity index of AMF taxa‡ | Persistence of *R. irregularis* inoculant§ |
| Inoculation (I) | *** | *** | *** | ** | *** | *** | *** | *** | ** | ** | - |
| Soil (S) | *** | *** | *** | *** | *** | *** | ** | ** | *** | ** | *** |
| Site (Si) | *** | *** | *** | *** | *** | *** | ******* | ****** | *** | ****** | ******* |
| I x S | *** | **ns** | **ns** | * | ** | ** | **ns** | | ** | ***** | **-** |
| I x Si | *** | **ns** | **ns** | * | ** | *** | ******* | **ns** | *** | ***** | **-** |
| S x Si | *** | **ns** | **ns** | * | ** | * | ****** | **ns** | * | **ns** | ****** |
| I x S x Si | ** | **ns** | **ns** | * | ** | ** | **ns** | **ns** | ** | **ns** | **-** |

Significant at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***). NS denotes non-significant.

†Arbuscular mycorrhizal fungal taxa consisting of *Rhizophagus, Funneliformis, Glomus, Septoglomus, Claroideoglomus, Diversispora, Archaeospora, entro* and *Paraglomus* detected by 18S rRNA gene pyrosequencing.

‡Shannon Diversity was determined using the abundance of indigenous AMF communities by the command 'collect.single' in MOTHUR bioinformatics pipeline, based on the formula by Shannon (1948).

§Persistence of commercial AMF strain: persistence was measured as the relative abundance of introduced *R. irregularis* inoculant in the trap roots grown in the soil samples from inoculated field soil cores.

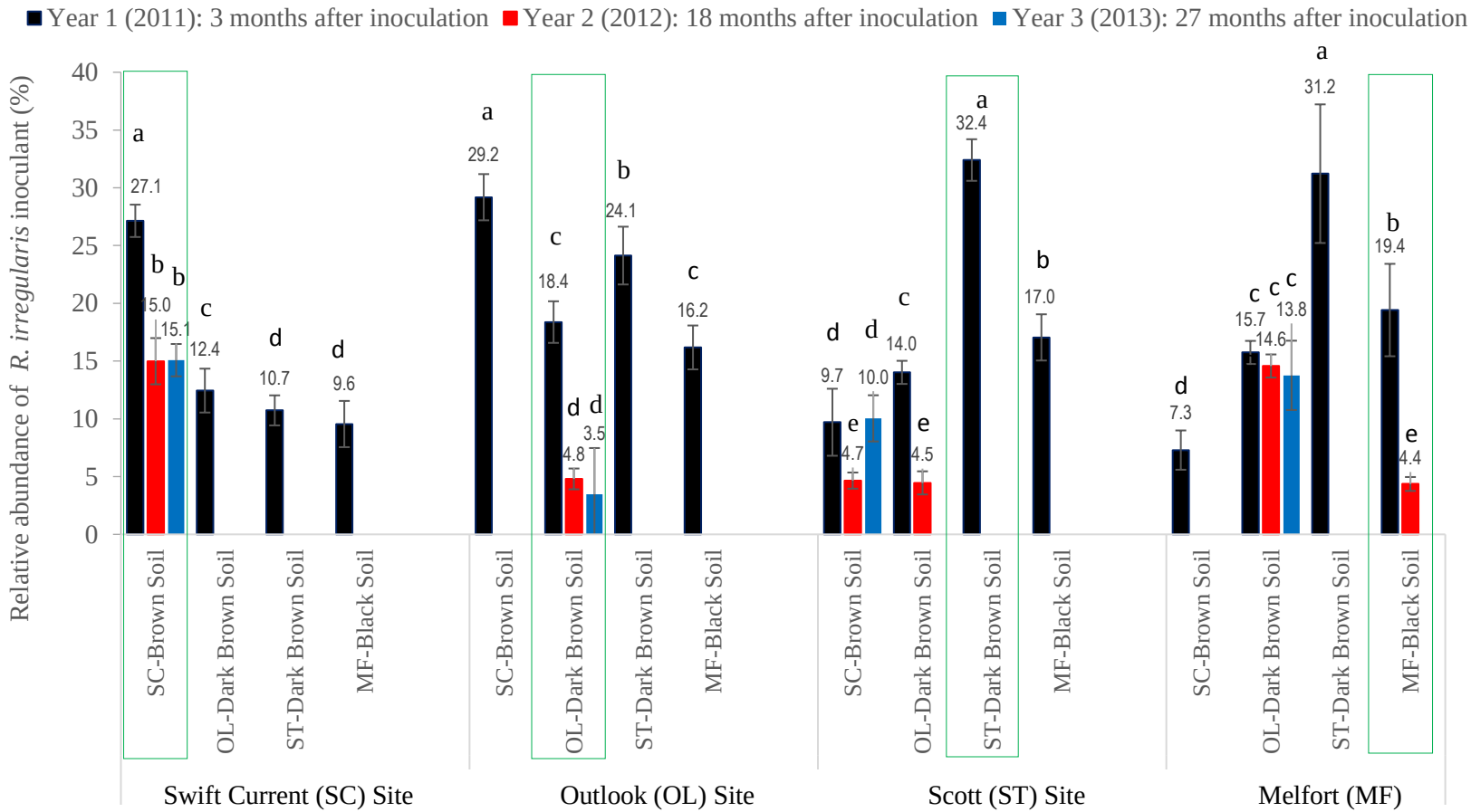


**Figure 3.6** Persistence (relative abundance) of an introduced commercial non-indigenous *R. irregularis* inoculant strain, associated with the trap roots of field pea, detected by pyrosequencing in year 1 (2011) 3 mo; year 2 (2012) 15 months; and year 3 (2013) 27 months after inoculation at the four sites. For a site, significant differences between soils ($P \leq 0.05$) are indicated by different letters. The soils demarcated by green rectangles are the original soils at original sites. The undemarcated soils are transplanted from other sites.

**Table. 3.5** The effect of inoculation with *R. irregularis* inoculant on the relative abundance of indigenous AMF taxa compared to uninoculated control, associated with the trap roots of field pea grown in the field incubated core soils, collected at harvest in 2011 cropping season, detected by 18S rRNA gene pyrosequencing.

| | | | **Indigenous arbuscular mycorrhizal fungal taxa** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **Site** | **Soil** | ***Rhizophagus*** | ***Glomus*** | ***Funneliformis*** | ***Septoglomus*** | ***Claroideoglomus*** | ***Diversispora*** | ***Archaeospora*** | ***Paraglomus*** | ***Entrophospora*** |
| 2011 | Swift Current | Swift Current | - | ns | ns | - | ns | - | ↓** | - | - |
| | | Outlook | ↓** | ↓** | ↑** | - | ns | - | - | ns | - |
| | | Scott | - | ↓* | ns | - | ns | - | - | ns | - |
| | | Melfort | ↓** | ns | ↑** | - | ns | - | - | ns | - |
| | Outlook | Swift Current | - | ↓** | ↑** | - | ↓** | ↓* | - | - | - |
| | | Outlook | ns | ns | ↑** | - | ns | ↓** | ns | ns | - |
| | | Scott | - | ns | ↑** | ↓* | ↑** | - | - | ↓** | - |
| | | Melfort | ↓** | ns | ns | - | ↑* | - | ↓* | ns | - |
| | Scott | Swift Current | - | ns | ns | - | ns | - | ns | - | - |
| | | Outlook | ↓** | ↓* | ↑** | ↓** | ↑** | ↓** | ↓** | ns | - |
| | | Scott | - | ns | ns | ↓** | ns | - | ↓** | ns | - |
| | | Melfort | ↓* | ns | ns | - | ns | - | ↑** | - | - |
| | Melfort | Swift Current | - | ↓* | ↑** | - | ns | - | - | - | - |
| | | Outlook | ↓** | ns | ns | - | ns | - | - | - | - |
| | | Scott | - | ns | ns | ↓** | ns | - | - | ns | - |
| | | Melfort | ↓** | ns | ↑* | - | ns | - | ↓** | ns | - |

Note: Paired mean comparisons using student's *t*-test at ($P$<0.05) and ($P$<0.01), marked as * and **, respectively was performed to assess the significant changes between inoculated and uninoculated treatments. ↓ indicates abundance decreased, ↑ indicates abundance increased, ns indicates non-significant and - indicates AMF taxa absent.

**Table 3.6** The effect of inoculation with *R. irregularis* inoculant on the relative abundance of indigenous AMF taxa compared to uninoculated control, associated with the trap roots of field pea grown in the field incubated core soils, collected at harvest in 2012 cropping season, detected by 18S rRNA gene pyrosequencing.

| | | | **Indigenous arbuscular mycorrhizal fungal taxa** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **Site** | **Soil** | ***Rhizophagus*** | ***Glomus*** | ***Funneliformis*** | ***Septoglomus*** | ***Claroideoglomus*** | ***Diversispora*** | ***Archaeospora*** | ***Paraglomus*** | ***Entrophospora*** |
| 2012 | Swift Current | Swift Current | - | ns | - | - | ↓* | - | - | ↓** | ↓** |
| | | Outlook | - | - | ↓* | - | ↑** | - | - | - | - |
| | | Scott | - | - | - | - | ns | ↓* | - | ns | - |
| | | Melfort | - | - | ns | - | ↑* | - | - | - | - |
| | Outlook | Swift Current | - | ↑** | ↓* | - | ↓** | - | - | - | - |
| | | Outlook | ↓* | - | ↓** | - | ns | ↑** | - | - | ns |
| | | Scott | - | - | - | - | ns | ns | - | ns | - |
| | | Melfort | - | - | ↓* | - | ↑* | - | - | - | - |
| | Scott | Swift Current | - | ↓** | - | - | ↑** | - | - | - | - |
| | | Outlook | - | - | ↓* | - | ↑** | - | - | - | - |
| | | Scott | - | - | - | - | ↑* | ns | - | ↓** | |
| | | Melfort | ↓** | - | ↑** | - | ↓* | - | - | - | - |
| | Melfort | Swift Current | - | ↓* | - | - | ↑* | - | - | - | - |
| | | Outlook | - | - | ↓** | - | ↑* | - | - | - | - |
| | | Scott | - | - | - | - | ns | - | - | ns | - |
| | | Melfort | ↓** | - | ns | - | ns | - | - | ↑** | - |

Note: Paired mean comparisons using student's *t*-test at ($P$<0.05) and ($P$<0.01), marked as * and **, respectively was performed to assess the significant changes between inoculated and uninoculated treatments. ↓ indicates abundance decreased, ↑ indicates abundance increased, ns indicates non-significant and – denotes AMF taxa absent.

**Table 3.7** The effect of inoculation with *R. irregularis* inoculant on the relative abundance of indigenous AMF taxa compared to uninoculated control, associated with the trap roots of field pea grown in the field incubated core soils, collected at harvest in 2013 cropping season, detected by 18S rRNA gene pyrosequencing.

| | | | **Indigenous arbuscular mycorrhizal fungal taxa** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Year** | **Site** | **Soil** | ***Rhizophagus*** | ***Glomus*** | ***Funneliformis*** | ***Septoglomus*** | ***Claroideoglomus*** | ***Diversispora*** | ***Archaeospora*** | ***Paraglomus*** | ***Entrophospora*** |
| 2013 | Swift Current | Swift Current | - | ↑** | ns | - | ↓** | ↓* | - | ns | ns |
| | | Outlook | - | ns | ↑* | - | ↓** | - | - | - | ↓** |
| | | Scott | - | ↓* | ↓* | - | ↓** | - | - | - | ↑** |
| | | Melfort | - | ↓* | ↓* | - | - | ns | - | - | ↓* |
| | Outlook | Swift Current | - | ↓* | ↓* | - | - | ns | - | ns | - |
| | | Outlook | ↓* | ↓* | ↓** | - | ↑** | ns | - | ↓* | ns |
| | | Scott | - | ↓* | ↑** | - | ↓* | ns | - | - | ↓** |
| | | Melfort | - | ↓** | ↓* | - | ↑** | ns | - | - | - |
| | Scott | Swift Current | ↓* | ns | ↑** | - | ns | ns | - | - | ↓* |
| | | Outlook | ↓* | - | ↑** | - | ↓* | ns | - | - | - |
| | | Scott | - | ↓* | - | - | - | - | - | ns | - |
| | | Melfort | - | ↓* | ns | - | ↑** | ns | - | - | ↓* |
| | Melfort | Swift Current | - | ns | ns | - | ns | ns | - | - | ns |
| | | Outlook | - | ↑** | ↓* | - | ns | - | - | - | - |
| | | Scott | - | ↑** | ↓** | - | ↑** | - | - | - | ns |
| | | Melfort | - | ↓** | ↑** | - | ns | - | - | ↑* | - |

Note: Paired mean comparisons using student's *t*-test at ($P$<0.05) and ($P$<0.01), marked as * and **, respectively was performed to assess the significant changes between inoculated and uninoculated treatments. ↓ indicates abundance decreased, ↑ indicates abundance increased, ns indicates non-significant and – denotes AMF taxa absent.

### 3.5.6 Effect of inoculation on composition and diversity of AMF communities

A total of 188 OTUs (86 in year 1, 30 in year 2 and 72 in year 3) belonging to nine AMF genera, namely *Rhizophagus, Glomus, Funneliformis, Septoglomus, Claroideoglomus, Diversispora, Archaeospora, Paraglomus* and *Entrophospora* were detected in field pea trap roots at four locations (Tables 3.5 to 3.7). Of the 188, *Septoglomus* and *Archaeospora* were absent in year 2 and year 3 and *Entrophospora* was absent in year 1. The three-way analyses of variance revealed that inoculation with *R. irregularis* significantly altered the indigenous AMF taxa in pea roots for all study years.

Low abundant AMF taxa, namely *Septoglomus*, *Diversispora*, *Archaeospora* and *Entrophospora* ranging from 1 to 25% were observed in trap roots from different soils and sites (Figs. 3.7 to 3.9). The relative abundance of *Archaeospora* (10%) in trap roots of uninoculated SC control soil was not detected in inoculated treatment in year 1 and was not detected either in the control or inoculated treatment following the year 2 and year 3 cropping seasons. Similarly, *Diversispora* persisted in trap roots at uninoculated SC soil in year 3 whereas it was not detected in year 1 and 2 (Figs. 3.7 to 3.9). An inconsistent pattern of distribution of *Diversispora* was found both in the OL and ST soils. For example, *Diversispora* was apparently displaced or suppressed in OL inoculated soil in year 1, although this genus was detected in year 2 but inoculation reduced the abundance in year 3 compared to control. *Paraglomus* was unaffected by inoculation, particularly at Melfort, where it was present in the inoculated trap roots in all three years (Figs. 3.7 to 3.9).

The composition of the AMF communities in different soils transplanted in different soil zones with variable climates is shown in Figs. 3.7 to 3.9. Moving soils from one location to another location caused a shift in the AMF community composition. For example, in year 1 *Glomus* was present in SC soils at SC, but undetected in SC soils transplanted to OL (Fig. 3.7). Similarly, *Entrophospora* was found both in the inoculated (2%) and uninoculated control (10%) in SC soil at SC in year 2 but *Entrophospora* was undetected in inoculated and uninoculated roots in SC soil transplanted to any other site (Fig. 3.8). Similarly, in year 1 MF Black soil the *Archaeospora* was detected in the uninoculated MF control and *Rhizophagus* was detected in the MF inoculated and control treatments but neither were detected when the MF soil was

transplanted at the SC site (Fig. 3.7). A similar trend was observed in year 2, where *Paraglomus* and *Rhizophagus* were not found in MF soil that was transplanted to either OL or SC sites.

In contrast, some taxa that were not detected at the site of origin were enhanced when transplanted to other sites. For example, in year 3 when the MF soil was transplanted in the SC site, *Entrophospora* colonized trap roots both in the inoculated and uninoculated soils, ranging from 49% to 61% abundance, whereas *Entrophospora* was absent in the original MF soil at the MF site. Also in year 3, the *Funneliformis* community shifted greatly both in the inoculated and uninoculated control soil in response to transplanting MF soil at any other sites. (Fig. 3.9).

The response to inoculation was assessed according to Shannon's diversity index ($H'$) (Figs. 3.10 to 3.12). According to the three-way ANOVA, significant effects of inoculation, soil and site on community diversity were identified (Table 3.4). There was a significant reduction of diversity across the soils and sites in response to inoculation in year 1. The effect of inoculation on the diversity index was less pronounced in year 2 and 3. In year 1, the Shannon diversity index varied from 2.47 at SC in the uninoculated control to 3.78 at MF in the uninoculated control. The Outlook ($H' = 2.58$) and Scott ($H' = 2.65$) soils showed moderate AMF diversity compared to the other two soils. In response to inoculation in year 1, a significant reduction of Shannon diversity was found in ST (from 2.65 to 2.0) followed by MF (3.78 to 3.0), OL (from 2.58 to 2.07) and SC (from 2.47 to 2.1) soils compared to uninoculated treatments (Fig. 3.10). The introduction of non-indigenous *R. irregularis* inoculant significantly affected root associated indigenous AMF diversity in the MF and ST soils over the three consecutive cropping seasons.

Transporting the soils to new environments had an impact on the AMF diversity in MF, ST and OL soils transplanted to SC in year 1. Inoculation significantly ($P \leq 0.05$) reduced the diversity index of all four soils including transplanted MF, ST, OL and SC soils in the SC site. All of the original soils transplanted at OL site showed no significant differences between inoculated and uninoculated cores; however, MF soil at the ST site and SC and OL soil at the MF site had significantly ($P \leq 0.05$) lower diversity in inoculated cores in year 1. Over time, the impact of inoculation on AMF diversity was reduced. For example, the Shannon diversity in the trap roots of SC and OL soils was unaffected by inoculation in year 2, whereas, inoculation significantly reduced diversity in year 1 in SC and OL soil. The diversity still remained

significantly ($P \leq 0.05$) lower in both the inoculated roots of the original MF and ST soils in response to inoculation.

Transplanting the original soils to the other sites had very little impact on diversity in response to inoculation in year 2. The patterns in diversity in inoculated roots remained unchanged in MF soil transplanted to the SC site and ST soil transplanted in the OL site. This result is not the same for SC and OL soils where a reduction of diversity was minimized in SC and OL soils transplanted to any site. A higher significant reduction of diversity was observed in the trap roots of ST (from 1.92 to 1.50), followed by MF (2.40 to 1.93) soils in a response to the prolonged existence of non-indigenous *R. irregularis* in incubated field soils in year 3 (Fig. 3.12).

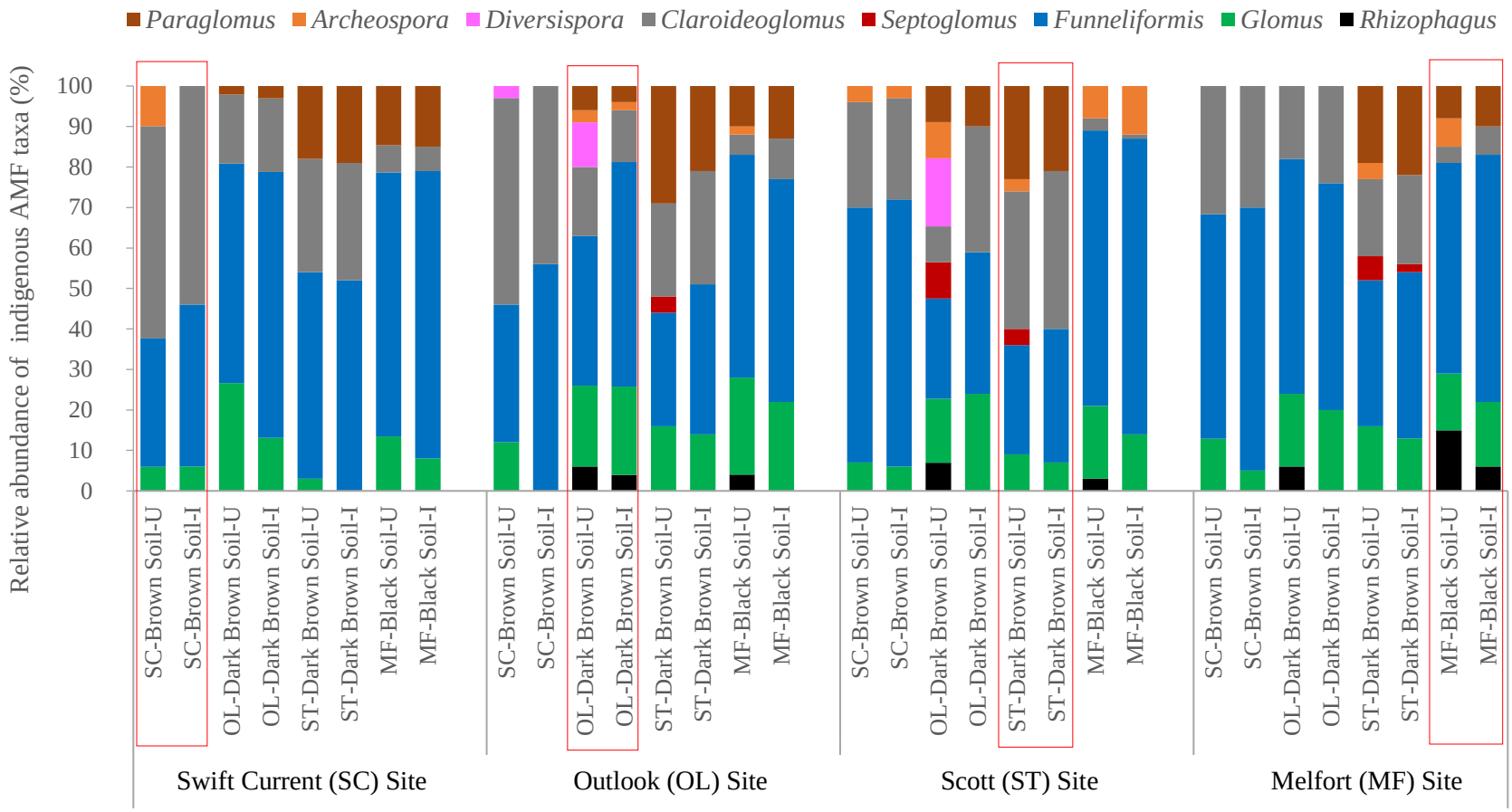


**Figure 3.7** The effect of inoculation with *R. irregularis* inoculant on distribution of relative abundance of indigenous AMF genera, associated with the trap roots of field pea, detected by pyrosequencing in year 1 (2011), 3 mo after inoculation in the four sites. Replicated (n=4), intact soil cores were extracted at four sites representing different soil zones, Swift Current (SC) Brown, Scott (ST) Dark Brown, Outlook (OL) Dark Brown and Melfort (MF) Black soil zones. The soils demarcated by red rectangles are the original soils at original sites. The undemarcated soils are transplanted soils from other sites. U: uninoculated and I: inoculated.

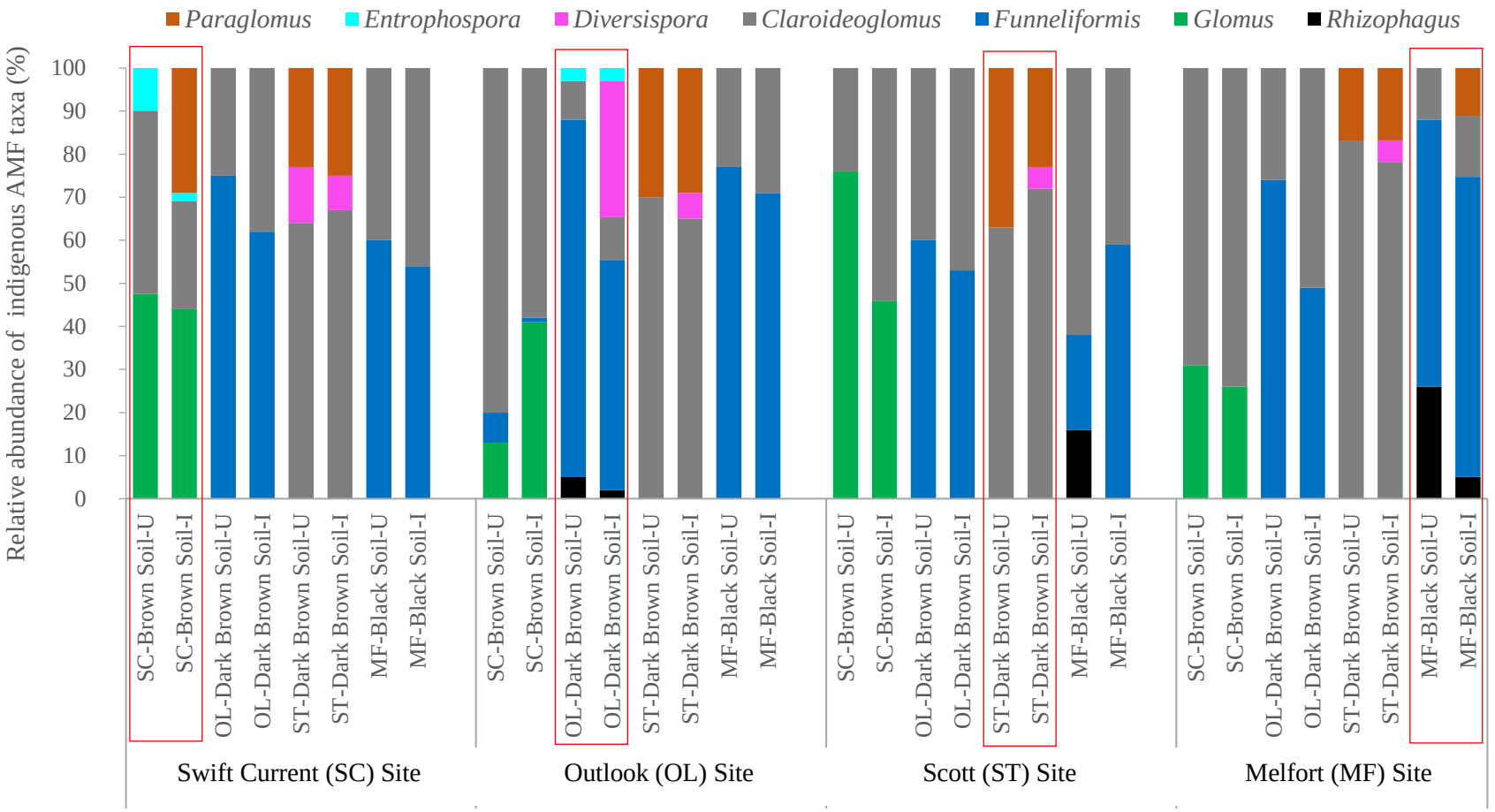


**Figure 3.8** The effect of inoculation with *R. irregularis* inoculant on distribution of relative abundance of indigenous AMF genera, associated with the trap roots of field pea, detected by pyrosequencing in year 2 (2012), 15 months after inoculation in the four sites. Replicated (n=4), intact soil cores were extracted at four sites representing different soil zones, Swift Current (SC) Brown, Scott (ST) Dark Brown, Outlook (OL) Dark Brown and Melfort (MF) Black soil zones. The soils demarcated by red rectangles are the original soils at original sites. The undemarcated soils are transplanted soils from other sites. U: uninoculated and I: inoculated.

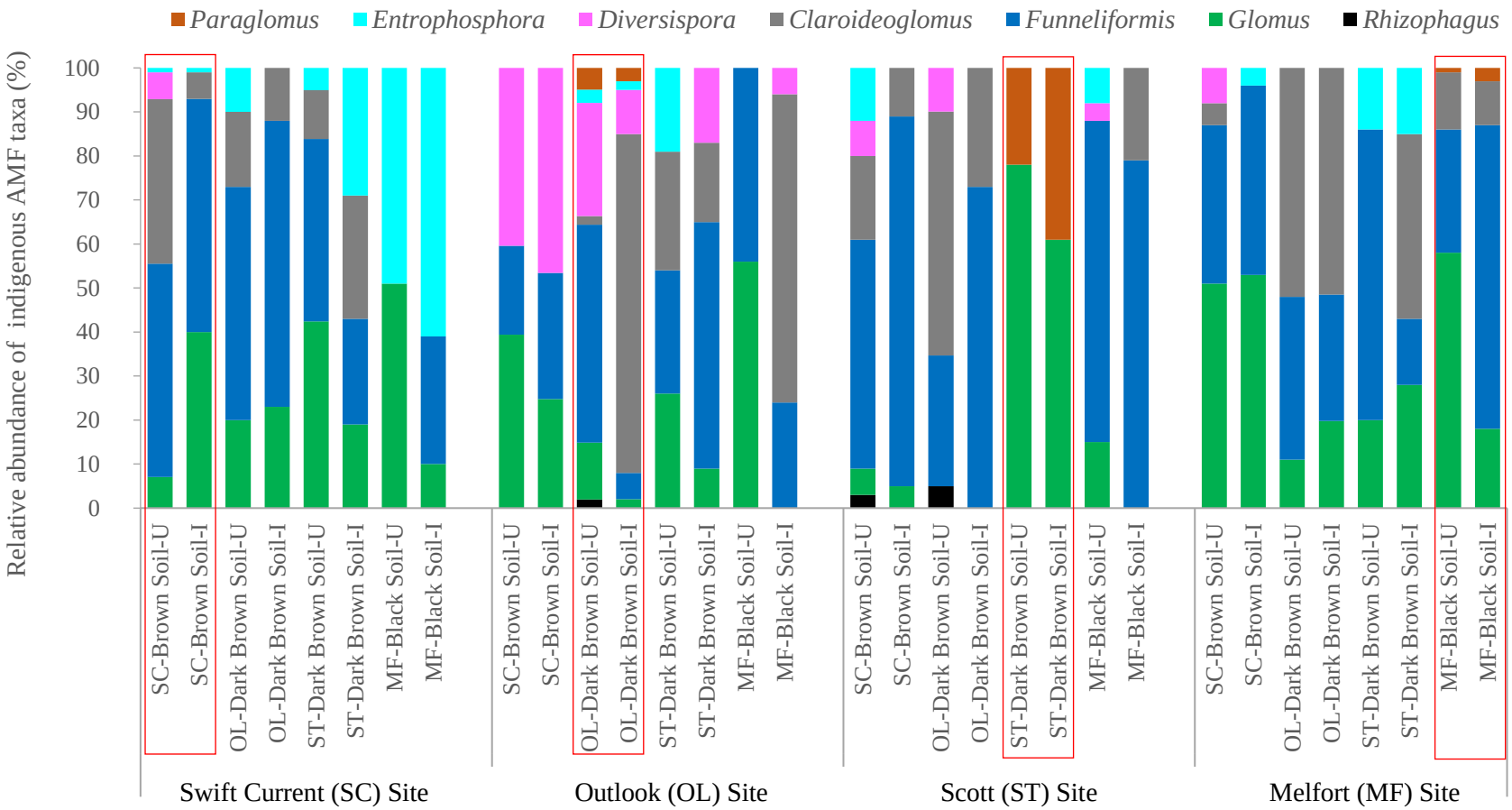


**Figure 3.9** The effect of inoculation with *R. irregularis* inoculant on distribution of relative abundance of indigenous AMF genera, associated with the roots of field pea, detected by pyrosequencing in year 3 (2013), 27 months after inoculation in the four sites. Replicated (n=4), intact soil cores were extracted at four sites representing different soil zones, Swift Current (SC) Brown, Scott (ST) Dark Brown, Outlook (OL) Dark Brown and Melfort (MF) Black soil zones. The soils demarcated by red rectangles are the original soils at original sites. The undemarcated soils are transplanted soils from other sites. U: uninoculated and I: inoculated.

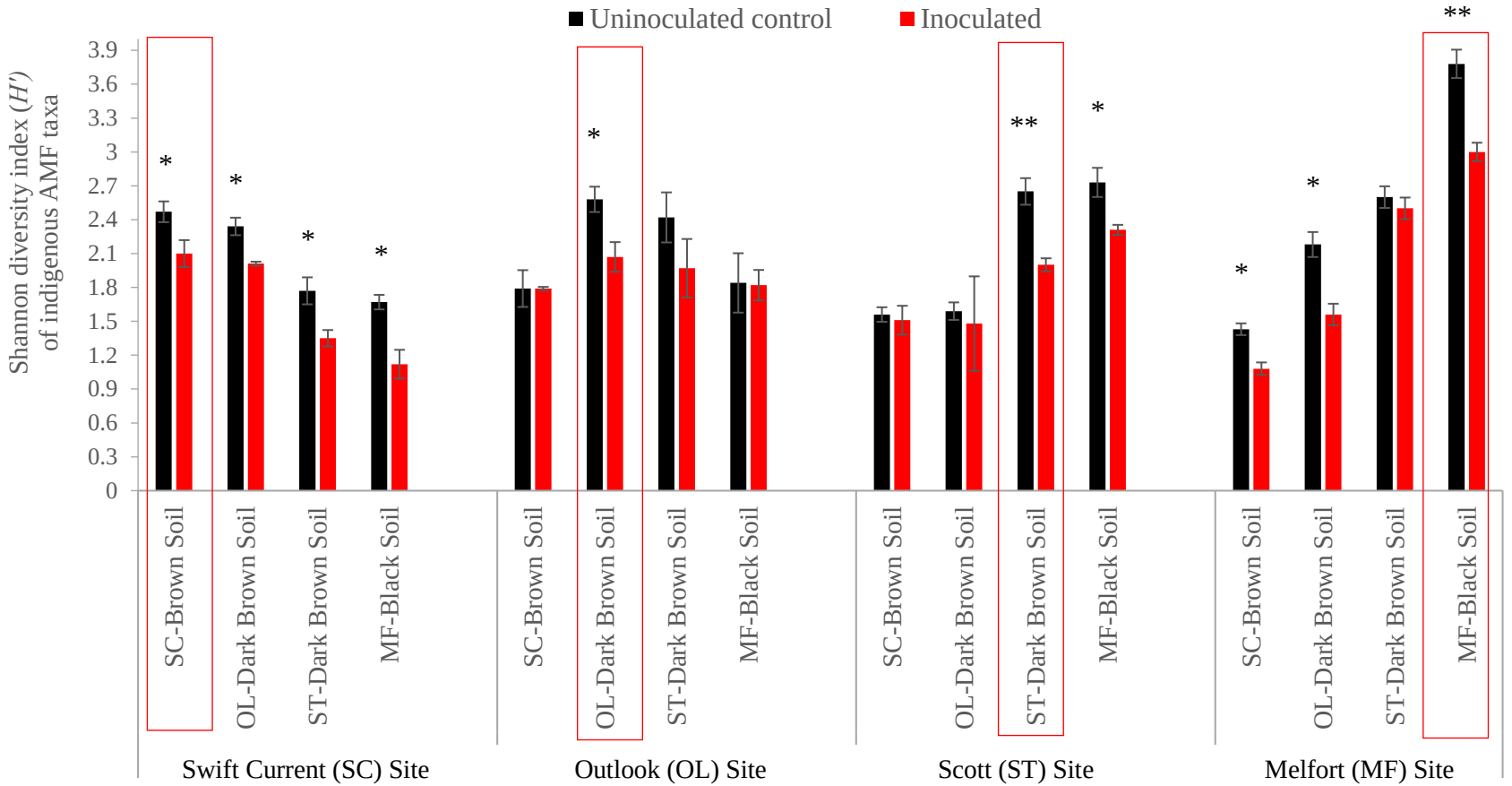


**Figure 3.10** The effect of inoculation with *R. irregularis* inoculant on Shannon diversity index of indigenous AMF communities, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in the four sites in year 1 (2011). Indigenous AMF genera consisting of *Rhizophagus, Claroideoglomus, Glomus, Funneliformis, Diversispora, Entrophospora, Archaeospora, and Paraglomus* were associated in the trap roots of the field pea. Tukey-Kramer honestly significant ($P<0.05$) and ($P<0.01$), marked as * and **, respectively was performed to assess the significance of differences between Shannon diversity index of inoculated and uninoculated treatment means. The demarcated red rectangles were the original soils at original sites. The undemarcated soils were transplanted soils from other sites.

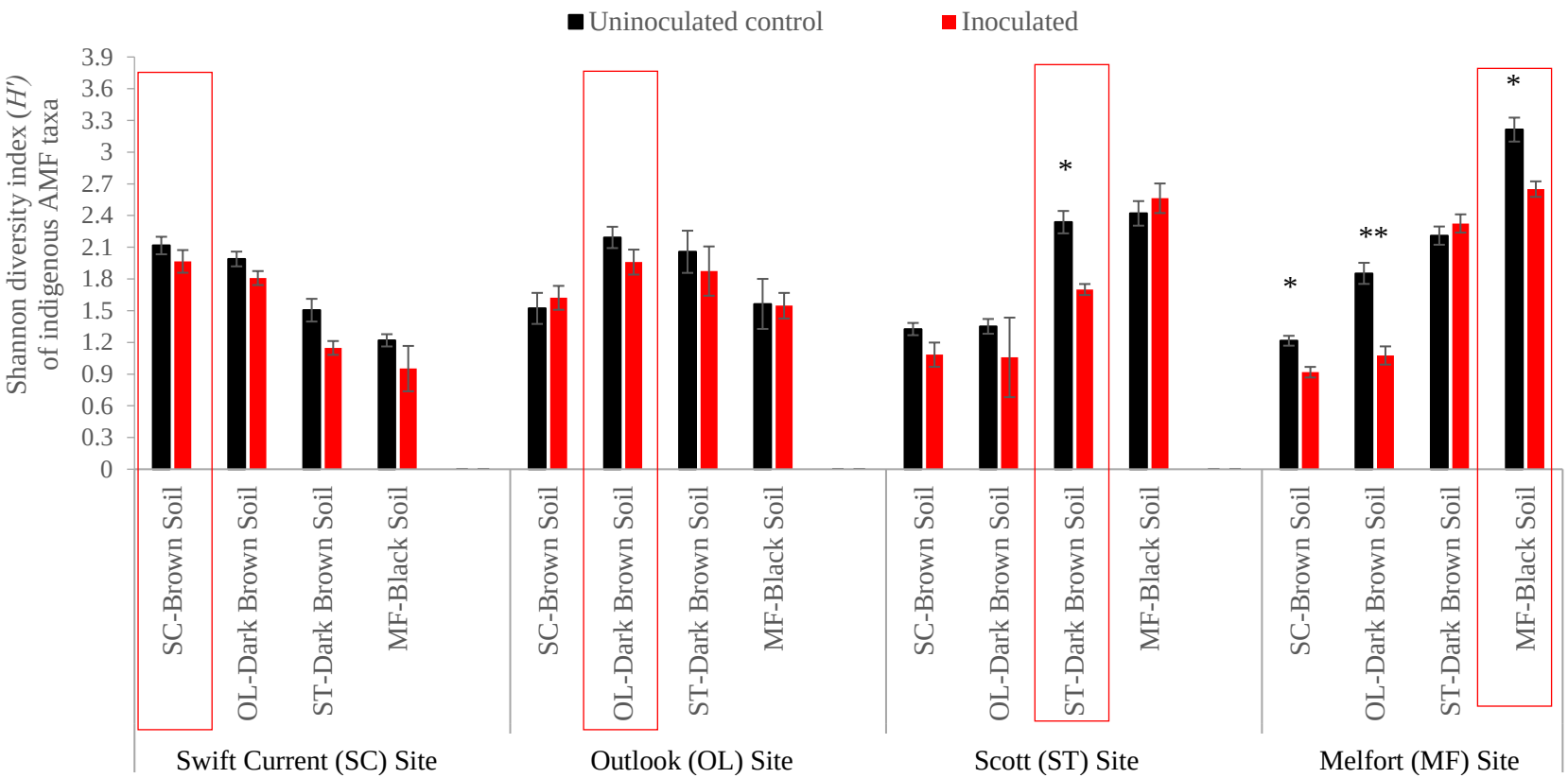


**Figure 3.11** The effect of inoculation with *R. irregularis* inoculant on Shannon diversity index of indigenous AMF communities, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in the four sites in year 2 (2012). Indigenous AMF genera consisting of *Rhizophagus, Claroideoglomus, Glomus, Funneliformis, Diversispora, Entrophospora, Archaeospora, and Paraglomus* were associated in the trap roots of the field pea. Tukey-Kramer honestly significant ($P<0.05$) and ($P<0.01$), marked as * and **, respectively was performed to assess the significance of differences between Shannon diversity index of inoculated and uninoculated treatment means. The demarcated red rectangles were the original soils at original sites. The undemarcated soils were transplanted soils from other sites.

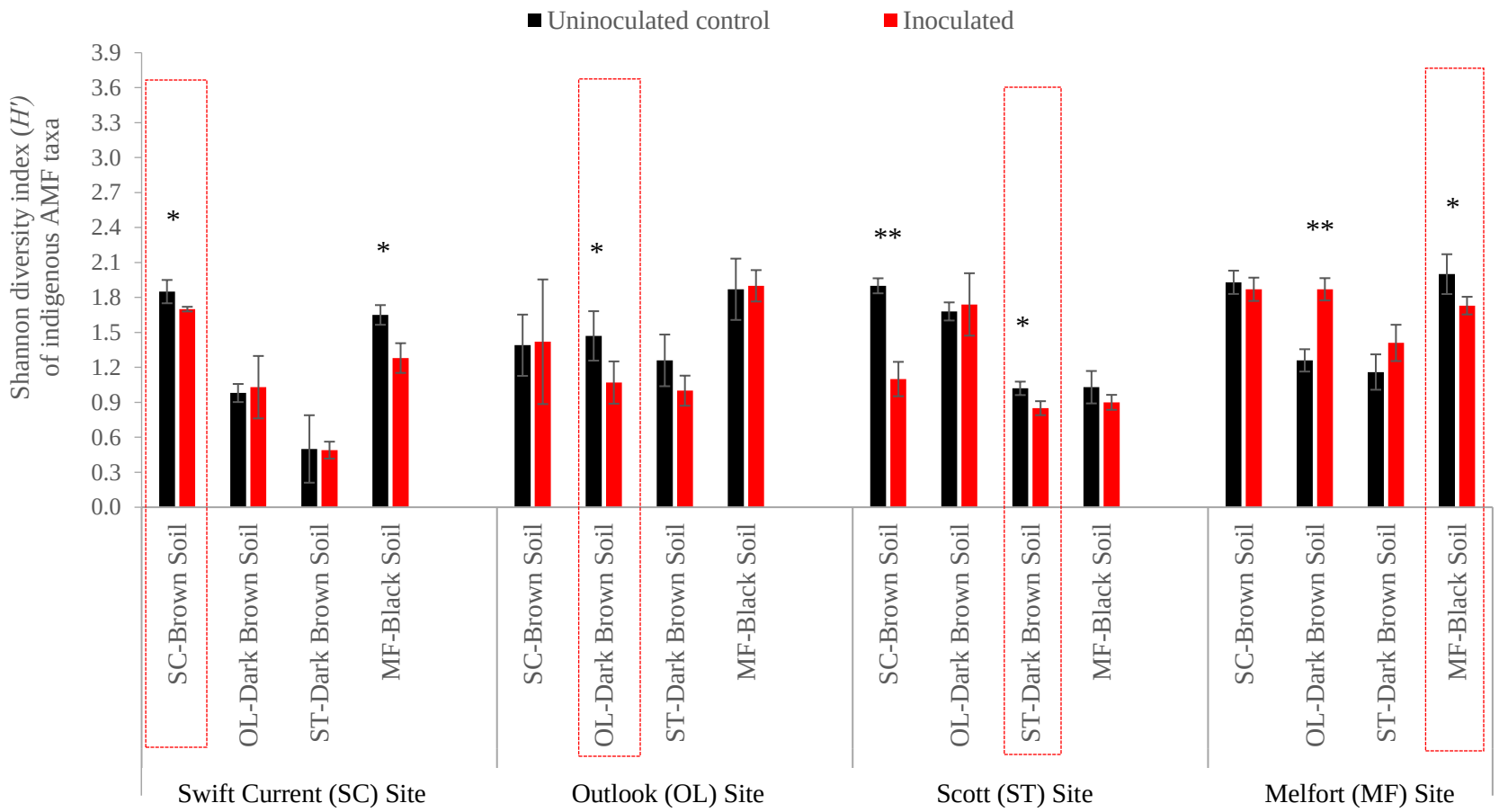


**Figure 3.12** The effect of inoculation with *R. irregularis* inoculant on Shannon diversity index of indigenous AMF communities, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in the four sites in year 3 (2013). Indigenous AMF genera consisting of *Rhizophagus, Claroideoglomus, Glomus, Funneliformis, Diversispora, Entrophospora, Archaeospora, and Paraglomus* were associated in the trap roots of the field pea. Tukey-Kramer honestly significant ($P<0.05$) and ($P<0.01$), marked as * and **, respectively was performed to assess the significance of differences between Shannon diversity index of inoculated and uninoculated treatment means. The demarcated red rectangles were the original soils at original sites. The undemarcated soils were transplanted soils from other sites.

## 3.6 Discussion

### 3.6.1 Persistence of introduced commercial non-indigenous inoculant, *R. irregularis*, in some Saskatchewan Prairie soils

*Rhizophagus irregularis* applied as an inoculant persisted for three years in Swift Current Brown and Outlook Dark Brown soils. Results suggest that the prolonged survival of a commercial non-indigenous AMF strain post inoculation depends on the soil and respective climate as well as indigenous AMF community structure and composition where the inoculant was introduced. This result is in agreement with Herrera-Peraza et al. (2011). They illustrated that root colonization by two commercial AMF inoculant strains (*Paraglomus occultum* and *Glomus mosseae*) was enhanced in relatively low organic matter and low nutrient Cuban soils. Others reported that local climate and resident AMF community composition and soils are important variables that must be compatible with introduced AMF inoculants for ultimate persistence and effectiveness (Oehl et al., 2010; Verbruggen and Toby Kiers, 2010; Verbruggen et al., 2012). Similarly, Pellegrino et al. (2012) reported that *Funneliformis mosseae* was detected in the inoculated field roots of *Medicago sativa* up to two years following inoculation.

Others have reported that the co-existence of a particular group of AMF varied between soil conditions and soil environments, illustrating differential adaptation of AMF (Helgason and Fitter, 2009a). Results of the current study indicate that SC soil harbored the *R. irregularis* inoculant over three growing seasons. The SC and OL contained lower organic matter and nutrient levels compared to ST and MF soils (Table 3.1). The pyrosequencing data from this field incubation study also showed the lowest number of absolute sequence reads of AMF communities in SC soil compared to the ST, OL, and MF soils in year 1 (Table A.3.1), similar to the findings shown by Dai et al. (2013).

Recent studies by Dai et al. (2013, 2014) show that Black and Dark Brown Chernozem soils of the Canadian Prairies host a diverse AMF community. Findings from the current study indicated that the persistence of commercial AMF *R. irregularis* inoculant was also greatly dependent on the level of existing resident AMF community diversity where the inoculant was introduced. This suggests that non-indigenous AMF strains are likely to persist for multiple

cropping seasons where the well-adapted local/indigenous AMF communities are less diverse and the competition for limited resources among the AMF communities is lower. Establishment of introduced non-indigenous taxa might be difficult if they are to compete with well-adapted indigenous communities. Therefore, inoculants should be tested using a wide range of soil types (Oehl et al., 2010), hosts (Öpik Maarja and Moora, 2012) and climatic conditions (Bellgard and Williams, 2011). The SC soil hosted relatively lower AMF diversity (SC: 2.47, OL: 2.58, ST: 2.65 and MF: 3.78) with most likely less competition, which could have led to a higher degree of persistence for the introduced AMF inoculant over the cropping seasons. Moreover, *R. irregularis* appeared to be a fast root colonizer (Jansa et al., 2003, 2008) and the low diversity of AMF at SC resulted in more unoccupied niches being available for the introduced inoculant.

Others have reported that the success of AMF inoculation mostly depends on soil type (Oehl et al., 2010), resident AMF community (Requena et al., 1997), functional variability among isolates (Pellegrino et al., 2012) and host plant type (Antunes et al., 2009). Researchers have reported that it is difficult to assess the impact of inoculant if the indigenous taxa similar to inoculant are present in soil (Antunes et al., 2009; Koch et al., 2011; Pellegrino et al., 2012; Sýkorová et al., 2012; Jin et al., 2013b). Until now, this has been an ongoing challenge as inoculant strains could not be differentiated from the number of genetic haplotypes and polymorphic variants within colonized roots (Börstler et al., 2008; Croll et al., 2009; Beaudet et al., 2014). Advanced massively parallel high-throughput pyrosequencing platform for profiling AMF communities in field pea trap roots provides a tool for examining AMF communities. This NGS technology was found to be efficient in minimizing the errors associated with AMF polymorphism and genetic manipulation among the populations (Öpik et al., 2009; Varshney et al., 2009). For example, introduced non-indigenous *G. irregulare* (currently named *R. irregularis*) inoculant strain was detected from the OTUs generated from indigenous AMF taxa in field pea and chickpea roots using pyrosequencing technology (Jin et al., 2013b).

In the current study, DNA from the spores of the *R. irregularis* inoculant was separately pyrosequenced to compare the differences in OTUs among indigenous and introduced non-indigenous *R. irregularis* taxa using neighbour-joining phylogenetic analysis (Figs. 3.3 to 3.5). It was apparent that both indigenous and introduced *R. irregularis* colonized pea trap roots. Pyrosequencing techniques used in this study discriminated between OTUs generated from the

indigenous and introduced *R. irregularis* strains. This was confirmed using AMF from uninoculated soil cores which presumably represented the indigenous AMF communities.

This introduced *R. irregularis* inoculant competed with those of indigenous *Rhizophagus* in OL and MF soils causing a reduction of 2% and 9% in the relative abundance of indigenous *Rhizophagus,* respectively, in both inoculated soils in the 2011 cropping season (Fig. 3.7). Furthermore, the abundance of closely related taxa to the *R. irregularis* inoculant, that is *Glomus,* was substantially decreased in 2013 due to inoculation in the trap roots of ST (17%) and MF (40%) soils compared to control (Fig. 3.9).

Indigenous *Rhizophagus* was not detected in SC soil. The absence of *Rhizophagus* may have reduced competition with introduced *R. irregularis* inoculant allowing it to persist and establish as a root colonizer in the second and third year of the study. The persistence of *R. irregularis* inoculant was minimal in OL soil, accounting for only 5% and 4% relative abundance in year 2 and year 3, respectively. Some competition between *Rhizophagus* communities might have occurred, as some indigenous *Rhizophagus* (6%) already existed in OL soil (Fig. 3.7). This explanation is supported by the supposition that the closely related species are suppressed for root colonization and facilitation by distantly related species (Maherali and Klironomos, 2007; Valiente-Banuet and Verdu, 2008). The higher persistence of the inoculant in SC soil shows a potentially successful inoculation approach where the soil may be missing closely related AMF families like *Rhizophagus* leaving a habitat "open". Such an approach to inoculation might result in enhanced establishment because of unoccupied niches (Verbruggen et al., 2013). For example, Farmer et al. (2007) compared three AMF inoculants and observed that the most successful colonizer, *Claroideoglomus etunicatum,* was not present in the experimental plot. They concluded that the absence of the particular AMF taxa likely contributed to inoculation success of this species. It follows that the inoculation success observed at SC soil over multiple cropping seasons may have been related to the absence of indigenous *Rhizophagus* at this site.

Understanding how the introduced non-indigenous AMF interact and coexist with the indigenous AMF community may be key to developing an efficient AMF inoculant for crop production systems. Pellegrino et al. (2012) observed the influence of two non-indigenous AMF strains and inoculant rate on yield performances under field conditions. They reported that one

out of two introduced AMF (*Funneliformis mosseae*) strains successfully persisted and became established up to two years following inoculation in maize roots. This observation indicates that species vary in their ability to persist or colonize in soil.

The persistence of introduced *R. irregularis* dramatically changed over three cropping seasons in response to different climatic conditions and soils; however, an obvious pattern of persistence was observed in transplanted soils (moving original soil core to another site) across the four locations. For example, the introduced *R. irregularis* never persisted in year 2 and 3 in ST and MF soils transplanted at other sites. The relative abundance of introduced *R. irregularis* inoculant was consistently higher over three consecutive cropping seasons in the original SC soil relative to the other soils, whereas when the original SC soil was transplanted to ST site, the relative abundance of AMF inoculant declined to as low as 5% in year 2 and absent when SC soil was moved to OL and MF sites in year 1, 2 and 3 (Fig. 3.6).

Persistence of introduced *R. irregularis* differed within soils depending on where the soil was transplanted (Fig. 3.6). For example, although all SC soil cores supported the inoculant strain in year 1, the relative abundance in subsequent years differed depending on the sites. This difference in persistence of inoculant may reflect differences in moisture levels. In particular, the variable precipitation events at the transplanted climates might have altered the soil moisture level. Similarly, original ST soil (cooler and moderately wet soil zone) transplanted to warmer and drier sites (OL or SC) or even in a cooler and wet MF site supported the inoculant persistence in trap roots only for year 1, and not for subsequent years. Others have reported that perennial cover hosts and gradients of soil moisture are key factors shaping the AMF structure and diversity and eventually determining ecosystem processes in the Canadian Prairie soils (Hamel et al., 2006; Dai et al., 2013). Regional climatic variation across the Saskatchewan Prairies results in increasing amounts of precipitation and lower average annual temperatures along a transect from the Swift Current Brown to Melfort Black soil zones over the cropping seasons (Figs. 3.1 and 3.2).

A positive correlation between soil moisture level and AMF community diversity was observed. Others also suggest the AMF are particularly important for plant growth under low moisture or moisture stress conditions (Schenck and Smith, 1982; Subramanian and Charest,

1999). Although enhanced diversity under high moisture conditions seems contradictory to these reports, it is possible that some AMF tend to dominate under low moisture, reducing the relative abundance of other species. The relative abundance of the inoculant was highest on year 1 at the wetter sites (i.e., MF 19% and ST 32%). Relative abundance declined significantly ($P$=0.001) when two cores (original ST and MF soils) were transplanted at drier sites (SC or OL). The *R. irregularis* inoculant persisted in the original MF soil for year 2 accounting for 4% of the relative abundance of the inoculant which was undetected while transplanted in either of the drier sites (i.e., SC or OL) (Fig. 3.6). This finding fully supports the previous results of Hamel et al. (2006), Wu et al. (2007) and Dai et al. (2013) who showed that the Black Chernozem soils harboured highly diverse AMF communities compared to soils from a drier region (i.e., Swift Current).

Recently, Hazard et al. (2013) suggested that specific environmental variables such as rainfall had a strong effect on AMF communities. In contrast, others have reported there was no significant correlation between climate variables (temperature and precipitation gradients) and AMF richness and diversity for epiphytic AMF communities suggesting AMF communities are less dependent on rainfall conditions (Torrecillas et al., 2013). The findings of Dai et al. (2013), Wu et al. (2007) and Hazard et al. (2013) are in agreement with the current results but contradictory with the results of Torrecillas et al. (2013). In the semiarid region of the SC site, the precipitation at SC for the period of May to September (three crop seasons total 148 mm) was less than MF site (three crop seasons total 158 mm) in all three years (Fig. 3.2A). The average three cropping seasons temperature at the SC site (16 °C) was higher than at the MF site (14 °C) (Fig. 3.2B). The wet MF and ST soil cores transplanted to a dry environment likely rapidly lost their available water to evaporation. Thus, both indigenous AMF and introduced *R. irregularis* community faced a drier climate. The decline in AMF persistence due to transplanting from wetter to drier sites suggests that AMF communities require an adaptation to the environment. Torrecillas et al. (2013) concluded that only drought tolerant AMF communities have the ability to colonize epiphytic plants and respond to the driest climatic conditions compared to other wet experimental sites. In the current study, the adaptation of the AMF inoculant to a particular environment is unknown. However, it is suggested that the origin of an introduced species is likely to influence the success of the species in a particular environment.

Among the sites, SC and OL experienced less precipitation and had lower AMF species diversity. The SC and OL soils exhibited increased persistence of the *R. irregularis* inoculant. The increased persistence of the inoculant supports the idea of an unoccupied niche and less competitive AMF species. A limitation of a particular AMF taxon closely related to inoculant species in a specific crop soils means more unoccupied niches available, which is most likely to increase inoculation success (Verbruggen et al., 2013). The inoculation success rate might increase in the AMF diverse soils (i.e., MF soil) if inoculant taxa are absent in those targeted soils. However, indigenous *Rhizophagus* was present in MF and absent in SC soil. *R. irregularis* has been documented to be ubiquitous, occurring in a wide range of environments, due to ecotypes adapted to different sets of environment (Börstler et al., 2010). Börstler et al. (2010) demonstrated that *R. irregularis* was found to preferentially inhabit an undisturbed low-nutrient grassland site which is very rarely observed in arable fields.

Thus, I conclude that the compatibility and choice of inoculant depends on prevailing environmental conditions. Most importantly, the crossing between individual populations from different introduced and indigenous AMF resulting in lower fitness (Verbruggen et al., 2012, 2013) or caused by genetic exchange of indigenous and non-indigenous AMF strains resulting in genetic manipulation and loss of genetic pool in the cropping soils could alter (both increase and decrease) the efficacy of symbiosis (Colard et al., 2011), and warrants further research investigation.

### 3.6.2 Composition and structure of the indigenous AMF communities in response to inoculation with *R. irregularis* inoculant

The impact of inoculation with *R. irregularis* over soils and climates on the relative abundance of AMF community composition was presented in the Tables. 3.5 to 3.7. The key findings of these three tables are simplified comparing changes of different indigenous AMF taxa following inoculation. The results suggest that the significant alteration of different AMF taxa in the roots persisted in different soils and sites and detectable even after three cropping seasons. *Glomus* and *Funneliformis* shifted inconsistently over cropping seasons in inoculated treatments. However, significant effect of inoculation on the abundance of these two major fungi was detected during year 3. On the other hand, low abundant indigenous AMF taxa such as

*Rhizophagus* were significantly reduced in response to inoculation with *R. irregularis* inoculant. Similarly, *Archaeospora* was completely suppressed and *Diversispora* was also undetectable except in SC soil by year 3. This is an overall measurement of AMF compositional changes in response to inoculation regardless of soils and climatic factors. The results indicate that inoculation had a long term impact on the indigenous AMF community.

Recently, the impact of inoculation with commercial AMF inoculant on the changes of AMF communities as a whole AMF phylotype rather than compositional changes at AMF taxa levels was assessed (Antunes et al., 2009; Koch et al., 2011). Antunes et al. (2009) demonstrated that introducing a commercial inoculant, *G. intraradices*, into the soil did not impact the structure of indigenous AMF communities; however, they concluded that the inoculant directly or indirectly interacted with the indigenous AMF communities since plant nutrition was increased following inoculation. Koch et al. (2011) conducted a greenhouse experiment with Canadian field soil to examine the impact of a commercial *G. intraradices* (renamed *R. irregularis*) inoculant on the indigenous AMF community. They found a drastic decrease of indigenous AMF in the roots of *Sorghum vulgare* roots in response to the commercial inoculant, *G. intraradices*; however, they did not report which of the AMF taxa were affected. Rather they examined total AMF community T-RF richness. Similarly, a recent study demonstrated the impact of *G. irregulare* (currently renamed *R. irregularis*) on AMF root colonization using a Swift Current Brown Chernozem soil (Jin et al., 2013a). They reported significant compositional changes of indigenous AMF communities in response to AMF inoculation using cloning and Sanger sequencing technology. However, they identified the compositional changes as different AMF taxa include *Glomus, Acaulospora, Scutellospora.* The occurrence of *Glomus* was significantly reduced in response to the introduction of *G. irregulare*. The pyrosequencing data from the current field incubation study were in agreement with the previous studies and revealed that the impact of inoculation with *R. irregularis* on the composition of indigenous AMF communities over the cropping seasons was highly significant ($P<0.001$). Similarly, significant effects of soil ($P<0.001$) and climate/site ($P<0.001$) on the compositional changes of AMF community taxa were also detected (Table 3.4).

Some taxonomic changes in the classification of Glomeromycota with the progress of molecular tools are used in phylogenetic analysis (Schüßler and Walker, 2010). Recent

classification (advanced molecular based phylogenetic lineage) was used to profile AMF communities using current pyrosequencing protocols. Some taxonomic groups such as the *Rhizophagus* and *Funneliformis* were recently renamed and previously not used in many of the published articles, although former taxonomic identification was used. Briefly, the order *Glomerales* is now separated into two families (Kruger et al., 2012). The family *Glomeraceae* now comprises the four genera *Glomus, Funneliformis, Rhizophagus* and *Sclerocystis*, and the family *Claroideoglomeraceae* includes one genus, *Claroideoglomus*, based on the former *Glomus claroideum*.

The high-throughput sequencing of this study obtained 37 405 AMF sequences generating 86 OTUs in year 1, 28 648 AMF sequences generating 30 OTUs from samples in year 2 and 42 174 AMF sequences generating 72 OTUs from samples in year 3 (Tables A.3.4 to A.3.6). This is consistent with the recent AMF field survey study findings by (Dai et al., 2013, 2014) and (Bainard et al., 2014a, 2014b) who used the pyrosequencing approach to examine AMF communities in Chernozemic soils of the Canadian Prairie region. Similarly, another recent study that used soil collected near the current SC site and grew field pea under controlled conditions used a pyrosequencing (18S rRNA gene of AMF) platform to characterize AMF community assemblages in trap field pea roots, produced 24 000 AMF sequences and generated 39 OTUs (Jin et al., 2013b). This current study suggests that the soils harboured phylogenetically diverse AMF communities, as has been reported by others.

Of the four sites, two sites (OL and MF) already had indigenous *Rhizophagu*s which is the same taxa as the introduced *R. irregularis* inoculant (Table. 3.5). It is apparent that indigenous *Rhizophagus* was established in field pea trap roots along with the *R. irregularis* inoculant. The relative abundance of indigenous *Rhizophagus* was greatly reduced in each of the sampling years in response to inoculation (Tables 3.5 to 3.7). Accordingly, in year 3, indigenous *Rhizophagus* had been completely suppressed from the AMF assemblage in inoculated roots in both OL and MF soils (Fig. 3.9). This finding indicates that the closest AMF taxa to the introduced *R. irregularis* inoculant could be affected when trying to compete with non-indigenous strains for root colonization. The introduced inoculant apparently interacts with the resident genotypes including those which were closely related. Thus, the introduced non-indigenous AMF strains might outcompete the indigenous taxa.

The AMF taxon *Funneliformis* was very abundant and distributed across all of the soils. The relative abundance of indigenous *Funneliformis* was very high and ranged from 40% to 66% of total AMF taxa in original four inoculated soils at harvest in year 1. Relative sequence reads of *Funneliformis* were significantly reduced in year 2 and significantly increased in year 3 ranged in response to inoculation, which may reflect the different host crops as wheat was grown in the cores in year 2 (Tables 3.5 to 3.7). The interactions between host roots and symbiotic microbes influence the composition of root exudates and the structure of the root microbiome is likely influenced by soil type (Vierheilig et al., 2008; Moebius-Clune et al., 2013). The abundance of indigenous *Funneliformis* varied in the different soils. For example, the abundance of indigenous *Funneliformis* increased in response to *R. irregularis* inoculation in SC and MF soils by 8% and 9% in year 1 and 5% and 41% in year 3, respectively (Figs. 3.7 and 3.9). A very irregular pattern of abundance of *Funneliformis* was identified in both ST and OL soils. *Funneliformis. mosseae* is known as a cosmopolitan species (Avio et al., 2009). *Funneliformis* is common and adapted to the environments throughout cultivated Canadian Prairie soils (Dai et al., 2013). The mechanisms of competition among AMF taxa within a community are not clear and need further exploration, focusing on the nature of competition for root colonization among isolates for local resources. Notably, *Funneliformis* was completely absent in both inoculated and uninoculated control trap roots grown in ST soil of year 2 and year 3 cropping seasons. The relative abundance of *Claroideoglomus* community was higher during the cropping seasons when *Funneliformis* was absent but this result was not seen in any of the other soils except in the SC soil in year 2 where no *Funneliformis* was detected (Figs. 3.8 and 3.9). Wheat was cultivated in year 2 (2012) and field pea in year 1 (2011) and year 3 (2013) as a crop rotational practice (Table 3.1), and the host crop may have influenced the AMF community composition.

The relative abundance of indigenous *Glomus* ranged from 6% to 22% of total AMF taxa in the inoculated soils at harvest in year 1. The relative abundance of *Glomus* was unaffected by inoculation both in year 1 and year 3 but significantly increased in relative abundance in year 2 (Table 3.6). Similar to *Funneliformis, Glomus* was completely absent in the inoculated and uninoculated control trap roots grown in ST soil in year 2. Instead, the *Claroideoglomus* was rich in abundance in year 2. This same pattern was not detected in other soils (Figs. 3.8 and 3.9). The explanation for the varying relative abundance of *Glomus* might be similar to that of

*Funneliformis.* The interactions among soil type, root exudates form different hosts and root microbes likely shaped the *Glomus* communities over the seasons (Vierheilig et al., 2008).

Overall, *Claroideoglomus* was one of the most abundant AMF community members ranging from 7% to 54% of total AMF taxa in original four inoculated soils at harvest in year 1. The impact of inoculation with *R. irregularis* on the distribution of abundance of *Claroideoglomus* was less pronounced at harvest, three months after inoculation. For example, the relative abundance of *Claroideoglomus* was unaffected in all soils except three transplanted soils (SC, ST at OL and OL at ST site) in year 1. The *Claroideoglomus* group are phylogenetically distant from *Rhizophagus* (Figs. 3.3 to 3.5); thus, less competition might have occurred because they are only a distantly related major AMF species detected in this study. Maherali and Klironomos (2007) and Valiente-Banuet and Verdu (2008) suggested that similarity among closely related taxa can promote coexistence because of reduced competition between distinct evolutionary lineages. The impact of inoculation in year 1 on the abundance of Claroideoglomus was pronounced in year 3. For example, in year 3, inoculation significantly ($P$<0.001) reduced the abundance of *Claroideoglomus* in SC and enhanced in OL soils, however, *Claroideglomus* was undetectable in ST soil and unaffected in MF soils (Fig. 3.9).

The abundance of minor indigenous AMF taxa particularly, *Septoglomus* and *Archaeospora,* over soils and sites in response to inoculation generally declined and eventually they were not detected in year 3 (Tables 3.5 to 3.7). The suppression of these AMF taxa likely occurred by direct or indirect pressure from inoculation with the non-indigenous *R. irregularis* inoculant. Although current results did not confirm the sole effect of inoculation with the *R. irregularis* inoculant, it is notable to observe the suppression of a few minor AMF genera from the root colonization in year 3 (27 months after inoculation). Wheat was grown in field incubated soil cores during the second cropping season (2012). Addition of crop residues might interfere with the soil microbial activities (Borie et al., 2002; Dai et al., 2013) although the residual biomass would act in a similar manner in both the inoculated and uninoculated control cores. It is predicted that minor taxa were unable to compete for root colonization with other existing AMF groups due to changes in root exudation, microbiological interactions and nutrient availability (Husband et al., 2002; Cheng, 2009). Root colonization by different AMF community members

also depended on host plant type and had varying selection pressure based on the community for competitive root colonization (Antunes et al., 2009).

The considerable AMF shift in the original soils exposed to different climates suggests that soil environment is one of the key driving factors shaping and altering the indigenous AMF structure, diversity and composition. Arbuscular mycorrhizal fungal function greatly depends on soil and environmental conditions (Helgason and Fitter, 2009). Some previous studies suggested that response to inoculation varied in different soils (Hamel et al., 1997; Paul Schreiner, 2007).

### 3.6.3 Inoculant persistence responding to AMF diversity over cropping seasons

The Shannon diversity index (*H′*) of AMF communities combines two components of diversity, i.e., species richness and evenness (Dejong, 1975). The diversity index value increases as the number of species increases and as the distribution of individuals among the species becomes more even (Ludwig and Reynolds, 1991). In year 1, indigenous *Archaeospora*, *Diversispora* and *Rhizophagus* were not detected in the inoculated trap roots of SC, OL and ST soils, respectively. The changes in the AMF diversity in response to inoculation might be due to compositional changes of indigenous AMF taxa (increase, decrease and suppression of AMF taxa) and level of nutrient contents in soils. Moreover, the crop rotation with wheat in year 2 instead of field pea may alter the Shannon diversity indices. For example, in year 1 and year 3 diversity was significantly ($P<0.05$) reduced at SC and OL soil in response to inoculation but in year 2, the diversity was unaffected in both SC and OL inoculated and uninoculated soils. The high yielding wheat cultivars may be non-responsive to major mycorrhizal taxa and soil P levels differed in the AMF community (Hetrick et al., 1996; Alguacil et al., 2012). Several studies have shown host preferences of AMF in different habitats (Sýkorová et al., 2007; Alguacil et al., 2009, 2011). The Shannon diversity indices from the Figs. 3.10 to 3.12 were simplified and presented in Table 3.8 to understand the pattern of diversity reduction at a glance in soil types over the cropping seasons.

During the experiment, several disturbance events occurred in the upper 15 cm soil layer during seeding, inoculant application, and soil sampling, all of which could have led to the disruption of indigenous AMF infective propagules, by breaking the hyphal network leading to

loss or reduced diversity (Mcgonigle and Miller, 1996; Xavier and Germida, 1999). Moreover, aluminum soil cores (37 cm depth, 20 cm diameter) would have restricted lateral water movement. Water movement causes changes in soil aggregation resulting in significant alteration of activities of microbial communities (Amézketa, 2008). Murphy et al. (2011) found a linear relationship between changes in physical, chemical and biological characteristics of soil. If one of the factors is altered, this may have consequences on the other factors resulting in changes to soil organisms in several way following every type of disturbance (Jasper et al., 1989a, 1989b). This could explain why the AMF Shannon diversity index of AMF communities was reduced sharply in the incubated field soils by year 3. The diversity index value ranged from $H'$ = 2.1 to 3.78 in year 1, whereas it varied from $H'$ = 1.1 to 2.0 in year 3. The current findings suggest that inoculation also influences the Shannon diversity index over three consecutive cropping seasons (Figs. 3.10 to 3.12). Moreover, introduced *R. irregularis* persisted with variable success for three years. For example, the inoculant persisted in SC to year 3, with a concomitant reduction in the diversity index from $H'$ = 1.85 in year 1 to $H'$ = 1.70 in year 3. Similarly, there was a reduction in diversity from $H'$ = 1.70 in year 1 to $H'$ = 1.05 in year 3 in OL soil; however, the diversity in the SC and OL soils was reduced in year 1 by 15% (from 2.47 to 2.1) and 20% (from 2.58 to 2.07), respectively (Fig. 3.10 and 3.12).

Inoculation greatly reduced the diversity index in ST and MF soils in year 1. However, the *R. irregularis* inoculant strain was not detected in ST soil in year 2 and year 3, or in MF soil in year 3. This indicates that the characteristics of both soils did not support the prolonged persistence of the non-indigenous *R. irregularis* inoculant (Fig. 3.6). These observations indicate that inoculation can disrupt indigenous AMF communities, and the changes can persist.

Environmental conditions had differential effects on AMF community composition and diversity. For example, MF soil transplanted at OL and ST sites had minimal changes in diversity both inoculated and uninoculated trap roots, but the reduction of diversity and alteration of composition were pronounced while the MF soil was exposed to the SC site. Moreover, early loss of AMF diversity in trap roots of SC and OL soils (i.e., from year 1 to year 2) following inoculation recovered in year 3. Very little is known about the adaptation strategy of AMF community taxa to environmental conditions (Johnson et al., 2013).

**Table 3.8** The effect of inoculation with *R. irregularis* inoculant on the Shannon diversity index (*H'*) over three cropping seasons.

| Soil Order: Chernozem | | Cropping Season | | |
|---|---|---|---|---|
| **Site/Location** | **Soil Type** | **2011** | **2012** | **2013** |
| Swift Current (SC) | Brown | ↓* | NS | ↓* |
| | OL-Dark Brown | ↓* | NS | NS |
| | ST-Dark Brown | ↓* | ↓* | NS |
| | Black | ↓* | NS | ↓* |
| Outlook (OL) | Brown | NS | NS | NS |
| | OL-Dark Brown | ↓* | NS | ↓* |
| | ST-Dark Brown | NS | NS | NS |
| | Black | NS | NS | NS |
| Scott (ST) | Brown | NS | ↓* | ↓** |
| | OL-Dark Brown | NS | NS | NS |
| | ST-Dark Brown | ↓** | ↓** | ↓* |
| | Black | ↓* | ns | NS |
| Melfort (MF) | Brown | ↓* | ↓* | NS |
| | OL-Dark Brown | ↓* | ↓** | ↓* |
| | ST-Dark Brown | NS | NS | NS |
| | Black | ↓** | ↓** | ↓* |

Significant effects are indicated by * ($P \leq 0.05$), ** ($P \leq 0.01$), NS (non-significant), and ↓ indicate diversity decreased.

Little is known about the combined effect of environmental conditions and the introduction of a non-indigenous AMF species on indigenous AMF community composition and subsequent diversity. The current results demonstrate persistence of *R. irregularis* inoculant for three consecutive cropping seasons in Swift Current Brown and Outlook Dark Brown soils. However, the level of persistence varied, and thus the impact of inoculation on the AMF community varied. Some ecological studies have confirmed that different species of AMF induce different effects and contribute different functionality on plant growth and yield (Van der Heijden et al., 1998; Klironomos, 2000, 2003). It seems likely that alterations to the indigenous AMF community could potentially alter the biomass and yield of a crop without changing overall colonization stages caused by an introduced AMF inoculant species.

### 3.7 Conclusions

The arbuscular mycorrhizal community structure and composition shifted in different soils in response to inoculation and this alteration of community compositions in pea trap roots

was detectable even 27 months after inoculation with non-indigenous *R. irregularis* inoculant (i.e., year 3). The inoculant persisted for three consecutive crop seasons in relatively drier and low organic matter soils such as Swift Current Brown and Outlook Dark Brown soils compared to Melfort soil (wet and high organic matter soil). None of the original soils transplanted at other sites was suitable for persistence of *R. irregularis* beyond year 2 (2012). Climatic conditions played a contributing role, shifting AMF communities over the cropping seasons in some soils. Transplanting soils to different climates stimulated minor or less abundant indigenous AMF taxa like *Entrophospora*. Major AMF species such as *Claroideoglomus* became predominant in abundance in year 2 over other two major genera (*Glomus* and *Funneliformis*), particularly when soils were transplanted to other climatic conditions. The current results raised several unanswered questions regarding the mechanism of competition between indigenous and non-indigenous AMF taxa in the context of different crops, soils, climates, and inoculant types over the long-term. Inoculation with a non-indigenous AMF strain should be further investigated to gain insight to the soil-inoculant-climate compatibility before an application of non-indigenous AMF strains in crop production systems occurs.

# CHAPTER 4

# IMPACT OF ARBUSCULAR MYCORRHIZAL FUNGAL INOCULANTS ON THE COMPOSITION AND DIVERSITY OF INDIGENOUS AMF COMMUNITIES, NUTRIENT UPTAKE AND BIOMASS ACCUMULATION BY LENTIL, CHICKPEA AND FIELD PEA

## 4.1 Preface

This chapter assess the impact of arbuscular mycorrhizal fungal (AMF) inoculants of different origins and genetics on indigenous AMF communities and their subsequent effectiveness for enhancing nitrogen (N) and phosphorus (P) uptake, and biomass accumulation in lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.), and field pea (*Pisum sativum* L.) under growth chamber conditions. In Chapter 3, the persistence of a commercial *Rhizophagus irregularis* inoculant and its impact on diversity and composition of indigenous AMF in pea roots at four locations of Saskatchewan was assessed. In this controlled growth chamber study, three AMF inoculants were assessed; two were isolated from Canadian soils and were designated SPARC (Semiarid Prairie Agricultural Research Centre) *Funneliformis mosseae* B04, GINCO (Glomeromycota In-vitro Collection) *F. mosseae* DAOM 221475. The third one was *R. irregularis* isolated from a commercial inoculant source (Premier Tech, Quebec, Canada, lot no. 4514535). The aim was to relate alterations in the AMF community due to inoculation with plant growth response.

## 4.2 Abstract

The influence of different AMF taxa inoculants on biomass accumulation and enhanced nutrient (N and P) uptake by lentil, chickpea and field pea was examined to understand the role of indigenous AMF communities and introduced inoculants on plant growth responses. The molecular phylogenetic analysis with high-throughput pyrosequencing was able to discriminate between introduced AMF inoculant strains and the indigenous AMF taxa in root assemblages. A two-way ANOVA and Tukey's test of significance showed that the non-indigenous commercial

inoculant (*R. irregularis* 4514535) significantly altered the composition of indigenous AMF taxa whereas no significant changes in the AMF taxa were detected in response to inoculation with SPARC (*F. mosseae* B04). The locally isolated SPARC inoculant significantly enhanced uptake of N and P, and shoot dry biomass in all three pulse crops with minimum disturbance to the indigenous AMF community composition and diversity. Inoculation with GINCO (*F. mosseae* DAOM 221475) also enhanced N uptake in chickpea; however, uptake of P and biomass response were variable between crops. The commercial inoculant, *R. irregularis* failed to result in significant growth promotion or enhanced nutrient uptake. Root occupancy by all three inoculant taxa was negatively correlated with the plant growth variables; however, strong positive correlations were detected between root occupancy by some highly abundant indigenous AMF taxa (*Rhizophagus*, *Funneliformis*) and growth performances which could be attributed to alterations in the AMF colonizing community as a consequence of inoculation. Significant negative correlations between the growth parameters and the relative abundance of indigenous *Glomus* and *Claroideoglomus* in treatments inoculated with commercial *R. irregularis* were detected. Increased abundance of indigenous *Glomus* following inoculation with *R. irregularis* inoculant may have acted as a carbon (C) sink without imparting growth benefits, thereby resulting in reduced plant growth performance. This requires validation to improve our understanding of the cause and effect of this change in the indigenous AMF communities in various cropping systems.

### 4.3 Introduction

Plant root systems typically are colonized by multiple species of arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota. The majority of the terrestrial plants, including many important grain legumes such as lentil (*Lens culinaris* L.), chickpea (*Cicer. arietinum* L.), and field pea (*Pisum sativum* L.), show mutualistic symbiosis with AMF (Kucey, 1987; Talukdar and Germida, 1993; Smith et al., 2011). The main benefit of AMF-crop symbiosis is enhanced nitrogen (N), phosphorus (P) and biomass accumulation through enhanced exploitation of the soil by the hyphal network (Giovannetti et al., 2001; Avio et al., 2006). The benefits from the symbiosis depends on a number of factors, including the dominant AMF genotypes and the environmental conditions under which symbionts co-exist (Jeffries et al., 2003; Klironomos,

2003; Koch et al., 2006). Plant growth responses to AMF may range from negative (parasitism) to positive (mutualism) (Dai et al., 2014).

The use of commercial AMF inoculants is increasing in horticultural and land reclamation applications worldwide (Gianinazzi and Vosátka, 2004) and could be an emerging production technology for field crops in Canada. The inoculated species can be isolated from local soils, which permits them to co-exist with other indigenous AMF populations (Klironomos, 2003; Johnson et al., 2010). The introduction of non-indigenous AMF isolates may alter the structure of the resident indigenous AMF communities through positive or negative interactions (Mummey et al., 2009; Koch et al., 2011). Inoculation of AMF in field soils often results in competition between the introduced non-indigenous AMF and existing indigenous AMF for colonization of host roots (Abbott and Robson, 1981). Monitoring the application of AMF inoculant in soil is important to distinguish AMF sources of root occupancy and to assess inoculant success. Some research indicates that introduced non-indigenous inoculants are less competitive than indigenous isolates (Klironomos, 2003; Mummey et al., 2009) and that introduction of non-indigenous commercial inoculant (e.g. *Rhizophagus irregularis*) did not affect the structure of the indigenous AMF communities when applied at the recommended dose (Antunes et al., 2009).

The ecological consequences of introducing non-indigenous isolates are still unclear. There currently is little understanding of the influence of host plant species on the performance of mass released non-indigenous AMF inoculants in competing with the resident indigenous isolates for root occupancy. Host plant species may have an important role in determining the efficacy of root occupancy by indigenous and non-indigenous AMF species. There is also little information available on how host plants affect resident indigenous AMF diversity and composition, and it is unknown how host plants influence the survival and effectiveness of root occupancy by commercial non-indigenous AMF co-existing with indigenous AMF communities. Additionally, little information is available about the effect of host plants and crop varieties on competitive interactions affecting root occupancy among indigenous and non-indigenous AMF.

Multiple species of AMF can co-exist in crop soils and co-occurring host plants can differ in their response to colonization by different AMF species. In greenhouse experiments, it was

observed that many species of AMF can colonize any plant species capable of forming mycorrhizas, suggesting that AMF are not host-specific (Eom et al., 2000). Others have noted several factors which may result in strong selection for host specificity including soil nutrient status, host types, land use patterns, and rhizosphere microbial community status (Harley et al., 1983; Clapp et al., 1995). Co-occurring plant species can differ significantly in their growth responses to AMF symbiosis (Wilson and Hartnett, 1998), and different AMF species differ significantly in their effects on host plant growth and protection (Newsham et al., 1995; Jeffries et al., 2003).

Significant progress has been made in understanding relationships between host plant taxa and AMF associations (Harley and Harley, 1987; Newman and Reddell, 1987) and what factors contribute the most towards successful AMF root establishment when competing for nutrients (Allen and Allen, 1984; Allen et al., 1995; Mahdi et al., 2010). However, the genetics of AMF can significantly influence growth, yield and yield attributes (Hart and Reader, 2002). Thus, when selecting an AMF inoculant strain, it is important to have a clear understanding about the response of the fungi to various soils, hosts and environmental conditions where the inoculants will be introduced. In addition, quality of infective propagules of inoculants, and the compatibility between AMF genotypes, soils, and host plants (Bever et al., 2003; Klironomos, 2003; Oehl et al., 2010; Verbruggen and Toby Kiers, 2010; Herrera-Peraza et al., 2011; Verbruggen et al., 2012) is essential for ensuring a positive AMF-plant relationship (Hart et al., 2003).

The first challenge in understanding the variable contribution of different types and/or sources of AMF inoculant strains or even variable indigenous AMF taxa on plant growth is to identify protocols which can discriminate between the introduced AMF strains and the indigenous strains colonizing roots if a similar AMF already exists in the soil. In a related field incubation study (Chapter 3), the persistence of an introduced *R. irregularis* was successfully monitored using high throughput pyrosequencing technology, even in soil with an indigenous *Rhizophagus* community. Recently, one study used cloning and sequencing protocols to demonstrate that two non-indigenous commercial isolates of *F. mosseae*, applied in field soils, appeared to have successfully competed with indigenous AMF communities as root colonizers and persisted for two years (Pellegrino et al., 2012). Still, very little information about the

challenge of monitoring AMF inoculants in field soils or greenhouse conditions currently exists. Without separating the introduced inoculant strains from the indigenous communities, it is difficult to identify the contribution of the inoculants to plant responses or evaluate the interaction between introduced AMF and the indigenous communities or observe synergistic effects on plant growth parameters. Thus, many questions about selecting suitable AMF inoculants exist, particularly in terms of what consequences will emerge following inoculation.

The aim of the present growth chamber study was to assess plant-growth response to multiple AMF inoculants. Additionally, the impact of AMF inoculation on the existing indigenous AMF communities was studied. By exploring the individual contribution of introduced and indigenous AMF root occupancy, it is possible to further understand how these AMF strains contribute to plant growth variables. Three AMF inoculants were used in the study. One was a locally isolated AMF strain, *F. mosseae* B04, isolated from Swift Current Brown Chernozem soil and cultured several months in the Outlook Dark Brown soil. A second AMF strain, *F. mosseae* DAOM 221475, was isolated from a geographically distant location (Ontario). The third strain, *R. irregularis*, was recovered from a commercial AMF inoculant formulation. All three strains were used to determine their effect on both plant growth variables and the composition and diversity of indigenous AMF communities. It was hypothesised that variable root occupancy would be achieved by different inoculant strains resulting in different contributions to enhance nutrient uptake and biomass accumulation. The relationship between the root occupancy (occurrence and abundance) of indigenous AMF taxa and three inoculants strains with plant growth variables was examined to determine if the response of lentil, chickpea and field pea was due to the impact of the inoculant strains or due to the alteration in the indigenous AMF community structure as a consequence of inoculation.

## 4.4. Materials and Methods

### 4.4.1 Experimental design and treatments

A pot culture experiment was conducted in a growth chamber at the University of Saskatchewan, Saskatoon, SK, Canada from September to November, 2014. The growth chamber conditions were as follows: ambient day, 24 °C and night 18 °C; 16 h day length; relative humidity during the day, 75%, and night, 85%. The experiment consisted of four AMF treatments as follows: 1) SPARC *F. mosseae* B04 strain; 2) GINCO *F. mosseae* DAOM 221475 strain; 3) spores isolated from the commercial formulation of *R. irregularis*; and 4) uninoculated control. Three crops were used as host plants, namely lentil, chickpea and field pea. The experimental design was a two factor (inoculant and crop) factorial completely randomized design (CRD). Each treatment was replicated four times. The soil was collected from the top 15 cm of Dark Brown Chernozem located at the Canada Saskatchewan Irrigation Diversification Center (CSIDC) research farm, Outlook, SK, Canada. The soil was dried and homogenized (2:1, w/w) with sterilized fine sand (Microcrystalline Silica CAS, Unimin Corp, USA). The fine sand was sterilized by autoclaving three times. All experimental pots were prepared with 1.5 kg of the soil-sand mix. Before seeding lentil (*Lens culinaris* L., var. CDC Maxim), chickpea (*Cicer arietinum* L., var. Frontier) and field pea (*Pisum sativum* L., var. CDC Meadow), the surface of the seeds were disinfected by immersing them in a 0.5% sodium hypochlorite solution for 20 min (Newsham et al., 1995) and then washing in sterile distilled $H_2O$. The disinfected seeds were germinated on moistened filter paper in Petri dishes for 3 d in continuous darkness. Three pre-germinated seeds were sown in each pot. After 7 d, two plants were confirmed established in each pot. Pots were irrigated as needed by weight and maintained at field capacity with daily addition of water. Hoagland and Arnon, (1950) solution (N: 211, S: 64, K: 236, Mg: 48, Ca: 200, B: 0.01, Cu: 0.01, Fe: 0.5, Mn: 0.1, Mo: 0.02, Zn: 0.01 µg $mL^{-1}$) without P was added (100 mL $kg^{-1}$ potting mix) onto growth substrate once during seed sowing. The nutrient solution and all other necessary materials used in this pot culture were sterilized to avoid any possible contamination. The properties of the soil-sand mix were determined as follows: pH (1:2 soil: water) 7.1; EC (1:2 soil: water extraction) 0.4dS$m^{-1}$; inorganic N ($NO_3^-$ and $NH_4^+$) 41 µg N $g^{-1}$; $NaHCO_3$ extractable P, 18 µg P $g^{-1}$; $CH_3COONH_4$ extractable K, 297 µg K $g^{-1}$; $K_2Cr_2O_7$-$H_2SO_4$ determined organic matter (OM), 28.1 g $g^{-1}$ (ALS Laboratory Group, Saskatoon, Canada).

### 4.4.2 Description of AMF inoculants and application in crop seedlings

Three different AMF strains were used. The first, *F. mosseae*, was isolated from a Brown Chernozemic soil at the Semiarid Prairie Agricultural Research Center (SPARC), Swift Current. The reference name of locally isolated AMF inoculant strain "*F. mosseae* B04" used throughout this study is "SPARC inoculant". The SPARC *F. mosseae* B04 was preserved and maintained by Dr. Chantal Hamel at SPARC. The other non-commercial strain *F. mosseae* DAOM 221475, was isolated from Rondeau Provincial Park, Ottawa, Ontario and preserved in Glomeromycota In-Vitro Collection (GINCO) as a reference species (*F. mosseae* DAOM 221475, Dr. Yolande Dalpé). The reference name of AMF inoculant strain "*F. mosseae* DAOM 221475" isolated from Rondeau Provincial Park, Ontario used throughout this study is "GINCO inoculant". The third inoculant was the commercial non-indigenous AMF species, *R. irregularis*, and was recovered from a commercial inoculant lot no. 4514535 (Primer Tech, Quebec, Canada). The reference name of commercial AMF inoculant strain "*R. irregularis* 4514535" used throughout this study is "commercial inoculant". The detailed background information of the three inoculants is summarized in Table 4.1.

The SPARC *F. mosseae* B04 strain pot culture was propagated and maintained in a sand-soil (Outlook soil) mix (1:1) in transparent Sunbags (Sigma-Aldrich, Germany) (Walker and Vestberg, 1994) with maize as the host crop for three months prior to use for this current growth chamber experiment. The GINCO *F. mosseae* DAOM 221475 strain was also propagated and maintained using the same sand soil (Swift Current soil) mix since January, 2014. Spores of each inoculant were extracted from the nurse cultures according to the methods by Daniels and Skipper (1982). Spores were rinsed in deionized water and 10 mL water suspension with 100 spores of each AMF inoculant per plant were used to inoculate 7 d old seedlings at the root zone.

### 4.4.3 Sampling plant roots for molecular analyses

Pulse crops were grown for eight weeks (approximately the end of the flowering) which is considered an optimum mycorrhizal colonization phase (Jakobsen and Nielsen, 1983). The roots from each pot were retrieved by washing with tap water followed by deionized water to

remove adhering soil particles. The cleaned roots were blotted dry with tissue papers, immersed in liquid N and preserved at – 80 °C until molecular analysis.

### 4.4.4 Determination of plant shoot nutrients (N and P) and biomass contents

Plants shoots were oven-dried at 60 °C to constant weight and biomass was determined. Dried shoots were ground to pass through a 2-mm mesh screen. The plant shoot powder was digested using $H_2SO_4$-$H_2O_2$ (Thomas et al., 1967). The acid digests were analysed for N and P concentration using a Technicon™ AutoAnalyzer (Technicon Industrial Systems, Tarrytown, USA) using standard methods (Thomas et al., 1967).

### 4.4.5 DNA extraction, 18S RNA gene pyrosequencing platforms, bioinformatics and phylogenetic analyses of AMF communities

A total of 48 replicated DNA samples from lentil, chickpea and field pea roots and 12 replicated DNA samples from the spores of the three AMF inoculants, SPARC inoculant (*F. mosseae* B04), GINCO inoculant (*F. mosseae* DAOM 221475) and commercial inoculant (*R. irregularis* 4514535) was used to perform 18S rRNA gene pyrosequencing analyses. The processing of raw sequence reads used bioinformatics tool, MOTHUR version 1.31 (Schloss et al., 2009) and a phylogenetic tree was constructed with 59 OTUs (from cleaned 24 459 sequence reads) generated from AMF taxa, associated with the roots of the above pulse crops including 9 OTUs from three introduced inoculants. The protocols for the above analyses are similar to the protocols used in field incubation study as described at section 3.4.6 to 3.4.10 in Chapter 3. Primers, tags and 454 Lib-L adaptors used for PCR amplification of 18S rRNA gene through pyrosequencing analyses were also listed in the Table 3.3 in Chapter 3.

**Table 4.1** Background information of three AMF inoculant strains including source, type, geographical location and habitat.

| | AMF inoculants | | |
|---|---|---|---|
| **Background information** | **Inoculant 1 (SPARC *F. mosseae* B04)** | **Inoculant 2 (GINCO *F. mosseae* DAOM 221475)** | **Inoculant 3 (Commercial *R. irregularis* 4514535)** |
| Taxonomic identity (microscopic) | *Glomus mosseae* | *Glomus mosseae* | *Glomus intraradices* |
| Code name of strain by collector | B04 | DAOM 221475 | Company bag/lot no: 4514535 |
| Formulation type | Non-commercial/In-vitro multiplication under growth chamber condition at Semiarid Prairie Agricultural Research Centre (SPARC), Saskatchewan, Canada | Non-commercial/In-vitro multiplication under growth chamber condition at U of S | Commercial formulation by Primer Tech, Quebec, Canada |
| Source soil (taxonomy) | Orthic Brown Chernozem | Luvisol | Unknown |
| Pure culture host | *Allium ampeloprasum* (Leek) | *Allium ampeloprasum* (Leek) | Unknown |
| Habitat | Wheat field | *Ammophila breviligulata* (American Beach Grass)/sand dune | Unknown |
| Origin of geographical location | SPARC of AAFC, SE 1/4 LSD 3 SW SEC 30 TWP 19 RG 28 W3RD), Swift Current, SK | Rondeau Provincial Park ( 42°16' 52.23" N 81°50' 27.38"), Ottawa, Ontario | Unknown |
| Country of origin | Saskatchewan (SK), Canada | Ontario (ON), Canada | Unknown |
| Soils/substrate of multiplication and maintenance before experimentation | Top 15 cm Dark Brown soil from Canada Saskatchewan Irrigation Diversification Centre (CSIDC) at Outlook with host: maize | Top 15 cm Brown Chernozem soil from SPARC, AAFC at Swift Current, SK with maize | Carrier materials: Peat materials |

**Table 4.1** Continued.

| Background information | AMF inoculants | | |
|---|---|---|---|
| | **Inoculant 1 (SPARC *F. mosseae* B04)** | **Inoculant 2 (GINCO *F. mosseae* DAOM 221475)** | **Inoculant 3 (Commercial *R. irregularis* 4514535)** |
| Collector and determiner | Dr. Chantal Hamel, SPARC, AAFC, Swift Current, SK | Dr. Yolande Dalpé, GINCO, AAFC, Ottawa, Ontario | Unknown |
| Year of collection | June, 2007 | September, 1989 | Unknown |
| Molecular identification (spore DNA 18S rRNA gene pyrosequencing) according to Krueger et al. (2012) | *Funneliformis mosseae* (closest match with GenBank ID: FR750227.1) | *Funneliformis mosseae* (closest match with GenBank ID: FR750227.1) | *Rhizophagus irregularis* (GenBank ID: HF968850.1) |
| Name of the OTUs in phylogenetic tree (Fig. 4.1) | SPARC *Funneliformis* B04 inoculant | GINCO *Funneliformis* 221475 inoculant | Commercial *Rhizophagus* inoculant |
| Reference name used throughout this study | SPARC *Funneliformis* B04 | GINCO *F. mosseae* DAOM 221475 | Commercial *R. irregularis* 4514535 |

### 4.4.6 Statistical analysis

The relative abundance of indigenous AMF taxa in the roots of lentil, chickpea and pea in response to inoculation were tested by subjecting the AMF OTUs abundance (sequence reads) data to two-way analysis of variance (ANOVA) in SAS v.5. (SAS institute Inc. Cary, NC). Two factors (factor 1: inoculation and factor 2: crop) were considered for determining the *P* value.

Two-way ANOVA was also used to test the effect of inoculants and crops on shoot biomass and N and P uptake. The significance of the differences in Shannon diversity, indigenous AMF taxa, three inoculants, and growth parameters (N, P uptake and biomass accumulation) was determined by Tukey's test of multiple comparison of means ($P \leq 0.05$) using SAS. Before analyses, all the parametric (plant growth variables) and non-parametric (percent relative abundance) data were subjected to a normality test. Skewness and kurtosis of data distribution were performed for the relative abundance of AMF communities.

Before statistical analyses, relative abundance of indigenous AMF and introduced inoculants was estimated from the absolute number of sequence reads of OTUs in each sample. To determine the relative abundance of the inoculants in roots, the absolute number of each inoculant strain (e.g., SPARC *F. mosseae* B04, GINCO *F. mosseae* DAOM 221475, and commercial *R. irregularis* 4514535) was divided by the total absolute number of indigenous AMF taxa to estimate the relative abundance of each inoculant. The calculation of relative abundance of indigenous AMF taxa and inoculants is presented in appendix B.

The Pearson correlation coefficient (*r*) and *P* value with linear model between the relative abundance of indigenous AMF taxa, inoculant strains, diversity index, and crop growth (N, P uptake and biomass accumulation) variables were calculated using Microsoft excel. The *r* and *P* value of each correlation analysis are included in the respective scatter plots.

## 4.5 Results

### 4.5.1 Description of the molecular AMF community data

Approximately 24 489 Glomeromycota (AMF) sequence reads of 18S rRNA gene from host root samples of all AMF inoculated and uninoculated control treatments of lentil, chickpea, and field pea were obtained from the GS-FLX+ pyrosequencing platform after cleaning and removing short, ambiguous and chimera sequences. Indigenous AMF taxa and three introduced inoculant strains generated 20 702 and 3 787 sequence reads, respectively. The 18S rRNA gene sequence length varied from 650 to 800 bp which was over 91% of the sequence length amplified by AML1 and AML2. A total of 59 AMF OTUs was identified based on 97% sequence similarity from 24 489 sequence reads, including nine OTUs generated from the three AMF inoculants (three OTUs from SPARC *F. mosseae* B04, two OTUs from GINCO *F. mosseae* DAOM 221475 and four OTUs from commercial *R. irregularis* 4514535 inoculants) (Fig. 4.1 and Table B.4.2). These indigenous 50 OTUs belonged to the Glomeraceae (*Rhizophagus*, *Funneliformis*, *Glomus* and *Septoglomus*), the Claroideoglomeraceae (*Claroideoglomus*), Diversisporaceae (*Diversispora*), Archaeosporaceae (*Archaeospora*) and Paraglomeraceae (*Paraglomus*) genera. Of the indigenous Glomeraceae sequence reads, 90.27 % represented 31 OTUs. These 31 OTUs included *Rhizophagus* (four OTUs), *Funneliformis* (11 OTUs), *Glomus* (14 OTUs) and *Septoglomus* (two OTUs) followed by 6.29% belonging to Claroideoglomeraceae (*Claroideoglomus* nine OTUs), 1.5% belonging to Paraglomeraceae (*Paraglomus* six OTUs), and 1.07% belonging to Diversisporaceae (*Diversispora* two OTUs) and less than 1% belonging to Archaeosporaceae (*Archaeospora* two OTUs). The details of absolute and relative sequence reads of each OTU per sample are presented in Appendix B (Table B.4.1 to B.4.2).

### 4.5.2 Identification and quantification of introduced AMF inoculant strains from the indigenous AMF communities

The 59 OTUs (indigenous AMF taxa and three introduced AMF inoculant strains) and 21 reference sequences from NCBI databases were used to construct a neighbour-joining phylogenetic tree to identify OTUs into AMF taxa (Fig. 4.1). According to the phylogenetic tree, the OTUs of SPARC inoculant (*F. mosseae* B04) and GINCO inoculant (*F. mosseae* DAOM

221475) were clustered with *Funneliformis* taxa, in which OTUs of two introduced inoculants (SPARC and GINCO) were closely matched (97% to 99%) with the GenBank reference sequences of *F. mosseae* (accession no AJ306438.1 and FR750227.1, respectively). Similarly, the commercial inoculant (*R. irregularis* 4514535) was also clustered with the *Rhizophagus* taxa and with a 99% similarity to *R. irregularis*, GenBank accession number HF968850.1 (Fig. 4.1).

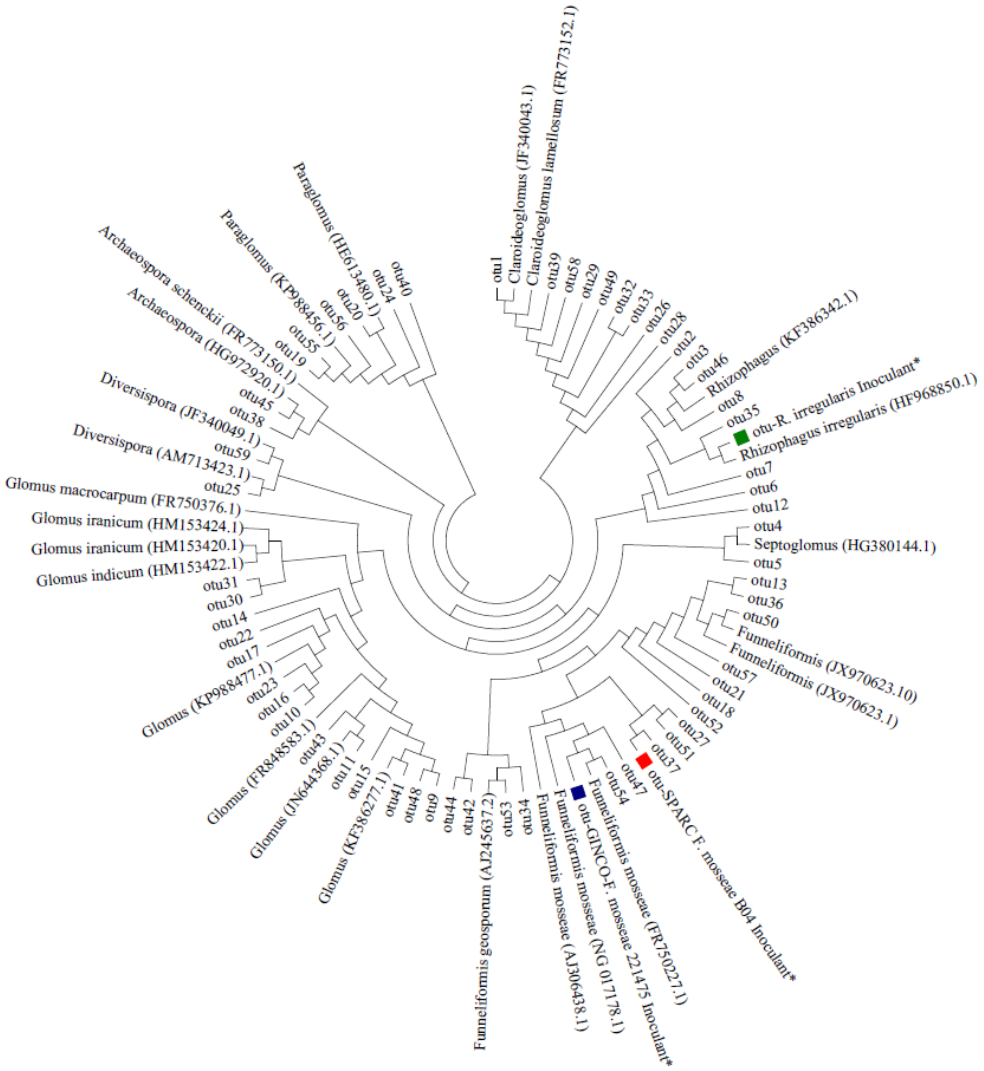


**Figure 4.1** Phylogenetic analysis of 59 AMF OTUs, associated with the roots of lentil, chickpea and field pea detected by pyrosequencing under control condition. AMF OTUs (indigenous and introduced inoculants) are clustered as *Rhizophagus*, *Glomus*, *Funneliformis*, *Septoglomus*, *Claroideoglomus*, *Diversispora*, *Archaeospora* and *Paraglomus* genera. Phylogenetic relationships are obtained by neighbor-joining analysis of AMF 18S rRNA gene with primer pairs (NS1/NS4 and AML1/AML2). GenBank reference sequences are indicated within a parenthesis. OTUs representing the introduced AMF inoculants, SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants are marked with red, blue and green rectangle and asterisk, respectively.

### 4.5.3 Influence of inoculants on the AMF community composition and diversity

A two-way ANOVA showed an overall significant influence of inoculants and crops on the relative abundance of indigenous AMF taxa and Shannon diversity index in roots of pulse crops (Table 4.2). Interaction between inoculants and crops also had a significant influence on the composition and diversity of indigenous AMF communities. Tukey's significant test of multiple comparisons ($P \leq 0.05$) between the three inoculants and crops was performed (Figs. 4.2 to 4.5).

No significant influence of SPARC (*F. mosseae* B04) and GINCO (*F. mosseae* DAOM 221475) inoculants on the relative abundance of indigenous *Rhizophagus* and *Funneliformis, Glomus* and *Claroideoglomus* sequence reads in any of the pulse crops was detected (Figs. 4.3 to 4.5). *Glomus* with the exception of which significantly increased from 6% to 13% in lentil in response to inoculation with GINCO inoculant (Fig. 4.3).

Commercial inoculant (*R. irregularis* 4514535) significantly ($P \leq 0.05$) reduced the relative abundance of *Rhizophagus* and *Funneliformis* in chickpea and field pea and of *Rhizophagus* in lentil. The abundance of indigenous *Rhizophagus* compared to control was reduced from 32% to 11% in lentil, 27% to 14% in chickpea and 29% to 13% in field pea (Figs. 4.2 to 4.5). Similarly, indigenous *Funneliformis* was reduced from 50% to 46% in lentil, 56% to 43% in chickpea and 61% to 37% in field pea (Fig. 4.2 to 4.5). In contrast, a significant increase of the relative abundance of *Glomus* and *Claroideoglomus* was observed in response to inoculation with commercial inoculant in lentil and field pea, and of G*lomus* in chickpea (Figs. 4.3 to 4.5). The relative abundance of indigenous *Glomus* increased from 6% to 15% in lentil, 4% to 36% in chickpea and 4% to 22% in field pea in response to commercial inoculant. Similar trends in increasing abundance of *Claroideoglomus* sequence reads in response to commercial inoculant were observed in lentil (4% to 23%) and in field pea (3% to 21%) (Fig. 4.2). No significant influence on *Claroideoglomus* in response to any of the inoculants was detected in chickpea crop (Fig. 4.4).

**Table 4.2** A two-way ANOVA showing the effect of inoculation and crop on relative abundance of indigenous AMF taxa, Shannon diversity index in roots, detected by 18S rRNA gene pyrosequencing and shoot N and P uptake and biomass accumulation in the lentil, chickpea and field pea.

| | **Root occupancy (relative abundance) (%) of indigenous AMF taxa**[†] | | | | | | | | | **Plant growth performances** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sources** | ***Rhizophagus*** | ***Funneliformis*** | ***Glomus*** | ***Claroideoglomus*** | ***Septoglomus*** | ***Diversispora*** | ***Archaeospora*** | ***Paraglomus*** | **Shannon diversity index of AMF taxa**[‡] | **Shoot N uptake** | **Shoot P uptake** | **Shoot dry biomass** |
| Inoculation (I) | ** | ** | *** | ** | *** | *** | ns | *** | ** | ** | ** | *** |
| Crop (C) | ns | ** | ** | * | *** | ** | *** | *** | * | ns | ** | ** |
| Interaction (I x C) | * | * | ** | ** | *** | ** | ** | *** | **ns** | **ns** | **ns** | **ns** |

Significant at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***). ns denotes non-significant.

[†]Arbuscular mycorrhizal fungal taxa consisting of *Rhizophagus*, *Funneliformis*, *Glomus, Septoglomus, Claroideoglomus, Diversispora, Archaeospora* and *Paraglomus* detected by 18S rRNA gene pyrosequencing and quantified as relative abundance of sequence reads in the roots of lentil, chickpea and field pea in response to AMF inoculation with SPARC inoculant (*F. mosseae* B04), GINCO inoculant (*F. mosseae* DAOM 221475) and commercial inoculant (*R. irregularis* 4514535).

[‡]Shannon diversity index was determined using the abundance of above indigenous AMF communities by the command 'collect.single' in MOTHUR bioinformatics pipeline, based on the formula by Shannon (1948).

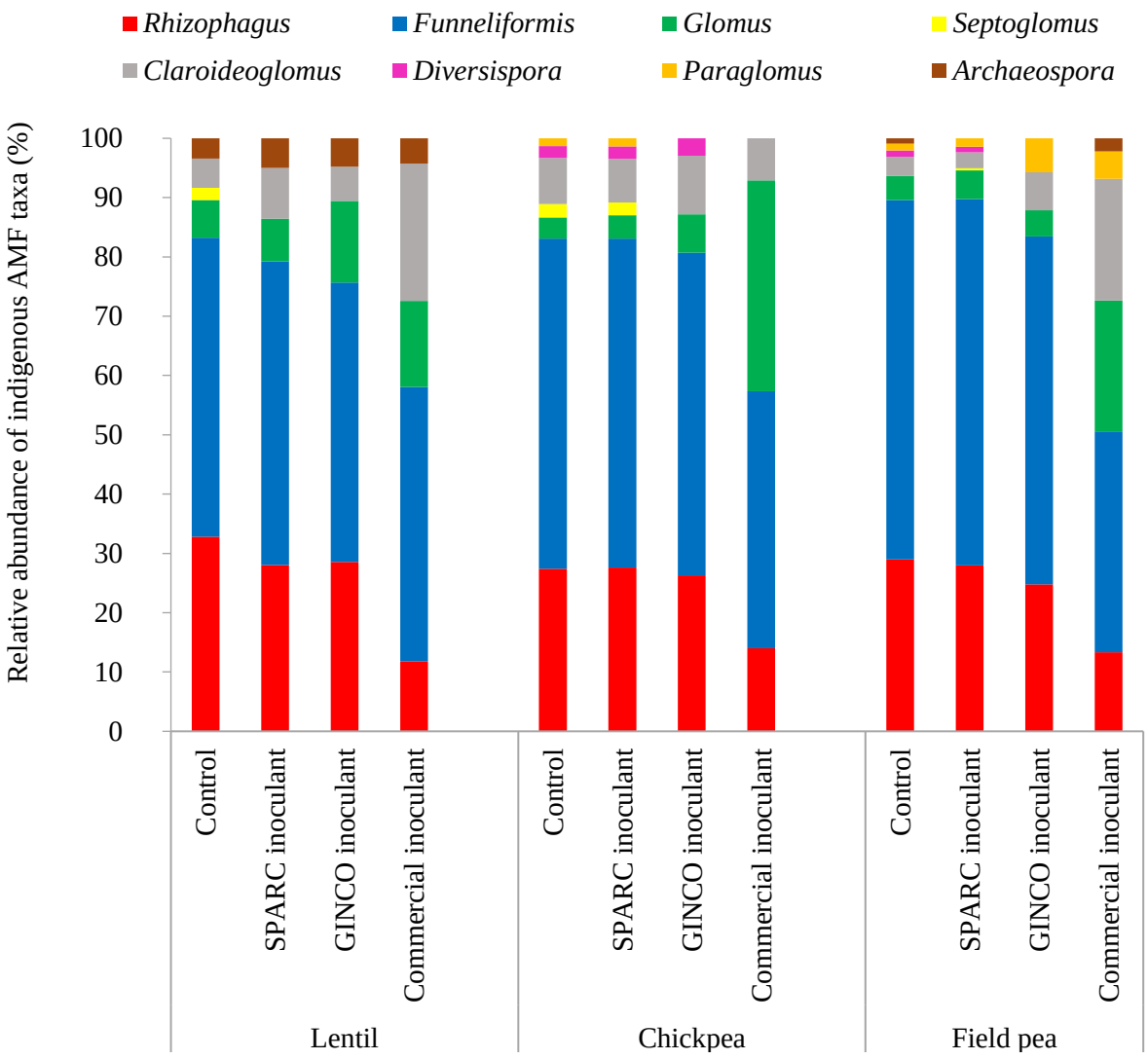


**Figure 4.2** Relative abundance of indigenous AMF genera, associated with the roots of lentil, chickpea and field pea, detected by pyrosequencing in response to AMF inoculation with SPARC inoculant (*F. mosseae* B04), GINCO inoculant (*F. mosseae* DAOM 221475) and commercial inoculant (*R. irregularis* 4514535).

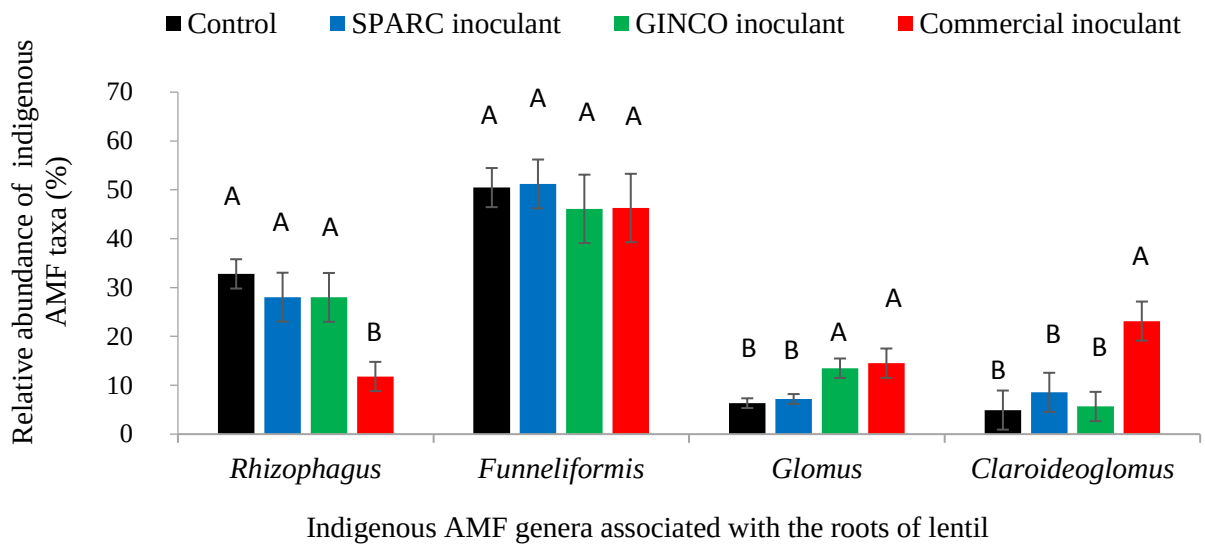


**Figure 4.3** The effect of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants on relative abundance of indigenous AMF genera *Rhizophagus*, *Funneliformis*, *Glomus* and *Claroideoglomus*, associated with the roots of lentil. Each value is a mean of four replicates (±SE). Different letters in each AMF taxa are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

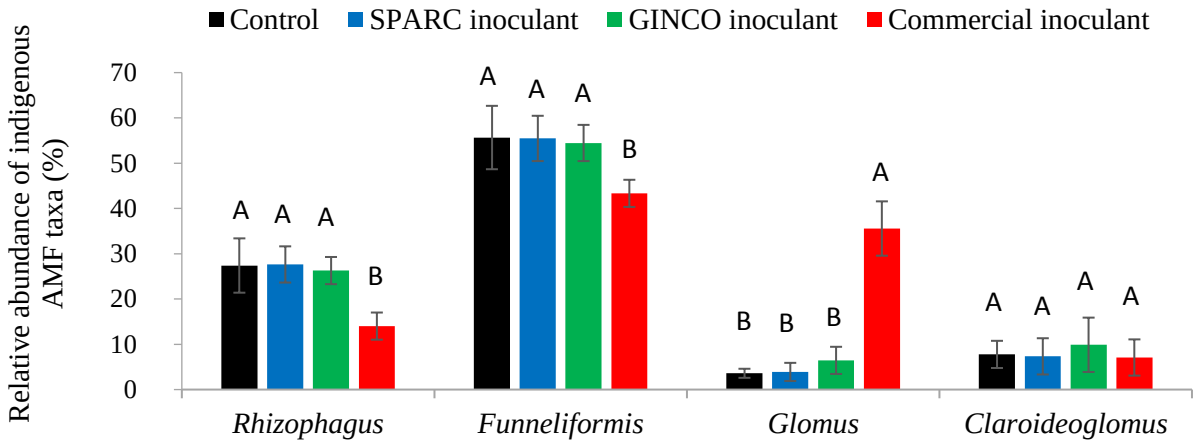


**Figure 4.4** The effect of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants on relative abundance of indigenous AMF genera *Rhizophagus*, *Funneliformis*, *Glomus* and *Claroideoglomus*, associated with the roots of chickpea, detected by pyrosequencing. Each value is a mean of four replicates (±SE). Different letters in each AMF taxa are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

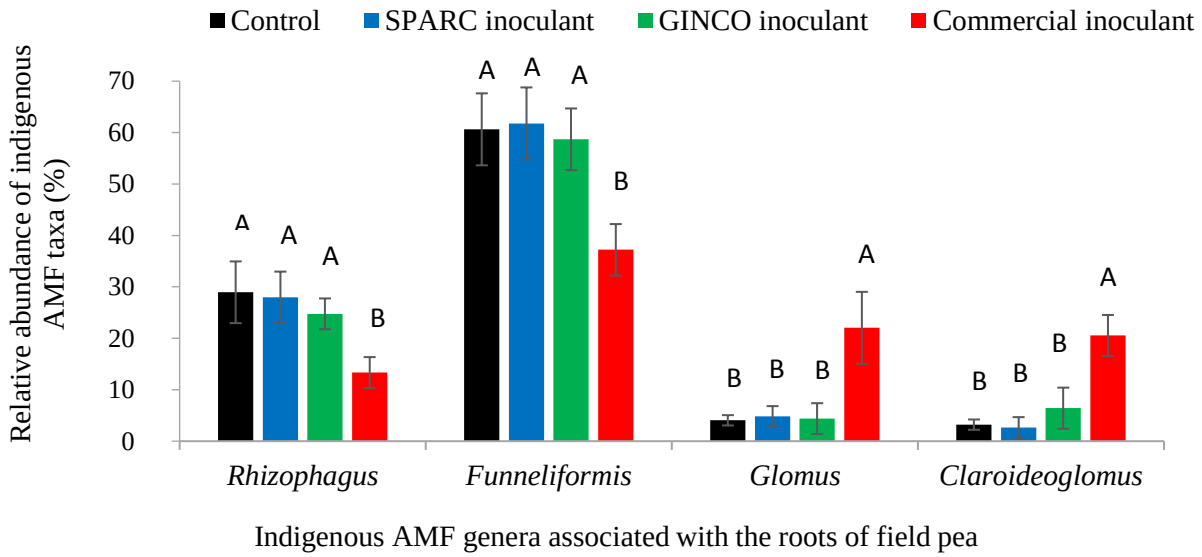


**Figure 4.5** The effect of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants on relative abundance of indigenous AMF genera *Rhizophagus*, *Funneliformis*, *Glomus* and *Claroideoglomus*, associated with the roots of field pea. Each value is a mean of four replicates (±SE). Different letters in each AMF taxa are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

The direction of the shift of AMF communities was variable depending on inoculants according to non-metric multi-dimensional scaling (NMDS) (Fig. 4.6). Each crop had different AMF community structures and the influence of inoculants on the structure of AMF was variable. The ordination scaling showed that the commercial inoculant had greater impact on the the association of AMF taxa in crop roots compared to controls. The lentil roots did not contain *Diversispora* and *Paraglomus*, while *Archaeospora* was absent in chickpea roots (Figs. 4.2 and 4.6). SPARC inoculant (*F. mosseae* B04) and GINCO inoculant (*F. mosseae* DAOM 221475) completely displaced or suppressed *Archaeospora* from field pea roots while the abundance of *Archaeospora* was enhanced in response to SPARC and GINCO inoculants in lentil roots. *Paraglomus* was also completely displaced or suppressed from the roots of chickpea in the presence of GINCO inoculant and commercial inoculant, while SPARC inoculant did not affect *Paraglomus* abundance compared to the control (Fig. 4.2). The indigenous *Rhizophagus* and *Funneliformis* abundance were dominant in SPARC and GINCO inoculated chickpea and field

pea roots. Bi-plot relationship between crops, inoculants and growth variables showed that biomass accumulation and N and P uptake were higher in response to SPARC and GINCO inoculants in both chickpea and field pea (Fig. 4.6).

The Shannon diversity index (*H'*) is commonly used to characterize species diversity in a community. The influence of inoculation ($P \leq 0.01$) and crop ($P \leq 0.05$) on Shannon (*H'*) diversity index of indigenous AMF community was significant but the impact of interaction on the Shannon (*H'*) diversity was non-significant according to two-way ANOVA (Table 4.2). Inoculation with GINCO, SPARC and commercial inoculants significantly ($P \leq 0.05$) reduced Shannon diversity of indigenous AMF community in three pulse crops except in chickpea where the diversity index was unaffected by SPARC inoculant (Fig. 4.7). The inoculation with commercial inoculant reduced indigenous AMF diversity by 16%, 23% and 31% compared to the control in lentil, chickpea and field pea, respectively. The non-commercial SPARC and GINCO inoculants reduced diversity by 26% and 28% in lentil, respectively and by 11%, 12% in field pea, respectively. A 25% reduction in diversity in chickpea was observed due to inoculation with GINCO inoculant while no reduction occurred in response to SPARC inoculant (Fig. 4.7).

Root occupancy was measured in terms of relative abundance of the 18S rRNA gene of inoculants using 454 pyrosequencing technology. The number of sequence reads of the respective OTUs generated from each of the inoculants was quantified separately. OTU27, OTU37 and OTU51 from SPARC inoculant (*F. mosseae* B04), OTU47 and OTU54 from GINCO inoculant (*F. mosseae* DAOM 221475) and OTU6, OTU7, OTU12 and OTU35 from commercial inoculant (*R. irregularis* 4514535) were identified (Fig. 4.1 and Table B.4.2). The relative abundance of commercial inoculant (*R. irregularis* 4514535) was significantly ($P \leq 0.05$) higher in all three crops compared to the other two inoculants. In chickpea and field pea, differences between the relative abundance of SPARC (*F. mosseae* B04) and GINCO (*F. mosseae* DAOM 221475) were insignificant (Fig. 4.8).

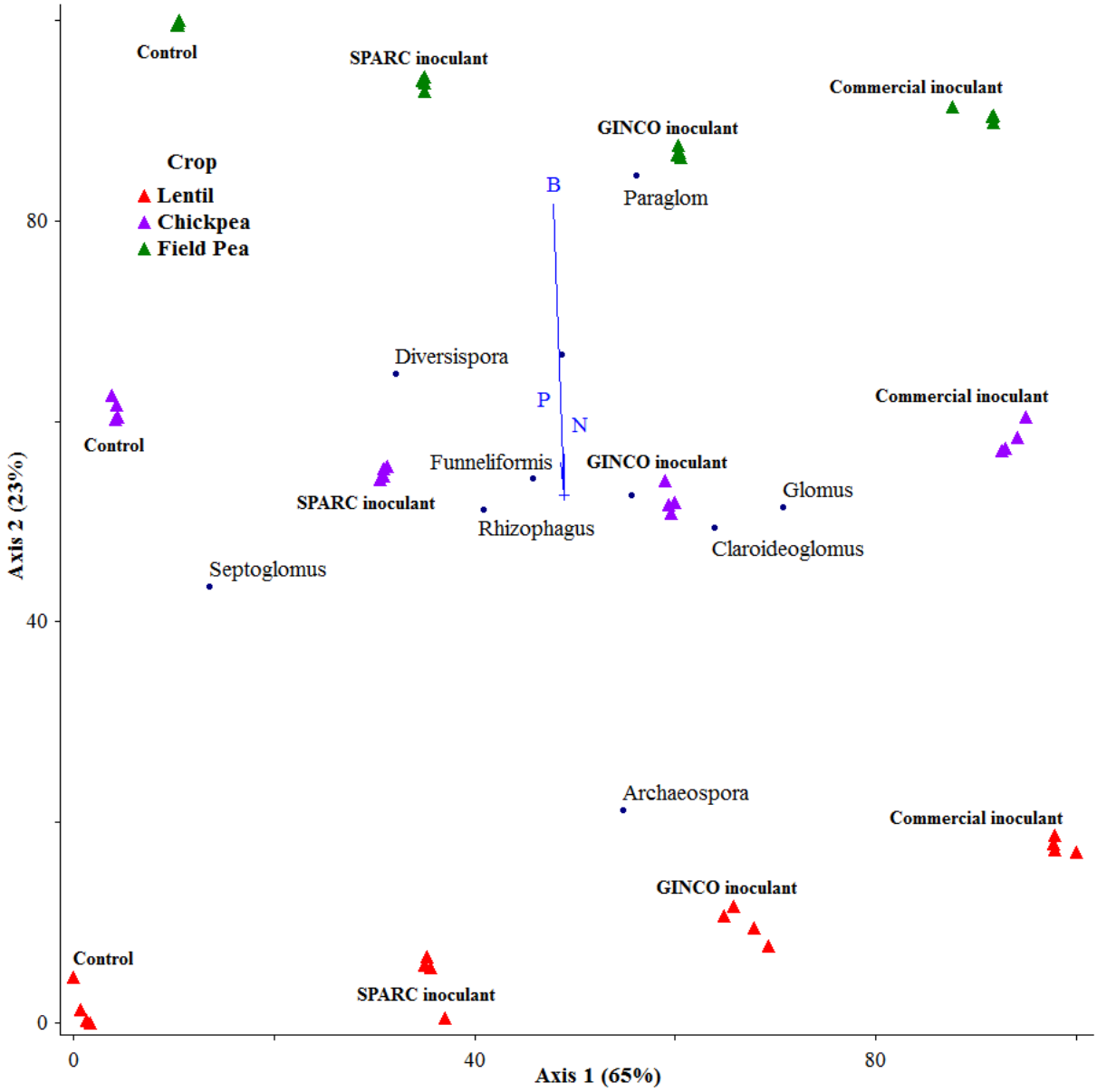


**Figure 4.6** Non-metric multi-dimensional scaling (NMDS) ordination between the relative abundance of indigenous AMF taxa, three different AMF inoculant strains and plant growth parameters of pot-cultured lentil, chickpea and field pea. The percentages between parentheses represents the contribution of each axis to the ordination solution (based on Bray-Curtis distance matrix). Nitrogen (N), phosphorus (P) uptake and biomass (B) accumulation correspond with the bi-plot blue lines within ordination graph. According to multi-response permutation process (MRPP), Crops: $P = 0.027$, A = 0.054, Inoculants: $P = 0.032$, A = 0.063. Final stress for 2-dimentional solution = 8.47. Inoculant strains are SPARC (*F. mosseae* B04) GINCO (*F. mosseae* DAOM 221475) and commercial inoculant (*R. irregularis* 4514535).

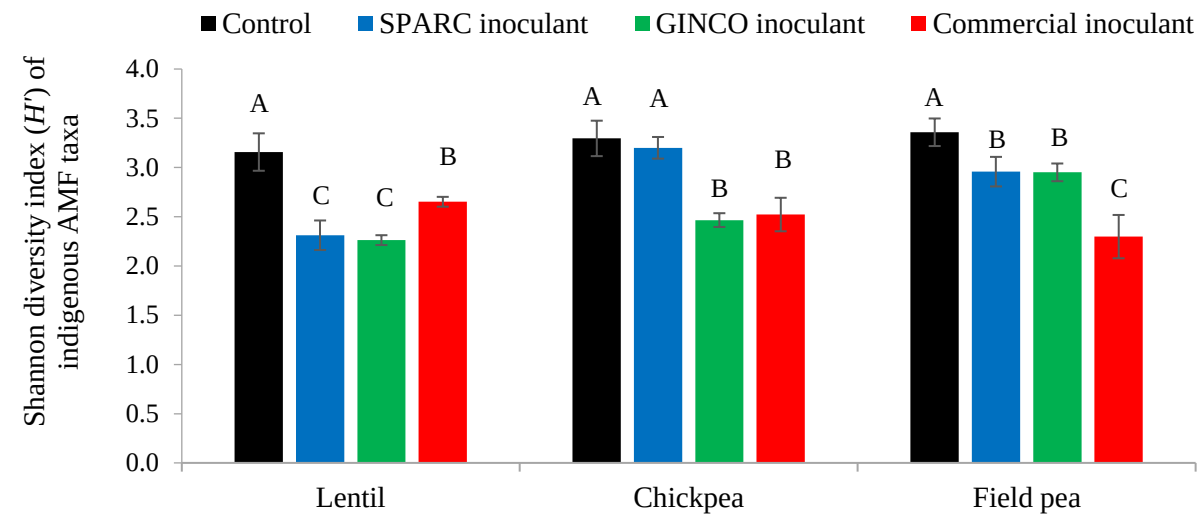


**Figure 4.7** The effect of inoculation with SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants on Shannon diversity index (*H'*) of AMF communities with the roots of lentil, chickpea and field pea, detected by pyrosequencing. Each value is a mean of four replicates ±SE. Values followed by different letters in plant are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

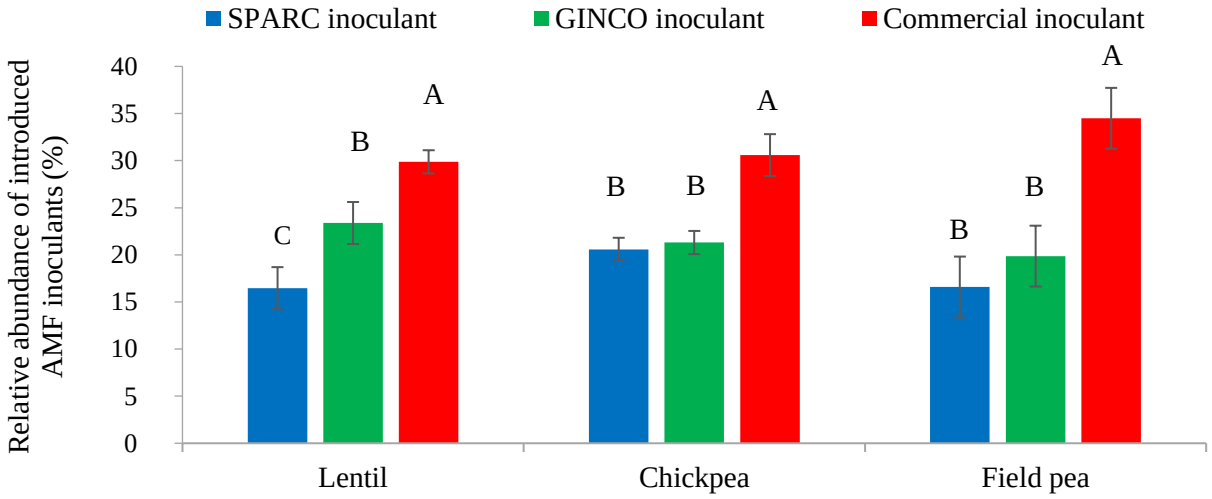


**Figure 4.8** Root occupancy (relative abundance) of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants associated with the roots of lentil, chickpea and field pea, detected by pyrosequencing. Each value is a mean of four replicates ±SE. Values followed by different letters in plant are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

The root occupancy (relative abundance) of the commercial inoculant (*R. irregularis* 4514535) (OTU6, OTU7, OTU12 and OTU35) was significantly higher in inoculated treatments, accounting for 29%, 31% and 34% of relative abundance in the roots of lentil, chickpea and field pea, respectively (Fig. 4.8). The relative abundance of SPARC and GINCO did not reach levels achieved by the commercial inoculant. Specifically, the abundance of GINCO inoculant (OTU47 and OTU54) accounted for 23% abundance in lentil, 21% in chickpea and 20% in field pea whereas SPARC inoculant (OTU27. OTU37 and OTU51) accounted for 16% in lentil, 20% in chickpea and, 17% in field pea (Fig. 4.8).

### 4.5.4 Shoot N and P uptake, and dry biomass accumulation in lentil, chickpea and field pea in response to inoculants

The main effect of inoculation and crops on growth performances was determined using a two-way ANOVA. Inoculation significantly influenced N uptake ($P \leq 0.01$), P uptake ($P \leq 0.01$) and shoot biomass ($P \leq 0.001$) in the three crops (Table 4.2). Similarly, crops also had significant impact on P uptake ($P \leq 0.01$) and biomass ($P \leq 0.01$) accumulation, but the impact of crop on N uptake was non-significant.

Inoculation with SPARC inoculant resulted in a significant ($P \leq 0.05$) increase of N uptake in lentil, chickpea and field pea compared to the uninoculated control (Fig. 4.9). The influence of GINCO inoculant on N uptake was found to be inconsistent among crop types, with only chickpea N uptake significantly ($P \leq 0.05$) increasing (1.5 times higher) compared to the control (Fig. 4.9). Inoculation with SPARC inoculant significantly ($P \leq 0.05$) increased P uptake by 1.4, 1.3 and 1.2 times in lentil, chickpea and field pea, relative to the control, respectively. A significant increase in P uptake also was observed in chickpea in response to GINCO inoculant (Fig. 4.10). A consistent positive impact of SPARC inoculant on biomass production was observed with increases of 1.2 times higher than the control in both lentil and chickpea, and 1.1 times higher in field pea. Neither GINCO nor commercial inoculant had a positive influence on biomass accumulation in any of the crops (Fig. 4.11).

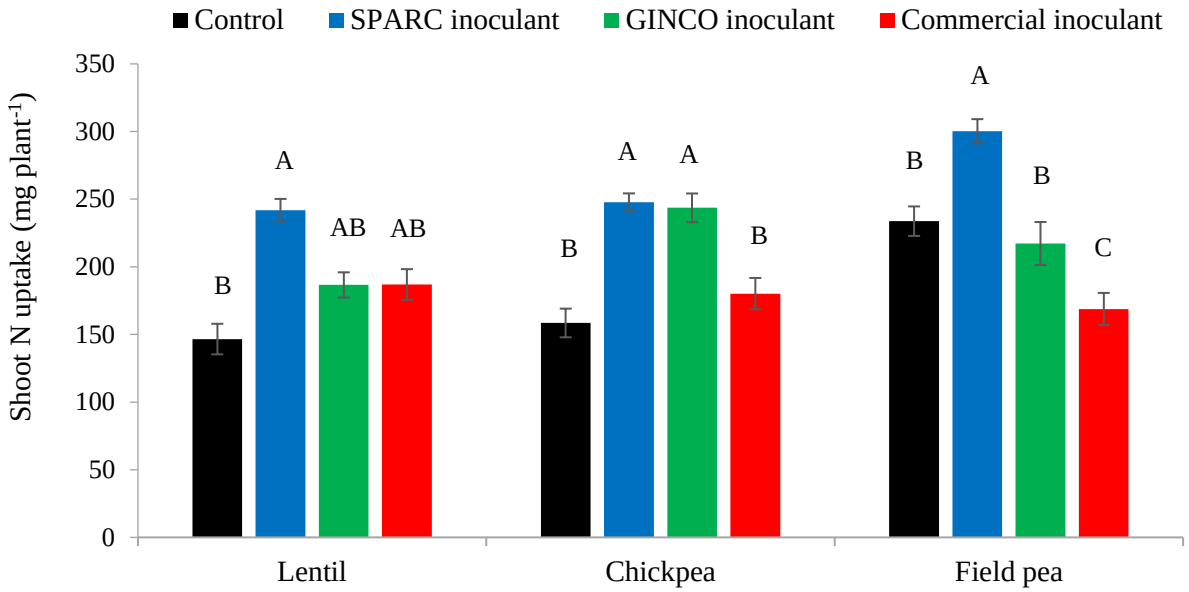


**Figure 4.9** The effect of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) on shoot nitrogen (N) uptake in lentil, chickpea and field pea. Each value is a mean of four replicates ±SE. Values followed by different letters in plant are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

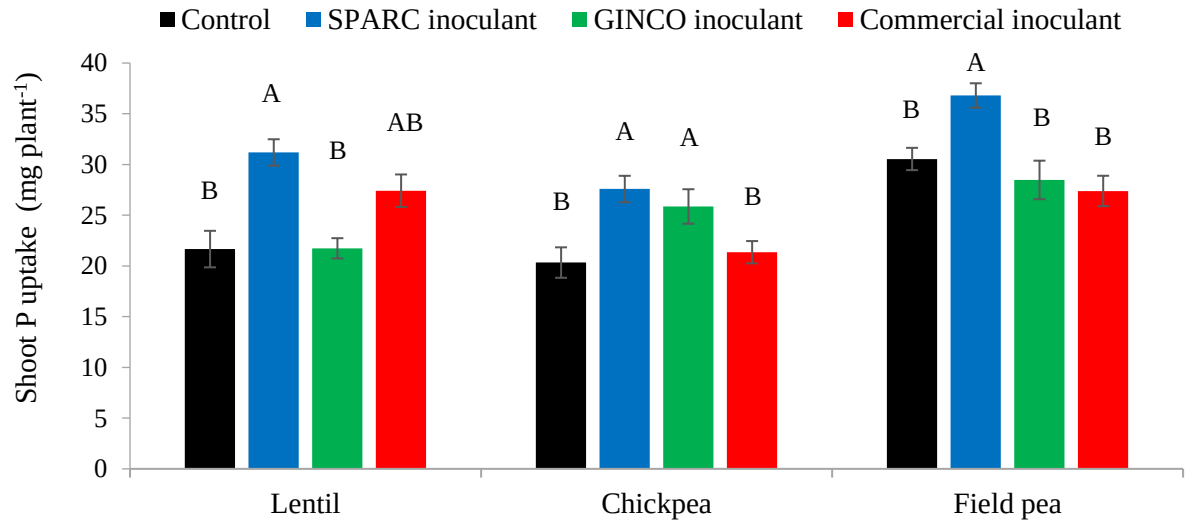


**Figure 4.10** The effect of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) on shoot phosphorus (P) uptake (mg plant$^{-1}$) in lentil, chickpea and field pea. Each value is a mean of four replicates ±SE. Values followed by different letters in plant are significantly different according to Tukey's test of multiple comparisons, $P \leq 0.05$.

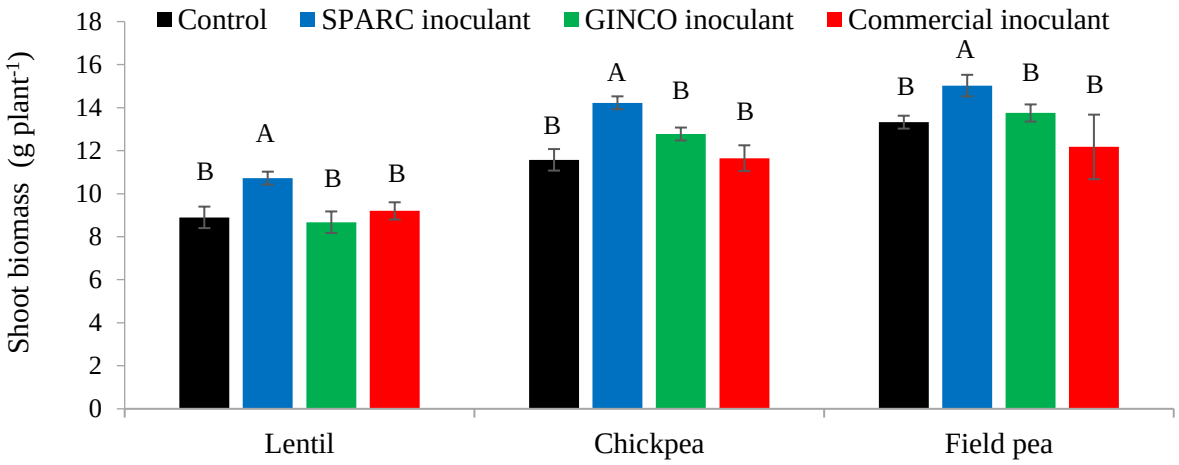


**Figure 4.11** The effect of SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) on shoot biomass uptake (g plant$^{-1}$) in lentil, chickpea and field pea. Each value is a mean of four replicates ±SE. Values followed by different letters in plant are significantly different according to Tukey's test of multiple comparisons, P≤0.05.

### 4.5.5 Relationship between the relative abundance of three inoculants, indigenous AMF taxa and plant growth performances

When three inoculant treatments were combined, significant negative correlations were detected between the relative abundance of the three introduced inoculants (root occupancy by introduced strains) and N uptake ($r$ = - 0.48, $P$ = 0.0005), P uptake ($r$ = - 0.34, $P$ = 0.0181), and biomass yield ($r$ = - 0.36, $P$ = 0.0119) in inoculated pulse crops (Fig. 4.12). There were significant positive correlations detected between the relative abundance of indigenous *Rhizophagus* and N uptake ($r$ = 0.26, $P$ = 0.0371) and biomass ($r$ = 0.29, $P$ = 0.0510). No significant correlations were detected between the relative abundance of indigenous *Rhizophagus* and P uptake ($r$ = 0.216, $P$ = 0.0698) (Fig. 4.13). The relative abundance of indigenous *Funneliformis* also showed significant positive correlations with N uptake ($r$ = 0.46, $P$ = 0.0010), P uptake ($r$ = 0.31, $P$ = 0.0320) and biomass yield ($r$ = 0.57, $P$ = 0.00007) (Fig. 4.14). In contrast, significant negative correlations were detected between indigenous *Glomus* and N uptake ($r$ = - 0.29, $P$ = 0.0455), and P uptake ($r$ = - 0.23, $P$ = 0.0070), and biomass yield ($r$ = - 0.313, $P$ = 0.0320) (Fig. 4.15). There were also significant negative correlations observed between the abundance of indigenous *Claroideoglomus* and N uptake ($r$ = - 0.26, $P$ = 0.0361) and biomass yield ($r$ = - 0.27, $P$ = 0.0317). There was no significant correlation between

*Claroideoglomus* and P uptake ($r$ = - 0.07, $P$ = 0.3181) (Fig. 4.16). There was significant positive correlation between the Shannon diversity index of altered indigenous AMF communities and shoot biomass accumulation ($r$ = 0.37, $P$ = 0.0110) (Fig. 4.17).

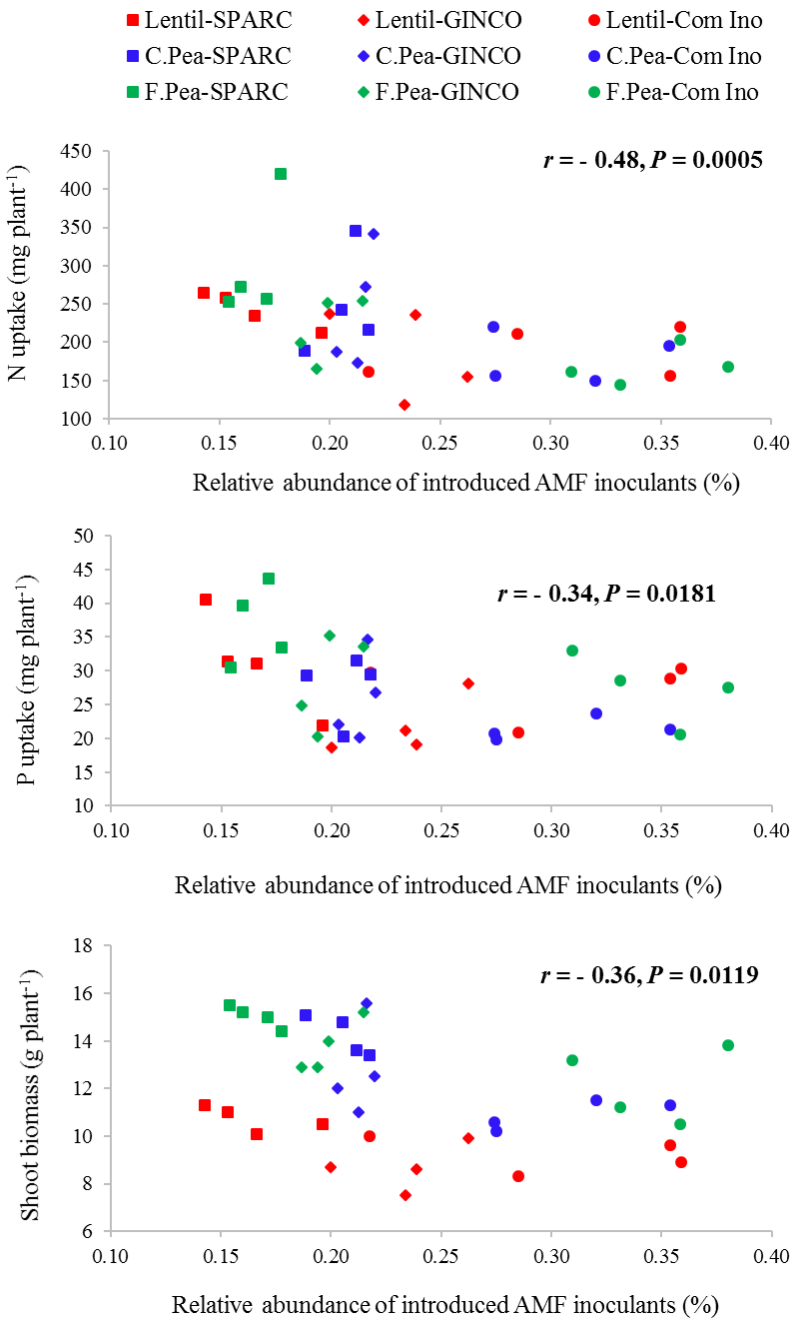


**Figure 4.12** Pearson correlation coefficient ($r$) between root occupancy (relative abundance) of three AMF inoculants in roots and N uptake (mg plant$^{-1}$), P uptake (mg plant$^{-1}$) and biomass (g plant$^{-1}$) accumulation in shoot of lentil, chickpea and field pea.

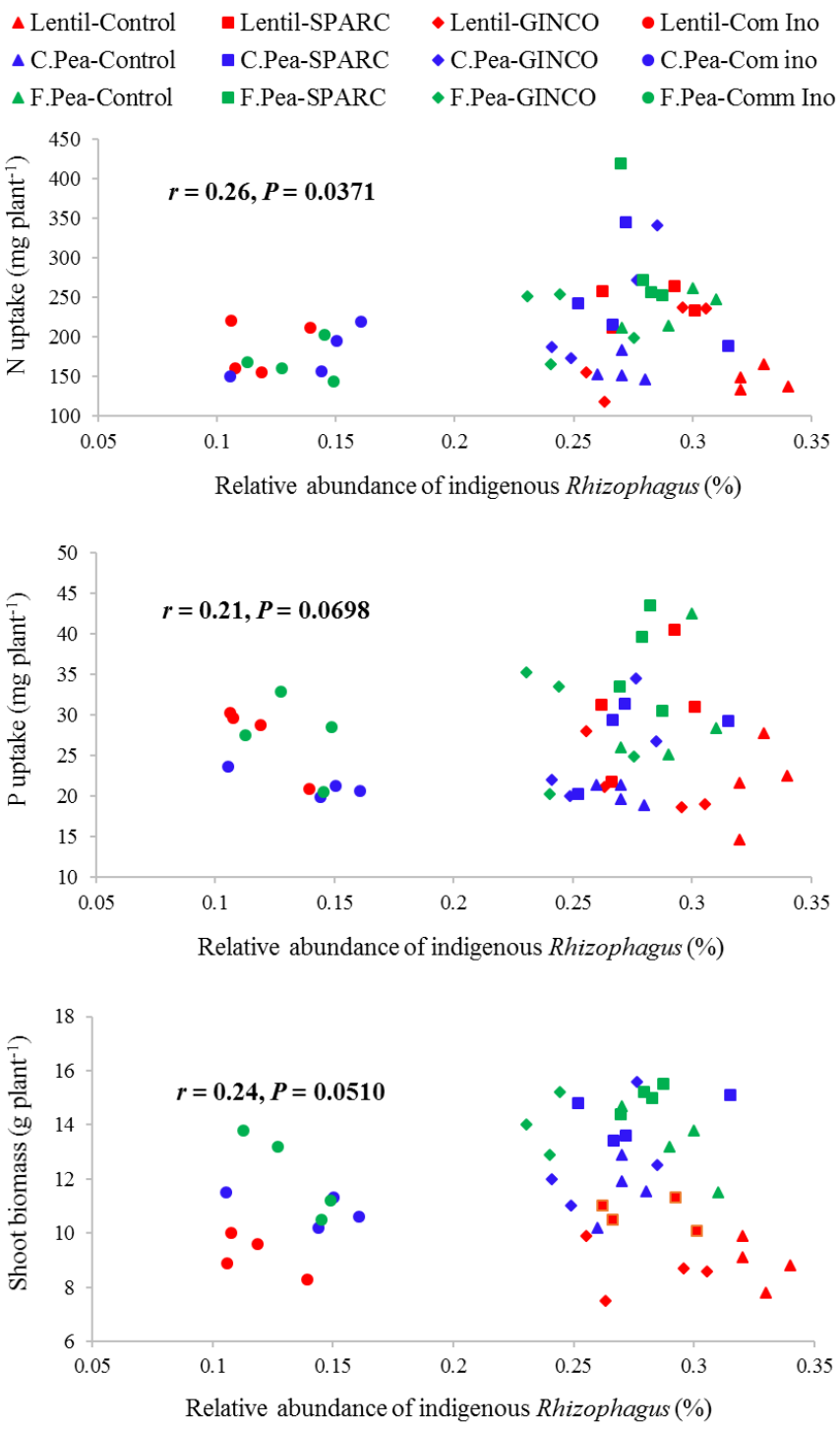


**Figure 4.13** Pearson correlation coefficient (*r*) between root occupancy (relative abundance) of indigenous *Rhizophagus* and shoot N uptake (mg plant$^{-1}$), shoot P uptake (mg plant$^{-1}$) and shoot biomass (g plant$^{-1}$) in lentil, chickpea and field pea in response to inoculation.

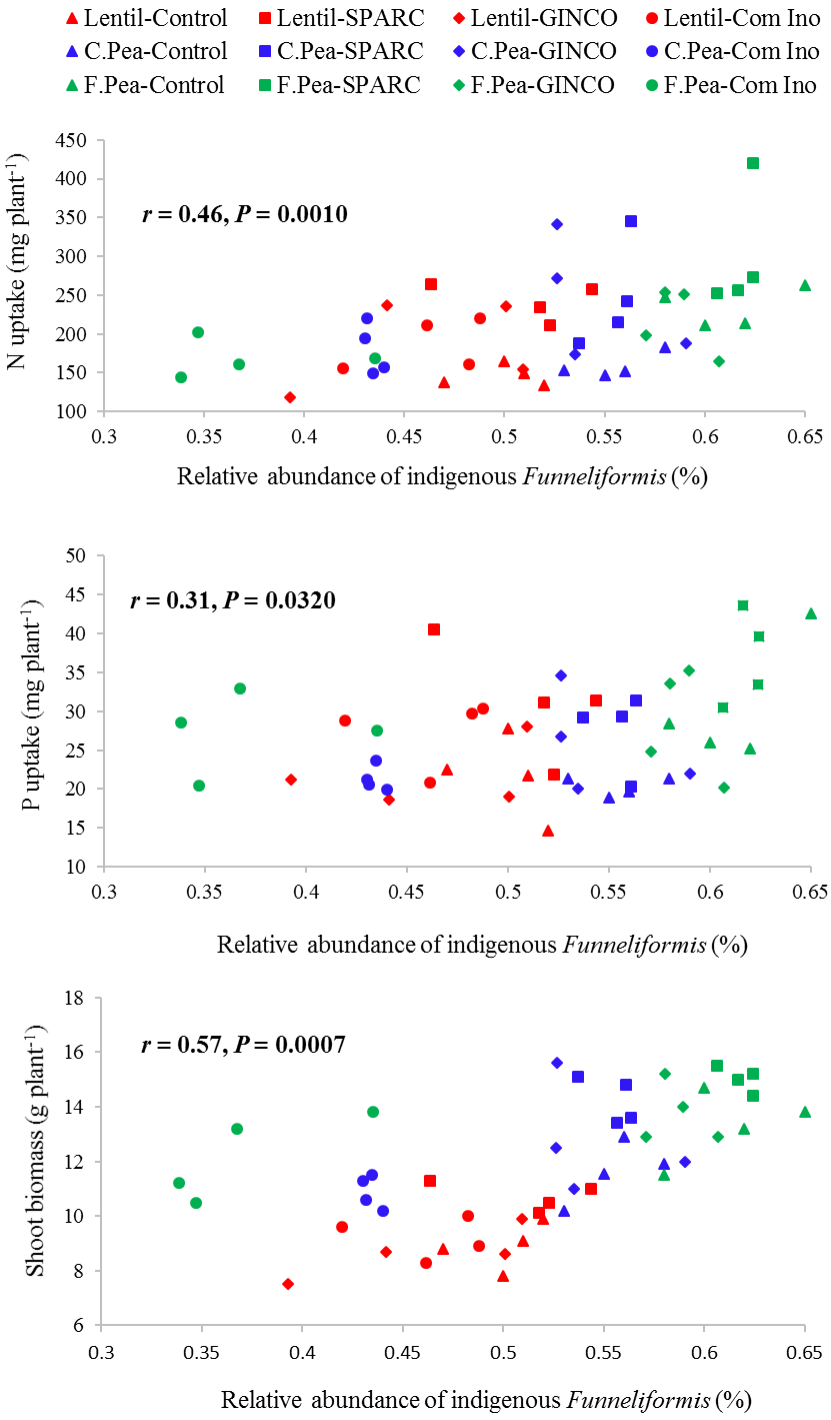


**Figure 4.14** Pearson correlation coefficient (*r*) between root occupancy (relative abundance) of indigenous *Funneliformis* and shoot N uptake (mg plant$^{-1}$), shoot P uptake (mg plant$^{-1}$) and shoot biomass (g plant$^{-1}$) in lentil, chickpea and field pea in response to inoculation.

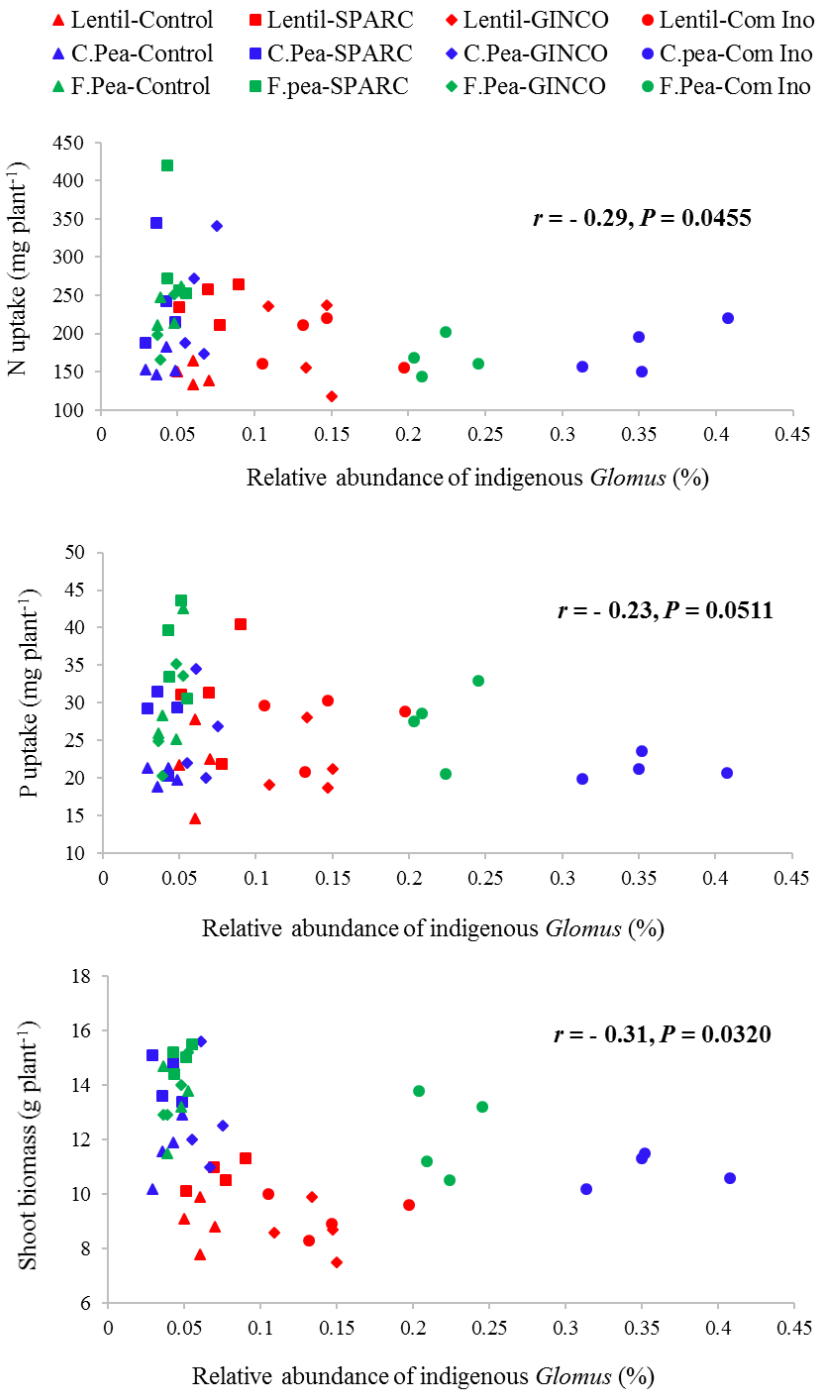


**Figure 4.15** Pearson correlation coefficient (*r*) between root occupancy (relative abundance) of indigenous *Glomus* and shoot N uptake (mg plant$^{-1}$), shoot P uptake (mg plant$^{-1}$) and shoot biomass (g plant$^{-1}$) in lentil, chickpea and field pea in response to inoculation.

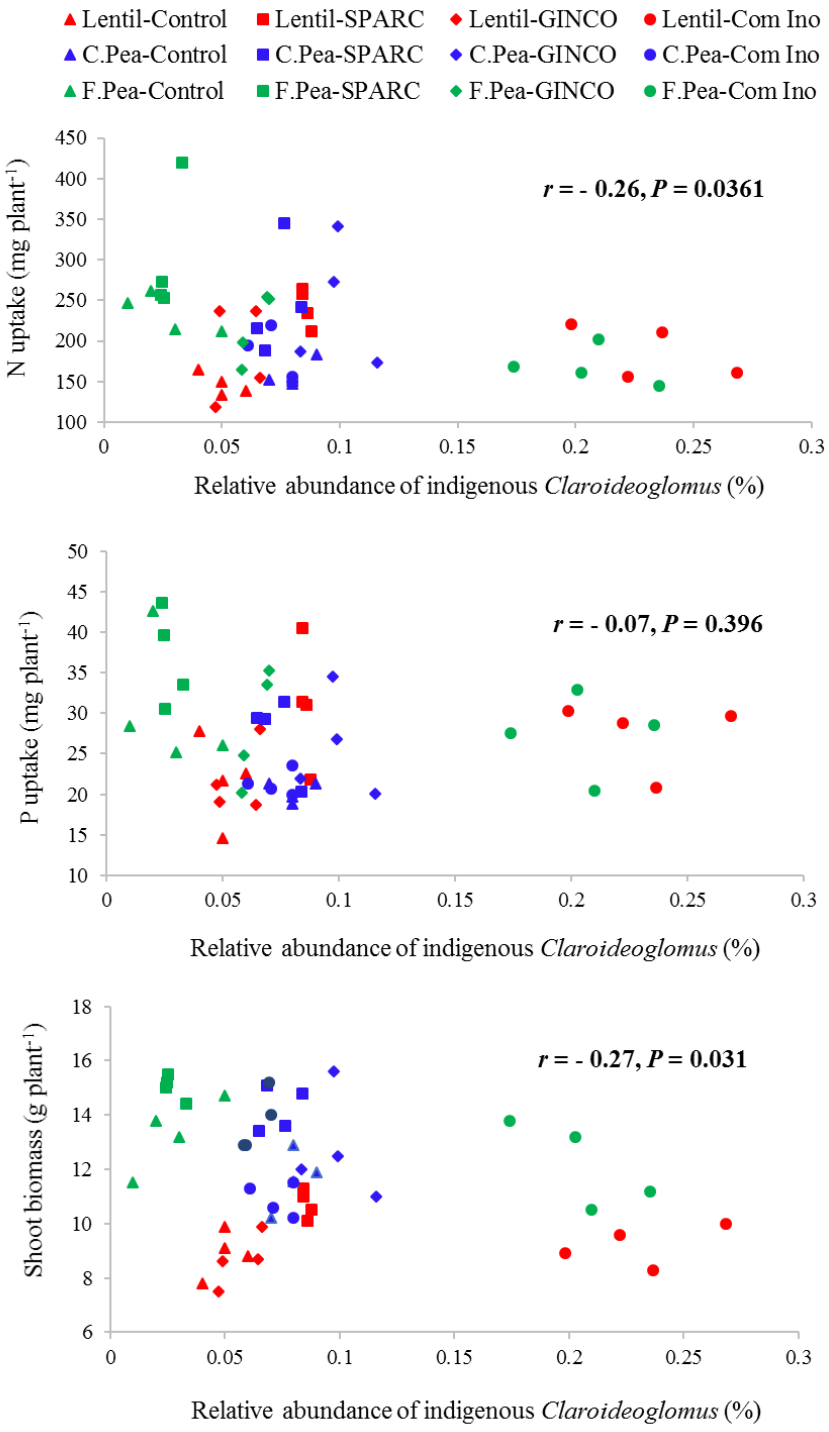


**Figure 4.16** Pearson correlation coefficient (*r*) between root occupancy (relative abundance) of indigenous *Claroideoglomus* and shoot N uptake (mg plant$^{-1}$), shoot P uptake (mg plant$^{-1}$) and shoot biomass (g plant$^{-1}$) in lentil, chickpea and field pea in response to inoculation.

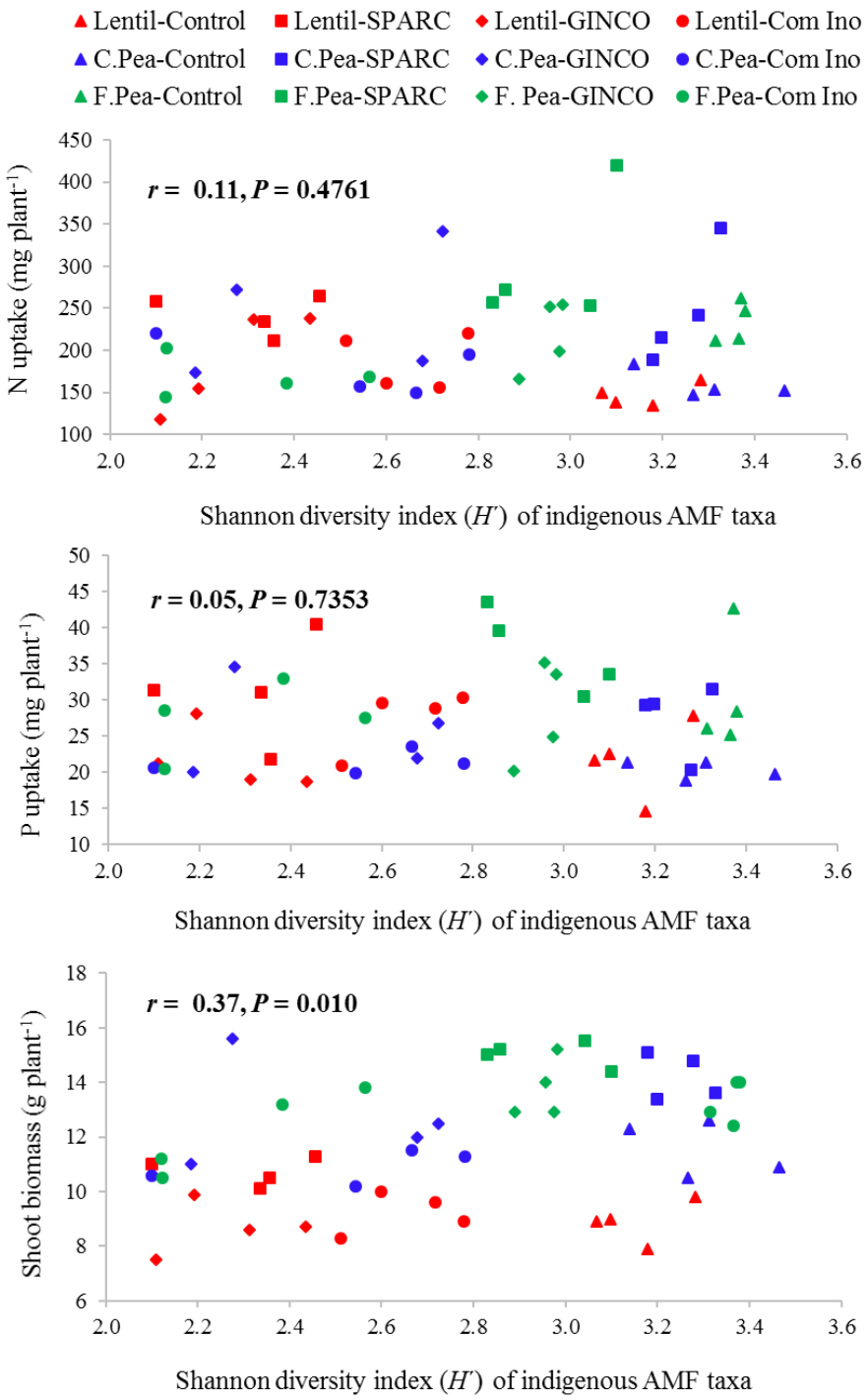


**Figure 4.17** Pearson correlation coefficient (*r)* between Shannon diversity index (*H'*) of AMF taxa **A.** shoot N uptake (mg plant$^{-1}$), **B.** shoot P uptake (mg plant$^{-1}$) and **C.** shoot biomass (g plant$^{-1}$) in lentil, chickpea and field pea in response to inoculation.

## 4.6 Discussion

This study assessed the impact of commercial and locally isolated AMF inoculants on shoot nutrient (N and P) uptake and biomass accumulation and the structure of indigenous AMF communities in pot-cultured roots of lentil, chickpea and field pea. Others have reported that commercial non-indigenous AMF strains can quickly colonize roots compared to many other AMF indigenous isolates (Jansa et al., 2008). Thus, it was expected that the indigenous AMF communities would be altered significantly. Additionally, the field incubation study (see Chapter 3) revealed different levels by which the indigenous AMF community composition was altered by AMF inoculation. Specifically, AMF inoculation reduced AMF diversity in field pea roots. Here, the impact of three different AMF inoculants of different origins on the composition and diversity of indigenous AMF communities was assessed and observed alterations in the AMF community were related to the subsequent growth performance of three pulse crops.

This study focused on determining whether the growth response to introduced AMF inoculation was directly related to root occupancy by the introduced taxa or if growth responses were indirectly related to the subsequent alteration in the existing indigenous AMF community assemblages in the crop roots. Alteration in indigenous AMF communities as a consequence of inoculation could alter plant growth. A recent study was able to identify the OTU of the introduced commercial inoculant, *Glomus irregulare* (currently named *R. irregularis*) from indigenous *Glomus* communities using pyrosequencing technology and estimated the alteration of indigenous AMF in response to inoculation in chickpea and field pea (Jin et al., 2013b). The challenge of separating the introduced from the indigenous strain has long been a microbial inoculant research issue, especially for AMF.

The AMF symbiosis is primarily involved in enhancing soil nutrient uptake for plant growth and productivity; however, there is a trade-off and cost for the AMF symbiosis. Both negative and positive contributions of indigenous and non-indigenous AMF taxa to nutrient uptake, biomass, yield and productivity responses have been reported (Wilson and Hartnett, 1998; Dai et al., 2014; Koziol et al., 2015). The present results showed that plant nutrient uptake could be attributed either to the direct effect of the introduced inoculant or due to the indirect effects on the resident AMF community. Crop growth parameters were positively correlated with

the root occupancy (relative abundance) of some indigenous AMF taxa (particularly, *Rhizophagus* and *Funneliformis*) that significantly shifted in response to inoculation. Significant negative correlations were also detected between some indigenous AMF taxa (*Glomus* and *Claroideoglomus*) and growth parameters. It was observed that root occupancy by inoculant strains and consequent growth parameters responses were frequently negatively correlated. This result is consistent with the results of several studies that similarly reported negative correlations between total mycorrhizal root colonization and plant growth variables both in field and controlled experiments (Wilson and Hartnett, 1998; Veiga et al., 2011, 2013; Dai et al., 2014).

### 4.6.1 Molecular phylogenetic discrimination between introduced AMF inoculants, from indigenous AMF taxa, and quantifying inoculation success rate in crops

The AMF inoculants of different origin used in this study (Table 4.1) successfully established in the roots of all test crops (i.e., lentil, chickpea and field pea). The pyrosequencing of AMF assemblages in eight-week-old plant roots revealed that the commercial inoculant (*R. irregularis* 4514535) was present in the roots of all three test crops with relative abundance varying from 26% to 36%. The other two inoculants, SPARC (*F. mosseae* B04) and GINCO (*F. mosseae* DAOM 221475) were also able to occupy crop roots ranging from 12% to 20% and 16% to 30%, respectively (Fig. 4.8). The result suggests that the occupancy of roots by inoculants competing with the indigenous AMF communities depends on the inoculant taxa.

Root occupancy levels of 34% (relative abundance) were achieved by commercial inoculant in field pea roots whereas SPARC inoculant achieved relative abundance of 17% and GINCO inoculant achieved relative abundance of 20%. The higher root occupancy by commercial inoculant than other two inoculants, SPARC and GINCO was perhaps due to the fast root colonizing nature of *Rhizophagus* (Jansa et al., 2003; Jansa et al., 2008). Moreover, the indigenous *Funneliformis* taxa were abundantly present in the pot soils compared to indigenous *Rhizophagus* taxa (Fig. 4.2). Others have argued that the absence of a AMF taxa similar to inoculant in a soil is often associated with greater inoculation success because more unoccupied niches are available (Verbruggen et al., 2013).

The success of AMF inoculation is affected by soil type (Karasawa et al., 2001), resident AMF community (Requena et al., 2001), functional variability among isolates (Pellegrino et al., 2012) and host plant type (Antunes et al., 2009). Recent studies reported that detection and quantification of introduced AMF inoculants is challenging if a group of fungi similar to the inoculant strain already exists in the soils and roots (Antunes et al., 2009; Koch et al., 2011; Pellegrino et al., 2012; Sýkorová et al., 2012). Some difficulties remain in AMF identification due to the genetically complex nature of genetic haplotypes and polymorphic variants within colonized roots (Börstler et al., 2008; Croll et al., 2009; Beaudet et al., 2014). However, the 454 pyrosequencing was able to discriminate between OTUs of the indigenous *F. mosseae* and *R. irregularis* from the OTUs generated from introduced SPARC (*F. mosseae* B04), GINCO (*F. mosseae* DAOM 221475) and commercial (*R. irregularis* 4514535) inoculants. For example, OTU6, OTU7, OTU12 and OTU35 were not found in the control roots but were abundant in the roots where commercial inoculant (*R. irregularis* 4514535) was added (Table. B.4.2). Concurrently, DNA from *R.* commercial inoculant spores were pyrosequenced and the resulting OTU (marked as "*Rhizophagus* inoculant" in the phylogenetic tree, Fig. 4.1) was run through neighbour–joining phylogenetic tree analysis and the closest match was with GenBank reference *R. irregularis (*HF968850.1). This confirmed that OTU6, OTU7, OTU12 and OTU35 (98% to 99% similar with GenBank reference sequences: HF968850.1 of *R. irregularis*) were generated from the commercial inoculant strain (*R. irregularis* 4514535) rather than the indigenous *R. irregularis* where none of the above OTUs were found in control roots. Similarly, OTU27, OTU37 and OTU51 from SPARC inoculant (*F. mosseae* B04) and OTU47 and OTU54 from GINCO inoculant (*F. mosseae* DAOM 221475) were the closest matches with the OTUs marked in phylogenetic tree as SPARC *Funneliformis* B04 inoculant and GINCO *Funneliformis* 221475 inoculant, based on the pyrosequencing results from the respective inoculant spores (Fig. 4.1).

Several studies have demonstrated that *R. irregularis* could evolve into several new progenies by anastomosing with genetically different populations of the same *R. irregularis* species population, which could then affect plant growth differently compared to those of the parent population (Croll et al., 2009; Angelard and Sanders, 2011; Colard et al., 2011). The present pyrosequencing data revealed that eight OTUs recovered from inoculated and uninoculated roots clustered with *Rhizophagus* genera. Consequently, there was a clear

indication that different OTUs were generated due to the interaction between inoculant strains and test crops. For instance, OTU6 and OTU12 were absent in the inoculated roots of chickpea and field pea, respectively, but lentil inoculated roots harbored all four OTUs (OTU6, OTU7, OTU12 and OTU35) (Table B.4.1). The distinct and different association of particular OTUs might be linked with the genetics of host-AMF symbiosis which regulate differential plant growth responses to inoculation (Angelard and Sanders, 2011; Colard et al., 2011).

### 4.6.2 Indigenous AMF community composition affected by inoculation

Significant alterations in the indigenous AMF taxa in pulse roots occurred where the relative abundance of the introduced inoculant was high (Fig. 4.8). The main effect of inoculation on the relative abundance of indigenous AMF taxa was significant, except *Archaeospora* (Table 4.2). The Tukey's test of significance showed that the commercial inoculant (*R. irregularis* 4514535) had a significant ($P \leq 0.05$) impact on different indigenous AMF taxa than the SPARC inoculant (*F. mosseae* B04), although GINCO inoculant (*F. mosseae* DAOM 221475) showed marginal impact on indigenous AMF community (Figs. 4.3 to 4.5). No significant changes in indigenous AMF taxa of all three crop roots in response to SPARC inoculant were observed (Table 4.3). This is probably due to lower occurrence of non-commercial inoculant, SPARC *F. mosseae* B04 in the roots compared to the commercial inoculant (Fig. 4.8). The inoculant that was locally isolated or indigenous to a particular target soil or site is often reported to be a more effective mutualists than non-indigenous AMF, apparently as a result of adaptation to edaphic factors such as soil nutrient concentrations, or to environmental factors (Stahl et al., 1988; Vosatka, 2002; Johnson et al., 2010; De Oliveira and de Oliveira, 2010). One study verified the impact of a commercial inoculant *G. intraradices* in agricultural soils on the structure of indigenous AMF community (Antunes et al., 2009). They confirmed that with recommended application rates, the structure of indigenous communities in maize roots was unaffected; however, they did not detect and quantify any particular AMF taxa affected but rather examined the richness of total AMF community terminal-restricted fragments (T-RF).

**Table 4.3** The effect of introduced inoculants on relative abundance and diversity index of indigenous AMF taxa, shoot N and P uptake and biomass accumulation in lentil, chickpea and field pea. The results extracted from the Figs. 4.3 to 4.5, 4.7 and 4.9 to 4.11.

| | | Diversity index and relative abundance of indigenous AMF taxa | | | | | Growth performance of pulse crops | | |
|---|---|---|---|---|---|---|---|---|---|
| Crops | Inoculants[§] | Shannon diversity | *Rhizophagus* | *Funneliformis* | *Glomus* | *Claroideoglomus* | Shoot N uptake | Shoot P uptake | Shoot biomass accumulation |
| Lentil | SPARC inoculant | ↓* | NS | NS | NS | NS | ↑* | ↑* | ↑* |
| | GINCO inoculant | ↓* | NS | NS | ↑* | NS | NS | NS | NS |
| | Commercial inoculant | ↓* | ↓* | NS | ↑* | ↑* | NS | NS | NS |
| Chickpea | SPARC inoculant | NS | NS | NS | NS | NS | ↑* | ↑* | ↑* |
| | GINCO inoculant | ↓* | NS | NS | NS | NS | ↑* | ↑* | NS |
| | Commercial inoculant | ↓* | ↓* | ↓* | ↑* | NS | NS | NS | NS |
| Field pea | SPARC inoculant | ↓* | NS | NS | NS | NS | ↑* | ↑* | ↑* |
| | GINCO inoculant | ↓* | NS | NS | NS | NS | NS | NS | NS |
| | Commercial inoculant | ↓* | ↓* | ↓* | ↑* | ↑* | ↓* | NS | NS |

Tukey's test of multiple comparisons at $P \leq 0.05$. ↑*: significant increase, ↓*: significant decrease and NS: non-significant.

[§]Inoculants: 1) SPARC-*F. mosseae* B04, 2) GINCO-*F. mosseae* DAOM 221475 and 3) Commercial-*R. irregularis* 4514535.

In contrast, both greenhouse and field experiments have demonstrated that in the short term, indigenous AMF communities were disturbed due to the inoculation with some strains of *G. intraradices* (Douds et al., 2011; Koch et al., 2011). These findings are in agreement with the current study. A recent study compared a commercial AMF strain, *G. irregulare* (currently identified as *R. irregularis*) and mixed AMF inoculants of *G. irregulare, G. mosseae* (currently identified as *F. mosseae*) and *G. clarum* (all the strains used from GINCO reference archive) and showed that the commercial strain significantly changed the compositions of indigenous AMF communities in field pea while no significant compositional changes were observed in response to inoculation with a non-commercial mixture of GINCO AMF reference isolates (Jin et al., 2013a). In the current study, non-commercial, locally isolated SPARC inoculant did not significantly impact the indigenous AMF community in crops (Table 4.3 and Figs. 4.3 to 4.5). In addition, there are numerous reports where non-indigenous commercial AMF have outperformed indigenous fungi (Trent et al., 1993; Calvente et al., 2004). Whether indigenous AMF are more effective symbionts than non-indigenous AMF in a particular soil and host remains unclear.

Indigenous *Rhizophagus* was established in lentil, chickpea and field pea roots along with the introduced commercial inoculant and non-commercial SPARC and GINCO inoculants (Figs. 4.2 to 4.5). The relative abundance of indigenous *Rhizophagus* was significantly ($P \leq 0.05$) reduced in response to commercial inoculant by 22%, 13% and 15% compared to the control in the roots of lentil, chickpea and field pea, respectively. Similar significant ($P \leq 0.05$) reductions of indigenous *Funneliformis* sequence reads of 4%, 10% and 23% in response to commercial inoculant were observed in lentil, chickpea and field pea, respectively. In contrast, non-commercial SPARC and GINCO inoculants were associated with reductions of only 1% to 4%, compared to the abundance of indigenous *Funneliformis* in uninoculated control roots (Figs. 4.3 to 4.5). This finding indicates that the closest indigenous AMF taxa to the introduced commercial inoculant could be affected and hampered when trying to compete with non-indigenous strains for root occupancy. AMF fungal inoculants interact with the resident indigenous genotypes which are closely related, and genetically distinct AMF anastomose and exchange genetic information (Croll et al., 2009); thus, the indigenous community members might outcompete the introduced strains. This reasoning and possible explanation are consistent with the commercial inoculant (*R. irregularis* 4514535) but not the SPARC (*F. mosseae* B04) and GINCO (*F.*

*mosseae* DAOM 221475) inoculants where indigenous *Funneliformis* abundance in all three crop roots was almost unchanged (Fig. 4.2). Specially, *F. mosseae* is known as a cosmopolitan species (Avio et al., 2009) and is common and adapted to the environments throughout the cultivated Canadian Prairie soils (Dai et al., 2013). The mechanisms of competition among indigenous AMF taxa within a community following introduction of AMF inoculant taxa are not clear and need further exploration focusing on the nature of competition for root occupancy among indigenous and different types of inoculant isolates.

### 4.6.3 Indigenous AMF community-mediated crop growth performance

Indigenous AMF communities (Requena et al., 2001) and functional differences among isolates (Pellegrino et al., 2012) have been considered imperative to the link between nutrient uptake and plant growth. Some ecological studies revealed that AMF community members, particularly different species, clearly showed different induced effects on plant growth performance (Van der Heijden et al., 1998). Changes in the indigenous AMF communities could potentially alter plant growth, nutrient uptake and eventually the yield and yield attributes without varying levels of total mycorrhizal root occupancy in response to introduced AMF inoculants. There is no clear evidence whether AMF inoculation directly enhances nutrient uptake in improved plant growth and yield or if the introduced AMF causes an indirect effect by altering the indigenous residence AMF communities (Rodriguez and Sanders, 2015).

The present data clearly indicated that there was a negative correlation between relative abundance of the three inoculants (root occupancy by inoculant strains) and N uptake, P uptake or shoot biomass accumulation (Fig. 4.12). The present findings demonstrated that the root occupancy by SPARC and GINCO inoculants was significantly lower compared to the commercial inoculant in all three test crops (Fig. 4.8). This lower root occupancy (relative abundance) was negatively correlated with N uptake, P uptake and biomass accumulation in lentil, chickpea and field pea (Figs. 4.9 to 4.11). The results suggest that the higher relative abundance (root occupancy) of commercial inoculant was unlikely to contribute to increasing growth parameters of the test crops. This indicates the altered indigenous AMF communities following inoculation contributed to enhanced plant growth parameters.

Root occupancy (colonization) by indigenous AMF might have an effective and functional relationship with growth parameters. Inoculation shifted indigenous AMF taxa at differential levels which caused a significant positive correlation between the abundance of indigenous *Rhizophagus*, *Funneliformis*, the diversity index after inoculation, and N, P and biomass variables (Figs. 4.13 to 4.14 and 4.17). Alternately, a significantly negative correlation coefficient was detected between the relative abundance of indigenous *Glomus*, *Claroideoglomus* and shoot N uptake, P uptake and biomass variables (Figs. 4.15 to 4.16). Inoculation with the commercial inoculant significantly altered the composition of indigenous AMF communities in roots. Both the abundance of indigenous *Funneliformis, Rhizophagus* and the diversity indices of indigenous AMF communities significantly declined following inoculation with commercial inoculant in all three crops (Table 4.3). The commercial inoculant probably disturbed nutrient uptake in crop plants by the alteration of indigenous AMF taxa. In contrast, the indigenous AMF communities (in particular, *Funneliformis* and *Rhizophagus*) were almost unaffected in response to inoculation with SPARC and GINCO inoculants (Table 4.3). This most likely caused higher nutrient supplies in SPARC and GINCO inoculant treated plants compared to commercial inoculant treated plants (Figs. 4.9 to 4.11). To support this hypothesis, the current results disclosed an important phenomenon related to indigenous AMF shifting from the influence of introduced inoculation. The relative abundance of indigenous *Glomus* was increased in commercial inoculant treated plant. Consequently, the increased abundance of *Glomus* communities in plants was significantly and negatively correlated with growth parameters (Fig. 4.15). The growth reduction in the plants with indigenous AMF (such as *Glomus*) could be attributed to decreased levels of carbon (C) availability for the growth of the host plant which caused by the increased AMF sink (Koide and Elliot, 1989).

One recent study confirmed that the abundance of two *Glomus* species (*G. iranicum* and *G. indicum*) was high in low yielding organic wheat compared to high yielding conventional wheat. The increased indigenous *Glomus* species might be a sink and hence drain C from the host plant more than other AMF species (Dai et al., 2014). Interestingly, in the current study, OTU30 and OTU31 were the closest match with GenBank reference sequences of *G. iranicum* (HM153420.1) and *G. indicum* (HM153422.1). These OTUs were high in abundance in commercial inoculant treated chickpea and field pea roots. The OTU30 and OTU31 were lower

in abundance in SPARC and GINCO inoculated plants compared to the plants inoculated with commercial inoculant (Table B.4.2). This result supports the idea that the cost of AMF symbiosis as a sink of some *Glomus* members resulted in reduced nutrient uptake and biomass accumulation in those plants where OTU30 and OTU31 were in high abundance. The relative abundance of indigenous *Claroideoglomus* also increased in lentil and field pea responding to commercial inoculant. The plants with high abundance of *Claroideoglomus* had reduced nutrient uptake and low biomass accumulation.

According to the NMDS ordination, indigenous *Funneliformis*, *Rhizophagus*, *Paraglomus* and *Diversispora* were dominant in SPARC *F. mosseae* B04 inoculated chickpea and field pea roots, and increased shoot biomass accumulation compared to uninoculated control was detected (Fig. 4.6). In contrast, *Glomus* and *Claroideoglomus* were apparently high in abundance in commercial *R. irregularis* 4514535 inoculated lentil and chickpea (Figs. 4.2 and 4.6). Little information about the functioning of *Paraglomus and Diversispora* communities on the plant growth is available; however, different AMF fungal species may provide different services to crop plants (Chagnon et al., 2013). Plant microbial communities are highly linked to each other, and plants have been shown to cultivate their own microbial communities (Berendsen et al., 2012). Chickpea and field pea root associated AMF communities were different than lentil communities (Figs. 4.2 and 4.6). A similar scenario was observed in a recent study in the Canadian Prairies where *Diversispora* and *Paraglomus* were higher in abundance in the conventional wheat cropping system than in the organic system, and had beneficial impacts on wheat production (Dai et al., 2014).

### 4.6.4 Relationship between AMF compositional diversity, functional diversity, and plant growth parameters

The N and P uptake varied between inoculant strains of the same species (*F. mosseae*) and different species (*R. irregularis*) (Figs. 4.9 to 4.10). Hence, the current results indicate that the effect of AMF communities on plant growth parameters could not be predicted based on the species composition of AMF because it is difficult to directly link the AMF compositional species diversity and AMF functional diversity. There was no correlation between the diversity indices and N uptake ($r$ = -0.042, $P$ = 0.476) and P uptake ($r$ = 0.047, $P$ = 0.748). However, a

significant positive correlation coefficient was detected between diversity indices and biomass accumulation ($r$ = 0.366, $P$ = 0.010), similar to the significantly positive correlation between indigenous AMF taxa (*Rhizophagus*, *Funneliformis*) and biomass accumulation (Figs. 4.13 to 4.14). Tilman et al. (1997) detected that microbial functional diversity rather than species diversity in a particular ecosystem was the key factors revealing effects of increased plant species diversity on plant productivity. Similarly, Vogelsang et al. (2006) suggested that AMF identity rather than diversity likely explains the impact of fungal diversity on plant productivity.

The functional significance of AMF communities is still unclear. Different AMF taxa vary in a wide range of characteristics, including the speed at which root occupancy occurs (Hart and Reader, 2002b), quantity of root colonization (Clark et al., 1999), spore production (Bever, 2002a), the frequency of hyphal fusions and the integrity of hyphal networks (Giovannetti et al., 1999; De La Providencia et al., 2005) and the physiological activities of nutrient uptake and transport pathways (Boddington and Dodd, 1999; Burleigh et al., 2002). The present results showed considerable increased N and P uptake and biomass accumulation compared to controls in chickpea crops where SPARC inoculant (*F. mosseae* B04) was added. Similarly, the Shannon diversity index was unaffected in chickpea and the alteration in indigenous AMF communities as a consequence of inoculation with SPARC inoculant was found to be non-significant (Fig. 4.7). The P uptake was high in SPARC inoculated lentil and field pea where no positive response of P uptake in GINCO inoculated plants was observed (Fig. 4.10). Different mycorrhizal fungal effects on plant growth performance suggest that AMF communities with a higher species diversity may have greater potential functional diversity. Consequently, high functional diversity in the indigenous AMF communities likely occurred (in chickpea) where SPARC inoculant resulted in consistently improved plant nutrient (N and P) and biomass. This could be due to the different combinations and interactions in symbiosis between host plant and AMF inoculants (Figs. 4.9 to 4.11). It is, however, still unclear whether such functional genes involved in AMF-host symbiosis can be used to explain the effects of indigenous AMF communities on plant nutrition, growth and yield components (Van Der Heijden and Scheublin, 2007). Nevertheless, few studies found direct correlations between the community composition and the functional significance of indigenous AMF for plant growth and ecosystem functioning (Rodriguez and Sanders, 2015).

### 4.6.5 Variation in inoculant genetic makeup and effectiveness in plant performance

Genetic variation exists within AMF inoculants assessed in the current study, particularly between SPARC (*F. mosseae* B04) and GINCO (*F. mosseae* DAOM 221475) strains. Both these strains showed sequence dissimilarity in phylogenetic analysis (Fig. 4.1). The strains (same species but different strains and source of geographical distribution and ecological habitat) showed different contributions to nutrient uptake and biomass acquisition. The SPARC inoculant strain displayed significantly higher N and P uptake and shoot biomass production in all three test crops (Figs. 4.9 to 4.11). This could be explained from the study of two genetically different *R. intraradices* strains that negatively impacted the growth of transformed roots of *Daucus carota* (Croll et al., 2009). Croll et al. (2009) attributed this negative influence as the cost of AMF colonization to the plant (Koch et al., 2006). This could also be concluded from the present observation where the significant reduction in N and P uptake by the GINCO inoculant strain occurred despite higher root occupancy compared to the SPARC inoculant strain. However, how the genetic variation in AMF strains contribute to the alteration of plant growth is unclear.

Colombo et al. (2013) reported that higher root colonization by mycorrhizal inoculant (GA5) was negatively correlated with plant biomass production. In the current study N and P uptake and shoot biomass accumulation by commercial inoculant (*R. irregularis* 4514535) was reduced with the exception of chickpea biomass. The GINCO inoculant (*F. mosseae* DAOM 221475) also showed significantly lower performance in N and P uptake and biomass accumulation in lentil and chickpea compared to SPARC inoculant (*F. mosseae* B04). This suggests different species and also different strains of a single species may have favourable or harmful effects on host plant development (Koch et al., 2006; Colombo et al., 2013). Mycorrhizal functions can range from mutualistic to parasitic with different host plants (Bever, 2002b; Klironomos, 2003; Jones and Smith, 2004). Numerous studies support the evidence of mycorrhiza-induced suppression of the plant P uptake pathway via root hairs and epidermis (Smith and Smith, 2011; Smith et al., 2011). Different species of *R. intraradices* have had almost complete suppression of the P uptake pathway in several plant species, including *Medicago truncatula* (Smith et al., 2004; Grunwald et al., 2009).

The positive influence of the AMF symbiosis on P uptake has long been known (Smith and Smith, 2011). However, N uptake by plant species as a contributory role of AMF-host symbiosis is still under debate (Smith and Smith, 2011). The current results, like many others, showed that the SPARC inoculant significantly enhanced N uptake in lentil, chickpea and field pea (Fig. 4.9). This study confirmed that the improved N uptake was caused by either indigenous or introduced inoculant AMF root occupancy or synergistic effects of the introduced and indigenous AMF. Some previous reports on the tripartite crop–mycorrhizae–rhizobia symbiosis showed stimulatory (Jin et al., 2010; Tajini et al., 2011) or inhibitory (Scheublin and Van Der Heijden, 2006; Franzini et al., 2010) effects on each other or on the growth of plants. Inoculation of common bean plants with *G. intraradice*s resulted in a significant increase in nodulation with N accumulation increasing by 42% compared to plants without mycorrhizal inoculation (Tajini et al., 2012). Moreover, an improved N status of mycorrhizal plants may simply be a consequence of an improved P nutrition in soils (Reynolds et al., 2005). The present results of increased N uptake are consistent with several studies where different AMF isolates increased the N content of the plants and induced a greater biomass response compared to non-inoculated controls in laboratory and field setting experiments (Toussaint et al., 2004; Tanaka and Yano, 2005; Ngwene et al., 2013; Nouri et al., 2014).

In this study, it was hypothesised that AMF inoculant taxa would differ in their ability to occupy host roots, and thus differ in their ability to promote plant growth parameters. However, the opposite was observed in terms of the plant growth performance responding to commercial inoculant. For instance, SPARC inoculant significantly enhanced N accumulation in all three pulses as well as P and biomass in lentil and field pea. The GINCO inoculant significantly increased N uptake in chickpea. None of the growth parameters was enhanced in response to the commercial inoculant in any of the crops; moreover, N uptake by field pea was reduced in response to commercial inoculant (Fig. 4.9). The growth performance in relation to inoculant taxa indicate the commercial non-indigenous inoculant was inefficient and incompatible in terms of providing nutrient uptake and biomass accumulation for the pulse hosts compared to AMF inoculant isolated locally. The SPARC inoculant was isolated from a Brown Chernozem from Swift Current, Saskatchewan (SK) and established for several months in the current experimental soils. The adaptation in soil perhaps allowed effective increased growth performance compared

to the non-commercial GINCO inoculant which was non-indigenous to experimental SK Chernozem soil and environments.

Calvente et al. (2004) demonstrated that *G. intraradices* (BEG 123), isolated from roots of target olive plantation, was a more efficient growth promoter than *G. intraradices* (EEZ 1) isolated from a different origin, suggesting the significance of the levels of physiological and ecological adaptability of an introduced inoculant strain for plant productivity (Requena et al., 1997; Jeffries and Barea, 2001). Some reports showed that two geographically different strains of *R. intraradices* had a differential response in their root occupancy (colonization) rates and had variable correlations (positive to negative) with the yield and plant growth components (Rasouli-sadaghiani et al., 2010; Colombo et al., 2013). There was, however, no conclusion on the mechanisms or genetics of host-microbe interactions and the subsequent cause and effect on plant growth and yield. The plants inoculated with different species than inoculated with different isolates of the same species contributed to a higher variation in plant growth (Klironomos and Hart, 2002). Large variations in plant P uptake have been observed due to inoculation with the isolates from different geographic origins (Munkvold et al., 2004).

### 4.7 Conclusions

Findings revealed significant variation of host plant biomass accumulation and nutrient uptake in response to different inoculant taxa. The SPARC inoculant (*F. mosseae* B04) isolated from Swift Current soil showed better performances to enhance growth in pulse crops compared to GINCO inoculant (*F. mosseae* DAOM 221475) isolated from Ontario Rondeau Provincial Park soil and commercial inoculant (*R. irregularis* 4514535). This result supports the possibility of locally isolated indigenous AMF that may form functionally efficient associations without significant or with minimal disturbance of indigenous AMF communities in crop root assemblages. A decisive explanation for the basis of this variation is beyond the scope of this controlled conditions study. The current research supports the hypothesis that plant growth performance can be mediated by changes in the abundance of indigenous AMF communities in roots as consequence of inoculation by introduced AMF inoculants rather than impact of root occupancy by inoculant strain alone.

# CHAPTER 5

# EFFECT OF POOLING REPLICATIONS OF PEA ROOT SAMPLES ON ESTIMATES OF RICHNESS, DIVERSITY AND COMPOSITION OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES USING 454 PYROSEQUENCING PLATFORM

## 5.1 Preface

The effect of pooling pea root sample replications on data generated from 454 pyrosequencing was examined using samples collected during the first (2011) and third (2013) year of a three-year field experiment, described in Chapter 3. Pooling replicates is used to reduce the number of samples analyzed because the sample preparation and subsequent 454 pyrosequencing is both time-consuming and costly. Replications may be pooled at some point during the sampling or sequencing process. Thus, the objective of Chapter 5 was to evaluate the impact of pooling prior to DNA extraction on the characterization of AMF communities. This chapter presents pyrosequencing analyses of pooled samples over two seasons including the outcome of data processing using MOTHUR bioinformatics pipeline, complete OTUs matrix, phylogenetic analyses, and relative abundance of AMF genera, estimating richness, and diversity indices for 32 treatment root samples. The major differences in the assessment of the composition and diversity of AMF communities using a pooled versus a non-pooled replicated sampling strategy and pyrosequencing technology were examined.

## 5.2 Abstract

The aim of this study was to evaluate the impact of pooled versus non-pooled replicated root samples prior to DNA extraction on the estimates of richness, diversity and composition of arbuscular mycorrhizal fungi in pea roots using an 18S rRNA gene pyrosequencing platform. Trap roots grown in field soil samples collected during the first (2011) and third (2013) year of a three-year study were used in this assessment. The pyrosequencing data revealed that the estimates of relative abundance of major AMF genera (i.e., ranging from 8% to 51% of the total

AMF taxa) namely *Glomus*, *Funneliformis*, *Claroideoglomus*, *Paraglomus* were similar for both sampling strategies; however, the abundance of minor AMF genera including *Septoglomus*, *Archaeospora*, *Entrophospora* and *Diversispora* **(**i.e., ranging from 0.3% to 7% of the total AMF taxa) were greatly affected by pooling and in some samples, three taxa were undetectable as a consequence of pooling replicates. Shannon diversity and Chao richness indices revealed variable shifts in community composition depending on the pooling strategy. The abundance of an introduced *Rhizophagus irregularis* inoculant strain was similar in 2011, irrespective of pooling. However, in 2013, differences between pooled and non-pooled estimates in the persistence of this inoculant strain were observed. Estimates of the diversity and richness of the AMF community composition were higher in non-pooled samples in both years. These results have important implications for future research in AMF community analyses. Pooling samples can reduce the analysis cost and reduce workloads, but it compromises estimates of community diversity, especially minor (low abundant) taxa.

## 5.3 Introduction

Next generation DNA sequencing (NGS) methods such as 454 pyrosequencing enable characterization of microbial communities in a wide range of environments. However, many factors influence the detection of patterns in the flood of sequences generated from these advanced metagenomics tools (Gilles et al., 2011; Yoccoz, 2012). Determination of the effectiveness of sampling procedures such as pooling field soil samples before and after DNA extractions, PCR events and sequencing run using different molecular techniques including the high-throughput pyrosequencing platform has been explored (Baker et al., 2009; Manter et al., 2010; Kennedy et al., 2014; Smith and Peay, 2014; Song et al., 2015). Preparation of samples influences fungal communities detected through NGS technology as the recovery of rare fungal species can be enhanced through compositing samples (Branco et al., 2013) and increasing the amount of soil used during DNA extraction (Zhou et al., 1996). Other studies show that adding some additional steps in the DNA extraction procedures increased DNA yield and captured more species (Martin-Laurent et al., 2001; Karakousis et al., 2006; Roh et al., 2006). Manter et al. (2010) examined three sampling strategies: 1) pooling samples prior to DNA extraction; 2) pooling prior to PCR amplification; and 3) non-pooled soil samples, and suggested that both pooling strategies negatively affected the fungal and bacterial phylotype richness compared to a

non-pooled (PCR amplification of nine biological replicates) sampling strategy, which detected an additional 67 fungal and 115 bacterial phylotypes using the automated ribosomal intergenic spacer analysis (ARISA) molecular protocol.

The impact of various sampling and processing strategies to characterize diversity and community composition has been examined, but not sufficiently investigated through a high-throughput NGS sequencing platform such as pyrosequencing. Several studies examined the appropriate size of the subsample that results in the lowest variation within a defined sampling area (Ranjard et al., 2003; Kang and Mills, 2006). Microbial communities in soils are extremely complex in nature. Multiple small samples collected from field plots are frequently homogenized by pooling samples to serve as a composite sample (Jenkins et al., 1997; Baker et al., 2009), which is then used to assess the microbial community. These composite samples and subsamples are assumed to be representative of the original field plot.

A meta-analysis of published articles in the leading peer-reviewed journals (e.g. FEMS Microbial Ecology, Applied Environmental Microbiology, Microbial Ecology) during 2009 revealed that more than 70% of the microbial research studies performed research without replications and analyzed microbial communities using different molecular techniques including finger-printing, microarray, clone library and pyrosequencing methods (Prosser, 2010). Ignoring basic principles of statistical analysis was widespread and common during the study period (2009) and beyond. Biological replicates are essential for any experiment involving microbial community profiling using high-throughput molecular technologies (Prosser, 2010; Zhou et al., 2011, 2015) and in particular for AMF characterization in field grown crop roots, and the same applied to the 454 pyrosequencing platform. In general, field samples within a single treatment are subjected to variation and thus biological replications are important for statistical significance and identifying sources of variations. Sequencing microbial communities such as AMF using a pyrosequencing platform (from DNA extraction to PCR library and sequence run) involves high costs varying from $50 to $100 per sample (from price quotation in 2014, invoice no. FCI031028 at Genome Quebec, Canada) based on the type and depth of sequence run (i.e., half/full/quarter run). Therefore, this study examined the effect of pooling four replications into a single composite root sample prior to DNA extraction on the richness, diversity and composition of 18S rRNA gene-pyrosequencing of AMF compared to non-pooled replicated samples.

## 5.4 Materials and Methods

### 5.4.1 Strategy of pooling and non-pooling replicated root samples and sampling flow charts

Root samples were obtained from an existing trap culture of field soil that has been described in Chapter 3. Briefly, a commercial non-indigenous AMF inoculant containing an *R. irregularis* strain was applied in field incubated soil cores in May 2011. Pea, wheat and pea as a rotation were grown in the aluminum soil cores during May to September in the 2011 and 2013 cropping seasons, respectively. There were four soils replicated four times maintaining inoculated and uninoculated control soil cores. We examined the effect of pooled and non-pooled sampling strategies prior to DNA extraction with 32 treatments from four sets of data (two pooled and two non-pooled in 2011 and 2013) on AMF community composition, richness and diversity using 454 pyrosequencing technology. The relative abundance of indigenous AMF taxa and the *R. irregularis* inoculant strains, indices of Chao richness and Shannon diversity indices of non-pooled replicates were averaged following analyses and compared to values obtained from pooled samples to estimate Pearson correlation *r* values. The details of sampling procedures, statistical design and treatments, soil conditions, procedure of inoculant application and crop cultivation practices during the study period and the methods of pyrosequencing analyses and data processing using bioinformatics were described in Chapter 3. Details of the sample processing are provided in Figs. 5.1 and 5.2 to illustrate the main events of pooling and non-pooling sampling strategy (organizing root samples, DNA extractions and pyrosequencing protocols) to computational data processing (cleaning sequence reads, harvesting OTUs, richness and diversity estimations) with MOTHUR (bioinformatics pipeline).

### 5.4.2 Statistical analysis

Pearson correlation coefficient values (*r*) were determined for variables arising from pooled and non-pooled samples (e.g., relative abundance of each AMF genus, Chao richness and Shannon diversity of 32 samples) from the 2011 and 2013 crop seasons. The relationships between two variables of 32 data points of relative abundance of different AMF taxa (e.g., *Glomus*) for pooled and non-pooled samples) were estimated. The *r* values close to one (1) determined a small difference between pooled and non-pooled data sets. The non-metric

multidimensional scaling (NMDS) ordination for both 2011 and 2013 data sets were analyzed based on Bray-Curtis distance matrix using PC-ORD v 6 (McCune and Mefford, 1999).

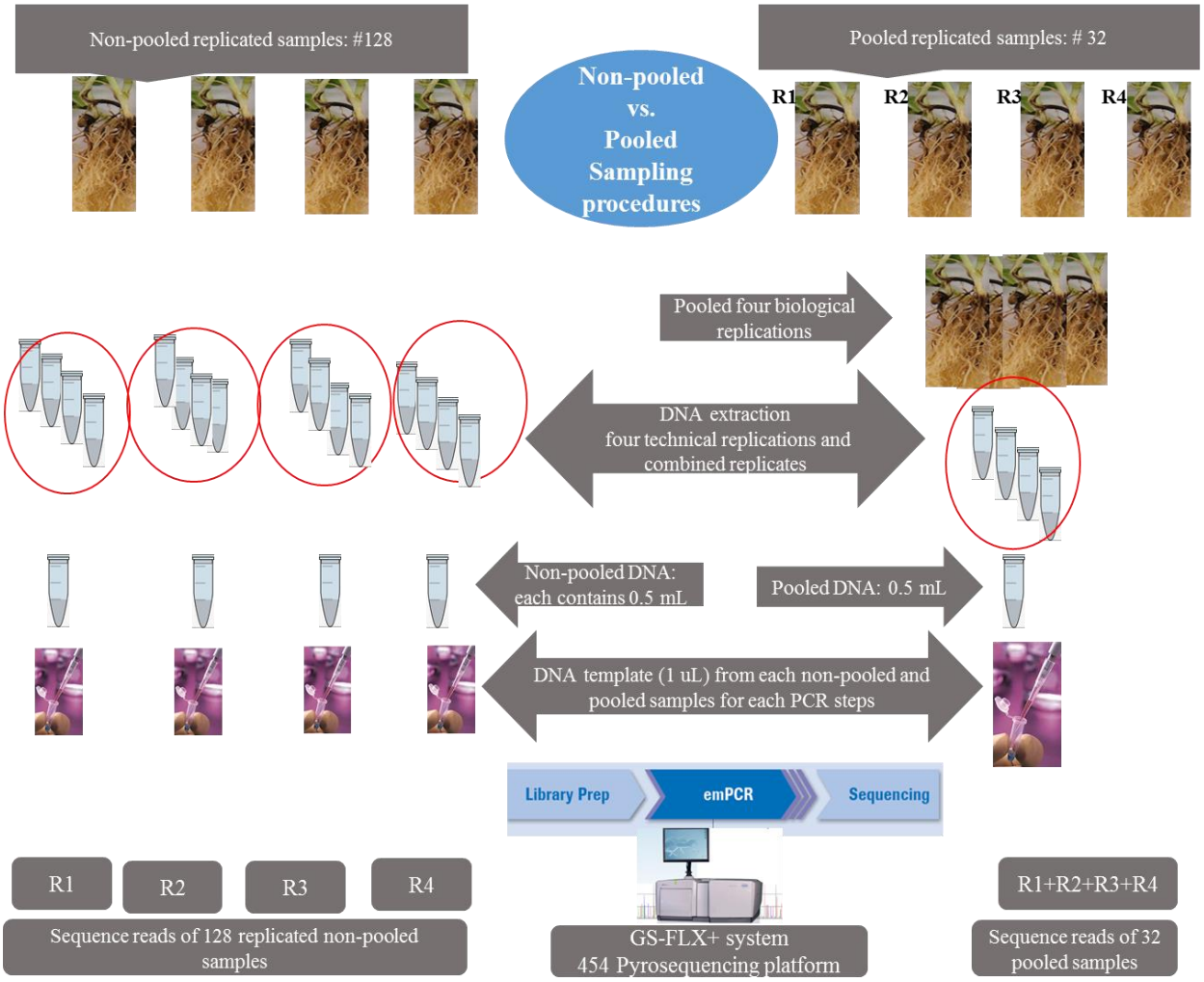


**Figure 5.1** Work-flow chart of the sampling strategy used to compare pooled and non-pooled replicates of pea trap root samples in characterizing AMF communities in 32 soil cores (128 replicates) at four locations of Saskatchewan Prairies using pyrosequencing technology. The red circle shows the pooling technical replicates.

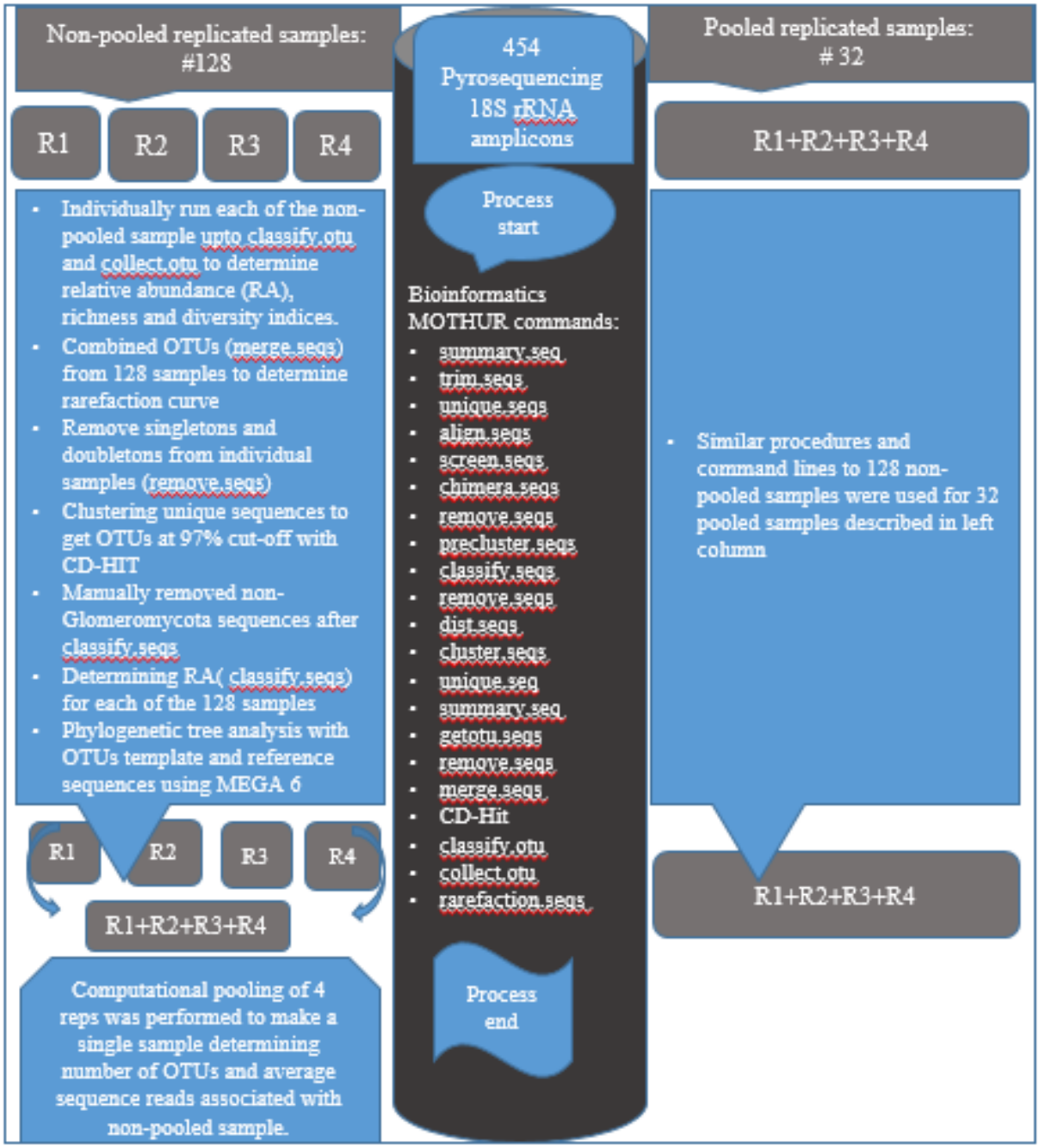


**Figure 5.2** Work-flow chart of bioinformatics analysis, MOTHUR pipeline with pooled and non-pooled samples in characterizing AMF communities, detected by 18S rRNA gene pyrosequencing.

## 5.5 Results

### 5.5.1 Arbuscular mycorrhizal fungal community sequence analysis using pyrosequencing platform, processing sequence data using bioinformatics tools for pooled versus non-pooled samples

A total of 37 405 (non-pooled) and 32 099 (pooled) AMF 18S rRNA gene sequence reads were obtained from pea roots associated with AMF communities following cleaning and removal of short, ambiguous, and chimera sequences in 2011. In 2013, 42 174 (non-pooled) and 29 010 (pooled) were obtained. The nested PCR protocol with the NS1/NS4 and AML1/AML showed 79% AMF specificity (on average 21% of sequences were from non-Glomeromycota microorganisms) for all pooled and non-pooled data from the 2011 and 2013 sampling years. The sequence reads were clustered based on 97% sequence similarity into 86 (non-pooled) and 70 (pooled) OTUs in 2011, and 72 (non-pooled) and 71 (pooled) OTUs in 2013, representing nine AMF genera (Figs. 5.3 and 5.4). The number of OTUs, Chao richness and Shannon diversity indices in each pooled and non-pooled samples of 2011 and 2013 are presented in Figs. 5.5, 5.6 and 5.7. *Rhizophagus*, *Glomus*, *Funneliformis*, *Claroideoglomus* and *Paraglomus* were found in both pooled and non-pooled samples from 2011 and 2013. In addition, *Septoglomus*, *Diversispora and Archaeospora* were detected in 2011 in the non-pooled samples, and *Entrophospora* was found in 2013 in the non-pooled samples whereas these were not found in the pooled samples (Tables A.3.1, A.3.3, C.5.1 and C.5.2).

Rarefaction curves were constructed to assess the effect of the sampling strategy (pooled versus non-pooled replicates) on the diversity and sequence abundance of AMF communities (Fig. 5.8) Relatively flat curves were obtained for both pooled and non-pooled samples in 2011 suggesting the number of OTUs recovered from those sampling strategies approached saturation and sampling efforts were adequate (Jin et al., 2013b). The pooled and non-pooled samples in 2013 resulted in steep rarefaction curves indicating a large fraction of the species diversity remained undetected. More OTUs could be recovered if the number of samples to be analyzed was increased (Chao et al., 2009).

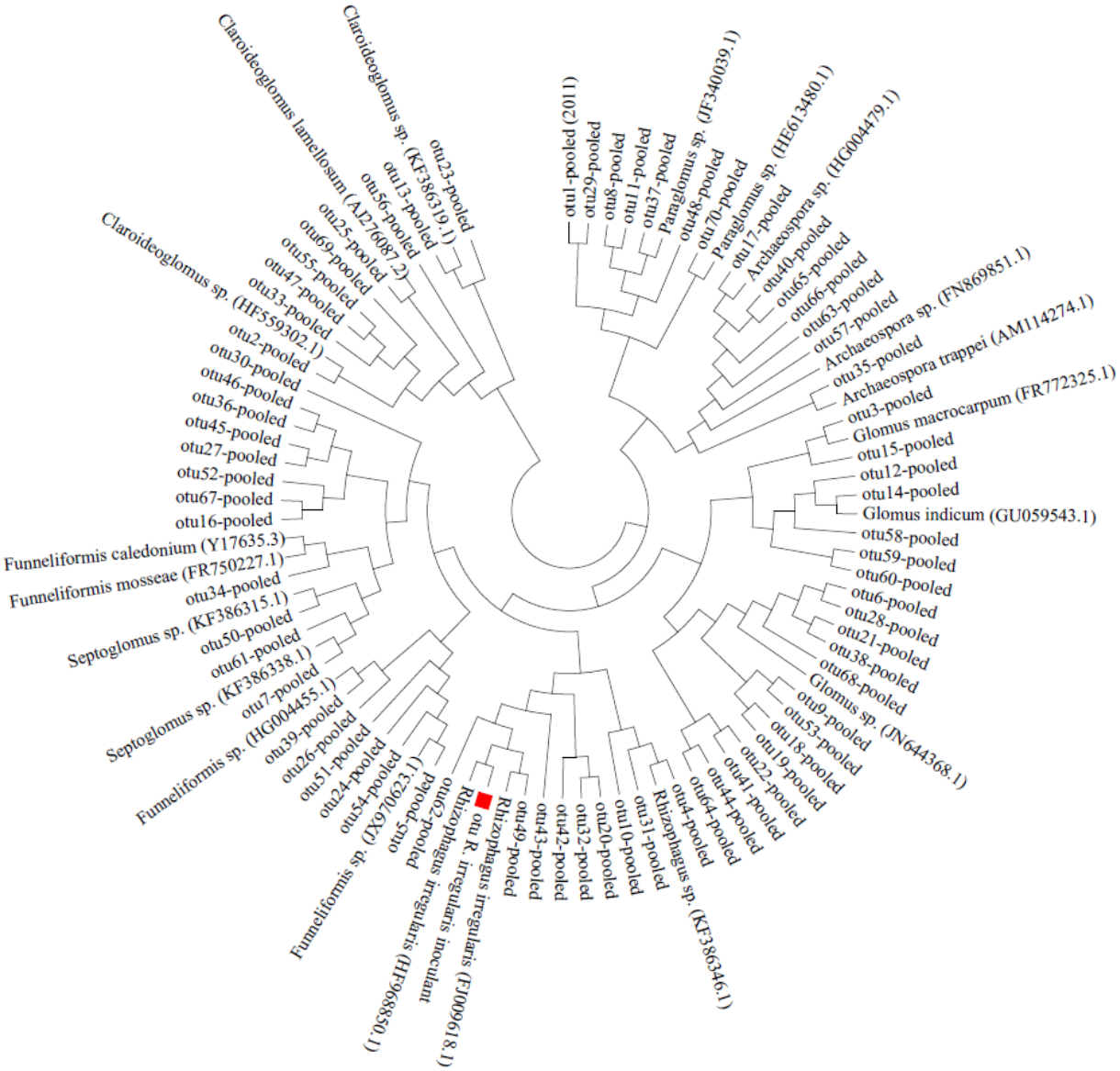


**Figure 5.3** Phylogenetic analysis of 70 AMF OTUs detected by pyrosequencing, year 1 (2011) from the pooled replicated trap root DNA of field pea. AMF OTUs are clustered as *Rhizophagus*, *Glomus*, *Funneliformis*, *Septoglomus*, *Claroideoglomus*, *Archaeospora* and *Paraglomus* groups. Phylogenetic relationships are obtained by neighbor-joining analysis of AMF 18S rRNA gene. GenBank reference sequences are indicated within a parenthesis. Sequence representing the commercial non-indigenous AMF inoculant strain, *R. irregularis* is marked with red rectangle.

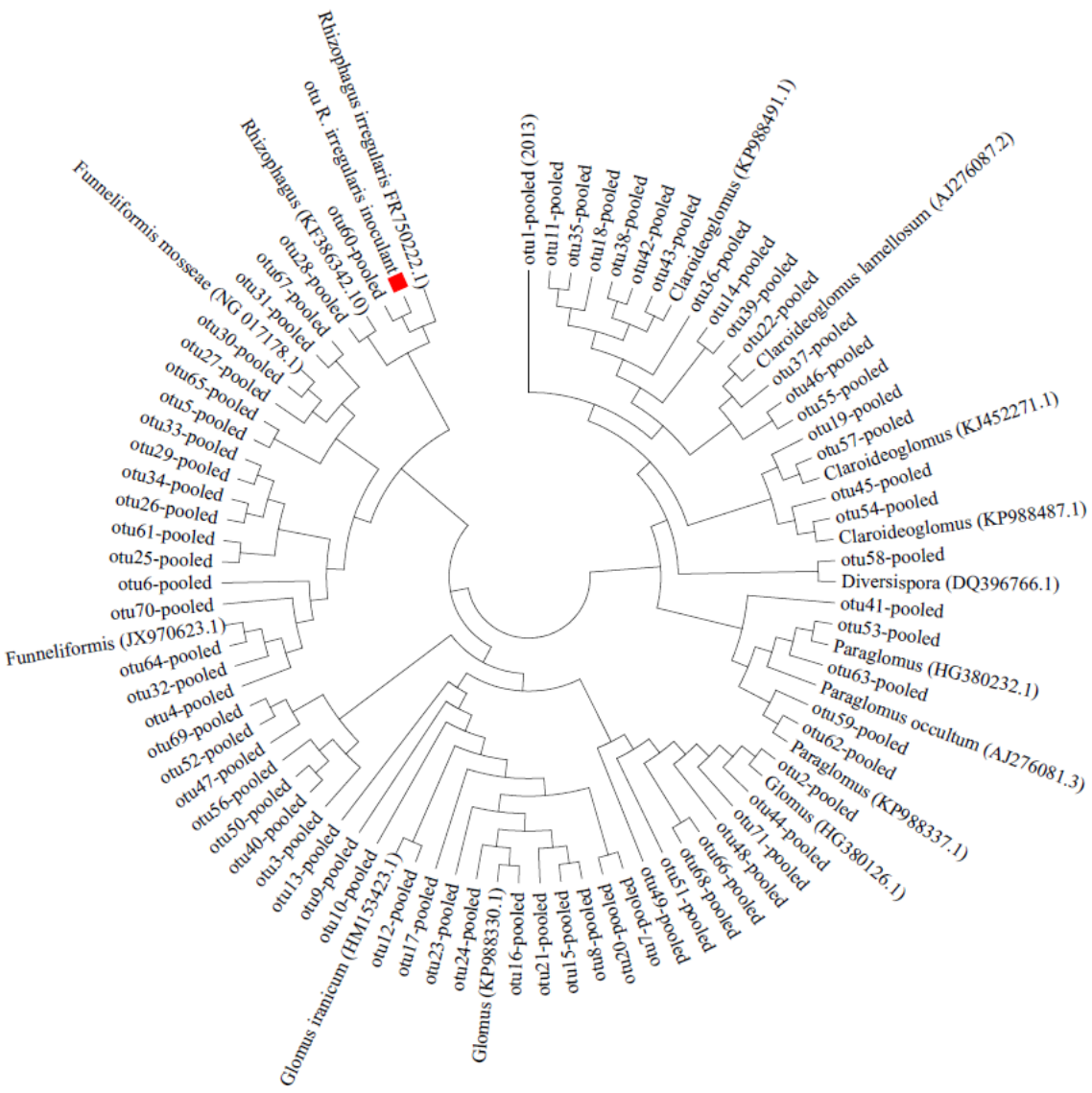


**Figure 5.4** Phylogenetic analysis of 71 AMF OTUs detected by pyrosequencing, in year 3 (2013) from the pooled replicated trap root DNA of field pea. AMF OTUs are clustered as *Rhizophagus, Glomus, Funneliformis, and Claroideoglomus, Diversispora,* and *Paraglomus* groups. Phylogenetic relationships are obtained by neighbor-joining analysis of AMF 18S rRNA gene. GenBank reference sequences are indicated within a parenthesis. Sequence representing the commercial non-indigenous AMF inoculant strain, *R. irregularis* is marked with red rectangle.

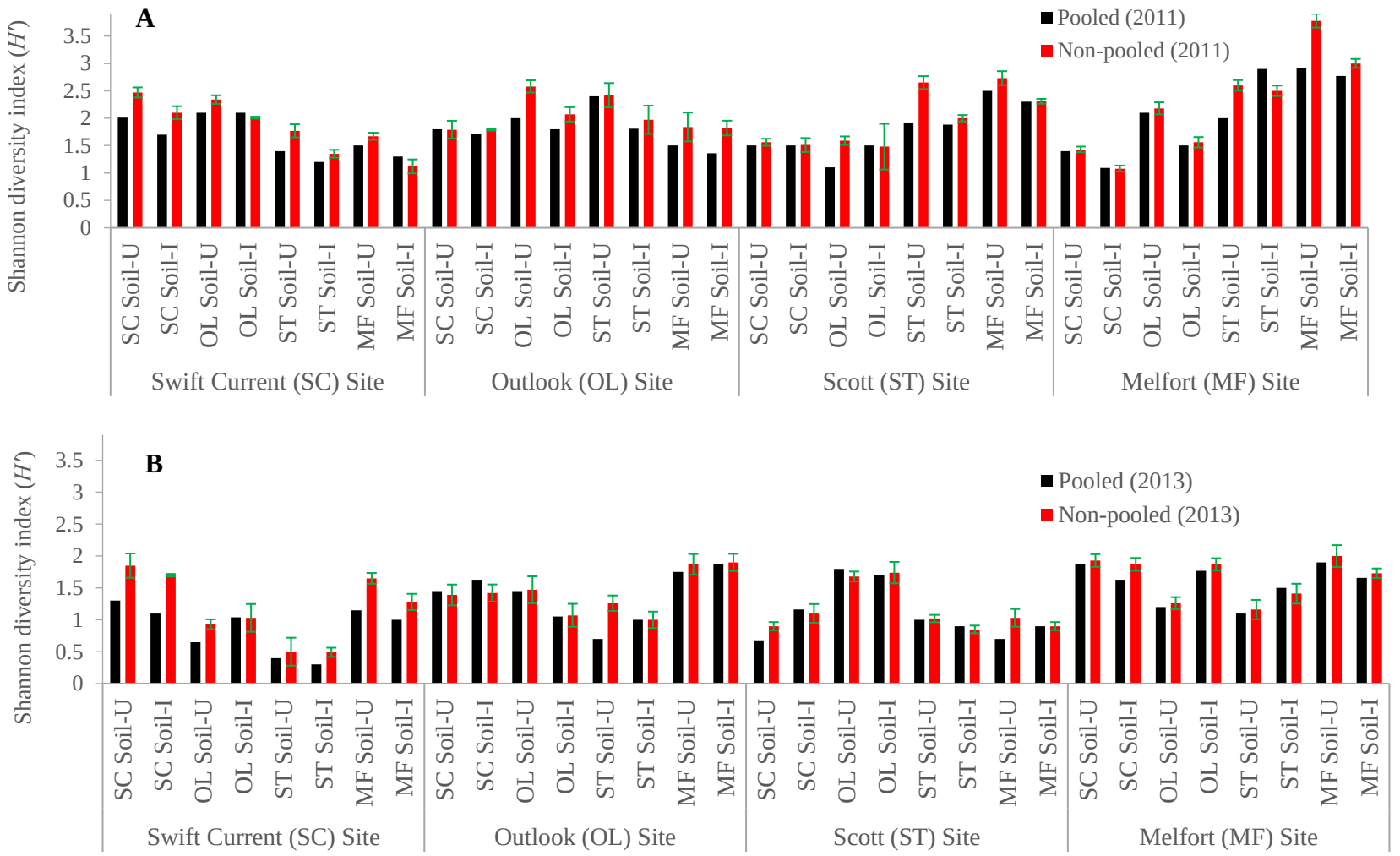


**Figure 5.5** The effect of pooling replications on the Shannon diversity index (*H'*) of AMF communities in field pea trap roots using 18S rRNA pyrosequencing platform across four sites in Saskatchewan in year 1 (2011) (**A**) and year 3 (2013) (**B**). Shannon diversity of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) and the standard error bars (±SE) of replicated non-pooled samples are presented. U: uninoculated and I: inoculated.

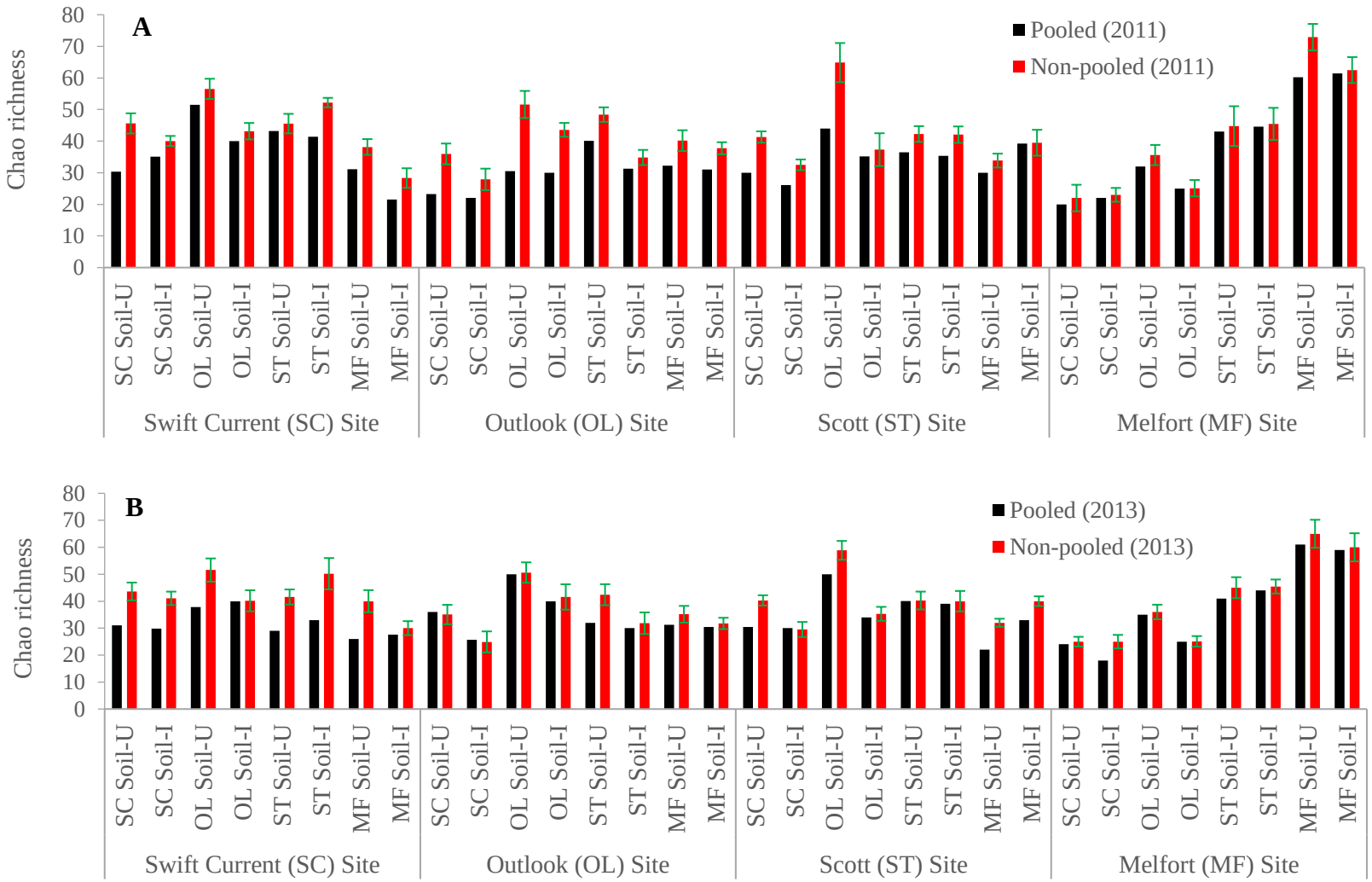




**Figure 5.6** The effect of pooling replications on the Chao richness of AMF communities in field pea trap roots using 18S rRNA pyrosequencing platform across four sites in Saskatchewan in year 1 (2011) (**A**) and year 3 (2013) (**B**). Chao richness of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) and the standard error bars (±SE) of replicated non-pooled samples are presented. U: uninoculated and I: inoculated.

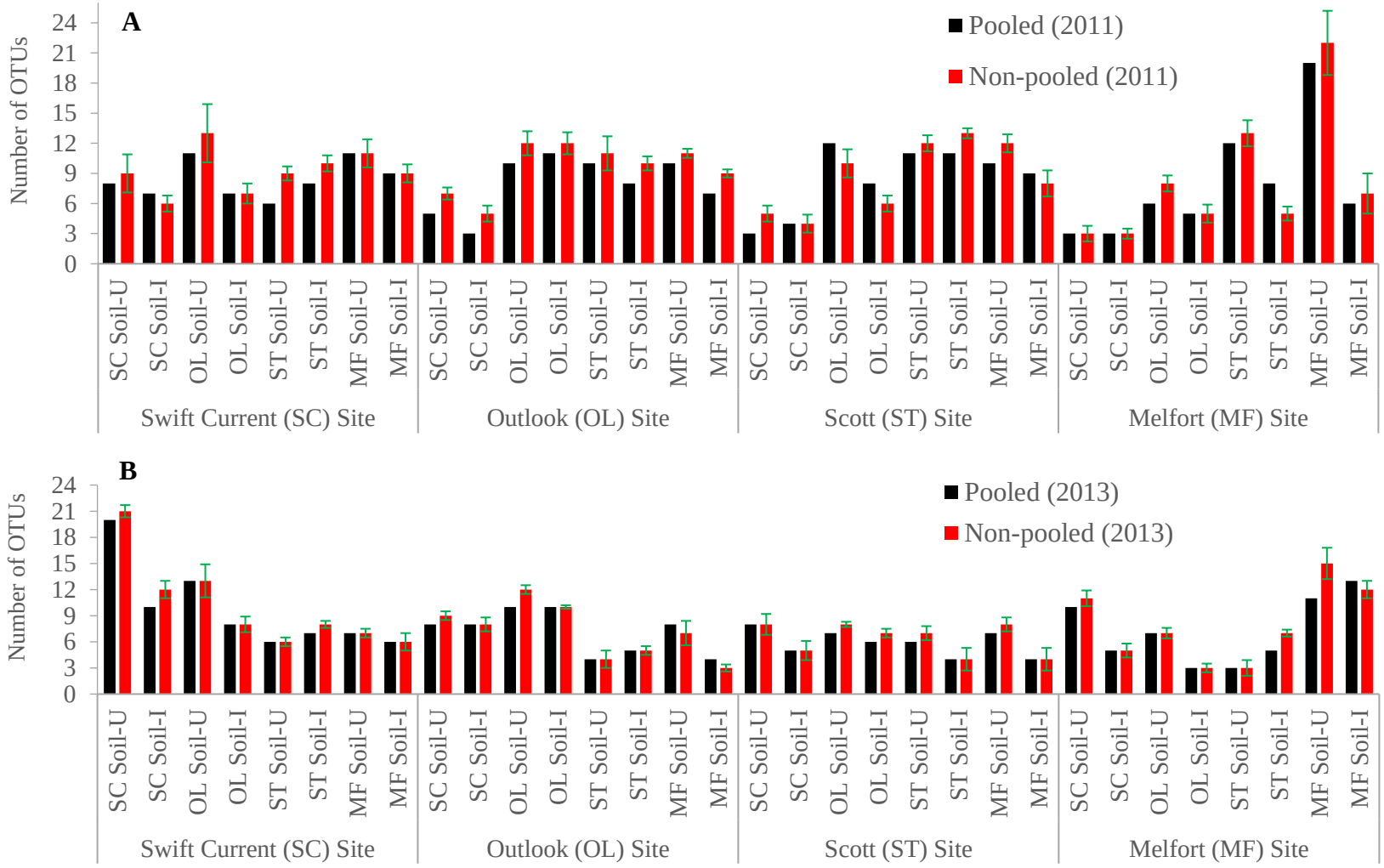


**Figure 5.7** The effect of pooling replications on the number of OTUs of AMF communities in field pea trap roots using 18S rRNA pyrosequencing platform across four sites in Saskatchewan in year 1 (2011) (**A**) and year 3 (2013) (**B**). Number of OTUs from pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) and the standard error bars (±SE) of replicated non-pooled samples are presented. U: uninoculated and I: inoculated.

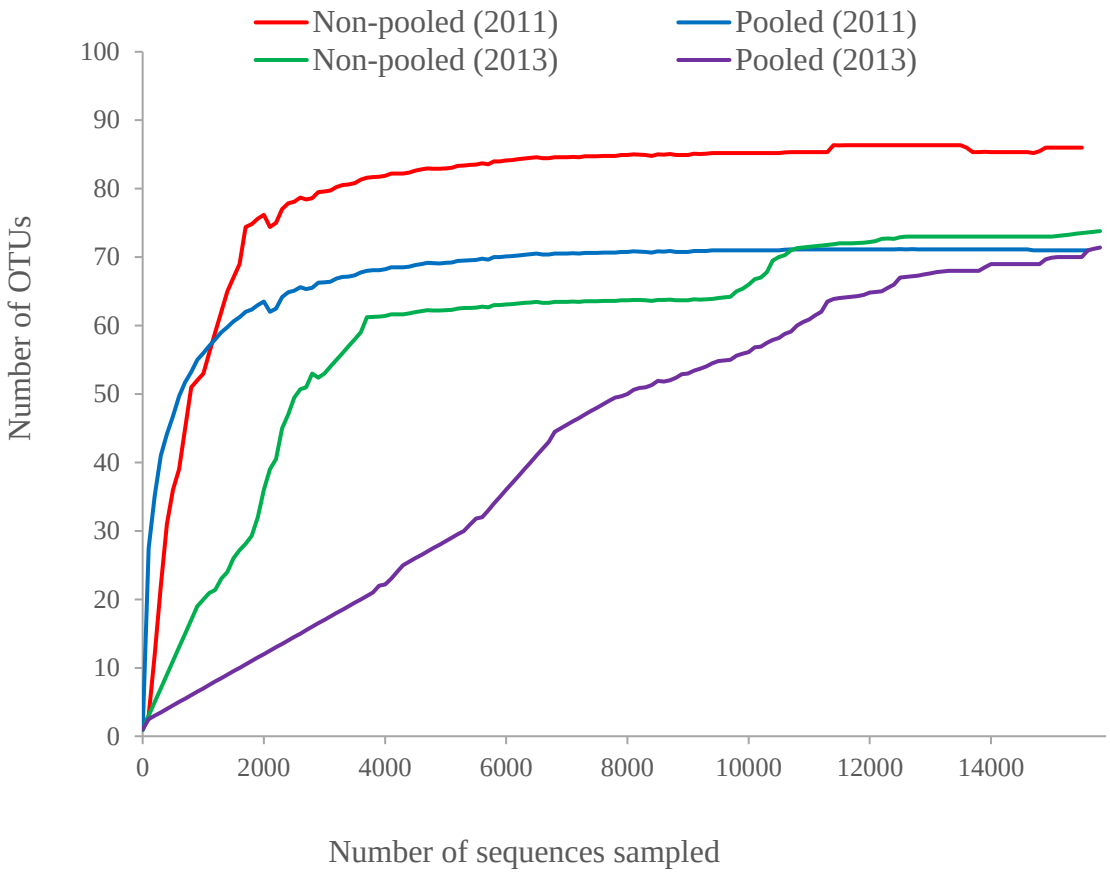


**Figure 5.8** Rarefaction curves from pyrosequencing analysis showing number of AMF OTUs and sequences sampled in pooled and non-pooled pea tap-roots over 2011 and 2013 cropping seasons.

The relative abundance of different AMF genera was compared between pooled and non-pooled replicates in 2011 and 2013. The effect of pooling replicates on the relative abundance of highly abundant AMF genera was negligible in 2011 with both strategies identifying *Funneliformis* (pooled: 51% and non-pooled: 50%), *Claroideoglomus* (pooled: 23% and non-pooled: 22%), *Paraglomus* (pooled: 8% and non-pooled: 9%) and *Glomus* (50% for both pooled and non-pooled) (Fig. 5.9). However, the abundance of *Glomus* sequence reads increased from 26% (non-pooled) to 31% (pooled) due to pooling in 2013 whereas two other highly abundant AMF genera, *Funneliformis* and *Claroideoglomus*, were largely unaffected by pooling in 2013 (Fig. 5.10).

The effect of pooling replicates on the less abundant AMF genera (i.e., those accounting for approximately 7% of sequence reads), in particular, *Archaeospora* and *Diversispora*, was notable in the 2011 cropping season (Fig. 5.10). For example, the relative sequence reads of *Archaeospora* were 0.02% in non-pooled samples and sharply declined to 0.005% due to pooling replicates. Similarly, the sequence reads of *Diversispora* was 1% in non-pooled samples, but no sequence reads were detected in pooled samples. The pooling had a negligible effect on the abundance of *Rhizophagus* (2%) and *Septoglomus* (< 1%) in 2011 (Fig. 5.10). In 2013, the relative abundance of *Diversispora* and *Paraglomus* declined from 6.0 % to 4.0 % and 2.0 % to 1.5 %, respectively, in response to pooling replicates. *Entrophospora* was undetectable in pooled samples in 2013 whereas 7% of the sequence reads in non-pooled replicated samples were associated with this taxon. *Paraglomus* was more abundant (8% to 9%) for both pooled and non-pooled samples in 2011 (Fig. 5.9) but in 2013, their frequency in both pooled and non-pooled samples were lower (1% to 2%) compared to 2011 (Fig. 5.10).

Pooling replicates resulted in a reduction in the persistence (relative abundance) associated with the inoculant treatments regardless of soils and climates. The abundance of introduced *R. irregularis* inoculant declined by 3% in 2011 and 2% in 2013 in response to pooling replicates (Fig. 5.11). Surprisingly, no inoculant was detected in pooled samples from Outlook and Swift Current soils in 2013 whereas the inoculant was detected in those two non-pooled samples (i.e., 3% and 10% abundance, respectively) (Table C.5.2 and A.3.3).

The details of the OTU clusters with AMF reference sequences for non-pooled samples in 2011 and 2013 can be found in the phylogenetic trees in Chapter 3 (Figs. 3.3 and 3.5) and the phylogenetic tree for pooled samples in 2011 and 2013 are presented in Figs. 5.3 and 5.4, respectively. The detailed absolute and relative abundance of AMF genera for non-pooled and pooled samples in 2011 and 2013 can be found in Appendix A and Appendix C, respectively.

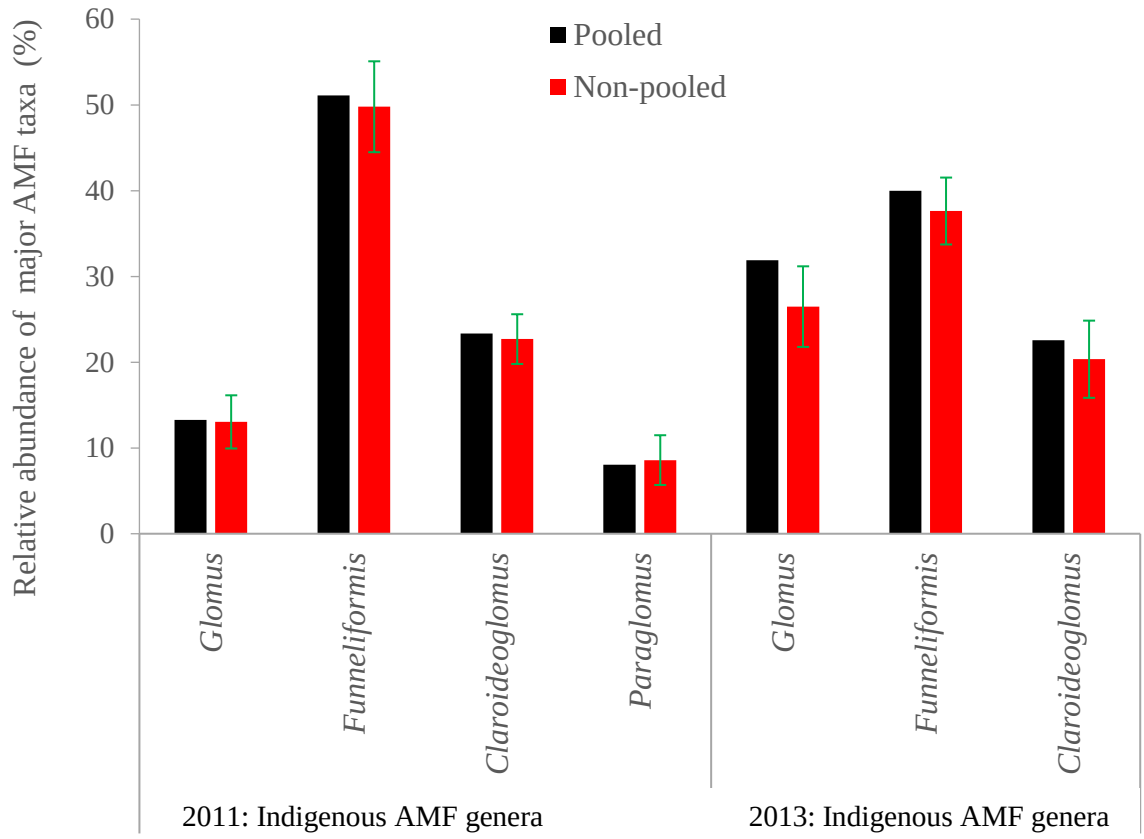


**Figure 5.9** The effect of pooling replications on the relative abundance of highly abundant AMF communities in field pea trap roots using 18S rRNA pyrosequencing platform across four sites of Saskatchewan in year 1 (2011) and year 3 (2013). Relative abundance of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) over all sites and the standard error bars (±SE) of replicated non-pooled samples are presented here.

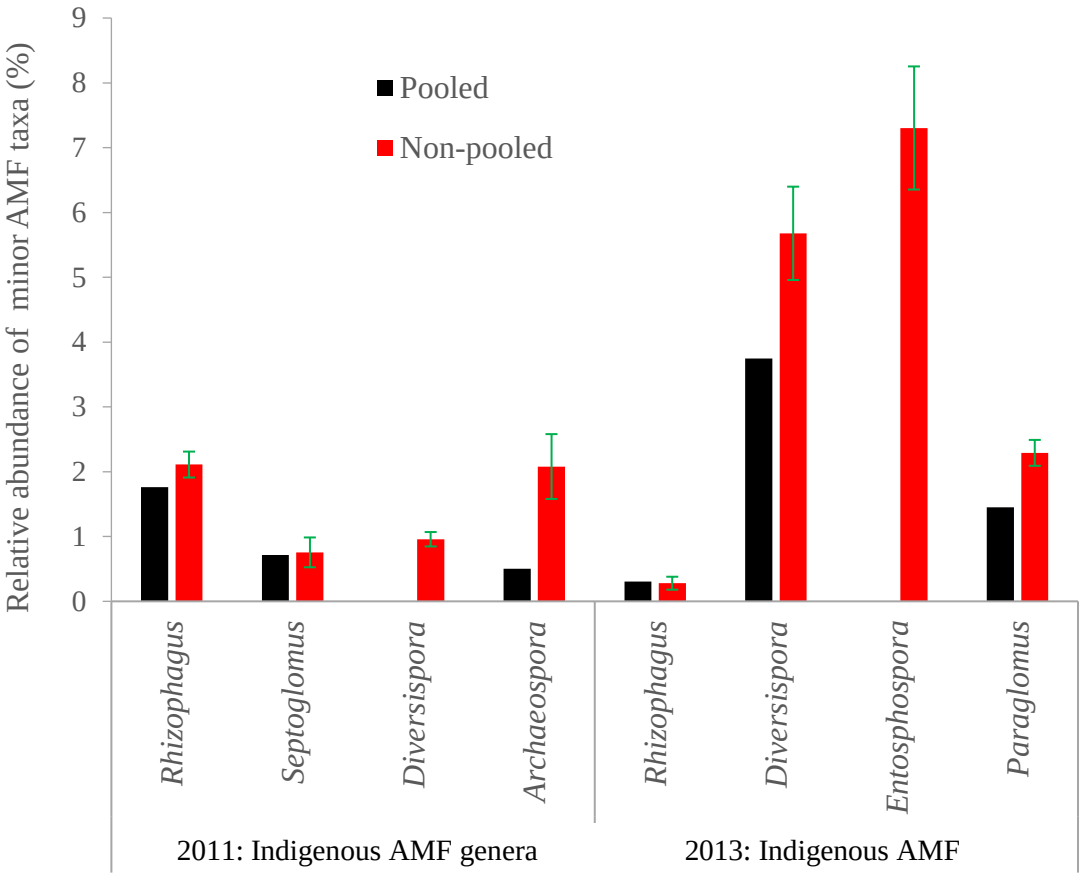


**Figure 5.10** The effect of pooling replications on the relative abundance of minor AMF communities in field pea trap roots using 18S rRNA pyrosequencing platform across four sites of Saskatchewan in year 1 (2011) and year 3 (2013). Relative abundance of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) over all sites and the standard error bars (±SE) of replicated non-pooled samples are presented here.

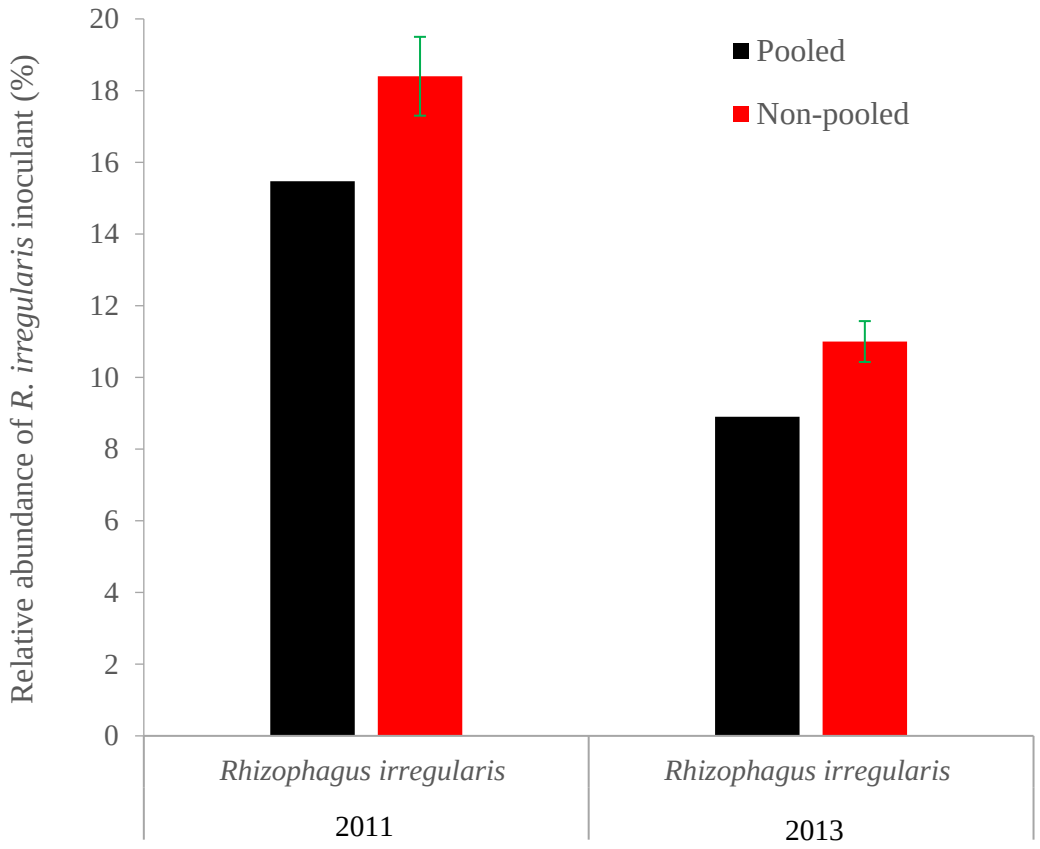


**Figure 5.11** Persistence (relative abundance) of introduced inoculant (*Rhizophagus irregularis*) in pooled and non-pooled samples year 1 (2011) and year 3 (2013). Relative abundance of inoculant from pooled (n=16) and non-pooled samples (n=64 became 16 by averaging four replicates) over all sites and the standard error bars (±SE) of replicated non-pooled samples are presented here.

### 5.5.2 Detection of the introduced commercial non-indigenous inoculant strain, *R. irregularis* from the indigenous *Rhizophagus* community in pooled and non-pooled replicated samples

Nine OTUs (OTU4, OTU10, OTU20, OTU31, OTU32, OTU42, OTU43, OTU49 and OTU62) were clustered together with high levels of similarities (97% to 99%) to the reference sequences of AMF genus *Rhizophagus* from the NCBI GenBank BLAST search in the phylogenetic analysis (Fig. 5.3). These nine OTUs were distributed throughout the year 1 pooled sample set. OTU49 was only detected in the inoculated soil cores. OTU49 was also the closest match with the inoculant OTU and the reference sequence from GenBank (accession no. FJ009618.1). Thus, OTU49 was confirmed as the non-indigenous *R. irregularis* generated from the introduced commercial inoculant in pooled samples (Table C.5.3). There were only two *Rhizophagus* OTUs (OTU60 and OTU28) clustered during phylogenetic analysis for the year 3 (2013) pooled samples (Fig. 5.4). Of these, OTU60 was concluded to be the inoculant OTU since it was not present in the uninoculated 2013 pooled samples (Table C.5.4).

### 5.5.3 Comparisons of relative abundance of *R. irregularis* inoculant, indigenous AMF genera, Chao richness and Shannon diversity between pooled and non-pooled replicated samples

The relative abundance of indigenous AMF genera as *Glomus* ($r$ = 0.974), *Funneliformis* ($r$ = 0.974), *Claroideoglomus* ($r$ = 0.967) and *Paraglomus* ($r$ = 0.967) between pooled and non-pooled sampling strategies were strongly correlated in year 1 (2011). Pooling had a clear effect on *Septoglomus*, *Archaeospora* and *Rhizophagus* in year 1 with $r$ values of 0.866, 0.607 and 0.907, respectively (Fig. 5.12). *Diversispora* was undetectable in the pooled data set from the 2011 cropping season, however it was detectable in 2011 non-pooled samples (Fig. 3.5, see Chapter 3). Similarly, in 2013, when comparing pooled versus non-pooled estimate, Pearson correlation coefficients revealed that pooling greatly influenced the relative abundance of *Rhizophagus* ($r$ = 0.836) and *Diversispora* ($r$ = 0.460); however, the pooling effect on relative abundance of *Claroideoglomus* ($r$ = 0.964) was minimum (Fig. 5.13). The correlation coefficient between pooled and non-pooled strategies indicated that the abundance of *Glomus* ($r$ = 0.932),

*Paraglomus* ($r$ = 0.932), and *Funneliformis* ($r$ = 0.895) was affected moderately in response to pooling samples (Fig. 5.13).

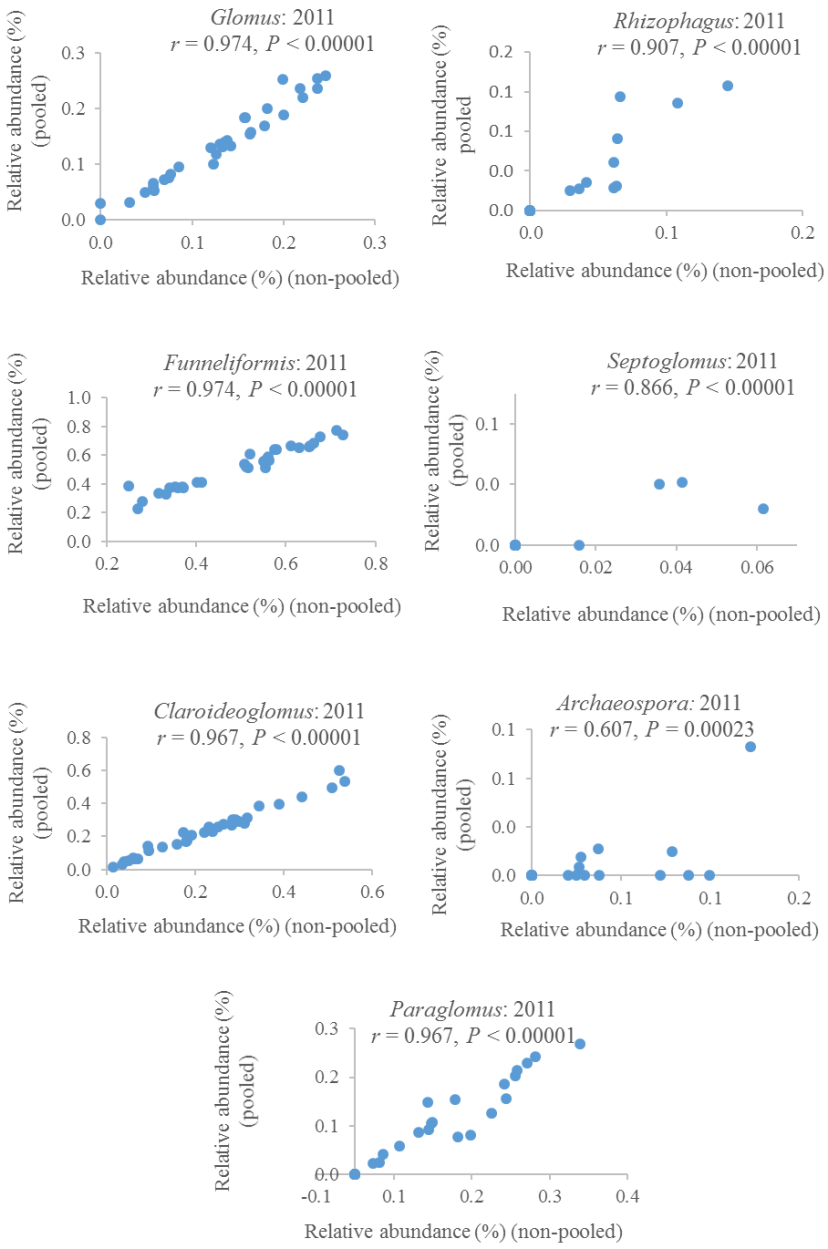


**Figure 5.12** Pearson correlation coefficients ($r$) between pooled and non-pooled relative abundance of indigenous *Glomus*, *Funneliformis*, *Claroideoglomus*, *Rhizophagus*, *Septoglomus*, *Archaeospora* and *Paraglomus* in 2011 samples. Relative abundance of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) is used to assess correlation coefficient.

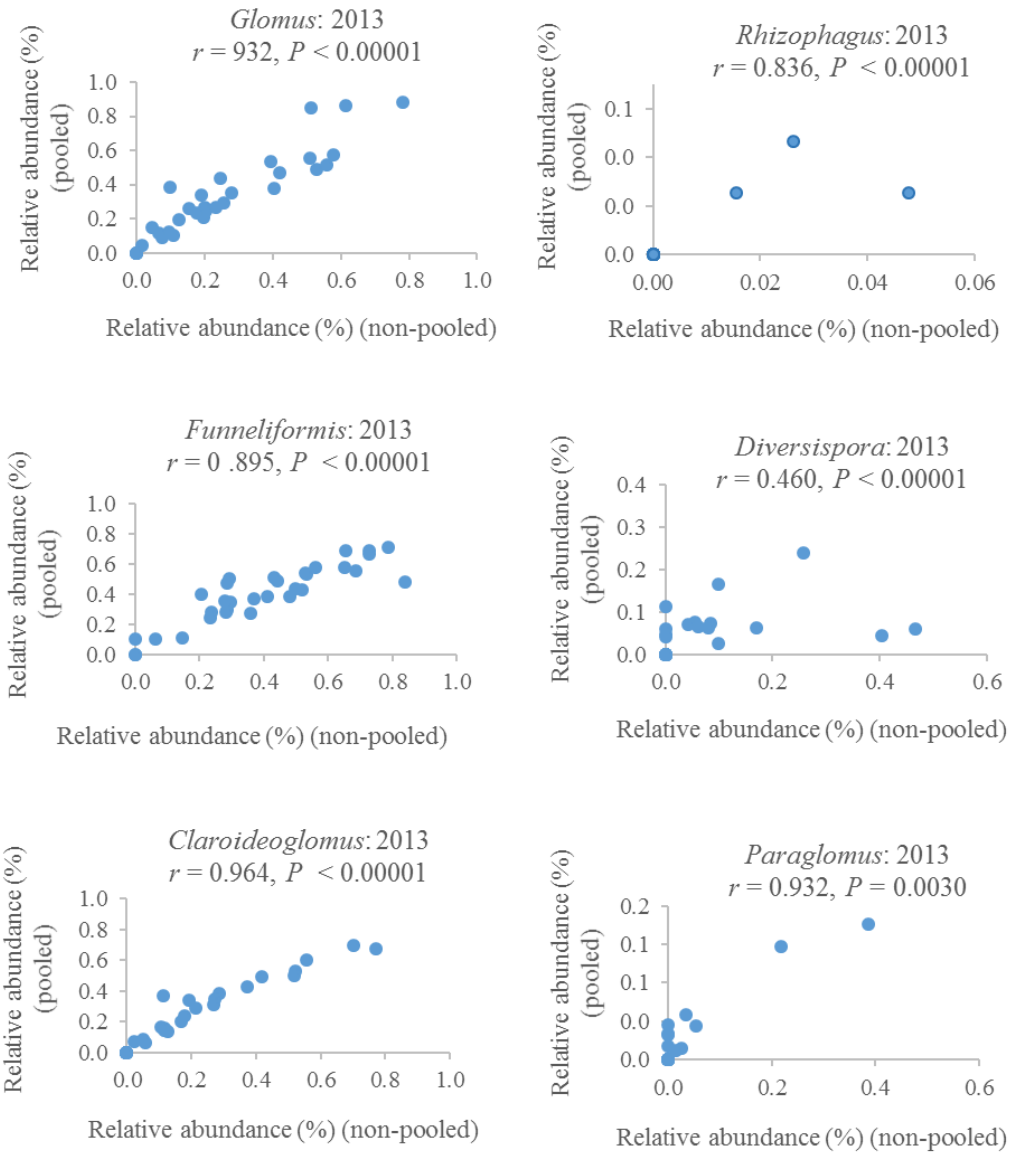


**Figure 5.13** Pearson correlation coefficients (*r*) between pooled and non-pooled relative abundance of indigenous *Glomus*, *Funneliformis*, *Claroideoglomus*, *Rhizophagus*, *Diversispora* and *Paraglomus* in year 2013 samples. Relative abundance of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) is used to assess correlation coefficient.

*Entrophospora* was also undetectable in the 2013 pooled data set whereas in non-pooled samples this taxon accounted for 7% of the sequences. The relationship between relative abundance of the inoculant (*R. irregularis*) in the pooled and non-pooled samples was $r = 0.914$ in 2011 and $r = 0.851$ in 2013, indicating that the estimated abundance of the inoculant in 2013 was affected by pooling samples compared to 2011 (Fig. 5.14).

The Chao richness and Shannon diversity indices were also compared to assess the relationship between pooled and non-pooled sampling strategies based on r values. The reduction of the Chao richness indices between pooled and non-pooled was $r = 0.771$ in 2011 and $r = 0.736$ in 2013 (Fig. 5.15). The Shannon diversity indices were also reduced due to pooling samples, accounting $r = 0.782$ in 2011 and $r = 0.736$ in 2013 (Fig. 5.15). For the 2011 and 2013 data sets, the *P* values for all the *r* values respective to the relative abundance of indigenous AMF genera, inoculant, richness and diversity were statistically significant and positive.

The abundance of AMF taxa in non-metric multi-dimensional scaling (NMDS) ordination based on Sorensen Bayer Curtis distance matrix is presented in Fig. 5.16. The NMDS with the combined 2011 and 2013 pooled and non-pooled data sets clearly shows that sampling strategies affected the estimation of AMF taxa occurrence in pooled and non-pooled samples. The pooled sample groups of 2011 and 2013 were comparatively distantly clustered and ordinated to each other (Fig. 5.16). The abundance of AMF taxa in non-pooled samples tended to be dispersed compared to pooled samples for both the years (Fig. 5.16). *Septoglomus* and *Archaeospora* were associated only in some 2011 non-pooled samples. Similarly, *Diversispora* and *Entrophospora* were associated with few samples of non-pooled replicates in 2013. The ordination graphs clearly show that *Septoglomus*, *Archaeospora*, *Diversispora* and *Entrophospora* occurred at a greater distance compared to other AMF genera within the pooled samples in both the years. This ordination indicates that those taxa were low in abundance in pooled samples. The bi-plot data in NMDS indicated that Chao richness indices are positively correlated to highly dispersed communities of non-pooled samples whereas the Shannon diversity index displayed a weaker positive relationship than richness (Fig. 5.16).

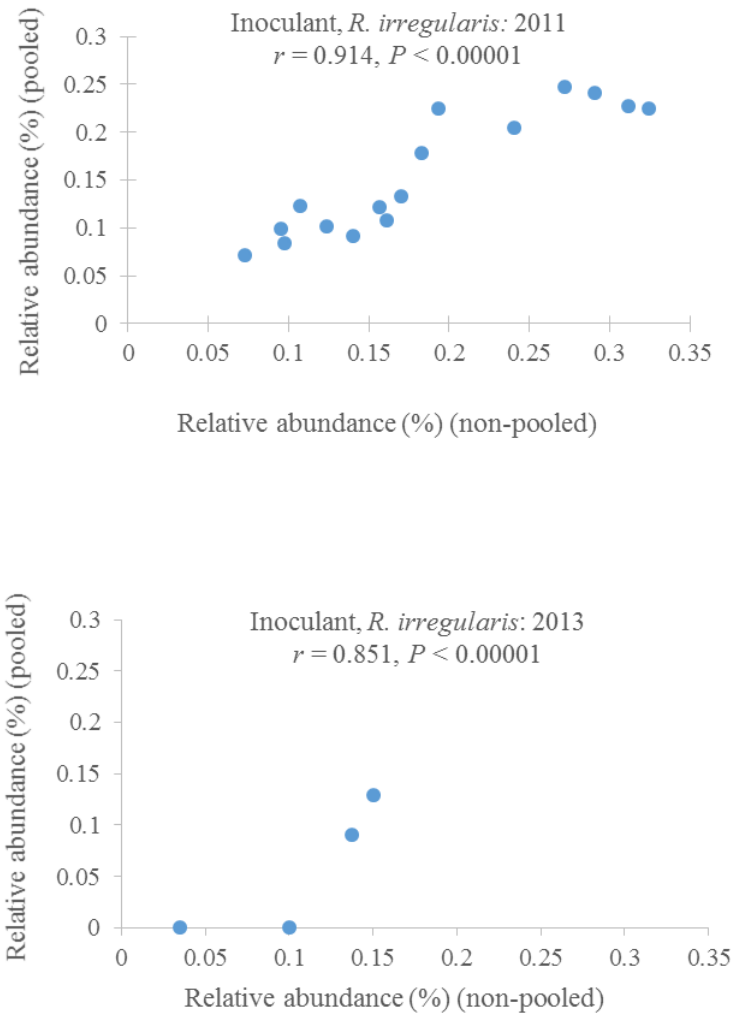


**Figure 5.14** Pearson correlation coefficient (*r*) between pooled and non-pooled relative abundance of introduced inoculant, *Rhizophagus irregularis* in year 2011 and year 2013 samples. Relative abundance of inoculant of pooled (n=16) and non-pooled samples (n=64 became 16 by averaging four replicates) is used to analyze correlation coefficient.

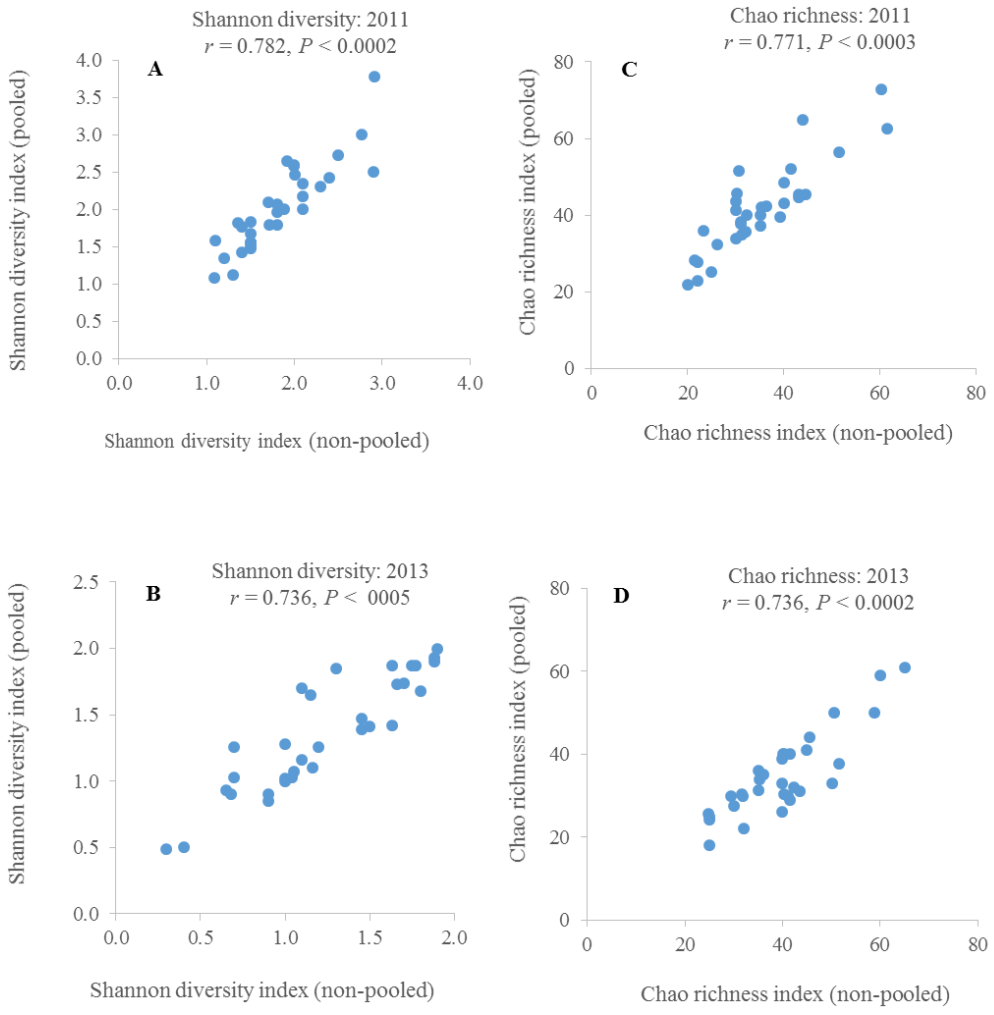


**Figure 5.15** Pearson correlation coefficient (*r*) between pooled and non-pooled **A.** Shannon diversity in year 1 (2011), **B.** Shannon diversity in year 3 (2013), **C.** Chao richness in year 1 (2011) and **D.** Chao richness in year 3 (2013) of AMF taxa. Richness and diversity of pooled (n=32) and non-pooled samples (n=128 became 32 by averaging four replicates) are used to analyze correlation coefficient.

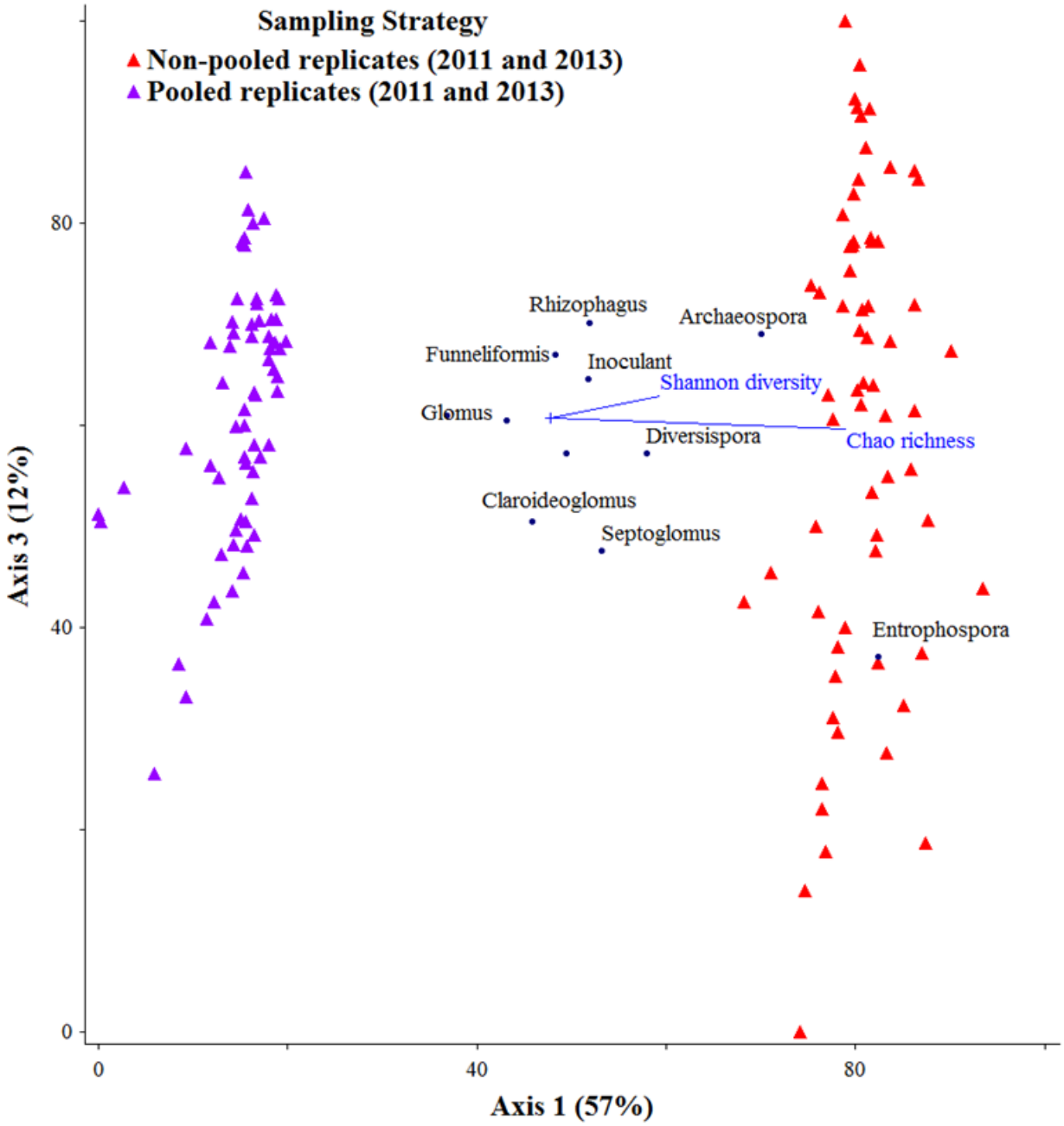


**Figure 5.16** Non-metric multidimensional scaling (NMDS) with 2011 and 2013 pooled and non-pooled samples. 9.69 = final stress for 3-dimentional solution. The percentages between parentheses represents the contribution of each axis to the ordination solution. Blue lines correspond with the bi-plot data set (diversity and richness indices). The relative abundance of non-pooled replicated samples (n=128 become 32 by averaging four replicates) for 2011 data is used in NMDS ordination with Bray-Curtis distance matrix.

## 5.6 Discussion

Pooling the replicated root samples prior to DNA extraction resulted in an apparent reduction in the abundance of minor AMF taxa including *Rhizophagus, Septoglomus, Archaeospora, Diversispora* and *Entrophospora* (Fig. 5.10) This pattern was consistent for both the 2011 and 2013 pooled data sets. *Diversispora* and *Entrophospora* were undetectable in 2011 and 2013 data sets, respectively, when the root samples were pooled. According to the higher *r* values (close to 1), associated with the pooled versus non-pooled data, the relative abundance of highly abundant AMF genera (*Glomus, Funneliformis* and *Claroideoglomus*) were relatively unaffected by pooling both in 2011 and 2013 samples (Figs. 5.12 to 5.13). These results are in partial agreement with those of Manter et al. (2010) who demonstrated that pooling nine replicated soil cores of a single plot from one agriculture field and two other forest sites significantly reduced the detectable phylotypes of both fungal and bacterial communities with differential effects within the sites.

In the current study, it is evident that pooling led to a loss of information as seen by the decreasing trend in the number of OTUs per pooled sample along with the reduction of Chao species richness and Shannon diversity indices compared to non-pooled samples (Figs. 5.5 to 5.7). The reduction in the OTUs detected, along with the concomitant reduction in the richness and diversity in pooled samples might be due to a reduced ability to detect the occurrence of *Septoglomus, Archaeospora, Diversispora* and *Entrophospora* and *Rhizophagus* genera which were undetectable in many pooled samples in both 2011 and 2013. This is in agreement with Engel et al. (2012) who concluded that pooling nine biological replicates prior to RNA extraction using SSU rRNA T-RFLP, masked the diversity in the ciliate community from intertidal sediment samples. The strong significant positive correlation (*r* values varying from 0.96 to 0.99) between the relative abundance of *Glomus, Funneliformis, Claroideoglomus,* and *Paraglomus* of AMF taxa in pooled and non-pooled samples was observed in both 2011 and 2013 (Figs. 5.12 to 5.13). These four AMF genera were high in relative abundance for both pooled and non-pooled samples and on average accounted for 92% of the total AMF communities detected in this study (Table C.5.1). According to others, pooling into larger homogenized samples was found to be efficient in capturing highly abundant bacterial communities compared to non-pooled replicated small samples (Kang and Mills, 2006; Manter et al., 2010). Manter et al. (2010) demonstrated

that the amount of starting DNA templates of pooled samples was positively correlated with the frequency of amplification of dominant fungal and bacterial phylotypes. This result supports our current findings of similar sequencing reads (relative abundance) of the highly abundant AMF taxa quantified both in pooled and non-pooled samples.

The coefficient of correlation analysis and NMDS ordination revealed that the relative abundance of dominant AMF was highly consistent in both pooled and non-pooled samples over 2011 and 2013 crop seasons (Figs. 5.12, 5.13 and 5.16). Interestingly, pooling reduced the total number of OTUs in most of the samples compared to the non-pooled samples, but this pattern was not observed for the relative abundance of highly abundant taxa, suggesting sample pooling may mask some of the minor species that are not very abundant. However, the more abundant species remained dominant both in pooled and non-pooled samples (Tables C.5.1 to C.5.4).

There were two PCR steps involved in amplification specifically, the first PCR amplification with a universal fungal primer set, and a second with an AMF specific primer set prior to the pyrosequencing run in the current protocol used in this study. On the basis of sampling strategies, each non-pooled sample (individual four replicates) passed through two PCR x four reps = eight PCR steps, whereas each pooled sample (4 reps pooled into one sample) had only two PCR x 1 sample (pooled 4 reps) = 2 steps for amplifying the targeted gene sequences. It is hypothesized that non-pooled samples had a greater opportunity to amplify targeted genes due to additional six PCR amplification steps compared to pooled samples. It is assumed that the minor AMF species likely occurred at very low levels or were absent in the initial DNA template for the pooled samples (Fig. 5.1). Therefore, the chance of amplification of species that were abundant at low level in non-pooled samples was greater than in the pooled sample. Few minor species (OTUs) were also detected only in some pooled samples whereas they were undetectable in non-pooled samples (Tables C.5.1, C.5.2, A.3.1 and A.3.3). Similar patterns of masking rare fungal species in pooling technical replicated samples was demonstrated by Avis et al. (2010). When they increased the number of clones in pooled samples the recovery rate of fungal species was high (average 90%) compared to non-pooled replicated samples. According to Avis et al. (2010), the significance of the large number of small samples, similar to several replicates of a single sample, seemed to be appropriate. Kang and Mills (2006) demonstrated that the composition of bacterial communities was variable among biological

replications which lead to detecting rare phyla. Conversely, a small number of bulked samples (i.e., a pooled sample) resulted in the detection of highly abundant microbial communities (Chandler et al., 1997).

Pooling replicates into one large sample reduces sample size and may also minimize the variability and source of heterogeneity. However, the results clearly indicate that pooling can influence the estimation of AMF species richness and diversity (Figs. 5.5 to 5.7). Manter et al. (2010) reported that pooling nine replicates of a soil sample from a single plot removed the spatial heterogeneity of many locally dominant fungal phylotypes. However, the overall rare fungal phylotypes were reduced in the final pooled sample, resulting in those phylotypes being undetectable. This explanation could explain the findings on the loss of minor species in pooled root samples. Pooling may remove spatial heterogeneity when roots were pooled and eventually the minor species became undetectable in the homogenized pooled samples, but they were detectable when individual replicated samples were taken.

One recent study suggested that robustness of microbial community amplification could be increased by addressing sampling related issues including pooling replicates of PCR products prior to sequencing (Lindahl et al., 2013). However, the experimental evidence that pooled replicates robustly captured the diversified microbial communities within the sample is unavailable. A recent study verified the effects of PCR replications and sequencing depth using two sequencing platforms (454 Pyrosequencing and Illumina Mi-Seq) for ecological inference of soil fungi (Smith and Peay et al., 2014). They concluded that pooling replicated PCR products prior to a sequencing run had no detectable effect on α and β diversity of soil fungal communities. They also suggested that molecular ecology might benefit by investing in robust sequencing technology rather than replicating PCR products of a single sample. The result of the current study suggests that multiple DNA extraction from replicated initial root samples resulted in a greater number of OTUs, sequence abundance, species richness, and diversity compared to DNA extraction from single pooled root samples (Figs. 5.5 to 5.7, Tables C.5.1 to C.5.4, A.3.1, A.3.3, A.3.4 and A.3.6). However, in the current study, the robustness of AMF taxa amplification between pooled and non-pooled samples was not examined to assess whether the pooled replicates prior to PCR or pooled after PCR (before sequencing) influence the level of detectable AMF taxa.

Pyrosequencing data revealed that a higher number of OTUs and sequence reads of taxa abundant at low levels was observed in most of the non-pooled samples (Fig. 5.7 and Tables C.5.3 to C.5.4). Amplification events occurred mostly during PCR steps and both pooled (32 samples) and non-pooled replicated DNA sample (128 samples) were exposed to PCR events. Only 1 µL of DNA was used as a representation of both pooled and non-pooled samples, but a greater opportunity for the amplification of taxa abundance at low levels could be associated with non-pooled replicated samples (Fig. 5.1). This is convincing because singleton (unique sequence present only once) and doubleton (unique sequence present only twice) sequences in non-pooled and pooled samples were 2321 and 995, respectively, in the 2011 data sets (data not shown). These sequences were removed from both the non-pooled and pooled data sets prior to the final OTU recovery during pyrosequencing data analysis using MOTHUR pipeline (Schloss et al., 2009). Singletons and doubletons in the NGS data set may be authentic rare species, though singletons and doubleton are thought to be a PCR artifact (Kauserud et al., 2012). Such erroneous singletons and doubletons influence diversity estimation and are sensitive to the artifact removal process under high throughput NGS sequencing technology (Unterseher et al., 2011; Zhan et al., 2014). Removal of singletons and doubletons are common practice prior to downstream statistical analyses (Tedersoo et al., 2010; Lindahl et al., 2013). The ratio of artifact sequence amplification between 2011 pooled and non-pooled samples supports the idea that the amplification of taxa abundant at low levels during PCR events could be three to four times higher for each non-pooled sample compared to pooled samples. This clarification supports the hypothesis that slight variations in the microbial population captured in the initial DNA template could lead to greater variation in multiple replicated sampling through PCR amplification events than in larger samples that are pooled (Tedersoo et al., 2010; Lindahl et al., 2013). This result also supports the idea that the amount of initial DNA template per pooled and non-pooled samples could be an important factor causing greater variation in community composition following pyrosequencing protocols.

Similarly, the ratio of artifact sequence (singleton and doubleton) amplification between 2011 pooled and non-pooled samples in current study was 3014 and 1515 in 2013 pooled and non-pooled samples, respectively. This higher number of artifacts in 2013 compared to 2011 samples could probably interfere with the estimation of sample diversity. Thus, the rarefaction

curves become inconsistent (Lindahl et al., 2013). Comparatively, higher occurrence of artifacts resulting in more singletons and doubletons both in pooled and non-pooled samples in 2013 caused a steep rarefaction curve and likely a large fraction of the species diversity remained undetectable (Fig. 5.8). Quince et al. (2009) demonstrated that the incidence of errors from PCR artifacts increased the number of singletons and doubletons, which markedly increased sequencing effort and species accumulation curves. The removal of singletons and doubletons from both 2011 and 2013 data sets was justified. The relevance of the estimation of Chao richness and Shannon diversity indices which rely on the frequency of artificial singletons and doubletons may be questioned in the process of high throughput NGS sequencing data ((Dickie, 2010). To what magnitude this depression could be reduced by efficient use of bioinformatics (e.g., removing singletons and doubletons) remains uncertain.

There are some inconsistencies in the number of sequences (abundance) in some pooled samples. For example, in the uninoculated Scott soil at the Outlook site and the Outlook soil at the Melfort site, a higher number of OTUs and sequence reads of *Septoglomus*, *Paraglomus* and *Rhizophagus* were generated from pooled samples compared to the same sample of non-pooled replicates (Tables C.5.3 to C.5.4). The reason for inconsistent trends in pooled samples is not clear; however, these variable results further suggest that pooling can lead to misleading interpretations of the actual community composition.

The current pyrosequencing results and the previous studies in the literature suggest that pooling replications, at least at the initial stage of sampling (i.e., prior to DNA extraction and prior to PCR), can mask some detectable fungal phyla which could thereby lead to underestimations when assessing certain quantitative microbial indices such as richness, evenness and diversity. It is recommended that researchers consider at least a few replications in designing fungal community analysis using next generation sequencing platforms such as 454 pyrosequencing to recover minor or rare species. Replications have been mostly neglected in many published results on fungal community analysis using high throughput 454 pyrosequencing analysis. According to Prosser (2010), only 29% of published articles, characterizing fungal diversity in peer-reviewed journals assessed in 2009 used true replications in microbial ecology community studies. The most suitable and efficient sampling strategy largely depends on the objective of the experiment. If the purpose is to examine the relationship between environmental

parameters and the community rather than addressing the analysis of the community diversity and structural composition, replicated large sample size may be compromised. The present results clearly indicate that pooling replicates reduced the number of OTUs per sample and the relative abundance of AMF taxa that were abundant at low levels. This reduction of infrequent taxa subsequently decreased the estimates of fungal richness and diversity of some samples in both 2011 and 2013 (Figs. 5.5 to 5.7).

The current data sets were taken from a project involving a three-year field incubation study to assess the persistence of a non-native AMF inoculant *R. irregularis,* and the impact of AMF inoculation on the diversity and composition of indigenous AMF communities. Overall abundance of inoculant regardless of the treatments of soil and climate revealed that pooling replicates reduced estimates of the persistence of the inoculant strain in 2011 by 3% and by 2% in 2013 compared to non-pooled replicated sampling strategy (Fig. 5.11). The inoculant in 2013 non-pooled samples was detected only in four of 16 inoculated root samples. Interestingly, pooling resulted in masking of the sequences from inoculant strains in two samples (Outlook soil at Outlook site and Swift Current soil at Scott site) (Table C.5.2). This result indicates that the persistence of the introduced AMF inoculant could be underestimated. Hundreds of community studies dealing with a large number of samples that employed pooling replicates or avoided replicates have been published (Prosser, 2010). Composite soil samples (i.e., pooling several replicates or bulking multiple soil samples) are a probable solution to reduce the cost and efforts of microbial community analyses ((Baker et al., 2009), and also lessen the analytical workload in the laboratory (Wollum, 1994) compared to handling individual sample analysis; however, they can result in inaccurate data interpretation.

The results from this study clearly indicate that non-pooled replicates resulted in a better understanding of the changes in indigenous AMF community composition and diversity compared to pooling replicates. Using non-pooled replicates resulted in the detection of minor AMF taxa, enhancing the estimates of diversity of the samples. Several recent studies that explored AMF communities associated with the crops grown across Chernozemic soils of western Canadian Prairies have shown highly diverse AMF communities in crop soils revealed by pyrosequencing and by maintaining replicated soil sampling procedures (Dai et al., 2013, 2014; Bainard et al., 2014a, 2014b).

## 5.7 Conclusions

High-throughput NGS platforms are effective for assessing AMF communities. However, misleading conclusions can occur without knowing the methodological biases, limitations and challenges of appropriate choices of molecular primers and platform during the handling of large-scale sequence reads like pyrosequencing. Moreover, recovering representative DNA samples can be enhanced by using more replicates particularly when the purpose of the study is to understand the variation in fungal community composition and diversity (Lindahl et al., 2013). In the future, automated sample processing together with reduced costs may escalate the scope, possibilities, and statistical power of ecological studies even further.

# CHAPTER 6

# SYNTHESIS, CONCLUSIONS AND FUTURE RESEARCH

This study addressed both the persistence of AMF inoculant, native AMF communities, and crop response to AMF inoculation under field and growth chamber conditions. The results in Chapter 3 suggest that introduction of commercial non-indigenous inoculant *Rhizophagus irregularis* altered the composition and diversity of the indigenous AMF communities in pea trap roots with different effects occurring at different locations. The commercial inoculant strain persisted over the cropping seasons with varying colonization (root occupancy) success in two locations. Chapter 4 demonstrated that application of three AMF inoculants with different geographical sources and genetics conferred different responses in terms of the magnitude of alteration in the indigenous AMF communities. Inoculation also altered the plant shoot nutrient (N and P) uptake and biomass accumulation patterns in lentil, chickpea, and pea. Chapter 5 used data sets from the 2011 and 2013 cropping seasons described in Chapter 3 to evaluate pooling versus non-pooling replicated sampling strategies for analyzing AMF community composition and diversity using pyrosequencing technology. Although pooling can reduce costs and labor, pooling replicated root samples prior to DNA extraction can underestimate the actual richness, diversity, and relative abundance of both indigenous and introduced non-indigenous commercial AMF inoculants compared to non-pooled replications.

## 6.1 Alteration of indigenous AMF communities in response to inoculation

Inoculation significantly altered the composition and diversity of indigenous AMF communities; however, the degree to which alterations occurred was varied and unpredictable given our current state of knowledge. This is because many factors influence indigenous AMF communities such as genetic identity and source of the inoculants, type of soil and climate, number of crop seasons used to assess the impact of inoculants, crop host, and the molecular tools used to assess community changes. In Chapter 3, a commercial inoculant, *R. irregularis* was assessed under field conditions to examine the alteration of indigenous communities associated with field pea crops. It was clear that the commercial, non-indigenous AMF inoculant

strain of *R. irregularis* significantly altered the composition and diversity of the indigenous AMF associated with the trap roots of field pea with varying responses to the different soils and climates. These impacts on the indigenous communities were consistent and observed for up to three consecutive crop seasons.

In Chapter 4, indigenous AMF communities were unaffected by non-commercial SPARC *F. mosseae* B04 strain isolated from Swift Current soil; however, the commercial non-indigenous strain, *R. irregularis* 4514535 significantly altered the composition and diversity of the indigenous AMF communities in field pea trap roots grown in soils collected from field incubated soil cores and lentil, chickpea and field pea under growth chamber conditions. These findings illustrate that the source and genetic identity of AMF inoculants can influence at variable levels to which indigenous AMF communities will be affected. For example, the introduction of the commercial *R. irregularis* inoculant significantly increased the relative abundance of indigenous *Claroideoglomus* and decreased the relative abundance of *Rhizophagus*. The impact of inoculation on these taxa was observed in both field and growth chamber experiments. Changes in abundance of other AMF taxa, such as *Glomus*, *Funneliformis*, and *Paraglomus* in response to commercial *R. irregularis* inoculation were inconsistent for both field and growth chamber conditions. No significant impact from two *F. mosseae* strains (SPARC and GINCO inoculants) on the abundance of indigenous *Rhizophagus, Glomus, Funneliformis,* and *Claroideoglomus* was observed. Regardless of inoculant source and genetic identity, suppression and or removal of minor AMF taxa such as *Septoglomus*, *Diversispora*, and *Archaeospora* in response to inoculation was common in field and growth chamber studies.

It can be concluded that for AMF inoculant, commercial non-indigenous sources have more of an impact on the existing indigenous communities compared to indigenous inoculants isolated or adapted to the target soils. However, introducing non-indigenous commercial AMF inoculants can significantly disrupt existing indigenous AMF communities over multiple cropping seasons. The magnitude of disruption varies depending on location which suggests that differences in soils or climatic conditions can have an impact of responses to inoculation. Additionally, the inoculant types can have an impact on responses to inoculation. Numerous studies demonstrated that the indigenous community diversity and composition were frequently and significantly altered due to various agricultural management practices. These management

practices were often physical disturbances such as ploughing, chemical application, fallowing, etc (Boddington and Dodd, 2000; Girvan et al., 2004; Kabir et al., 2004; Johnson et al., 1993). Inoculation with an AMF strain is a biological disturbance, releasing new genetic materials into a soil habitat. A common effect of introducing new genetic material is that crossing between introduced and local populations results in generation of a population with lower fitness (Edmands, 1999; Colard et al., 2011; Verbruggen et al., 2012). Depression in communities usually caused by genetic exchange between indigenous and non-indigenous AMF strains can result in the loss of local genetic resources in cropped soils. The loss of genetic resources can either increase or decrease the AMF-host symbiotic association (Colard et al., 2011) and this warrants further research investigation.

### 6.2 Impact of inoculation and subsequent changes of indigenous AMF on crop productivity

The ultimate purpose of AMF inoculation is to enhance crop productivity by increasing nutrient uptake and biomass accumulation. Inoculation in cropping systems has increased in recent years, although the impact on the existing indigenous AMF communities remains unknown. Most of the colonization between AMF and crops in nature is inherently beneficial for cropping systems. AMF inoculation is required to stimulate root colonization, especially in degraded and low fertility soils, when the density of indigenous AMF communities is low (Verbruggen et al., 2013).

In Chapter 4, it was demonstrated that the root occupancy of inoculant in crop roots was negatively correlated with nutrient uptake and biomass accumulation. This result suggests that a root colonization due to inoculation may not always lead to increased growth, nutrient uptake, and biomass production. Results demonstrated that the altered abundance of indigenous AMF taxa (in particular *Rhizophagus* and *Funneliformis*) and AMF diversity were significantly and positively correlated with shoot N, P uptake and biomass accumulation. These relationships were consistent for three test crops (lentil, chickpea and field pea). In contrast, the relative abundance of *Glomus* following inoculation was significantly and negatively correlated with the biomass accumulation of lentil, chickpea, and field pea. These findings suggest that inoculation may not directly contribute to plant productivity but may contribute indirectly from the alteration of the indigenous communities. This study separately estimated the abundance of introduced AMF

inoculant strains, *R. irregularis*, SPARC *F. mosseae* and GINCO *F. mosseae* from the abundance of indigenous AMF communities in roots. Thus, it established a potential relationship between the relative abundance of introduced and indigenous taxa, and the crop growth parameters.

The abundance of indigenous AMF communities in root assemblages, rather than the abundance of introduced inoculants following inoculation apparently influenced crop responses. Importantly, SPARC *F. mosseae* did not significantly affect the indigenous communities although, shoot nutrients (N and P) and biomass accumulation were enhanced. In contrast, inoculation with a commercial non-indigenous *R. irregularis* strain did not promote plant growth parameters and significantly altered the indigenous AMF communities in root assemblages. Moreover, some reports indicate non-indigenous commercial AMF outperformed indigenous fungi (Trent et al., 1993; Calvente et al., 2004). Inoculants that are locally isolated on-farm or indigenous to a particular soil or site are often more effective mutualists than non-indigenous fungi (Douds et al., 2000).

### 6.3 The fate and significance of introduced AMF inoculant strain and its impact on the indigenous AMF communities and plant productivity

The importance of monitoring introduced AMF inoculants in soil has increased in recent years in order to verify inoculation success and to identify the contribution to plant productivity. However, it is difficult to detect the introduced strain in colonized plant roots due to the complex genetic nature, especially when polymorphism occurs from interactions between indigenous and non-indigenous strains in soils and roots (Pawlowska and Taylor, 2005; Croll et al., 2009; Beaudet et al., 2015). Pellegrino et al. (2012) used T-RFLP-cloning and Sykovora et al. (2012) used PCR-mt-LSU-cloning together with sequencing technologies to detect introduced inoculant strains in field conditions. A recent study demonstrated that an introduced AMF inoculant (*R. irregularis*) was detected among the OTUs generated from indigenous AMF taxa in field pea and chickpea roots three months after inoculation using 18S rRNA gene pyrosequencing technology (Jin et al., 2013b). In the current research, the 454 pyrosequencing technology was useful to identify OTUs generated from introduced inoculants since those OTUs were not detected in field pea, lentil, and chickpea roots of uninoculated treatments in field and growth chamber conditions.

In Chapter 3, the inoculant, *R. irregularis*, applied in soil cores at four locations across Saskatchewan Prairies was successfully detected at harvest in the first two consecutive cropping seasons (2011 and 2012) at all four locations. The persistence of the introduced inoculant in pea trap roots in terms of relative abundance gradually decreased over the crop seasons. The degree of persistence also varied between sites. These results clearly demonstrated that prolonged survival of commercial non-indigenous AMF inoculant depends on the soil properties and climate conditions where soil cores were installed. The persistence of the inoculant for three consecutive crop seasons was limited to the Swift Current Brown and Outlook Dark Brown soils. Scott Dark Brown and Melfort Black soils supported inoculant persistence for year 1 and year 2, but not year 3. Organic matter and moisture gradient increases from the southern west side (Brown soil) to northern east side (Black soil) of Saskatchewan Prairies suggest that cooler temperature and higher soil moisture content support high organic matter accumulation and decomposition (Less Fuller, 2010). Various reports have indicated that AMF root colonization decreases with increasing soil fertility (Smith and Read, 1997), especially with high P levels (Sanders, 1975; Jasper et al., 1979; Olsson et al., 2002). The prolonged existence of *R. irregularis* from the initial inoculation for 27 months at Swift Current (Brown) and Outlook (Dark Brown) supports the hypothesis that the high organic matter and highly fertile Melfort (Black) and Scott (Dark Brown) soils do not support the persistence of commercial AMF inoculants over multiple seasons.

It is important to be able to detect introduced inoculants in the presence of indigenous AMF. Without being able to distinguish the introduced AMF taxa, it is hard to determine the contribution from introduced inoculants to plant growth and yield. In the growth chamber study (Chapter 4), the individual abundance of three introduced inoculants in the roots of three crops nine weeks after inoculation was successfully estimated. Thus, it was concluded that the relative abundance of inoculant strains was significantly and negatively correlated with shoot nutrient (N and P) uptake and biomass accumulation. A significant and positive correlation was established between the abundance of indigenous *Rhizophagus* and *Funneliformis* and nutrient uptake and biomass accumulation. Moreover, separating the abundance of each inoculant strain from the indigenous AMF taxa facilitating the assessment of how the indigenous AMF taxa responded to each introduced inoculant compared to the uninoculated control treatments. The results

demonstrated that the introduction of commercial *R. irregularis* inoculant altered the indigenous communities and reduced the AMF diversity index. This apparently resulted in lower nutrient (N and P) uptake and biomass accumulation compared to uninoculated controls in lentil, chickpea, and field pea. In contrast, lower abundance of the inoculant, SPARC *F. mosseae* B04 strain, which was isolated locally, caused minimum disruption to the indigenous AMF communities and had little effect on the diversity indices. As a consequence, inoculated lentil, chickpea and field pea had higher nutrient (N and P) uptake and biomass accumulation. These results indicate that root occupancy by AMF inoculant may not influence plant growth characteristics and may affect these characteristics by altering the indigenous AMF community assemblages. Further long-term investigations focusing on how the altered indigenous communities relate to the promotion of plant productivity are required.

### 6.4 Assessing the impact of pooled and non-pooled replicated samples on estimates of AMF communities using high throughput pyrosequencing technology

Assessing the impact of AMF inoculants on indigenous AMF communities, examining the effect of the interaction between inoculants and indigenous communities on crop productivity, and estimating the persistence of introduced inoculants separated from indigenous AMF taxa in crop roots were undertaken using 454 pyrosequencing technology. An extensive survey reported that during 2009, more than 70% of the published peer-reviewed research articles on microbiology did not use biological replications or combine replicated samples when using molecular methods including pyrosequencing technology (Prosser, 2010). It is important to determine whether the differences between pooled versus non-pooled replications are significant. In Chapter 5, two sampling strategies were evaluated namely, pooling four biological replications of root samples and non-pooling replications before DNA extraction and pyrosequencing from two data sets (2011 and 2013).

This methods study showed that estimates of the relative abundance of major indigenous AMF taxa such as *Glomus*, *Funneliformis*, *Claroideoglomus* were unaffected by pooling and non-pooling root sampling procedures prior to DNA extraction. However, many of the minor AMF taxa such as *Diversispora*, *Archaeospora*, *Septoglomus*, and *Entrophospora* were undetected in pooled samples compared to the non-pooled replicated samples. As a result, the

relative abundance of these less abundant AMF taxa significantly differed between the two sampling strategies. Consequently, the Chao richness and Shannon diversity indices of AMF communities were greatly reduced in the pooled replicated samples. The number of total OTUs, OTUs per sample, and OTUs generated from the introduced inoculant *R. irregularis* were reduced in response to pooling replications. The reduction in the parameters of community analysis was consistent for both data sets from year 1 (2011) and year 3 (2013).

As a consequence of pooling, estimates of the persistence of the introduced inoculants were reduced. For example, *R. irregularis* was detected in 4 out of 16 samples in 2013 from the non-pooled samples compared to 2 out of 16 in pooled samples. The average inoculant persistence in 16 samples was reduced by 11% and 50% in response to pooling in the 2011 and 2013 cropping seasons, respectively.

When analyzing AMF communities in field pea trap roots, the non-pooled replications revealed greater diversity, richness and compositional abundance of indigenous AMF compared to pooled replicates. However, this study did not identify the stage during pyrosequencing at which the variations occurred between pooled and non-pooled replications. The cost, effort, and time for each sample was approximately four times higher when analyzing four replications compared to pooling. It would be reasonable to pool samples when the costs and efforts are minimized and the nature of the study does not include assessing less abundant taxa.

Renker at al. (2006) evaluated three AMF sampling methods. Method 1 used 50 non-pooled DNA replicates, method 2 used 50 pooled amplicon replicates separated following PCR of 50 root DNA samples, and method 3 used 50 pooled DNA replicates passed through separate PCR cycles. Method 2 revealed the highest recovered AMF diversity, was the most economical, and required the least amount of time. However, their results were not verified with high-throughput sequencing technology. These results are supported by Chandler et al. (1997), Reed et al. (2002) and Webster (2003) who assessed bacterial diversity by16S rRNA gene amplification. In this thesis, I did not determine whether pooling each replicated PCR product or pooling replicated DNA prior to PCR would result in the alteration of more AMF taxa and thereby enhance assessing estimates. Thus, it is possible that pooling at a later stage such as after PCR amplification but before sequencing may be advantageous in terms of cost, effort, and time.

Smith and Peay et al. (2014) demonstrated that estimates of α and β diversity of soil fungi were unaffected by pooling PCR replicates prior to sequencing compared to sequencing non-pooled replications. Future research should aim to evaluate more pooling options at different points of sample processing within the pyrosequencing protocol to allow others to make a better decision on sampling efforts.

The results suggested that optimizing the number of replications and pooling those replications at a particular point of analysis have significant implications for future research on efficient AMF community analysis. Whereas pooling samples reduces the analysis cost significantly and decreases efforts for sample processing both in the field and the laboratory, it can result in data misinterpretation. Issues can arise if pooling is not performed at the appropriate stage of analysis, in particular, when community richness, diversity and composition are key variables for the microbial research.

### 6.5 Recommendations and future research directions

The first consideration for inoculation is if the application of AMF inoculant is beneficial, since most agricultural soils harbor sufficient AMF communities to impact plant growth (Abbot and Robson, 1991; Olsson et al., 1999). In particular, diverse indigenous AMF communities are present across Chernozemic soils of the Saskatchewan Prairies (Dai et al., 2013; Dai et al., 2014; Hamel et al., 2013; Bainard et al., 2014a, 2014b). It is important to investigate the feasibility and necessity of AMF inoculant application in order to maximize yields for pulse production systems. There are several simple methods available for testing the level of indigenous AMF inoculum potentials in crop soils such as most probable number (MPN). If the tests support the introduction of more AMF strains, inoculation options should be considered to promote sustainable cropping systems.

This thesis demonstrated that commercial non-indigenous AMF significantly alter the indigenous AMF communities with negative plant growth outcomes. It was beyond the scope of our research to explore the exact cause and mechanisms of how the alterations occurred. How introduced and local AMF populations interact should be further investigated in long-term

research settings focusing on the genetic materials exchange between the introduced and indigenous AMF communities and their consequences on crop productivity.

This research suggested that there was a link between altered indigenous AMF communities, as a consequence of inoculation, and subsequent crop growth and nutrient uptake. Future research should investigate the direct and indirect phenomenon of how AMF inoculants contribute to crop productivity. To address this issue, a comprehensive strategy will be needed to explore how the symbiotic genes are triggered both in AMF strains and host following inoculation. Identification and characterization of the genes will allow for an improved understanding of the actual contribution to functionality (productivity) either from the introduced or indigenous AMF communities, or synergistic effects from both.

The results suggest that local indigenous AMF strains had minimal impact on the indigenous AMF communities, which eventually enhanced crop nutrient uptake and biomass accumulation. Conservation of indigenous AMF resources for the management of biotic integrity in soil has previously been recommended (Trappe 1977; Abbott and Robson, 1982; Douds et al., 2000). Based on the current results, AMF strains isolated from local soils could be used to evaluate growth and yield potentials under different crop, soil and climate conditions.

Inoculation success of commercial *R. irregularis* was higher in the soils in which indigenous *Rhizophagus* was either absent or present only at low levels of abundance. It appears that lower competition led to minimal alteration of the indigenous AMF communities. Previous research reported that selecting AMF taxa for inoculation which taxa are absent in local soils could be an option for promoting AMF inoculation success by filling unoccupied niches (Verbruggen et al., 2012).

Other research demonstrated that AMF inoculation affected rhizosphere bacterial communities associated with pea and tomato roots as well as reduced rhizosphere respiration and protozoa communities (Marschner and Baumann, 2003; Wamberg et al., 2003; Marschner and Timonen, 2005; Lioussanne et al., 2010). It is necessary to continue investigating the influence of AMF inoculants of various sources on the crop rhizosphere microbial communities including indigenous AMF groups and subsequent crop productivity.

Following inoculation and three crop seasons, the commercial strain, *R. irregularis* still persisted in pea trap roots and resulted in the continued alteration of the indigenous AMF taxa. It is not clear what, if any future consequences of this inoculant persistence may have on the indigenous AMF community and crop productivity. Studies of several crop rotation cycles beyond three years should be performed to understand the impacts of AMF inoculation on the indigenous microbial community structure and the subsequent crop yield. Permanent research plots under commercial cropping management systems should be established to investigate this topic. Conservation of indigenous AMF genetic resources is important for environmental and sustainable management of crop production systems. It is still not known whether genetic exchange between indigenous and introduced strains could lead to a change in local genetic resources (indigenous genetic pool), which are inherently beneficial for cropping systems.

From the findings of Chapter 5 on the pooling and non-pooling sampling strategy, it should be noted that high throughput pyrosequencing technology produces millions of sequence reads which are challenging to process. Biases and errors are common at various points throughout the sample collection to sequences (OTUs) recovery using bioinformatics. In microbiology experiments, it is important to increase the number of biological and technical replications. At the later stage of sample processing for PCR library preparation, the biological replications could be pooled; however, it is important to understand the mechanism of how variations occurred between pooled and non-pooled replicated sampling strategies. In particular, at which stage of molecular events in pyrosequencing protocols, pooling replications can be performed with efficient manner warrants further investigation.

## 7.0 References


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# Appendices

**Appendix A:** Absolute and relative abundance of AMF taxa (indigenous and introduced inoculant) in 2011 to 2013 field incubation study.

**Table A.3.1**. Relative abundance of indigenous and introduced AMF inoculant taxa representing 86 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in year 1 (2011) at four sites in Saskatchewan.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Septoglomus* | *Claroideoglomus* | *Diversispora* | *Archaeospora* | *Paraglomus* | Absolute abundance of indigenous AMF taxa | Relative abundance of inoculant | Relative abundance of indigenous AMF taxa | Absolute abundance of inoculant | Total absolute abundance of AMF taxa |
| SC | SC | U | 0.00 | 0.06 | 0.32 | 0.00 | 0.53 | 0.00 | 0.10 | 0.00 | 782 | 0 | 0 | 0 | 782 |
| | SC | I | 0.00 | 0.06 | 0.40 | 0.00 | 0.54 | 0.00 | 0.00 | 0.00 | 596 | 0.27 | 0.73 | 219 | 815 |
| | OL | U | 0.06 | 0.25 | 0.51 | 0.00 | 0.16 | 0.00 | 0.00 | 0.02 | 1272 | 0 | 0 | 0 | 1272 |
| | OL | I | 0.00 | 0.13 | 0.65 | 0.00 | 0.18 | 0.00 | 0.00 | 0.03 | 796 | 0.12 | 0.88 | 112 | 908 |
| | ST | U | 0.00 | 0.03 | 0.51 | 0.00 | 0.28 | 0.00 | 0.00 | 0.18 | 722 | 0 | 0 | 0 | 722 |
| | ST | I | 0.00 | 0.00 | 0.52 | 0.00 | 0.29 | 0.00 | 0.00 | 0.19 | 679 | 0.11 | 0.89 | 81 | 760 |
| | MF | U | 0.11 | 0.12 | 0.58 | 0.00 | 0.06 | 0.00 | 0.00 | 0.13 | 942 | 0 | 0 | 0 | 942 |
| | MF | I | 0.00 | 0.08 | 0.71 | 0.00 | 0.06 | 0.00 | 0.00 | 0.15 | 733 | 0.10 | 0.90 | 77 | 810 |
| OL | SC | U | 0.00 | 0.12 | 0.34 | 0.00 | 0.51 | 0.03 | 0.00 | 0.00 | 647 | 0 | 0 | 0 | 647 |
| | SC | I | 0.00 | 0.00 | 0.56 | 0.00 | 0.44 | 0.00 | 0.00 | 0.00 | 555 | 0.29 | 0.71 | 224 | 779 |
| | OL | U | 0.06 | 0.20 | 0.37 | 0.00 | 0.17 | 0.11 | 0.03 | 0.06 | 1166 | 0 | 0 | 0 | 1166 |
| | OL | I | 0.04 | 0.22 | 0.56 | 0.00 | 0.13 | 0.00 | 0.02 | 0.04 | 977 | 0.17 | 0.83 | 206 | 1183 |
| | ST | U | 0.00 | 0.16 | 0.28 | 0.04 | 0.23 | 0.00 | 0.00 | 0.29 | 1256 | 0 | 0 | 0 | 1256 |
| | ST | I | 0.00 | 0.14 | 0.37 | 0.00 | 0.28 | 0.00 | 0.00 | 0.21 | 985 | 0.23 | 0.77 | 297 | 1282 |
| | MF | U | 0.04 | 0.24 | 0.55 | 0.00 | 0.05 | 0.00 | 0.02 | 0.10 | 1378 | 0 | 0 | 0 | 1378 |
| | MF | I | 0.00 | 0.22 | 0.55 | 0.00 | 0.10 | 0.00 | 0.00 | 0.13 | 1093 | 0.16 | 0.84 | 201 | 1294 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort. U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF).

**Table A.3.1** Continued.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Septoglomus* | *Claroideoglomus* | *Diversispora* | *Archaeospora* | *Paraglomus* | Absolute abundance of indigenous AMF taxa | Relative abundance of inoculant | Relative abundance of indigenous AMF taxa | Absolute abundance of inoculant | Total absolute abundance of AMF taxa |
| ST | SC | U | 0.00 | 0.07 | 0.63 | 0.00 | 0.26 | 0.00 | 0.04 | 0.00 | 505 | 0 | 0 | 0 | 505 |
| | SC | I | 0.00 | 0.06 | 0.66 | 0.00 | 0.25 | 0.00 | 0.03 | 0.00 | 438 | 0.10 | 0.90 | 48 | 486 |
| | OL | U | 0.07 | 0.16 | 0.25 | 0.09 | 0.09 | 0.17 | 0.09 | 0.09 | 1230 | 0 | 0 | 0 | 1230 |
| | OL | I | 0.00 | 0.24 | 0.35 | 0.00 | 0.31 | 0.00 | 0.00 | 0.10 | 585 | 0.15 | 0.85 | 100 | 685 |
| | ST | U | 0.00 | 0.09 | 0.27 | 0.04 | 0.34 | 0.00 | 0.03 | 0.23 | 2431 | 0 | 0 | 0 | 2431 |
| | ST | I | 0.00 | 0.07 | 0.33 | 0.00 | 0.39 | 0.00 | 0.00 | 0.21 | 1825 | 0.33 | 0.67 | 895 | 2720 |
| | MF | U | 0.03 | 0.18 | 0.68 | 0.00 | 0.03 | 0.00 | 0.08 | 0.00 | 1375 | 0 | 0 | 0 | 1375 |
| | MF | I | 0.00 | 0.14 | 0.73 | 0.00 | 0.01 | 0.00 | 0.12 | 0.00 | 976 | 0.17 | 0.83 | 201 | 1177 |
| MF | SC | U | 0.00 | 0.13 | 0.56 | 0.00 | 0.32 | 0.00 | 0.00 | 0.00 | 576 | 0 | 0 | 0 | 576 |
| | SC | I | 0.00 | 0.05 | 0.65 | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 | 478 | 0.07 | 0.93 | 37 | 515 |
| | OL | U | 0.06 | 0.18 | 0.58 | 0.00 | 0.18 | 0.00 | 0.00 | 0.00 | 1187 | 0 | 0 | 0 | 1187 |
| | OL | I | 0.00 | 0.20 | 0.56 | 0.00 | 0.24 | 0.00 | 0.00 | 0.00 | 876 | 0.15 | 0.85 | 157 | 1033 |
| | ST | U | 0.00 | 0.16 | 0.36 | 0.06 | 0.19 | 0.00 | 0.04 | 0.19 | 1202 | 0 | 0 | 0 | 1202 |
| | ST | I | 0.00 | 0.13 | 0.41 | 0.02 | 0.22 | 0.00 | 0.00 | 0.22 | 944 | 0.32 | 0.68 | 444 | 1388 |
| | MF | U | 0.15 | 0.14 | 0.52 | 0.00 | 0.04 | 0.00 | 0.07 | 0.08 | 3057 | 0 | 0 | 0 | 3057 |
| | MF | I | 0.06 | 0.16 | 0.61 | 0.00 | 0.07 | 0.00 | 0.00 | 0.10 | 1484 | 0.19 | 0.81 | 358 | 1842 |
| **Total absolute sequence reads (indigenous AMF and inoculant):** | | | | | | | | | | | **33 748** | | | **3 657** | **37 405** |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort. U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF). Skewness: 1.63, (<1.96 at α=0.05%, data is normally distributed), Kurtosis: 1.82, Ref.: Kim (2013).

**Table A.3.2.** Relative abundance of indigenous and introduced AMF inoculant taxa representing 30 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in year 2 (2012) at four sites in Saskatchewan.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Claroideoglomus* | *Diversispora* | *Entrophospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of AMF taxa |
| SC | SC | U | 0.00 | 0.48 | 0.00 | 0.43 | 0.00 | 0.10 | 0.00 | 376 | 0 | 0 | 0 | 376 |
| | SC | I | 0.00 | 0.44 | 0.00 | 0.25 | 0.00 | 0.02 | 0.29 | 2794 | 0.15 | 0.85 | 491 | 3285 |
| | OL | U | 0.00 | 0.00 | 0.75 | 0.25 | 0.00 | 0.00 | 0.00 | 1204 | 0 | 0 | 0 | 1204 |
| | OL | I | 0.00 | 0.00 | 0.62 | 0.38 | 0.00 | 0.00 | 0.00 | 89 | 0 | 0 | 0 | 89 |
| | ST | U | 0.00 | 0.00 | 0.00 | 0.64 | 0.13 | 0.00 | 0.23 | 329 | 0 | 0 | 0 | 329 |
| | ST | I | 0.00 | 0.00 | 0.00 | 0.67 | 0.08 | 0.00 | 0.25 | 1245 | 0 | 0 | 0 | 1245 |
| | MF | U | 0.00 | 0.00 | 0.60 | 0.40 | 0.00 | 0.00 | 0.00 | 566 | 0 | 0 | 0 | 566 |
| | MF | I | 0.00 | 0.00 | 0.54 | 0.46 | 0.00 | 0.00 | 0.00 | 1451 | 0 | 0 | 0 | 1451 |
| OL | SC | U | 0.00 | 0.13 | 0.07 | 0.80 | 0.00 | 0.00 | 0.00 | 245 | 0 | 0 | 0 | 245 |
| | SC | I | 0.00 | 0.41 | 0.01 | 0.58 | 0.00 | 0.00 | 0.00 | 1546 | 0 | 0 | 0 | 1546 |
| | OL | U | 0.05 | 0.00 | 0.83 | 0.09 | 0.00 | 0.03 | 0.00 | 2220 | 0 | 0 | 0 | 2220 |
| | OL | I | 0.02 | 0.00 | 0.54 | 0.10 | 0.32 | 0.03 | 0.00 | 412 | 0.05 | 0.95 | 21 | 433 |
| | ST | U | 0.00 | 0.00 | 0.00 | 0.70 | 0.00 | 0.00 | 0.30 | 267 | 0 | 0 | 0 | 267 |
| | ST | I | 0.00 | 0.00 | 0.00 | 0.65 | 0.06 | 0.00 | 0.29 | 925 | 0 | 0 | 0 | 925 |
| | MF | U | 0.00 | 0.00 | 0.77 | 0.23 | 0.00 | 0.00 | 0.00 | 1033 | 0 | 0 | 0 | 1033 |
| | MF | I | 0.00 | 0.00 | 0.71 | 0.29 | 0.00 | 0.00 | 0.00 | 1539 | 0 | 0 | 0 | 1539 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. Abbreviation: SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort. U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF).

**Table A.3.2.** Continued

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Claroideoglomus* | *Diversispora* | *Entrophospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of AMF taxa |
| ST | SC | U | 0.00 | 0.76 | 0.00 | 0.24 | 0.00 | 0.00 | 0.00 | 394 | 0 | 0 | 0 | 394 |
| | SC | I | 0.00 | 0.46 | 0.00 | 0.54 | 0.00 | 0.00 | 0.00 | 438 | 0.05 | 0.95 | 21 | 459 |
| | OL | U | 0.00 | 0.00 | 0.60 | 0.40 | 0.00 | 0.00 | 0.00 | 578 | 0 | 0 | 0 | 578 |
| | OL | I | 0.00 | 0.00 | 0.53 | 0.47 | 0.00 | 0.00 | 0.00 | 164 | 0.04 | 0.96 | 7 | 171 |
| | ST | U | 0.00 | 0.00 | 0.00 | 0.63 | 0.00 | 0.00 | 0.37 | 208 | 0 | 0 | 0 | 208 |
| | ST | I | 0.00 | 0.00 | 0.00 | 0.72 | 0.05 | 0.00 | 0.23 | 1366 | 0 | 0 | 0 | 1366 |
| | MF | U | 0.16 | 0.00 | 0.22 | 0.62 | 0.00 | 0.00 | 0.00 | 637 | 0 | 0 | 0 | 637 |
| | MF | I | 0.00 | 0.00 | 0.59 | 0.41 | 0.00 | 0.00 | 0.00 | 1063 | 0 | 0 | 0 | 1063 |
| MF | SC | U | 0.00 | 0.31 | 0.00 | 0.69 | 0.00 | 0.00 | 0.00 | 286 | 0 | 0 | 0 | 286 |
| | SC | I | 0.00 | 0.26 | 0.00 | 0.74 | 0.00 | 0.00 | 0.00 | 1169 | 0 | 0 | 0 | 1169 |
| | OL | U | 0.00 | 0.00 | 0.74 | 0.26 | 0.00 | 0.00 | 0.00 | 1419 | 0 | 0 | 0 | 1419 |
| | OL | I | 0.00 | 0.00 | 0.49 | 0.51 | 0.00 | 0.00 | 0.00 | 100 | 0.15 | 0.85 | 17 | 117 |
| | ST | U | 0.00 | 0.00 | 0.00 | *0.83* | 0.00 | 0.00 | 0.17 | 372 | 0 | 0 | 0 | 372 |
| | ST | I | 0.00 | 0.00 | 0.00 | 0.79 | 0.05 | 0.00 | 0.17 | 741 | 0 | 0 | 0 | 741 |
| | MF | U | 0.26 | 0.00 | 0.62 | 0.12 | 0.00 | 0.00 | 0.00 | 961 | 0 | 0 | 0 | 961 |
| | MF | I | 0.05 | 0.00 | 0.69 | 0.14 | 0.00 | 0.00 | 0.11 | 1868 | 0.04 | 0.96 | 86 | 1954 |
| **Total absolute sequence reads (indigenous AMF and inoculant):** | | | | | | | | | | **28 005** | | | **643** | **28 648** |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. Abbreviation: SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort. U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF).

**Table A.3.3**. Relative abundance (proportional sequence reads) of indigenous and introduced AMF inoculant taxa representing 72 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in year 3 (2013) at four sites in Saskatchewan.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Claroideoglomus* | *Diversispora* | *Entrophospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of AMF taxa |
| SC | SC | U | 0.00 | 0.07 | 0.48 | 0.37 | 0.06 | 0.01 | 0.00 | 2311 | 0 | 0 | 0 | 2311 |
| | SC | I | 0.00 | 0.40 | 0.53 | 0.06 | 0.00 | 0.01 | 0.00 | 2043 | 0.15 | 0.85 | 361 | 2404 |
| | OL | U | 0.00 | 0.20 | 0.53 | 0.17 | 0.00 | 0.10 | 0.00 | 2430 | 0 | 0 | 0 | 2430 |
| | OL | I | 0.00 | 0.23 | 0.65 | 0.12 | 0.00 | 0.00 | 0.00 | 2068 | 0 | 0 | 0 | 2068 |
| | ST | U | 0.00 | 0.42 | 0.41 | 0.11 | 0.00 | 0.05 | 0.00 | 890 | 0 | 0 | 0 | 890 |
| | ST | I | 0.00 | 0.19 | 0.24 | 0.28 | 0.00 | 0.29 | 0.00 | 790 | 0 | 0 | 0 | 790 |
| | MF | U | 0.00 | 0.51 | 0.00 | 0.00 | 0.00 | 0.49 | 0.00 | 689 | 0 | 0 | 0 | 689 |
| | MF | I | 0.00 | 0.10 | 0.29 | 0.00 | 0.00 | 0.61 | 0.00 | 645 | 0 | 0 | 0 | 645 |
| OL | SC | U | 0.00 | 0.39 | 0.20 | 0.00 | 0.40 | 0.00 | 0.00 | 1158 | 0 | 0 | 0 | 1158 |
| | SC | I | 0.00 | 0.25 | 0.29 | 0.00 | 0.47 | 0.00 | 0.00 | 1515 | 0 | 0 | 0 | 1515 |
| | OL | U | 0.02 | 0.13 | 0.50 | 0.02 | 0.26 | 0.03 | 0.05 | 2337 | 0 | 0 | 0 | 2337 |
| | OL | I | 0.00 | 0.02 | 0.06 | 0.77 | 0.10 | 0.02 | 0.03 | 2245 | 0.03 | 0.97 | 81 | 2326 |
| | ST | U | 0.00 | 0.26 | 0.28 | 0.27 | 0.00 | 0.19 | 0.00 | 698 | 0 | 0 | 0 | 698 |
| | ST | I | 0.00 | 0.09 | 0.56 | 0.18 | 0.17 | 0.00 | 0.00 | 657 | 0 | 0 | 0 | 657 |
| | MF | U | 0.00 | 0.56 | 0.44 | 0.00 | 0.00 | 0.00 | 0.00 | 1560 | 0 | 0 | 0 | 1560 |
| | MF | I | 0.00 | 0.00 | 0.24 | 0.70 | 0.06 | 0.00 | 0.00 | 804 | 0 | 0 | 0 | 804 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort. U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF).

**Table A.3.3**. Continued.

| Site | Soil | Inoculation | Relative abundance of indigenous AMF taxa (%) | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Rhizophagus* | *Glomus* | *Funneliformis* | *Claroideoglomus* | *Diversispora* | *Entrophospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of inoculant and indigenous taxa |
| ST | SC | U | 0.03 | 0.06 | 0.52 | 0.19 | 0.08 | 0.12 | 0.00 | 842 | 0 | 0 | 0 | 842 |
| | SC | I | 0.00 | 0.05 | 0.84 | 0.11 | 0.00 | 0.00 | 0.00 | 1177 | 0.10 | 0.90 | 131 | 1308 |
| | OL | U | 0.05 | 0.00 | 0.30 | 0.56 | 0.10 | 0.00 | 0.00 | 774 | 0 | 0 | 0 | 774 |
| | OL | I | 0.00 | 0.00 | 0.73 | 0.27 | 0.00 | 0.00 | 0.00 | 699 | 0 | 0 | 0 | 699 |
| | ST | U | 0.00 | 0.78 | 0.00 | 0.00 | 0.00 | 0.00 | 0.22 | 320 | 0 | 0 | 0 | 320 |
| | ST | I | 0.00 | 0.61 | 0.00 | 0.00 | 0.00 | 0.00 | 0.39 | 212 | 0 | 0 | 0 | 212 |
| | MF | U | 0.00 | 0.15 | 0.73 | 0.00 | 0.04 | 0.08 | 0.00 | 1495 | 0 | 0 | 0 | 1495 |
| | MF | I | 0.00 | 0.00 | 0.79 | 0.21 | 0.00 | 0.00 | 0.00 | 1132 | 0 | 0 | 0 | 1132 |
| MF | SC | U | 0.00 | 0.51 | 0.36 | 0.05 | 0.08 | 0.00 | 0.00 | 2140 | 0 | 0 | 0 | 2140 |
| | SC | I | 0.00 | 0.53 | 0.43 | 0.00 | 0.00 | 0.04 | 0.00 | 1364 | 0 | 0 | 0 | 1364 |
| | OL | U | 0.00 | 0.11 | 0.37 | 0.52 | 0.00 | 0.00 | 0.00 | 1488 | 0 | 0 | 0 | 1488 |
| | OL | I | 0.00 | 0.20 | 0.29 | 0.52 | 0.00 | 0.00 | 0.00 | 1469 | 0.14 | 0.86 | 234 | 1703 |
| | ST | U | 0.00 | 0.20 | 0.66 | 0.00 | 0.00 | 0.14 | 0.00 | 607 | 0 | 0 | 0 | 607 |
| | ST | I | 0.00 | 0.28 | 0.15 | 0.42 | 0.00 | 0.15 | 0.00 | 640 | 0 | 0 | 0 | 640 |
| | MF | U | 0.00 | 0.58 | 0.28 | 0.13 | 0.00 | 0.00 | 0.01 | 2587 | 0 | 0 | 0 | 2587 |
| | MF | I | 0.00 | 0.18 | 0.69 | 0.10 | 0.00 | 0.00 | 0.03 | 1581 | 0 | 0 | 0 | 1581 |
| **Total absolute sequence reads (indigenous AMF and inoculant):** | | | | | | | | | | **41 367** | | | **807** | **42 174** |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort. U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF). Skewness: 1.49 and Kurtosis: 1.22.

**Table A.3.4**. Absolute sequence reads of indigenous and introduced AMF inoculant taxa representing 86 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in year 1 (2011) at four sites in Saskatchewan.

| AMF | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| R | 55 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42 | 21 |
| R | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 |
| R | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0 |
| R | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 56 |
| R | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 0 |
| R | 34 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 127 | 14 |
| R | 13 | 0 | 0 | 25 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 41 | 0 |
| R | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 75 | 0 | 0 | 0 | 41 | 0 |
| R | 75 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 75 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 |
| G | 69 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 0 | 3 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 |
| G | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| G | 5 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 213 | 0 | 136 | 325 | 142 | 0 | 25 | 90 | 44 | 100 | 100 | 55 | 0 | 0 | 0 | 46 | 0 | 0 | 0 | 0 | 100 |
| G | 72 | 0 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 98 |
| G | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 |
| G | 17 | 0 | 0 | 102 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 | 0 |
| G | 74 | 0 | 0 | 73 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 4 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 11 | 0 | 0 | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 207 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 103 | 0 |
| G | 64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 65 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 175 | 190 | 123 | 78 | 0 |
| G | 58 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 61 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 |
| G | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 |
| G | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| G | 43 | 15 | 14 | 0 | 106 | 12 | 0 | 0 | 23 | 78 | 0 | 108 | 0 | 0 | 0 | 0 | 100 | 35 | 0 | 104 | 0 | 108 | 36 | 52 | 36 | 73 | 23 | 46 | 0 | 0 | 0 | 56 | 0 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, and P: *Paraglomus*.

**Table A.3.4.** Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| F | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 401 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 37 | 0 | 0 | 225 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 0 |
| F | 39 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 130 | 0 | 0 | 0 | 0 | 0 | 0 | 83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 57 | 0 | 0 | 0 | 0 | 0 | 66 | 0 | 0 | 0 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 | 97 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 |
| F | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 359 | 0 | 0 | 0 | 291 | 0 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 52 | 100 | 0 | 0 | 0 | 0 | 102 | 0 | 0 | 0 | 170 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 117 | 0 | 0 | 143 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 227 |
| F | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 0 | 667 | 0 |
| F | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 179 | 155 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 359 | 632 |
| F | 79 | 100 | 0 | 650 | 520 | 0 | 0 | 532 | 348 | 0 | 0 | 0 | 0 | 0 | 365 | 349 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 0 | 0 | 0 | 0 | 0 | 0 | 49 | 0 |
| F | 22 | 0 | 0 | 0 | 0 | 367 | 0 | 10 | 158 | 0 | 0 | 0 | 0 | 0 | 0 | 403 | 0 | 0 | 0 | 0 | 206 | 199 | 307 | 930 | 0 | 320 | 312 | 687 | 0 | 0 | 0 | 45 | 0 |
| F | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 290 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 491 | 0 | 0 | 36 | 0 |
| F | 41 | 48 | 240 | 0 | 0 | 0 | 150 | 0 | 0 | 0 | 0 | 0 | 55 | 352 | 0 | 0 | 105 | 318 | 0 | 105 | 0 | 156 | 0 | 0 | 179 | 0 | 0 | 0 | 0 | 432 | 389 | 337 | 0 |
| S | 77 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 71 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 |
| S | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| S | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | 0 |
| S | 85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| S | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 |
| S | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 95 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 |
| C | 27 | 210 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 245 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 |
| C | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 29 | 201 | 0 | 201 | 135 | 205 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 247 | 0 | 0 | 0 | 0 | 0 | 0 | 838 | 0 | 0 | 0 | 183 | 143 | 0 | 43 | 230 | 208 | 118 | 0 |
| C | 31 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 279 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 45 | 0 | 151 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 231 | 0 | 0 | 0 | 0 | 0 | 0 | 182 | 0 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 160 | 0 | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 110 | 113 | 0 | 0 | 61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 24 | 0 | 149 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 123 | 0 | 0 | 0 | 0 |
| C | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 140 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 81 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 106 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 47 | 0 | 0 | 0 | 10 | 0 | 197 | 57 | 0 | 0 | 0 | 168 | 0 | 0 | 0 | 67 | 68 | 0 | 0 | 0 | 0 | 0 | 108 | 47 | 13 | 0 | 0 | 212 | 0 | 0 | 0 | 0 | 104 |
| D | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 128 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 206 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, and P: *Paraglomus*.

**Table A.3.4.** Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| A | 67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | 33 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 44 | 0 | 19 | 13 | 68 | 0 | 20 | 0 | 28 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 |
| A | 59 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 64 | 0 |
| A | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | 66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 33 | 0 |
| A | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 10 | 0 |
| A | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |
| A | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 |
| A | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| A | 49 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 52 | 0 |
| P | 73 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 10 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 0 | 0 | 0 | 0 | 0 | 0 | 43 | 0 | 0 | 0 |
| P | 6 | 0 | 0 | 0 | 0 | 0 | 19 | 16 | 0 | 0 | 0 | 0 | 0 | 67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 42 | 0 | 0 | 0 | 0 | 17 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 |
| P | 86 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 8 | 0 | 0 | 0 | 10 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 |
| P | 82 | 0 | 0 | 0 | 8 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 |
| P | 25 | 0 | 0 | 9 | 0 | 45 | 16 | 83 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 |
| P | 2 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 109 | 0 | 0 | 0 | 0 | 19 | 23 | 134 | 0 | 0 | 0 | 116 | 0 | 504 | 123 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 209 | 0 | 0 |
| P | 62 | 0 | 0 | 11 | 0 | 24 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 |
| P | 83 | 0 | 0 | 0 | 7 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 123 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 124 | 0 | 0 | 0 |
| P | 35 | 0 | 0 | 10 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 67 | 36 | 0 | 0 | 0 | 141 | 0 | 0 | 0 | 58 | 0 | 65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 251 | 141 |
| IG-A | | 782 | 596 | 1272 | 796 | 722 | 679 | 942 | 733 | 647 | 555 | 1166 | 977 | 1256 | 985 | 1378 | 1093 | 505 | 438 | 1230 | 585 | 2431 | 1825 | 1375 | 976 | 576 | 478 | 1187 | 876 | 1202 | 944 | 3057 | 1484 |
| IC-A | 1 | 0 | 185 | 0 | 36 | 0 | 22 | 0 | 12 | 0 | 52 | 0 | 81 | 0 | 125 | 0 | 48 | 0 | 21 | 0 | 23 | 0 | 23 | 0 | 78 | 0 | 15 | 0 | 59 | 0 | 58 | 0 | 0 |
| IC-A | 3 | 0 | 23 | 0 | 25 | 0 | 24 | 0 | 32 | 0 | 73 | 0 | 88 | 0 | 0 | 0 | 66 | 0 | 0 | 0 | 0 | 0 | 422 | 0 | 61 | 0 | 22 | 0 | 53 | 0 | 79 | 0 | 105 |
| IC-A | 37 | 0 | 11 | 0 | 26 | 0 | 28 | 0 | 33 | 0 | 99 | 0 | 37 | 0 | 114 | 0 | 87 | 0 | 27 | 0 | 32 | 0 | 233 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 99 | 0 | 34 |
| IC-A | 84 | 0 | 0 | 0 | 25 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 217 | 0 | 62 | 0 | 0 | 0 | 0 | 0 | 208 | 0 | 219 |
| IC-AT | | 0 | 219 | 0 | 112 | 0 | 81 | 0 | 77 | 0 | 224 | 0 | 206 | 0 | 297 | 0 | 201 | 0 | 48 | 0 | 100 | 0 | 895 | 0 | 201 | 0 | 37 | 0 | 157 | 0 | 444 | 0 | 358 |
| (IG-A+IC-A)-T | | 782 | 815 | 1272 | 908 | 722 | 760 | 942 | 810 | 647 | 779 | 1166 | 1183 | 1256 | 1282 | 1378 | 1294 | 505 | 486 | 1230 | 685 | 2431 | 2720 | 1375 | 1177 | 576 | 515 | 1187 | 1033 | 1202 | 1388 | 3057 | 1842 |
| **Total absolute sequence reads (indigenous AMF and inoculant): 37 405** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*. IG-A: Absolute abundance of indigenous sequence reads, IC-A: Absolute abundance of inoculant sequence reads, IC-AT: Absolute abundance of inoculant sequence reads total and (IC-A+IG-A)-T: Inoculant and indigenous absolute abundance sequence reads total.

**Table A.3.5**. Absolute sequence reads of indigenous and introduced AMF inoculant taxa representing 30 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in year 2 (2012) at four sites in Saskatchewan.

| AMF | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| R | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 253 | 100 |
| G | 1 | 38 | 165 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 155 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 5 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 2 | 0 | 167 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 4 | 142 | 839 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 479 | 0 | 0 | 0 | 0 | 0 | 0 | 277 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 207 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 3 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 20 | 0 | 0 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 135 | 0 | 0 | 0 | 327 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 57 |
| F | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 107 | 0 | 0 | 0 | 146 | 0 | 0 | 0 | 146 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 204 |
| F | 26 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 0 | 19 | 72 | 0 | 0 | 0 | 256 | 0 | 0 | 0 | 90 | 0 | 0 | 0 | 0 | 110 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 244 |
| F | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 43 | 0 | 0 | 269 | 109 | 0 | 0 | 0 | 165 | 0 | 0 | 0 | 35 | 0 | 0 | 97 | 0 | 0 | 0 | 86 | 0 | 0 | 0 | 0 | 0 |
| F | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 59 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 123 | 0 |
| F | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 6 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 134 |
| F | 6 | 0 | 0 | 156 | 0 | 0 | 0 | 54 | 231 | 0 | 0 | 106 | 0 | 0 | 0 | 63 | 119 | 0 | 0 | 259 | 52 | 0 | 0 | 42 | 457 | 0 | 0 | 779 | 49 | 0 | 0 | 435 | 659 |
| F | 17 | 0 | 0 | 680 | 55 | 209 | 0 | 145 | 457 | 0 | 0 | 1089 | 106 | 0 | 0 | 0 | 793 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 0 | 0 | 135 | 0 | 0 | 0 | 0 | 0 |
| C | 14 | 0 | 131 | 0 | 0 | 0 | 0 | 0 | 500 | 0 | 246 | 201 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 304 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 |
| C | 29 | 0 | 0 | 0 | 0 | 0 | 230 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 124 | 0 | 0 | 0 | 0 | 77 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 16 | 0 | 0 | 0 | 0 | 0 | 63 | 0 | 161 | 0 | 258 | 0 | 0 | 0 | 0 | 103 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 |
| C | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 347 | 0 | 215 | 0 | 0 | 229 | 0 | 0 | 0 | 0 | 0 | 0 | 862 | 0 | 0 | 307 | 0 | 0 | 259 |
| C | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 146 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 196 | 0 | 0 | 0 | 0 | 107 | 37 | 0 |
| C | 15 | 0 | 334 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 226 | 0 | 235 | 0 | 0 | 131 | 679 | 0 | 431 | 0 | 0 | 376 | 51 | 0 | 0 | 13 | 0 |
| C | 30 | 160 | 235 | 301 | 34 | 0 | 537 | 224 | 0 | 196 | 243 | 0 | 40 | 188 | 256 | 0 | 0 | 96 | 0 | 0 | 0 | 0 | 0 | 398 | 0 | 0 | 0 | 0 | 0 | 0 | 476 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, and P: *Paraglomus*.

**Table A.3.5**. Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| D | 8 | 0 | 0 | 0 | 0 | 0 | 98 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 21 | 0 | 0 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 121 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 34 | 0 | 0 |
| E | 13 | 36 | 62 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 18 | 0 | 0 | 0 | 0 | 0 | 179 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 9 | 0 | 354 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 153 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| P | 11 | 0 | 124 | 0 | 0 | 0 | 127 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 103 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 0 | 10 |
| P | 12 | 0 | 328 | 0 | 0 | 77 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 164 | 0 | 0 | 0 | 0 | 0 | 0 | 77 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 65 | 44 | 0 | 201 |
| IG-A | | 376 | 2794 | 1204 | 89 | 329 | 1245 | 566 | 1451 | 245 | 1546 | 2220 | 412 | 267 | 925 | 1033 | 1539 | 394 | 438 | 578 | 164 | 208 | 1366 | 637 | 1063 | 286 | 1169 | 1419 | 100 | 372 | 741 | 961 | 1868 |
| IC-A | 25 | 0 | 491 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 86 |
| (IG-A+IC-A)-T | | 376 | 3285 | 1204 | 89 | 329 | 1245 | 566 | 1451 | 245 | 1546 | 2220 | 433 | 267 | 925 | 1033 | 1539 | 394 | 459 | 578 | 171 | 208 | 1366 | 637 | 1063 | 286 | 1169 | 1419 | 117 | 372 | 741 | 961 | 1954 |
| **Total absolute sequence reads (indigenous AMF and inoculant): 28 648** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*. IG-A: Absolute abundance of indigenous sequence reads, IC-A: Absolute abundance of inoculant sequence reads, and (IC-A+IG-A)-T: Inoculant and indigenous absolute abundance sequence reads total.

**Table A.3.6**. Absolute sequence reads of indigenous and introduced AMF inoculant taxa representing 72 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing in year 3 (2013) at four sites in Saskatchewan.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| R | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 68 | 55 | 226 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 215 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 37 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 63 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 26 | 0 | 0 | 0 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 456 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 52 | 0 |
| G | 38 | 0 | 90 | 0 | 0 | 0 | 0 | 179 | 0 | 156 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 135 | 0 |
| G | 44 | 0 | 0 | 0 | 0 | 136 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 12 | 0 |
| G | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 179 | 0 | 0 | 0 | 0 | 0 | 0 | 130 | 0 | 0 | 225 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 126 | 0 | 0 | 0 |
| G | 53 | 24 | 0 | 275 | 0 | 0 | 0 | 96 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 7 | 0 | 0 | 0 | 0 | 237 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 8 | 0 | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 320 | 0 |
| G | 61 | 0 | 0 | 0 | 61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 221 | 0 | 160 | 0 | 0 | 0 | 82 | 0 | | |
| G | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| G | 18 | 0 | 0 | 150 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | | |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, and P: *Paraglomus*.

**Table A.3.6**. Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| G | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 110 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 201 | 0 | 0 | 0 | 0 | 0 | 124 | 0 | 0 | 0 |
| G | 28 | 44 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 143 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 720 | 0 | 0 | 0 | 0 | 891 | 0 |
| G | 60 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 62 | 0 | 0 | 0 | 0 | 0 | 0 | 289 | 0 | 0 | 0 | 0 |
| G | 17 | 0 | 499 | 50 | 345 | 0 | 95 | 0 | 42 | 0 | 267 | 150 | 37 | 179 | 62 | 431 | 0 | 0 | 54 | 0 | 0 | 106 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 278 |
| F | 3 | 0 | 557 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 59 | 257 | 230 | 510 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 13 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 147 | 0 |
| F | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 91 | 221 | 89 | 550 | 0 | 0 | 0 | 0 | 0 |
| F | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 38 | 0 |
| F | 42 | 0 | 73 | 89 | 172 | 0 | 0 | 0 | 0 | 0 | 0 | 221 | 0 | 0 | 0 | 449 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 358 | 225 | 0 | 0 | 0 | 0 | 400 | 399 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 9 | 0 | 0 | 0 | 0 | 110 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 |
| F | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 72 | 40 | 199 | 409 | 440 | 257 | 53 | 0 | 189 | 236 | 185 | 0 | 0 | 0 | 287 | 0 | 0 | 0 | 456 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 22 | 0 | 0 | 256 | 556 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 194 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 587 | 401 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 |
| F | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 137 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 |
| F | 2 | 451 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 198 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 64 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 420 | 398 | 0 | 0 | 475 |
| F | 25 | 622 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 66 | 435 | 0 | 0 | 0 | 94 | 24 | 0 |
| F | 69 | 0 | 239 | 462 | 178 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 453 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | 5 | 0 | 0 | 0 | 0 | 0 | 133 | 0 | 0 | 0 | 249 | 642 | 0 | 0 | 0 | 241 | 189 | 0 | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 447 | 578 |
| C | 70 | 66 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 117 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 54 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 145 | 0 | 0 | 0 | 0 | 0 |
| C | 56 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 883 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 14 | 116 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 59 | 77 | 56 | 59 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 258 | 0 | 0 | 0 | 0 | 0 |
| C | 62 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 66 | 226 | 19 | 0 | 241 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 228 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, and P: *Paraglomus*.

**Table A.3.6**. Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | Scot | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| C | 15 | 22 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 57 | | 0 | 101 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 147 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 108 | 0 | 0 |
| C | 41 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 161 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 138 | 0 | 0 | 0 | 0 | 241 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 65 | 0 | 0 | 85 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 223 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 135 | 0 | 0 | 0 | 0 | 0 |
| C | 34 | 0 | 41 | 166 | 0 | 0 | 58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 146 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 160 | 331 | 100 |
| C | 1 | 215 | 0 | 0 | 0 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 202 | 190 | 0 | 0 | 565 | 0 | 134 | 0 | 16 | 0 | 0 | 0 | 0 | 110 | 0 | 40 | 760 | 0 | 0 | 0 | 65 |
| D | 20 | 86 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 29 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 68 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 107 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 224 | 0 | 111 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 322 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 24 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 228 | 256 | 600 | 0 | 0 | 0 | 0 | 50 | 71 | 0 | 40 | 0 | 0 | 0 | 62 | 0 | 170 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E | 34 | 32 | 23 | 235 | 0 | 48 | 229 | 336 | 392 | 0 | 0 | 65 | 52 | 135 | 0 | 0 | 0 | 98 | 0 | 0 | 0 | 0 | 0 | 115 | 0 | 0 | 56 | 0 | 0 | 85 | 99 | 0 | 0 |
| P | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 124 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 |
| P | 67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 |
| P | 71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 59 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 |
| IG-A | | 2311 | 2043 | 2430 | 2068 | 890 | 790 | 689 | 645 | 1158 | 1515 | 2337 | 2245 | 698 | 657 | 1560 | 804 | 842 | 1177 | 774 | 699 | 320 | 212 | 1495 | 1132 | 2140 | 1364 | 1488 | 1469 | 607 | 640 | 2587 | 1581 |
| IC-A | 31 | 0 | 203 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 0 | 0 | 0 | 0 | 0 | 98 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 145 | 0 | 0 | 0 | 0 |
| IC-A | 50 | 0 | 158 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 |
| IC-AT | | 0 | 361 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 81 | 0 | 0 | 0 | 0 | 0 | 131 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 234 | 0 | 0 | 0 | 0 |
| (IG-A+IC-A)-T | | 2311 | 2404 | 2430 | 2068 | 890 | 790 | 689 | 645 | 1158 | 1515 | 2337 | 2326 | 698 | 657 | 1560 | 804 | 842 | 1308 | 774 | 699 | 320 | 212 | 1495 | 1132 | 2140 | 1364 | 1488 | 1703 | 607 | 640 | 2587 | 1581 |
| **Total absolute sequence reads (indigenous AMF and inoculant): 42 174** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the average of four replications. **Abbreviation:** U: Uninoculated, I: Inoculated, SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*. IG-A: Absolute abundance of indigenous sequence reads, IC-A: Absolute abundance of inoculant sequence reads, IC-AT: Absolute abundance of inoculant sequence reads total and (IC-A+IG-A)-T: Inoculant and indigenous absolute abundance sequence reads total.

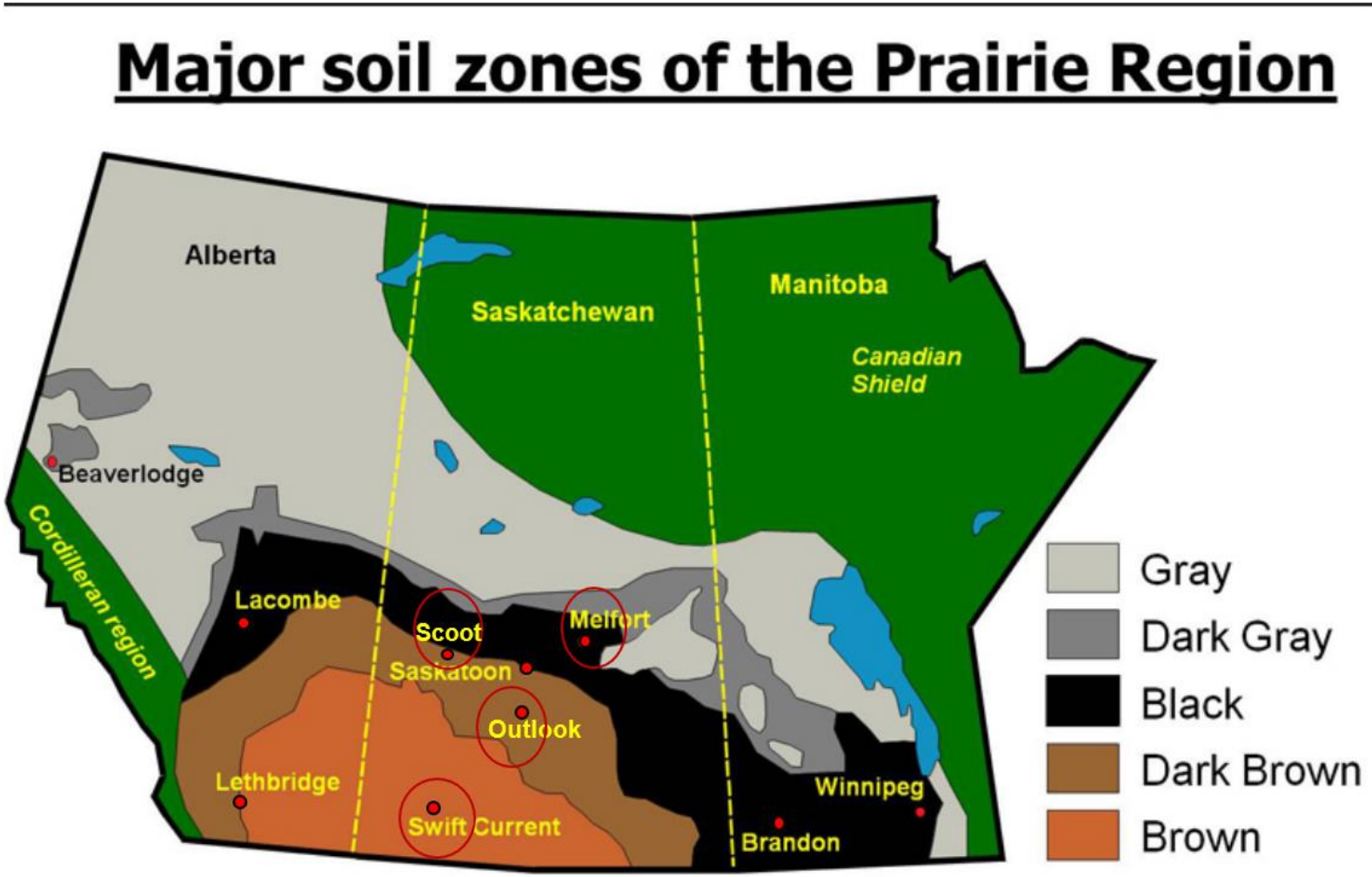


**Figure A.3.1.** Map of the four experimental sites located at three soil zones of Canadian (Saskatchewan) Prairie. Sites are demarcated with red circles (Acknowledgement to https://www.google.ca/webhp?sourceid=chrome-instant&ion=1&espv=2&ie=UTF-8#q=map+of+canada+prairie+soil+zone).


**Figure A.3.2** Layout of three-year field incubation study (Swift Current location) with 32 aluminum soil cores and the distinct colors of core soils.

**Figure A.3.3** Extraction, transportation and installation of aluminum soil cores using hydraulic mountain truck at different study sites.

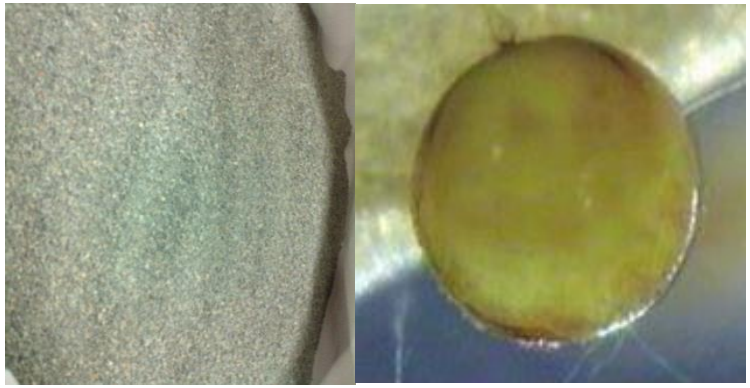

**Figure A.3.4**. Granular formulation containing infective propagules (spore under microscope) of commercial non-indigenous AMF inoculant (*Rhizophagus irregularis*).



**Figure A.3.5.** Commercial non-indigenous *R. irregularis* inoculant applied into soil cores in 2011 in which field pea (2011)-wheat (2012)-field pea were subsequently grown.

**Figure A.3.6** Crop (field pea) grows in soil cores during 2011, 2012 and 2013 cropping seasons at four locations.

**Figure A.3.7** Sampling soils (0-15 cm) for trap culture at harvest in AAFC Scott Research Farm.

**Figure A.3.8** AMF trap culture and sampling roots for DNA extraction and subsequent pyrosequencing analyses.

**Appendix B:** Absolute and relative abundance of AMF taxa (indigenous and introduced inoculants) in growth chamber study.

**Table B.4.1** Relative abundance of indigenous AMF genera and three introduced inoculants (SPARC *F. mosseae* B04, GINCO *F. mosseae* DAOM 221475 and commercial inoculant, *R. irregularis* 4514535) of AMF taxa, representing 59 OTUs, associated with the roots of lentil, chickpea and pea, detected by pyrosequencing after eight weeks of inoculation under growth chamber conditions.

| | | Relative abundance of indigenous AMF taxa (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Crops | Inoculants | *Rhizophagus* | *Glomus* | *Funneliformis* | *Septoglomus* | *Claroideoglomus* | *Diversispora* | *Paraglomus* | *Archaeospora* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of AMF taxa |
| Lentil | Control | 0.33 | 0.06 | 0.51 | 0.02 | 0.05 | 0.00 | 0.00 | 0.03 | 1766 | 0 | 1.0 | 0 | 1766 |
| | IC 1 | 0.28 | 0.07 | 0.51 | 0.00 | 0.09 | 0.00 | 0.00 | 0.05 | 1525 | 0.16 | 0.84 | 300 | 1825 |
| | IC 2 | 0.28 | 0.13 | 0.46 | 0.00 | 0.06 | 0.00 | 0.00 | 0.05 | 502 | 0.23 | 0.77 | 153 | 655 |
| | IC 3 | 0.12 | 0.15 | 0.46 | 0.00 | 0.23 | 0.00 | 0.00 | 0.04 | 951 | 0.30 | 0.70 | 405 | 1356 |
| Chickpea | Control | 0.27 | 0.04 | 0.56 | 0.02 | 0.08 | 0.02 | 0.01 | 0.00 | 1752 | 0 | 1.0 | 0 | 1752 |
| | IC 1 | 0.28 | 0.04 | 0.55 | 0.02 | 0.07 | 0.02 | 0.01 | 0.00 | 1316 | 0.21 | 0.79 | 340 | 1656 |
| | IC 2 | 0.26 | 0.06 | 0.54 | 0.00 | 0.10 | 0.03 | 0.00 | 0.00 | 886 | 0.21 | 0.79 | 240 | 1126 |
| | IC 3 | 0.14 | 0.36 | 0.43 | 0.00 | 0.07 | 0.00 | 0.00 | 0.00 | 929 | 0.31 | 0.69 | 409 | 1338 |
| Pea | Control | 0.29 | 0.04 | 0.61 | 0.00 | 0.03 | 0.01 | 0.01 | 0.01 | 4250 | 0 | 1.0 | 0 | 4250 |
| | IC 1 | 0.28 | 0.05 | 0.62 | 0.00 | 0.03 | 0.01 | 0.01 | 0.00 | 3644 | 0.17 | 0.83 | 727 | 4371 |
| | IC 2 | 0.25 | 0.04 | 0.59 | 0.00 | 0.06 | 0.00 | 0.06 | 0.00 | 1618 | 0.20 | 0.80 | 397 | 2015 |
| | IC 3 | 0.13 | 0.22 | 0.37 | 0.00 | 0.21 | 0.00 | 0.05 | 0.02 | 1563 | 0.34 | 0.66 | 816 | 2379 |
| **Total absolute sequence reads:** | | | | | | | | | | **20 702** | | | **37 87** | **24 489** |

**NB:** 18S rRNA gene pyrosequencing data presented here are the average of 4 replications. Abbreviation: R: *Rhizophagus*, F: *Funneliformis*, G: *Glomus*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, P: *Paraglomus*. IC1: SPARC *F. mosseae* B04, IC2: GINCO *F. mosseae* DAOM 221475, and IC3: commercial inoculant *R. irregularis* 4514535. Skewness: 1.46 and Kurtosis: 1.18.

**Table B.4.2** Absolute sequence reads of indigenous AMF genera and three introduced inoculants (SPARC *F. mosseae* B04, GINCO *F. mosseae* DAOM 221475 and commercial inoculant, *R. irregularis* 4514535) of AMF taxa associated with the roots of lentil, chickpea and pea, detected by 18S rRNA gene pyrosequencing after eight weeks of inoculation under growth chamber conditions.

| AMF | | Crops | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lentil | | | | Chickpea | | | | Field pea | | | |
| Genera | OTUs | Control | IC 1 | IC 2 | IC 3 | Control | IC 1 | IC 2 | IC 3 | Control | IC 1 | IC 2 | IC 3 |
| R | OTU2 | 100 | 150 | 0 | 0 | 309 | 90 | 243 | 0 | 255 | 206 | 0 | 0 |
| R | OTU8 | 105 | 121 | 127 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 34 | 0 |
| R | OTU46 | 304 | 123 | 0 | 188 | 38 | 0 | 190 | 157 | 0 | 0 | 0 | 0 |
| R | OTU3 | 0 | 15 | 0 | 0 | 0 | 103 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 276 | 0 | 0 | 0 |
| F | OTU36 | 32 | 401 | 207 | 0 | 296 | 0 | 0 | 0 | 0 | 328 | 0 | 0 |
| F | OTU50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 855 | 0 | 239 | 0 |
| F | OTU57 | 306 | 267 | 102 | 0 | 220 | 47 | 254 | 0 | 0 | 226 | 0 | 0 |
| F | OTU18 | 0 | 0 | 0 | 360 | 0 | | 0 | 107 | 226 | 0 | 0 | 0 |
| F | OTU52 | 0 | 109 | 0 | 0 | 196 | 250 | 0 | 0 | 98 | 127 | 0 | 0 |
| F | OTU21 | 322 | 0 | 0 | 101 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 321 |
| F | OTU34 | 0 | 26 | 0 | 0 | 11 | 360 | 0 | 70 | 0 | 103 | 0 | 0 |
| F | OTU42 | 205 | 0 | 0 | 0 | 29 | 0 | 0 | 76 | 0 | 0 | 0 | 155 |
| F | OTU44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 207 | 0 | 0 | 0 | 89 |
| F | OTU53 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 321 | 0 | 0 |
| G | OTU9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 109 | 0 | 0 | 26 |
| G | OTU11 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 13 | 0 | 87 |
| G | OTU43 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 350 | 0 | 0 | 0 |
| G | OTU15 | 0 | 0 | 0 | 0 | 14 | 12 | 0 | 0 | 221 | 20 | 0 | 27 |
| G | OTU48 | 0 | 22 | 0 | 0 | 0 | 25 | 0 | 0 | 123 | 0 | 0 | 0 |
| G | OTU41 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing data presented here are the average of 4 replications. **Abbreviation:** R: *Rhizophagus*, F: *Funneliformis*, G: *Glomus*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, P: *Paraglomus*, IC 1: SPARC *F. mosseae* B04, IC 2: GINCO *F. mosseae* DAOM 221475, IC 3: commercial *R. irregularis* 4514535 inoculant.

**Table B.4.2** Continued.

| AMF | | Crops | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lentil | | | | Chickpea | | | | Field pea | | | |
| Genera | OTUs | Control | IC 1 | IC 2 | IC 3 | Control | IC 1 | IC 2 | IC 3 | Control | IC 1 | IC 2 | IC 3 |
| G | OTU10 | 30 | 0 | 0 | 0 | 0 | 32 | 0 | 50 | 0 | 32 | 0 | 0 |
| G | OTU16 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 156 | 0 | 0 | 0 |
| G | OTU23 | 0 | 0 | 0 | 0 | 33 | 0 | 0 | 0 | 0 | 399 | 0 | 0 |
| G | OTU17 | 12 | 56 | 0 | 0 | 0 | 76 | 0 | 0 | 38 | 0 | 0 | 38 |
| G | OTU22 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU14 | 8 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 50 | 248 | 0 | 0 |
| G | OTU30 | 0 | 30 | 0 | 31 | 0 | 0 | 0 | 103 | 0 | 0 | 42 | 58 |
| G | OTU31 | 0 | 0 | 0 | 40 | 0 | 0 | 29 | 137 | 0 | 134 | 0 | 93 |
| S | OTU4 | 35 | 0 | 0 | 0 | 97 | 32 | 0 | 0 | 0 | 80 | 0 | 0 |
| S | OTU5 | 0 | 0 | 0 | 0 | 36 | 51 | 0 | 0 | 109 | 0 | 0 | 0 |
| C | OTU1 | 14 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 64 | 0 | 0 |
| C | OTU39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 98 | 0 | 259 | 0 |
| C | OTU58 | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU29 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 39 | 159 | 34 | 0 |
| C | OTU32 | 66 | 0 | 0 | 88 | 0 | 0 | 95 | 0 | 55 | 109 | 349 | 0 |
| C | OTU33 | 34 | 100 | 0 | 0 | 0 | 41 | 0 | 0 | 65 | 159 | 217 | 502 |
| C | OTU49 | 0 | 0 | 0 | 65 | 0 | 0 | 75 | 0 | 237 | 0 | 0 | 0 |
| C | OTU26 | 0 | 0 | 0 | 0 | 187 | 0 | 0 | 0 | 81 | 358 | 269 | 0 |
| C | OTU28 | 0 | 35 | 0 | 0 | 0 | 45 | 0 | 0 | 224 | 0 | 0 | 0 |
| D | OTU25 | 0 | 0 | 0 | 0 | 39 | 79 | 0 | 0 | 168 | 102 | 0 | 0 |
| D | OTU59 | 0 | 0 | 0 | 0 | 70 | 0 | 0 | 0 | 82 | 100 | 57 | 0 |
| A | OTU38 | 78 | 70 | 23 | 0 | 0 | 0 | 0 | 0 | 124 | 145 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing data presented here are the average of 4 replications. **Abbreviation:** R: *Rhizophagus*, F: *Funneliformis*, G: *Glomus*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, P: *Paraglomus*, IC 1: SPARC *F. mosseae* B04, IC 2: GINCO *F. mosseae* DAOM 221475, IC 3: commercial *R. irregularis* 4514535 inoculant.

**Table B.4.2** Continued.

| AMF | | Crops | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lentil | | | | Chickpea | | | | Field pea | | | |
| Genera | OTUs | Control | IC 1 | IC 2 | IC 3 | Control | IC 1 | IC 2 | IC 3 | Control | IC 1 | IC 2 | IC 3 |
| A | OTU45 | 0 | 0 | 43 | 14 | 0 | | 0 | 0 | 16 | 0 | 0 | 0 |
| P | OTU19 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 32 | 60 | 0 | 0 |
| P | OTU55 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 65 | 0 | 105 |
| P | OTU56 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 65 | 0 | 0 | 0 |
| P | OTU20 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 54 | 0 | 62 |
| P | OTU24 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 98 | 0 | 118 | 0 |
| P | OTU40 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 32 | 0 | 0 |
| IG-A | | 1766 | 1525 | 502 | 951 | 1752 | 1316 | 886 | 929 | 4250 | 3644 | 1618 | 1563 |
| IC-1 | OTU37 | 0 | 115 | 0 | 0 | 0 | 169 | 0 | 0 | 0 | 276 | 0 | 0 |
| IC-1 | OTU51 | 0 | 95 | 0 | 0 | 0 | 171 | 0 | 0 | 0 | 301 | 0 | 0 |
| IC-1 | OTU27 | 0 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 150 | 0 | 0 |
| IC-2 | OTU54 | 0 | 0 | 105 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 198 | 0 |
| IC-2 | OTU47 | 0 | 0 | 48 | 0 | 0 | 0 | 83 | 0 | 0 | 0 | 199 | 0 |
| IC-3 | OTU35 | 0 | 0 | 0 | 305 | 0 | 0 | 0 | 150 | 0 | 0 | 0 | 359 |
| IC-3 | OTU7 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 127 | 0 | 0 | 0 | 151 |
| IC-3 | OTU12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 132 | 0 | 0 | 0 | 0 |
| IC-3 | OTU6 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 306 |
| IC-A | | 0 | 300 | 153 | 405 | | 340 | 240 | 409 | 0 | 727 | 397 | 816 |
| (IG-A+IC-A)-T | | 1766 | 1825 | 655 | 1356 | 1752 | 1656 | 1126 | 1338 | 4250 | 4371 | 2015 | 2379 |
| **Total absolute sequence reads (indigenous AMF and inoculants): 24 489** | | | | | | | | | | | | | |

**NB:** 18S rRNA gene pyrosequencing data presented here are the average of 4 replications. **Abbreviation:** R: R: *Rhizophagus*, F: *Funneliformis*, G: *Glomus*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, P: *Paraglomus*, IG-A: Total Absolute sequence reads of indigenous AMF genera, IC-A: Absolute sequence reads of inoculant, (IC-A+IG-A)-T: Total absolute sequence reads of inoculant and indigenous AMF, IC 1: SPARC *F. mosseae* B04, IC 2: GINCO *F. mosseae* DAOM 221475, IC 3: commercial *R. irregularis* 4514535 inoculant.


**Figure B.4.1.** Three pulse crops (lentil, chickpea and field pea) treated with different AMF inoculants in growth chamber conditions.

**Appendix C:** Absolute and relative abundance of AMF taxa (indigenous and introduced inoculant) in pooled replicates of field pea trap roots (2011 and 2013 field study).

**Table C.5.1**. Relative abundance of indigenous and introduced AMF inoculant taxa, representing 70 OTUs, associated with the trap roots of field pea, detected by 18S rRNA gene pyrosequencing (pooled replications) in year 1 (2011) at four sites in Saskatchewan.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Septoglomus* | *Claroideoglomus* | *Diversispora* | *Archaeospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of inoculant and indigenous taxa |
| SC | SC | U | 0.00 | 0.07 | 0.33 | 0.00 | 0.60 | 0.00 | 0.00 | 0.00 | 684 | 0 | 0 | 0 | 684 |
| | SC | I | 0.00 | 0.05 | 0.41 | 0.00 | 0.54 | 0.00 | 0.00 | 0.00 | 561 | 0.25 | 0.75 | 185 | 746 |
| | OL | U | 0.05 | 0.26 | 0.52 | 0.00 | 0.15 | 0.00 | 0.00 | 0.02 | 1191 | 0 | 0 | 0 | 1191 |
| | OL | I | 0.00 | 0.13 | 0.67 | 0.00 | 0.18 | 0.00 | 0.00 | 0.02 | 764 | 0.10 | 0.90 | 86 | 850 |
| | ST | U | 0.00 | 0.03 | 0.54 | 0.00 | 0.30 | 0.00 | 0.00 | 0.13 | 624 | 0 | 0 | 0 | 624 |
| | ST | I | 0.00 | 0.03 | 0.51 | 0.00 | 0.30 | 0.00 | 0.00 | 0.16 | 627 | 0.12 | 0.88 | 88 | 715 |
| | MF | U | 0.11 | 0.10 | 0.64 | 0.00 | 0.07 | 0.00 | 0.00 | 0.08 | 700 | 0 | 0 | 0 | 700 |
| | MF | I | 0.00 | 0.08 | 0.77 | 0.00 | 0.07 | 0.00 | 0.00 | 0.08 | 678 | 0.10 | 0.90 | 75 | 753 |
| OL | SC | U | 0.00 | 0.13 | 0.37 | 0.00 | 0.50 | 0.00 | 0.00 | 0.00 | 538 | 0 | 0 | 0 | 538 |
| | SC | I | 0.00 | 0.00 | 0.56 | 0.00 | 0.44 | 0.00 | 0.00 | 0.00 | 535 | 0.24 | 0.76 | 170 | 705 |
| | OL | U | 0.07 | 0.25 | 0.38 | 0.00 | 0.22 | 0.00 | 0.01 | 0.06 | 802 | 0 | 0 | 0 | 802 |
| | OL | I | 0.02 | 0.24 | 0.57 | 0.00 | 0.14 | 0.00 | 0.00 | 0.04 | 902 | 0.18 | 0.82 | 195 | 1097 |
| | ST | U | 0.00 | 0.16 | 0.28 | 0.04 | 0.26 | 0.00 | 0.00 | 0.27 | 1127 | 0 | 0 | 0 | 1127 |
| | ST | I | 0.00 | 0.14 | 0.37 | 0.00 | 0.27 | 0.00 | 0.00 | 0.21 | 955 | 0.20 | 0.80 | 246 | 1201 |
| | MF | U | 0.03 | 0.26 | 0.56 | 0.00 | 0.05 | 0.00 | 0.00 | 0.11 | 1274 | 0 | 0 | 0 | 1274 |
| | MF | I | 0.00 | 0.22 | 0.51 | 0.00 | 0.11 | 0.00 | 0.00 | 0.15 | 920 | 0.11 | 0.89 | 112 | 1032 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the pooled of 4 replicated sample. **Abbreviation**: SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF).

**Table C.5.1.** Continued.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Septoglomus* | *Claroideoglomus* | *Diversispora* | *Archaeospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of inoculant and indigenous taxa |
| ST | SC | U | 0.00 | 0.07 | 0.65 | 0.00 | 0.27 | 0.00 | 0.00 | 0.00 | 486 | 0 | 0 | 0 | 486 |
| | SC | I | 0.00 | 0.06 | 0.68 | 0.00 | 0.26 | 0.00 | 0.00 | 0.00 | 425 | 0.08 | 0.92 | 39 | 464 |
| | OL | U | 0.11 | 0.18 | 0.39 | 0.14 | 0.14 | 0.00 | 0.00 | 0.15 | 785 | 0 | 0 | 0 | 785 |
| | OL | I | 0.00 | 0.24 | 0.38 | 0.00 | 0.28 | 0.00 | 0.00 | 0.11 | 545 | 0.09 | 0.91 | 55 | 600 |
| | ST | U | 0.00 | 0.10 | 0.23 | 0.04 | 0.38 | 0.00 | 0.01 | 0.24 | 2045 | 0 | 0 | 0 | 2045 |
| | ST | I | 0.00 | 0.08 | 0.33 | 0.00 | 0.39 | 0.00 | 0.00 | 0.20 | 1799 | 0.23 | 0.77 | 523 | 2322 |
| | MF | U | 0.02 | 0.20 | 0.73 | 0.00 | 0.03 | 0.00 | 0.02 | 0.00 | 1168 | 0 | 0 | 0 | 1168 |
| | MF | I | 0.00 | 0.14 | 0.74 | 0.00 | 0.01 | 0.00 | 0.11 | 0.00 | 946 | 0.13 | 0.87 | 146 | 1092 |
| MF | SC | U | 0.00 | 0.12 | 0.57 | 0.00 | 0.31 | 0.00 | 0.00 | 0.00 | 508 | 0 | 0 | 0 | 508 |
| | SC | I | 0.00 | 0.05 | 0.66 | 0.00 | 0.29 | 0.00 | 0.00 | 0.00 | 458 | 0.07 | 0.93 | 35 | 493 |
| | OL | U | 0.02 | 0.17 | 0.64 | 0.00 | 0.17 | 0.00 | 0.00 | 0.00 | 983 | 0 | 0 | 0 | 983 |
| | OL | I | 0.00 | 0.19 | 0.59 | 0.00 | 0.23 | 0.00 | 0.00 | 0.00 | 809 | 0.12 | 0.88 | 112 | 921 |
| | ST | U | 0.00 | 0.18 | 0.38 | 0.02 | 0.21 | 0.00 | 0.02 | 0.18 | 1092 | 0 | 0 | 0 | 1092 |
| | ST | I | 0.00 | 0.14 | 0.41 | 0.00 | 0.22 | 0.00 | 0.00 | 0.23 | 972 | 0.23 | 0.77 | 285 | 1257 |
| | MF | U | 0.13 | 0.13 | 0.61 | 0.00 | 0.05 | 0.00 | 0.00 | 0.09 | 2067 | 0 | 0 | 0 | 2067 |
| | MF | I | 0.02 | 0.16 | 0.66 | 0.00 | 0.07 | 0.00 | 0.00 | 0.09 | 1378 | 0.22 | 0.78 | 399 | 1777 |
| **Total absolute sequence reads:** | | | | | | | | | | | **29 348** | | | **2 751** | **32 099** |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the pooled of 4 replicated sample. **Abbreviation**: SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF). Skewness: 1.66 and Kurtosis: 1.78.

**Table C.5.2**. Relative abundance of indigenous and introduced AMF inoculant taxa, representing 71 OTUs, associated with the trap roots of field pea, detected by pyrosequencing (pooled replications) in year 3 (2013) at four sites in Saskatchewan.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF taxa | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Claroideoglomus* | *Diversispora* | *Entrophospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of inoculant and indigenous taxa |
| SC | SC | U | 0.00 | 0.09 | 0.38 | 0.42 | 0.08 | 0.00 | 0.03 | 2115 | 0 | 0 | 0 | 2115 |
| | SC | I | 0.00 | 0.38 | 0.54 | 0.07 | 0.00 | 0.00 | 0.01 | 1960 | 0.13 | 0.87 | 290 | 2250 |
| | OL | U | 0.00 | 0.26 | 0.53 | 0.20 | 0.00 | 0.00 | 0.00 | 1698 | 0 | 0 | 0 | 1698 |
| | OL | I | 0.00 | 0.27 | 0.58 | 0.15 | 0.00 | 0.00 | 0.00 | 1622 | 0 | 0 | 0 | 1622 |
| | ST | U | 0.00 | 0.47 | 0.39 | 0.14 | 0.00 | 0.00 | 0.00 | 540 | 0 | 0 | 0 | 540 |
| | ST | I | 0.00 | 0.34 | 0.28 | 0.38 | 0.00 | 0.00 | 0.00 | 449 | 0 | 0 | 0 | 449 |
| | MF | U | 0.00 | 0.85 | 0.11 | 0.00 | 0.04 | 0.00 | 0.00 | 447 | 0 | 0 | 0 | 447 |
| | MF | I | 0.00 | 0.39 | 0.50 | 0.00 | 0.11 | 0.00 | 0.00 | 249 | 0 | 0 | 0 | 249 |
| OL | SC | U | 0.00 | 0.53 | 0.40 | 0.00 | 0.04 | 0.00 | 0.03 | 519 | 0 | 0 | 0 | 519 |
| | SC | I | 0.00 | 0.43 | 0.47 | 0.00 | 0.06 | 0.00 | 0.04 | 475 | 0 | 0 | 0 | 475 |
| | OL | U | 0.03 | 0.19 | 0.44 | 0.07 | 0.24 | 0.00 | 0.04 | 1340 | 0 | 0 | 0 | 1340 |
| | OL | I | 0.00 | 0.05 | 0.10 | 0.68 | 0.17 | 0.00 | 0.01 | 1236 | 0 | 0 | 0 | 1236 |
| | ST | U | 0.00 | 0.29 | 0.36 | 0.35 | 0.00 | 0.00 | 0.00 | 543 | 0 | 0 | 0 | 543 |
| | ST | I | 0.00 | 0.12 | 0.58 | 0.24 | 0.06 | 0.00 | 0.00 | 377 | 0 | 0 | 0 | 377 |
| | MF | U | 0.00 | 0.51 | 0.49 | 0.00 | 0.00 | 0.00 | 0.00 | 1113 | 0 | 0 | 0 | 1113 |
| | MF | I | 0.00 | 0.00 | 0.24 | 0.70 | 0.06 | 0.00 | 0.00 | 784 | 0 | 0 | 0 | 784 |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the pooled of 4 replicated sample. **Abbreviation:** SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF).

**Table C.5.2**. Continued.

| | | | Relative abundance of indigenous AMF taxa (%) | | | | | | | Absolute and relative abundance of inoculant and indigenous AMF total | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | *Rhizophagus* | *Glomus* | *Funneliformis* | *Claroideoglomus* | *Diversispora* | *Entrophospora* | *Paraglomus* | Absolute abundance of indigenous taxa | Relative abundance of inoculant | Relative abundance of indigenous taxa | Absolute abundance of inoculant | Total absolute abundance of inoculant and indigenous taxa |
| ST | SC | U | 0.05 | 0.11 | 0.43 | 0.34 | 0.07 | 0.00 | 0.00 | 472 | 0 | 0 | 0 | 472 |
| | SC | I | 0.00 | 0.15 | 0.48 | 0.37 | 0.00 | 0.00 | 0.00 | 364 | 0 | 0 | 0 | 364 |
| | OL | U | 0.03 | 0.00 | 0.35 | 0.60 | 0.03 | 0.00 | 0.00 | 665 | 0 | 0 | 0 | 665 |
| | OL | I | 0.00 | 0.00 | 0.69 | 0.31 | 0.00 | 0.00 | 0.00 | 581 | 0 | 0 | 0 | 581 |
| | ST | U | 0.00 | 0.88 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 170 | 0 | 0 | 0 | 170 |
| | ST | I | 0.00 | 0.86 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | 128 | 0 | 0 | 0 | 128 |
| | MF | U | 0.00 | 0.26 | 0.67 | 0.00 | 0.07 | 0.00 | 0.00 | 880 | 0 | 0 | 0 | 880 |
| | MF | I | 0.00 | 0.00 | 0.71 | 0.29 | 0.00 | 0.00 | 0.00 | 801 | 0 | 0 | 0 | 801 |
| MF | SC | U | 0.00 | 0.56 | 0.27 | 0.09 | 0.06 | 0.00 | 0.00 | 1210 | 0 | 0 | 0 | 1210 |
| | SC | I | 0.00 | 0.49 | 0.51 | 0.00 | 0.00 | 0.00 | 0.00 | 1219 | 0 | 0 | 0 | 1219 |
| | OL | U | 0.00 | 0.10 | 0.37 | 0.53 | 0.00 | 0.00 | 0.00 | 1396 | 0 | 0 | 0 | 1396 |
| | OL | I | 0.00 | 0.21 | 0.29 | 0.50 | 0.00 | 0.00 | 0.00 | 1391 | 0.09 | 0.91 | 139 | 1530 |
| | ST | U | 0.00 | 0.25 | 0.69 | 0.00 | 0.06 | 0.00 | 0.00 | 289 | 0 | 0 | 0 | 289 |
| | ST | I | 0.00 | 0.35 | 0.11 | 0.49 | 0.05 | 0.00 | 0.00 | 483 | 0 | 0 | 0 | 483 |
| | MF | U | 0.00 | 0.57 | 0.28 | 0.14 | 0.00 | 0.00 | 0.01 | 1787 | 0 | 0 | 0 | 1787 |
| | MF | I | 0.00 | 0.23 | 0.56 | 0.16 | 0.00 | 0.00 | 0.05 | 1378 | 0 | 0 | 0 | 1378 |
| **Total absolute sequence reads:** | | | | | | | | | | **28 681** | | | **429** | **29 110** |

**NB:** 18S rRNA gene pyrosequencing reads presented here are the pooled of 4 replicated samples. **Abbreviation:** SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, U: uninoculated control (indigenous AMF only), I: inoculated (*R. irregularis* inoculant + indigenous AMF). Skewness: 1.88 and Kurtosis: 2.12.

**Table C.5.3.** Absolute sequence reads of indigenous and introduced AMF inoculant taxa representing 70 OTUs, associated with the trap roots of field pea, detected by pyrosequencing (pooled replications) in year 1 (2011) at four sites in Saskatchewan.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil | SC | Soil | OL | Soil | ST | Soil | MF | Soil |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| R | OTU62 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 |
| R | OTU20 | 0 | 0 | 0 | 0 | 0 | 0 | 57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0 |
| R | OTU32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 |
| R | OTU42 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 43 | 0 |
| R | OTU43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 32 |
| R | OTU4 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42 | 0 |
| R | OTU31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 36 | 0 |
| R | OTU10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 |
| G | OTU6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 0 | 39 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 30 |
| G | OTU28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 |
| G | OTU21 | 0 | 0 | 0 | 51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 136 | 22 | 45 | 0 | 25 | 50 | 52 | 100 | 0 | 0 | 28 | 0 | 0 | 46 | 0 | 0 | 0 | 0 | 0 |
| G | OTU38 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 95 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 |
| G | OTU68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 67 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 |
| G | OTU9 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 41 | 0 |
| G | OTU53 | 0 | 0 | 113 | 0 | 0 | 0 | 0 | 7 | 32 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 123 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 123 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU19 | 0 | 18 | 123 | 0 | 0 | 0 | 30 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU22 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 157 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 146 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 7 | 144 | 146 | 0 |
| G | OTU3 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 60 | 0 | 0 | 0 | 56 | 0 | 0 | 0 |
| G | OTU15 | 0 | 12 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 89 | 0 |
| G | OTU59 | 0 | 0 | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 257 | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing data presented here are the pooled of 4 replications. **Abbreviation:** U: Uninoculated control (indigenous AMF only), I: Inoculated (introduced *R. irregularis* inoculant + indigenous AMF), SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*.

**Table C.5.3.** Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| G | OTU60 | 25 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU58 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 78 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 152 | 0 | 0 | 0 | 84 |
| F | OTU5 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 232 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 0 | 256 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 |
| F | OTU54 | 0 | 0 | 0 | 65 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 130 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 0 | 0 | 0 | 101 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 125 | 0 |
| F | OTU24 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 221 | 78 |
| F | OTU26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 246 | 0 | 0 | 0 | 145 | 0 | 112 | 0 | 0 | 0 | 0 | 203 | 0 | 0 | 0 | 0 | 0 | 105 | 0 | 103 | 0 |
| F | OTU39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 105 | 0 | 0 | 153 | 100 | 0 | 0 | 0 | 0 | 0 | 221 | 0 | 0 | 227 |
| F | OTU30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 256 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 218 | 0 |
| F | OTU34 | 75 | 0 | 357 | 0 | 159 | 0 | 0 | 200 | 0 | 231 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 159 | 356 | 100 | 0 | 0 | 0 | 0 | 0 | 45 | 206 | 453 | 56 |
| F | OTU16 | 0 | 0 | 245 | 359 | 0 | 0 | 325 | 148 | 0 | 0 | 0 | 0 | 0 | 248 | 712 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 301 | 0 | 0 | 0 | 0 | 0 | 0 | 49 | 260 |
| F | OTU67 | 0 | 0 | 0 | 0 | 178 | 0 | 0 | 128 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 142 | 135 | 17 | 347 | 0 | 290 | 302 | 626 | 0 | 0 | 0 | 35 | 240 |
| F | OTU52 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 69 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 | 0 | 0 | 12 | 0 | 0 | 33 | 0 | 0 | 0 | 473 | 0 | 0 | 29 | 0 |
| F | OTU27 | 0 | 123 | 18 | 0 | 0 | 159 | 0 | 0 | 0 | 0 | 0 | 68 | 235 | 0 | 0 | 72 | 318 | 0 | 70 | 0 | 0 | 50 | 0 | 79 | 0 | 0 | 0 | 0 | 0 | 205 | 0 | 0 |
| F | OTU45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 98 | 0 | 0 | 0 | 0 | 0 | 64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU36 | 135 | 0 | 0 | 86 | 0 | 0 | 81 | 0 | 0 | 0 | 0 | 60 | 46 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU46 | 0 | 84 | 0 | 0 | 0 | 64 | 0 | 0 | 0 | 0 | 72 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 |
| S | OTU7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 62 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| S | OTU61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 |
| S | OTU50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU13 | 246 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 235 | 0 | 0 | 0 | 0 | 0 | 104 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 |
| C | OTU23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing data presented here are the pooled of 4 replications. **Abbreviation:** U: Uninoculated control (indigenous AMF only), I: Inoculated (introduced *R. irregularis* inoculant + indigenous AMF), SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*.

**Table C.5.3.** Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| C | OTU56 | 37 | 156 | 74 | 135 | 189 | 0 | 0 | 45 | 268 | 0 | 0 | 0 | 0 | 245 | 0 | 0 | 0 | 0 | 0 | 0 | 238 | 0 | 0 | 0 | 158 | 133 | 0 | 52 | 259 | 208 | 95 | 0 |
| C | OTU25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 107 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU2 | 128 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 302 | 279 | 0 | 0 | 0 | 0 | 166 | 0 | 0 | 0 | 0 | 90 |
| C | OTU69 | 0 | 0 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 231 | 0 | 0 | 0 | 0 | 0 | 0 | 152 | 80 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU33 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 78 | 34 | 0 | 0 | 0 | 0 | 0 | 110 | 43 | 0 | 0 | 360 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU47 | 0 | 145 | 0 | 0 | 0 | 189 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 17 | 0 | 133 | 0 | 35 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 88 | 0 | 0 | 0 | 0 |
| C | OTU55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 20 | 0 | 0 | 0 | 30 | 0 | 0 | 46 | 37 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | OTU35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | OTU57 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | OTU63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | OTU40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | OTU65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A | OTU66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 |
| A | OTU17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 |
| P | OTU8 | 0 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 303 | 0 | 0 | 141 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 74 | 0 | 45 | 0 |
| P | OTU11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 |
| P | OTU37 | 0 | 0 | 25 | 0 | 0 | 0 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 0 | 100 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 29 | 32 | 127 |
| P | OTU48 | 0 | 0 | 0 | 18 | 0 | 78 | 30 | 0 | 0 | 0 | 47 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 20 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 23 | 0 |
| P | OTU1 | 0 | 0 | 0 | 0 | 22 | 0 | 10 | 54 | 0 | 0 | 0 | 0 | 0 | 64 | 53 | 0 | 0 | 0 | 50 | 0 | 301 | 155 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 |
| P | OTU29 | 0 | 0 | 0 | 0 | 0 | 20 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 58 | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 78 | 78 | 0 |
| P | OTU70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 121 | 0 | 0 | 0 | 0 | 16 | 0 | 124 | 178 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 0 | 0 | 0 |
| IG-A | | 684 | 561 | 1191 | 764 | 624 | 627 | 700 | 678 | 538 | 535 | 802 | 902 | 1127 | 955 | 1274 | 920 | 486 | 425 | 785 | 545 | 2045 | 1799 | 1168 | 946 | 508 | 458 | 983 | 809 | 1092 | 972 | 2067 | 1378 |
| IC-A | OTU49 | 0 | 185 | 0 | 86 | 0 | 88 | 0 | 75 | 0 | 170 | 0 | 195 | 0 | 246 | 0 | 112 | 0 | 39 | 0 | 55 | 0 | 523 | 0 | 146 | 0 | 35 | 0 | 112 | 0 | 285 | 0 | 399 |
| (IG-A + IC-A)-T | | 684 | 746 | 1191 | 850 | 624 | 715 | 700 | 753 | 538 | 705 | 802 | 1097 | 1127 | 1201 | 1274 | 1032 | 486 | 464 | 785 | 600 | 2045 | 2322 | 1168 | 1092 | 508 | 493 | 983 | 921 | 1092 | 1257 | 2067 | 1777 |
| **Total absolute sequence reads (indigenous AMF and inoculant): 32 099** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**NB:** 18S rRNA gene pyrosequencing data presented here are the pooled of 4 replications. **Abbreviation:** U: Uninoculated control (indigenous AMF only), I: Inoculated (introduced *R. irregularis* inoculant + indigenous AMF), SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*. IG-A: Absolute abundance of indigenous sequence reads, IC-A: Absolute abundance of inoculant sequence reads, (IC-A+IG-A)-T: Inoculant and indigenous absolute abundance sequence reads total.

**Table C.5.4.** Absolute sequence reads of indigenous and introduced AMF taxa, representing 71 OTUs, associated with the trap roots of field pea, detected by pyrosequencing (pooled replications) in year 3 (2013) at four sites across Saskatchewan.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Gnera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| R | OTU28 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU2 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 24 | 24 | 131 | 0 | 45 | 56 | 43 | 89 | 72 | 24 | 225 | 22 |
| G | OTU44 | 0 | 0 | 42 | 14 | 55 | 0 | 0 | 0 | 0 | 0 | 179 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU71 | 30 | 40 | 0 | 0 | 0 | 0 | 350 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 |
| G | OTU48 | 0 | 0 | 25 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 15 | 0 | 0 | 0 | 42 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 123 | 0 |
| G | OTU51 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 25 | 0 | 0 | 17 | 0 | 0 |
| G | OTU66 | 19 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU68 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 35 | 0 | 0 | 44 | 0 | 0 | 147 | 0 |
| G | OTU49 | 29 | 120 | 0 | 76 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 26 | 0 | 0 | 0 | 335 | 0 | 0 | 0 | 0 | 0 | 0 | 25 |
| G | OTU3 | 0 | 0 | 52 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 |
| G | OTU40 | 0 | 111 | 0 | 0 | 0 | 0 | 31 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 0 | 38 | 0 | 0 | 0 | 0 |
| G | OTU50 | 30 | 0 | 43 | 156 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 159 | 0 |
| G | OTU56 | 0 | 53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 206 | 0 | 0 | 0 | 0 | 0 | 0 | 65 |
| G | OTU47 | 0 | 0 | 25 | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU52 | 77 | 53 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 125 | 0 | 29 | 0 | 0 | 358 | 0 |
| G | OTU69 | 0 | 0 | 26 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 0 | 0 |
| G | OTU7 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 145 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 |
| G | OTU8 | 0 | 69 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU15 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 256 | 0 | 0 | 0 | 0 | 0 | 210 |
| G | OTU21 | 0 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 135 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 |
| G | OTU23 | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 |
| G | OTU16 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 122 | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU24 | 0 | 55 | 0 | 83 | 0 | 0 | 0 | 0 | 131 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 0 | 0 | 108 | 0 | 0 | 0 | 17 | 0 | 0 |
| G | OTU9 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 243 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing data presented here are the pooled of 4 replications. **Abbreviation:** U: Uninoculated control (indigenous AMF only), I: Inoculated (introduced *R. irregularis* inoculant + indigenous AMF), SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*.

**Table C.5.4.** Continued.

| AMF | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| G | OTU10 | 0 | 45 | 0 | 0 | 0 | 97 | 0 | 36 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU13 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 15 | 25 | 0 | 24 | 0 | 0 |
| G | OTU12 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 71 | 0 | 0 | 0 | 0 | 146 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | OTU17 | 0 | 121 | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 42 | 0 | 0 | 10 | 0 |
| F | OTU4 | 0 | 145 | 123 | 258 | 18 | 0 | 0 | 0 | 28 | 0 | 325 | 0 | 0 | 0 | 67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 356 | 28 | 0 | 35 | 0 | 0 | 54 | 0 | 263 |
| F | OTU64 | 106 | 0 | 0 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 125 | 0 | 0 | 123 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU32 | 35 | 0 | 321 | 147 | 0 | 0 | 0 | 23 | 85 | 0 | 0 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 111 | 0 | 0 | 0 |
| F | OTU70 | 0 | 256 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 261 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 125 | 0 | 61 | 0 | 0 | 0 | 0 | 0 | 325 |
| F | OTU25 | 58 | 0 | 125 | 0 | 0 | 32 | 0 | 0 | 0 | 112 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 256 | 0 | 0 | 0 | 252 | 0 |
| F | OTU61 | 0 | 0 | 0 | 123 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 126 | 0 | 0 | 0 | 0 | 175 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU26 | 0 | 278 | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 132 | 0 | 0 | 102 | 0 | 54 | 0 | 0 | 0 | 0 |
| F | OTU34 | 88 | 0 | 147 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 194 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 125 |
| F | OTU29 | 0 | 0 | 0 | 159 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 111 | 0 | 229 | 0 | 0 | 0 | 0 | 0 |
| F | OTU33 | 0 | 0 | 123 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 139 | 0 |
| F | OTU6 | 258 | 225 | 0 | 0 | 0 | 24 | 0 | 0 | 45 | 0 | 0 | 0 | 0 | 17 | 225 | 0 | 0 | 0 | 0 | 102 | 0 | 0 | 258 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU5 | 0 | 0 | 52 | 152 | 0 | 0 | 47 | 0 | 0 | 112 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 176 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 0 | 0 | 356 | 0 | 0 | 0 | 53 |
| F | OTU65 | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 189 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 235 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU27 | 77 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 127 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 115 | 0 |
| F | OTU30 | 0 | 56 | 0 | 99 | 123 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 123 | 0 | 0 | 0 | 178 | 0 | 103 | 0 | 0 | 0 | 89 | 0 | 0 | 0 |
| F | OTU31 | 189 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 225 | 0 | 0 | 0 | 0 | 0 | 0 |
| F | OTU67 | 0 | 65 | 15 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU1 | 146 | 0 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 325 | 0 | 0 | 0 | 0 |
| C | OTU11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 259 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**NB:** 18S rRNA gene pyrosequencing data presented here are the pooled of 4 replications. **Abbreviation:** U: Uninoculated control (indigenous AMF only), I: Inoculated (introduced *R. irregularis* inoculant + indigenous AMF), SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*.

**Table C.5.4.** Continued.

| | | Swift Current (SC) Site | | | | | | | | Outlook (OL) Site | | | | | | | | Scott (ST) Site | | | | | | | | Melfort (MF) Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMF | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | | SC Soil | | OL Soil | | ST Soil | | MF Soil | |
| Genera | OTUs | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I | U | I |
| C | OTU35 | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 214 | 0 | 0 | 0 | 0 | 0 |
| C | OTU38 | 0 | 130 | 0 | 0 | 0 | 71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 181 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 238 | 0 | 0 |
| C | OTU42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 51 | 0 | 0 | 0 | 0 |
| C | OTU43 | 325 | 0 | 63 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 329 | 0 | 0 | 0 | 256 | 0 | 0 | 200 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 132 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU18 | 0 | 0 | 0 | 0 | 76 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 56 | 0 | 0 | 0 | 0 |
| C | OTU36 | 0 | 0 | 35 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 190 | 0 | 0 | 0 | 161 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 241 | 0 |
| C | OTU39 | 256 | 0 | 0 | 251 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 98 |
| C | OTU22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 0 | 0 | 0 | 0 | 206 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 110 | 0 | 286 | 0 | 0 | 0 | 0 | 0 |
| C | OTU37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 91 | 0 | 0 | 0 | 134 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU46 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 127 | 0 | 0 | 0 | 0 | 0 | 0 | 201 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 125 | 0 | 0 | 0 | 0 |
| C | OTU55 | 0 | 0 | 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 72 |
| C | OTU19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 | 0 | 0 | 0 | 0 | 0 |
| C | OTU57 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | OTU45 | 0 | 0 | 101 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 120 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 135 | 0 | 0 | 0 | 56 |
| C | OTU54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | OTU58 | 159 | 0 | 0 | 0 | 0 | 0 | 19 | 28 | 23 | 28 | 320 | 204 | 0 | 24 | 0 | 50 | 34 | 0 | 17 | 0 | 0 | 0 | 62 | 0 | 76 | 0 | 0 | 0 | 17 | 22 | 0 | 0 |
| P | OTU41 | 25 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 11 |
| P | OTU53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 10 |
| P | OTU63 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 |
| P | OTU59 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| P | OTU62 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 |
| IG-A | | 2115 | 1960 | 1698 | 1622 | 540 | 449 | 447 | 249 | 519 | 475 | 1340 | 1236 | 543 | 377 | 1113 | 784 | 472 | 364 | 665 | 581 | 170 | 128 | 880 | 801 | 1210 | 1219 | 1396 | 1391 | 289 | 483 | 1787 | 1378 |
| IC-A | OTU60 | 0 | 290 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 139 | 0 | 0 | 0 | 0 |
| (IG-A + IC-A)-T | | 2115 | 2250 | 1698 | 1622 | 540 | 449 | 447 | 249 | 519 | 475 | 1340 | 1236 | 543 | 377 | 1113 | 784 | 472 | 364 | 665 | 581 | 170 | 128 | 880 | 801 | 1210 | 1219 | 1396 | 1530 | 289 | 483 | 1787 | 1378 |
| **Total absolute sequence reads (indigenous AMF and inoculant): 29 110** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**NB:** 18S rRNA gene pyrosequencing data presented here are the pooled of 4 replications. **Abbreviation:** U: Uninoculated control (indigenous AMF only), I: Inoculated (introduced *R. irregularis* inoculant + indigenous AMF), SC: Swift Current, OL: Outlook, ST: Scott, MF: Melfort, R: *Rhizophagus*, G: *Glomus*, F: *Funneliformis*, S: *Septoglomus*, C: *Claroideoglomus*, D: *Diversispora*, A: *Archaeospora*, P: *Paraglomus*. IG-A: Absolute abundance of indigenous sequence reads, IC-A: Absolute abundance of inoculant sequence reads, (IC-A+IG-A)-T: Inoculant and indigenous absolute abundance sequence reads total.

## Appendix: D

**Table D.3.1.** A three-way ANOVA with *P* value was performed to test the effect of inoculant, soil and site on the plant growth performances across the soil zones of Saskatchewan for three consecutive cropping seasons.

| | **Year 1: 2011 cropping season (field pea)** | | | **Year 2: 2012 cropping season (wheat)** | | | **Year 3: 2013 cropping season (field pea)** | | |
|---|---|---|---|---|---|---|---|---|---|
| Sources | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) |
| Inocuation (I) | 0.0216 | <0.001 | <0.001 | 0.968 | 0.071 | 0.069 | <0.001 | 0.0631 | 0.0471 |
| Soil (S) | 0.010 | 0.008 | <0.001 | 0.125 | 0.244 | 0.341 | <0.001 | 0.043 | 0.561 |
| Site (Si) | 0.20 | 0.951 | <0.001 | 0.062 | 0.669 | 0.442 | 0.073 | 0.051 | 0.723 |
| I x S | 0.020 | 0.075 | <0.001 | 0.012 | 0.013 | 0.011 | 0.001 | 0.039 | 0.041 |
| I x Si | 0.241 | 0.303 | 0.223 | 0.819 | 0.323 | 0.564 | 0.332 | 0.034 | 0.052 |
| S x Si | 0.253 | 0.877 | <0.001 | 0.832 | 0.709 | 0.231 | 0.173 | 0.182 | 0.117 |
| I x S x Si | 0.641 | 0.573 | <0.001 | 0.171 | 0.224 | 0.113 | 0.841 | 0.557 | 0.231 |

Note: *P* values of different sources of variance are presented here.

**Table D.3.2.** The effect of inoculation with non-indigenous *R. irregularis* on the plant growth performances across the soil zones of Saskatchewan for three consecutive cropping seasons.

| | | | **Year 1: 2011 cropping season (field pea)** | | | **Year 2: 2012 cropping season (wheat)** | | | **Year 3: 2013 cropping season (field pea)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Soil | Inoculation | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) |
| Swift Current | Swift Current | U | 11.0 | 169.0 | 25.0 | 4.8 | 156.2 | 22.9 | 10.1 | 181.0 | 24.0 |
| | | I | 12.0 | 192.0 | 29.0 | 5.4 | 188.5 | 28.2 | 10.4 | 187.8 | 25.7 |
| | Outlook | U | 10.2 | 222.1 | 28.0 | 4.7 | 135.6 | 22.5 | 9.3 | 217.1 | 24.9 |
| | | I | 10.8 | 240.1 | 37.0 | 5.1 | 139.2 | 28.0 | 10.9 | 235.0 | 33.0 |
| | Scott | U | 8.8 | 204.1 | 33.0 | 5.3 | 112.7 | 21.7 | 8.1 | 199.5 | 29.3 |
| | | I | 9.8 | 201.1 | 36.0 | 4.3 | 122.3 | 22.4 | 9.0 | 196.6 | 32.0 |
| | Melfort | U | 10.0 | 200.1 | 31.0 | 4.5 | 135.5 | 20.2 | 9.2 | 195.6 | 27.5 |
| | | I | 11.2 | 201.1 | 30.0 | 4.8 | 130.0 | 20.7 | 11.0 | 196.6 | 25.7 |
| Outlook | Swift Current | U | 9.0 | 152.0 | 33.0 | 4.7 | 143.2 | 23.6 | 8.3 | 148.7 | 29.3 |
| | | I | 8.8 | 215.1 | 36.0 | 4.0 | 142.2 | 33.7 | 8.1 | 210.3 | 32.0 |
| | Outlook | U | 9.4 | 201.1 | 38.0 | 4.2 | 144.6 | 29.8 | 8.6 | 190.0 | 30.0 |
| | | I | 13.0 | 255.1 | 51.0 | 4.0 | 181.2 | 30.3 | 13.1 | 244.6 | 45.3 |
| | Scott | U | 11.9 | 197.0 | 25.0 | 5.5 | 143.0 | 28.4 | 10.9 | 192.7 | 22.2 |
| | | I | 12.1 | 205.1 | 46.0 | 5.6 | 140.1 | 23.8 | 11.1 | 200.5 | 40.8 |
| | Melfort | U | 14.4 | 195.0 | 46.0 | 5.0 | 135.0 | 19.7 | 13.3 | 190.7 | 25.7 |
| | | I | 9.4 | 214.1 | 29.0 | 5.0 | 132.4 | 21.4 | 12.0 | 209.3 | 27.4 |
| Fisher LSD (U versus I) | | | 2.1 | 23.7 | 8.4 | 0.74 | 20.7 | 7.6 | 1.6 | 23.2 | 8.1 |

Note: Average data of four replicates are presented here. U: Uninoculated control and I: Inoculated.

**Table D.3.2**. Continued.

| Site | Soil | Inoculation | Year 1: 2011 cropping season (field pea) | | | Year 2: 2012 cropping season (wheat) | | | Year 3: 2013 cropping season (field pea) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) | Seed wt. (g plant$^{-1}$) | Shoot N uptake (mg plant$^{-1}$) | Shoot P uptake (mg plant$^{-1}$) |
| Scott | Swift Current | U | 11.0 | 178.0 | 38.0 | 5.2 | 178.9 | 27.4 | 10.1 | 174.1 | 33.7 |
| | | I | 13.5 | 196.0 | 49.0 | 4.8 | 189.2 | 34.5 | 12.4 | 191.7 | 43.5 |
| | Outlook | U | 11.7 | 175.0 | 30.0 | 5.4 | 135.3 | 26.3 | 10.7 | 171.2 | 26.6 |
| | | I | 14.1 | 202.1 | 45.0 | 5.3 | 133.5 | 25.1 | 13.7 | 200.8 | 39.9 |
| | Scott | U | 8.7 | 199.1 | 30.0 | 4.3 | 122.1 | 20.0 | 8.0 | 194.6 | 26.6 |
| | | I | 11.1 | 235.1 | 32.0 | 5.3 | 121.9 | 20.2 | 10.2 | 229.8 | 28.4 |
| | Melfort | U | 11.0 | 197.0 | 24.0 | 5.7 | 186.0 | 35.6 | 10.1 | 192.7 | 21.3 |
| | | I | 13.2 | 266.1 | 34.0 | 5.1 | 200.4 | 31.7 | 12.5 | 260.1 | 28.8 |
| Melfort | Swift Current | U | 11.8 | 187.0 | 35.0 | 5.3 | 189.4 | 20.4 | 10.9 | 182.9 | 31.1 |
| | | I | 13.9 | 209.1 | 39.0 | 5.4 | 201.3 | 22.7 | 12.8 | 204.4 | 34.6 |
| | Outlook | U | 12.0 | 188.0 | 40.0 | 4.8 | 165.6 | 26.2 | 11.0 | 188.0 | 35.0 |
| | | I | 15.0 | 210.1 | 58.0 | 4.4 | 169.7 | 25.4 | 14.8 | 205.4 | 51.5 |
| | Scott | U | 7.4 | 201.1 | 38.0 | 5.1 | 132.8 | 17.7 | 6.8 | 196.6 | 33.7 |
| | | I | 8.7 | 239.1 | 32.0 | 5.8 | 145.6 | 21.3 | 8.0 | 233.7 | 28.4 |
| | Melfort | U | 12.0 | 214.1 | 24.0 | 5.2 | 152.6 | 28.5 | 11.0 | 209.3 | 21.3 |
| | | I | 12.2 | 217.1 | 38.0 | 5.3 | 155.6 | 27.7 | 13.4 | 216.3 | 33.7 |
| Fisher LSD (U versus I) | | | 2.1 | 23.7 | 8.4 | 0.7 | 20.7 | 7.6 | 1.6 | 23.2 | 8.1 |

Note: Average data of four replicates are presented here. U: Uninoculated control and I: Inoculated.