Université de Montréal

Impacts de la fertilisation phosphatée sur la biodiversité microbienne de sols agricoles.

par

Marie-Soleil Beauregard

Département de Sciences Biologiques

Institut de recherche en biologie végétale

Faculté des Arts et des Sciences

Thèse présentée à la Faculté des études supérieures

en vue de l'obtention du grade de Philosophiæ Doctor (Ph.D.) en sciences biologiques

janvier, 2010

Université de Montréal
Faculté des études supérieures

Cette thèse intitulée :

Impacts de la fertilisation phosphatée sur la biodiversité microbienne de sols agricoles.

présentée par :
Marie-Soleil Beauregard

a été évaluée par un jury composé des personnes suivantes :

Jacques Brisson, président-rapporteur
Marc St-Arnaud, directeur de recherche
Chantal Hamel, co-directrice
Mohamed Hijri, membre du jury
John Klironomos, examinateur externe
Représentant du doyen de la FES

Résumé

La fertilisation phosphatée est très répandue dans les pratiques agricoles Nord-Américaines. Bien que généralement très efficace pour augmenter la production végétale, son utilisation peut engendrer certaines contaminations environnementales. Afin de diminuer ce problème, plusieurs pratiques de gestion sont envisagées. Parmi celles-ci, on retrouve l'intéressante possibilité de manipuler la flore microbienne car cette dernière est reconnue pour son implication dans bons nombres de processus fondamentaux liés à la fertilité du sol.

Cette étude a démontré que lors d'essais en champs, la forme de fertilisant ajouté au sol ainsi que la dose de phosphore (P) appliquée avaient un impact sur la distribution des microorganismes dans les différentes parcelles.

Une première expérience menée sur une culture de luzerne en prairie semi-aride a montré que les échantillons provenant de parcelles ayant reçu différentes doses de P présentaient des différences significatives dans leurs communautés bactériennes et fongiques. La communauté de CMA est restée similaire entre les différents traitements.

Une deuxième expérience fut menée pendant trois saisons consécutives afin de déterminer l'effet de différentes formes de fertilisation organiques et minérale ajustées selon une dose unique de P sur les populations bactériennes et fongiques d'une culture intensive de maïs en rotation avec du soja. Les résultats des analyses ont montrés que les populations varient selon le type de fertilisation reçu et que les changements sont indépendants du type de végétaux cultivé. Par contre, les populations microbiennes subissent une variation plus marquée au cours de la saison de culture. La technique de

DGGE a permis d'observer les changements frappant la diversité microbienne du sol mais n'a permis d'identifier qu'une faible proportion des organismes en cause.

Parallèlement à cette deuxième étude, une seconde expérience au même site fut menée sur la communauté de champignons mycorhiziens à arbuscules (CMA) puisqu'il s'agit d'organismes vivant en symbiose mutualiste avec la majorité des plantes et favorisant la nutrition de même que l'augmentation de la résistance aux stress de l'hôte. Ceci permit d'identifier et de comparer les différents CMA présents dans des échantillons de sol et de racines de maïs et soja. Contrairement aux bactéries et aux champignons en général, les CMA présentaient une diversité très stable lors des différents traitements. Par contre, au cours des trois années expérimentales, il a été noté que certains ribotypes étaient significativement plus liés au sol ou aux racines.

Finalement, l'ensemble de l'étude a démontré que la fertilisation phosphatée affecte la structure des communautés microbiennes du sol dans les systèmes évalués. Cependant, lors de chaque expérience, la date d'échantillonnage jouait également un rôle prépondérant sur la distribution des organismes. Plusieurs paramètres du sol furent aussi mesurés et ils présentaient aussi une variation au cours de la saison. L'ensemble des interactions possibles entre ces différents paramètres qui, dans certains cas, variaient selon le traitement appliqué, aurait alors probablement plus d'impact sur la biodiversité microbienne que la seule fertilisation.

Mots Clés

Diversité microbienne, fertilisation phosphatée, agriculture, champignons mycorhiziens, DGGE.

Abstract

Phosphorus fertilization is a widespread practice in North American agriculture. Although it is generally efficient to increase yields, its use can also induce some environmental contaminations. Several management practices are considered in order to decrease this problem. Among these possibilities there is the challenging one of manipulating microbial flora, which is well known for its implication in many processes related to soil fertility.

We have demonstrated in field trials that both the form of fertilizer added to soil and the applied P amounts impact microbial distribution in plots.

A first experiment performed on alfalfa monocultures in semi-arid prairie conditions demonstrated that samples coming from plots that had received different doses of P fertilizer presented significant differences on their bacterial and fungal communities. AMF population remained stable between treatments.

A second experiment was conducted over three growing season of an intensive maize/soybean rotation cropping system. It aimed to determine the effect of different organic and mineral fertilizers containing equal P amount on bacterial and fungal populations. It was demonstrated that these communities varied according to the fertilizer type applied. Changes are independent from the grown crop. However, microbial populations have undergone greater variation within each growing season. DGGE approach allowed to observe changes occurring in soil microbial diversity but have only permit to identify a small proportion of organisms.

A second experiment in the latter study was performed on the same site and focused on arbuscular mycorrhizal fungi (AMF) as they are organisms living in a mutualistic

symbiosis with most land plants and increasing host nutrition and resistance to stresses. It led to the identification and comparison of the different AMF found in maize and soybean soil and root samples. In opposition to what was observed with bacteria and fungi previously, AMF presented a very stable diversity between the different treatments. However, some ribotypes were significantly more present in soil or roots during each growing season.

Finally, our whole project demonstrated that P fertilization affected microbial community structure on studied sites. Nevertheless, in each experiment, sampling time also played a substantial role in the organism distribution. Many soil parameters were also monitored and presented a seasonal variation. The sum of possible interactions between these parameters, which in some cases varied according to treatment, would thus have more impact on microbial diversity that the sole fertilization.

Key words

Microbial diversity, AMF, P fertilization, agriculture, DGGE.

Table des matières

Liste des tableaux

Liste des figures

Liste des sigles et abréviations

ADN: acide désoxyribonucléique (DNA)
AEM : anion exchange membrane
AIA: acide indole acétique
AM: arbuscular mycorrhizæ (al)
AMF: arbuscular mycorrhizal fungi
ANOVA: analysis of variance
ARN: acide ribonucléique
c.v.: cultivar
C: carbone
Ca: calcium
$CaCl_2$: chlorure de calcium
$CaCO_3$: carbonate de calcium
CCA: canonical correspondance analysis
Cd: cadmium
Cl: chlore
cm: centimètre
CM: champignon mycorhizien
CMA: champignon mycorhizien à arbuscules
Cu : Cuivre
ddH_2O: eau bidistillée
DGGE : denaturing gradient gel electrophoresis
DMC: dichloromethane
dNTP: désoxyribonucléotide triphosphates
EC: electrical conductivity
FAME : fatty acid methyl esters
FAO : Food and Agriculture Organization
FID: flame ionization detector
Fig.: figure
g : gramme
h. : heure
H_2O: eau
ha : hectare
HCl: acide chlorhydrique
HSD : Honestly Significant Difference
INVAM : International culture collection of vesicular arbuscular mycorrhizal fungi
K : potassium
KCl : chlorure de potassium
kg: kilogramme
km : kilomètre
L. : Carl von Linné
LF: light fraction
M : molaire
m: mètre

MeOH: méthanol
Merr. : Elmer Drew Merrill
mg: milligramme
Mg: magnésium
min: minute
ml : millilitre
mm: millimètre
mM: millimolaire
mS: milliSiemens
N : nord
N: normale
N: nitrogen (azote)
N_2: azote atmosphérique
NaCl: chlorure de sodium
NaI: iodide de sodium
NaOH: hydroxyde de sodium
NCBI: National center for biotechnology information
NLFA: neutral lipid fatty acids
nm: nanomètre
NO_3: nitrate
NRC: National Research Council
NSERC: Natural Sciences and Engineering Research Council of Canada
O.D.: densité optique
P: phosphore
P_2O_5: anhydride de phosphate
pb: paire de bases (bp)
Pb: plomb
PCR: polymerase chain reaction
PGPR: plant growth promoting rhizobacteria/ rhizomicroorganisms
Pi: phosphore inorganique
PLFA: phospholipids fatty acids
PNP: p-nitrophenyl phosphate solution
Po: phosphore organique
PO_4: phosphate
RDA: representational difference analysis
rRNA: ARN ribosomal
sec: seconde
SPARC: Semiarid prairie agricultural research center
spp.: species (espèce)
SSCP: single-strand conformation polymorphism
SSU: small subunit
TAE: Tris-acetate-EDTA
TGGE: temperature gradient gel electrophoresis
TPF: triphénylformazan
T-RFLP: terminal restriction fragment length polymorphism
TTC: triphenyl tetrazolium chloride
U: unité

USDA: United States Department of Agriculture
UV : ultraviolet
v : volume
var.: variété
w : weight
W : ouest
yr : year (année)
Zn: zinc
µl: micro litre
µM: micro molaire
µm^3: micro mètre cube

Remerciements

En premier lieu je dois remercier mes co-directeurs : Marc St-Arnaud et Chantal Hamel. Marc a su trouver les arguments pour me convaincre de commencer ce projet, Chantal me motiver à le finir malgré toutes les embûches.

Également un immense merci à Keith, Atul, Tandra et Wai Ma en Saskatchewan de même qu'à Isabelle, Marie-Pierre et Stéphane Daigle de l'IRBV pour leur support et leur aide technique.

Je tiens aussi à remercier les étudiants de l'IRBV qui ont contribué au maintient de ma santé mentale au cours des dernières années. Merci aux deux Éric C., à Olivier, Jonathan, Mathieu et Julie.

Un merci tout particulier à Philippe Séguin qui m'a donné une chance dans le merveilleux monde des études graduées et qui m'a certainement aidé à développer les qualités pour passer à travers!

Sur le plan plus personnel, mais ô combien lié au bon déroulement de ce projet, j'aimerais remercier mes beaux-parents, Andreas et Ulrike Illi; mes parents, Élaine et Pierre Beauregard et ma sœur Suzelle pour leur compréhension mais surtout pour avoir eu si souvent le cœur à jouer avec Jules alors que je devais m'installer devant l'ordinateur.

Finalement, mes plus sincères remerciements à mon mari David qui a non seulement soutenu tant financièrement que moralement une éternelle étudiante, mais qui, en plus a été présent et souriant chaque jour depuis 5 ans.

Avant-propos

Le travail effectué au cours de ce projet de doctorat a été regroupé en cinq chapitres correspondant à une introduction générale du sujet ainsi qu'à quatre articles publiés ou rédigés en vue de l'être.

Le premier chapitre consiste donc en une introduction présentant la problématique, les objectifs et les hypothèses de ce travail.

Le second chapitre est composé de deux parties. Premièrement, un résumé d'une exhaustive revue de littérature sur les principaux sujets abordés dans l'ensemble du travail de doctorat. Une partie de ce texte est la traduction d'un chapitre de livre publié en 2009 sous le titre : '*Advances in Mycorrhizal Biotechnology: a Canadian Perspective*'. L'ensemble de ce chapitre de livre fut écrit en collaboration avec Dre Laetitia Lioussanne mais j'ai écrit le texte traduit et utilisé dans cette thèse. J'ai rédigé seule les sections de ce chapitre ne faisant pas partie de cette publication et elles furent révisées par mes co-directeurs, les Dre Chantal Hamel et Dr Marc St-Arnaud.

La deuxième partie du second chapitre est un article de synthèse publié en 2008 sous forme d'un chapitre du livre 'Mycorrhizae: Sustainable Agriculture and Forestry'. J'ai écrit l'essentiel de cet article et des corrections furent apportées par mes co-directeurs. Il résume bien l'importance des CMA en agriculture et la raison de la sélection de ce groupe d'organismes pour l'étude.

Les autres articles furent également rédigés par moi-même et corrigés par mes co-directeurs. De plus, certaines extractions d'ADN, amplifications PCR et gels de DGGE ont été effectués par Mme. Marie-Pierre Gauthier dans l'expérience sur les CMA (cf., chapitre V). Les essais à Harrow furent effectués par M. Tom Welacky et Dr Chin S.

Tan et les résultats transmis avec les informations sur le protocole appliqué par Mme. Mary-Anne Reeb sous la direction du Dr Tiequan Zhang (Agriculture et Agroalimentaire Canada, Harrow). Les échantillons de sol de Swift Current de même que les données sur les divers paramètres du sol furent mesurés au laboratoire de Dre Chantal Hamel et transmis par MM. Atul-Nayyar et Keith Hanson (respectivement étudiant au doctorat et technicien chez Agriculture et Agroalimentaire Canada à Swift Current). Mme. Isabelle Degongre (anciennement étudiante à la maîtrise à l'Institut de recherche en biologie végétale) et le Dr Wai Ma (University of Saskatchewan) m'ont également aidé à mettre en place le protocole de DGGE. Stéphane Daigle (statisticien de l'Institut de recherche en biologie végétale) m'a finalement guidé dans le choix des analyses statistiques présentes dans les chapitres III, IV et V.

Chapitre I

Introduction générale

Depuis longtemps, l'agriculture intensive fait appel à la fertilisation afin de hausser les rendements. Les fertilisants ajoutés au sol représentent non seulement une dépense supplémentaire pour les agriculteurs mais ils provoquent également la pollution des sols et des cours d'eau. Par exemple, le phosphore (P) présent dans les sols affecte positivement la production agricole mais contribue également à l'eutrophisation des eaux de surface (Cahoon and Ensign 2004; Sharpley et al. 2004). Il semble maintenant clair qu'une réduction substantielle des risques environnementaux liés à l'agriculture et le maintient du fort rendement des productions agricoles ne peuvent que passer par de meilleures pratiques de gestion (« *best management practices* »). Cependant, ces pratiques sont difficiles à définir car très peu d'informations sont présentement disponibles concernant les mécanismes qui influencent les cycles de l'azote (N), du C et du P dans le sol ainsi que sur les effets à long terme des différentes pratiques culturales. Bien que certaines méthodes de travail du sol soient maintenant utilisées afin de diminuer les problèmes liés à l'application de fertilisants, l'idée de manipuler directement les microorganismes qui jouent un rôle primordial dans la fertilité des sols en milieu agricole semble de plus en plus intéressante. En effet, il devient de plus en plus clair qu'en influençant la diversité microbienne d'un sol, il serait possible d'améliorer sa fertilité. Bien que plusieurs recherches portant sur des microorganismes du sol furent publiées au cours de la dernière décennie, celles-ci ont plutôt décrit les impacts de différentes pratiques de gestion de culture sur des groupes fonctionnels ou restreints d'organismes (Castillo et al. 2006; da Silva and Nahas 2002; Oehl et al. 2003). Cependant, les facteurs influençant la croissance des ces organismes et les variations de leur communauté sont nombreux à agir en même temps et leurs effets combinés sont

difficiles à prédire. L'ensemble des bactéries et champignons présents dans le sol a donc été pris en compte lors de la présente étude afin d'obtenir un portrait plus global de leur comportement suite à la fertilisation. Le groupe des champignons mycorhiziens à arbuscules (CMA) a aussi été ciblé plus spécifiquement puisque ces organismes sont particulièrement intéressants en agriculture (voir chap. II).

Plusieurs mécanismes impliqués dans les changements au niveau des communautés microbiennes sont toujours relativement inconnus surtout à cause d'un manque d'informations sur la véritable composition des sols agricoles. Il existe un vaste choix de méthodes d'analyse des communautés microbiennes mais l'analyse des communautés à l'aide d'une méthode de biologie moléculaire semble plus appropriée pour observer les variations d'un grand nombre d'organismes souvent difficiles à cultiver et identifier visuellement.

Le projet présenté ici avait donc pour but d'évaluer les impacts d'une pratique agricole fort répandue (i.e., la fertilisation phosphatée) sur différents paramètres liés à la fertilité des sols de même que sur l'ensemble de la biodiversité microbienne afin d'acquérir de nouvelles connaissances sur la dynamique de ce milieu complexe. Dans un premier cas (chap. III), l'effet provoqué par l'ajout de différentes doses de P sur la composition du sol a été étudié. Les variations entraînées chez les bactéries et les champignons du sol en général et chez les CMA plus spécifiquement ont donc été rapportées dans un seul chapitre. Ces mêmes groupes ont été ciblés au cours de la deuxième expérience qui étudiait, cette fois, l'impact de la forme de P appliquée sur la composition du sol. Ici il a été possible d'identifier les CMA à partir d'échantillons de sol mais aussi de racines. Par contre, les bactéries et champignons n'étaient analysés qu'à partir d'échantillons de sol. Les résultats présentant les variations des communautés bactériennes et fongiques ont

donc été présentés seuls (chap. IV) afin que ceux comparant les CMA retrouvés dans le sol et les racines soient mis en évidence dans un autre chapitre (chap. V).

Les objectifs de cette étude étaient donc :

1. Identifier les principaux représentants bactériens, fongiques et endomycorrhiziens présents dans le sol par l'approche moléculaire de PCR-DGGE.
2. Comparer l'impact de la fertilisation en P chez les CMA retrouvés dans les racines d'une plante hôte et dans sa rhizosphère.
3. Évaluer l'effet à long terme de l'ajout de différentes doses de P minéral sur la composition du sol.
4. Évaluer l'effet à court terme de l'ajout d'une même dose de fertilisation phosphatée mais appliquée sous différentes formes (minérale et organiques) sur la composition du sol.

Les facteurs affectant la biodiversité d'un sol en culture étant particulièrement nombreux, les effets de la fertilisation sont difficilement prévisibles.

Néanmoins, nos hypothèses de départ étaient :

1. La technique de PCR-DGGE, une technique de biologie moléculaire utilisée depuis environ une dizaine d'années, permettrait de façon simple et rapide d'étudier les communautés bactérienne et fongique dans leur ensemble. En présentant de nombreux avantages par rapport aux techniques culturales conventionnelles qui sont laborieuses et donnent lieu à une identification limitée de la communauté bactérienne, nous supposions que cette technique s'avèrerait être un outil d'identification intéressant en vue de la compréhension des interactions très complexes entre les microorganismes du sol.

2. Huit années de différentes doses d'application de P sur une culture de luzerne en continue influenceraient la biodiversité microbienne car une augmentation de P entraînerait une augmentation de la biomasse végétale et, par la suite, une diminution de la diversité microbienne.

Il n'existe cependant pas d'indication claire permettant de savoir si, avec l'apport de nutriments, il y aura ou non augmentation des taxons microbiens ou si seulement le nombre d'organismes déjà présent variera. Nous supposions que seulement un nombre limité de taxons les plus compétitifs à assimiler les différents nutriments présents dans le sol suite à l'augmentation de la biomasse végétale et de la fertilisation continueraient à augmenter la biomasse de leur communauté, diminuant ainsi la biodiversité microbienne détectée par le PCR-DGGE.

Également, comme il existe assez peu d'espèces constituant le groupe des CMA dans le monde entier, la proportion pouvant donc croître dans les conditions nord-américaines et dans des champs cultivés (souvent en monoculture) est encore plus limitée (i.e., on suggère même qu'une dizaine d'espèces seulement serait présente dans les champs cultivés de régions tempérées) (Jansa et al. 2002; Oehl et al. 2003). Nous envisagions une diminution des taxons détectés lors de la fertilisation à plus forte teneur en P.

3. L'application de doses équivalentes de P sous 3 différentes formes organiques et une minérale modifieraient la diversité et les taxons dominants de la rhizosphère (court terme) car l'apport d'autres éléments nutritifs en proportions différentes favoriserait certains groupes microbiens.

Seul le P était dosé pour chacune des fertilisations et cela entraîne inévitablement des variations dans la disponibilité d'autres éléments essentiels dans le sol. Par exemple, le taux de N disponible varie beaucoup entre le fumier et le lisier de porc. Habituellement la forme minérale d'engrais est appliquée afin d'être tout de suite disponible à la plante, permettant ainsi une croissance plus rapide. Nous croyions donc qu'il y aurait eu une certaine pression pour conserver les taxons microbiens les plus adaptés aux conditions entourant la croissance de la plante. Les taxons dominants auraient aussi varié d'un type de fertilisation à un autre compte tenu des variations apportées par la présence en différentes quantités des autres éléments essentiels.

Nous supposions également que l'alternance dans le type de culture (i.e., maïs-soja) entraînerait une augmentation de la diversité et modifierait la dominance de certains taxons au cours des différentes saisons.

Chapitre II

Analyse de la littérature

Les sections II.I à II.IV de ce chapitre comportent une traduction partielle d'un texte publié selon la référence suivante :

Laëtitia Lioussanne[1], Marie-Soleil Beauregard[1, 2], Chantal Hamel[2], Mario Jolicoeur[3], Marc St-Arnaud[1]. 2009. Interactions between arbuscular mycorrhizal fungi and soil microorganisms. pp. 51-69. Dans *Advances in Mycorrhizal Science and Technology.* D. Khasa, Y. Piché, A.P. Coughlan (Eds.), Chapitre 4. NRC Research Press, Ottawa, Canada.

La section II.V de ce chapitre a été publiée sous forme de chapitre de livre selon la référence suivante :

Marie-Soleil Beauregard[1, 2], Chantal Hamel[2], Marc St-Arnaud[1]. 2008. AM fungi communities in major intensive North American grain productions. pp. 135-158. Dans *Mycorrhizae: Sustainable Agriculture and Forestry.* Z.A. Siddiqui et al. (Eds.), Chapter 5, Springer, Dortrecht, The Netherlands.

[1]Institut de recherche en biologie végétale, Jardin botanique de Montréal, 4101, Sherbrooke Est, Montréal, Québec, Canada, H1X 2B2;
[2]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, 1 Airport Road, Box 1030, Swift Current, Saskatchewan, Canada, S9H 3X2;
[3]École Polytechnique Montréal, Université de Montréal, 2900 Boul. Édouard-Montpetit, Montréal, Québec, Canada, H3T 1J4

Le sol représente un habitat unique et complexe où vivent des milliers d'organismes. Parmi ceux-ci, les microorganismes tels que les algues, bactéries, champignons, nématodes et protozoaires forment une majeure partie de la population. La plupart d'entre eux dépendent de la matière organique comme source de carbone (C) et d'énergie et c'est pourquoi ils prolifèrent dans les premiers 10 à 15 cm de la surface du sol où les résidus organiques et les racines des végétaux sont le plus abondants.

Les microorganismes sont justement impliqués dans bons nombres de fonctions telles que l'élimination de toxines et la circulation du P, N et C, rendant leur diversité cruciale au maintient de la santé du sol. Ils jouent un rôle important dans la structure du sol. Parallèlement, l'activité microbienne est elle-même régulée par les diverses espèces et stades de croissance de plantes ainsi que par les conditions reliées à la santé du sol (Girvan et al. 2004; Schloter et al. 2003; van Elsas and Garbeva 2002).

II.I Interactions entre les microorganismes et les propriétés du sol

La majorité des 10^6 à 10^9 propagules microbiennes retrouvées dans un seul gramme de sol sont des bactéries et des champignons. Les communautés bactériennes du sol comprennent une multitude d'individus unicellulaires ayant un volume d'environ 1 μm^3. Les champignons quant à eux sont presque exclusivement retrouvés sous forme filamenteuse. Leurs hyphes sont souvent organisées de façon grossière et impliquées dans un large réseau de mycélium (Brodie et al. 2003). Ils constituent donc la plus importante proportion de la biomasse microbienne du sol (Atlas and Bartha 1997; Brodie et al. 2003; Domsch et al. 1980). Il existe plusieurs preuves que l'hétérogénéité de l'organisation sol/microorganismes donnerait au sol sa structure caractéristique (Young et al. 2001). Cette structure permet la co-existence de l'air ainsi que de l'eau et

détermine entre autres la facilité avec laquelle la plante pourra extraire l'eau et le taux de diffusion de certains composés à travers la matrice du sol (Young and Crawford 2004). Avec leur mycélium extra-racinaire, les champignons mycorhiziens à arbuscules (CMA) favorisent notamment l'agrégation des particules de sol et l'apport en eau et minéraux (Six et al. 2004; Subramanian and Charest 1998).

Il est reconnu que bien que les populations microbiennes interviennent elles-mêmes dans la structure du sol, elles sont influencées à leur tour par la nature et les propriétés de celui-ci. En effet, la disponibilité des nutriments essentiels tels que le P et N joue un rôle important dans la croissance des plantes mais également des microorganismes présents dans le sol. Les caractéristiques chimiques et physiques telles que l'humidité, la texture, le pH et le contenu en matière organique ont un impact sur la taille et la composition des communautés ainsi que sur leurs activités (Lundquist et al. 1999; Steenwerth et al. 2008). Par exemple, il a été démontré que dans certains cas de cultures vivaces le ratio champignons : bactéries du sol augmentait parallèlement à l'ajout d'intrants carbonés, de la hausse de N et de l'humidité (Allison et al. 2005; Bailey et al. 2002). Finalement, si le sable, l'argile et le limon ont tous des structures différentes, les microorganismes pour lesquels la croissance y sera favorisée le seront également.

Évidemment les propriétés des sols cultivés varient comparativement à celles de milieux non perturbés. La quantité et la nature des espèces de microorganismes de même que le nombre d'individus dans le sol sont clairement affectées par différents stress environnementaux et par certaines pratiques agricoles (Bardgett et al. 1999; Borneman et al. 1996). En agriculture, les sols sont soumis plus fréquemment à différents stress mécaniques ou chimiques et la matière organique récoltée en fin de saison n'est qu'en faible partie retournée au sol. Les différentes pratiques de gestion du sol appliquées en

agriculture influencent donc de façon importante les populations microbiennes puisqu'elles ont un impact direct sur la dynamique du milieu. Des pratiques comme les rotations de cultures, les cultures en continu et les labours induisent des changements chez les communautés microbiennes qui peuvent persister longtemps après que les traitements aient eu lieu. Les méthodes mécaniques employées pour travailler les sols représentent des éléments fortement perturbateurs pour les communautés microbiennes comme celles-ci se trouvent principalement dans les premiers centimètres à la surface du sol. Des études démontrent également que les pesticides peuvent contribuer à diminuer la diversité microbienne (Borneman et al. 1996; Martinez-Toledo et al. 2005).

L'apport de substances fertilisantes a donc une grande influence sur les communautés microbiennes du sol. Les fumiers et les fertilisants minéraux apportent différentes formes et quantités d'éléments essentiels à la croissance végétale qui pourront ou devront, dans certains cas, être assimilés et transformés par les microorganismes. Les bactéries ont tendance à réagir plus rapidement à l'apport de composés simples comme les sucres et les acides aminés très présents dans la matière organique. Par contre, les champignons continuent à décomposer du matériel organique complexe bien après que les bactéries aient arrêté (Brady and Weil 2002). L'arrêt de la fertilisation provoque généralement un changement rapide de dominance des populations bactériennes aux populations fongiques dans le sol, en changeant subitement la disponibilité en nutriments (Bardgett et al. 1999).

Les différentes pratiques de culture créent en effet des conditions environnementales pour les organismes du sol favorisant certains groupes fonctionnels (Ge et al. 2008; Girvan et al. 2004; Oehl et al. 2004).

II.II Interactions entre les différents microorganismes et les plantes

La nature de même que le nombre d'espèces végétales présentes dans un écosystème ont aussi une importante influence sur la biodiversité microbienne à cause de leur implication dans l'apport en sources de C et en énergie (van Elsas and Garbeva 2002). Ces interactions souterraines ont lieu entre les racines des plantes et les microorganismes et sont particulièrement complexes. Les racines, en sécrétant certains composés organiques spécifiques, enrichissent la rhizosphère de façon à favoriser les microorganismes adaptés pour utiliser ces substances. Les exsudats racinaires sont en fait une source très importante de C pour les microorganismes et ils peuvent représenter de 10 à 40% du C fixé par les plantes lors de la photosynthèse (Bertin et al. 2003; Bowen and Rovira 1999). Les substances exsudées comprennent notamment des sucres, des acides organiques et des acides aminés qui constituent donc une source de C et d'énergie dans le sol (Knee et al. 2001). La nature des exsudats racinaires varie selon l'espèce végétale, son stade de croissance et son état (Innes et al. 2004; Weisskopf et al. 2006) ce qui exerce une sélection différente sur les microorganismes qui se développent dans le sol (Broeckling et al. 2008; Ibekwe and Kennedy 1998). D'un autre côté, les microorganismes de la rhizosphère libèrent également des substances qui restent plus ou moins longtemps au niveau du sol accessibles aux autres organismes ou sont absorbés par la plante. Ces produits qui incluent des molécules telles que des régulateurs de croissance, des acides organiques, des antibiotiques, des hormones, et des enzymes, influencent à leur tour la croissance végétale (Rai 2006).

L'exsudation de différentes substances tant par les plantes que par les microorganismes favorisent certaines interactions entre ceux-ci. Bon nombre

d'interactions impliquent des liens directs entre les deux types d'individus et peuvent avoir des impacts majeurs sur la croissance et la productivité végétale. Par exemple, les maladies végétales d'origines microbiennes sont nombreuses. Plusieurs trouvent leur source directement dans le sol puisque de nombreux pathogènes attaquent la plante en pénétrant les racines (Baute 2002).

Il est également reconnu qu'il existe un nombre considérable d'espèces microbiennes capables d'exercer un effet bénéfique sur la plante. L'ensemble de ces microorganismes est appelé PGPR (*plant growth promoting rhizomicroorganisms*). Ils jouent un rôle primordial dans le maintient de l'équilibre écologique du sol et la durabilité des écosystèmes agricoles. Une demi-douzaine d'études ont démontrée que des souches d'*Azospirillum* augmentaient de façon significative la croissance du maïs, du blé et du millet en champ non seulement parce qu'elles fournissent de l'azote assimilable aux plantes mais aussi parce qu'elles produisent de l'AIA (*acide indole acétique*), une phytohormone impliquée dans la croissance (Dobbelaere et al. 2001; Jacoud et al. 1998; Lucy et al. 2004). Le *Burkholderia cepacia* inhibe la germination des spores d'*Alternaria*, un champignon pathogène des pommiers (Holmes et al. 1998). La fixation de N atmosphérique par les nodules racinaires est également un exemple évident. Ce processus complexe, qui permet ultimement la formation d'une structure fixant le N, implique une séquence bien précise de molécules excrétées par les plantes puis par des bactéries spécifiques. Il est maintenant bien connu que ce type d'association joue un rôle crucial en agriculture puisque certaines espèces bactériennes arrivent, de façon très spécifique, à fixer le N atmosphérique pour des légumineuses et ainsi augmenter la production de feuillage servant à la pâture des animaux (Seguin et al. 2001). D'ailleurs, en agriculture biologique, le fait de cultiver du soja à intervalles de temps réguliers entre

les cultures de maïs permet également d'augmenter la teneur en N du sol puisque le soja vit en symbiose avec le *Bradyrhizobium* qui fixe l'azote atmosphérique alors que le maïs ne forme pas ce type d'association et est donc dépendant des quantités de nitrates disponibles dans le sol pour sa croissance (Ferreira et al. 2000).

Les champignons mycorhiziens (CM) vivent en symbiose avec la majorité des plantes terrestres. Parmi ceux-ci, les champignons mycorhiziens arbusculaires (CMA) sont l'objet de plusieurs recherches. Ils formeraient avec les racines de 80% des plantes terrestres, les plus anciennes et omniprésentes symbioses mycorhiziennes. En fait, les CMA sont des symbiotes obligatoires vivant en association avec les racines de la majorité des plantes et affectant positivement leur croissance et leur nutrition (Schloter et al. 2003). Plusieurs études ont démontré une augmentation significative de l'assimilation du P chez les plantes mycorhizées. Les associations mycorhiziennes favoriseraient aussi l'assimilation d'autres éléments comme le N, le cuivre (Cu) et le zinc (Zn) (Giri et al. 2005; Jones et al. 1998; Karandashov and Bucher 2005). En fait, ils agissent comme une extension des racines, augmentant ainsi sensiblement le volume de sol exploité par la plante. Leur rôle dans la circulation des nutriments est indéniable puisqu'ils se développent à l'interface entre la racine et le sol (Dodd 2000; Hodge 2000). La productivité des écosystèmes est aussi grandement influencée par la symbiose mycorhizienne à cause de son impact sur la compétitivité de l'hôte (Klironomos 2002). Les champignons mycorhiziens affectent à la fois le sol et la plante et sont donc un outil à considérer pour la gestion de systèmes agricoles.

II.III Interactions entre les différentes communautés microbiennes

Les microorganismes du sol interagissent, favorablement ou non, entre eux. La présence d'une espèce en particulier peut, en effet, provoquer des variations qualitatives, quantitatives et spatiales chez d'autres communautés. Le développement d'interactions positives permet aux communautés microbiennes impliquées d'utiliser les ressources disponibles plus efficacement. Ces interactions permettent d'occuper des niches où, souvent, une seule population ne pourrait se développer. Certains types de microorganismes sont, par exemple, en mesure de produire des enzymes essentielles au métabolisme de substrat utilisés par d'autres. La détoxification de composés toxiques à une partie de la communauté peut aussi être effectuée par d'autres espèces. Évidemment, les interactions négatives sont également possibles et la compétition se produit fréquemment lorsque deux populations microbiennes utilisent la même ressource dans un espace où les nutriments sont limités (Atlas and Bartha 1997).

Plusieurs études ont révélé que l'association mycorhizienne influençait le taux de croissance bactérien (Christensen and Jakobsen 1993; Marschner et al. 1997a). La présence de mycélium mycorhizien dans le sol aurait aussi une influence positive ou négative sur les champignons saprophytes ou pathogènes (Filion et al. 1999; Green et al. 1999; Larsen et al. 1998). À l'inverse, certains champignons influenceraient le développement des certaines souches de CMA (Martinez et al. 2004). Il a également été démontré que l'efficacité de la symbiose fixatrice de N chez les légumineuses pouvait être augmentée en présence de colonisation mycorhizienne de la plante (Azcón-Aguilar and Barea 1981; Xavier and Germida 2002).

Plusieurs microorganismes sont maintenant étudiés dans le but de combattre de façon biologique certains pathogènes présents dans le sol. En effet, divers microorganismes du sol ont également un fort potentiel comme agents de biocontrôle et les mécanismes permettant le contrôle des agents pathogènes par un autre microorganisme sont variés. Par exemple, certaines souches de *Bacillus subtilis* produisent plus d'une soixantaine d'antibiotiques dont plusieurs ont des propriétés anti-fongiques. Il a été démontré que l'utilisation de ces souches arrivait à inhiber, *in vitro*, cinq souches du pathogène du soja *Sclerotinia sclerotiorum* (Araujo et al. 2005).

II.IV Analyse de la biodiversité microbienne

Les communautés microbiennes du sol jouent donc un rôle essentiel dans la dynamique de celui-ci et l'intérêt qui leur est porté ne cesse de croître. Il est cependant toujours difficile d'affirmer avec exactitude combien d'espèces microbiennes (bactéries et champignons confondus) se retrouvent dans le sol. Certaines études estiment qu'il y aurait environ $4x10^3$ à 10^4 espèces / g de sol alors que d'autres avancent des chiffres de $1x10^9$ uniquement de bactéries / g de sol appartenant à 10 000 espèces (Borneman et al. 1996; Schloter et al. 2003). Pendant plusieurs années, les microorganismes furent étudiés en culture ou par extraction directe à partir du sol de spores fongiques (i.e., technique de filtration sur membrane). Par contre, les analyses microscopiques ont ensuite démontré que seulement 1 à 10% des microorganismes du sol seraient cultivables et ceux qui le sont requièrent souvent des conditions de croissance très particulières rendant leur observation difficile (Bardgett et al. 1999; Duineveld et al. 2001). Bien que la culture des bactéries et champignons ait énormément contribué à l'acquisition de connaissances, elle reste une méthode longue, parfois coûteuse et qui

donne très peu, voire aucune information sur la distribution spatiale des microorganismes.

Aujourd'hui, certaines méthodes biochimiques et microbiologiques sont utilisées pour étudier les communautés microbiennes. Les méthodes biochimiques utilisées dans l'étude des profils microbiens incluent entre autres les analyses métaboliques qui mesurent les métabolites totaux produits par une communauté et les analyses lipidiques qui se basent sur la distribution de divers lipides. Par exemple, les méthodes d'analyse des PLFA (*phospholipids fatty acids*) et FAME (*fatty acid methyl esters*) sont des approches dans lesquelles la nature et la distribution de divers lipides membranaires sont utilisées pour construire les profils phylogéniques et d'activités métaboliques de communautés microbiennes. Les lipides membranaires peuvent fournir de l'information quant à l'état physiologique d'une communauté puisque la composition en lipides de la membrane des microorganismes peut être altérée en réponse à différents stress (Misko and Germida 2002). Certaines informations taxonomiques peuvent également être tirées de la nature et de la distribution de divers lipides mais à cause du nombre limité de marqueurs spécifiques (surtout pour les champignons), les PLFA sont plutôt utilisés pour comparer les biomasses des échantillons entres eux.

La majorité des études récentes portant sur la biodiversité microbienne fait appel à des techniques de biologie moléculaire puisqu'elles permettent l'identification d'organismes directement à partir d'échantillons environnementaux sans tenir compte de leur morphologie ou de leur stade de développement. Pour les techniques faisant appel à l'utilisation de fragments des différents génomes microbiens d'échantillons complexes, l'ADN (ou ARN) doit d'abord être extrait puis amplifié à l'aide d'une réaction de

polymérase en chaîne (PCR). La justesse des résultats et la reproductibilité des méthodes employées en biologie moléculaire sont particulièrement dépendantes d'une bonne extraction mais aussi de l'amplification du fragment génomique étudié. La rectitude des techniques basées sur le polymorphisme génétique repose donc largement sur la qualité de l'extraction de l'ADN et de la PCR. Il a d'ailleurs été démontré que l'évaluation de la diversité microbienne pouvait varier selon les différentes techniques d'extraction (Martin-Laurent et al. 2001). Ceci souligne la difficulté de comparer des données obtenues à partir de méthodes différentes. La grosseur de l'échantillon de sol utilisé pour l'extraction d'ADN aurait aussi de l'importance. Une complication supplémentaire, dans le cas de l'étude des champignons, réside dans la présence potentielle de spores et de mycélium dans un même échantillon rendant l'étude de la population fongique active plus difficile. Au niveau de l'amplification, la contamination de l'habitat par plusieurs inhibiteurs comme les acides humiques et fulviques est aussi un facteur très important dans le cas du sol et peut freiner l'efficacité de certaines réactions (Felske et al. 1996; Landeweert et al. 2003). Évidemment la complémentarité des amorces face au gène ou à la région étudiée influence grandement l'amplification. Le défi du design d'amorces est de trouver des parties du génome assez différentes pour différencier spécifiquement le type d'organisme étudié.

Il existe toute une variété de techniques moléculaires pour l'analyse des communautés microbiennes possédant chacune leurs avantages et inconvénients (Kirk et al. 2004). Parmi les techniques les plus utilisées pour l'étude de la biodiversité de la rhizosphère mentionnons seulement le SSCP (*single-strand conformation polymorphism*), le T-RFLP (*terminal-restriction fragment length polymorphism*) et le DGGE ou TGGE (*denaturing gradient gel electrophoresis* et *temperature gradient gel electrophoresis*) (Duineveld et

al. 2001; Lukow et al. 2000; Muyzer et al. 1993; Stach et al. 2001). Le DGGE, utilisé au cours des expériences présentées ici, semble être une des techniques les plus efficaces pour observer la diversité, la richesse et l'uniformité de la communauté microbienne (Yu and Morrison 2004). Il s'agit d'une approche moléculaire qui permet de séparer des fragments de même taille mais de composition différentes en nucléotides. Cette technique consiste en une séparation des séquences amplifiées sur des gels de polyacrylamide contenant un gradient linéaire de dénaturants chimiques (urée et formamide) de l'ADN. Le gradient de concentration utilisé varie en fonction de la taille des amplicons à séparer (idéalement < 500 pb). Les variations de séquences entre les différents amplicons vont influencer leur dénaturation et par conséquent leur distance de migration électrophorétique. Des profils complexes représentatifs de la diversité des fragments amplifiés sont ainsi obtenus. Les bandes formées peuvent ensuite être excisées du gel et séquencées afin d'identifier les organismes. La qualité de l'information recueillie à l'aide de cette méthode dépend donc à la fois du nombre de variants et de la résolution des amplicons dans le gradient dénaturant (Muyzer et al. 1993; Yu and Morrison 2004). La technique d'analyse microbienne par DGGE est généralement considérée comme une approche avantageuse parce que beaucoup moins longue, fastidieuse et coûteuse que l'approche de clonage/ séquençage utilisée dans certaines études (Filion et al. 2004). En effet, le produit peut, dans certains cas, être excisé directement du gel, réamplifié et séquencé. Autrement, il est nécessaire de d'abord cloner le produit PCR puis analyser tous les clones obtenus. Le clonage offre une meilleure résolution et identification des espèces plus rares alors que le DGGE permet d'analyser rapidement et à moindre coût plusieurs échantillons et de comparer leur contenu microbien sur des différences temporelles et géographiques (Diez et al.

2001; Muyzer et al. 1993). Par contre, il a été démontré que des bandes migrant à une position identique n'appartenaient pas nécessairement à la même espèce microbienne et que comme le DGGE représente davantage les groupes dominants, certains effets de la rhizosphère pourraient être masqués. Finalement, le DGGE est une approche qualitative ne permettant pas d'estimer le nombre de microorganismes présents dans l'échantillon de départ. D'autres méthodes doivent donc être employées afin de les quantifier.

En résumé, les microorganismes jouent un rôle primordial dans le concept de fertilité des sols en milieu agricole. Les facteurs influençant leur croissance et les variations de leur communauté sont nombreux à agir en même temps sur le sol et leurs effets combinés sont difficiles à prédire. Il devient de plus en plus clair qu'en manipulant la diversité microbienne d'un sol, il serait possible d'améliorer sa fertilité. Par contre, plusieurs mécanismes impliqués dans des tels changements sont relativement inconnus surtout à cause d'un manque d'informations sur la véritable composition des sols agricoles. Il existe un vaste choix de méthodes d'analyse des communautés microbiennes mais l'analyse des communautés à l'aide des séquences ribosomales semble une méthode de choix puisque le nombre de ribosomes dans les cellules physiologiquement actives est plus élevé et qu'une bonne quantité d'ADN est requise au départ à cause de la présence dans le sol de plusieurs inhibiteurs naturels. Le projet présenté ici avait donc pour but d'évaluer les impacts d'une pratique agricole fort répandue (i.e., la fertilisation phosphatée) sur différents paramètres liés à la fertilité des sols de même que sur l'ensemble de la biodiversité microbienne afin d'acquérir de nouvelles connaissances sur la dynamique de ce milieu complexe.

II.V AM fungi communities in major intensive North American grain productions

Abstract

With population increase, urban sprawl on some of the best agricultural soils and the interest for biofuels, serious pressures have been created on grain and oilseeds production in North America. Fertilizers are the main expense in intensive agricultural management practices. P fertilization is often closely related with soil degradation and contamination of surface water, causing eutrophication and accumulation of blue-green algae in certain locations of Canada. Arbuscular mycorrhizal symbioses have been shown to benefit plant growth in large part due to the very extensive hyphal network development in soil, exploiting nutrients more efficiently and improving plant uptake. AM symbiosis also increases resistance to stress and reduces disease incidence, representing a key solution in sustainable agriculture. Appropriate management of mycorrhizae in agriculture should allow a substantial reduction in chemical use and production costs. This chapter will review the effects of various fertilization practices on AMF community structure and crop productivity in major North American grain productions (i.e., corn, soybean, wheat, barley), and their reaction to other common management practices (i.e., tillage, rotation, pesticide use).

K**ey words**: Arbuscular mycorrhizal fungi, intensive agriculture, grain production.

Introduction

With population increase, urban sprawl on some of the best agricultural soils and growing interest for biofuels, serious pressures have been created on grain and oilseeds

production in North America. However, agriculture still occupies an important part of North American territory and remains an important part of the economy (Table I).

Many forms of crop management have been used systematically by farmers since many decades with the aim of rapidly increasing crop productivity. Among them mineral fertilizers now represent one of the main expenses in intensive agricultural management practices (Heffer and Prud'homme 2006). Organic fertilizers such as manures and composts are easily available for most farmers but their nutrient content is often uneven and unpredictable from year to year. No matter which form of fertilizer is applied, conventional farming generates large nitrogen (N) and phosphorus (P) surplus, which can lead to both N and P leaching (Brady and Weil 2002). Not only is there a cost for farmers associated to this loss, but the phenomenon has also been related to soil contamination, and can be a major threat for aquatic systems through surface and groundwater degradation (Kirchmann and Thorvaldsson 2000). The role of P in anthropogenic eutrophication of water bodies is well known since many decades (Imboden 1974). More recently, fertilizer runoff from agricultural fields was emphasize among the causes of excessive cyanobacteria growth and increasing of potentially harmful blooms leading to restricted access to lakes in certain locations of Canada and United States.

Arbuscular mycorrhizal fungi (AMF) are estimated to associate with over 90% of vascular plants, including most agricultural crops (Read et al. 1976; Smith and Read 1997). Their extensive soil hyphal network development has been largely studied. These obligatory symbionts have been demonstrated to benefit the growth of numerous plant species by improving their nutrient uptake as well as increasing their resistance to abiotic stresses and reducing damages caused by pathogenic microorganisms (Barea et

al. 2002; Clark and Zeto 2000; Schloter et al. 2003; Smith and Read 1997; St-Arnaud and Vujanovic 2007). A more appropriate management of mycorrhizae in agriculture is expected to allow a substantial reduction in the amount of minerals used without losses in productivity, whereas permitting a more sustainable production management. This chapter will review the AMF contribution to crop productivity and effects of various fertilization practices on AMF community structure in major North American grain productions (i.e., corn, soybean, wheat, barley), and their response to other common management practices (i.e., tillage, rotation, fallow).

Table I. North American cereal cropping importance according to the number of cultivated hectares (ha) and tonnes harvested in 2003.

Crops		Canada	Mexico	USA
Barley	ha	49 894 000	N.A.	2 000 000
	tonnes	12 327 600	1 109 424	6 011 080
Maize	ha	1 226 100	7 780 880	28 789 240
	tonnes	9 587 300	19 652 416	256 904 560
Soybean	ha	10 528 000	N.A.	29 000 000
	tonnes	2 268 300	75 686	65 795 340
Wheat	ha	10 467 400	626 517	21 383 410
	tonnes	23 552 000	3 000 000	63 589 820

Source: (FAO 2004; Statistics Canada 2007; USDA 2007); N.A.: data not available

Arbuscular mycorrhizae contribution to grain production

Several studies have been conducted to confirm AM symbiosis positive influence on major edible plants (Table II). A survey published in 1988 reported that in 78 field trials, increased AMF colonization resulted in an average yield increase of 37% (McGonigle 1988). A more recent meta-analysis of 290 field and greenhouse studies published between 1988 and 2003 confirmed this relation between colonization extent and crop productivity, and determined that increased colonization resulted in an overall 23% yield

increase (Lekberg and Koide 2005). It has been concluded that soybean, maize, barley and wheat yields were all increased by AMF colonization in greenhouse trials (Ilbas and Sahin 2005; Karagiannidis and Hadjisavvazinoviadi 1998; Lekberg and Koide 2005; Nourinia et al. 2007).

Crop plants show variations in their dependence on mycorrhizae for nutrient uptake meaning that the ones with roots that cannot seek P efficiently receive the most benefit from mycorrhizal symbiosis. Other factors such as root surface area, root hair abundance and length, growth rate, response to soil conditions and exudations can be related to the plant dependency on AM symbiosis for nutrient uptake. Some crops are considered as facultative mycotrophs, while others are seen as obligate mycotrophs (Smith and Read 1997). Various plants, such as leek or corn, are highly dependent on mycorrhizae to meet their basic P requirements, while others like wheat, barley and oat, benefit from the symbiosis but are less dependent (Plenchette 1983; Ryan and Angus 2003). Ryan *et al.* (2005) even noticed that high colonization by AMF was associated with reduced growth of winter wheat in low-P condition, strengthening the hypothesis that different plant species do not benefit equally from AMF because some of them acquire more nutrients from the symbiosis than others (Smith and Read 1997). Screening of maize inbred lines to study their tolerance to low-P stress conditions has brought evidence that there are genetic variations in P uptake efficiency (da Silva and Gabelman 1992). Many experiments conducted on North America intensively grown crops have also showed that responsiveness to mycorrhizal colonisation changes with plant cultivars (Baon et al. 1993; Khalil et al. 1994; Zhu et al. 2001).

Table II. Recent studies showing beneficial impacts of AMF on various edible plants cultivated in North America.

Type of plant	Conclusions	References
Citrus	• enhanced drought tolerance of tangerine • increased citrus P, Zn, and Cu contents • increased root length of lemon	(Fidelibus et al. 2001; Ortas et al. 2002; Wu and Xia 2006)
Cereals	• enhanced growth of corn in compacted soil • increased biomass production of corn in low P soil • increased K, Ca, Mg uptake of corn • enhanced growth of millet • alleviated the adverse effects of chlorothalonil on rice • exerted protective effects against toxicity of Cu, Zn, Pb, and Cd in contaminated soil on rice	(Bagayoko et al. 2000; Liu et al. 2002; Miransari et al. 2007; Zhang et al. 2005; Zhang et al. 2006)
Legumes	• reduced the development of pea root-rot • increased growth and yield of peanut • decreased incidence of peanut pod rot	(Abdalla and Abdel-Fattah 2000; Quilambo et al. 2005; Thygesen et al. 2004)
Vegetables	• enhanced development of pepper plants • reduced *Phytophthora* blight in green pepper • decreased *Fusarium* wilt incidence in cucumber	(Hao et al. 2005; Ozgonen and Erkilic 2007)

Studies have been conducted to investigate the effects of AMF on plant competition to support the hypothesis that the presence and abundance of such fungi can influence plant species dominance or mediate coexistence (Hamel et al. 1992; Hart et al. 2003; Marler et al. 1999; West 1996; Yao et al. 2005). For example, Feldmann and Boyle (1998) found that the AMF benefits to maize yield come from maintaining a diverse weed cover crop.

A winter wheat cover crop was compared to a dandelion cover and found that dandelion produced higher AMF colonization, P-uptake and yield in the following maize crop showing that in some cases, weeds may provide an effective support to AMF between cropping periods (Kabir and Koide 2000). Jordan et al. (2000) hypothesized that specific AMF could reduce the prevalence of non-host species in weed communities. Some studies have highlighted the capability of some AMF to strongly change the relative abundance of some important agricultural weeds. In fact, it has been reported that early growth rate of non-host weedy species were reduced in the presence of AMF (Francis and Read 1994; Johnson 1998). Conversely, there are also some indications that non-mycorrhizal plants may actively antagonistize AMF, e.g. via inhibitory compounds released into soil (Fontenla et al. 1999). It is thus interesting to note that many common North American agricultural weeds belong to families that appear to be predominantly non-host (Francis and Read 1994; Jordan et al. 2000).

Data also support the idea that the influence of AM associations on plant competition is dependent not only on the presence but also on the identity of AMF (van der Heijden et al. 2003; Vogelsang et al. 2006). As the symbiosis between plants and AMF is non-specific, plant response to mycorrhizal colonization also varies according to the organism they are in symbiosis with. Therefore, biomass and P acquisition depend on the specific plant–AMF combination (Klironomos et al. 2000; Scheublin et al. 2007; Smith et al. 2004). To illustrate this, a greenhouse study conducted on wheat demonstrated an increase in plant growth 42 days after inoculation with *Scutellospora calospora* but a significant decrease after inoculations with *Glomus* spp. or *Gigaspora decipiens,* under low-P condition (Graham and Abbott 2000).

However, the whole dynamics of field soils in such plant production brings many more variables that should be taken into consideration. In fact, the degree to which AMF increase yields is greatly dependent on various factors such as soil type, nutrient status, crop, management practices, and soilborne microorganisms (Karagiannidis and Hadjisavvazinoviadi 1998). Agricultural management practices, soil nature, abiotic stresses and other soil microorganisms are factors impacting plants and, thus, influencing AMF development and/or colonization, or *vice versa*.

Alleviation of major abiotic stresses

AMF can adapt to a wide range of environments. They are found in soils with very different water regimes including very arid habitats. In these regions, low level of soil moisture can sometimes be compensated by increased root system area for water uptake through hyphal ramification in the soil (Ahmad Khan et al. 2003). It has been shown that mycorrhizal fungi can improve water use efficiency and sustain drought stress in wheat (Al-Karaki et al. 2004), oat (Ahmad Khan *et al*., 2003), and corn (Subramanian and Charest 1995, 1999; Subramanian et al. 1995, 1997). Colonized soybeans had higher leaf water potential and relative water content than non-mycorrhizal plants under water stress conditions (Aliasgarzad et al. 2006). AMF colonization also increased onion yield under water deficit condition (Bolandnazar et al. 2007). In fact, mycorrhizal plants have, in general, higher water uptake due to hyphal extraction of soil water (Al-Karaki 1998; Bethlenfalvay et al. 1988; Ruiz-Lozano et al. 1995) and higher root hydraulic conductivity than non-mycorrhizal plants (Augé and Stodola 1990). Some results also suggest that AM association enhances N assimilation by maize which enables the host

plant to more efficiently withstand drought conditions and recover after stress is relieved (Subramanian and Charest 1998).

There are also some evidences in the literature that AMF colonization can protect crops such as barley, cotton and lettuce against the negative effects of salt (Nourinia et al. 2007; Ruiz-Lozano and Azcon 2000; Tian et al. 2004). Moreover, in acidic soil conditions, growth and mineral acquisition of maize has been positively associated with AM root colonization (Alloush and Clark 2001; Clark 1997). Similarly, in alkaline soil, an experiment performed on durum wheat inoculated with *Glomus mosseae* also showed greater grain yields in mycorrhizal plants than in controls (Al-Karaki and Al-Omoush 2002). Soil compaction is another important soil characteristic that greatly affects crop yields. Soil compaction can rapidly lead to reduced root density which can result in decreased water and nutrient uptake (Pardo et al. 2000). Corn growth was increased by AM colonization in compacted soil, though the effectiveness of AMF-derived growth increase varied with the level of soil compaction, the AMF strain, and interaction with other soil microorganisms (Miransari et al. 2007).

Effect of AMF on plant pathogens

AMF may impact crop growth by affecting some soil microbial populations also present in the agroecosystems. Many researches focused on these relationships, showing that interactions between AMF and other soil microorganisms can be either detrimental or favourable to plant pathogens, other rhizosphere microbes, AMF or to mycorrhizal plants (Calvet et al. 1992; Elsen et al. 2001; Filion et al. 1999; Gryndler et al. 2002; Meyer and Linderman 1986a, 1986b; Paulitz and Linderman 1989; Rousseau et al. 1996; St-Arnaud and Elsen 2005; St-Arnaud et al. 1995; Talavera et al. 2001; Vigo et al.

2000). The effect of plants on soil biota may be related to the amount and quality of root exudates released in the soil. Ability to access and metabolize different nutrient sources vary from one microbial species to the other (Baudoin et al. 2003). Plant root exudation pattern and therefore its impact on soil biological environment can be greatly modified by mycorrhizal colonization (Linderman 1992). It has often been demonstrated that changes in the amount, quality or pattern of release of root exudates by mycorrhizal colonization could influence the other microorganisms present in the rhizosphere (de Boer et al. 2005; Filion et al. 1999; Graham 2001; Lioussanne 2007; Sood 2003).

Several studies have investigated the changes occurring in the bacterial communities of mycorrhizal plants' rhizosphere. There are numerous reports of AMF influencing bacterial growth rate (Christensen and Jakobsen 1993; Marschner and Crowley 1996a, 1996b; Marschner et al. 1997b). Experiments also demonstrated that mycorrhizal associations could be related to qualitative, quantitative and spatial shifts in those populations (Andrade et al. 1997; Linderman and Paulitz 1990; Meyer and Linderman 1986b; Posta et al. 1994). AM colonization can influence the species composition of the soil microbial community by increasing some groups and decreasing others (Christensen and Jakobsen 1993; Vazquez et al. 2000).

Such an important influence on soil microorganisms has rapidly raised interest on the possible role of AMF in bioprotection. Consequently, the number of research works conducted on the interactions between AMF and pathogens in diverse agricultural systems has exploded in the last decade. Even if mycorrhizal inoculation was sometimes associated with neutral effects (Bødker et al. 2002) or enhanced disease symptoms, with some AMF isolates (Garmendia et al. 2004), in most studies AMF inoculation reduced pathogen damages revealing the potential of AMF as a biological control agent (Li et al.

2007; Selim et al. 2005). It is also important to mention that although most studies have reported a decrease in fungal or nematode-induced root disease severity in mycorrhizal treatments (Borowicz 2001; Castillo et al. 2006; Graham 2001; Matsubara et al. 2001; St-Arnaud and Vujanovic 2007), there are only very few reports of such behaviour associated to most North American agronomic crop plants. For example, *Cochliobolus sativus*, currently one of the dominant pathogen of barley in Canada (Ghazvini and Tekauz 2007), has been shown to be suppressed by various AMF independently of P availability and water stress (Boyetchko and Tewari 1988, 1990; Rempel 1989), as was transmission of *B. sorokiniana* in aerial parts of barley plants in Sweden (Sjöberg 2005). Severity of take-all caused by *Gaeumannomyces graminis* var. *tritici* in wheat was also reduced by AMF inoculation in P-deficient soil but was not affected at a higher P level (Graham and Menge 1982). Conversely, stem rust was more severe in mycorrhizal wheat plants inoculated with *Puccinia graminis* urediospores at the two-leaf stage, compared to non-mycorrhizal plantlets (Rempel 1989). Despite its great potential to be used as a key component of sustainable agriculture, AMF effectiveness as disease control agents depends on many other factors, such as temperature, soil nutrient and water contents, time of mycorrhizal inoculation, amount of mycorrhizal inoculum, pathogen virulence, parameters which are all very difficult to study, not to mention control, in the field. On the other hand, management of soil microbial components, including AMF, definitely represents a promising direction toward the control of plant diseases.

Effect of AMF on plant beneficial microorganisms

Several types of microorganisms like N_2-fixing bacteria (de Varennes and Goss 2007; Powell et al. 2007), P-solubilizing bacteria and fungi (Barea *et al*., 2002), antagonist of plant pathogens (Budi et al. 1999) and soil aggregating bacteria (Rillig et al. 2005) are associated with the rhizosphere of mycorrhizal plants. These organisms are generally grouped under the name 'plant growth promoting rhizosphere microorganisms' (PGPR) because they are able to exert beneficial effects on plant growth. Many leguminous plants have the ability to create a symbiosis with rhizobia, which can fix atmospheric nitrogen (N_2) and hence increase plant access to N sources. Various studies suggested that a specific interaction that influences both the nodulation and mycorrhizal colonization processes occurs between AMF and the N_2-fixing rhizobia in legumes (Ibijbijen et al. 1996; Saxena et al. 1997; Xavier and Germida 2002; Xavier and Germida 2003). Growth and productivity of the legumes were always dependent on the combination of selected AMF and rhizobia, revealing that positive interactions between compatible symbionts could significantly increase growth and yields. Pot experiments done with soybean demonstrated that under controlled environment conditions, N_2-fixation in mycorrhizal plants is generally greater than in non-mycorrhizal plants, with more nodules and greater nodule dry weight (Goss and de Varennes 2002). However, it seems that under field conditions N_2-fixation is not always promoted even if the tripartite symbiosis formed by indigenous arbuscular mycorrhizae, *Bradyrhizobium* and soybean is established (Antunes et al. 2006).

Synergistic effect of associative diazotroph bacteria on AMF activity has often been reported (Barea et al. 2002; Sala et al. 2007). Inoculation of barley with *Glomus mosseae*

or *G. fasciculatum* together with *Azospirillum brasilense* produced a synergic effect on dry matter and grain yield, in a greenhouse study (Subba Rao 1985). Biró *et al.* (2000) also noted a beneficial effect on soybean of co-inoculation with *Azospirillum brasilense, Rhizobium meliloti* and *Glomus fasciculatum,* while Russo *et al.* (2005) concluded after pot and field tests that an indirect effect of *Azospirillum* on mycorrhization can be assumed on corn and wheat plants as a consequence of the positive effect on root growth. Co-inoculation of wheat with strains of *Pseudomonas* and *Glomus clarum* have also shown a positive dry matter response (Walley and Germida 1997). However, in this study, inoculation did not result in any increase in root dry weight or length. Another greenhouse trial conducted on maize inoculated with a biofertilizer containing *Glomus*, *Azotobacter* and *Bacillus* strains resulted in a significant increase of plant growth (Wu et al. 2005). Inoculation also improved soil properties, such as organic matter content and total N in soil as well as it increased the nutritional assimilation of plant (total N, P and K).

Higher nutrient assimilation can often be related to the beneficial effects of P- and K-solubilizing bacteria (Rodriguez and Fraga 1999). They may indeed enhance mineral uptake by plants through solubilizing insoluble forms of P and K, and making them available in soil to plant roots. Many microorganisms are thus able to improve plant growth by solubilizing rock phosphate into plant available P form (Reyes et al. 2002; Rodriguez and Fraga 1999; Whitelaw 2000). Among reports that showed synergistic interactions between P-solubilizing microorganisms and AMF (Artursson et al. 2006; Hamel 2004; Villegas and Fortin 2001, 2002), a few studies have been conducted on wheat plants. In field trials performed in southern Egypt, the highest significant effect on *Triticum aestivum* L. yield and P content was observed when seeds were inoculated with

a mixture of *Glomus constrictum* and two fungal isolates (*Aspergillus niger* and *Penicillium citrinum*), which are known as phosphate rock-solubilizing fungi (Omar 1998). A recent work proposed by Babana and Antoun (2006) also showed that by inoculating wheat seeds with phosphate rock-solubilizing microorganisms and *Glomus intraradices* under field conditions, it is possible to obtain grain yields comparable to those produced by using diammonium phosphate fertilizer.

However, the application of microbial fertilizers has not resulted in constant effects. The mechanisms and interactions among crops, microbes and abiotic factors are still not well understood but there are great expectations regarding the fact that biofertilizers may complement mycorrhizal activities in sustainable agricultural systems.

Impact of land use on AMF abundance and diversity

Crop management practices such as tillage, pesticide application, crop rotations and fertilization can impact the AM association, both directly, by damaging the AMF network, and indirectly, by modifying soil conditions essential to their survival and development. In general, agricultural practices affect the occurrence of AMF, with resulting effects on soil biological activity (Collins Johnson et al. 1992; Helgason et al. 1998; Johnson and Pfleger 1992; Menendez et al. 2001). These impacts have raised a large interest in the scientific community.

A major effect of conventional crop management in field studies was the reduction of AMF biodiversity. To illustrate this, less than 10 different AMF species were identified in conventional agricultural soils (Cousins et al. 2003; Talukdar and Germida 1993), while more than 20 were found in grassland (Bever et al. 1996) using trap cultures, both in Canada and USA. Oehl *et al.* (2005) recently supported this fact in Switzerland by

demonstrating a significant difference between the number of species found in intensively managed maize fields and grasslands. Many studies have indeed indicated that AMF abundance and effectiveness are declining upon agricultural intensification (Douds and Millner 1999; Gosling et al. 2006; Oehl et al. 2003). In major cropping systems, the diversity of host plant is by far lower than in an undisturbed ecosystem. Monoculture, which is very common in grain production, seems to create a selective pressure on AMF species leading to both a spore population decrease and a shift in the community (Collins Johnson et al. 1992; Oehl et al. 2003; Rao et al. 1995). For example, Bedini *et al.* (2007) reported that no more than six AMF spore morphotypes were detected in a maize monoculture. Only spores related to the genus *Glomus* were recovered, confirming data on its predominance in managed soils. Several studies reported prevalence of *Glomus* spp. in cropped soils, in contrast to rich AMF communities containing *Gigaspora* spp., *Scutellospora* spp. and *Acaulospora* spp. in uncultivated soils (Blaszkowski 1993; Hamel et al. 1994; Helgason et al. 1998; Talukdar and Germida 1993).

Long fallow periods or non-mycorrhizal crop plants have a profound effect on AMF activity and diversity. For example, a study conducted by Karasawa *et al.* (2002) indicated an increase in AMF colonization and growth of maize following a sunflower crop as compared to maize following mustard, a non-mycorrhizal crop. Earlier, Gavito and Miller (1998) observed a delay of more than 60 days in AMF colonization of corn following canola, a non-AMF host species, as compared to a previous crop of bromegrass and alfalfa (both mycorrhizal). Similar AMF spore numbers were detected in wheat field whether the previous crop was corn or canola (Jansa et al. 2002). Hamel et

al. (2006a) however reported that cropping frequency did not influence AMF abundance in a wheat-based rotation in Canadian prairies, according to PLFA analysis.

During fallow periods, the viable AMF hyphal network decreases over time leading to a lower mineral uptake and growth of the subsequent mycorrhizal crop (Kabir et al. 1999). It has been demonstrated that AM colonization and P uptake decrease with increasing length of a preceding fallow (Kabir and Koide 2000; Kabir et al. 1999). In fact, Kabir *et al.* (1999) have shown that a 90-day fallow in maize decreases AMF active hyphae by 57%, root colonization by 33 %, and P, Zn, and Cu uptake by 19%, 54%, and 61% respectively. Another study conducted on maize and soybean has shown that AMF spore number increased after three years of continuous cropping. Under fallow, spore number declined during the first year, and then stabilised at a low level (Troeh and Loynachan 2003). In some cases the adverse effect of fallow periods on AMF inoculum potential can be avoided by growing a cover crop. For example, Boswell *et al.* (1998) found that growing winter wheat in comparison to fallow could increase AM inoculum potential and the growth and yield of maize. Authors have suggested that the absence of host plant during fallow negatively impacts AMF through energy source exhaustion. Moreover, in some part of North America, soil freezing and thawing during winter can directly disrupt extraradical hyphae (Boswell et al. 1998).

More than a decade ago, it has been suggested that tillage disturb mycorrhizal activity in soil and therefore, plants nutrient uptake (Boddington and Dodd 2000; Miller et al. 1995; Mozafar et al. 2000). Soil disturbance has often been shown to reduce the density of AMF spores, species richness and the length of extraradical mycelium of AMF relative to undisturbed soil (Boddington and Dodd 2000). In a study conducted by Jansa *et al.* (2002) on wheat plants, it has been observed that AMF community composition

was affected by tillage treatments. Fifteen AMF species were detected in the no-till fields, 14 in those under chisel treatments and 13 under conventional tillage conditions. Also, significantly more AMF spores were observed in soil from the no-tilled plots than from the tilled plots (when in rotation with canola). In this case, other factors, such as weed roots may have supported AMF development because canola is a non-mycorrhizal plant. Other research showing that soil disturbance by tillage in maize fields causes physical disruption of the fungus mycelium and therefore decreases the absorptive abilities of the mycorrhizae, have been summarized by Miller (2000). In reduced tillage systems, heavy P fetilization may not be as necessary as in heavily tilled systems because the intact mycorrhizal network increases the effective surface area for crop P uptake (Miller *et al*., 1995; Miller, 2000).

Impact of fertilizers on AMF

The addition of nutrients to the soil is a common practice in every intensive grain production in North America. In intensively managed agricultural systems, soil is often fertilized with N, P and K, and much of the plant biomass is harvested and not returned to the soil at the end of the growing season, which contributes to create very unique types of ecosystems. Various forms of fertilizer effectively increase crop yields (Lithourgidis et al. 2007; Schmidt et al. 2001). Mineral fertilizers are largely applied but several forms of more ‘natural’ fertilizers are also used. Mineral fertilizers are expensive and in some occasions, application of organic amendments to cultivated field reduces their necessity (Singer et al. 2004). The nutrient content of organic amendments is not as consistent as the one of mineral fertilizers, which is less practical. Studies have been conducted with dozens of different types of manure or compost as well as mineral

fertilizers on major agronomic plants. For example, swine bedding materials (Liebman et al. 2004), crushed cotton gin compost (Tejada and Gonzalez 2006), and urban refuse compost (Bazzoffi et al. 1998) have been tried in corn production. In Canada, swine liquid compost application have been shown to benefit both barley and soybean in a crop rotation (Carter and Campbell 2006). Both mineral and organic fertilizers bring to soil nutrients essential either to plants or soil microorganisms. It is a well known fact that AMF are important for the efficient uptake of nutrients, such as P and N (Smith and Read 1997). Many authors have stressed increased P and N uptake by mycorrhizal plants but mostly under limiting availability conditions (Cruz et al. 2004; Kanno et al. 2006; Li et al. 2006; Schreiner 2007).

Fertilizer-AMF interactions are complex and difficult to predict. While in some cases manure addition leads to an increase of AM colonization (Tarkalson et al. 1998), Ellis *et al.* (1992) found greater AM colonization in sorghum plants when neither manure nor fertilizer was added to soil. It appears from the literature that AMF directly affect N absorption and N assimilation (Barea et al. 1987), particularly in neutral to slightly alkaline soils (Azcón et al. 2001). The ability of mycorrhizal plants to better exploit soil N resources can occur directly through the uptake of organic molecules by AMF or, as stated before in this chapter, through different interactions with other soil microorganisms (i.e. competition with heterotrophic microbes for mineral N, enhancement of mineralization, better N_2-fixation, etc.). The arbuscular mycorrhizal symbiosis can both enhance decomposition of, and increase N capture from complex organic material in soil. Increased hyphal growth of the fungal partner was noted in the presence of the organic material, independently of the host plant (Hodge et al. 2001). Feng *et al.* (2002) have also demonstrated that mycorrhizal fungi had significantly

increased N uptake derived from soil in mycorrhizal cotton plants, while no significant influence on uptake of N derived from fertilizer was observed. Colonization also increased the amount of soil available N after fertilization treatment meaning that AMF may facilitate plant acquisition of nitrogen from sources which are otherwise not or less available to non-mycorrhizal plants. However this contribution of AMF to plant nutrition and growth would likely be more significant in organic farming systems or in unfertilized soil-plant systems than in highly N-fertilized agricultural fields.

Similarly, several reports have established the potential of arbuscular mycorrhizas to increase uptake of P by crops in otherwise P-deficient soils (Mohammad et al. 2004; Powell 1981). Plants with a moderate stress related to nutrient deficiency tend to release more soluble carbohydrate in their root exudates than unstressed plants (Schwab et al. 1991). A selective pressure is then exerted on AMF strains that are more aggressively acquiring plant carbohydrates. But in North America, cereal crop plants are rarely under such stress as most growers are unwilling to risk low production and largely fertilize their fields.

Although it has been reported that the ability of AMF to improve plant P uptake is largest when the P source is organic (Feng et al. 2003), which could be explained by the fact that much of the P applied to the soil through mineral fertilizers is rapidly fixed into insoluble forms, demonstration has been made that AMF from fertilized soils produce fewer hyphae and arbuscules than fungi from unfertilized soils (Johnson 1993). Moreover, AM colonization is generally severely limited by the high P-inputs used in vegetable production systems (Ryan and Graham 2002). However, in a large-scale survey of 40 asparagus fields conducted in eastern Canada, PLFA 16:1ω5 in soil, used as an estimation of extraradical AM fungal development, was positively correlated with

soil available P (Hamel et al. 2006b). This may be related to the higher plant growth and higher plant-derived C therefore available to sustain AMF hyphal network development. Nevertheless, at the moment in intensive crop productions, unless P-supply is balanced carefully with plant requirements, management practices favouring AM fungal activity may risk crop growth depression and profits reduction. Low-input agricultural systems have gained attention in many industrialized countries due to rising interest for the conservation of natural resources, reduction of environmental degradation, and escalating price of fertilizers. Conventional farming systems with lower application of fertilizers and pesticides have been developed (Mäder et al. 2002). Under these conditions, plants are more dependent on an effective AMF symbiosis (Galvez et al. 2001; Scullion et al. 1998). AMF communities were generally impoverished in species composition in intensively managed agricultural lands (Galvez et al. 2001; Jansa et al. 2002; Johnson and Pfleger 1992; Oehl et al. 2003; Oehl et al. 2004), supporting the idea that organic farming could rely on a higher soil microbial biodiversity.

As P and N availability in agricultural systems is not as limiting to plant productivity as in other soil-plant systems, the main impacts of AMF may also be different. Hamel (2004) suggested that ‘under these conditions, the major impact of AMF, which are root extensions and regulators of photosynthesis-derived C input to soil, could well be on microbial processes and soil quality.’

Conclusion

Since most intensively cultivated crops in North America are mycorrhizal, all possible interactions influencing plant growth must be considered. As conventional farming are now definitely high inputs agricultural systems generating large N and P surplus, the main benefit of AMF in the rhizosphere may not so much be related to nutrient uptake. AMF, by their interactions with soil particles and other organisms, represent an important component of soil quality. A better understanding of soil system would probably lead to a better management of AMF contribution to soil fertility and, may be, to a more sustainable agriculture, even in high yielding grain productions.

Chapitre III

Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa

Ce chapitre a été accepté pour publication dans la revue Microbial Ecology et est actuellement sous-presses (DOI: 10.1007/s00248-009-9583-z):

M.S. Beauregard[1, 2], C. Hamel[2], Atul-Nayyar[2], M. St-Arnaud[1]

[1]Institut de recherche en biologie végétale, Université de Montréal and Jardin botanique de Montréal, 4101 Sherbrooke Street East, Montreal, Quebec, Canada, H1X 2B2;
[2]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, 1 Airport Road, Box 1030, Swift Current, Saskatchewan, Canada, S9H 3X2

Les résultats de ce chapitre ont également été présentés lors du congrès suivant :

St-Arnaud, M, M.S. Beauregard, C. Hamel, A. Nayyar. Long-term phosphorus fertilization impacts on AM fungi and other soil microbial communities in alfalfa rhizosphere. Pp. 67-68 In Abstracts of the 6th International conference on mycorrhiza. ICOM6. Belo Horizonte, Brazil. August 9-14, 2009.

Abstract

Soil function may be affected by cropping practices impacting the soil microbial community. The effect of different phosphorus (P) fertilization rates (0, 20, or 40 kg P_2O_5 ha^{-1}) on soil microbial diversity was studied in 8-year-old alfalfa monocultures. The hypothesis that P fertilization modifies soil microbial community was tested using denaturing gradient gel electrophoresis (DGGE) and phospholipids fatty acid (PLFA) profiling to describe soil bacteria, fungi and arbuscular mycorrhizal (AM) fungi diversity. Soil parameters related to fertility (soil phosphate flux, soluble P, moisture, phosphatase and dehydrogenase assays, and carbon and nitrogen content of the light fraction of soil organic matter) were also monitored and related to soil microbial ribotype profiles. Change in soil P fertility with the application of fertilizer had not effect on crop yield in 8 years, but on the year of this study, was associated with shifts in the composition of fungal and bacterial communities without affecting their richness, as evidenced by the absence of effect on the average number of ribotypes detected. However, variation in soil P level created by a history of differential fertilization did not significantly influence AM fungi ribotype assemblages. Fertilization increased P flux and soil soluble P level but reduced soil moisture and soil microbial activity, as revealed by dehydrogenase assay. Results suggest that soil P fertility management could influence soil processes involving soil microorganisms. Seasonal variations were also recorded in microbial activity, soil soluble P level as well as in the abundance of specific bacterial and fungal PLFA indicators of soil microbial biomass.

Introduction

Soil microorganisms are important components of ecosystems. They mediate nutrient cycling, organic matter decomposition, soil aggregate formation, plant disease promotion and biocontrol (Artursson et al. 2006; Biró et al. 2000; Kennedy 1999). Agricultural soils are special ecosystems as they are often intensively managed. In Canada alone, more than 35 millions hectares of land were in crop production in 2006. More than 25 millions of them received mineral fertilizers (Statistics Canada 2007).

The natural P fertility of soils varies greatly with location and, in agricultural settings, it is altered by nutrient exportation in crop yield and by fertilization. Fertilization is a common cropping practice. Repeated mineral P application to soil can directly or indirectly induce changes in soil chemical, physical, and biological properties as it increases soil P saturation level and general P fertility. In the long term, such modifications may result in shifts in the quality and functionality of soils (Acton and Gregorich 1995). Some microorganisms may be suppressed and others may proliferate in vacant ecological niches. Crop fertilization may lead to important variations in the composition of the soil nutrient pools (Stevenson and Cole 1999). As different groups of microorganisms can vary in their ability to process the various nutrient forms found in soil, fertilization can surely affect their growth competitiveness. The availability of nutrients such as C, N and P can influence soil microbial growth and activity (Broeckling et al. 2008; da Silva and Nahas 2002). Soil fertility has been shown to influence in different ways the diversity, biomass and activities of the soil microbial community (Fox and MacDonald 2003; Liu et al. 2008a; Ryan and Graham 2002; Wei et al. 2008).

This study was conducted to test the long-term impact of P fertilization on the diversity of soil microorganisms, using biochemical and molecular fingerprinting, and exploratory multivariate analysis. Trials were conducted using a 8-year long-term field experiment comprised of differentially P-fertilized alfalfa stands located in the mixed grass prairie ecozone of Saskatchewan. These alfalfa stands were examined at three points in time during the growing season.

Materials and methods

Study site and experimental design

The study was conducted in 2004 on a Brown Chernozem located at the Semiarid Prairie Agricultural Research Centre in Swift Current, Saskatchewan, Canada (latitude: 50°16.2'N, longitude: 107°43.8'W, elevation 825 m). The soil was a loamy clay with 2.28 % organic matter, slightly acidic with pH of 6.62 and EC of 0.32 mS cm^{-1}. The climate is semiarid with mean (30-year average) annual temperature and precipitations of 3.9°C and 349 mm (Environment Canada 2008). The field experiment was initiated in 1997, i.e. 8 years before the present study. Alfalfa (*Medicago sativa* L. cv. Rangerlander) was seeded in 1.8 m × 6 m plots with a plot seeder at the rate of 25 seeds 30 cm^{-1} and at 30 cm width row spacing. It was fertilized annually with one of three different amounts of super phosphate: 0, 20, or 40 kg P_2O_5 ha^{-1}. Phosphorus was applied in early May with a plot seeder disk drill that placed the P at about the 1.25 cm depth in between the rows and disturbs the soil in a band less than 1.25 cm wide. The experiment was set up in a randomized complete block design where the different P fertilization rates were replicated in four complete blocks. The weather station collecting precipitation and temperature data was located at approximately 1 km from the experimental plots.

Plant and soil sampling

Various samples were collected three times: on June 30, August 30 and September 29. Plants were harvested as a hay crop on June 30 and on August 30, and plant shoot samples were taken from two randomly selected 1-meter rows in each plot, only on the

two dates, as there was no re-growth between September 1 and 30. The plant material was dried at 40°C until constant weight, and dry weight was recorded.

The 0–7.5-cm layer of the soil was sampled with a bulb planter between the row and the fertilizer band to analyze broad microbial diversity using fatty acid methyl ester analysis and at a finer scale using PCR-DGGE analysis of rDNA gene fragments. A first set of five cores taken from different locations in each plot and pooled in plastic bags was used for lipid analysis, while a second set was used for DNA analysis. All samples were placed on ice in a cooler and refrigerated upon arrival in the laboratory. The set for DNA analysis was frozen and stored at –20°C until analysis. The other set was sieved through 2 mm and parsed into two sub-samples. One was frozen at –20°C until lipid extraction, and the other was weighed and oven dried at 105°C to constant weight. The dried sample was weighed, and gravimetric soil water content was calculated by difference and expressed as a percentage, on a dry soil weight basis (Topp 1993).

Soil PO_4-P flux was measured *in situ* using 6.5 cm × 2.25 cm anion-exchange membranes (AEMs). A set of three AEMs was placed between the row and the fertilizer band in each plot. The AEMs (#200253, Ionics, Watertown, Massachusetts) were inserted vertically in the soil (2–7 cm depth) through the slit opened with a small shovel. These AEMs were inserted in the soil of each plot on May 17, and were replaced on June 30, and September 1. Once removed from the soil, the membranes were rinsed with distilled water to remove the adhering soil particles, immediately placed in tubes containing 25 ml of 1 M KCl and brought to the laboratory for analysis as described below.

Fatty acid methyl ester (FAME) analysis

The amount of microbial fatty acid biomarkers in the phospholipid (PLFA) and neutral lipid (NLFA) fractions of soil lipid extracts was used as a measure of active soil microbial biomass and storage material of fungal origin, respectively, using the method described in Hamel et al. (2006a). Total soil lipids were extracted from 4 g of soil (dry weight equivalent) in dichloromethane (DMC): methanol (MeOH): citrate buffer (1:2:0.8 v/v). Lipid-class separation was conducted in silica gel columns. The neutral, glyco- and phospholipids fractions were eluted by sequential leaching with DCM, acetone and MeOH, respectively. The glycolipid fraction was discarded. The neutral and phospholipid fractions were dried under a flow of N_2 at 37°C in the fume hood, dissolved in 2 ml of MeOH for PLFA or DCM for NLFA and stored at -20°C. Fatty acid methyl esters were created through mild acid methanolysis. Ten microliters of methyl nonadecanoate (19:0; Sigma–Aldrich) was added to serve as internal standard and samples were dried under a flow of N_2 at 37°C in the fume hood. Samples dissolved in 50 ml of hexane were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a CP-8400 auto sampler and a flame ionization detector (FID). Helium was the carrier gas (30 ml min^{-1}) and the column was a 50-m Varian Capillary Select FAME # cp7420. Sample (2 ml) injection was in 5:1 split mode. The injector was held at 250°C and the FID at 300°C. The initial oven temperature, 140°C, was held for 5 min, raised to 210°C at a rate of 2°C min^{-1}, raised from 210 to 250°C at a rate of 5°C min^{-1}, and held for 12 min.

Identification of peaks was based on comparison of retention times to known standards (Supelco Bacterial Acid Methyl Esters #47080-U, plus MJS Biolynx #MT1208 for 16:1ω5). The abundance of individual PLFAs was expressed as mg PLFA g^{-1} dry soil.

Amounts were derived from the relative area under specific peaks, as compared to the internal standard (19:0) peak value, which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane.

Individual fatty acids have been used as signatures for various groups of micro-organisms (Hamel et al. 2006a; Pankhurst et al. 2002). The FAME 18:1c was used as indicators of fungal biomass (Petersen and Klug 1994) and FAME 16:1ω5, as indicator of AM fungi (Balser et al. 2005). FAMEs a-15:0, i-15:0, 15:0, 2OH-14:0, i-16:0, i-17:0, 2OH-16:0 and 18:1t were chosen to represent bacterial PLFAs based on the bacterial standards used.

Dehydrogenase assay

Dehydrogenase activity was assayed by a method from Casida et al. (1964) with few modifications. Amounts of fresh soil equivalent to 20 g oven dried soil were mixed with 0.2 g $CaCO_3$. This mixture was parsed into three 16 x 125 mm test tubes and enough dd H_2O was added to bring soil to field capacity. One ml of 3 % 2, 3, 5-triphenyl tetrazolium chloride (TTC) and 2.5 ml of ddH_2O were added to each tube before they were inverted a few times and incubated at 37°C for 24 hours. A blank contained all the amendments except the TTC. Each soil sample was extracted with 10 ml of methanol after incubation. The extract was filtered through a Whatman No. 5 filter paper. The optical density (O.D.) of the filtrate was read at 485 nm in a spectrophotometer.

Phosphatase assay

Acid phosphatase activity was determined on amount of fresh soil equivalent to 1 g of oven dried soil according to the method of Eivazi and Tabatabai (1977). Samples were

mixed with 0.2 ml toluene, 4 ml of modified universal buffer, and 1 ml p-nitrophenyl phosphate solution (PNP) before being incubated 1 hour at 37°C. One ml of 0.5M $CaCl_2$ and 4 ml of 0.5 M NaOH were then added to tubes. The extract was filtered through a Whatman No. 5 filter paper. The O.D. of the filtrate (diluted 5:1 in ddH_2O) was read at 400 nm.

Light fraction of soil organic matter

Light fraction (LF) of soil organic matter was measured following the method of Strickland and Sollins (1987). Ten gram of air-dried soil was added to sodium iodide (NaI) solution (1.6 g cm^{-3}) in a centrifuge tube. The mixture was shaken for 30 min and centrifuged. The LF in the supernatant was recovered by filtration, washed with a 0.01 M $CaCl_2$ solution and deionised water, and analyzed for total-C and total-N content with a Carlo Erba N-C-S analyzer (Baccanti and Colombo 1992).

Soil PO_4-P flux and available P determinations

PO_4-P flux was monitored using anion exchange membranes prepared and extracted as described by Ziadi et al. (1999). AEM strips were saturated with Cl^- by immersion in 1 M HCl solution and agitation in 1 M NaCl solution for 1 h. Membranes were then thoroughly rinsed and kept in distilled water until soil placement. After recovery from the soil, membranes were extracted in 25 ml of 1 M KCl in test tubes by shaking for 1 h. The extract was filtered and its P concentration was determined colorimetrically by the molybdate–ascorbic acid method (Anonymous 1976) using a Technicon segmented flow autoanalyzer (AAII system).

Available P at time of sampling was also analysed by resin extraction following a protocol modified from Hedley and Stewart (1982). Briefly, 0.5 g of soil was placed into 50 ml plastic centrifuge tube with 1 exchange membrane and 25 ml of ddH_2O. Tubes were shaken overnight. The membranes were removed, rinsed with H_2O, and placed into a 50 ml plastic centrifuge tube with 20 ml of 0.5 N HCl. Tubes were set aside for 1 hour for gas to escape, then capped and shaken for 16 hours. On the next day, the resin extract was decanted into clean, appropriately marked vials. P concentration was determined by colorimetry (molybdate–ascorbic acid method) on the autoanalyzer.

DNA extraction and PCR amplification

Total genomic DNA was extracted from 600 mg of soil using the UltraClean^{tm} Soil DNA isolation kit (MoBio Laboratories, Inc.). Final elutions were kept at -20°C until amplification. Three different primer sets were used to separately analyze the diversity of soil fungi, AM fungi and bacteria, targeting the SSU rRNA gene region. Fungi were amplified using primers FF390/FR1-GC (Vainio and Hantula 2000). For AM fungi and bacteria, a nested PCR strategy was used. AM fungi were amplified using primers NS1/NS41 first, and AM1/NS31-GC in the second round of amplification as described in Yergeau et al. (2006) modified from Kowalchuk et al. (2002). For bacteria, primers pA/pH (Edwards et al. 1989) were used first, and the primer pair 341F-GC/534R (Muyzer et al. 1993) in the second round. To improve gel resolution and the separation of closely similar fragments in DGGE, a GC-clamp was added to primer FR1, NS31, and 341F (Myers et al. 1985). Primer sequences and reaction conditions are detailed in Table 3. All reactions were carried out in a Mastercycler ep S gradient thermocycler

(Eppendorf, NY, USA) in a 25 µl volume containing 2.5 µl of 10 × PCR buffer, 2.5 µl of each primer (5 µM), 0.5 µl of dNTPs mix (10 mM), and 1.25 U of Taq polymerase (QIAgen). To amplify DNA fragment from AM fungi and from fungi, 1 µl of a 1:10 dilution of the extract was used, while 1 µl of a 1:20 dilution was added to the PCR mix for bacteria. Twice the amount of DNA was used for samples that failed to amplify or gave very faint PCR product. Amplicons were analysed by agarose gel electrophoresis (1%) using ethidium bromide staining and UV light. For nested PCR, products with a visible band were diluted 1:100 (products with a faint band were undiluted) and 1 µl were used as template in subsequent amplification using the reaction mixture described above.

Denaturing gradient gel electrophoresis (DGGE) analysis

Amplicons were analysed using a DCODE Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) with 16 × 18 cm glass plates and 1-mm spacers. The different DGGE conditions corresponding to the three types of microorganisms studied are listed in Table III. In all cases, a 3 ml stacking gel containing no denaturant was added before polymerization and DGGE were conducted in 1× TAE electrophoresis buffer. Gels were stained with SYBR Gold (Invitrogen) diluted 1:10000, visualized on a UV transilluminator and photographed (GelDoc, Bio-Rad Laboratories). Pictures were visually inspected and bands that migrated to different positions were considered as different ribotypes. The presence of each ribotype was noted for all PCR products that produced bands on DGGE and a presence–absence matrix was built for statistical analyses.

Statistical analyses

The Shannon–Weaver diversity index (Rosenzweig 1995) was calculated from the number of AM fungal, bacterial and fungal bands detected on DGGE to compare the ribotypes diversity between P fertilization treatments and sampling dates. Canonical correspondence analyses (CCA) were performed on the taxa presence/absence matrix of DGGE banding patterns using Canoco 4.5 (Ter Braak and Smilauer 2002) to evaluate the relationships between the DGGE patterns of the different samples and the possible influence of fertilization treatment and sampling date. Parameters related to soil fertility, PLFA biomarkers, dehydrogenase activity and ribotypes abundance data were subjected to analysis of variance (ANOVA) using JMP 6.0 (SAS Institute, Cary, NC) to test the significance of P fertilization effects. The probability level of 5% was adopted for accepting or rejecting Null hypotheses. Tukey's HSD all-pairwise comparisons were calculated after ANOVA to compare treatments means.

Table III. Primers and PCR conditions used in this study.

Microbial group	Primers (5' – 3') [1]	PCR conditions	DGGE conditions
Bacteria	**pA** AGAGTTTGATCCTGGCTCAG **pH** AAGGAGGTGATCCAGCCGCA	3 min at 94°C, 35 cycles of 30 sec at 94°C, 30 sec at 58°C, 1 min at 72°C and final extension for 5 min at 72°C.	8% (w/v) polyacrylamide gels with a denaturant gradient of 43-70% (100% is defined as 40% formamide and 7 M urea) at 60°C. 60V for 18h.
	341F-GC (GC clamp) TACGGGAGGCAGCAG **534R** ATTACCGCGGCTGCTGG	1 min at 94°C, 45 sec at 65°C, 1 min at 72°C; decreased annealing temperature by 1°C every 2 cycles until 55°C, final extension for 5 min at 72°C.	
Fungi	**FF390** CGATAACGAACGAGACCT **FR1-GC** (GC clamp) AICCATTCAATCGGTAIT	3 min at 95°C, then 35 cycles of 30 sec at 95°C, 45 sec 50°C, 1 min at 72°C and final extension for 10 min at 72°C.	7.5% (w/v) polyacrylamide gels with a denaturant gradient of 40-63% at 58°C. 100V for 20 min., then 80V for an additional 16h.
AM fungi	**NS1** GTAGTCATATGCTTGTCTC **NS41** CCCGTGTTGAGTCAAATTA	3 min at 94°C, then 30 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C and final extension for 7 min at 72°C.	6% (w/v) polyacrylamide gels with a denaturant gradient of 38-50% (100% is defined as 40% formamide and 7 M urea). A 3 ml stacking gel containing no denaturant added before polymerization. DGGE conducted in electro 1× TAE at 60°C. Voltage was first set at 80V for 20 min., and then lowered to 45V for 16h.
	AM1 GTTTCCCGTAAGGCGCCGAA **NS31-GC** (GC clamp) TTGGAGGGCAAGTCTGG TGCC	3 min at 94°C, then 28 cycles of 45 sec at 94°C, 45 sec at 58°C and 45 sec at 72°C and final extension for 7 min at 72°C.	

[1] For bacteria and AM fungi, primer pairs used in the first round of nested PCR are presented first

Results

Microbial diversity

P fertilization treatments and sampling dates had no effect on the Shannon-Weaver diversity index of the bacteria, fungi and AM fungi (Table IV) and on soil microbial taxonomic richness, i.e. the number of different ribotypes detected in samples. Taxonomic richness varied however between the three groups of microorganisms studied. DGGE revealed the presence of eight different bands corresponding to AM fungi sequences, with only three of them present in more than 20% of the samples. Only three ribotypes were counted in the samples with the highest richness. The majority presented two or less ribotypes. The universal primers for fungi amplified sequences that migrated as twenty-four bands in acrylamide gel. Four to twelve ribotypes per samples were found, but the average sample contained seven ribotypes. Each sequence variants was detected in 6% to 100% of the soil samples. Thirty-two bacterial ribotypes were detected after band patterns analysis. The various ribotypes were detected in 6% to 100% of the total number of sample from which DNA amplification had been successfully performed (i.e. 33 of 36 samples). The average number of ribotype identified per sample was fifteen, but patterns containing more than twenty-five bands were found. This is about twice as much as the number obtained for the global fungal community.

Table IV. Diversity of AM fungi, bacteria and fungi communities associated with different P fertilization rates and sampling times in 2004.

P fertilizer rate	Shannon-Weaver diversity index [1]		
	AM fungi	Bacteria	Fungi
0 kg P_2O_5 ha^{-1}	1.94	3.22	2.67
20 kg P_2O_5 ha^{-1}	1.89	3.24	2.73
40 kg P_2O_5 ha^{-1}	1.82	3.25	2.66
Sampling date			
June 30	1.44	3.18	2.71
August 30	1.64	3.30	2.60
Sept. 29	1.95	3.22	2.70

[1] Shannon-Weaver index, $H' = -\sum p_i \ln p_i$

Effect of P fertilizer rate and time of sampling on the soil environment

Yields were not affected by fertilization rate (Table V) although soil available P levels (i.e. available P and PO_4-P flux) were increased while soil moisture and dehydrogenase activity were decreased with P dose (Table VI). Soil acid phosphatase activity, and the amount of C and N in the light fraction of organic matter were not influenced by P fertilization treatments and times of sampling (data not shown), indicating that the supply of C and N to the soil microbial community and the system's need for P did not change with P fertilization treatments. Available P was the only measured environment descriptor influenced by time; it decreased as the season proceeded (Table VII). Precipitations were most abundant in spring, but the end of June was dry resulting in very dry soil at the first sampling (Fig. 1). Mid-summer was warm and dry, but some substantial rainfall events in the last 11 days of August had residual effect on the moisture level of the soil sampled on August 30. September was cool and dry.

Table V. Average crop yield collected from plots receiving different P fertilizer rates.

P fertilizer rate	Date of harvest [1]	Yield (kg ha^{-1}) [2]
0 kg P_2O_5 ha^{-1}	June 30th	4907.76a
20 kg P_2O_5 ha^{-1}	June 30th	5638.76a
40 kg P_2O_5 ha^{-1}	June 30th	5298.52a
0 kg P_2O_5 ha^{-1}	August 30th	842.61a
20 kg P_2O_5 ha^{-1}	August 30th	879.59a
40 kg P_2O_5 ha^{-1}	August 30th	871.43a

[1] Plants were harvested as a hay crop two times during the season

[2] Within each date of harvest, different letters represent significantly different values (Tukey's HSD all-pairwise comparisons, $\alpha = 0.05$)

Table VI. Environmental factors influenced by P fertilizer rate.

P fertilizer rate	PO_4-P flux [1] (µg cm^{-2} day^{-1})	Soil moisture (%)	Available P [2] (mg kg^{-1}) ($\times 10^{-2}$)	Dehydrogenase (µg TPF day^{-1})
0 kg P_2O_5 ha^{-1}	0.053 b [3]	9.20 a	0.84 b	83.66 a
20 kg P_2O_5 ha^{-1}	0.059 b	8.76 ab	1.73 b	73.23 ab
40 kg P_2O_5 ha^{-1}	0.253 a	8.69 b	6.93 a	64.51 b

[1] Monitored using anion exchange membranes

[2] Analysed by resin extraction

[3] Different letters represent significantly different values (Tukey's HSD all-pairwise comparisons, $\alpha = 0.05$).

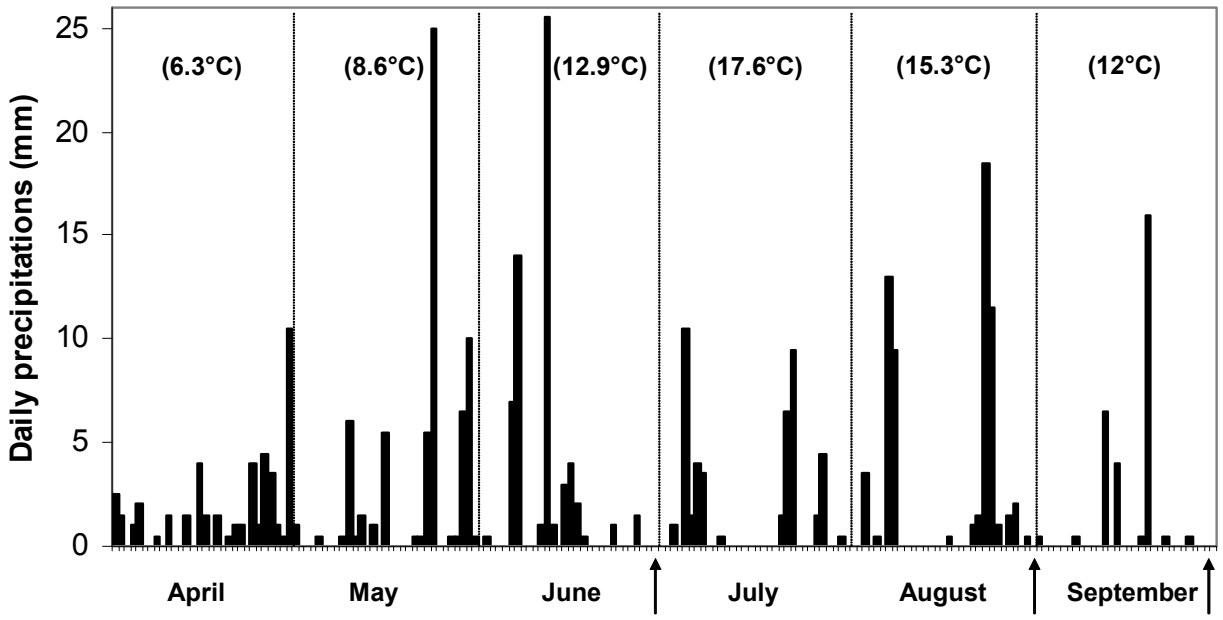
Table VII. Soil and microbial descriptors influenced by sampling date.

Date	Phospholipid fatty acid biomarkers (μg g^{-1}) [1]						Available P	Dehydrogenase
	Bacteria					Fungi	(mg kg^{-1}) [2]	(μg TPF day^{-1})
	i-15:0	a-15:0	i-16:0	i-17:0	Sum	18:1c	($\times 10^{-2}$)	
June 30	10.30 a	1.60 ab	13.76 a	2.98 a	50.03 a	7.35 a	4.36 a	65.65 b
Aug. 30	3.97 b	0.65 b	3.84 b	0.98 b	20.11 b	3.26 b	3.00 ab	78.80 a
Sept. 29	12.16 a	1.97 a	12.16 a	2.11 ab	52.33 a	6.16 ab	2.14 b	76.95 ab

[1] Between sampling dates, different letters represent significantly different values (Tukey's HSD all-pairwise comparisons, $\alpha = 0.05$)

[2] Analysed by resin extraction

Figure 1. Daily rainfall and average monthly temperature (in brackets) during the 2004 growing season. Arrows represent sampling dates.



Effect of P fertilizer rate and time of sampling on soil microbial communities

The influence of P treatments and time of sampling on ribotypes detection frequency varied with the type of organism. CCA revealed substantial alteration of bacterial (Fig. 2; P=0.03) and fungal (Fig. 3; P=0.01) communities with regards to time of sampling and fertilization rates. Time of sampling had a larger influence than P rate for bacteria ribotypes but a level of influence similar to fertilization treatments on fungi ribotypes detection frequency. On the other hand, AM fungi detection frequency (Fig. 4; P=0.07) was barely shifted according with these parameters. AM fungi CCA ordination yielded a principal axis direction clearly related with sampling time showing that AM fungi ribotype frequencies were mostly influenced this factor, yet not significantly. Microbial PLFA biomarkers (Fig. 5; P=0.1) were not significantly shifted according with P treatments and time of sampling.

ANOVA indicated that soil bacterial and fungal biomasses, as indicated by PLFA amounts, were affected by time of sampling (Table V), but not by P fertilizer rates. The abundance of all fungal and bacterial biomarkers were high in early season, decreased in late summer and increased again in fall, although the effect of time was significant only for four bacterial PLFA biomarkers and the fungal PLFA biomarker. Dehydrogenase activity decreased with P dose and was high in late summer. The biomass of AM fungi, as revealed by the PLFA biomarker 16:1ω5, remained constant throughout the season.

Figure 2. CCA biplot depicting the relationship between bacteria ribotypes with regard to sampling time and P fertilizer rate (P=0.03). Dates of sampling and fertilizer effects are represented by vectors (June=June 30; August=August 30; Sept. =September 29; P0=no P_2O_5 applied; P20 and P40=20 or 40 kg P_2O_5 ha-1). Ribotypes are represented by triangles and named according to the migration position on the gels.

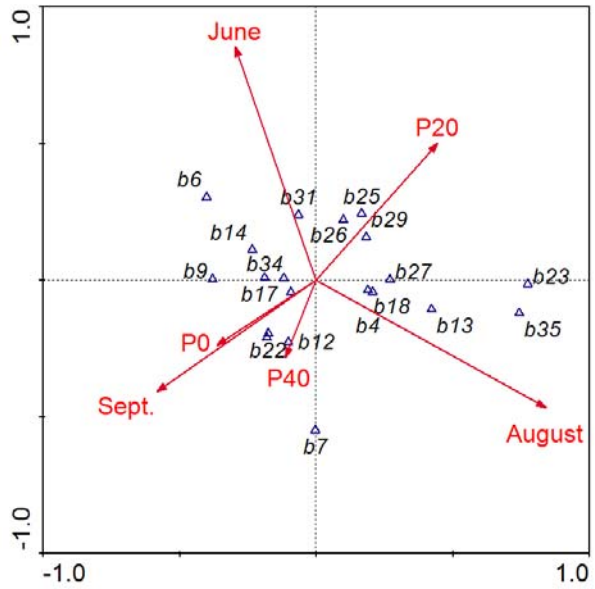


Figure 3. CCA biplot depicting the relations between fungi ribotypes with regard to sampling time and P fertilizer rate (P=0.01). Dates of sampling and fertilizer effects are represented by vectors (June=June 30; August=August 30; Sept. =September 29; P0=no P_2O_5 applied; P20 and P40=20 or 40 kg P_2O_5 ha-1). Ribotypes are represented by triangles and named according to the migration position on the gels.

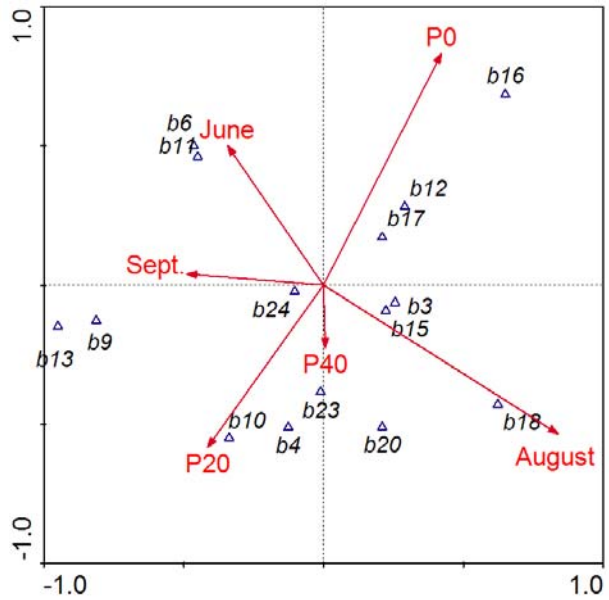


Figure 4. CCA biplot depicting the relations between AM fungi ribotypes with regard to sampling time and P fertilizer rate (P=0.07). Dates of sampling and fertilizer effects are represented by vectors (June=June 30; August=August 30; Sept. =September 29; P0=no P_2O_5 applied; P20 and P40=20 or 40 kg P_2O_5 ha^{-1}). Ribotypes are represented by triangles and named according to the migration position on the gels.

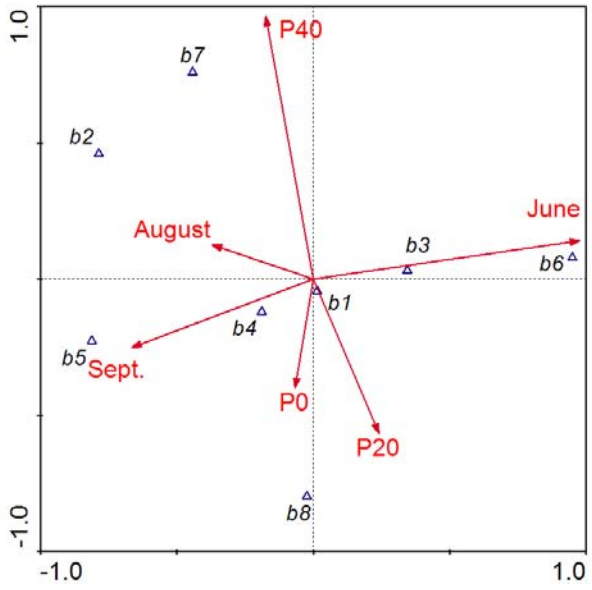
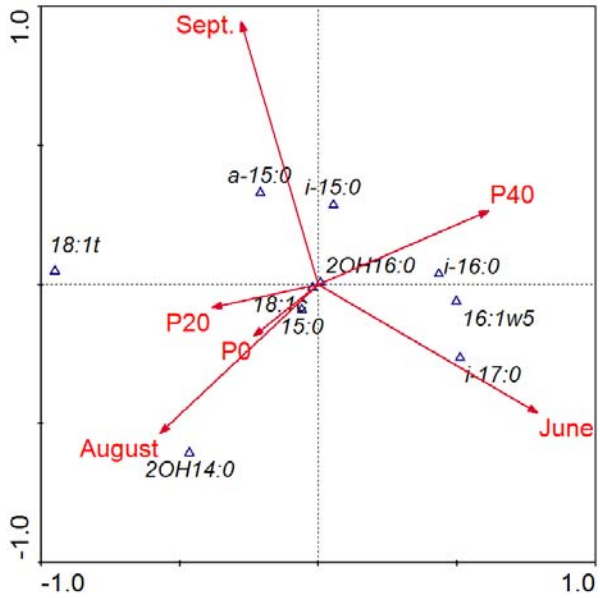


Figure 5. CCA biplot depicting the relations between microbial PLFA biomarkers according to sampling time and P fertilizer rate (P=0.1). Dates of sampling and fertilizer effects are represented by vectors (June=June 30; August=August 30; Sept. =September 29; P0=no P_2O_5 applied; P20 and P40=20 or 40 kg P_2O_5 ha^{-1}). Ribotypes are represented by triangles and named according to the migration position on the gels.



Discussion

This study demonstrated that soil P fertility management can modify the composition of the soil microbial community in an alfalfa monoculture submitted to long-term P fertilization. It was also found that in contrast to bacterial and whole fungal communities, the composition of the AM fungi community was not influenced by P fertilizer rates ranging from 0 to 40 kg P_2O_5 ha^{-1} yr^{-1}, but changed as the season proceeded.

Influence of P fertilization on soil microbial community

P is an essential element for both plants and microorganisms, thus, fertilization applied to crops should influence soil microorganisms. In our study, differential P fertilization during eight years had a profound effect on the soil microbial community. Phospholipid fatty acids are useful indicators of the biomass of broad microbial taxonomic groups (Fliessbach and Widmer 2006). Modification in the PLFA biomarkers profiles of soils fertilized differently would have reflect P-induced changes either in the total biomass of the whole bacteria, fungi or AM fungi groups, or else structural changes in the bacteria community at a broad taxonomic scale. The absence of P effect on these biomarkers noted here may indicate that an 8-12× increase in P availability (Table IV) in soil did not influence the size of soil microbe communities, or that an influence on specific taxa not changing the whole community size was not accounted for by this approach. Changes at a finer taxonomic scale were revealed by the PCR-DGGE analysis of whole fungal and bacterial 18S gene fragments in soil extracts, supporting this hypothesis. The PCR-DGGE method detected the most abundant genotypes in soil microbial communities, as

DGGE analysis is known to show sequence variants forming more than 1% of the target sequences present in the extract (Muyzer et al. 1993). In our study, fungal and bacterial taxa dominating communities in plots with a different history of P fertilization were different and some ribotypes could be associated with a specific P rate. Some specific ribotypes were found mostly in fertilized soils suggesting favourable conditions for the proliferation and activity of specific microbial taxa. Soil P fertility can directly influence soil microorganisms, but the effect of fertilization may also be attributable to changes in plant metabolism. Root exudation represents an important source of soil carbon for microorganisms and is influenced by plant P status (Singh and Pandey 2003).

If compared to the dozens of organisms that could be found in soil, relatively low numbers of ribotypes for all the microbial groups under study were detected. Although such numbers are comparable to what was found in other studies using the DGGE approach (Ge et al. 2008; Ma et al. 2005; Sun et al. 2004b), there are now evidences through other detection methods that it underestimates total diversity of large group of organisms. In fact, the DGGE method was considered here as an initial step to discriminate among global microbial communities.

It was reported that different land management practices produced a selective influence on microbial community diversity (Hagn et al. 2003; Wu et al. 2008). The response of total soil microbial community to P mineral fertilization is poorly documented as most studies focused either on N fertilization (Benizri and Amiaud 2005), organic fertilizers (Ros et al. 2006), or on specific microbial taxa or functional groups (Mäder et al. 2000). Recent studies conducted on cultivable soil microorganisms have shown that bacterial and fungal counts changed with soil P content (He et al. 2008;

Zhong and Cai 2007). Other studies also concluded that fertilization caused large shifts in both the active bacterial and fungal community structures which additionally resulted in a decrease in the heterogeneity of the bacterial community (da Silva and Nahas 2002; Girvan et al. 2004). Our study demonstrated that in the case of mineral P fertilization, the applied dose of fertilizer lead to specific bacterial and fungal community structures although the data does not indicate in which taxa the changes were occurring.

Soil microorganisms are key factors in many soil processes. It is possible, thus, that P fertilization indirectly impacts the various services mediated by the soil microflora. The observation of reduced dehydrogenase activity at high P fertilizer rate supports this hypothesis. Dehydrogenase activity is an indicator of the general oxidative metabolism, thus, a good indicator of the activity of the soil microbial biomass (Chu et al. 2007; Quilchano and Marañón 2002). While it is impossible here to identify the soil processes most affected, it seems that the overall contribution of the soil microbial biomass was decreased.

Variations in the soil microbial community, as a result of differential fertilization regime, have possible impact on soil biochemical processes (Cruz et al. 2009). Fertilizer use in semi-natural grasslands have produced a decrease in plant diversity (Santos et al. 2006; van der Heijden et al. 1998) which has also been related to variations in soil microbial populations (Johnson et al. 2003; Wieland et al. 2001). Our study confirms the hypothesis that changes produced by P addition to soil produce certain shifts in the population of bacteria and general fungi. Long-term fertilization can influence soil microorganism through modification in root exudation (Bais et al. 2006; Vierheilig et al. 1998a), which is influenced by plant nutritional status (Neumann and Römheld 2001).

Phosphorus fertilization affected neither AM fungal community structure estimated by ribotype profiling nor AM fungi biomass measured with the PLFA biomarker 16:1ω5, but results obtained from the same long-term experiment showed a reductio in AM root colonization with P fertilization (Atul-Nayyar et al. 2008). The lower AM colonization of roots in P fertilized soil reflects that plants adjust their investment in symbiotic development to the level of soil P availability, in order to fulfill their requirement for P in the most energy efficient way (Smith and Read 2008). It appears that P fertilization does impact the soil microbial community. The fact that P fertilization never improved alfalfa yield at this site suggests that the microbial community in low P soil is involved in efficient P cycling.

Seasonal dynamics and functionality under varying soil conditions

Seasonal effects on the soil microbial community were often reported (Dunfield and Germida 2003; Hamel et al. 2006a; Houlden et al. 2008). Whereas sporulation in AM fungi was known to vary seasonally, the composition of the AM fungal community under a perennial plant cover was not known to vary. Studies have showed that plant phenology can influence mycorrhizal development (Kabir et al. 1997; Li et al. 2005) and functionality (Carvalho et al. 2001; Idoia et al. 2004). Here, it was also observed that the community structure slightly changed from early summer to fall, an effect that could be attributable to variations in plant physiological status. The alfalfa plants grew well up to the end of June using snow melt water and good early season precipitations, but produced very little growth from the end of June harvest to fall, due to droughty conditions. It is also possible that the hot and dry soil conditions of late summer directly

favoured the proliferation of AM fungi taxa adapted to such conditions, which might have replaced species adapted to cool and moist soil conditions. There are studies concluding that AM fungi act on plant water uptake (Ahmad Khan et al. 2003; Al-Karaki et al. 2004). Pressure caused by dry conditions could lead to the selection of specific taxa or strains allowing better water uptake. There are only few report on climate adaptation in AM fungi, but we know that AM fungi root colonization respond to temperature (Rillig et al. 2002) and that the dominance of different species varies with regional temperature (Koske 1987).

The taxonomic composition of the community under study changed from early to late season, at our study site, but the richness of that community, i.e. the number of taxa detected, did not vary. This stability of community richness suggests that the hot and dry climate normally prevailing in late season does not negatively impact the AM fungi community. The observed shift in taxonomic composition appears as an adaptation of the AM fungi community to the climate of the mixed-grass prairie ecozone, which insures the effectiveness of the arbuscular mycorrhizae living in this environment throughout the growing season. Soil microbial succession is triggered by seasonal variation linked to variations in plant phenology and environmental conditions. Changes in soil microbial communities correspond to variations in the materials released by the roots as well as by soil nutrient cycling (Atlas and Bartha 1997). Fungi and most soil bacteria are heterotrophs and depend on exogenous carbon for growth. Labile organic matter and plant root exudates, which include many carbon-containing primary and secondary metabolites, have a profound influence on soil microbial community structure (Bais et al. 2006; Broeckling et al. 2008). Plant biomass production and root exudate composition differ with plant growth stage and health status, thereby affecting

rhizosphere microorganisms (Filion et al. 2004; Grayston et al. 1996). Considering the two cuts of alfalfa hay taken at the end of June and August, changes in the microbial communities during the season is not surprising. The removal of plant above ground material by cutting or grazing has a large impact on plant physiology and triggers changes in root exudation (O'Leary 1965; Tracy and Frank 1998).

Microorganisms found in soil surrounding plant roots may also be under nutrient stress, as growing plants are better competitor for N (Hodge et al. 2000) and water (Cruz et al. 2009). Plants may be the major source of stress affecting microbial community diversity and activity (Cruz et al. 2009; Liu et al. 2000). Drought stress is associated with reduced substrate diffusion in dry soils and increased microbial demands for C and N. Microorganisms rapid turnover in time results in 'leakyness' of the microbial biomass pool and constant remobilization of resources, which are used to build up plants as they grow. This is supported by our observation of reduced fungal and bacterial biomass after a warm and dry mid-summer period over which alfalfa regrew. In such situation, higher dehydrogenase level could indicate that soil microorganisms diverted more energy from growth into maintenance as stress increased (Killham 1985).

In this study, P fertilization increased the level of soil available P, but did not affect plant productivity. It suggests that microbial communities in low P soils improve the efficiency of nutrient cycling. Phosphorus fertilizer influences the composition of soil bacteria and fungi communities probably by impacting the soil environment, at least on the long-term, but did not influence the AM fungi community. The natural evolution of the growing season has a large impact on all these communities

Acknowledgments

The authors would like to acknowledge the work of Stéphane Daigle at IRBV with statistical analyses and Keith G. Hanson at SPARC for providing essential help and environmental data. This work was supported by an Agriculture and Agri-Food Canada GAPS Grant #348 allocated to C. Hamel and collaborators and by a NSERC discovery grant to M. St-Arnaud.

Chapitre IV

Different forms of P fertilization impact bacterial and fungal diversity in a maize-soybean rotation system

Ce chapitre sera soumis prochainement pour publication dans une revue internationale:

M.S. Beauregard[1, 2], C. Hamel[2], T. Zhang[3], T. Welacky[3], C.S. Tan[3], M. St-Arnaud[1]

[1]Institut de recherche en biologie végétale, Université de Montréal and Jardin botanique de Montréal, 4101 Sherbrooke Street East, Montreal, Quebec, Canada, H1X 2B2;
[2]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, 1 Airport Road, Box 1030, Swift Current, Saskatchewan, Canada, S9H 3X2
[3]Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, 2585 County Rd 20, Harrow, Ontario, Canada, N0R 1G0.

Abstract

Soil nutrient availability can influence plants as well as soil microbial growth and activities, which are involved in several processes related to soil fertility. The changes in soil nutrient concentrations are then likely to play an important role in shaping microbial community composition. The effect of different forms of phosphorus (P) fertilizers (organic and mineral) on soil microbial diversity was studied in a typical Canadian maize/soybean rotation. Denaturing gradient gel electrophoresis (DGGE) and fatty acid methyl ester (FAME) analysis were used to study soil bacterial and fungal communities. Soil parameters related to fertility were also monitored and related to soil microbial ribotypes patterns. The hypothesis that the form of P fertilization applied to soil modifies microbial community structure or size was tested. Microbial composition of samples varied according to P treatment and time of sampling. However, bacterial and fungal taxonomic richness based on the number of ribotypes detected remained stable. Fertilization also induced significant changes in microbial activity, specific microbial biomass and soil P-related variables.

Introduction

In intensive grain production, fertilization is a common practice used to optimize crop productivity. Organic and inorganic fertilizers that are used primarily to increase P availability to plants and promote their productivity, surely affect soil microorganisms as well. The addition of P under different forms (i.e., mineral fertilizer, manure, etc.) to agricultural soils has been shown to increase yields but also to be responsible of deleterious environmental effects (Brady and Weil 2002; Kirchmann and Thorvaldsson 2000). Soil quality is indeed a major factor effecting crop growth in agriculture and even if some cropping management practices have been proved to help maintaining more P available to plants and reduce losses, other alternatives should be proposed (Moore et al. 1999; Schaller et al. 2007).

The area surrounding plant roots is a site of high microbial activity (Paul 2007) and microorganisms carry out essential processes that contribute to nutrient cycling, soil structure, and plant health (Hamel 2004; St-Arnaud and Vujanovic 2007; Whitelaw 2000). Changes in their diversity, activity and composition induced by various biotic and abiotic stresses (Calvaruso et al. 2007; Galvez et al. 2001; Scheublin et al. 2004; Staddon et al. 1998) thus impact soil quality in agroecosystems.

Consequently, recent comparative microbial community analyses utilizing molecular approaches have been conducted to obtain a better understanding of community structure and function in complex environments such as soils (Schmalenberger and Tebbe 2003; Smalla et al. 2007; Yergeau et al. 2005). However, at this point, managing soil biological and chemical activities would still require a better knowledge of mechanisms influencing soil P cycling and its effects on microorganisms.

As most studies have focused on small-size population (Bhadalung et al. 2005; Yergeau et al. 2006), little is still known about the factors affecting global soil microbial biodiversity. This study aimed at understanding the impact of different P fertilizer forms, and growth season evolution on soil total microbial diversity. Field trials were conducted in Harrow, Ontario over three years of a maize/soybean rotation. Plots were submitted to five different fertilizations including mineral and organic forms and were sampled three times corresponding to specific plant growth stages every year. Identification of rRNA gene sequences of bacteria and fungi was performed. The experiment was conducted using denaturant gradient gel electrophoresis (DGGE) prior to sequencing as it avoids many inconvenients of time-consuming methods (i.e., culture and isolation). FAME analysis was also used to monitor the biomass changes within different microbial taxonomic groups. The impacts of the applied P form as well as the time of sampling on various other soil and plant parameters were also investigated.

Changes in field conditions were predicted to affect the microbial populations. Our hypotheses were that both environmental variations occurring within a season but also between the years (because of the two different cropping systems in place) and P applications would affect bacterial and fungal communities. To our knowledge, there is no report on soil global microbial diversity combining both P fertilization and time variables on such a long time period. These informations about indigenous microbial populations are crucial to understand the impact of fertilization on soil quality and eventually improve management practices, while maintaining high yields.

Materials and methods

Experimental design and sampling

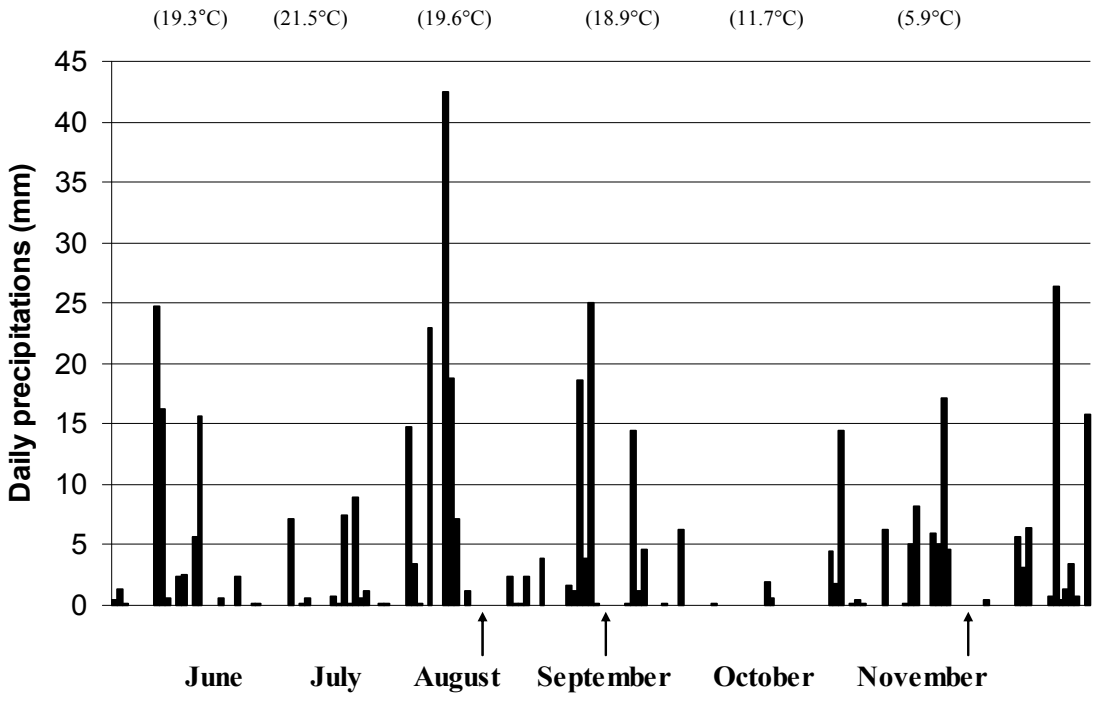
The experiment was conducted from 2004 to 2006 in a field located at the Greenhouse and Processing Crops Research Centre, Woodslee, Ontario, Canada (42° 13' N lat., 82° 44' W long.). The soil was an orthic-humic gleysol in the Canadian soil classification system. The climate is humid, and cool-temperate, with a mean (on 15 years) annual air temperature of 8.7°C, and an average annual precipitation of 875.5 mm. The soil contained 280 g kg^{-1} sand, 35 g kg^{-1} silt, 37 g kg^{-1} clay, 22.7 g kg^{-1} organic C; 1.95 g kg^{-1} total N; 12 mg kg^{-1} soil test P (Olsen P), and 131 mg kg^{-1} soil test K, with a pH of 5.8. The experimental design was a randomized complete block design in 3 blocks and involved a rotation from maize in 2004 to soybean in 2005 and maize in 2006. Maize plants (*Zea mays* L. var. N29A2) were seeded at a rate of 76,855 seeds ha^{-1}, while soybean plants (*Glycine max* (L.) Merr. var. S24-K4) were seeded at a rate of 486,800 seeds ha^{-1}. Fertilization treatments were applied to the corn phase, in 2004 and 2006. Fertilizers were inorganic phosphorus (super phosphate; IP), solid manure (SM), manure compost (MC) and liquid manure (LM) (Table VIII). The amounts used were adjusted to provide 50 kg P_2O_5 ha^{-1}, i.e. twice the amount of the control treatment which corresponded to the recommended dose in this area. The control treatment, as the IP treatment, was applied under the form of inorganic P (0-46-0).

Soil samples were collected at three specific growth stages during the season, corresponding to different dates each year but to identical growth stages. The 6-leaf stage (V6), tasseling and harvest for corn in 2004 and 2006, and to the reproductive stages of full bloom (R2), full size seed in top four nodes (R6) and full maturity (R8) for

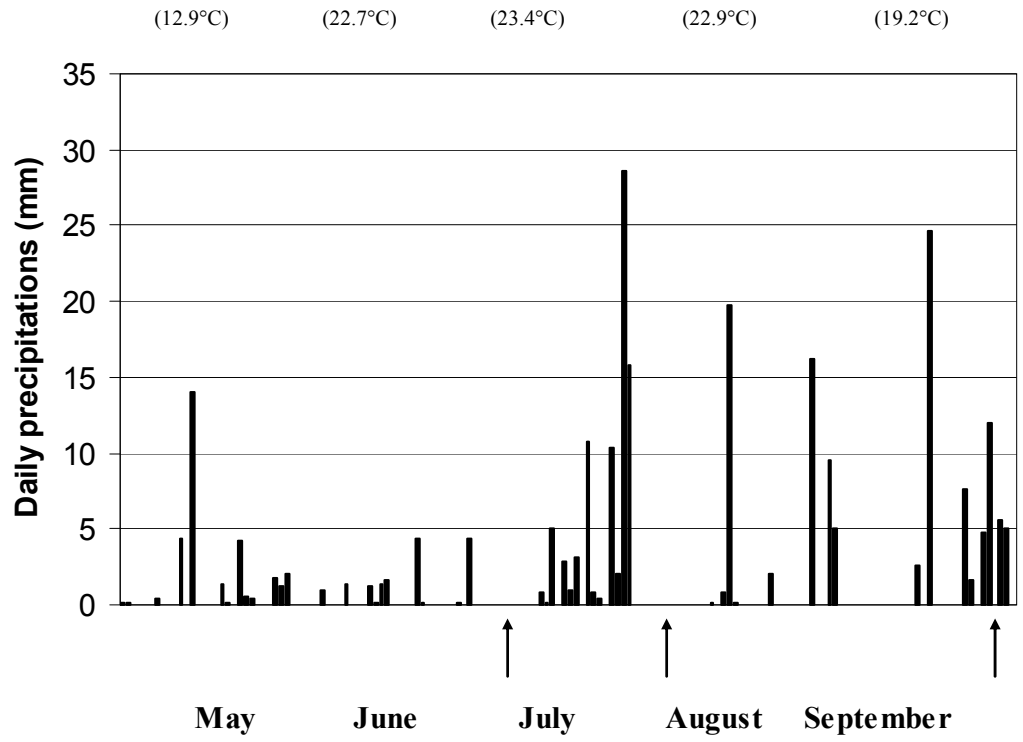
soybean in 2005. Specific sampling dates are represented by arrows on figure 6. Five soil samples per plot were taken from the crop row with 7.5 cm long probes, mixed thoroughly to obtain one representative sample per plot, kept on ice, brought back to the laboratory and frozen at - 20°C within a few hours. They were sent on dry ice to the different laboratories performing the analyses.

Figure 6. Daily rainfall and average monthly temperature (in brackets) during the 2004 (a), 2005 (b) and 2006 (c) growing seasons. Arrows represent sampling dates.

A)



B)


(12.9°C)
(22.7°C)
(23.4°C)
(22.9°C)
(19.2°C)
Daily precipitations (mm)
0
5
10
15
20
25
30
35
May
June
July
August
September

C)

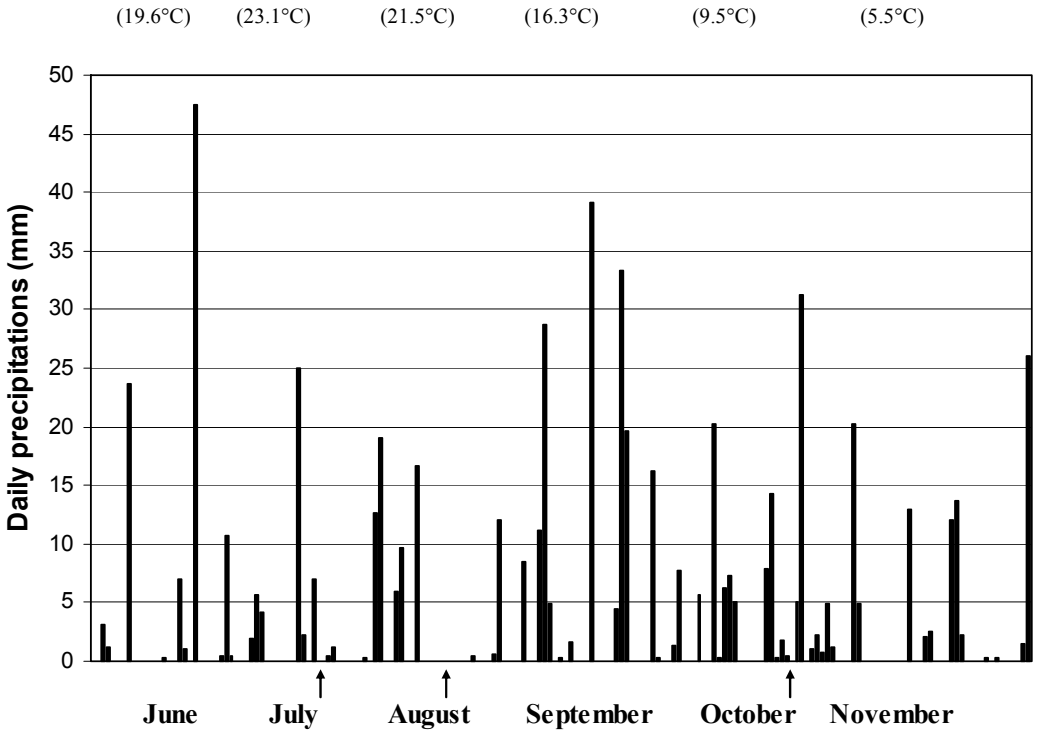

(19.6°C)
(23.1°C)
(21.5°C)
(16.3°C)
(9.5°C)
(5.5°C)
Daily precipitations (mm)
0
5
10
15
20
25
30
35
40
45
50
June
July
August
September
October
November

Table VIII. Chemical analysis of manures applied in the corn phase.

Parameter	LM		SM		MC	
	2004	2006	2004	2006	2004	2006
	g kg^{-1} ± SD[1]					
Organic C	48.0 ± 0.60	28.6 ± 0.60	65.6 ± 1.94	188 ± 6.99	94.7 ± 4.38	88.0 ± 5.08
Total N	9.57 ± 0.12	5.89 ± 0.08	5.26 ± 0.17	23.7 ± 5.88	8.97 ± 0.29	18.3 ± 1.12
Total P	3.14 ± 0.74	1.73 ± 0.01	3.30 ± 0.17	3.79 ± 0.42	1.61 ± 0.08	2.91 ± 0.42
Total K	3.80 ± 0.01	1.77 ± 0.01	6.02 ± 0.42	7.84 ± 0.75	3.96 ± 0.23	4.18 ± 0.22
pH	7.8	7.7	7.2	8.2	7.4	6.6

[1]SD = standard deviation.

DNA extraction and PCR amplification

Total genomic DNA was extracted from 600 mg of soil using the UltraClean^{tm} Soil DNA isolation kit (MoBio Laboratories, Inc.). Final elutions were kept at -20°C until use. Two different primer sets were used to separately analyze the diversity of soil bacteria and fungi. The latter were amplified using primers FF390/FR1-GC (Vainio and Hantula 2000). For bacteria, a nested PCR strategy used with primers pA/pH (Edwards et al. 1989) first, and the primer pair 341F-GC/534R (Muyzer et al. 1993) in the second round. To improve gel resolution and the separation of closely similar fragments in DGGE, a GC-clamp was added to primer FR1 and 341F (Myers et al. 1985). Primer sequences and reaction conditions are detailed in Table IX. All reactions were carried out in a Mastercycler ep S gradient thermocycler (Eppendorf, NY, USA) in a 25 µl volume containing 2.5 µl of 10 × PCR buffer, 2.5 µl of each primer (5 µM), 0.5 µl of dNTPs mix (10 mM), and 1.25 U of Taq polymerase (QIAgen). To amplify DNA fragment from fungi, 1 µl of a 1:10 dilution of the extract was used, while 1 µl of a 1:20 dilution was added to the PCR mix for bacteria. Twice the amount of DNA was used for

samples that failed to amplify or gave very faint PCR product. Amplicons were analysed by agarose gel electrophoresis (1%) using ethidium bromide staining and UV light. For nested PCR, products with a visible band were diluted 1:100 (products with a faint band were undiluted) and 1 μl were used as template in subsequent amplification using the reaction mixture described above.

Denaturing gradient gel electrophoresis (DGGE) analysis

Amplicons were analysed using a DCODE Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) with 16 × 18 cm glass plates and 1-mm spacers. The different DGGE conditions corresponding to the two types of microorganisms studied are listed in Table IX. In all cases, a 3 ml stacking gel containing no denaturant was added before polymerization and DGGE were conducted in 1× TAE electrophoresis buffer. Gels were stained with SYBR Gold (Invitrogen) diluted 1:10000, visualized on a UV transilluminator and photographed (GelDoc, Bio-Rad Laboratories). Pictures were visually inspected and bands that migrated to different positions were considered as different ribotypes. The presence of each ribotype was noted for all PCR products that produced bands on DGGE and a presence–absence matrix was built for statistical analyses. Bands corresponding to different ribotypes were excised from gel, reamplified and submitted to sequencing in triplicate. Results of clear sequences were aligned and compared to the NCBI database in order to find the organisms with the higher level of similarity.

Table IX. Primer sets, primer sequences, amplified region and PCR conditions used in this study. Primer sets are written in the order they are used in case of nested PCR.

Organisms	Primers (5' – 3')	Targeted region	PCR conditions	DGGE conditions
Bacteria	**pA** AGAGTTTGATCCTGGCTCAG **pH** AAGGAGGTGATCCAGCCGCA	16S rRNA	3 min at 94°C, then 35 cycles of 30 sec at 94°C, 30 sec at 58°C, 1 min at 72°C and final extension for 5 min at 72°C. 2 cycles of 1 min at 94°C, 45 sec at 65°C, 1 min at 72°C; decreased annealing temperature by 1°C every 2 cycles until 55°C (10 cycles), final extension for 5 min at 72°C.	8% (w/v) polyacrylamide gels with a denaturant gradient of 43-70% (100% is defined as 40% formamide and 7 M urea) at 60°C. 60V for 18h.
	341F-GC (GC clamp) TACGGGAGGCAGCAG **534R** ATTACCGCGGCTGCTGG			
Fungi	**FF390** CGATAACGAACGAGACCT **FR1-GC** (GC clamp) AICCATTCAATCGGTAIT	18S rRNA	3 min at 95°C, then 35 cycles of 30 sec at 95°C, 45 sec 50°C, 1 min at 72°C and final extension for 10 min at 72°C.	7.5% (w/v) polyacrylamide gels with a denaturant gradient of 40-63% at 58°C. 100V for 20 min., then 80V for an additional 16h.

Fatty acid methyl ester (FAME) analysis

The amount of microbial fatty acid biomarkers in the phospholipid (PLFA) and neutral lipid (NLFA) fractions of soil lipid extracts was used as a measure of active soil microbial biomass and storage material of fungal origin, respectively, using the method described in Hamel et al. (2006a). Total soil lipids were extracted from 4 g of soil (dry weight equivalent) in dichloromethane (DMC): methanol (MeOH): citrate buffer (1:2:0.8 v/v). Lipid-class separation was conducted in silica gel columns. The neutral, glyco- and phospholipids fractions were eluted by sequential leaching with DCM,

acetone and MeOH, respectively. The glycolipid fraction was discarded. The neutral and phospholipid fractions were dried under a flow of N_2 at 37°C in the fume hood, dissolved in 2 ml of MeOH for PLFA or DCM for NLFA and stored at -20°C. Fatty acid methyl esters were created through mild acid methanolysis. Ten microliters of methyl nonadecanoate (19:0; Sigma–Aldrich) was added to serve as internal standard and samples were dried under a flow of N_2 at 37°C in the fume hood. Samples dissolved in 50 ml of hexane were analyzed using a Varian 3900 gas chromatograph (GC) equipped with a CP-8400 auto sampler and a flame ionization detector (FID). Helium was the carrier gas (30 ml min^{-1}) and the column was a 50-m Varian Capillary Select FAME # cp7420. Sample (2 ml) injection was in 5:1 split mode. The injector was held at 250°C and the FID at 300°C. The initial oven temperature, 140°C, was held for 5 min, raised to 210°C at a rate of 2°C min^{-1}, raised from 210 to 250°C at a rate of 5°C min^{-1}, and held for 12 min.

Identification of peaks was based on comparison of retention times to known standards (Supelco Bacterial Acid Methyl Esters #47080-U, plus MJS Biolynx #MT1208 for 16:1ω5). The abundance of individual PLFAs was expressed as mg PLFA g^{-1} dry soil. Amounts were derived from the relative area under specific peaks, as compared to the internal standard (19:0) peak value, which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane.

Individual fatty acids have been used as signatures for various groups of micro-organisms (Hamel et al. 2006a; Pankhurst et al. 2002). The FAME 18:1c was used as indicators of fungal biomass (Petersen and Klug 1994) and FAME 16:1ω5, as indicator of AM fungi (Balser et al. 2005). FAMEs a-15:0, i-15:0, 15:0, 2OH-14:0, i-16:0, i-17:0,

2OH-16:0 and 18:1t were chosen to represent bacterial PLFAs based on the bacterial standards used.

Plant and soil variables

Soil moisture was determined from 50 g of soil which was dried at 105°C for 24 h.

Soil PO_4 flux was measured *in situ* using 6.5 cm x 2.25 cm anion-exchange membranes (AEMs). A set of three AEMs was placed between the row and the fertilizer band in each plot. The AEMs (#200253, Ionics, Watertown, Massachusetts) were inserted vertically in the soil (2–7 cm deep) through the slit opened with a small shovel. These AEMs were inserted in the soil of each plot on June 24th, May 27th and June 14th in 2004, 2005 and 2006 respectively. Membranes were replaced on August 9th, July 5th and July 18th as well as on September 3rd, August 5th and August 8th. Once removed from the soil, the membranes were rinsed with distilled water to remove the adhering soil particles, placed in tubes containing 25 ml of 1 M KCl and brought to the laboratory for analysis.

AEMs were also used to monitor soil mean daily nitrate- N fluxes during the growth seasons. They were prepared and extracted as described by Ziadi et al. (1999) with modifications as mentioned in Hamel et al. (2006a).

Soil available P was analysed by resin extraction following Hedley and Stewart (1982) with modifications. Briefly, 0.5 g of soil was placed into 50 ml plastic centrifuge tube with 1 exchange membrane and 25 ml of ddH_2O. Tubes were shaken overnight. The membranes were removed, rinsed with H_2O, and placed into a 50 ml plastic centrifuge tube with 20 ml of 0.5 N HCl. Tubes were set aside for 1 hour for gas to escape, then

capped and shaken for 16 hours. On the next day, the resin extract was decanted into clean, appropriately marked vials. P concentration was determined by colorimetry (molybdate–ascorbic acid method) on the autoanalyzer Technicon segmented flow AAII system.

Dehydrogenase activity was assayed following Casida et al. (1964) with modifications. Fresh weight equivalent (FWE) of 20 g oven dried soil was mixed with 0.2 g $CaCO_3$. FWE of 6 g of this mixture was placed into three test tubes with ddH_2O. One ml of 3% 2,3,5-triphenyl tetrazolium chloride (TTC) and 2.5 ml of ddH_2O were added to each tube before they were inverted a few times and incubated at 37°C for 24 hours. Blank contained all the amendments except the TTC. Each soil sample was extracted with 10 ml of methanol after incubation. The extract was filtered and the O.D. of the filtrate was read at 485 nm.

Acid phosphatase activity was measured according to Eivazi and Tabatabai (1977). The equivalent of 1 g (oven dried) of soil samples were reacted with p-nitrophenyl phosphate solution during 1 h at 37°C and the p-nitrophenol concentration in the filtrate was analyzed with a spectrophotometer at 400 nm. Phosphatase activity was determined based on a standard curve of p-nitrophenol.

Root mycorrhizal colonization extent was assessed from randomly selected 0.3 g fresh root samples cut into 1-cm fragments and boiled 10 min in a 10% KOH solution before staining 3 min in an ink–vinegar solution (Vierheilig et al. 1998b). The percentage of root length bearing arbuscular mycorrhizal structures was estimated with the gridline intersect method under a dissecting microscope (Giovannetti and Mosse 1980).

Plants were dried at 40°C to constant weight. Ground plant tissue digestion (Thomas et al. 1967) was completed and tissue N and P concentrations were measured by the

salicylate/nitroprusside, and the acidic molybdate/ascorbic acid method (Anonymous 1976) on the Technicon segmented flow autoanalyzer (AAII system).

Statistical analyses

Soil and plant variables were subjected to one-way analysis of variance (ANOVA) followed by the Tukey's HSD test at the 5% level to evaluate the effect of P treatments, plant growth stage and year of sampling on selected environmental variables (i.e., soil moisture, plant N and P content, soil P as measured by anion exchange membrane and resin methods, phosphatase and dehydrogenase levels) to use in multivariate analyses. Possible interactions between fertilization treatments and time of sampling, treatments and year of sampling or time of sampling and year have also been evaluated for each variable. Canonical correspondence analysis (CCA) were performed on the taxa presence/absence matrix of DGGE banding patterns using Canoco 4.5 for Windows to assess the influence of fertilization treatments, plant growth stage and year of sampling on DGGE patterns. The discriminant analysis procedure of JMP 6.0 (SAS institute) was also used to define possible data grouping between treatments or sampling dates. As the data exhibited a linear response in the CCA, a canonical redundancy analysis (RDA) was performed using Canoco 4.5 to study the interplay of environmental variables and DGGE patterns. This technique finds the portion of the variability that can be explained by experimental treatments. The matrix of dependent variables contained fungal or bacterial ribotypes detected by DGGE, while the matrix of independent variables contained soil moisture, plant N and P content, soil P as measured by anion exchange membrane and resin methods, phosphatase and dehydrogenase levels (see methods above). RDA was performed only on the data collected in 2004 and 2005 as several

measures were missing in 2006 due to equipment failures. Rare or ubiquitous ribotypes were excluded from the analysis because of the distortion they provoke to this type of ordination.

Results

Diversity and identification of microorganisms

The analysis of DGGE patterns revealed thirty-four and twenty-four different bands for bacteria and fungi, respectively, among the 135 samples studied in this experiment. Bands located at different position on acrylamide gel were considered as different ribotypes as it was confirmed by sequencing.

A great variability was noted among the number of detected microbial ribotypes per sample. Six to 28 bacterial and 1 to 14 fungal ribotypes per amplicon were found. However, most samples contained around 6 fungal and 15 bacterial ribotypes. Microbial richness, as measured by the average number of ribotypes detected, was similar across fertilization treatments, plant growth stage and years of sampling. The presence of a ribotype was never exclusive to a specific P treatment or date of sampling. However, their detection frequency, as detected by DGGE, indicated that some of them were more frequently found after a certain fertilization treatment, at a specific moment of the season or year of sampling (see table X). The positive correlation of a specific ribotype to a fertilization treatment or plant growth stage was not recurrent from one year to the other though.

Only a few microbial ribotypes could be classified as ubiquitous and their dominance, as measured by their frequency of detection in the samples, was stable over the testing period (Table X). Fourteen fungal and 8 bacterial ribotypes were detected in less than

10% of samples. Most of these fungal ribotypes were shared between the years, which was not the case for rare bacterial ribotypes as they are different each year.

Seventy-two percent of fungal ribotypes produced sequences of good quality that matched sequences from Genbank database. However the number of bacterial ribotype dropped to twelve percent. All identified ribotypes corresponded to soil microorganisms (Table X).

Table X. Associated organisms according to the BlastN results and detection frequency of the different coding band number as measured by DGGE in 2004, 2005 and 2006.

Band #	Most similar organism	Similarity %	Accession number	Detection frequency		
				2004[1]	2005	2006[1]
bacteria						
HB1	Poor quality DNA sequence	-	-	30%	33%	20%
HB2	*Sphingobacteriaceae* bacterium BR5-29	100%	EU370957.1	35%	51%	24%
HB4	Poor quality DNA sequence	-	-	65%	65%	53%
HB5	Poor quality DNA sequence	-	-	47%	56%	49%
HB6	Poor quality DNA sequence	-	-	40%	51%	7%
HB7	Poor quality DNA sequence	-	-	16%	54%	53%
HB8	Poor quality DNA sequence	-	-	12%	21%	62%
HB9	Poor quality DNA sequence	-	-	79%	40%	38%
HB11	Poor quality DNA sequence	-	-	58%	74%	53%
HB12	Poor quality DNA sequence	-	-	44%	42%	27%
HB13	Poor quality DNA sequence	-	-	30%	74%	73%
HB14	Poor quality DNA sequence	-	-	56%	70%	49%
HB15	Poor quality DNA sequence	-	-	72%	58%	60%
HB16	Poor quality DNA sequence	-	-	28%	58%	33%
HB17	*Sphingopyxis* sp. S25 alpha proteobacterium O11 several uncultured soil bacteria	100%	AY190144.1 FM211710.1	100%	98%	89%
HB18	Poor quality DNA sequence	-	-	44%	74%	53%
HB19	Poor quality DNA sequence	-	-	14%	9%	16%
HB20	Several uncultured soil bacteria	100%		49%	65%	49%
HB21	Poor quality DNA sequence	-	-	42%	61%	56%
HB22	Poor quality DNA sequence	-	-	61%	84%	69%
HB23	Poor quality DNA sequence	-	-	70%	54%	60%
HB24	Poor quality DNA sequence	-	-	65%	88%	58%
HB25	Poor quality DNA sequence	-	-	23%	5%	38%
HB26	Poor quality DNA sequence	-	-	54%	33%	22%
HB27	Poor quality DNA sequence	-	-	35%	40%	47%
HB28	Poor quality DNA sequence	-	-	26%	21%	31%
HB29	Uncultured bacterium clone BPH3C45002	99%	DQ221537.1	72%	98%	80%
HB30	Poor quality DNA sequence	-	-	56%	26%	27%

Band #	Most similar organism	Similarity %	Accession number	Detection frequency		
				2004	2005	2006
HB31	Poor quality DNA sequence	-	-	30%	12%	40%
HB32	Poor quality DNA sequence	-	-	30%	30%	27%
HB33	Poor quality DNA sequence	-	-	54%	30%	51%
HB34	Poor quality DNA sequence	-	-	0%	23%	27%
HB35	Poor quality DNA sequence	-	-	0%	9%	20%
HB36	Poor quality DNA sequence	-	-	0%	37%	18%
fungi						
HF1	Uncultured chytridiomycete	100%	AJ506018.1	31%	11%	22%
HF2	*Rhizophlyctis rosea* AFTOL-43	100%	AY635829.1	31%	11%	49%
	Catenomyces sp. AFTOL-47	100%	AY635830.1			
HF3	*Rhizopus oryzae*	100%	AB250174.1	44%	36%	22%
	Amylomyces rouxii	100%	AB250171.1			
HF4	*Rhizophlyctis rosea* AFTOL-43	99%	AY635829.1	38%	78%	29%
	Catenomyces sp. AFTOL-47	99%	AY635830.1			
HF5	*Mortierella chlamydospora*	100%	AF157173.1	98%	98%	82%
	Mortierella alpina	100%	AJ271630.1			
HF6	*Entophlyctis helioformis* AFTOL-40	95%	AY635826.1	44%	89%	4%
HF7	Poor quality DNA sequence	-	-	16%	11%	18%
HF8	*Spizellomycete* sp.	100%	DQ536490.1	18%	16%	22%
	Powellomyces sp.	100%	AF164245.2			
	Powellomyces variabilis	100%	AF164244.1			
HF10	*Entophlyctis confervae-glomeratae*	100%	EF014367.1	31%	24%	38%
HF11	Poor quality DNA sequence	-	-	31%	9%	40%
HF12	Uncultured fungus	97%	DQ244008.1	67%	47%	29%
HF13	Poor quality DNA sequence	-	-	20%	2%	44%
HF14	Several *Alternaria* sp.	100%	-	0%	29%	11%
HF15	*Vollutella colletotrichoides*	100%	AJ301962.1	2%	11%	4%
HF16	*Microdochium nivale*	99%	AF548077.1	2%	4%	2%
HF17	Uncultured fungus	100%	AJ510049.1	98%	100%	84%
HF18	*Phyllachora graminis*	99%	AF064051.1	7%	9%	47%
HF19	Several *Clavipitaceae* sp.	100%	-	9%	13%	18%
HF20	*Dothideomycete* sp.	100%	AY275186.1	2%	18%	33%
	Pleospora sp.	100%	AY392129.1			
	Phoma sp.	100%	AB252869.1			
	Microphaeropsis olivacea	100%	AY642519.1			
HF21	*Pleosporales* sp.	99%	AB255234.1	7%	16%	31%
HF22	*Acremonium* sp.	100%	AB167384.1	20%	38%	27%
HF23	Poor quality DNA sequence	-	-	29%	4%	9%
HF24	Poor quality DNA sequence	-	-	13%	0%	7%
HF25	Poor quality DNA sequence	-	-	22%	4%	27%

[T] maize was grown

Impact of P treatment and time of sampling on microbial population and environmental variables

ANOVA performed on environmental data have shown that there was no significant difference in the level of most measured PLFAs biomarker according to P fertilization treatments, plant growth stages or years of sampling. However, some biomarkers corresponding to bacteria and fungi varied significantly between treatments (Table XI). General bacterial biomarkers 15:0 and 2OH:16:0 as well as fungal biomarker 18:1c showed significantly higher values in 2005. They all also presented significantly higher values at GS2 and after solid manure treatment. Gram positive biomarkers i-16:0 and i-17:0 followed the same trend according to fertilization treatment and sampling date. Soil soluble P, P and N fluxes as well as levels of microbial activity and plant P content were significantly affected by fertilization treatments. Although all variables did not react exactly the same to the different treatments, measures were generally higher when manure compost was applied. Significant differences in phosphatase levels, soil moisture, plant N and P content as well as in P and N fluxes were noted between the different samplings. Phosphatase level was significantly increased at harvest and during the last growing season. Plant nutrient content as soil P and N fluxes decreased within growing season.

Amending the soil with fertilizer forms differing in their P bioavailability did not modify significantly the effect of time on the recorded soil parameters and microbial biomass over the growing seasons. There was no notable interaction between fertilization treatments and year of sampling either.

Bacterial ribotypes composition of samples showed significant difference every year between plots treated with inorganic P, manure compost as well as control plots. Fungal

population from samples treated with solid or liquid manure was always different from the control (Table XII).

However, CCA clearly showed that the year of sampling had the greatest impact on microbial ribotypes detection frequency (P=0.002). Sampling date had more influence than fertilization treatments regarding the fungal distribution, while both factors have very similar impact for bacteria (Fig. 6).

Relationships between environmental variables and ribotypes presence

RDA results reflected that soil is a dynamic medium with complex interactions. In analyses conducted in 2004 and 2005, most of the microbial ribotypes were positively correlated. However, their correlations to selected soil fertility indicators are variable. Bacterial and fungal ribotypes were similarly correlated to environmental variables.

RDAs were conducted with environmental parameters affected by either P fertilization or time. They turned out to be poor predictors of microbial community composition. In fact, community variance explained on the first two canonical axes was of 4.3% and 10% in 2004 and 2005 respectively. However, the model created with 2005 data appeared to be stronger (P= 0.01) than that of 2004 (P= 0.3) (Fig. 7).

RDA performed using PLFA measures as species data gave results very similar to these using ribotypes in their graphic representation as well as in their variance explanation which were of 10% (P= 0.8) and 20% (P= 0.5) in 2004 and 2005 respectively (graph not shown).

Table XI. PLFA biomarkers and soil parameters that were submitted to significant variations. Data are reported as averages for 27, 45 and 45 replicates for fertilization treatments (TRT), sampling time (GS) and year respectively.

	15:0	2OH:16:0	18:1c	i-16:0	i-17:0	Ptase[1] (μg PNP/g/h)	Soil Moisture (%)	Resin P (ppm)	Dase (μg TPU/g/day)	Plant N (%)	Plant P (%)	PO_4-P flux (μg/cm^2/day)	NO_3-N flux (μg/cm^2/day)
Treatment													
Ctrl	4.86B[2]	4.87B	6.85B	11.05B	2.23B	85.85A	19.76A	0.19C	166.89AB	1.74A	0.16B	7.90E-5B	0.0013B
IP	4.96B	4.67B	6.75B	10.61B	2.43B	90.55A	19.33A	0.74A	151.09B	2.00A	0.19AB	1.13E-4B	0.0032A
MC	6.18B	5.47B	6.55B	12.36B	2.58B	88.22A	20.07A	1.00A	203.63A	1.75A	0.22A	9.39E-4A	0.0012B
SM	45.87A	48.42A	48.01A	57.96A	15.3A	100.23A	19.01A	0.89A	161.83B	2.02A	0.20AB	8.78E-4A	0.0027AB
LM	5.41B	5.59B	7.74B	10.81B	2.34B	96.56A	18.50A	0.43B	178.92AB	1.75A	0.16B	2.49E-4B	0.0019AB
Sampling date													
GS1	4.98B	6.00B	8.28B	9.20B	1.41B	82.88B	15.58B	0.67A	165.22A	3.00A	0.32A	2.91E-4B	0.03A
GS2	28.16A	29.65A	34.58A	36.57A	9.91A	84.58B	21.05A	0.67A	174.67A	1.77B	0.17B	7.52E-4A	0.03A
GS3	5.83B	4.49B	11.10B	14.07B	3.05B	111.10A	21.79A	0.63A	180.87A	0.79C	3.1E-4C	3.13E-4B	0.01B
Year of sampling													
2004	3.33B	4.79B	6.93B	7.27A	1.28A	77.75B	21.54A	1.08A	174.72A	1.46A	0.19A	8.20E-4A	0.03A
2005	26.91A	28.30A	28.96A	32.64A	8.82A	85.25B	19.33B	0.46B	158.43A	2.24B	0.18A	8.39E-5B	0.01B
2006	9.01B	7.11B	8.52B	20.93A	4.55A	115.57A	17.55B	0.42B	187.62A	-	-	-	-

[1] Ptase= levels of acid phosphatase; resin P= soil available P analysed by resin extraction; Dase= levels of dehydrogenase; P or N flux= monitored using anion exchange membranes.

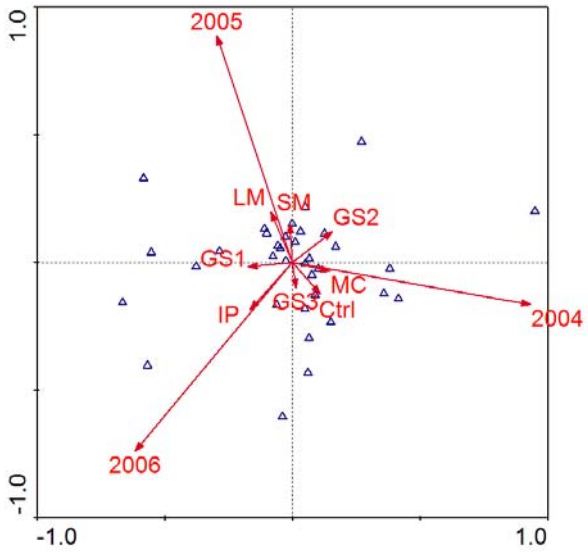
[2] Within lines, significant differences between fertilization treatments, sampling time and year are indicated by different letters and were determined by a Tukey's HSD test at the 5% level after a one-way ANOVA

Table XII. Results from discriminant analysis. Different letters means that samples fertilized with different treatments (ctrl= control; IP= inorganic P; MC= manure compost; SM= solid manure; LM= liquid manure) clustered in significantly different groups (a to d) according to their ribotypes composition (as verified by Wilks' lambda values).

Samples	Ctrl	IP	MC	SM	LM
Bacteria 2004	a	b	c	d	a
Bacteria 2005	a	b	c	a	d
Bacteria 2006	a	b	c	a	d
Fungi 2004	a	a	a	b	c
Fungi 2005	a	bc	ab	b	c
Fungi 2006	a	ab	c	b	d

Figure 7. CCA depicting the relationship between the detection of the various ribotypes (triangles) and variables such as year of sampling (2004, 2005, 2006); plant growth stage (GS1, GS2, GS3) and fertilization treatment (ctrl= control; IP= inorganic P; MC= manure compost; SM= solid manure; LM= liquid manure). A) analysis on bacterial ribotypes (P=0.002), b) fungal ribotypes (P=0.002).

a)



b)

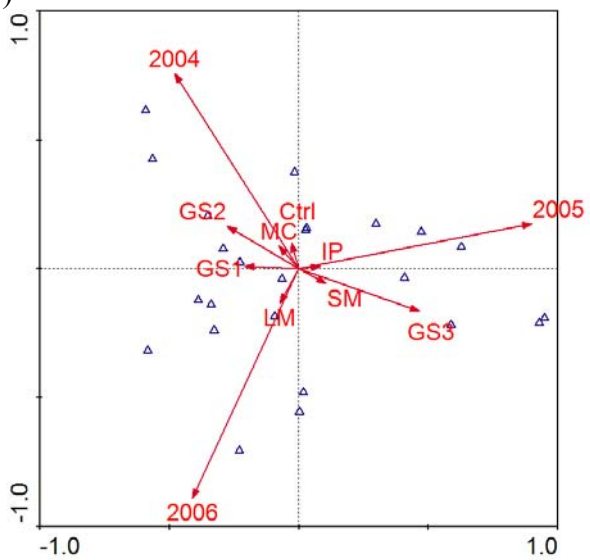
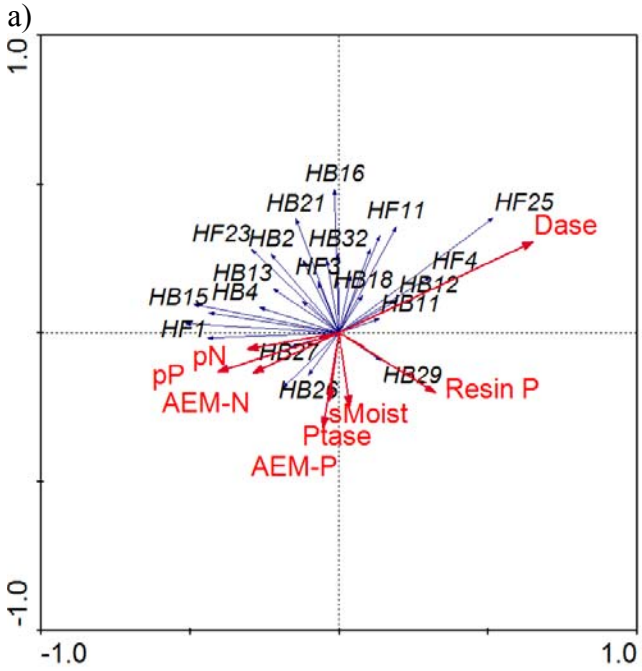
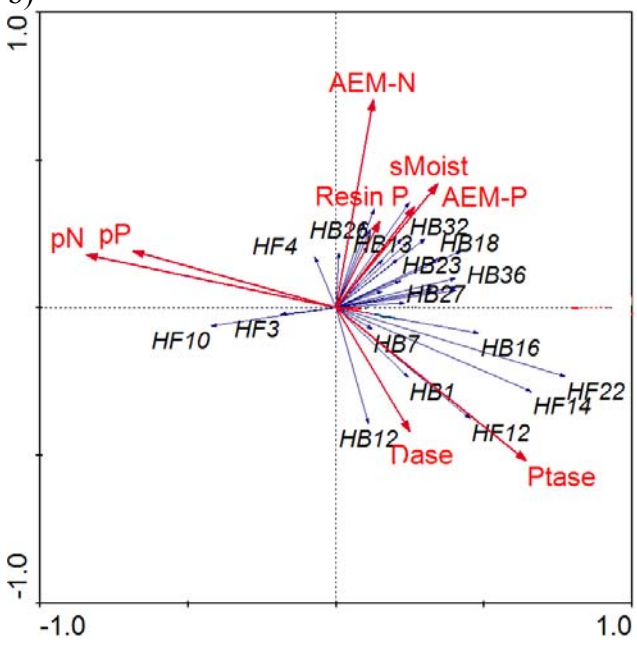


Figure 8. RDA biplot representing the correlations between bacterial and fungal ribotypes and different parameters related to soil fertility in a) 2004 (P= 0.3) and b) 2005 (P= 0.01). Ribotypes are coded by the letter B (bacteria) or F (fungi) depending on their nature and numbers indicating the order they were identified. Other parameters are coded as: Dase = dehydrogenase; Ptase = acid phosphatase; Resin P = soluble P measured by resin; AEMp = P flux measured by anion exchange membrane; AEM-N = N flux measured by anion exchange membrane; pN= plant N content; pP= plant P content; smoist= soil moisture.

a)



b)



Discussion

The experiment demonstrated that both total bacterial and fungal communities of samples were affected by fertilization treatments and time of sampling. However, the microbial ribotypes richness was not affected as the average number of detected ribotypes remained similar. Measures of PLFAs biomarkers revealed that solid manure fertilization treatment significantly increased specific bacterial and fungal biomass, which was also higher at the second soil sampling period of the season. Despite the fact that different types of fertilizers changed soil microbial composition, the time of sampling (within a single growing season or between years) had more influence. It was not possible to observe significant interactions between the form of fertilizer applied and time. Such hierarchy in variations have been observed recently at different levels in other farming management systems (Ge et al. 2008; Hagn et al. 2003). However, this is the first study depicting large scale variations on soil microbes following P fertilization on such a long period of time.

In opposition to our first assumption, it has been impossible to identify specific organisms corresponding to most ribotypes detected by DGGE. Other studies provided evidences that soil microbial diversity is high (Lynch and Thorn 2006; Rösch and Bothe 2009). Low ribotype diversity recorded in this study leads to the conclusion that DGGE is useful as an initial step to discriminate among global microbial communities, not reveal fine scale taxa variations. The use of universal primer sets may not totally suit global soil microbial diversity experiment. Even if the primer pair FF390/FR1-GC used in fungal DNA amplification has been demonstrated to produce quite similar results among several variables (Kang and Mills 2006), it was first designed and used in

forestry studies where fungal community is different from agricultural soil (Lilja et al. 2005; Perkiömäki and Fritze 2003; Vainio and Hantula 2000). For bacteria, the DNA band pattern obtained by DGGE of the bacterial V3 region was an attractive way to study complex communities in environmental samples because it has been shown that most bacterial species produce a single band on acrylamide gel (Dilly et al. 2004). As both bacterial and fungal DNA sequences were short (less than 500 bp), identical DNA sequences from different closely related species may have been amplified and produced only one band on DGGE, contributing to the reduction of measured fungal diversity in this case. It turned out that in this specific experiment, bacterial DNA sequences of good quality were rare once extracted from the gel. Overlapping bands complicate the acuity of band patterns interpretation and make direct sequencing impossible (Rolleke et al. 1999). Profiles reflecting the whole microbial community obtained by DGGE in soils are thus relevant, sometimes more than results obtained by culture-dependent methods (He et al. 2008). However, DGGE mostly observe major community changes unless bands are cloned before sequencing in order to identify specific organisms. Observations done by DGGE remain real and this culture-independent approach should still be used to study soil microorganisms. Focus could be made on smaller functional groups in order to optimize direct species identification though. Primer sets targeting such groups exist and allow a more precise evaluation of specific variations (Green et al. 2004; Yergeau et al. 2005).

Changes in microbial patterns have been observed even if most ribotypes remained unidentified. The nature of P fertilizer was strongly expected to have an influence on soil microorganisms as it had different nutritional composition and was adjusted according to P_2O_5 dose. The form under which it was applied must have indeed affected various

mechanisms in soil that were not all monitored in this study. Mineral fertilizer compared to manures has a stable composition because it comes from industrial fabrication. It first brings readily available P to living organisms. However within a few days after being dissolved from the superphosphate granules, P is precipitated by various cations, which changes soil properties (Havlin et al. 1999). Precipitated P is largely unavailable to plants. It can be mobilized from organic matter through mineralization, a process mediated by fungi and bacteria but that is not immediate (Brady and Weil 2002). Distribution of P forms in manures varies meaning that their chemical composition can affect their potential of P bioavailability (Zhongqi et al. 2004). Manures are highly heterogeneous mixture of fodder, litter, and animal cells, and their properties are extremely dependent on the age of the pigs, the type of fodder, the age of the manure, and the storage conditions (Kirchgessner et al. 1991). The composition of the soil microbial community also varies with the soil organic matter level or C availability and can be modified by fertilization (Tiquia et al. 2002). Immediate and long-term effects provoked by different type of fertilizers in soil are then likely to vary. In this study, however, there was no evidence that the form under which P was applied to soil modified the effects of season evolution on microbial communities and soil parameters as there was no interaction between both variables.

Organic fertilizers often present major positive effects on soil physical properties compared to mineral fertilizer (Celik et al. 2004). In the present report these fertilizers brought different doses of substrates which were likely to influence soil dynamics. Although it could not be concluded here that all forms of organic fertilizers changed soil properties enough to impact microbial composition, the results still suggested that it was

the case for the form of manure containing the highest dose of organic material (i.e., manure compost).

Solid manure presented higher levels of nutrients when compared to other organic fertilizers in 2006 and it probably explains why both fungal and Gram positive bacterial biomasses increased impressively following this treatment. However, the microbial activity as measured by dehydrogenase levels did not follow the same pattern. It was influenced by the form of fertilization but composted manure treatment had shown the highest microbial activity. Applying organic amendments has already been shown to increase soil microbial activity (Liu and Ristaino 2003), diversity (Girvan et al. 2004) and biomass (Okur et al. 2009). Increased microbial activity in plots treated with high organic matter substrates is logical as fungi and Gram positive bacteria include main decomposers.

Plots treated with solid and liquid manure also showed regular differences in their bacterial and fungal composition. This might be related to the fact that the liquid manure contained less nutrient that solid manure. Though it was not exactly the case in this study, average swine liquid manure contains about half the N and a quarter of the K found in solid manure (Troeh and Thompson 2005). These nutrient can impact growing soil microbial populations (Kaufman and Williams 1964; Sarathchandra et al. 2001). Fungal composition of plots in this study appeared to be less affected by the addition of different nutrients to soil. However, the PCR reaction might have amplified DNA from resting fungal spores which were indeed present in soil but inactive. Such fragments also produce a visible band on gel meaning that although the composition of the total fungal community (including active and resting organisms) remained similar, active taxa might have changed.

The fact that both bacterial and fungal ribotypes richness did not vary with fertilization treatments refutes the belief that the use of mineral fertilizer decreases drastically soil microbial diversity (Oehl et al. 2004; Sun et al. 2004a). This could support the hypothesis that the whole potential microbial community (including spores) is almost entirely uninfluenced by the present investigated factors, whereas active populations show a response to environmental changes (Hagn et al. 2003). As DGGE is a culture-independent approach based on DNA amplification, it detects resting organisms that are present but inactive just as much as active organisms in the soil system. Other management practices common to intensive agriculture such as tillage and herbicides application were used on the studied fields and probably contributed to the pressure leading to this low microbial diversity (Garbeva et al. 2004).

Bacterial ribotypes composition of samples showed significant differences every year between plots treated with inorganic P and control plots. Such variations suggest that P dose also had an influence on bacterial structure as inorganic P treatment and control had the same composition but twice the P rate. This supports the results of a recent study conducted in field conditions demonstrating that bacterial community, as measured by DGGE, is influenced by the application of different dose of mineral P fertilizer (Beauregard et al. in press).

Part of temporal changes in soil microbial communities is likely due to uncontrollable environmental conditions such as moisture and temperature (Fig. 6). Temperatures in 2004 and 2006 were comparable to regular growing season averages but precipitations, therefore soil moisture, varied greatly between months and years (Canada Weather Office 2009). For example, Harrow research station received 215.40 mm of rain in growing season 2005 against 423.70 in 2006. Unusual arid soil conditions have been

created in 2005 as the average temperatures were relatively high with very low levels of precipitations during the growing season. It is known that growth of legume is depressed under severe drought conditions (Serraj et al. 1999) and that N_2 fixation by root nodules can also be affected (Streeter 2003), leading to modifications in soil carbon (C) sources. Consequently, microbial populations surrounding soybean plants could have been impacted. Three PLFAs microbial biomarkers measuring general bacterial and fungal biomass have shown significantly higher values that year, suggesting that the dry conditions may have favoured the proliferation of soil microorganisms. The nature of the crop may also be implicated in the increased microbial biomass as soybean was grown only in 2005 during the experimentation.

Plant root growth that has to be rapid in the case of highly productive annual crops probably played an important role in the modification of microbial communities. Plant growth creates relocalisation of plant main C income (Larcher 2003). Plant phenology has been determined to influence C translocation and the quality of root exudates released in the rhizosphere (Grayston et al. 1996). The soil conditions indeed created a more selective environment for microbial development than the type of fertilizer considered alone. Several studies have noticed important seasonal changes in soil microbial communities (Beauregard et al. in press; Dunfield and Germida 2003; Hamel et al. 2006a; Houlden et al. 2008). Both bacterial and fungal ribotypes richness remained unchanged over time. However, as suggested earlier, although the same ribotypes were detected changes that might have occurred in the active population could have been missed. The PCR-DGGE is a qualitative approach. It detects the targeted DNA fragments if present (even in a very small amount) but gives no indication in which

quantity. Therefore, the detected ribotype's proportion of the total DNA extracted from samples may vary.

Conclusion

Even if the ribotypes richness was stable during the experiment, there were evidences that switches in terms of microbial composition happened. Both P fertilization form and time caused changes in the total soil microbial composition under maize as well as soybean cropping. Bacterial and fungal communities include various functional groups of individual and are unlikely to react in the same way when changes occur in their environment as they fulfill different roles in soil.

Acknowledgements

The authors would like to thank Stéphane Daigle for his help with the statistical analyses, Keith G. Hanson for providing the environmental data. This work was supported by an Agriculture and Agri-Food Canada GAPS team grant allocated to C. Hamel and collaborators and by a NSERC discovery grant to M. St-Arnaud.

Chapitre V

Various forms of organic and inorganic P fertilizers did not negatively affect AM fungi communities and biomass in a maize-soybean rotation system

Ce chapitre sera soumis prochainement pour publication dans une revue internationale:

M.S. Beauregard[1, 2], M.-P. Gauthier[1], C. Hamel[2], T. Zhang[3], T. Welacky[3], C.S. Tan[3], M. St-Arnaud[1]

[1]Institut de recherche en biologie végétale, Université de Montréal and Jardin botanique de Montréal, 4101 Sherbrooke Street East, Montreal, Quebec, Canada, H1X 2B2;
[2]Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, 1 Airport Road, Box 1030, Swift Current, Saskatchewan, Canada, S9H 3X2
[3]Greenhouse and Processing Crops Research Centre, Agriculture and Agri-Food Canada, 2585 County Rd 20, Harrow, ON, Canada, N0R 1G0.

Les résultats ont été présentés sous forme de présentations orales selon les références suivantes :

M.S. Beauregard, C. Hamel, M. St-Arnaud. Impacts of P fertilisation on mycorrhizal diversity in a maize/soybean rotation. International conference on mycorrhiza. ICOM5. Granada, Spain. July 23-27, 2006.

M.S. Beauregard, C. Hamel, M. St-Arnaud. Characterization and comparison of fungal and mycorrhizal communities following various P-fertilization in a maize/soybean rotation. 2007 Annual Meeting of CPS-SCP (with Plant Canada 2007), Saskatoon, Saskatchewan, Canada, June 10-14 2007, p. SG3-5.

Abstract

Arbuscular mycorrhizal (AM) fungi are key components of most agricultural ecosystems. Therefore, understanding the impact of agricultural practices on their community structure is essential to improve nutrient mobilization and reduce plant stress in the field. The effects of five different organic or mineral P fertilizations on AM fungi diversity were assessed in a maize/soybean rotation system over a three-year period. Total DNA was extracted from root and soil samples collected at three different plant growth stages. An AM fungi specific DNA fragment was amplified and taxa were detected and identified using denaturing gradient gel electrophoresis (DGGE) followed by sequencing. AM fungal biomass was estimated by fatty acid methyl ester (FAME) analysis. Soil fertility parameters were also monitored and analyzed for possible changes related with fertilization or growth stages with multivariate analysis. Seven AM fungal ribotypes have been detected. Fertilizations significantly modified soil P and N fluxes, and plant P content, but had no effect on AM fungi community structure, except between plots treated with inorganic P in the third year of the experiment. There was no difference in AM fungi community between plant growth stages. Specific ribotypes have however been associated to root or soil samples but their detection frequency greatly varied between seasons. AM fungal biomass remained stable between the various P treatments and samplings within growing seasons. This study demonstrated that soil and AM fungal diversity were very stable over time even if submitted to different forms of fertilizations but that root and soil environments are dominated by specific ribotypes.

Keywords

AM fungi, Glomeromycota, *Zea mays*, *Glycine max*, DGGE, phosphorus

Introduction

Global soil quality relies on many complex relationships between plants, soil microbes and the organic and inorganic soil components. There are several evidences that key interactions involving soil microorganisms occur. Different microbial taxa in soil can influence positively, neutrally or negatively soil fertility by directly affecting the plant or the soil quality (Artursson et al. 2006; Caesar-Tonthat 2002; Sala et al. 2007; St-Arnaud and Elsen 2005). Microbes are closely related to several processes such as nutrient cycling and soil aggregation determining soil quality. In agricultural systems, arbuscular mycorrhizal (AM) fungi form a major component of soil fungal communities, accounting for about 30% of whole microbial biomass (Hamel 2007; Olsson et al. 1999) and up to 80% of the fungal biomass in certain agricultural soils (Kabir et al. 1997). They benefit the growth of numerous plant species by improving nutrient uptake, resistance to abiotic stresses or by reducing damages caused by pathogenic microorganisms (Schloter et al. 2003; Smith and Read 1997; St-Arnaud and Vujanovic 2007). Productivity of a particular plant species may depend on the presence of one to several specific AM fungi (Graham and Abbott 2000; Scheublin et al. 2007). The composition of vegetation was also shown to significantly affect the AM fungal taxa (Kabir and Koide 2000). Diversity of soil microorganisms, including AM fungi, present in plant rhizosphere has thus been identified as a key-factor influencing crop development (Kernaghan 2005; van der Heijden et al. 1998). The positive effect that fungal richness may have on plant is thought to be due to increased chances of "functionally compatible" symbiotic combinations (Read 1998). Several authors have however reported very low levels of mycorrhizal diversity related to intensification of

agriculture which may actually diminish AM fungi role in highly productive agriculture (Bedini et al. 2007; Gosling et al. 2006; Oehl et al. 2004).

Nevertheless, recent progresses in recognizing microbial species through direct molecular fingerprinting instead of isolation by culture have provided evidences that diversity is much greater that what was previously known. AM fungi are no exception as they are obligatory symbionts for which culture is demanding, time-consuming and requires high-skill knowledge for morphological identification. Moreover, it has been shown that genetically different nuclei co-exist in individual AM fungal mycelia (Kuhn et al. 2001). Because of the considerable genetic diversity within morphologically recognizable species, there is still confusion about what constitutes an AM fungus species. There is even questions about what really constitutes an individual (Rosendahl 2008).

Cultural practices such as fertilization is believe to have impact on the occurrence and activity of AM fungi, which in turn may result in effects on global soil biological activity (Rubio et al. 2003). However, the influence of several soil parameters on AM fungal communities is still unclear. The addition of phosphorus (P) under different forms to the soil is a common practice in intensive grain production and as been shown to influence the both bacterial and fungal communities (Cruz et al. 2009; Toljander et al. 2008). Soils receiving high amounts of inorganic P tend to reduce mycorrhizal root colonization (Ryan and Graham 2002). Nevertheless, the application of fertilizer containing variable doses of available P and organic matter can also stimulate AM fungi as well as other soil microbes' growth and activities, which can contribute to enhance soil fertility (Tarkalson et al. 1998). P fertilization has often been closely related to soil and water degradation (Imboden 1974; Kirchmann and Thorvaldsson 2000). Several

cropping practices aim to reduce those negative impacts. Since most intensively cultivated crops are mycorrhizal and these organisms are closely related to P cycling, a more appropriate management of AM fungi in agriculture is expected to allow a substantial reduction in the amount of minerals used without losses in productivity, whereas permitting a more sustainable agricultural production.

Knowledge on the presence and diversity of AM fungi in a specific area is essential for their management in any application. Most studies on AM fungi populations have focused on either mycorrhizal roots (Sykorova et al. 2007; Vallino et al. 2006) or spores recovered from soil (Bhadalung et al. 2005; Schloter et al. 2003) although when they where analysed from both type of samples, their diversity varied (Hempel et al. 2007).

The goals of this study were thus to test the effect of different organic and mineral P fertilizers on AM fungi community structure in a maize-soybean rotation cropping system, and to compare the taxa assemblages in roots and in the surrounding soil. The AM fungal community was assessed at three plant growth stages from spring to fall during three consecutive years using denaturant gradient gel electrophoresis (DGGE) from total genomic DNA extracted directly from root or soil samples. The impact of fertilizers on AM fungi community structure and relationship with soil and plant variables was investigated using multivariate analysis.

Material and methods

Experimental design and sampling

The experiment was conducted from 2004 to 2006 in a field located at the Greenhouse and Processing Crops Research Centre, Woodslee, Ontario, Canada (42° 13' N lat., 82° 44' W long.). The soil was an orthic-humic gleysol in the Canadian soil classification system (Agriculture and Agrifood Canada 1998). The climate is humid, and cool-temperate, with a mean (on 15 years) annual air temperature of 8.7°C, and an average annual precipitation of 875.5 mm. The soil contained 280 g kg^{-1} sand, 35 g kg^{-1} silt, 37 g kg^{-1} clay, 22.7 g kg^{-1} organic C; 1.95 g kg^{-1} total N; 12 mg kg^{-1} soil test P (Olsen P), and 131 mg kg^{-1} soil test K, with a pH of 5.8. The experimental design was a randomized complete block design in 3 blocks and involved a rotation from maize in 2004 to soybean in 2005 and maize in 2006. Maize plants (*Zea mays* L. var. N29A2) were seeded at a rate of 76855 seeds ha^{-1}, while soybean plants (*Glycine max* (L.) Merr. var. S24-K4) were seeded at a rate of 486800 seeds ha^{-1}. Fertilization treatments were applied to the corn phase, in 2004 and 2006. Fertilizers were inorganic phosphorus (super phosphate; IP), solid manure (SM), manure compost (MC), liquid manure (LM) and a control treatment which corresponded to the recommended dose in this area (Table VIII, chapitre IV). The amounts used were adjusted to provide 50 kg P_2O_5 ha^{-1}, i.e. twice the amount of the control treatment.

Soil and root samples were collected at three specific growth stages during the season, corresponding to different dates each year but to identical growth stages: the 6-leaf stage (V6), tasseling and harvest for corn in 2004 and 2006, and to the reproductive stages of full bloom (R2), full size seed in top four nodes (R6) and full maturity (R8) for soybean

in 2005. Specific dates of sampling are noted in figure 6. All soil and maize roots were collected with 7.5 cm long probes. Five soil samples per plot were taken from the crop row, mixed thoroughly to obtain one representative sample per plot, kept on ice and stored at - 20°C within a few hours. The root samples were taken beside the corn stalk; the plant was not cut or removed. When the crop was soybeans, the plants were clipped at ground level and the probe centered over the plant stem for sampling. Samples were kept on ice, brought back to the laboratory and 1-2 g of root pieces were randomly picked out and frozen at - 20°C. They were sent on dry ice to the different laboratories performing the analyses.

DNA extraction, amplification, DGGE and sequence analyses

Total DNA was extracted from 600 mg of soil using the UltraClean^{tm} Soil DNA isolation kit (MoBio Laboratories, Inc.) following the manufacturer's instructions, or from 400 mg of roots using the UltraClean^{tm} Microbial DNA isolation kit (MoBio Laboratories, Inc.) with the following modifications. Root tips were mixed with 100 µl of inhibitor removal solution (IRS from MoBio Laboratories, Inc.) in provided microbead tubes. Tubes were incubated 10 min. at - 20°C instead of 5 min. at 4°C. Final elutions were kept at -20°C until amplification. All reactions were carried out on a Mastercycler ep gradient S thermocycler (Eppendorf, NY, USA) in a 25 µl volume containing 2.5 µl of 10× PCR buffer, 2.5 µl of each primer (5 µM), 0.5 µl of dNTPs mix (10 mM), 1.25 U of Taq polymerase (Qiagen), and 1 µl of a 1:10 dilution of the DNA extract. Dilutions of 1:5 were used for samples that failed to amplify or gave very faint PCR product. A nested PCR strategy was used as described in Yergeau et al. (2006)

modified from Kowalchuk et al. (2002). The primer pair NS1/NS41 was used first and AM1/NS31-GC in the second round of amplification. Cycling conditions included an initial denaturation of 3 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, 1 min at 72°C and a final extension of 7 min at 72°C (first round) or 28 cycles of 45 sec at 94°C, 45 sec at 58°C and 45 sec at 72°C (second round). Amplicons were analysed by agarose gel electrophoresis (1%) after the first round. Products with a visible band were diluted 1:100 while those with a faint band were undiluted, and 1 μl was used as template in the subsequent PCR. Final PCR products were analysed similarly to confirm the presence of a fragment of the expected size (≈600 bp).

Eight μl of each amplicon were used for DGGE analysis using a DCODE Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) with 16 × 18 cm glass plates and 1-mm spacers. Gels contained 6% (w/v) polyacrylamide (acrylamide/bis-acrylamide 37.5:1) with a denaturant gradient of 38-50% for soil samples and 25-50% for root samples (100% is defined as 40% (v/v) formamide and 7 M urea). A 3 ml stacking gel containing no denaturant was added before polymerization. DGGE was run at 80V for 20 min., then lowered to 45V for an additional 16h, in TAE (1×) electrophoresis buffer maintained at 60°C. Gels were stained with SYBR Gold (Invitrogen) diluted 1:10 000, and digitized using a gel imaging system (GelDoc, Bio-Rad Laboratories).

Pictures were visually inspected and each migration position was binary coded in a presence–absence matrix. Clear bands corresponding to all positions were selected in triplicate (when possible) to be sequenced. Bands were excised and DNA was eluted from the acrylamide gel by incubation in 30 μl sterilized ddH_2O at room temperature

overnight. Eluted DNA was used for PCR amplification as described above, and products analyzed again by DGGE using a narrower gradient (40-50%) to confirm the presence of single band. PCR products from eluted DNA were sent to the Genome Quebec Innovation Centre (Montreal, Canada) to be sequenced. Similarity comparisons of the sequences were performed using the National Centre for Biotechnology Information (NCBI) online standard nucleotide BLAST (Basic Local Alignment Search Tool) program (http://www.ncbi.nlm.nih.gov/). The sequences were aligned with CLUSTALX 2.0.10 for Windows, and a distance tree was produced with PAUP* 4.0 using the sequences obtained here, and with closely or distantly related AM fungal taxa sequences from the GenBank database. The confidence of branching was assessed using 1000 bootstrap resamplings in distance analysis with a neighbour joining approach. All sequences generated in this study were deposited in GenBank under accession numbers FJ827125-FJ827131.

Fatty acid methyl ester (FAME) analysis

Fatty acids in the soil phospholipid (PLFAs) and neutral lipid (NLFAs) fractions were extracted and analyzed as a measure of active soil microbial biomass and storage material of fungal origin, respectively, using the method described in Hamel et al. (2006). Total soil lipids were extracted from 4 g of soil (dw equivalent) in dichloromethane (DMC): methanol (MeOH): citrate buffer (1:2:0.8 v/v). Lipid-class separation was conducted in silica gel columns. The neutral, glyco- and phospholipids fractions were eluted by sequential leaching with DCM, acetone and MeOH, respectively. The glycolipid fraction was discarded. The neutral and phospholipid fractions were dried under a flow of N_2 at 37 °C in the fume hood, dissolved in 2 ml of

MeOH for PLFA or DCM for NLFA and stored at -20 °C. Fatty acid methyl esters were created through mild acid methanolysis. Ten µl of methyl nonadecanoate fatty acid (19:0; Sigma–Aldrich) was added to serve as internal standard and samples were dried under a flow of N_2 at 37 °C in the fume hood. Samples dissolved in 50 ml of hexane were analyzed using a Varian 3900 gas chromatograph equipped with a CP-8400 auto sampler and a flame ionization detector (FID). Helium was the carrier gas (30 ml min^{-1}) and the column was a 50-m Varian capillary select FAME # cp7420. Sample (2 ml) injection was in 5:1 split mode. The injector was held at 250 °C and the FID at 300 °C. The initial oven temperature, 140 °C, was held for 5 min, raised to 210 °C at a rate of 2 °C min^{-1}, and then raised from 210 to 250 °C at a rate of 5 °C min^{-1}, and held for 12 min.

Identification of peaks was based on comparison of retention times to known standards (Supelco bacterial acid methyl esters #47080-U, plus MJS Biolynx #MT1208 for 16:1ω5). The abundance of individual PLFAs was expressed as mg PLFA g^{-1} dry soil. Amounts were derived from the relative area under specific peaks, as compared to the internal standard (19:0) peak value, which was calibrated according to a standard curve made from a range of concentrations of the 19:0 FAME standard dissolved in hexane. Individual fatty acids have been used as signatures for various groups of micro-organisms (Hamel et al. 2006a; Pankhurst et al. 2002). The FAME 16:1ω5 is used as an indicator of AM fungi biomass (Balser et al. 2005).

Plant and soil variables

Soil moisture was determined from 50 g of soil which was dried at 105°C for 24 h.

Soil PO_4 flux was measured *in situ* using 6.5 cm x 2.25 cm anion-exchange membranes (AEMs). A set of three AEMs was placed between the row and the fertilizer

band in each plot. The AEMs (#200253, Ionics, Watertown, Massachusetts) were inserted vertically in the soil (2–7 cm deep) through the slit opened with a small shovel. These AEMs were inserted in the soil of each plot on June 24th, May 27th and June 14th in 2004, 2005 and 2006 respectively. Membranes were replaced on August 9th, July 5th and July 18th as well as on September 3rd, August 5th and August 8th. Once removed from the soil, the membranes were rinsed with distilled water to remove the adhering soil particles, placed in tubes containing 25 ml of 1 M KCl and brought to the laboratory for analysis.

AEMs were also used to monitor soil mean daily nitrate- N fluxes during the growth seasons. They were prepared and extracted as described by Ziadi et al. (1999) with modifications as mentioned in Hamel et al. (2006a).

Soil available P was analysed by resin extraction following Hedley and Stewart (1982) with modifications. Briefly, 0.5 g of soil was placed into 50 ml plastic centrifuge tube with 1 exchange membrane and 25 ml of ddH_2O. Tubes were shaken overnight. The membranes were removed, rinsed with H_2O, and placed into a 50 ml plastic centrifuge tube with 20 ml of 0.5 N HCl. Tubes were set aside for 1 hour for gas to escape, then capped and shaken for 16 hours. On the next day, the resin extract was decanted into clean, appropriately marked vials. P concentration was determined by colorimetry (molybdate–ascorbic acid method) on the autoanalyzer.

Dehydrogenase activity was assayed following Casida et al. (1964) with modifications. Fresh weight equivalent (FWE) of 20 g oven dried soil was mixed with 0.2 g $CaCO_3$. FWE of 6 g of this mixture was placed into three test tubes with ddH_2O. One ml of 3% 2,3,5-triphenyl tetrazolium chloride (TTC) and 2.5 ml of ddH_2O were added to each tube

before they were inverted a few times and incubated at 37°C for 24 hours. Blank contained all the amendments except the TTC. Each soil sample was extracted with 10 ml of methanol after incubation. The extract was filtered and the O.D. of the filtrate was read at 485 nm.

Acid phosphatase activity was measured according to Eivazi and Tabatabai (1977). Soil samples were reacted with p-nitrophenyl phosphate solution during 1 h at 37°C and the p-nitrophenol concentration in the filtrate was analyzed with a spectrophotometer at 400 nm. Phosphatase activity was determined based on a standard curve of p-nitrophenol.

Root mycorrhizal colonization extent was assessed from randomly selected 0.3 g fresh root samples cut into 1-cm fragments and boiled 10 min in a 10% KOH solution before staining 3 min in an ink–vinegar solution (Vierheilig et al. 1998b). The percentage of root length bearing arbuscular mycorrhizal structures was estimated with the gridline intersect method under a dissecting microscope (Giovannetti and Mosse 1980).

Plants were dried at 40°C to constant weight. Ground plant tissue digestion (Thomas et al. 1967) was completed and tissue N and P concentrations were measured by the salicylate/nitroprusside, and the acidic molybdate/ascorbic acid methods (Anonymous 1976) on the autoanalyzer.

Statistical analyses

A Chi-square test was performed to assess differences in frequencies of the different ribotypes between soil and root samples within each year. Soil and plant variables were subjected to one-way analysis of variance (ANOVA) followed by the Tukey's HSD test at the 5% level to evaluate the variation between P treatments, plant growth stage and

year of sampling. Environmental variables that were significantly affected by fertilizer treatment or time of sampling were selected to use in multivariate analyses (see Table XV). Canonical correspondence analysis (CCA) were performed on the taxa presence/absence matrix of DGGE banding patterns using Canoco 4.5 for Windows (Ter Braak and Smilauer, 2002) to assess the influence of fertilization treatments, plant growth stage, year of sampling and sample type (soil, root) on DGGE patterns. The discriminant analysis procedure of JMP 6.0 (SAS institute, Cary, NC, USA) was also used to define possible data grouping between treatments or sampling dates. As the data exhibited a linear response in the CCA, a canonical redundancy analysis (RDA) was performed using Canoco 4.5 to study the interplay of environmental variables and DGGE patterns. This technique finds the portion of the variability that can be explained by experimental treatments. The matrix of dependent variables contained seven AM fungi ribotypes detected by DGGE, while the matrix of independent variables contained the environmental variables that were measured for soil or root samples. Rare ribotypes were excluded from the analysis because of the distortion they provoke to this type of ordination. Four independent analyses were performed as soil and roots AM fungi communities have been considered separately for the first 2 years of the experiment. RDA of 2006 was not considered as there were many important variables that were not recorded on that year, which yielded in a weak comparison with both other years.

Results

Identity and diversity of AM fungi ribotypes

DGGE patterns revealed a low diversity of AM fungi ribotypes in the soil and root samples. Seven different sequence variants belonging to AM fungi, each corresponding to a single migration position, were identified among the 270 samples analyzed. A maximum of four ribotypes per sample was found, while most produced only two bands on DGGE gels. The most frequent ribotype in both soil and root samples was found 100% similar to a *Glomus intraradices*/*Glomus fasciculatum* (and newly introduced *G. irregulare)* complex rRNA gene sequence. Another band was identified as 99% similar to a *Gigaspora margarita* sequence while the other five were 99-100% similar to various uncultured *Glomus* sequences (Table XIII).

Table XIII. Identity of DGGE bands from root and soil samples of a maize/soybean rotation system.

Sequence designation	Most closely related taxa from GenBank	% similarity	Sequence accession number
B1	Uncultured *Glomus* clone OC6_29F2X	99%	EF177638.1
	Uncultured *Glomus* clone OC4_06F2X		EF177598.1
	Uncultured *Glomus* clone OC6_35F2X		EF177642.1
	Uncultured *Glomus* clone K287c6		EU152169.1
	Uncultured *Glomus* clone K309c5		EU152184.1
	Uncultured *Glomus* clone K321c4		EU152190.1
	Uncultured *Glomus* ID19		EU169405.1
B2	Uncultured *Glomus* clone G14.1	100%	AY916397.1
B3	Uncultured *Glomus* clone A10	100%	DQ388658.1
B4	Uncultured *Glomus* clone p3693	100%	AJ563872.1
B5	*Gigaspora margarita* isolate UY278.9	99%	AJ567844.1
	Gigaspora sp. 0715-4		EU332724.1
	Gigaspora margarita clone 8_18.M-SUB		AM415114.1
B6	Uncultured *Glomus* clone p4098	99%	AJ563899.1
B7	*Glomus fasciculatum* isolate BEG53, clone pKL5-3a	100%	Y17640.2
	Glomus intraradices clone 13		FJ009602.1
	Glomus irregulare clone 14		FJ009618.1
	Uncultured *Glomus* isolate Glo8		EF041065-8.1

These sequences were compared to the most similar sequences and to sequences from other selected AM fungi taxa from GenBank in a distance analysis (Figure 9). The alignment shows that most sequences identified as AM fungi in this study belonged to the *Glomus* A subgroup based on Schüßler et al. (2001) classification. None corresponded to subgroup B. It also confirmed that ribotype B5 was more related to the *Gigasporaceae* family.

The detection frequency of specific sequence variants significantly differed ($P<0.05$) between soil and root samples within each year (Table XIV). However, the amount and identity of the ribotypes detected at a significantly higher frequency in each sample type changed between years. Only the B1 ribotype was found in similar proportion each year in both soil and root samples. The ribotypes B3 and B5 were detected in the root at a significantly higher frequency in 2004 and 2006, in association with the maize phase. The average number of ribotypes detected in maize was significantly higher than in soybean roots, but not in soil surrounding the roots (Table XV).

Figure 9. Distance tree depicting the relationship between the various ribotypes identified in this study and selected AM fungal sequences. Bootstrap values over 50% from 1000 replications are shown on the branches. Sequences obtained in the present study are given in bold (with identifications in Table XIII).

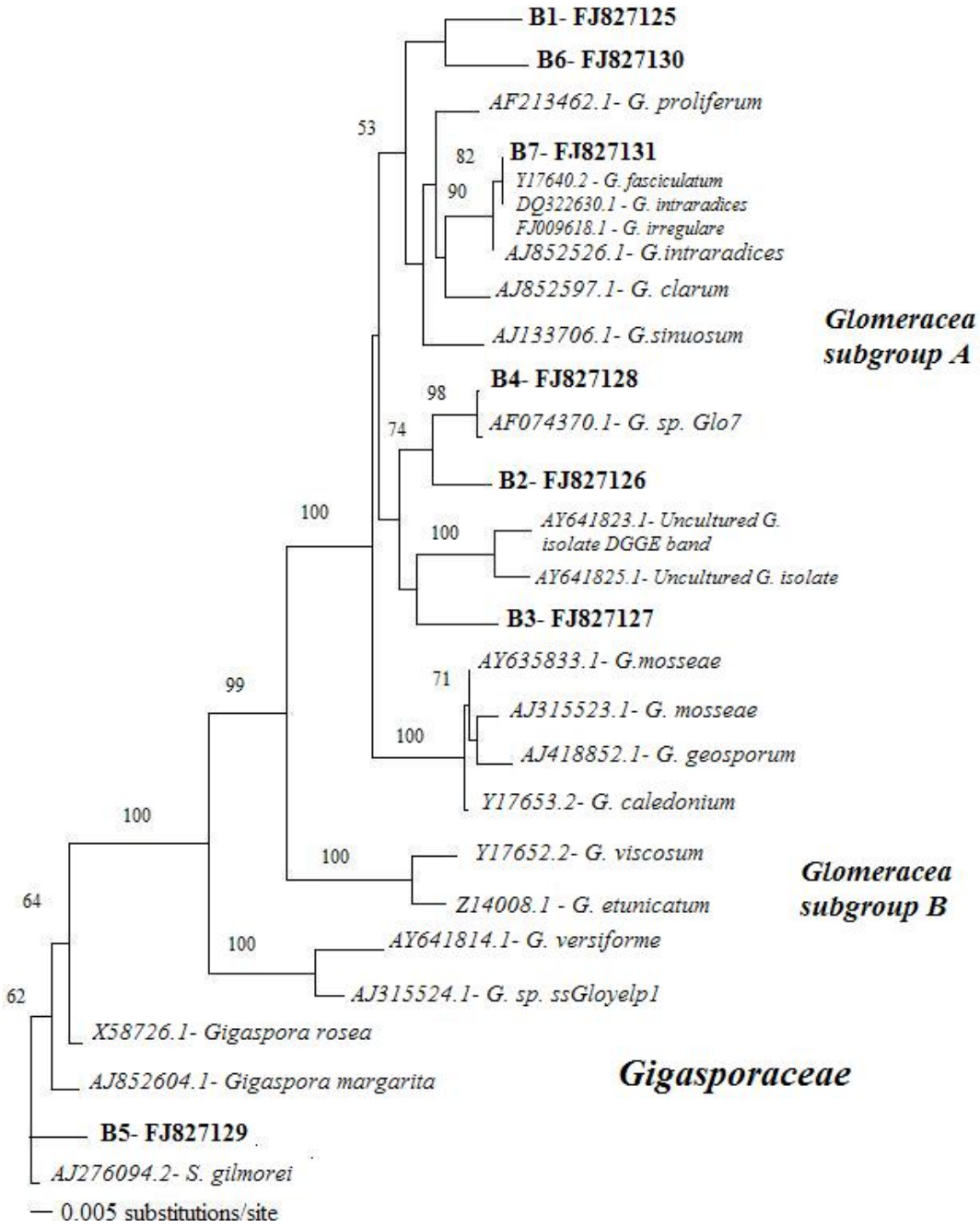


Table XIV. Relative abundance of the various ribotypes in root and soil samples of a maize/soybean rotation system.

Ribotypes	Relative abundance of ribotypes (%)					
	2004[1]		2005		2006[1]	
	roots	soil	roots	soil	roots	soil
B1	15	9	19	9	24	25
B2	24	42*	3	27*	24	25
B3	41*	15	19	35*	27*	16
B4	29	42	13	27*	47*	28
B5	44*	24	36	24	35*	19
B6	70*	36	36	35	53	47
B7	50*	27	55	71*	77	88

* indicates that the detection frequency is significantly ($P < 0.05$) higher in this type of sample at the specified growing season as measured by Qui-square test.

[1] indicates when maize was grown.

Effect of manure or mineral P fertilizer application

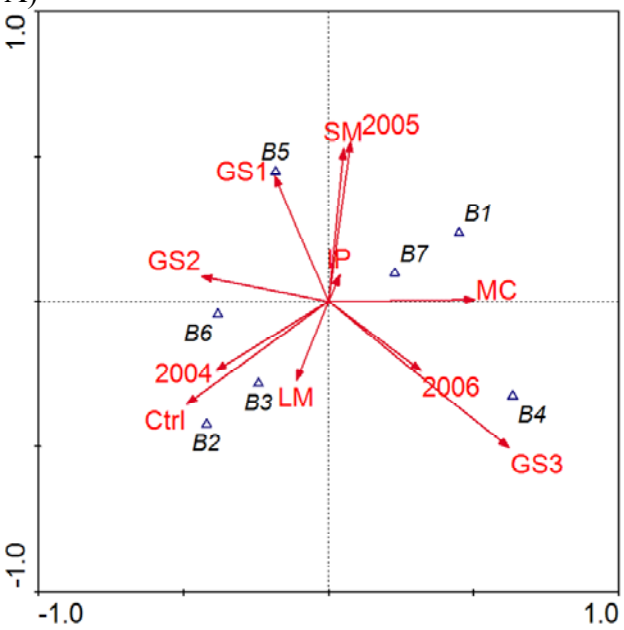
According to CCA (Figure 10), AM communities vary according to P fertilizer treatment, plant growth stage and year of sampling. There was a similar overall effect of these factors on AM fungi ribotypes distribution in samples. Communities found at GS1 and GS2 were, in both type of samples, more similar than ribotype assemblage detected at the end of growing season. Levels of the indicator for AMF storage lipids, NLFA 16:1w5 was higher in 2004. AM fungal biomass, as measured by PLFA 16:1ω5 biomarker remained unchanged within P fertilization treatments and plant growth stages. However, this variable was much greater on the third year of the experiment with a significant 6 x increase over the two previous years (Table XV).

A discriminant analysis revealed that the sequence assemblages were not significantly different between P fertilization treatment or sampling dates in root samples overall in all years of the study. Sampling at different plant growth stages in soil samples also led to similar community structure within each year. However, in 2006, soil samples treated

with inorganic P and the ones coming from the control plots presented significantly different AM fungal community structures (Figure 11).

Figure 10. Canonical correspondence analysis depicting the relationship between the detection of the various ribotypes (Δ), year of sampling (2004, 2005, 2006), plant growth stage (GS1, GS2, GS3) and fertilization treatment (ctrl= control; IP= inorganic P; MC= manure compost; SM= solid manure; LM= liquid manure). A) DNA extracted from root samples (*P*= 0.003), B) DNA extracted from soil samples (*P*= 0.007).

A)



B)

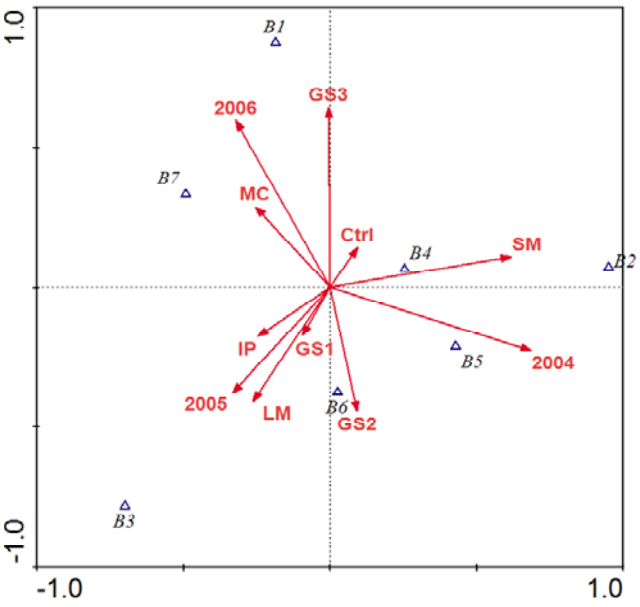


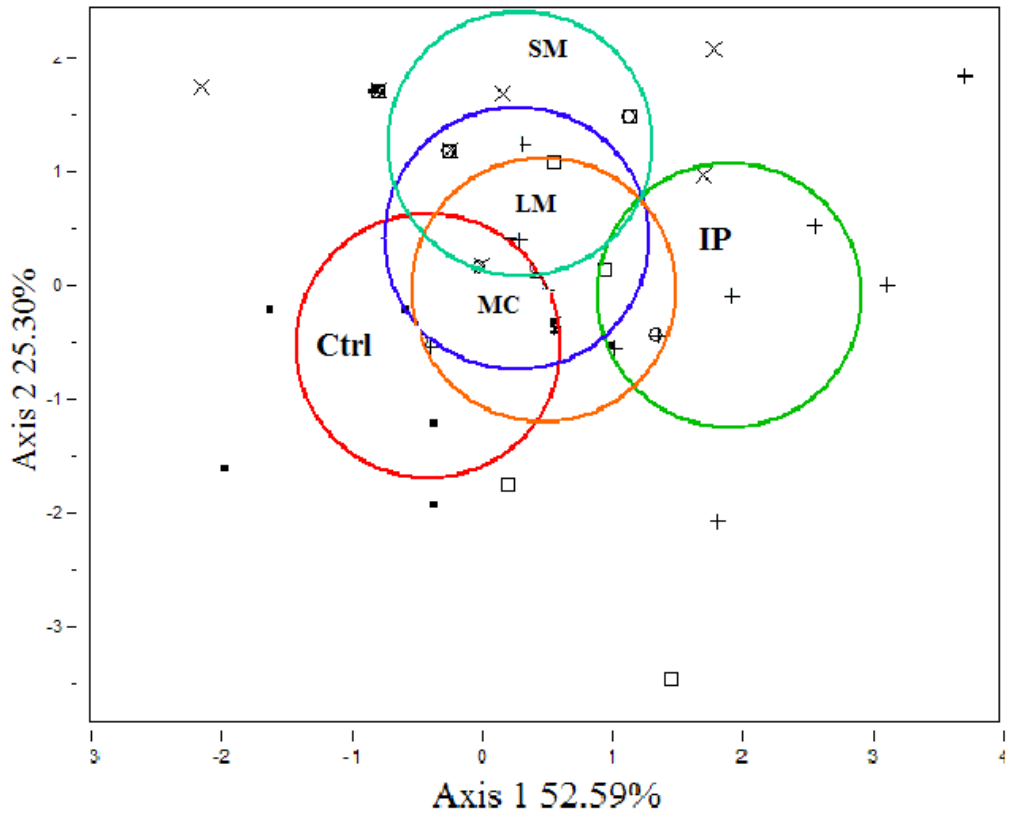
Table XV. Soil and plant parameters as well as average AM fungal number of ribotypes and yields of plots submitted to different fertilization treatments. Data are reported as averages for 27, 45 and 45 replicates for fertilization treatments, sampling time and year respectively.

	NLFA 16w5	PLFA 16w5	Ptase[1] (µg PNP/g/h)	% AMF col.	Soil Moisture (%)	Soil P (ppm)	Dase (µg TPU/g/day)	pN (%)	pP (%)	AEM-P 10^{-4} (µg/cm^2/day)	AEM-NO_3 (µg/cm^2/day)	# rib. roots	# rib. soil	Yield (t/ ha)
Fertilization														
Ctrl	6.67A[2]	6.71A	85.85A	32.73A	19.76A	0.19C	166.89AB	1.74A	0.16B	0.79B	0.013B	1.96A	1.74A	3.08C
IP	6.48A	4.14A	90.55A	31.68A	19.33A	0.74A	151.09B	2.00A	0.19AB	1.13B	0.031A	2.00A	1.52A	5.94A
MC	2.89A	5.73A	88.22A	32.64A	20.07A	1.00A	203.63A	1.75A	0.22A	9.39A	0.012B	1.92A	1.74A	3.88BC
SM	3.20A	3.10A	100.23A	28.60A	19.01A	0.89A	161.83B	2.02A	0.20AB	8.78A	0.027AB	1.70A	1.85A	5.15AB
LM	4.37A	5.22A	96.56A	39.85A	18.50A	0.43B	178.92AB	1.75A	0.16B	2.49B	0.019AB	1.51A	1.33A	5.99A
Sampling date														
GS1	1.64A	3.14A	82.88B	17.08B	15.58B	0.67A	165.22A	3.00A	0.32A	2.91B	0.03A	1.64A	1.49A	-
GS2	5.70A	20.13A	84.58B	36.22A	21.05A	0.67A	174.67A	1.77B	0.17B	7.52A	0.03A	2.00A	1.91A	-
GS3	6.11A	8.61A	111.10A	40.67A	21.79A	0.63A	180.87A	0.79C	3.1E-4C	3.13B	0.01B	1.87A	1.51A	-
Year of sampling														
2004	3.73A	2.27B	77.75B	30.88A	21.54A	1.08A	174.72A	1.46A	0.19A	8.20A	0.03A	2.10A	1.44A	5.48A
2005	1.75B	2.58B	85.25B	29.48A	19.33B	0.46B	158.43A	2.24B	0.18A	0.84B	0.01B	1.24B	1.71A	3.07B
2006	1.81B	11.89A	115.57A	33.61A	17.55B	0.42B	187.62A	1.01A	0.20A	-	-	2.16A	1.75A	5.86A

[1] Ptase= levels of acid phosphatase; % AMF col.= root colonization; soil P= soil available P analysed by resin extraction; Dase= levels of dehydrogenase; pN= plant N content; pP= plant P content; AEM= monitored using anion exchange membranes.

[2] Within lines, significant differences between fertilization treatments, sampling time and year are indicated by different letters and were determined by a Tukey's HSD test at the 5% level after a one-way ANOVA

Figure 11. Discriminant analysis of AM fungal distribution at Harrow in 2006 plots submitted to P fertilization treatments (■ = control; + = inorganic P (IP); □ = manure compost (MC); o = liquid manure (LM); x= solid manure (SM)). P = 0.03 and N = 45.



Fertilization treatments significantly influenced specific plant and soil parameters (Table XV). Plant yield was significantly increased with the use of inorganic P, solid manure and liquid manure. Manure compost led to yield similar to the control treatment. P concentration in the soil solution at sampling, measured with the resin-extraction technique, was the lowest in the control plots, intermediate with liquid manure and highest but similar in plots fertilized with inorganic P, manure compost and solid manure. Soil available P was significantly higher in 2004. P flux values in soil measured with AEM followed a similar trend, with lower values in control, inorganic P and liquid

manure treated-plots compared to manure compost and solid manure treated soils. There were also significant seasonal and annual variations, with higher values at mid-season and in 2004 compared to 2005 (2006 samples not analyzed).

N flux in soil as measured with AEM presented higher values when plots were treated with inorganic P. Measures collected at the last sampling date were significantly lower. Nutrient contents of both crops were also modified by fertilization treatments. N concentration was significantly higher in soybean plants, while the P concentration was similar between crops. Plant N content remained similar in all treatments but was significantly higher at the first sampling date and decreased significantly with time. Plant P content was significantly higher when manure compost, solid manure or inorganic P were applied, and lower with the liquid manure and control treatments. As with N content, the higher levels were monitored at the beginning of the season and decreased thereafter.

Variations in the levels of acid phosphatase were noted only according to time: they were higher at the end of the growing season and during the last year of the experiment.

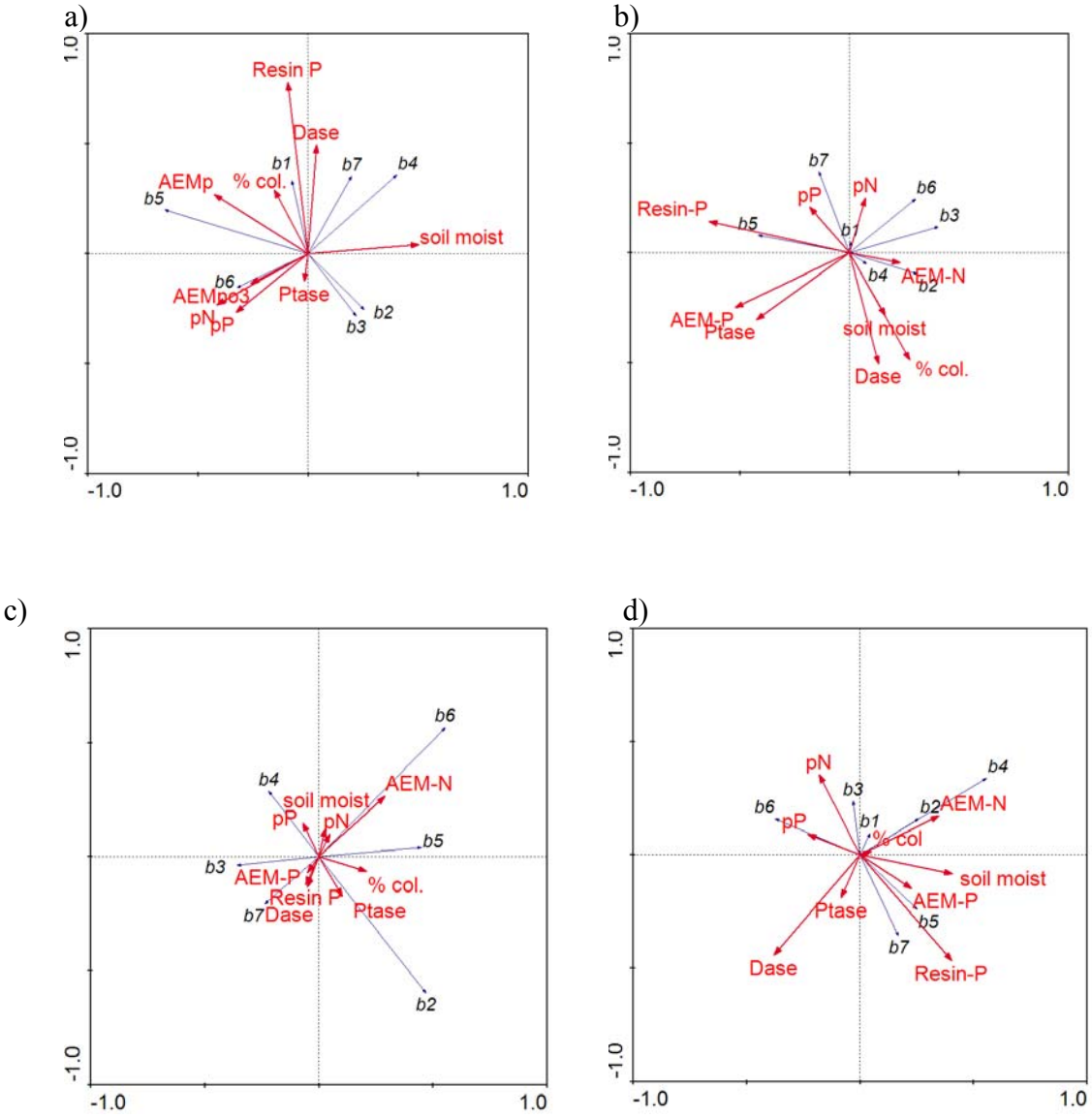
Higher microbial activity was found in plots treated with manure compost according to dehydrogenase activity. Mycorrhizal root colonization extent and soil moisture were not significantly different between fertilization treatments. However, both were significantly lower at the beginning of the season.

Relationship between environmental variables and AM fungal community structure

RDA showed that soil soluble P had the strongest correlation with AM fungal ribotypes distribution in maize and soybean root samples, as well as in soybean soil samples (Fig.

12). The strength of other factors varied from soil to root samples or between the years. Moreover, correlations between specific ribotypes and environmental parameters showed important variations between years and between root or soil samples. Only the negative correlation between ribotypes B3 and B5 was consistent in both years and sample types. The model results however showed that the chosen environmental variables were weak predictors of soil AM fungal community composition, as measured by DGGE analysis, with only 10 % in 2004 and 12 % in 2005 of the community variance explained on the first two canonical axes and a marginal significance of the model only in 2004 (P= 0.05).

Figure 12. RDA biplot representing the correlations between AMF ribotypes and different parameters related to soil fertility and soil P levels. Ribotypes are identified by the letter b and numbers indicating their migration position of gel. Other parameters are coded as: Dase= dehydrogenase; Ptase= acid phosphatase; Resin P = soluble P measured by resin extraction; AEMp = P flux measured by anion exchange membrane; AEM-no3 = NO_3 flux measured by anion exchange membrane; pN= plant N content; pP= plant P content; soil moist= soil moisture and % col.= percentage of root colonization by AMF. Evaluation conducted on a)maize roots 2004 (P= 0.06), b)soybean roots 2005 (P= 0.4), c)maize soil 2004 (P= 0.05), d)soybean soil 2005 (P= 0.4).



Discussion

The effect of three different organic and two mineral P fertilizations on AM fungi ribotypes assemblages was evaluated in roots and the surrounding soil in a maize/soybean rotation system at three different growth stages, over three consecutive years. The results demonstrated that AM fungal communities remained stable. While the whole community did not show large variations, the frequency of specific ribotypes however varied between the type of samples (soil or roots) and with the host plant.

Despite an important variability in the ribotypes identity between years, significant associations between some of them and root or soil samples have been found in this research on specific years. There are few studies conducted on the overlap of AM fungal species in soil and roots. It was recently showed that AM fungi forming mycelium in soil differed markedly than those found in roots in a grassland ecosystem, with significant differences in AM fungal families and species groups between sample types (Hempel et al. 2007). Johnson et al. (2003) postulated that roots only recruit a fraction of the AM fungal taxa pool present as spores in soils. As the grown plant and environmental conditions varied each year in the present study, one might have expected a large influence on selected ribotypes. On the contrary, the composition of AM fungi ribotypes was quite stable seasonally and over the three years despite the crop rotation occurring every year. This observation is enforced by the fact that the dominance of specific ribotypes in root or soil is inconsistent between years as only two ribotypes have been more frequently associated to maize roots. Literature present conflicting results about the impact of host plant on AM fungi community composition. While, many studies tend to demonstrate that different host plant species are colonized by different

AM fungi communities (Scheublin et al. 2004; Vandenkoornhuyse et al. 2003), a lack of host specificity was reported by others (Öpik et al. 2003; Santos et al. 2006). In this study, the ribotype assemblage has not shown similar reactions to treatments on both years when corn was grown. This suggests that the nature of the plant alone was not the predominant key factor responsible for the AM fungi selection. Results analyzed here announced that the combination of multiple environmental factors probably have more impact.

The variation in the presence (or absence) of each ribotype across and within growing seasons supports the idea that soil is a dynamic medium. It demonstrated the fact that environmental factors have impacts on soil microbial diversity. The evaluation of soil microorganisms previously conducted on this experimental field confirmed that the evolution of each growing season globally influences the communities of bacteria and fungi (see chap. IV). It seems that this can also be observed for groups of organisms counting as few members as AM fungi does.

The addition of different forms of organic fertilizer brought various amounts of nutrients to soil. Applying organic fertilizers is often related to important increases in bacterial population size and diversity (Farrell et al. 2010; Liu et al. 2008b). It has also been demonstrated that the global fungal community appears to be more diversified when manure is applied to soil (He et al. 2008). Though there was no changes observed in the taxonomic richness of those communities in the studied soil, fungal biomass was increased following solid manure treatment (see Table XI). Such variation in biomass was not reflected in the AM fungal sub-group and there was no modification in the AM fungal composition following the application of different forms of fertilizers either. It is possible that soil nutrient status provided by this agroecosystem created a low level of

stress on host plants, therefore avoiding species presenting differences in their ability to acquire nutrients to be selected.

Organic fertilizers contain inorganic P (Pi) as well as organic P (Po). The Po in these materials is gradually mineralized and released as Pi, which may explain the higher P flux recorded when manure compost and solid manure were applied (Herath et al. 2007). Changes in P-related soil parameters brought by different P fertilizations reflected the fact that these fertilizers provided different forms of P to the soil. However, fertilization treatments were also expected to create variations in other soil parameters as there were several differences in their nutrient content. Manure compost, which brings much organic matter to soil, was the treatment that had the strongest impact on soil P levels as it significantly increased soluble P as well as P flux compared to the control treatment. It also increased P content in plants when compared to control. Literature reports that mineral fertilization usually lead to greater P uptake by corn and soybean (Puno 1991). This assertion was not supported here as IP treatment lead to similar plant P content as liquid manure and solid manure application.

Substantial amounts of Po are released from organic matter by acid phosphatase activity, which is an extra-cellular enzyme responsible for the hydrolyzing of Po and has often been noticed to increase by organic fertilization (Lee et al. 2004). However in this study, changes in the soluble P concentration were not related to variations in the acid phosphatase levels. Conflicting results from previous studies demonstrated that the link between acid phosphatase and the kind of fertilizer applied is complex (Clarholm 1993; Lopez-Hernandez et al. 2004; Wang et al. 2008). The increase in acid phosphatase levels related to the evolution of the growing season might be explained by the fact that phosphatase enzymes are produced when P is the most growth-limiting element (Spiers

and McGill 1978). In this experiment, fertilization was applied at the beginning of the season when higher amounts of available P are present. An increase in levels of phosphatase is also suggesting an increased need of P.

The increase in root colonization with time is typical of that seen after initial exposure to mycorrhizae (Henry and Kosola 1999). This reflects the fact that the studied crops were seeded at the beginning of the season and were newly colonized each spring. Also, host's nutrient needs vary within growing season. Mycorrhizal fungi are well known to play a key role in nutrient uptake and therefore the root colonization is usually stimulated at specific time, depending on P availability (Grant et al. 2005; Nogueira and Nogueira Cardoso 2007). The type of fertilization applied to soil had no impact on mycorrhizal root colonization nor did the dose of fertilizer, since plants receiving single and double doses of fertilizer showed similar levels of colonization. Soil AM fungal biomass was also similar between fertilization treatments during the three years of the experiment. This contrasts with the generally-accepted belief that fertilizers reduce mycorrhizal growth and colonization. Such results have already been reported in clover though (Joner 2000). Root colonization levels found in this study were lower that what is usually observed in field studies conducted on maize and soybean (Carrenho et al. 2001; Nogueira and Nogueira Cardoso 2003; Oliveira et al. 2009). The field plots studied in our experiment were located on a site used for several years to study land development and cropping management strategies. Previous experiment conducted on that land have however lead to an initial low assimilable P content, which even with P application may not get high enough to turn on the mechanisms responsible for spore germination causing reduced mycorrhizal colonization upon P fertilization (Joner 2000; Thomson et al. 1991).

The low diversity of ribotypes that was detected in this field supports the findings of previous studies that suggested low richness of AMF in highly productive agricultural soils (Bedini et al. 2007; Cousins et al. 2003). Our field was tilled, fertilized and treated with glyphosate herbicide. The immediate stress caused by these management practices used to intensify productivity in broadacre crops surely pressures microbial populations. The effects are various on the different microbial communities but have often been shown to lower diversity of AM fungal strains colonizing different hosts (Beauregard et al. 2008).

Because of the considerable genetic diversity within morphologically recognizable species, there is still confusion about what constitutes an AMF species or individual (Kuhn et al. 2001; Rosendahl 2008; Stukenbrock and Rosendahl 2005). This study provides information about the presence of different 18S sequence variants within root and soil samples from plots fertilized with P from various organic or mineral forms. In a field with annual crops, new mycorrhizal networks are forming each year from germinating spores or from vesicles in dead roots from the previous growth season. It is thought that the germinating hyphae will anastomose with compatible hyphae of other mycelium and form a common mycelial network, which would favoured the dominant genotypes (Rosendahl 2008). It is not possible to know the proportion of DNA that was extracted from resting spores or active mycelia. Variations in sequence variant assemblages would support the idea that in a heterogeneous environment, variance in local conditions leads to the evolution of niche-adapted fungal genotypes (Goddard et al. 2005). However, our results did not support such a selection of specific ribotypes. This may be explained by the fact that during the growing seasons studied, soil conditions remained quite stable or simply because the variation in nutrient availability as measured

in this experiment has not as much influence on AM fungi taxa selection as other factors.

The composition of the AMF community in our study is dominated by taxa belonging to the genus *Glomus*, more specifically belonging to the *Glomus* A subgroup based on Schüßler et al. (2001) classification. Identification of five of the seven sequence variants recovered as close matches with 'uncultured *Glomus*' supports the idea that molecular approach to AM fungal community ecology will reveal an important unknown diversity (Liang et al. 2008; Rosendahl and Stukenbrock 2004). The most frequent ribotype in both root and soil samples was the one corresponding equally to one strain of *Glomus fasciculatum, G. intraradices* and recently identified *G. irregulare* (Blaszkowski et al. unpublished data). Considering slightly lower scores in our search, it was noted that all Genbank sequences identified as *G. fasciculatum* 18S were closer match to ribotype B7 that those identified as other strains of *G. intraradices*. *G. fasciculatum* has not been identified as prevalent in any other recent studies conducted on Canadian agricultural soils, but has been described in 1987 as 'perhaps the most commonly reported AM fungus from soil surveys' (Walker and Koske 1987). The fact that this ribotype was so often detected in samples was indeed unexpected. Other studies have demonstrated that agricultural soils are colonized mostly by *Glomus* species (Mathimaran et al. 2005; Oehl et al. 2004; Sjöberg et al. 2004) and this species has in fact been identified in various types of agricultural soils (Johnson et al. 1991; Öpik et al. 2006), but not often with this level of dominance (Allen et al. 1981; Porras-Alfaro et al. 2007). However, even though more literature has been published using *G. fasciculatum* as the experimental organism than any other arbuscular fungal species (496 reports according to INVAM website database in 2008), yet all cultures accessed in INVAM labelled as *G. fasciculatum*

proved to group with the reference culture of *G. intraradices,* which appears to be ubiquitous (INVAM 2008). As all the identifications in this study were only based on molecular approaches, there was no morphological character that could be used to confirm the identity of the ribotype. It is questionable though that all Genbank reference sequences identified as *G. fasciculatum* came from good identification at the first place.

No taxa belonging to *Glomus* B subgroup were observed in our study. This could be caused by its absence from our samples; however, the AM1 primer have shown mismatches to single priming sites in at least some taxa belonging to this subgroup (Husband et al. 2002). More, it was only rarely represented in studies using the AM1 primer (Santos et al. 2006; Uhlmann et al. 2004). No sequences from AM taxa belonging to the families *Paraglomaceae* or *Archaeosporaceae* were detected either, as the AM1 primer is known for not amplifying sequences from these two families (Redecker et al. 2000).

Although no significant differences in AM fungal biomass and in the number of ribotypes detected were recorded in plots submitted to different P fertilization treatments or sampled at different plant growth stages, it does not mean that the importance of one or a few ribotypes did not vary. DGGE provides information about the presence/absence of dominating ribotypes, while PLFA shows the variation in the whole AM fungi community size. So it is probable that variations in the representation of each ribotype in the global community occurred seasonally, annually or in response to the fertilization treatments but were missed. Methods measuring changes in the amounts of DNA belonging to a single ribotype will need to be used to solve this problem.

In conclusion, it was possible to observe that the various forms of P added to soil in this study did not impact soil conditions enough to have a measurable influence on AM

fungi ribotypes detection frequency. It was also clear that the use of P fertilizers, whether under organic or mineral forms, did not lead to negative effects on both AM fungal biomass and ribotypes richness.

Acknowledgements

The authors are grateful to Stéphane Daigle for help with statistical analyses, Keith G. Hanson and Mary-Anne Reeb for help with the soil and plant variables data; Brian Hohner, George Stasko, Karl Rinas, and farm crew at the GPCRC for field plots establishment, management and samplings. This work was supported by an Agriculture and Agri-Food Canada GAPS team grant allocated to C. Hamel and collaborators and by a NSERC discovery grant to M. St-Arnaud.

Chapitre VI

Discussion générale et conclusion

Cette thèse s'est penchée sur l'influence de la dose ainsi que de la forme de fertilisants phosphatés sur la diversité microbienne de sols en monoculture de luzerne et en rotation maïs-soja. Les résultats obtenus lors d'études antérieures menées sur la microbiologie du sol indiquaient que des rapports complexes régissaient le comportement de plusieurs populations microbiennes différentes face à divers apports en nutriments. Cependant, il n'avait pas été clairement démontré comment l'apport en P pouvait influencer la fertilité d'un sol en incluant l'ensemble de sa flore microbienne. À notre connaissance, s'agit donc de la première étude ayant évalué les impacts de l'apport en P sur la biodiversité microbienne totale et, parallèlement, sur divers paramètres liés à la fertilité des sols agricoles. Le nombre d'échantillons étudiés lors de ces expériences est également beaucoup plus élevé que celui d'études utilisées à titre de comparaison.

Au cours de la présente étude, il a été montré qu'à la fois la quantité et la forme sous laquelle est appliquée le P a un impact sur les structures bactérienne et fongique du sol mesurées par PCR-DGGE. La dynamique de fertilité des différents sols étudiés, telle que mesurée par une dizaine de paramètres, a aussi été influencée par l'apport en P. De plus, l'évolution de la saison de culture s'est également avérée avoir une grande influence sur la structure des communautés de microorganismes de même que sur les niveaux des différentes variables du sol qui ont été mesurées.

VI.I Limite des méthodes utilisées pour analyser la structure des communautés microbiennes du sol et alternatives possibles.

Il existe plusieurs méthodes de biologie moléculaire pouvant mesurer la diversité microbienne. Chacune de ces méthodes comporte une série d'avantages et inconvénients

pour l'étude des milieux complexes comme le sol. L'approche d'identification par PCR-DGGE des microorganismes du sol est de plus en plus utilisée (Ma et al. 2005; Smalla et al. 2007; Yergeau et al. 2005). Cette méthode présente de grands avantages notamment à cause du nombre élevé d'échantillons pouvant être traités en même temps et de la reproductibilité des patrons de bandes obtenus sur gel. Cependant, son utilisation lors d'expérience étudiant de très larges communautés microbiennes est discutable.

La faible diversité de ribotypes mesurée lors de cette étude suggère que le DGGE pourrait être utile en tant que première étape de discrimination entre les groupes de larges communautés. La combinaison de la technique de séparation des amplicons (le DGGE) avec le choix des amorces s'est avérée, dans cette étude, insuffisamment discriminante pour déceler des variations à une fine échelle taxonomique.

En effet, l'observation de communautés regroupant un nombre d'espèce particulièrement important requiert l'utilisation d'amorces universelles pour l'amplification PCR. En admettant que ces paires d'amorces soient totalement spécifiques au groupe recherché, elles visent souvent des séquences d'ADN très similaires, voire identiques, entre plusieurs espèces et/ou genre microbiens ce qui fait que ces organismes ne peuvent être spécifiquement identifiés par une bande unique sur le gel de DGGE. Comme les fragments d'ADN bactérien et fongique ciblés dans cette étude étaient très courts (moins de 500 pb) pour permettre une séparation claire sur DGGE, des séquences identiques provenant de différentes espèces proches ont probablement été amplifiées et ont produits seulement une bande sur gel, contribuant ainsi à une sous-estimation de la diversité microbienne réelle des échantillons.

La migration identique de fragments différents d'ADN amplifié est un autre problème qui a déjà été décelé suite à l'utilisation du DGGE (Rolleke et al. 1999). Ici, les

fragments d'ADN de plusieurs bandes ont été excisés, réamplifiés et soumis au séquençage avec un résultat montrant plus d'une séquences qui semblaient se superposer à la lecture du chromatogramme. Évidemment, ceci complique l'interprétation des patrons de bandes et le séquençage direct. Dans ce cas, une étape de clonage précédent celle du séquençage pourrait démontrer la présence de plusieurs taxons migrant à la même position. Les profils des communautés microbiennes globales du sol obtenus par la technique de DGGE seraient donc plus adéquats afin d'observer les changements majeurs.

Pour augmenter la résolution taxonomique sans passer par la méthode de clonage, il serait toujours possible d'utiliser le DGGE mais en ciblant de plus petits groupes fonctionnels pour lesquels il existe des amorces visant des fragments d'ADN moins généraux (Green et al. 2004; Yergeau et al. 2005). Des approches métagénomiques (*metagenomic studies*) sont maintenant de plus en plus courantes lors de l'étude de la diversité microbienne d'échantillons provenant de milieux complexes. Comme leur nom l'indique, elles permettent d'analyser une collection de séquences génétiques. Ces méthodes ne sont, pour l'instant, pas envisageables pour détecter les populations les moins dominantes du sol en raison du nombre trop imposant de séquençage que cela demanderait. Par contre, elles permettent aujourd'hui de produire un profil réaliste de la diversité microbienne d'échantillons provenant de milieux environnementaux (Hugenholtz and Tyson 2008).

La méthode d'analyse des profils de PLFA/NLFA est un bon choix d'approche d'évaluation des communautés microbiennes complémentaire au DGGE. Cette méthode biochimique est rapide et permet, contrairement au DGGE, de comparer de façon quantitative certaines communautés. Les phospholipides sont des éléments retrouvés

seulement dans les cellules vivantes et permettent donc une mesure relativement fiable de la biomasse (Zelles 1999). Par contre, l'identification taxonomique à l'aide des acides gras est délicate et limitée. Parfois les acides gras reconnus pour être davantage associés à un groupe d'organismes peuvent être retrouvés en petites quantités chez d'autres organismes totalement différents et venir compliquer l'interprétation de données issues de milieux environnementaux aussi complexes que le sol (Bååth 2003; Frostegård and Bååth 1996). La technique restreint donc l'observation des variations de la biomasse à certains groupes d'organismes particuliers pour lesquels les lipides membranaires doivent être le plus spécifique possible.

VI.II La fertilisation minérale, appliquée pendant plusieurs années, a un impact sur la diversité bactérienne et fongique d'une culture de luzerne.

La disponibilité des nutriments essentiels tels que le C, N et P est reconnue pour avoir de l'impact sur les taux de croissance et l'activité microbienne (Broeckling et al. 2008; da Silva and Nahas 2002). Dans le même ordre d'idée, la fertilité d'un sol, elle-même grandement influencée par la teneur en nutriments, contribue à faire varier la diversité, la biomasse et l'activité microbienne (Fox and MacDonald 2003; Liu et al. 2008a; Ryan and Graham 2002; Wei et al. 2008).

Les applications répétées de P minéral comme dans le cas étudié (chapitre III), provoquent généralement l'augmentation du niveau de saturation en P du sol de même que de sa fertilité phosphorique. À long terme, de telles modifications peuvent induire des changements dans la qualité et le fonctionnement des sols (Acton and Gregorich 1995) en causant d'importantes variations dans leur composition en nutriments

essentiels (Stevenson and Cole 1999). Lors de cette étude, il a en effet été observé que la fertilisation en P minéral a permis de hausser significativement les flux de P et le P soluble du sol mais aussi de diminuer le degré d'activité microbienne tel que mesuré par le niveau d'activité de la déhydrogénase. Des modifications au niveau de certains biomarqueurs PLFA permettent de détecter des changements soit dans les biomasses bactérienne, fongique ou mycorhizienne totales soit dans la communauté bactérienne. Cette méthode rend donc possible l'observation de modifications structurelles à une large échelle taxonomique. Par contre, l'absence d'effet au niveau des biomarqueurs PLFA dans cette étude pourrait indiquer qu'une substantielle augmentation (huit à douze fois) de la disponibilité en P dans le sol n'a pas influencé la taille de la population microbienne ou qu'un changement chez un taxon en particulier n'a pas fait varier la taille de la communauté. Cette hypothèse est supportée par les changements enregistrés chez les populations bactériennes et fongiques à une plus fine échelle taxonomique par l'analyse en PCR-DGGE.

En effet, bien que les changements induits par l'apport en P minéral n'aient pas modifié significativement le rendement de la luzerne ni la richesse taxonomique des communautés bactérienne et fongiques, ils furent tout de même associés à des variations dans la composition de ces dernières. Les taxons dominants les diverses communautés microbiennes dans les parcelles ayant un différent historique de fertilisation en P étaient différents et certains ribotypes pouvaient être associés à un taux de P en particulier. Certains ribotypes étaient identifiés principalement dans les sols fertilisés, suggérant des conditions favorables à leur développement dans ces parcelles. Par contre, la variation des niveaux de P créée par l'historique de fertilisation n'a pas influencé la distribution des ribotypes détectés de CMA.

VI.III Différentes formes de fertilisation phosphatée influence la diversité bactérienne et fongique dans une rotation maïs-soja.

Au fil du temps, la fertilisation en P (aussi bien minérale qu'organique) est devenue une pratique agricole répandue afin d'optimiser les rendements. D'abord utilisée dans le but d'augmenter la quantité de P disponible pour la plante et donc de hausser la productivité de la culture, la fertilisation joue directement sur la dynamique des sols en culture intensive. Il a été rapporté que les différentes pratiques de gestion des sols pouvaient favoriser certains microorganismes par rapport à d'autres et ainsi changer l'équilibre microbien initialement en place (Ge et al. 2008; Johnson 1993). Les interactions entre les différents groupes de microorganismes du sol sont primordiales et plusieurs études ont été menées récemment afin d'acquérir une meilleure connaissance des structures et fonctions de ces populations (Schmalenberger and Tebbe 2003; Smalla et al. 2007; Yergeau et al. 2005). Comme la plupart de ces recherches ont ciblé des populations microbiennes de petite taille, les facteurs influençant l'ensemble de la biodiversité microbienne d'un sol sont encore très peu connus.

Les fertilisants utilisés dans cette expérience (chapitre IV) étant de natures variées, ils contenaient différentes quantités d'éléments nutritifs essentiels susceptibles d'avoir un impact sur la dynamique du sol. Effectivement, la composition des fertilisants minéraux qui proviennent de fabrication industrielle est relativement simple comparativement à celle des engrais organiques qui sont plutôt un mélange très hétérogène d'excréments et de litière, et leurs propriétés sont extrêmement dépendantes de l'âge des animaux, de la nourriture et des conditions d'entreposage (Kirchgessner et al. 1991).

La présente étude a d'ailleurs démontré que dans ce cas-ci, la composition des échantillons en terme de structure des communautés de bactéries et de champignons était influencée par les traitements de fertilisation. Parallèlement, les différentes formes de fertilisants ajoutées au sol ont eu un impact sur l'activité microbienne et la biomasse de certains groupes microbiens dont les champignons et les bactéries Gram positives. Par contre, la richesse taxonomique bactérienne et fongique est demeurée stable entre les échantillons de sol soumis à différents traitements de fertilisation. L'apport de nutriments en quantités variables contenus dans les différents fertilisants n'a pas induit de différence significative dans les niveaux de phosphatase, une enzyme liée à l'hydrolyse du P organique dans le sol.

VI.IV L'application de différentes formes organiques et inorganiques de fertilisants phosphatés ne diminue pas la biomasse et affecte très peu la structure de la communauté de CMA d'une rotation maïs-soja.

Comme les CMA se développent en symbiose avec une plante hôte, ils dépendent totalement des apports en C provenant de la photosynthèse. Ils sont donc susceptibles d'être influencés par la fertilisation visant à modifier le rythme de croissance et la productivité des végétaux. En fait, il existe plusieurs études démontrant la présence ou l'absence d'effet du P tant sur leur diversité, leur croissance que sur leur capacité de coloniser les racines d'un hôte (Bhadalung et al. 2005; Grant et al. 2005; Nogueira and Nogueira Cardoso 2007). Par contre, la vaste majorité de ces études se basaient uniquement soit sur l'identification des spores extraites du sol soit sur l'intensité de colonisation des racines mycorhizées.

Notre approche a permis de comparer la diversité mycorhizienne d'échantillons de racines et de sol et de démontrer que ce type de communauté fongique demeurait stable malgré les différents types de fertilisation appliqués. Bien que l'ensemble de la communauté n'ait pas connu d'importants changements, la fréquence de détection de certains ribotypes variait selon le type d'échantillon étudié mais également selon la plante hôte. La biomasse mycorhizienne n'a, elle non plus, pas présenté de variation significative entre les différents types de traitements en P; ce qui contraste avec la croyance voulant que la fertilisation réduise la croissance mycorhizienne de même que la colonisation. Justement, la colonisation racinaire n'a pas été significativement modifiée ni par le type ni par la dose de fertilisant (Carrenho et al. 2001; Nogueira and Nogueira Cardoso 2003; Oliveira et al. 2009).

Comme les champs où ont été installé les parcelles se trouvent sur un site où le sol est étudié depuis de nombreuses années pour le développement de stratégies de gestion agricoles, il est possible que l'état nutritionnel de ce système ait créé un bas niveau de stress sur les plantes hôtes et donc empêché la sélection d'espèces présentant des différences dans leurs habilités à acquérir des nutriments. D'ailleurs, d'autres expériences menées sur ce site ont entraîné un niveau initial de P assimilable très peu élevé. Même avec la fertilisation, il est possible que ce niveau soit toujours trop bas pour enclencher les mécanismes nécessaires à la colonisation (Joner 2000).

VI.V La structure de la communauté microbienne varie considérablement avec l'évolution de la saison de culture dans les systèmes agricoles étudiés.

Lors de cette étude, il a été démontré que la communauté microbienne du sol était grandement influencée par l'évolution de la saison au sein des deux systèmes agricoles étudiés. En fait, la date de l'échantillonnage représentant différents stades de croissance de la plante cultivée au moment de l'expérience avait généralement plus d'impact que le traitement de fertilisation sur la fréquence de détection des ribotypes des groupes de microorganismes étudiés. Des variations saisonnières significatives, mais différentes d'un système à l'autre, ont par ailleurs été mesurées en ce qui concerne les niveaux des différents paramètres du sol mesurés. Par exemple, lors de l'application de différentes doses de P (chap. III), l'avancement de la saison de culture a entraîné des différences significatives dans les quantités de P soluble du sol, de l'activité microbienne et de la biomasse de certains groupes microbiens tandis qu'après l'application de différents types de fertilisant (chap. IV), ce sont plutôt les niveaux de phosphatase et de flux de P et $N0_3$ qui ont connu une variation.

Comme la composition des fertilisants utilisés au cours de l'expérience menée sur la rotation maïs-soja (chapitres IV et V) était différente, la vitesse à laquelle leur contenu étaient assimilés par les plantes et transformés par les microorganismes pouvait varier. En effet, la proportion des formes de P présentes dans les engrais organiques varie selon le type d'engrais. Cela signifie que leur composition chimique peut affecter leur potentiel à apporter du P disponible rapidement (Zhongqi et al. 2004). Le temps nécessaire à l'assimilation et la transformation des différents éléments nutritifs par les plantes et les microorganismes peut donc entraîner des modifications de la teneur en

nutriments sur une certaine période de temps. Par conséquent, il est normal que les effets immédiats et à long terme sur les flux de N et P ou les quantités d'enzymes retrouvées dans le sol suite à différents types de fertilisation aient variés. Il est intéressant de noter que l'apport de différentes doses de matières organiques a provoqué des changements assez importants dans le sol pour mener à des variations dans la structure des communautés microbiennes. Par contre, comme aucune interaction n'a été notée entre le traitement et la date d'échantillonnage, il semble que la nature des fertilisants n'ait pas modifié suffisamment la composition du sol pour avoir un impact sur les effets possibles de l'avancement de la saison de croissance sur les différents paramètres du sol mesurés dans le cas étudié ici.

Il va de soi que le sol est constamment soumis à plusieurs facteurs extérieurs susceptibles d'influencer l'ensemble de sa dynamique. L'effet saisonnier sur la communauté microbienne du sol a donc souvent été rapporté dans la littérature (Dunfield and Germida 2003; Hamel et al. 2006a; Houlden et al. 2008) et bien que le contenu en P du sol puisse directement influencer les microorganismes, il est également possible que l'effet observé chez les communautés microbiennes soit attribuable aux changements dans le métabolisme de la plante cultivée. Plusieurs études ont démontré l'effet des exsudats libérés par les racines des plantes sur certains changements dans les communautés microbiennes du sol (Bais et al. 2006; Baudoin et al. 2003). Ces exsudats représentent une importante source de C pour les microorganismes et sont, entre autre, influencés par le statut en P, l'état de santé et le stade de croissance de la plante qui évoluent évidemment au cours de la saison de culture (Balser et al. 2005; Singh and Pandey 2003).

En considérant qu'il y a eu deux coupes de luzerne au cours de la saison étudiée en Saskatchewan (chapitre III) et que l'élimination de la partie aérienne de la plante a un fort impact sur la physiologie de la plante et sur son exsudation racinaire (O'Leary 1965; Tracy and Frank 1998), des changements saisonniers au niveau de la composition taxonomique des communautés ne sont pas surprenants. Par contre le nombre de taxons microbiens détectés est resté similaire. Cette stabilité dans la richesse taxonomique a permis de conclure que des conditions climatiques chaudes et sèches correspondant à un milieu semi-aride n'ont pas d'impact négatif sur la diversité des communautés étudiées. En effet, l'expérience menée en Ontario, un milieu soumis à des conditions beaucoup moins arides que les prairies, révélait une richesse taxonomique très similaire. Les variations dans les différentes communautés apparaissent plutôt comme une adaptation de certains ribotypes à des conditions environnementales particulières. Chez les CMA plus spécifiquement, la stabilité de la richesse taxonomique suggère que les conditions climatiques particulières n'affectent pas négativement la diversité de la communauté probablement parce que les organismes sont davantage adaptés à leur environnement. C'est cette adaptation qui assurerait l'efficacité des CMA présent dans cet environnement pendant toute la saison.

La réduction observée dans la biomasse fongique et bactérienne après une période chaude et sèche renforce l'hypothèse voulant que la sécheresse du climat soit associée à une diffusion réduite des nutriments dans le sol et à une demande accrue en C et N. Les ressources nutritionnelles seraient d'abord utilisées par les plantes pour leur croissance. Dans une telle situation, un plus haut niveau de déhydrogénase pourrait indiquer que les microorganismes du sol dédient plus d'énergie à leur maintient qu'à leur croissance (Killham 1985).

VI.VI Conclusion

En résumé, il a été démontré que la dose de fertilisant phosphaté de même que la forme sous laquelle il est appliqué affectent les communautés bactériennes et fongiques globales du sol dans les systèmes agricoles étudiés, mais très peu la communauté des CMA. Ces traitements favorisent les modifications au niveau de l'environnement des organismes ce qui, de toute évidence, entraîne des changements (d'ampleurs variables) dans la distribution, l'activité et/ou la biomasse des différentes communautés. Cependant, malgré l'importance de la fertilisation, l'avancement de la saison de culture entraîne davantage de changements au niveau de ces paramètres.

Comme la communauté microbienne globale du sol est sensible aux différents apports en P, il serait maintenant intéressant de se pencher sur l'impact de ces traitements sur de plus petits groupes fonctionnels. Dans ce cas-ci, l'utilisation du DGGE serait plus appropriée puisque ces groupes contiennent un nombre plus restreint d'organismes. L'effet de la fertilisation pourrait par exemple être étudié sur des groupes de champignons pathogènes des plantes alors en culture afin d'observer si la fertilisation affecte leur présence dans la rhizosphère. Les organismes reconnus comme bénéfiques à la croissance des plantes pourraient aussi être étudiés. Dans le futur, la gestion de la fertilisation qui prendrait en compte l'effet des doses et de la nature des fertilisants sur ces communautés pourrait bien s'avérer une pratique qui permettrait de réduire l'impact de l'agriculture sur l'environnement.

Références

Abdalla, M. E. and G. M. Abdel-Fattah (2000). "Influence of the endomycorrhizal fungus *Glomus mosseae* on the development of peanut pod rot disease in Egypt." Mycorrhiza **10**: 29-35.

Acton, D. F. and L. J. Gregorich (1995). La santé de nos sols: vers une agriculture durable au Canada. Centre de recherches sur les terres et les ressources biologiques. Ottawa, Agriculture et Agroalimentaire Canada.

Agriculture and Agrifood Canada (1998). The Canadian System of Soil Classification, 3rd edition. Ottawa**:** 187 pp.

Ahmad Khan, I., S. Ahmad and N. Ayub (2003). "Response of oat (*Avena sativa*) to inoculation with vesicular arbuscular mycorrhizae (VAM) in the presence of phosphorus." Asian Journal of Plant Sciences **2**: 371-373.

Al-Karaki, G., B. McMichael and J. Zak (2004). "Field response of wheat to arbuscular mycorrhizal fungi and drought stress." Mycorrhiza **14**: 263-269.

Al-Karaki, G. N. (1998). "Benefit, cost and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress." Mycorrhiza **8**: 41-45.

Al-Karaki, G. N. and M. Al-Omoush (2002). "Wheat response to phosphogypsum and mycorrhizal fungi in alkaline soil." Journal of Plant Nutrition **25**: 873-883.

Aliasgarzad, N., M. R. Neyshabouri and G. Salimi (2006). "Effects of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on drought stress of soybean." Biologia **61**: 324-328.

Allen, M. F., W. K. Smith, T. S. Moore and M. Christensen (1981). "Comparative water relation and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis*." New Phytologist **88**: 683-693.

Allison, V. J., M. R. Miller, J. D. Jastrow, R. Matamala and D. R. Zak (2005). "Changes in soil microbial community structure in a tallgrass prairie chronosequence." Soil Science Society of America Journal **69**: 1412-1421.

Alloush, G. A. and R. B. Clark (2001). "Maize response to phosphate rock and arbuscular mycorrhizal fungi in acidic soil." Communications in Soil Science & Plant Analysis **32**: 231-254.

Andrade, G., K. L. Mihara, R. G. Linderman and G. J. Bethlenfalvay (1997). "Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi." Plant and Soil **192**: 71-79.

Anonymous (1976). Inorganic phosphorus in water and wastewater. Industrial method no. 93-70W/B. Tarrytown, NY., Technicon Industrial Systems.

Antunes, P. M., A. de Varennes, T. Zhang and M. J. Goss (2006). "The tripartite symbiosis formed by indigenous arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* and soya bean under field conditions." Journal of Agronomy & Crop Science **192**: 373-378.

Araujo, F. F., A. Henning and M. Hungria (2005). "Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development." World Journal of Microbiology & Biotechnology **21**: 1639-1645.

Artursson, V., R. D. Finlay and J. K. Jansson (2006). "Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth." Environmental Microbiology **8**: 1-10.

Atlas, R. M. and R. Bartha, Eds. (1997). Microbial ecology: fundamentals and applications. , California: Benjamin Cummings Science Publishing.

Atul-Nayyar, C. Hamel, T. Forge, F. Selles, P. G. Jefferson, K. Hanson and J. J. Germida (2008). "Arbuscular mycorrhizal fungi and nematodes are involved in negative feedback on a dual culture of alfalfa and Russian wildrye." Applied Soil Ecology **40**: 30-36.

Augé, R. M. and A. J. W. Stodola (1990). "An apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted Rosa plants." New Phytologist **115**: 285-295.

Azcón-Aguilar, C. and J. M. Barea (1981). "Field inoculation of *Medicago* with V-A mycorrhiza and *Rhizobium* in phosphate-fixing agricultural soil." Soil Biology & Biochemistry **13**: 19-22.

Azcón, R., J. Ruiz-Lozano and R. Rodriguez (2001). "Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (N-15) under increasing N supply to the soil." Canadian Journal of Botany **79**: 1175-1180.

Bååth, E. (2003). "The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi." Microbial Ecology **45**: 373-383.

Babana, A. H. and H. Antoun (2006). "Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (*Triticum aestivum* L.) in Mali." Plant and Soil **287**: 51-58.

Baccanti, M. and B. Colombo (1992). A new method for the automatic and selective determination of total organic carbon in sediments, soils, compost, particles in air, etc. Milano, Italy, Carlo Erba Instruments.

Bagayoko, M., E. George, V. Romheld and A. B. Buerkert (2000). "Effects of mycorrhizae and phosphorus on growth and nutrient uptake of millet, cowpea and sorghum on a West African soil." Journal of Agricultural Science **135**: 399-407.

Bailey, V. L., J. L. Smith and H. Bolton (2002). "Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration." Soil Biology & Biochemistry **34**: 997-1007.

Bais, H. P., T. L. Weir, L. G. Perry, S. Gilroy and J. M. Vivanco (2006). "The role of root exudates in rhizosphere interactions with plants and other organisms." Annual Review in Plant Biology **57**: 233-266.

Balser, T. C., K. K. Treseder and M. Ekenler (2005). "Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence." Soil Biology & Biochemistry **37**: 601-604.

Baon, J. B., S. E. Smith and A. M. Alston (1993). "Mycorrhizal responses of barley cultivars differing in P-efficiency." Plant and Soil **157**: 97-105.

Bardgett, R. D., P. J. Hobbs and S. C. Jarvis (1999). "Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands." Soil Biology & Biochemistry **31**: 1021-1030.

Barea, J. M., C. Azcón-Aguilar and R. Azcón (1987). "Vesicular-arbuscular improve both symbiotic N2 fixation and N uptake from soil as assessed with a N-15 technique under field conditions." New Phytologist **106**: 717-726.

Barea, J. M., R. Azcón and C. Azcón-Aguilar (2002). "Mycorrhizosphere interactions to improve plant fitness and soil quality." Antonie van Leeuwenhoek International Journal of General & Molecular Microbiology **81**: 343-351.

Baudoin, E., E. Benizri and A. Guckert (2003). "Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere." Soil Biology & Biochemistry **35**: 1183-1192.

Baute, T. (2002). Agronomy guide for field crops. Toronto, Ministry of Agriculture, Food and Rural Affairs. **publication 811**.

Bazzoffi, P., S. Pellegrini, A. Rocchini, M. Morandi and O. Grasselli (1998). "The effect of urban refuse compost and different tractors tires on soil physical properties, soil erosion and maize yield." Soil & Tillage Research **48**: 275-286.

Beauregard, M.-S., C. Hamel, Atul-Nayyar and M. St-Arnaud (in press). "Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa." Microbial Ecology.

Beauregard, M.-S., C. Hamel and M. St-Arnaud (2008). AM fungi communities in major intensive North American grain productions. Mycorrhizae: Sustainable Agriculture and Forestry. Z. A. Siddiqui, S. Akhtar and K. Futai. The Netherlands, Springer**:** 135-158.

Bedini, S., L. Avio, E. Argese and M. Giovannetti (2007). "Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein." Agriculture Ecosystems & Environment **120**: 463-466.

Benizri, E. and B. Amiaud (2005). "Relationship between plants and soil microbial communities in fertilized grasslands." Soil Biology & Biochemistry **37**: 2055-2064.

Bertin, C., X. H. Yang and L. A. Weston (2003). "The role of root exudates and allelochemicals in the rhizosphere." Plant & Soil. **256**: 67-83.

Bethlenfalvay, G. J., M. S. Brown, R. N. Ames and R. S. Thomas (1988). " Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphate uptake." Physiol Plant **72**: 565-571.

Bever, J. D., J. B. Morton, J. Antonovics and P. A. Schultz (1996). "Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland." Journal of Ecology **84**: 71-82.

Bhadalung, N. N., A. Suwanarit, B. Dell, O. Nopamornbodi, A. Thamchaipenet and J. Rungchuang (2005). "Effects of long-term NP-fertilization on abundance and diversity of arbuscular mycorrhizal fungi under a maize cropping system." Plant & Soil **270**: 371-382.

Biro, B., K. Koves-Pechy, I. Voros, T. Takacs, P. Eggenberg and R. J. Strasser (2000). "Interrelations between Azospirillum and Rhizobium nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa at sterile, AMF-free or normal soil conditions." Applied Soil Ecology **15**: 159-168.

Biró, B., K. Koves-Pechy, I. Voros, T. Takacs, P. Eggenberg and R. J. Strasser (2000). "Interrelations between *Azospirillum* and *Rhizobium* nitrogen-fixers and abuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions." Applied Soil Ecology **15**: 159-168.

Blaszkowski, J. (1993). "Comparative studies of the occurrence of arbuscular fungi and mycorrhizae (*Glomales*) in cultivated and uncultivated soils of Poland." Acta Mycologia **28**: 93-140.

Blaszkowski, J., B. Czerniawska, T. Wubet, T. Schaefer, F. Buscot and C. Renker (unpublished data). *Glomus irregulare*, a new arbuscular mycorrhizal fungus in the *Glomeromycota*.

Boddington, C. L. and J. C. Dodd (2000). "The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol." Plant and Soil **218**: 137-144.

Bødker, L., R. Kjøller, K. Kristensen and S. Rosendahl (2002). "Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea." Mycorrhiza **12**: 7-12.

Bolandnazar, S., N. Aliasgarzad, M. R. Neishabury and N. Chaparzadeh (2007). "Mycorrhizal colonization improves onion (*Allium cepa* L.) yield and water use efficiency under water deficit condition." Scientia Horticulturae **114**: 11-15.

Borneman, J., P. W. Skroch, K. M. Osullivan, J. A. Palus, N. G. Rumjanek, J. L. Jansen, J. Nienhuis and E. W. Triplett (1996). "Molecular microbial diversity of an agricultural soil in Wisconsin." Applied & Environmental Microbiology **62**: 1935-1943.

Borowicz, V. A. (2001). "Do arbuscular mycorrhizal fungi alter plant-pathogen relations?" Ecology **82**: 3057-3068.

Boswell, E. P., R. T. Koide, D. L. Shumway and H. D. Addy (1998). "Winter wheat cover cropping, VA mycorrhizal fungi and maize growth and yield." Agriculture Ecosystems & Environment **67**: 55-65.

Bowen, G. D. and A. D. Rovira (1999). "The rhizosphere and its management to improve plant growth." Advances in Agronomy **66**: 1-102.

Boyetchko, S. M. and J. P. Tewari (1988). "The effect of VA mycorrhizal fungi on infection by *Bipolaris sorokiniana* in barley." Canadian Journal of Plant Pathology **10**: 361.

Boyetchko, S. M. and J. P. Tewari (1990). Effect of phosphorus and VA mycorrhizal fungi on common root rot of barley. Innovation and integration. Innovation and integration. Proceedings of the 8th North American Conference on Mycorrhizae, Jackson, Wyoming.

Brady, N. C. and R. R. Weil (2002). The nature and properties of soils. NJ, Prentice Hall.

Brodie, E., S. Edwards and N. Clipson (2003). "Soil fungal community structure in a temperate upland grassland soil." FEMS Microbiology Ecology **45**: 105-114.

Broeckling, C. D., A. K. Broz, J. Bergelson, D. K. Manter and J. M. Vivanco (2008). "Root exudates regulate soil fungal community composition and diversity." Applied & Environmental Microbiology **74**: 738-744.

Budi, S. W., D. van Tuinen, G. Martinotti and S. Gianinazzi (1999). "Isolation from the *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens." Applied & Environmental Microbiology **65**: 5148-5150.

Caesar-Tonthat, T. C. (2002). "Soil binding properties of mucilage produced by a basidiomycete fungus in a model system." Mycological research **106**: 930-937.

Cahoon, L. B. and S. H. Ensign (2004). "Spatial and temporal variability in excessive soil phosphorus levels in eastern North Carolina." Nutrient Cycling in Agroecosystems **69**: 111-125.

Calvaruso, C., M. P. Turpault, E. Leclerc and P. Frey-Klett (2007). "Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes." Microbial Ecology **54**: 567-577.

Calvet, C., J. M. Barea and J. Pera (1992). "*In vitro* interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates." Soil Biol. Biochem. **24**: 775-780.

Canada Weather Office. (2009). "National climate data and information archive." http://www.climate.weatheroffice.ec.gc.ca/climateData.

Carrenho, R., E. Schunk Silva, E. F. Botelho Trufem and V. L. Ramos Bononi (2001). "Successive cultivation of maize and agricultural practices on root colonization, number of spores and species of arbuscular mycorrhizal fungi." Brazilian Journal of Microbiology **32**: 262-270.

Carter, M. R. and A. J. Campbell (2006). "Influence of tillage and liquid swine manure on productivity of a soybean-barley rotation and some properties of a fine sandy loam in Prince Edward Island." Canadian Journal of Soil Science **86**: 741-748.

Carvalho, L. M., I. Cacador and M. A. Martins-Loucao (2001). "Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal)." Mycorrhiza **11**: 303-309.

Casida, L. E., D. A. Klein and T. Santoro (1964). "Soil dehydrogenase activity." Soil Science **98**: 371-378.

Castillo, C. G., R. Rubio, J. L. Rouanet and F. Borie (2006). "Early effects of tillage and crop rotation on arbuscular mycorrhizal fungal propagules in an ultisol." Biology & Fertility of Soils **43**: 83-92.

Celik, I., I. Ortas and S. Kilic (2004). "Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil " Soil and Tillage Research **78**: 59-67.

Christensen, H. and I. Jakobsen (1993). "Reduction of bacterial growth by a vesicular-arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L)." Biology and Fertility of Soils **15**: 253-258.

Chu, H., X. Lin, T. Fujii, S. Morimoto, K. Yagi, J. Hu and J. Zhang (2007). "Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management." Soil Biology & Biochemistry **39**: 2971-2976.

Clarholm, M. (1993). "Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers." Biology and Fertility of Soils **16**: 287-292.

Clark, R. B. (1997). "Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH." Plant and Soil **192**: 15-22.

Clark, R. B. and S. K. Zeto (2000). "Mineral acquisition by arbuscular mycorrhizal plants." Journal of Plant Nutrition **23**: 867-902.

Collins Johnson, N., D. Tilman and D. Wedin (1992). "Plant and soil controls on mycorrhizal fungal communities." Ecology **73**: 2034-2042.

Cousins, J. R., D. Hope, C. Gries and J. C. Stutz (2003). "Preliminary assessment of arbuscular mycorrhizal fungal diversity and community structure in an urban ecosystem." Mycorrhiza **13**: 319-326.

Cruz, A. F., C. Hamel, K. Hanson, F. Selles and R. P. Zentner (2009). "Thirty-seven years of soil nitrogen and phosphorus fertility management shapes the structure and function of the soil microbial community in a Brown Chernozem." Plant and Soil **315**: 173-184.

Cruz, C., J. J. Green, C. A. Watson, F. Wilson and M. A. Martins-Loucao (2004). "Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity." Mycorrhiza **14**: 177-184.

da Silva, A. E. and W. H. Gabelman (1992). "Screening maize inbred lines for tolerance to low-P stress condition." Plant and Soil **146**: 181-187.

da Silva, P. and E. Nahas (2002). "Bacterial diversity in soil in response to different plants, phosphate fertilizers and liming." Brazilian Journal of Microbiology **33**: 304-310.

de Boer, W., L. B. Folman, R. C. Summerbell and L. Boddy (2005). "Living in a fungal world: impact of fungi on soil bacterial niche development." FEMS Microbiology Reviews **29**: 795-811.

de Varennes, A. and M. J. Goss (2007). "The tripartite symbiosis between legumes, rhizobia and indigenous mycorrhizal fungi is more efficient in undisturbed soil." Soil Biology & Biochemistry **39**: 2603-2607.

Diez, B., C. Pedros-Alio, T. L. Marsh and R. Massana (2001). "Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques." Applied & Environmental Microbiology **67**: 2942-2951.

Dilly, O., J. Bloem, A. Vos and J. C. Munch (2004). "Bacterial Diversity in Agricultural Soils during Litter Decomposition." Applied and Environmental Microbiology **70**: 468-473.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, J. Vanderleyden, P. Dutto, C. Labandera-Gonzalez, J. Caballero-Mellado, J. F. Aguirre, Y. Kapulnik, S. Brener, S. Burdman, D. Kadouri, S. Sarig and Y. Okon (2001). "Responses of agronomically important crops to inoculation with Azospirillum." Australian Journal of Plant Physiology **28**: 871-879.

Dodd, J. C. (2000). "The role of arbuscular mycorrhizal fungi in agro- and natural ecosystems." Outlook on Agriculture **29**: 55-62.

Domsch, K. H., W. Gams and T. H. Anderson (1980). Compendium of soil fungi. London, Academic Press.

Douds, D. D. and P. Millner (1999). "Biodiversity of arbuscular mycorrhizal fungi in agroecosystems." Agriculture Ecosystems & Environment **74**: 77-93.

Duineveld, B. M., G. A. Kowalchuk, A. Keijzer, J. D. van Elsas and J. A. van Veen (2001). "Analysis of bacterial communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA." Applied & Environmental Microbiology **67**: 172-178.

Dunfield, K. E. and J. J. Germida (2003). "Seasonal changes in the rhizosphere microbial communities associated with fieldgrown genetically modified canola (*Brassica napus*)." Applied & Environmental Microbiology **69**: 7310-7318.

Edwards, U., T. Rogall, H. Blöcker, M. Emde and E. C. Böttger (1989). "Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA." Nucleic Acids Research **17**: 7843-7853.

Eivazi, F. and M. A. Tabatabai (1977). " Phosphatases in soils." Soil Biology & Biochemistry **9**: 167-172.

Ellis, J. R., W. Roder and S. C. Mason (1992). "Grain sorghum-soybean rotation and fertilization influence on vesicular-arbuscular mycorrhizal fungi." Soil Science Society of America Journal **56**: 789-794.

Elsen, A., S. Declerck and D. De Waele (2001). "Effects of *Glomus intraradices* on the reproduction of the burrowing nematode (*Radopholus similis*) in dixenic culture." Mycorrhiza **11**: 49-51.

Environment Canada. (2008). "Historical weather." from http://www.weatheroffice.gc.ca/ canada_e.html.

FAO. (2004). "FAO Statistical Yearbook. Table B1. Production of cereals and share in world." 2007, from http://www.fao.org/statistics/yearbook/vol_1_1/index.asp.

Farrell, M., G. W. Griffith, P. J. Hobbs, W. T. Perkins and D. Jones (2010). "Microbial diversity and activity are increased by compost amendment of metal-contaminated soil." FEMS Microbiology Ecology **71**.

Feldmann, F. and C. Boyle (1998). "Weed-mediated stability of arbuscular mycorrhizal fungi effectiveness in maize monocultures." Journal of Applied Botany **73**: 1-5.

Felske, A., B. Engelen, U. Nubel and H. Backhaus (1996). "Direct ribosome isolation from soil to extract bacterial rRna for community analysis." Applied & Environmental Microbiology **62**: 4162-4167.

Feng, G., Y. C. Song, X. L. Li and P. Christie (2003). "Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil." Applied Soil Ecology **22**: 139-148.

Feng, G., F. S. Zhang, X. L. Li, C. Y. Tian, C. X. Tang and Z. Rengel (2002). "Uptake of nitrogen from indigenous soil pool by cotton plant inoculated with arbuscular mycorrhizal fungi." Communications in Soil Science and Plant Analysis **33**: 3825-3836.

Ferreira, M. C., D. D. Andrade, L. M. D. Chueire, S. M. Takemura and M. Hungria (2000). "Tillage method and crop rotation effects on the population sizes and diversity of bradyrhizobia nodulating soybean." Soil Biology & Biochemistry **32**: 627-637.

Fidelibus, M. W., C. A. Martin and J. C. Stutz (2001). "Geographic isolates of *Glomus* increase root growth and whole-plant transpiration of citrus seedlings grown with high phosphorus." Mycorrhiza **10**: 231-236.

Filion, M., R. C. Hamelin, L. Bernier and M. St-Arnaud (2004). "Molecular profiling of rhizosphere microbial communities associated with healthy and diseased black spruce (*Picea mariana*) seedlings grown in a nursery." Applied & Environmental Microbiology **70**: 3541-3551.

Filion, M., M. St-Arnaud and J. A. Fortin (1999). "Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms." New Phytologist **141**: 525-533.

Fliessbach, A. and F. Widmer (2006). Estimating soil microbial biomass. Microbiological methods for assessing soil quality. J. Bloem, D. W. Hopkins and A. Benedetti. Wallingford, UK; Cambridge, MA, CABI Publishing**:** 307 p.

Fontenla, S., I. Garcia-Romera and J. A. Ocampo (1999). "Negative influence of non-host plants on the colonization of *Pisum sativum* by the arbuscular mycorrhizal fungus *Glomus mosseae*." Soil Biology & Biochemistry **31**: 1591-1597.

Fox, C. A. and K. B. MacDonald (2003). "Challenges related to soil biodiversity research in agroecosystems—Issues within the context of scale of observation." Canadian Journal of Soil Science **83**: 231-244.

Francis, R. and D. J. Read (1994). "The contributions of mycorrhizal fungi to the determination of plant community structure." Plant and Soil **159**: 11-25.

Frostegård, A. and E. Bååth (1996). "The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil." Biology and Fertility of Soils **22**: 59-65.

Galvez, L., D. D. Douds, L. E. Drinkwater and P. Wagoner (2001). "Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize." Plant and Soil **228**: 299-308.

Garbeva, P., J. A. van Veen and J. D. van Elsas (2004). "Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness." Annu. Rev. Phytopathol. **42**: 243-270.

Garmendia, I., N. Goicoechea and J. Aguirreolea (2004). "Effectiveness of three *Glomus* species in protecting pepper (*Capsicum annuum* L.) against verticillium wilt." Biological Control **31**: 296-305.

Gavito, M. E. and M. H. Miller (1998). "Early phosphorus nutrition, mycorrhizae development, dry matter partitioning and yield of maize." Plant and Soil **199**: 177-186.

Ge, Y., J. B. Zhang, L. M. Zhang, M. Yang and J. Z. He (2008). " Long-term fertilization regimes and diversity of an agricultural affect bacterial community structure soil in northern China. ." Journal of Soils & Sediments. **8**: 43-50.

Ghazvini, H. and A. Tekauz (2007). "Reactions of Iranian barley accessions to three predominant pathogens in Manitoba." Canadian Journal of Plant Pathology **29**: 69-78.

Giovannetti, M. and B. Mosse (1980). "An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots." New Phytologist **84**.

Giri, B., R. Kapoor and K. G. Mukerji (2005). "Effect of the arbuscular mycorrhizae *Glomus fasciculatum* and *G. macrocarpum* on the growth and nutrient content of *Cassia siamea* in a semi-arid Indian wasteland soil." New Forests **29**: 63-73.

Girvan, M. S., J. Bullimore, A. S. Ball, J. N. Pretty and A. M. Osborn (2004). "Responses of active bacterial and fungal communities in soils under winter wheat to different fertilizer and pesticide regimens." Applied & Environmental Microbiology **70**: 2692-2701.

Goddard, M. R., H. C. Godfray and A. Burt (2005). "Sex increases the efficacy of natural selection in experimental yeast populations." Nature **434**: 636-640.

Gosling, P., A. Hodge, G. Goodlass and G. D. Bending (2006). "Arbuscular mycorrhizal fungi and organic farming." Agriculture Ecosystems & Environment **113**: 17-35.

Goss, M. J. and A. de Varennes (2002). "Soil disturbance reduces the efficacy of mycorrhizal associations for early soybean growth and N-2 fixation." Soil Biology & Biochemistry **34**: 1167-1173.

Graham, J. H. (2001). "What do root pathogens see in mycorrhizas?" New Phytologist **149**: 357-359.

Graham, J. H. and L. K. Abbott (2000). "Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi." Plant and Soil **220**: 207-218.

Graham, J. H. and J. A. Menge (1982). "Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat." Phytopathology **72**: 95-98.

Grant, C., S. Bittman, M. Montreal, C. Plenchette and C. Morel (2005). "Soil and fertilizer phosphorus:Effects on plant P supply and mycorrhizal development." Canadian Journal of Plant Science **85**: 3-14.

Grayston, S. J., D. Vaughan and D. Jones (1996). "Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability." Applied Soil Ecology **5**: 29-56.

Green, H., J. Larsen, P. A. Olsson, D. F. Jensen and I. Jakobsen (1999). " Suppression of the biocontrol agent *Trichoderma harzianum* by mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* in root-free soil." Applied & Environmental Microbiology **65**: 1428-1434.

Green, S. J., S. Freeman, Y. Hadar and D. Minz (2004). "Molecular tools for isolate and community studies of Pyrenomycete fungi." Mycologia **96**: 439-451.

Gryndler, M., M. Vosatka, H. Hrselova, V. Catska, I. Chvatalova and J. Jansa (2002). "Effect of dual inoculation with arbuscular mycorrhizal fungi and bacteria on growth and mineral nutrition of strawberry." Journal of Plant Nutrition **25**: 1341-1358.

Hagn, A., K. Pritsch, M. Schloter and J. C. Munch (2003). "Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp." Biology and Fertility of Soils **38**: 236-244.

Hamel, C. (2004). "Impact of arbuscular mycorrhizal fungi on N and P cycling in the root zone." Canadian Journal of Soil Science **84**: 383-395.

Hamel, C. (2007). Extraradical arbuscular mycorrhizal mycelia: shadowy figures in the soil. Mycorrhizae in crop production. C. Hamel and C. Plenchette. Binghampton, New York, Haworth Press**:** 1-36.

Hamel, C. and D. Angers (2004). "Mycorrhiza and soil quality." Canadian Journal of Soil Science **84**: 353.

Hamel, C., Y. Dalpé, C. Lapierre, R. R. Simard and D. L. Smith (1994). "Composition of the vesicular-arbuscular mycorrhizal fungi population in an old meadow as affected by ph, phosphorus and soil disturbance." Agriculture Ecosystems & Environment **49**: 223-231.

Hamel, C., V. Furlan and D. L. Smith (1992). "Mycorrhizal effects on interspecific plant competition and nitrogen transfer in legume grass mixtures." Crop science **32**.

Hamel, C., K. Hanson, F. Selles, A. F. Cruz, R. Lemke, B. McConkey and R. Zentner (2006a). "Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotations of the Canadian prairie." Soil Biology & Biochemistry **38**: 2104-2116.

Hamel, C., V. Vujanovic, R. Jeannotte, A. Liu, A. Nakano and M. St-Arnaud (2006b). Variation in arbuscular mycorrhizal fungi extraradicular biomass along a climatic gradient in an agricultural zone of Quebec, Canada. . 5th International Symbiosis Society Congress, Vienna, Austria.

Hao, Z. P., P. Christie, L. Qin, C. X. Wang and X. L. Li (2005). "Control of fusarium wilt of cucumber seedlings by inoculation with an arbuscular mycorrhical fungus." Journal of Plant Nutrition **28**: 1961-1974.

Hart, M. M., R. J. Reader and J. N. Klironomos (2003). "Plant coexistence mediated by arbuscular mycorrhizal fungi." Trends in Ecology & Evolution **18**: 418-423.

Havlin, J. L., J. d. Beaton, S. L. Tisdale and W. L. Nelson (1999). Soil fertility and fertilizers. NY, MacMillian Publishing Co.

He, J. Z., Y. Zheng, C. R. Chen, Y. Q. He and L. M. Zhang (2008). "Microbial composition and diversity of an upland red soil under long-term fertilization treatments as revealed by culture-dependent and culture-independent approaches." Journal of Soils & Sediments **8**: 349-358.

Hedley, M. J. and J. W. B. Stewart (1982). "Method to measure microbial phosphate in soils." Soil Biology & Biochemistry **14**: 377-385.

Heffer, P. and M. Prud'homme (2006). Medium-term outlook for global fertilizer demand, supply and trade, 2006-2010, summary report. presented at the 74th IFA Annual Conference, Cape Town, June 2006. Paris, France, International Fertilizer Industry Association.

Helgason, T., T. J. Daniell, R. Husband, A. H. Fitter and J. P. W. Young (1998). "Ploughing up the wood-wide web." Nature **394**: 431.

Hempel, S., C. Renker and F. Buscot (2007). "Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem." Environmental Microbiology **9**: 1930-1938.

Henry, A. and K. R. Kosola (1999). "Root age and phosphorus effects on colonization of Andropogon gerardii by mycorrhizal fungi " Soil Biology & Biochemistry **31**: 1657-1660

Herath, A., T. Q. Zhang, C. Hamel, C. S. Tan, T. W. Welacky and M. J. Goss (2007). Immediate and residual effects of swine manure and its compost on soil phosphorus fractions in a clay loam soil under corn-soybean rotation. In Annual Meeting of CPS-SCP (with Plant Canada 2007). Saskatoon, SK, Canada**:** SF3-4.

Hodge, A. (2000). "Microbial ecology of the arbuscular mycorrhiza." FEMS Microbiology Ecology **32**: 91-96.

Hodge, A., C. D. Campbell and A. H. Fitter (2001). "An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material." Nature **413**: 297-299.

Hodge, A., D. Robinson and A. Fitter (2000). "Are microorganisms more effective than plants at competing for nitrogen?" Trends in Plant Science **5**: 304-308.

Holmes, A., J. Govan and R. Goldstein (1998). "Agricultural Use of *Burkholderia* (*Pseudomonas*) cepacia: A Threat to Human Health?" Emerging infectious diseases **4**: 221-227.

Houlden, A., T. M. Timms-Wilson, M. J. Day and M. J. Bailey (2008). "Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. ." FEMS Microbiology Ecology. **65**: 193-201.

Hugenholtz, P. and G. W. Tyson (2008). "Metagenomics." Nature **455**: 481-483.

Husband, R., E. A. Herre, S. Turner, R. Galler and J. P. W. Young (2002). "Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest." Molecular Ecology **11**: 2269-2278.

Ibekwe, A. M. and A. C. Kennedy (1998). "Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils." Plant & Soil. **206**: 151-161.

Ibijbijen, J., S. Urquiaga, M. Ismaili, B. J. R. Alves and R. M. Boddey (1996). "Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*)." New Phytologist **134**: 353-360.

Idoia, G., G. Nieves and A. Jone (2004). "Plant phenology influences the effect of mycorrhizal fungi on the development of *Verticillium*-induced wilt in pepper." European Journal of Plant Pathology **110**: 227-238.

Ilbas, A. I. and S. Sahin (2005). "*Glomus fasciculatum* inoculation improves soybean production." Acta Agriculturae Scandinavica Section B-Soil & Plant Science **55**: 287-292.

Imboden, D. M. (1974). "Phosphorus model of lake eutrophication." Limnol. Oceanogr. **19**: 297-304.

Innes, L., P. J. Hobbs and R. D. Bardgett (2004). "The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility." Biology and Fertility of Soils **40**: 7-13.

INVAM. (2008). "*Voucher specimens of Glomus fasciculatum*." http://invam.caf.wvu.edu/fungi/taxonomy/Glomaceae/Glomus/fasciculatum/fascic.htm.

Jacoud, C., D. Faure, P. Wadoux and R. Bally (1998). "Development of a strain-specific probe to follow inoculated *Azospirillum lipoferum* CRT1 under field conditions and enhancement of maize root development by inoculation." FEMS Microbiology Ecology **27**: 43-51.

Jansa, J., A. Mozafar, T. Anken, R. Ruh, I. R. Sanders and E. Frossard (2002). "Diversity and structure of AMF communities as affected by tillage in a temperate soil." Mycorrhiza **12**: 225-234.

Johnson, D., P. J. Vandenkoornhuyse, J. R. Leake, L. Gilbert, R. E. Booth and J. P. Grime (2003). "Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms." New Phytologist **161**: 503-515.

Johnson, N. C. (1993). "Can fertilization of soil select less mutualistic mycorrhizae?" Ecological applications **3**: 749-757.

Johnson, N. C. (1998). "Responses of *Salsola kali* and *Panicum virgatum* to mycorrhizal fungi, phosphorus and soil organic matter - Implications for reclamation." Journal of Applied Ecology **35**: 86-94.

Johnson, N. C. and F. L. Pfleger (1992). Vesicular-arbuscular mycorrhizae and cultural practices. In Mycorrhizae in Sustainable Agriculture. G. J. Bethlenfalvay and R. G. Linderman. Madison, ASA, CSSA, and SSSA. **54:** 71-99.

Johnson, N. C., D. R. Zak, D. Tilman and F. L. Pfleger (1991). "Dynamics of vesicular-arbuscular mycorrhizae during old field succession." Oecologia **86**: 349-358.

Joner, E. J. (2000). "The effect of long-term fertilization with organic or inorganic fertilizers on mycorrhiza-mediated phosphorus uptake in subterranean clover." Biology and Fertility of Soils **32**: 435-440.

Jones, M. D., D. M. Durall and P. B. Tinker (1998). "A comparison of arbuscular and ectomycorrhizal Eucalyptus coccifera: growth response, phosphorus uptake efficiency and external hyphal production." New Phytologist **140**: 125-134.

Jordan, N. R., J. Zhang and S. Huerd (2000). "Arbuscular-mycorrhizal fungi: potential roles in weed management." Weed Research **40**: 397-410.

Kabir, Z. and R. T. Koide (2000). "The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize." Agriculture Ecosystems & Environment **78**: 167-174.

Kabir, Z., I. P. O'Halloran and C. Hamel (1999). "Combined effects of soil disturbance and fallowing on plant and fungal components of mycorrhizal corn (*Zea mays* L.)." Soil Biology & Biochemistry **31**: 307-314.

Kabir, Z., I. P. O'Halloran, J. W. Fyles and C. Hamel (1997). "Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: Hyphal density and mycorrhizal root colonization." Plant and Soil **192**: 285-293.

Kang, S. and A. L. Mills (2006). "The effect of sample size in studies of soil microbial community structure." Journal of microbiological methods **66**: 242-250.

Kanno, T., M. Saito, Y. Ando, M. C. M. Macedo, T. Nakamura and C. H. B. Miranda (2006). "Importance of indigenous arbuscular mycorrhiza for growth and phosphorus uptake in tropical forage grasses growing on an acid, infertile soil from the brazilian savannas." Tropical Grasslands **40**: 94-101.

Karagiannidis, N. and S. Hadjisavvazinoviadi (1998). "The mycorrhizal fungus *Glomus mosseae* enhances growth, yield and chemical composition of a durum wheat variety in 10 different soils." Nutrient Cycling in Agroecosystems **52**: 1-7.

Karandashov, V. and M. Bucher (2005). "Symbiotic phosphate transport in arbuscular mycorrhizas." Trends in Plant Science **10**: 22-29.

Karasawa, T., Y. Kasahara and A. Takebe (2002). "Differences in growth responses of maize to preceding cropping caused by fluctuation in the population of indigenous arbuscular mycorrhizal fungi." Soil Biology & Biochemistry **34**: 851-857.

Kaufman, D. D. and S. L. E. Williams (1964). "Effect of mineral fertilization and soil reactions on soil fungi." Phytopathology **54**.

Kennedy, A. C. (1999). "Bacterial diversity in agroecosystems." Agriculture, Ecosystems and Environment **74**: 65-76.

Kernaghan, G. (2005). "Mycorrhizal diversity: Cause and effect?" Pedobiologia **49**: 511-520.

Khalil, S., T. E. Loynachan and M. A. Tabatabai (1994). "Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars." Agronomy Journal **86**: 949-958.

Killham, K. (1985). "A physiological determination of the impact of environmental stress on the activity of microbial biomass." Environmental Pollution **38**: 283-294.

Kirchgessner, M., M. Kreuzer and F. X. Roth (1991). "Bestimmungsfaktoren der Güllecharakteristik beim Schwein. 1. Einfluß von Leistungsrichtung, -stadium und - intensität sowie Futteraufnahme und Geschlecht unter üblichen Fütterungsbedingungen." Agribiological Research **44**: 299-324.

Kirchmann, H. and G. Thorvaldsson (2000). "Challenging targets for future agriculture." European Journal of Agronomy **12**: 145-161.

Kirk, J. L., L. A. Beaudette, M. Hart, P. Moutoglis, J. N. Klironomos, H. Lee and J. T. Trevors (2004). "Methods of studying soil microbial diversity." Journal of microbiological methods **58**: 169-188.

Klironomos, J. N. (2002). "Feedback with soil biota contributes to plant rarity and invasiveness in communities." Nature **417**: 67-70.

Klironomos, J. N., J. McCune, M. Hart and J. Neville (2000). "The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity." Ecology Letters **3**: 137-141.

Knee, E. M., F. C. Gong, M. S. Gao, M. Teplitski, A. R. Jones, A. Foxworthy, A. J. Mort and W. D. Bauer (2001). "Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source." Molecular Plant-Microbe Interactions **14**: 775-784.

Koske, R. E. (1987). "Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient." Mycologia **79**: 55-68.

Kowalchuk, G. A., F. A. De Souza and J. A. van Veen (2002). " Community analysis of arbuscular mycorrhizal fungi associated with *Ammophila arenaria* in Dutch coastal sand dunes." Molecular Ecology **11**: 571-581.

Kuhn, G., M. Hijri and I. R. Sanders (2001). "Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi." Nature **414**: 745-748.

Landeweert, R., P. Leeflang, T. W. Kuyper, E. Hoffland, A. Rosling, K. Wernars and E. Smit (2003). "Molecular identification of ectomycorrhizal mycelium in soil horizons." Applied & Environmental Microbiology **69**: 327-333.

Larcher, W. (2003). Ecophysiology and stress physiology of functional groups. Physiological Plant Ecology. Berlin, Science Press, Springer.

Larsen, J., P. A. Olsson and I. Jakobsen (1998). "The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the saprotrophic fungus *Fusarium culmorum* in root-free soil." Mycol. Res. **102**: 1491-1496.

Lee, J. J., R. D. Park, Y. W. Kim, J. H. Shim, D. H. Chae, Y. S. Rim, B. K. Sohn, T. H. Kim and K. Y. Kim (2004). "Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth." Bioresource Technology **93**: 21-28.

Lekberg, Y. and R. T. Koide (2005). "Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003." New Phytologist **168**: 189-204.

Li, B., S. Ravnskov, G. L. Xie and J. Larsen (2007). "Biocontrol of *Pythium* damping-off in cucumber by arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus*." BioControl **52**: 863-875.

Li, H. Y., S. E. Smith, R. E. Holloway, Y. G. Zhu and F. A. Smith (2006). "Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses." New Phytologist **172**: 536-543.

Li, L., A. Yang and Z. Zhiwei (2005). "Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China." FEMS Microbiology Ecology **54**: 367-373.

Liang, Z., R. A. Drijber, D. J. Lee, I. M. Dwiekat, S. D. Harris and D. A. Wedin (2008). "A DGGE-cloning method to characterize arbuscular mycorrhizal community structure in soil." Soil Biology and Biochemistry **39**: 956-966.

Liebman, M., F. D. Menalled, D. D. Buhler, T. L. Richard, D. N. Sundberg, C. A. Cambardella and K. A. Kohler (2004). "Impacts of composted swine manure on weed and corn nutrient uptake, growth, and seed production." Weed Science **52**: 365-375.

Lilja, A., M. Poteri, M. Vuorinen, T. Kurkela and J. Hantula (2005). "Cultural and PCR-based identification of the two most common fungi from cankers on container-grown Norway spruce seedlings." Canadian Journal of Forest Research **35**: 432-439.

Linderman, R. G. (1992). Vesicular-arbuscular mycorrhizae and soil microbial interactions. Mycorrhizae in sustainable agriculture. G. J. Bethlenfalvay and R. G. Linderman. Madison, Wisconsin, American Society of Agronomy. **Special Publ. No. 54:** 45-70.

Linderman, R. G. and T. C. Paulitz (1990). Mycorrhizal-rhizobacterial interactions. Biological control of soil-born Plant Pathogens. D. Hornby, R. J. Cook, Y. Heniset al. Wallingford, UK, CAB International**:** 261-283.

Lioussanne, L. (2007). Rôles des modifications de la microflore bactérienne et de l'exsudation racinaire de la tomate par la symbiose mycorhizienne dans le biocontrôle sur le *Phytophthora nicotianae*. . Biological sciences. Montréal, Université de Montréal,. **Ph.D.:** 264.

Lithourgidis, A. S., T. Matsi, N. Barbayiannis and C. A. Dordas (2007). "Effect of liquid cattle manure on corn yield, composition, and soil properties." Agronomy Journal **99**: 1041-1047.

Liu, A., C. Hamel, A. Elmi, C. Costa, B. Ma and D. L. Smith (2002). "Concentrations of K, Ca and Mg in maize colonized by arbuscular mycorrhizal fungi under field conditions." Canadian Journal of Soil Science **82**: 271-278.

Liu, A., C. Hamel, T. Spedding, T. Q. Zhang, R. Mongeau, G. R. Lamarre and G. Tremblay (2008a). "Soil microbial carbon and phosphorus as influenced by phosphorus fertilization and tillage in a maize-soybean rotation in south-western Quebec." Canadian Journal of Soil Science **88**: 21-30.

Liu, B. and J. B. Ristaino (2003). "Microbial community structure in soils from organic and conventional agroecosystems." Phytopathology **96**: S53.

Liu, F., W. Liang, X. Zhang, Y. Jiang and J. Wang (2008b). "Changes in soil microbial biomass and bacterial community in a long-term fertilization experiment during the growth of maize." Advances in Environmental Biology **2**: 1-8.

Liu, X., W. C. Lindemann, W. G. Whitford and R. L. Steiner (2000). "Microbial diversity and activity of disturbed soil in the northern Chihuahuan Desert." Biology and Fertility of Soils **32**: 243-249.

Lopez-Hernandez, D., Y. Araujo, A. Lopez, I. Hernandez-Valencia and C. Hernandez (2004). "Changes in soil properties and earthworm populations induced by long-term organic fertilization of a sandy soil in the Venezualan amazonia." Soil Science **169**: 188-194.

Lucy, M., E. Reed and B. R. Glick (2004). "Applications of free living plant growth-promoting rhizobacteria." Antonie van Leeuwenhoek **86**: 1-25.

Lukow, T., P. F. Dunfield and W. Liesack (2000). "Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants." FEMS Microbiology Ecology **32**: 241-247.

Lundquist, E. J., K. M. Scow, L. E. Jackson, S. L. Uesugi and C. R. Johnson (1999). "Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle." Soil Biology & Biochemistry **31**: 1661-1675.

Lynch, M. D. J. and R. G. Thorn (2006). "Diversity of basidiomycetes in Michigan agricultural soils." Appl. Environ. Microbiol. **72**: 7050-7056.

Ma, W. K., S. D. Siciliano and J. J. Germida (2005). "A PCR-DGGE method for detecting arbuscular mycorrhizal fungi in cultivated soils." Soil Biology & Biochemistry. **37**: 1589-1597.

Mäder, P., S. Edenhofer, T. Boller, A. Wiemken and U. Niggli (2000). "Arbuscular mycorrhizae in along-term field trial comparing low-input (organic, biological) and high-put (conventional) farming systems in a crop rotation." Biology and Fertility of Soils **31**: 150-156.

Mäder, P., A. Fliessbach, D. Dubois, L. Gunst, P. Fried and U. Niggli (2002). "Soil fertility and biodiversity in organic farming." Science **296**: 1694-1697.

Marler, M. J., C. A. Zabinski and R. M. Callaway (1999). "Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass." Ecology **80**: 1180-1186.

Marschner, H., E. A. Kirkby and C. Engels (1997a). "Importance of cycling and recycling of mineral nutrients within plants for growth and development." Botanica Acta **110**: 265-273.

Marschner, P. and D. E. Crowley (1996a). "Physiological activity of a bioluminescent *Pseudomonas fluorescens* (strain 2-79) in the rhizosphere of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L)." Soil Biology & Biochemistry **28**: 869-876.

Marschner, P. and D. E. Crowley (1996b). "Root colonization of mycorrhizal and nonmycorrhizal pepper (*Capsicum annuum*) by *Pseudomonas fluorescens* 2-79RL." New Phytologist **134**: 115-122.

Marschner, P., D. E. Crowley and R. M. Higashi (1997b). "Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L)." Plant and Soil **189**: 11-20.

Martin-Laurent, F., L. Philippot, S. Hallet, R. Chaussod, J. C. Germon, G. Soulas and G. Catroux (2001). "DNA extraction from soils: old bias for new microbial diversity analysis methods." Applied & Environmental Microbiology **67**: 2354-2359.

Martinez-Toledo, M. V., V. Salmeron, B. Rodelas, C. Pozo and J. Gonzalez-Lopez (2005). "Studies on the effects of the herbicide Simazine on microflora of four agricultural soils." Environmental Toxicology and Chemistry **15**: 1115-1118.

Martinez, A., M. Obertello, A. Pardo, J. A. Ocampo and A. Godeas (2004). "Interactions between *Trichoderma pseudokoningii* strains and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*." Mycorrhiza **14**: 79-84.

Mathimaran, N., R. Ruh, P. Vullioud, E. Frossard and J. Jansa (2005). "*Glomus intraradices* dominates arbuscular mycorrhizal communities in a heavy textured agricultural soil." Mycorrhiza. **16**: 61-66.

Matsubara, Y., N. Ohba and H. Fukui (2001). "Effect of arbuscular mycorrhizal fungus infection on the incidence of fusarium root rot in asparagus seedlings." Journal of the Japanese Society for Horticultural Science **70**: 202-206.

McGonigle, T. P. (1988). "A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi." Functionnal ecology **2**: 473-478.

Menendez, A. B., J. M. Scervino and A. M. Godeas (2001). "Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina." Biology & Fertility of Soils **33**: 373-381.

Meyer, J. R. and R. G. Linderman (1986a). "Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*." Soil Biology & Biochemistry **18**: 185-190.

Meyer, J. R. and R. G. Linderman (1986b). "Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*." Soil Biology & Biochemistry **18**: 191-196.

Miller, M. H. (2000). "Arbuscular mycorrhizae and the phosphorus nutrition of maize: A review of Guelph studies." Canadian Journal of Plant Science **80**: 47-52.

Miller, M. H., T. P. McGonigle and H. D. Addy (1995). "Functional ecology of vesicular arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem." Critical Reviews in Biotechnology **15**: 241-255.

Miransari, M., H. A. Bahrami, F. Rejali, M. J. Malakouti and H. Torabi (2007). "Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth." Soil Biology & Biochemistry **39**: 2014-2026.

Misko, A. L. and J. J. Germida (2002). "Taxonomic and functional diversity of pseudomonads isolated from the roots of field-grown canola." FEMS Microbiology Ecology **42**: 399-407.

Mohammad, A., B. Mitra and A. G. Khan (2004). "Effects of sheared-root inoculurn of *Glomus intraradices* on wheat grown at different phosphorus levels in the field." Agriculture Ecosystems & Environment **103**: 245-249.

Moore, P. A., T. C. Daniel and D. R. Edwards (1999). "Reducing phosphorus runoff and improving poultry production with alum." Poultry Science **78**: 692-698.

Mozafar, A., T. Anken, R. Ruh and E. Frossard (2000). "Tillage intensity, mycorrhizal and nonmycorrhizal fungi, and nutrient concentrations in maize, wheat, and canola." Agronomy Journal **92**: 1117-1124.

Muyzer, G., E. C. de Waal and A. G. Uitterlinden (1993). "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analyses of polymerase chain reaction-amplified genes for 16S rRNA." Applied & Environmental Microbiology **59**: 695-700.

Myers, R., L. Lerman and T. Maniatis (1985). "Nearly all single base substitutions in DNA fragments joined to a GC-clamp can be detected by denaturating gradient gel electrophoresis." Nucleic Acids Research **13**: 3131-3145.

Neumann, G. and V. Römheld (2001). The release of root exudates as affected by the plant physiological status. The Rhizosphere: biochemistry and organic substances at the soil-plant interface. R. Pinton, Z. Varanini and Z. Nannipieri. New York, USA, Marcel Dekker**:** 41-94.

Nogueira, M. A. and E. J. B. Nogueira Cardoso (2003). "Mycorrhizal effectiveness and manganese toxicity in soybean as affected by soil type and endophyte." Scientia Agricola **60**: 329-335.

Nogueira, M. A. and E. J. B. Nogueira Cardoso (2007). "Phosphorus availability changes the internal and external endomycorrhizal colonization and affects symbiotic effectivenes." Scientia Agricola **64**: 295-300.

Nourinia, A. A., E. Faghani, F. Rejali, A. Safarnezhad and M. Abbasi (2007). "Evaluation effects of symbiosis of mycorrhiza on yield components and some physiological parameters of barley genotypes under salinity stress." Asian Journal of Plant Sciences **6**: 1108-1112.

O'Leary, J. W. (1965). "Root-pressure exudation in woody plants." Botanical Gazette **126**: 108-115.

Oehl, F., E. Sieverding, K. Ineichen, P. Mader, T. Boller and A. Wiemken (2003). "Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe." Applied & Environmental Microbiology **69**: 2816-2824.

Oehl, F., E. Sieverding, K. Ineichen, E. A. Ris, T. Boller and A. Wiemken (2005). "Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems." New Phytologist **165**: 273-283.

Oehl, F., E. Sieverding, P. Mader, D. Dubois, K. Ineichen, T. Boller and A. Wiemken (2004). "Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi." Oecologia **138**: 574-583.

Okur, N., A. Altindisli, M. Cengel, S. Gocmez and H. H. Kayikcioglu (2009). "Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems." Turkish Journal of Agriculture & Forestry. **33**: 413-423.

Oliveira, C. A., N. M. H. Sá, E. A. Gomes, E. M. Ivanildo, M. R. Scotti, C. T. Guimarães, R. E. Schaffert and V. M. C. Alves (2009). "Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L.) genotypes contrasting for phosphorus efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE)." Applied Soil Ecology **41**: 249-258.

Olsson, P. A., I. Thingstrup, I. Jakobsen and F. Baath (1999). "Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field." Soil Biology & Biochemistry. **31**: 1879-1887.

Omar, S. A. (1998). "The role of rock-phosphate-solubilizing fungi and vesicular-arbusular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate." World Journal of Microbiology & Biotechnology **14**: 211-218.

Öpik, M., M. Moora, J. Liira, U. Koljalg, M. Zobel and R. Sen (2003). "Divergent arbuscular mycorrhizal fungal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forest and grassland soils." New Phytologist **160**: 581-593.

Öpik, M., M. Moora, J. Liira and M. Zobel (2006). "Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe." Journal of Ecology **94**: 778-790.

Ortas, I., D. Ortakci and Z. Kaya (2002). "Various mycorrhizal fungi propagated on different hosts have different effect on citrus growth and nutrient uptake." Communications in Soil Science and Plant Analysis **33**: 259-272.

Ozgonen, H. and A. Erkilic (2007). "Growth enhancement and Phytophthora blight (*Phytophthora capsici* Leonian) control by arbuscular mycorrhizal fungal inoculation in pepper." Crop Protection **26**: 1682-1688.

Pankhurst, C. E., A. Pierret, B. G. Hawke and J. M. Kirby (2002). "Microbiological and chemical properties of soil associated with macropores at different depths in a red-duplex soil in NSW Australia." Plant & Soil. **238**: 11-20.

Pardo, A., M. Amato and F. Q. Chiaranda (2000). "Relationships between soil structure, root distribution and water uptake of chickpea (*Cicer arietinum* L.). Plant growth and water distribution." European Journal of Agronomy **13**: 39-45.

Paul, E. A. (2007). Soil microbiology, ecology and biochemistry. Amsterdam, Academic Press.

Paulitz, T. C. and R. G. Linderman (1989). "Interactions between fluorescent pseudomonads and VA mycorrhizal fungi." New Phytologist **113**: 37-45.

Perkiömäki, J. and H. Fritze (2003). "Does simulated acid rain increase the leaching of cadmium from wood ash to toxic levels to coniferous forest humus microbes?" FEMS Microbiology Ecology. **44**: 27-33.

Petersen, S. O. and M. J. Klug (1994). "Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community." Applied Environmental Microbiology **60**: 2421-2430.

Plenchette, C. (1983). "Growth responses of several plant species to mycorrhizae in a soil of moderate P fertility." Plant and Soil **70**: 199-209.

Porras-Alfaro, A., J. Herrera, D. O. Natvig and R. L. Sinsabaugh (2007). "Effect of long-term nitrogen fertilization on mycorrhizal fungi associated with a dominant grass in a semiarid grassland." Plant & Soil. **296**: 65-75.

Posta, K., H. Marschner and V. Römheld (1994). "Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize." Mycorrhiza **5**: 119-124.

Powell, C. L. (1981). " Inoculation of barley with efficient mycorrhizal fungi stimulates seed yield." Plant and Soil **59**: 487-489.

Powell, J. R., R. H. Gulden, M. M. Hart, R. G. Campbell, D. J. Levy-Booth, K. E. Dunfield, K. P. Pauls, C. J. Swanton, J. T. Trevors and J. N. Klironomos (2007). "Mycorrhizal and rhizobial colonization of genetically modified and conventional soybeans." Applied & Environmental Microbiology **73**: 4365-4367.

Puno, H. K. (1991). Calibration of soil test P with crop response to applied phosphorus. Structural Engineering. Tandojam, Pakistan, Sindh Agriculture University. **Ph.D.:** 172.

Quilambo, O. A., I. Weissenhorn, H. Doddema, P. J. C. Kuiper and I. Stulen (2005). "Arbuscular mycorrhizal inoculation of peanut in low-fertile tropical soil. II. Alleviation of drought stress." Journal of Plant Nutrition **28**: 1645-1662.

Quilchano, C. and T. Marañón (2002). "Dehydrogenase activity in Mediterranean forest soils " Biology and Fertility of Soils **35**: 102-107.

Rai, M. K. (2006). Handbook of microbial biofertilizers, Haworth Press.

Rao, A. V., J. C. Tarafdar, S. K. Sharma and R. H. Aggarwal (1995). "Influence of cropping systems on soil biochemical properties in an arid rainfed environment." J. Arid Environ **31**: 237-244.

Read, D. J. (1998). "Plants on the web." Nature **396**: 22-23.

Read, D. J., H. K. Koucheki and J. Hodgson (1976). "Vesicular-arbuscular mycorrhiza in natural vegetation systems." New Phytologist **77**: 641-653.

Redecker, D., J. B. Morton and T. D. Bruns (2000). "Ancestral lineages of arbuscular mycorrhizal fungi (*Glomales*)." Molecular Phylogenetics and Evolution **14**: 276-284.

Rempel, C. B. (1989). Interactions between vesicular-arbuscular mycorrhizae (VAM) and fungal pathogens in wheat. Winnipeg, University of Manitoba. **M.Sc. thesis:** 134p.

Reyes, I., L. Bernier and H. Antoun (2002). "Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*." Microbial Ecology **44**: 39-48.

Rillig, M. C., E. R. Lutgen, P. W. Ramsey, J. N. Klironomos and J. E. Gannon (2005). "Microbiota accompanying different arbuscular mycorrhizal fungal isolates influence soil aggregation." Pedobiologia **49**: 251-259.

Rillig, M. C., S. F. Wright, M. R. Shaw and C. B. Field (2002). "Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland " Oikos **97**: 52-58.

Rodriguez, H. and R. Fraga (1999). "Phosphate solubilizing bacteria and their role in plant growth promotion." Biotechnology Advances **17**: 319-339.

Rolleke, S., C. Gurtner, U. Drewello, R. Drewello, W. Lubitz and R. Weissmann (1999). "Analysis of bacterial communities on historical glass by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA." Journal of microbiological methods **36**: 107-114.

Ros, M., S. Klammer, B. Knapp, K. Aichberger and H. Insam (2006). "Long-term effects of compost amendment of soil on functional and structural diversity and microbial activity." Soil Use and Management **22**: 209-218.

Rösch, C. and H. Bothe (2009). "Diversity of total, nitrogen-fixing and denitrifying bacteria in an acid forest soil." European Journal of Soil Science **60**: 883-894.

Rosendahl, S. (2008). "Communities, populations and individuals of arbuscular mycorrhizal fungi." New Phytologist **178**: 253-266.

Rosendahl, S. and E. Stukenbrock (2004). " Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences." Molecular Ecology **13**: 3179-3186.

Rosenzweig, M. (1995). Species diversity in space and time. Princeton, NJ, Cambridge University Press.

Rousseau, A., N. Benhamou, I. Chet and Y. Piché (1996). "Mycoparasitism of the extramatrical phase of *Glomus intraradices* by *Trichoderma harzianum*." Phytopathology **86**: 434-443.

Rubio, R., F. Boriea, C. Schalchlia, C. Castilloa and R. Azcón (2003). "Occurrence and effect of arbuscular mycorrhizal propagules in wheat as affected by the source and amount of phosphorus fertilizer and fungal inoculation." Applied Soil Ecology **23**: 245-255.

Ruiz-Lozano, J. M. and R. Azcon (2000). "Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity." Mycorrhiza **10**: 137-143.

Ruiz-Lozano, J. M., R. Azcón, M. Gomez and (1995). "Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses." Appl. Environ. Microbiol. **61**: 456-460.

Russo, A., C. Felici, A. Toffanin, M. Gotz, C. Collados, J. M. Barea, Y. Moenne-Loccoz, K. Smalla, J. Vanderleyden and M. Nuti (2005). "Effect of *Azospirillum* inoculants on arbuscular mycorrhiza establishment in wheat and maize plants." Biology & Fertility of Soils **41**: 301-309.

Ryan, M. H. and J. F. Angus (2003). "Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield." Plant and Soil **250**: 225-239.

Ryan, M. H. and J. H. Graham (2002). "Is there a role for arbuscular mycorrhizal fungi in production agriculture?" Plant and Soil **244**: 263-271.

Ryan, M. H., A. F. van Herwaarden, J. F. Angus and J. A. Kirkegaard (2005). "Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation by arbuscular mycorrhizal fungi." Plant and Soil **270**: 275-286.

Sala, V. M. R., S. D. Freitas and A. P. D. da Silveira (2007). "Interaction between arbuscular mycorrhizal fungi and diazotrophic bacterial in wheat plants." Pesquisa Agropecuaria Brasileira **42**: 1593-1600.

Santos, J. C., R. D. Finlay and A. Tehler (2006). "Molecular analysis of arbuscular mycorrhizal fungi colonising a semi-natural grassland along a fertilisation gradient." New Phytologist **172**: 159-168.

Sarathchandra, S. U., A. Ghani, G. W. Yeates, G. Burch and N. R. Cox (2001). "Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils." Soil Biology & Biochemistry **33**: 953-964.

Saxena, A. K., S. K. Rathi and K. Tilak (1997). "Differential effect of various endomycorrhizal fungi on nodulating ability of green gram by *Bradyrhizobium* sp. (vigna) strain s24." Biology & Fertility of Soils **24**: 175-178.

Schaller, B. T., T. Nemecek, B. Streit, U. Zihlmann, A. Chervet and W. G. Sturny (2007). "Bilan écologique comparatif du semis direct et du labour." Revue suisse Agric. **39**: 73-79.

Scheublin, T. R., K. P. Ridgway, J. P. W. Young and M. G. A. van der Heijden (2004). " Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. ." Applied & Environmental Microbiology **70**: 6240-6246.

Scheublin, T. R., R. S. P. Van Logtestijn and M. G. A. Van der Heijden (2007). "Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species." Journal of Ecology **95**: 631-638.

Schloter, M., O. Dilly and J. C. Munch (2003). "Indicators for evaluating soil quality." Agriculture Ecosystems & Environment **98**: 255-262.

Schmalenberger, A. and C. C. Tebbe (2003). "Bacterial diversity in maize rhizospheres: conclusions on the use of genetic profiles based on PCR-amplified partial small subunit rRNA genes in ecological studies " Molecular Ecology **12**: 215-262.

Schmidt, J. P., J. A. Lamb, M. A. Schmitt, G. W. Randall, J. H. Orf and H. T. Gollany (2001). "Soybean varietal response to liquid swine manure application." Agronomy Journal **93**: 358-363.

Schreiner, R. P. (2007). "Effects of native and nonnative arbuscular mycorrhizal fungi on growth and nutrient uptake of 'Pinot noir' (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus." Applied Soil Ecology **36**: 205-215.

Schüßler, A., H. Gehrig, D. Schwarzott and C. Walker (2001). "Analysis of partial *Glomales* SSU rRNA gene sequences: implications for primer design and phylogeny." Mycol. Res. **105**: 5-15.

Schwab, S. M., J. A. Menge and P. B. Tinker (1991). "Regulation of nutrient transfer between host and fungus in vesicular-arbusculare mycorrhizas." New Phytologist **117**: 387-398.

Scullion, J., W. R. Eason and E. P. Scott (1998). "The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grassland and grass-arable rotations." Plant & Soil **204**: 243-254.

Seguin, P., C. C. Sheaffer, N. J. Ehlke, M. P. Russelle and P. H. Graham (2001). "Nitrogen fertilization and rhizobial inoculation effects on kura clover growth." Agronomy Journal **93**: 1262-1268.

Selim, S., J. Negrel, C. Govaerts, S. Gianinazzi and D. van Tuinen (2005). "Isolation and partial characterization of antagonistic peptides produced by Paenibacillus sp strain B2 isolated from the sorghum mycorrhizosphere." Applied & Environmental Microbiology **71**: 6501-6507.

Serraj, R., T. R. Sinclair and L. C. Purcell (1999). "Symbiotic N2 fixation response to drought." Journal of Experimental Botany **50**: 143-155.

Sharpley, A., P. Kleinman and J. Weld (2004). "Assessment of best management practices to minimise the runoff of manure-borne phosphorus in the United States." New Zealand Journal of Agricultural Research **47**: 461-477.

Singer, J. W., K. A. Kohler, M. Liebman, T. L. Richard, C. A. Cambardella and D. D. Buhler (2004). "Tillage and compost affect yield of corn, soybean, and wheat and soil fertility." Agronomy Journal **96**: 531-537.

Singh, B. and R. Pandey (2003). "Differences in root exudation among phosphorus-starved genotypes of maize and green gram and its relationship with phosphorus uptake " Journal of Plant Nutrition **26**: 2391-2401.

Six, J., H. Bossuyt, S. Degryze and K. Denef (2004). "A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics." Soil & Tillage Research **79**: 7-31.

Sjöberg, J. (2005). Arbuscular mycorrhizal fungi: occurrence in Sweden and interaction with a plant pathogenic fungus in barley. Dept. of Crop Production Ecology. Sweden, Acta Universitatis agriculturae Sueciae**:** 53.

Sjöberg, J., P. Persson, A. Martensson, L. Mattsson, A. Adholeya and S. Alstrom (2004). "Occurrence of *Glomeromycota* spores and some arbuscular mycorrhiza fungal species in arable fields in Sweden." Acta Agric Scand B Soil Plant Sci **54**: 202-212.

Smalla, K., M. Oros-Sichler, A. Milling, H. Heuer, S. Baumgarte, R. Becker, G. Neuber, S. Kropf, A. Ulrich and C. C. Tebbe (2007). "Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments : Do the different methods provide similar results?" Journal of microbiological methods **69**: 470-479.

Smith, S. E. and D. J. Read (1997). Mycorrhizal Symbiosis, Harcourt Brace and Company.

Smith, S. E. and D. J. Read (2008). Mycorrhizal Symbiosis, Harcourt Brace and Company.

Smith, S. E., F. A. Smith and I. Jakobsen (2004). "Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake." New Phytologist **162**: 511-524.

Sood, S. G. (2003). "Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants." FEMS Microbiology Ecology **45**: 219-227.

Spiers, G. A. and W. B. McGill (1978). "Effects of phosphorus addition and energy supply on acid phosphatase activity in soils." Soil Biology & Biochemistry **11**: 3-8.

St-Arnaud, M. and A. Elsen (2005). Interaction or arbuscular-mycorrhizal fungi with soil-borne pathogens and non-pathogenic rhizosphere micro-organisms. In vitro culture of mycorrhizas. S. Declerck, D.-G. Strullu and J. A. Fortin. Berlin Heidelberg, Springer-Verlag**:** 217-231.

St-Arnaud, M., C. Hamel, B. Vimard, M. Caron and J. A. Fortin (1995). "Altered growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an *in vitro* dual culture system with the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots." Mycorrhiza **5**: 431-438.

St-Arnaud, M. and V. Vujanovic (2007). Effects of the arbuscular mycorrhizal symbiosis on plant diseases and pests. Mycorrhizae in crop production. C. Hamel and C. Plenchette. New York, Haworth Press**:** 67-122.

Stach, J. E. M., S. Bathe, J. P. Clapp and R. G. Burns (2001). "PCR-SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods." FEMS Microbiology Ecology **36**: 139-150.

Staddon, W. J., J. T. Trevors, L. C. Duchesne and C. A. Colombo (1998). "Soil microbial diversity and community structure across a climatic gradient in western Canada." Biodiversity and Conservation **7**: 1081-1092.

Statistics Canada. (2007). "Field and specialty crops (seeded area)." Retrieved Table 001-0010, 2008, from http://www40.statcan.ca/l01/cst01/prim11a.htm.

Steenwerth, K. L., R. E. Drenovsky, J. J. Lambert, D. A. Kluepfel, K. M. Scow and D. R. Smart (2008). "Soil morphology, depth and grapevine root frequency influence microbial communities in a Pinot noir vineyard." Soil Biology & Biochemistry **40**: 1330-1340.

Stevenson, F. J. and M. A. Cole (1999). Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients, John Wiley & Sons, Inc.

Streeter, J. G. (2003). "Effects of drought in nitrogen fixation in soybean root nodules." Plant, Cell and Environment **26**: 1199-1204.

Strickland, T. C. and P. Sollins (1987). "Improved method for separating light and heavy fraction organic matter from soil." Soil Science Society of America Journal **51**: 1390-1393.

Stukenbrock, E. H. and S. a. Rosendahl (2005). "Clonal diversity and population genetic structure of arbuscular mycorrhizal fungi (*Glomus* spp.) studied by multilocus genotyping of single spores.
." Molecular Ecology **14**: 743-752.

Subba Rao, N. S. (1985). "Effect of combined inoculation of *Azospirillum brasilense* and vesicular arbuscular mycorrhiza on pearl millet (*Pennisetum americanum*)." Plant and Soil **81**: 283-286.

Subramanian, K. S. and C. Charest (1995). "Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress." Mycorrhiza **5**: 273-278.

Subramanian, K. S. and C. Charest (1998). "Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery." Psysologia Plantarum **102**: 285-296.

Subramanian, K. S. and C. Charest (1999). "Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in

maize under drought-stressed and well-watered conditions." Mycorrhiza **9**: 69-75.

Subramanian, K. S., C. Charest, L. M. Dwyer and R. I. Hamilton (1995). "Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling." New Phytologist **129**: 643-650.

Subramanian, K. S., C. Charest, L. M. Dwyer and R. I. Hamilton (1997). "Effects of arbuscular mycorrhizae on leaf water potential, sugar content, and P content during drought and recovery of maize." Canadian Journal of Botany **75**: 1582-1591.

Sun, H. Y., S. P. Deng and W. R. Raun (2004a). "Bacterial community structure and diversity in a century-old manure-treated agroecosystem." Applied & Environmental Microbiology **70**.

Sun, H. Y., S. P. Deng and W. R. Raun (2004b). " Bacterial community structure and diversity in a century-old manure-treated agroecosystem." Applied & Environmental Microbiology **70**: 5868-5874.

Sykorova, Z., K. Ineichen, A. Wiemken and D. Redecker (2007). "The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment." Mycorrhiza. **18**: 1-14.

Talavera, M., K. Itou and T. Mizukubo (2001). "Reduction of nematode damage by root colonization with arbuscular mycorrhiza (*Glomus* spp.) in tomato-*Meloidogyne incognita* (Tylenchida : Meloidognidae) and carrot-*Pratylenchus penetrans* (Tylenchida : Pratylenchidae) pathosystems." Applied Entomology and Zoology **36**: 387-392.

Talukdar, N. C. and J. J. Germida (1993). "Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of saskatchewan, canada." Canadian Journal of Microbiology **39**: 567-575.

Tarkalson, D. D., V. D. Jolley, C. W. Robbins and R. E. Terry (1998). "Mycorrhizal colonization and nutrition of wheat and sweet corn grown in manure-treated and untreated topsoil and subsoil." Journal of Plant Nutrition **21**: 1985-1999.

Tejada, M. and J. L. Gonzalez (2006). "Crushed cotton gin compost on soil biological properties and rice yield." European Journal of Agronomy **25**: 22-29.

Ter Braak, C. J. F. and P. Smilauer (2002). CANOCO reference manual and canodraw for windows User's guide: Sofware for Canonical Community Ordination (version 4.5). Ithaca, NY, USA, Microcomputer Power.

Thomas, R. L., R. W. Shearc and J. R. Moyer (1967). "Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion." Agronomy Journal **59**: 240-243.

Thomson, B. D., A. D. Robson and L. K. Abbott (1991). "Soil-mediated effects of phosphorus supply on the formation of mycorrhiza by *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders on subterranean clover." New Phytologist **118**: 463-470.

Thygesen, K., J. Larsen and L. Bodker (2004). "Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by *Aphanomyces euteiches* using oospores as pathogen inoculum." European Journal of Plant Pathology **110**: 411-419.

Tian, C. Y., G. Feng, X. L. Li and F. S. Zhang (2004). "Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants." Applied Soil Ecology **26**: 143-148.

Tiquia, S. M., J. Lloyd, D. A. Hermsb, H. A. J. Hoitink and F. C. Michel (2002). "Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes." Applied Soil Ecology **21**: 31-48.

Toljander, J. F., J. C. Santos-Gonzalez, A. Theler and R. D. Finlay (2008). "Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial." FEMS Microbiology Ecology **65**: 323-338.

Topp, G. C. (1993). Soil water content. Soil sampling and methods of analysis. M. R. Carter. Boca Raton, FL, Lewis Publishers**:** 541-557.

Tracy, D. F. and D. A. Frank (1998). "Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem: Yellowstone National Park." Oecologia **114**: 556-562.

Troeh, F. R. and L. M. Thompson (2005). Soils and Soil Fertility 6th edition, Blackwell Publishing.

Troeh, Z. I. and T. E. Loynachan (2003). "Endomycorrhizal fungal survival in continuous corn, soybean, and fallow." Agronomy Journal **95**: 224-230.

Uhlmann, E., C. Gorke, A. Petersen and F. Oberwinkler (2004). "Arbuscular mycorrhizae from semiarid regions of Namibia." Canadian Journal of Botany **82**: 645-653.

USDA. (2007). "National statistics, crops and plants." 2008, from http://www.nass.usda.gov/About_NASS/index.asp.

Vainio, E. J. and J. Hantula (2000). "Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA." Mycological Research **104**: 927-936.

Vallino, M., N. Massa, E. Lumini, V. Bianciotto, G. Berta and P. Bonfante (2006). "Assessment of arbuscular mycorrhizal fungal diversity in roots of *Solidago gigantea* growing in a polluted soil in Northern Italy. ." Environmental Microbiology **8**: 971-983.

van der Heijden, M. G. A., J. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken and I. Sanders (1998). " Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity." Nature **69-72**.

van der Heijden, M. G. A., A. Wiemken and I. R. Sanders (2003). "Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant." New Phytologist **157**: 569-578.

van Elsas, J. D. and P. Garbeva (2002). "Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens." Biodegradation **13**: 29-40.

Vandenkoornhuyse, P., K. P. Ridgway, I. J. Watson, A. H. Fitter and J. P. W. Young (2003). "Co-existing grass species have distinctive arbuscular mycorrhizal communities." Molecular Ecology **12**: 3085-3095.

Vazquez, M. M., S. Cesar, R. Azcon and J. M. Barea (2000). "Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum,*

Pseudomonas, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants." Applied Soil Ecology **15**: 261-272.

Vierheilig, H., B. Bago, C. Albrecht, M.-P. Poulin and Y. Piché (1998a). Flavonoids and arbuscular mycorrhizal fungi. Flavonoids in the living system. J. A. Manthey and B. S. Buslig, Plenum Press**:** 9-33.

Vierheilig, H., A. P. Coughlan, U. Wyss and Y. Piche (1998b). "Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. ." Applied & Environmental Microbiology. **64**: 5004-5007.

Vigo, C., J. R. Norman and J. E. Hooker (2000). "Biocontrol of the pathogen *Phytophthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on infection loci." Plant Pathology **49**: 509-514.

Villegas, J. and J. A. Fortin (2001). "Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NH4+ as nitrogen source." Canadian Journal of Botany **79**: 865-870.

Villegas, J. and J. A. Fortin (2002). "Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NO3- as nitrogen source." Canadian Journal of Botany **80**: 571-576.

Vogelsang, K. M., H. L. Reynolds and J. D. Bever (2006). "Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system." New Phytologist **172**: 554-562.

Walker, C. and R. E. Koske (1987). "Taxonomic concepts in the endogonaceae: IV. *Glomus fasciculatum* redescribed." Mycotaxon: 253-262.

Walley, F. L. and J. J. Germida (1997). "Response of spring wheat (*Triticum aestivum*) to interactions between pseudomonas species and *Glomus clarum* NT4." Biology & Fertility of Soils **24**: 365-371.

Wang, Q. K., S. L. Wang and Y. X. Liu (2008). "Responses to N and P fertilization in a young Eucalyptus dunnii plantation: Microbial properties, enzyme activities and dissolved organic matter." Applied Soil Ecology **40**: 484-490.

Wei, D., Q. Yang, J. Z. Zhang, S. Wang, X. L. Chen, X. L. Zhang and W. Q. Li (2008). "Bacterial community structure and diversity in a black soil as affected by long-term fertilization." Pedosphere **18**: 582-592.

Weisskopf, L., N. Tomasi, D. Santelia, E. Martinoia, N. B. Langlade, R. Tabacchi and E. Abou-Mansour (2006). "Isoflavonoid exudation from white lupin roots is influenced by phosphate supply, root type and cluster-root stage." New Phytologist **171**: 657-668.

West, H. M. (1996). "Influence of arbuscular mycorrhizal infection on competition between *Holcus lanatus* and *Dactylis glomerata*." Journal of Ecology **84**: 429-438.

Whitelaw, M. A. (2000). "Growth promotion of plants inoculated with phosphate-solubilizing fungi." Advances in Agronomy **69**: 99-151.

Wieland, G., R. Neumann and H. Backhaus (2001). "Variation of microbial communities in soil, rhizosphere, and rhizoplane in response to crop species, soil type, and crop development." Applied & Environmental Microbiology **67**: 5949-5855.

Wu, Q. S. and R. X. Xia (2006). "Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions." Journal of Plant Physiology **163**: 417-425.

Wu, S. C., Z. H. Cao, Z. G. Li, K. C. Cheung and M. H. Wong (2005). "Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial." Geoderma **125**: 155-166.

Wu, T. H., D. O. Chellemi, J. H. Graham and E. N. Rosskopf (2008). " Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems." Soil Biology & Biochemistry **40**: 1967-1970.

Xavier, L. J. C. and J. J. Germida (2002). "Response of lentil under controlled conditions to co-inoculation with arbuscular mycorrhizal fungi and rhizobia varying in efficacy." Soil Biology & Biochemistry **34**: 181-188.

Xavier, L. J. C. and J. J. Germida (2003). "Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. *viceae* enhance pea yield and nutrition." Biology & Fertility of Soils **37**: 261-267.

Yao, Q., H. H. Zhu, J. Z. Chen and P. Christie (2005). "Influence of an arbuscular mycorrhizal fungus on competition for phosphorus between sweet orange and a leguminous herb." Journal of Plant Nutrition **28**: 2179-2192.

Yergeau, E., M. Filion, V. Vujanovic and M. St-Arnaud (2005). "A PCR-denaturing gradient gel electrophoresis (DGGE) approach to assess Fusarium diversity in asparagus." Journal of microbiological methods **60**: 143-154.

Yergeau, E., V. Vujanovic and M. St-Arnaud (2006). "Changes in communities of *Fusarium* and arbuscular mycorrhizal fungi as related to different asparagus cultural factors." Microbial Ecology **52**: 104-113.

Young, I. M. and J. W. Crawford (2004). "Interactions and self-organization in the soil-microbe complex." Science **304**: 1634-1637.

Young, I. M., J. W. Crawford and C. Rappoldt (2001). "New methods and models for characterising structural heterogeneity of soil." Soil and Tillage Research **61**: 33-45.

Yu, Z. T. and M. Morrison (2004). "Comparisons of different hypervariable regions of rrs genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis." Applied & Environmental Microbiology **70**: 4800-4806.

Zelles, L. (1999). "Fatty acid patterns of phospholipids and lipololysaccharides in the characterisation of microbial communities in soil: a review." Biology & Fertility of Soils **29**: 111-129.

Zhang, X. H., Y. G. Zhu, B. D. Chen, A. J. Lin, S. E. Smith and F. A. Smith (2005). "Arbuscular mycorrhizal fungi contribute to resistance of upland rice to combined metal contamination of soil." Journal of Plant Nutrition **28**: 2065-2077.

Zhang, X. H., Y. G. Zhu, A. J. Lin, B. D. Chen, S. E. Smith and F. A. Smith (2006). "Arbuscular mycorrhizal fungi can alleviate the adverse effects of chlorothalonil on *Oryza sativa* L." Chemosphere **64**: 1627-1632.

Zhong, W. H. and Z. C. Cai (2007). "Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay." Applied Soil Ecology **36**: 84-91.

Zhongqi, H., T. S. Griffin and W. C. Honeycutt (2004). "Phosphorus distribution in dairy manures." Journal of environmental quality **33**: 1528-1534

Zhu, Y. G., S. E. Smith, A. R. Barritt and F. A. Smith (2001). "Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars." Plant and Soil **237**: 249-255.

Ziadi, N., R. R. Simard, G. Allard and G. Lafond (1999). "Field evaluation of anion exchange membranes as a N soil testing method for grasslands." Canadian Journal of Soil Science **79**: 281-294.