DIMINUTION DES PERTES DU NITRATE PAR LIXIVIATION ET AUGMENTATION DE LA DIVERSITÉ MICROBIENNE DANS LES SYSTÈMES AGROFORESTIERS

par

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L'agroforesterie de type culture intercalaire (CI) est une pratique agricole alternative alliant rangées d'arbres et cultures conventionnelles. Supprimer la dichotomie entre agriculture et foresterie pourrait contribuer à rétablir une production domestique de bois de feuillu chancelante en plus de créer des bénéfices environnementaux. Parmi ces avantages on compte une augmentation de la qualité du sol. Cette dernière est souvent définie comme un amalgame de fertilité, biodiversité, contribution à la santé environnementale, qualité physique et absence de pathogènes.

Le présent travail s'inscrit au sein de l'effort d'un collectif de chercheurs tentant d'étudier les possibles avantages de l'adoption des CI au Canada. Plus particulièrement, les pages qui suivent s'intéressent à deux aspects de la qualité du sol: la diversité microbienne et à la réduction du lessivage des nitrates dans les sols des CI.

Dans le chapitre 1, l'utilisation des acides gras phospholipidiques des communautés microbiennes révèle une plus grande hétérogénéité spatiale (beta-diversité) microbienne dans une CI que dans une culture conventionnelle lorsqu'il y a une part d'argile assez importante dans le sol et/ou que les racines des arbres utilisés prolifèrent en surface. Bien que des tentatives de lier cette hétérogénéité à des propriétés physico-chimiques du sol et à une plus grande stabilité des communautés microbiennes demeurent pour l'instant infructueuses, ce travail a mis en lumière une présence accrue des mycorhizes arbusculaires dans les CI.

Dans le chapitre 2, l'utilisation de lysimètres permet de détecter, pour la période de mai à mi-octobre, une diminution d'au moins 81% des nitrates lessivant dans l'eau du sol lorsque les racines des arbres d'une CI sont préservées, en comparaison avec une section où les racines sont tranchées et isolées du système. Ce travail suggère que l'adoption des CI pourrait contribuer à diminuer le lessivage de ce polluant dans les zones agricoles du Québec.

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INTRODUCTION

En 2005 le Canada demeurait le plus important exportateur de produits forestiers au monde avec 17,3% du marché global (Ressources naturelles Canada, 2007). Bien qu'on associe traditionnellement la foresterie canadienne à la forêt boréale, un secteur contribuant de façon significative à cette industrie utilise le bois des feuillus. En effet, l'industrie du meuble crée 28000 emplois au pays et offre 30 fois plus d'emplois par unité de bois que tout autre secteur forestier (AFMQ, 2004). Cependant, ces industries font face à une compétition grandissante provenant de pays émergeants comme la Chine. En augmentant de 40% entre 1995 et 2002, les importations en bois de feuillu ont dépassé la production domestique canadienne et ceci reste un obstacle à la préservation d'une autosuffisance et d'une industrie forte (Plourde, 2000). Le déclin de cette autosuffisance est dû à différents facteurs. Par exemple, on estime qu'au Québec 200 plants de conifères sont mis en terre pour chaque feuillu planté alors que les produits provenant du bois de ces derniers représentent 25% des exportations du Canada (Parent et Fortin, 2003). Le problème principal auquel fait face la production de bois de feuillu est que l'habitat naturel de ces arbres se retrouve au sud du pays, une zone qui est déjà urbanisée mais surtout déjà exploitée par une agriculture intensive qui exclut les arbres.

Il existe une quantité importante de terres abandonnées au Québec. Des 3,2 millions d'hectares de terres agricoles utilisées en 1961 il ne restait qu'environ 50% exploitées en 2001 (Ruiz et Domon, 2005). Il serait envisageable d'introduire des plantations de feuillus de valeur sur ces terres marginales qui ne sont plus utilisées pour l'agriculture. Toutefois, on retrouve une dichotomie institutionnelle entre les secteurs de la forêt et de l'agriculture résultant des difficultés historiques dans l'attribution des territoires pour ces domaines et du fait que les parties concernées sont peu disposées à changer le zonage actuel. Pourtant, plusieurs peuples ont montré que ces deux activités ne s'excluent pas et les pratiques agroforestières combinant agriculture et arbres sur une même terre sont traditionnelles dans plusieurs pays d'Afrique et émergeantes en Europe. Combiner arbres et plantes agricoles pourrait contribuer à rétablir la production de feuillus, diminuer la pollution résultant de l'agriculture intensive et, surtout à la suite de certaines modifications aux programmes de subventions gouvernementales, augmenter les profits des producteurs (Graves *et al.*, 2007).

L'agroforesterie regroupe plusieurs pratiques : les pratiques sylvopastorales (arbres et bétail), les brises vents, les bandes riveraines, la récolte de produits forestiers non ligneux retrouvés dans les sous-bois et finalement les cultures intercalaires (CI) (Garrett et Buck, 1997). Parmi ces systèmes agroforestiers, les CI ont récemment été proposées dans l'est du Canada pour augmenter la production de feuillus et faire une meilleure utilisation des terres en friche (Rivest et Olivier, 2007). On mentionne plusieurs avantages environnementaux des CI comprenant une qualité du sol accrue (Garrett et McGraw, 2000). La définition de « qualité du sol » varie dépendamment de l'usage qu'on veut faire de celui-ci mais, outre les qualités physiques associées au drainage et à la structure, les écologistes réfèrent souvent aux caractéristiques biologiques de cette ressource;

- Fertilité; richesse et disponibilité des nutriments pour la croissance des plantes
- Santé; absence d'agents pathogènes dommageables
- Biodiversité; présence d'une variété d'espèces de micro-, méso- et macrofaune
- Contribution à la santé de l'environnement; capacité de filtrage ou de captage de polluants agricoles ou industriels

Ces aspects sont étudiés dans le cadre d'un projet tentant de démontrer les bienfaits des CI dans l'est du Canada. Lors de mes études à la maîtrise, j'ai participé à ce projet en contribuant à approfondir les connaissances scientifiques à propos des deux derniers points listés ci-dessus, soit la biodiversité du sol et la contribution du sol à la santé de l'environnement. En premier lieu, j'avais comme objectif de comparer la biodiversité des communautés microbiennes du sol des CI avec celle des communautés provenant de systèmes conventionnels. En deuxième lieu, je me suis intéressé à la possible réduction du lessivage des nitrates, un polluant agricole néfaste pour l'environnement, grâce à la présence d'arbres dans les CI. Ces deux objectifs représentent les deux chapitres de ce mémoire.

CHAPITRE 1

AVANT-PROPOS

Ce travail tente de répondre à trois questions reliées à la diversité microbienne des sols :

- Les cultures intercalaires augmentent-elles l'hétérogénéité spatiale (beta-diversité) des communautés microbiennes du sol? Et si oui;
    - Est-ce que ceci est dû à une hétérogénéité de certaines propriétés physico-chimiques du sol qui pourrait être créée par les arbres?
    - En résulte-t-il une plus grande stabilité des communautés microbiennes?

La beta-diversité est au niveau du paysage. Donc, on se pose la question « est-ce que la communauté diffère plus *d'un échantillon de sol à l'autre* dans la culture intercalaire? ». Cette hétérogénéité spatiale diverge du concept d'alpha-diversité. Dans ce dernier cas nous nous aurions posé la question « Y'a t'il généralement plus d'espèces (plus grande richesse) *à l'intérieur des échantillons de sol* de la culture intercalaire ». Le portrait des communautés microbiennes du sol a été obtenu à l'aide des acides gras phospholipidiques (PLFA) microbiens. Contrairement aux méthodes génétiques, les PLFAs ne permettent pas l'identification des espèces même si certains PLFAs sont associés à de grands groupes de microorganismes. Par contre, la méthode nous permet d'observer que le profil microbien de deux échantillons est différent et de tirer des conclusions sur l'abondance de ces grands groupes (bactéries Gram positif et Gram négatif, champignons, mycorhizes arbusculaires, etc.). De plus, l'étude des PLFAs peut se faire rapidement et était bien adaptée à la quantité d'échantillons à l'étude. À notre connaissance, il s'agit de la première étude comparant l'hétérogénéité spatiale des communautés microbiennes du sol de cultures intercalaires avec celle de cultures agricoles conventionnelles.

Dans tous les échantillons, nous avons aussi mesuré 11 propriétés physico-chimiques du sol (pH, matière organique, humidité, azote total, ratio carbone-azote, nitrates, ammonium et la concentration totale de quatre cations). Sachant que les arbres ont une influence sur leur environnement pédologique, l'acquisition de ces données avait pour but de trouver des mécanismes par lequel les arbres d'une culture intercalaire pourraient augmenter la beta-diversité microbienne. Malheureusement, les variables choisies n'ont pu expliquer la variation des profils de PLFAs.

Un autre aspect original de ce travail est le lien qu'il tente de faire entre la beta-diversité et la stabilité des communautés microbiennes. Comme il est mentionné dans l'introduction de l'article proposé qui suit, des chercheurs ont observé qu'une plus grande diversité microbienne dans un échantillon de sol offre plus de chances que certains groupes pouvant survivre à des conditions extrêmes seront présents. S'il s'avérait vrai qu'il existe une plus grande beta-diversité dans les cultures intercalaires, on pourrait alors poser l'hypothèse que cette hétérogénéité spatiale engendre une plus grande tolérance face à un stress. Nous avons donc utilisé des mélanges aléatoires d'échantillons de sols pour « capturer » la beta-diversité dans les deux systèmes et pour tester leur tolérance à un stress. Nous avons traité ces mélanges avec un taux grandissant de cuivre (métal lourd) et nous avons mesuré la biomasse microbienne afin de voir lesquels toléraient mieux ce stress. Bien que le résultat ne fut pas celui escompté, nous considérons l'idée et la méthode suffisamment intéressantes pour poursuivre cette initiative.

Pour ce travail, j'ai développé les hypothèses avec mon superviseur Dr. Robert L. Bradley et j'ai pris en charge tout le travail d'échantillonnage et de laboratoire avec l'aide de stagiaires. J'ai fait la plupart des analyses de données en plus d'être le rédacteur principal. J'ai utilisé la méthode d'extraction des acides gras dans le laboratoire de Dr. Bradley après l'avoir apprise dans le laboratoire de Dr. Chantal Hamel au SPARC d'Agriculture et Agroalimentaire Canada à Swift Current, Saskatchewan. Les Dr. Carole Beaulieu et Dr. William F.J. Parsons ont respectivement offert leurs connaissances en microbiologie et en statistiques. Le manuscrit sera soumis pour publication prochaine dans une édition spéciale sur l'agroforesterie du périodique « Agriculture, Ecosystems & Environment ».

DO TREE-BASED INTERCROPPING SYSTEMS INCREASE THE DIVERSITY AND STABILITY OF SOIL MICROBIAL COMMUNITIES?

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Abstract : We tested the hypothesis that tree-based intercropping (TBI) increases the diversity and stability of soil microbial communities compared to conventional mono-cropping systems (CC). Quadrats of TBI research plots in Saint-Rémi (Québec) and Guelph (Ontario) were intensively sampled along 56 point grid patterns and compared to adjacent CC systems. Phospholipid fatty-acids (PLFAs) were extracted from each sample, purified and methylated, and subsequently analyzed by gas chromatography. The spatial heterogeneity (i.e. microbial beta-diversity) of whole PLFA profiles from each field were analyzed using multivariate statistical methods (PCA, PERMDISP), and those of individual PLFAs were analyzed using Levene's and Moses' tests. Multiple regression analysis failed to reveal any soil physico-chemical variable that could significantly predict the PLFA data . We compared the concentrations of broad microbial groups in both cropping systems using T-tests and found a higher incidence of vesicular arbuscular mycorhizal (AM) fungi (both sites) and a greater Gram+ to Gram- ratio (St-Rémi site only) in TBI than in CC systems. In order to determine the stability of soil microbial communities, we monitored changes in microbial biomass of bulked soil samples from the sampling grids after these had been exposed to varying concentrations of a heavy metal (Cu) contaminant. Data were then fitted to decreasing exponential functions and first-order rate constants were used as indices of microbial tolerance (i.e. stability). At both sites, we found no significant difference in soil microbial stability of TBI and CC systems. Future research should investigate the possible role of trees in TBI systems to provide a nursery of AM inocula to the alley crops.

Key words: AM fungi, microbial diversity, microbial stability, PLFA, soil respirometry, tree-based intercropping

1.1. Introduction

Tree-based intercropping (TBI) systems represent a land-use approach incorporating rows of trees and traditional agricultural field crops. These have been proposed in eastern Canada as a means of increasing an insufficient hardwood production and optimising the use of abandoned fields (Rivest and Olivier, 2007). TBI systems are also expected to improve soil quality by increasing fertility and decreasing non-point source pollution (Garrett and McGraw, 2000). One aspect of soil quality that has so far been neglected is microbial diversity. In a previous study, it was shown that soil properties such as organic matter content and nitrogen mineralization were spatially structured according to the distribution of the different plant species (Thevathasan and Gordon, 2004). In addition, different plant species can be associated with different soil microbial communities (Nehl et al., 1997) and TBI systems have the added presence of trees. Consequently, we hypothesized that TBI systems increase the spatial heterogeneity (beta-diversity) of soil microbial communities compared to conventional cropping (CC) monoculture systems, and that this may be a result of a higher degree of spatial variation in TBI soil physico-chemical conditions. Here we also hypothesized that this higher microbial diversity would provide an array of species that may subsist over a wider range of environmental conditions. The link between diversity and stability has been observed in various studies (Griffiths et al., 2004; Girvan et al., 2005).

One way of characterizing soil microbial diversity is by analyzing soil extractable phospholipid fatty acids (PLFA). This is because different groups of microbes contain different proportions of specific PLFAs (Frostegård et al., 1993), and because these PLFAs do not persist in soil for long when microbial cells die. While PLFA profiles may vary over very small scales (< 1 cm soil distance) (Cavigelli et al., 1995), it may be presumed that these variations repeat themselves at larger scales in a homogeneous landscape such as conventional agricultural fields. In more complex plant communities, such as forests, microbial community structure may vary predictably at much larger scales according to the vegetation and other landscape features (Saetre and Bååth, 2000; Lamarche et al., in press). Hence, compared to CC systems, we expect a greater beta-diversity of PLFA patterns in TBI systems where different tree species and annual crops alternate over scales of several metres. In addition to allowing the test of heterogeneity, the PLFA data gives us the opportunity to compare the abundance of Gram positive bacteria, Gram negative bacteria, fungi and, more specifically, arbuscular mycorrhizae (AM) in CC and TBI systems because those broad microbial groups are associated with specific PLFAs (see Methods). Particularly, we can test the hypothesis that the alternative TBI agricultural land management should show higher rates of AM fungi. These microorganisms may survive better in TBI systems because of shallower tillage (Kabir et al., 1997) and the presence of perennial species that may act as AM inoculum nurseries (Kabir and Koide, 2000).

There has been evidence in the past that certain PLFAs were affected by soil physico-chemical properties. For instance, Ritz et al. (2004) found PLFA concentrations to be sensitive to the presence of sheep urine in the soil as well as pH. This is why, in the present study, every soil sample was also tested for a set of physico-chemical properties including acidity and nitrate concentrations. Since plants can change the physico-chemical environment of the soil (George et al., 2005), it is reasonable to expect a higher heterogeneity of these properties in TBI systems. An objective of this experiment was then to link heterogeneous physico-chemical properties to heterogeneous PLFA distribution. Based on the above examples, we at least expected PLFA profiles, and thus microbial diversity, to be sensitive to nitrates and/or pH.

Our study was also interested in testing the diversity-stability relationship that could exist in soil microbial communities of TBI and CC systems. We defined "microbial stability" as the tolerance of microbial communities to different levels of stress. Orwin and Wardle (2005) showed a relationship between plant community composition and the resilience-tolerance of soil microbial communities to a drying disturbance. Our experiment, however, attempts to first observe microbial diversity brought by TBI systems and then find a resulting ecological stability. We wanted the same samples used in the heterogeneity facet of the study to be used in the stress tolerance part of the experiment as we believed this would provide presumptive evidence that such a diversity-stability relationship exists. We consequently mixed randomly selected subsamples of the samples collected for the first experiment because mixtures of within-site soils would incorporate the inherent microbial beta-diversity of TBI and CC systems. Since our hypothesis was that TBI systems had a higher beta-diversity, we could now hypothesize that TBI systems would also have a higher microbial stability. The mixtures were treated with increasing concentrations of a heavy metal (Cu) and microbial biomass was later assessed. A biomass maintaining itself was considered the result of a more stable community.

1.2. Materials and methods

1.2.1. Study sites and sampling

The study compared soil microbial communities in a TBI and an adjacent CC field, at two study sites. The first study site is located near the town of Saint-Rémi (45° 16' N, 73° 36' W), Québec, Canada. Mean annual temperature is 6.2 °C and mean annual precipitation is 978.9 mm, of which 22% falls as snow (Environment Canada, 2006). The TBI field was created in 2000 using alternating rows of hybrid poplar (*Populus trichocarpa x deltoides* TD-3230, *Populus nigra x maximowiczii* NM-3729, *Populus deltoids x nigra* DN-3308) and hardwood tree species (*Juglands nigra, Fraxinus americana*) with 8 m spacings between rows (Rivest et al., 2005). Soybean (*Glycine max*) was grown between tree rows since 2004. This field was paired with a CC field, situated 1500 m northeast, which had been planted with a soybean monoculture for the last 3 years.

The second study site is located near the City of Guelph (43° 32' N, 80° 12' W), Ontario, Canada. Mean annual temperature is 7.5 °C and mean annual precipitation is 792.7 mm, of which 15% falls as snow (Environment Canada, 2006). The TBI field was created in 1987 using 10 tree species. The section sampled was bordered with rows of black walnut (*Juglans nigra*) and silver maple (*Acer saccharinum*) with 12.5 m spacings between rows (Thevathasan and Gordon, 2004). This field was paired with a CC field, situated 300 m southwest, which had been planted with the same crop rotation of maize, soybean, winter wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) as the TBI field. A summary of each field's site characteristics and physico-chemical properties is shown in Table 1.

In each field, a rectangular grid of 56 (7x8) sampling points was established between tree rows, with 1 m spacings between neighbouring sampling points. In late-August (St-Rémi) and early-September (Guelph) 2006, one 10 cm wide and 15 cm deep soil core was collected at each sampling point. The soil samples were immediately sieved (4 mm mesh) and transported on ice in coolers to the University of Sherbrooke where they were kept at –20 °C until analyzed.

1.2.2. Soil PLFA profiles and physico-chemical properties

PLFAs were extracted from all 224 soil samples within three months of freezing following the method detailed by Hamel et al. (2006). Briefly, field-moist soil subsamples (4 g dry-wt equiv.) were first extracted with 9.5 mL dicholoromethane (DCM):methanol (MeOH):citrate (1:2:0.8 v/v) buffer for 2 h, and then with 2.5 mL DCM plus 10 mL saturated sodium hydroxide solution for 5 min. The mixture was then centrifuged at 1000 g for 10 min and the lipid layer transferred to a vial. Soil subsamples were extracted a second time with 5 mL DCM:MeOH (1:1 v/v), and the lipid layer combined with the first extract. PLFAs were then collected by eluting with 2 mL MeOH through a silica-gel column after discarding other fractions eluted with 2 mL DCM and 2 mL acetone. The unlinked fatty acids (FAs) were N_2 gas-dried and methylated by adding approximately 0.5 mL of 1:25 (v/v) sulphuric acid:MeOH solution and heating at 80 °C for 10 min. Two mL of hexane were added to the vial and the mixture was vortexed for 30 sec, then the aqueous fraction was discarded. The same procedure was repeated with ultra-pure water before a known concentration of FA 19:0 (Sigma Aldrich) was added to the sample and used as an internal standard to quantify concentrations of the 30 detected and identified indigenous FAs. The extracts were dried down under N_2 gas and

Table 1: Site characteristics and means of soil properties in each experimental field (CC = conventional cropping; TBI = tree-based intercropping) measured in 2006. Values in parentheses are standard deviations (n=56).

	Site characteristics				Physico-chemical properties										
	%						%			$\mu g\ g\ soil^{-1}$		Total base cations $mg\ g\ soil^{-1}$			
	clay	sand	2006 crop	Tree spacing (m)	pH KCl	C:N	N	H_20	org. mat.	NO_3	NH_4	Ca	K	Mg	Na
St. Rémi															
CC	35	35	Soybean	-	6.82 (0.09)	10.79 (1.36)	0.20 (0.02)	0.17 (0.01)	4.08 (0.29)	2.64 (1.00)	1.08 (0.71)	8.08 (1.58)	9.01 (1.25)	4.06 (1.28)	3.10 (1.00)
TBI	35	35	Soybean	8	6.05 (0.17)	10.13 (1.58)	0.35 (0.24)	0.23 (0.03)	5.97 (0.49)	5.49 (1.73)	1.37 (0.81)	2.99 (0.59)	7.20 (1.47)	3.35 (0.68)	2.52 (1.39)
Guelph															
CC	20	50	Maize	-	7.14 (0.07)	13.36 (3.17)	0.19 (0.07)	0.15 (0.01)	4.51 (0.53)	2.06 (0.72)	0.64 (0.97)	8.76 (2.06)	2.84 (0.37)	5.17 (1.51)	1.69 (0.30)
TBI	20	50	Maize	12.5	7.10 (0.07)	15.57 (1.90)	0.16 (0.02)	0.14 (0.02)	4.61 (0.49)	2.60 (1.06)	0.58 (0.28)	9.57 (1.98)	3.05 (0.62)	8.49 (1.55)	1.20 (0.29)

dissolved in 100 μL hexane, then injected in a HP 6890 gas chromatograph equipped with a flame ionization detector and He as carrier gas. Microbial community structure at each sampling point was thus based on the concentrations of 30 identified FAs.

Each sample was also analysed for a variety of physico-chemical properties. Soil moisture was determined by weight loss after drying subsamples in a air-draft oven (101 °C) for 24 h. Soil pH was measured in 0.01 N KCl (1:3 v/v) using a standard hydrogen probe. Total C and N were obtained using a Macro Elemental CN analyser carbon-nitrogen analyser (Elementar Analysensysteme GmbH, Hanau, Germany). Organic matter content was estimated from total C using a conversion ratio of 1.9 (Nelson and Sommers, 1994). KCl (1.0 N) extractable NO_3^- and NH_4^+ concentrations were measured colorimetrically using a Technicon Autoanalyzer (Pulse Instrumentations, Saskatoon, Canada). Total base cations (K, Ca, Mg, Zn) were measured using an Analyst-100 atomic absorption spectrometer (Perkin-Elmer Corporation, Norwalk, U.S.A.).

1.2.3. Microbial stability

To assess microbial stability, we developed a test that monitors changes in microbial biomass (MB) after treating five bulk soil samples from each field (N=20) to increasing concentrations of a heavy metal (Cu) contaminant. Copper is a contaminant that could possibly be found in agricultural fields as it is a component of certain fungicides. Each bulked sample was comprised of five randomly chosen soil samples within each sampling grid. Each bulked sample was divided into 24 subsamples (24 g dry wt equiv.), and paired subsamples were then treated with 9 mL of aqueous solutions containing 0.000, 0.006, 0.012, 0.018, 0.024, 0.036, 0.060, 0.090, 0.120, 0.150, 0.180 or 0.240 g of $CuCl_2$. The soils were mixed with sterilised sand to retain air-filled pores and prevent water saturation. The optimal quantity of sand depended on soil texture in each field and was determined experimentally beforehand. The treated soils were left to incubate for 7 d after which microbial biomass was determined by substrate induced respirometry (SIR) (Anderson and Domsch, 1978). Briefly, 500 mg of a

22:3 talc:glucose mixture were mixed into each sample, these were sealed in jars and left to incubate for 100 min. The headspace was then flushed for 5 min, jars were sealed and left to incubate for another 30 min. Headspace air from each jar was sampled with a needle and a syringe for CO_2 concentration using a gas chromatograph (Chrompack Micro GC CP-2002P, Chrompack, Bergen op Zoom, Netherlands). CO_2 production was obtained by subtracting ambient CO_2 concentration from sampled CO_2 concentration. Microbial biomass was calculated using the equation developed by Anderson and Domsch (1978). The resulting data were fitted to decreasing exponential functions (Fig. 1), and the reciprocal of the estimated first-order rate constants (1/k) were used to denote microbial tolerance (i.e. stability) to heavy metal (Cu) stress.

1.2.4. Data analyses

PLFA data from Saint-Rémi and Guelph were analyzed separately by principal component analysis (PCA), and ordination biplots projecting the within-grid sampling locations on the first two principal components were used as a graphic measure of the degree of spatial heterogeneity within each field. To do so, the scores on the first two components of each field's samples were averaged and the standard deviation obtained. Six rare FAs were removed from this analysis because they contributed many zero values to the data matrix. In addition, the multivariate data set from each site was analyzed for dispersion with the PERMDISP software (Anderson, 2004a), a non-parametric and multivariate permutational extension of Levene's test for equality of variances. This test was performed on untransformed data using Euclidian distances. Finally, individual FAs within each sampling grid were analysed for equality of variance and dispersion using both Levene's test and Moses' test. The former is more robust than Bartlett's test for homogeneity of variance in the case of a departure from normality in the data and the latter is entirely non-parametric. The PCA and PERMDISP procedures were repeated on the set of 11 physico-chemical variables that were measured. However, this set of data was first standardised because the physico-chemical variables had widely different ranges and used different units. We then used DISTLM v.5 (Anderson, 2001; McArdle and Anderson, 2001; Anderson, 2004b) to try to link physico-chemical data to microbial (PLFA) data through multiple regressions as it is a multivariate data analysis software that could function with Euclidian distances (or any distance measure). This part of the analysis was limited to the St-Rémi sites where PLFA results concurred with our first hypothesis. CC and TBI fields were analysed independently to see if the same properties would control PLFA profiles. The PLFA concentrations of known microbial groupings (Table 2) were compared between field types at both locations using Student T-tests. Data was log-transformed when necessary to meet assumption of normality and homoscedasticity. Finally, indices of microbial stability (1/k values) in TBI and CC systems were also compared using a Student T-test.

Table 2: Soil PLFAs associated with specific microbial groupings.

Gram positive bacteria	i14:0, a15:0, i15:0, i16:0, i17:0, a17:0, br17:0, 10Me16:0, 10Me18:0
Gram negative bacteria	cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω7
Fungi	18:2ω6
Arbuscular mycorrhizae (AM)	16:1ω5

(Sources : Fostergard and Bååth, 1996; Sundh et al., 1997; Zelles ,1999; Hill et al., 2000; Madan et al., 2002; Diaz-Ravina et al., 2006; Balser et al. 2005; Hamel et al. 2006; Rinnan et al. 2007)

1.3. Results

The average total concentration of identified FAs in the 224 soil samples was 72 ng g^{-1}. Results from PCA showed TBI and CC samples from the St-Rémi site were segregated along the first principal component, with a greater dispersion of TBI samples along the second axis (Fig. 2.A). At the Guelph site, TBI and CC sample scores along both principal components had similar means and standard deviations (Fig. 2.B). The first two components at the St-Rémi and Guelph sites respectively explained 90% and 80% of the total variance in the data set. Results from PCA showed a clear segregation of samples from CC and TBI fields at both study sites locations, based on soil physico-chemical properties (Fig. 2.C and 2.D). The first

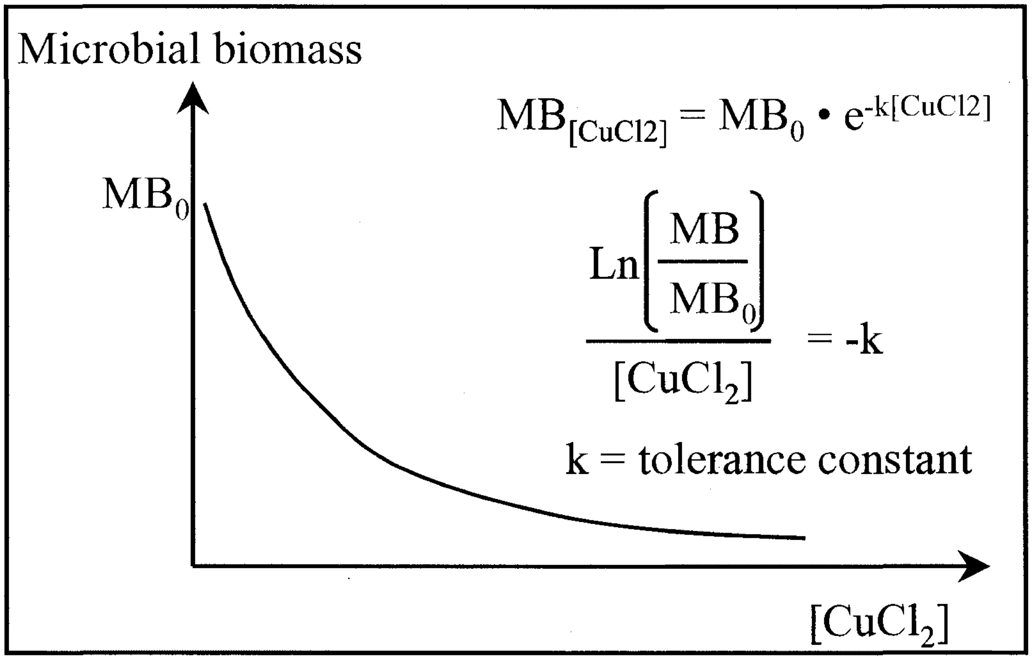

Fig. 1: Theoretical Relationship between soil microbial biomass (MB) and $CuCl_2$ concentration.

two components of the PCA procedure explained 60% of the variation in St-Rémi and 45% in Guelph. Biplot scores for the TBI field in St-Rémi appeared to be slightly more dispersed than in the CC field (Fig. 2.C), but the PERMDISP software revealed this difference to be only marginal (P=0.058). Dispersion in each field was not significantly different in Guelph (Fig. 2.D) and PERMDISP confirmed this (P=0.708). DISTLM v.5 performed on Saint-Rémi data reduced all FA variables to a few composite variables explaining most of the variation and regressed these on a model which used all 11 measured soil physico-chemical properties. Those multiple regressions linking physico-chemical data to PLFA profiles were insignificant.

According to T-tests, samples from the two TBI fields contained significantly higher concentrations of AM fungi than their corresponding CC fields (Fig. 3.A). In addition, soil samples from the St-Rémi TBI system had a significantly higher ratio of PLFAs originating from Gram positive bacteria (Fig. 3.B). Saint-Rémi TBI soils generally contained a higher concentration of all PLFAs than the other fields, consequently, T-tests that only revealed significantly larger amounts for this field were not shown. The G+:G- comparison was kept because it is a ratio which effectively eliminates the Saint-Rémi TBI higher PLFA concentration bias.

Microbial tolerance at the Guelph site could not be assessed because data failed to produce a significant fit to the decreasing exponential curve necessary to derive "k" values. In Saint-Rémi, "k" values were derived but did not differ significantly between CC and TBI fields (t=-0.334, P=0.747).

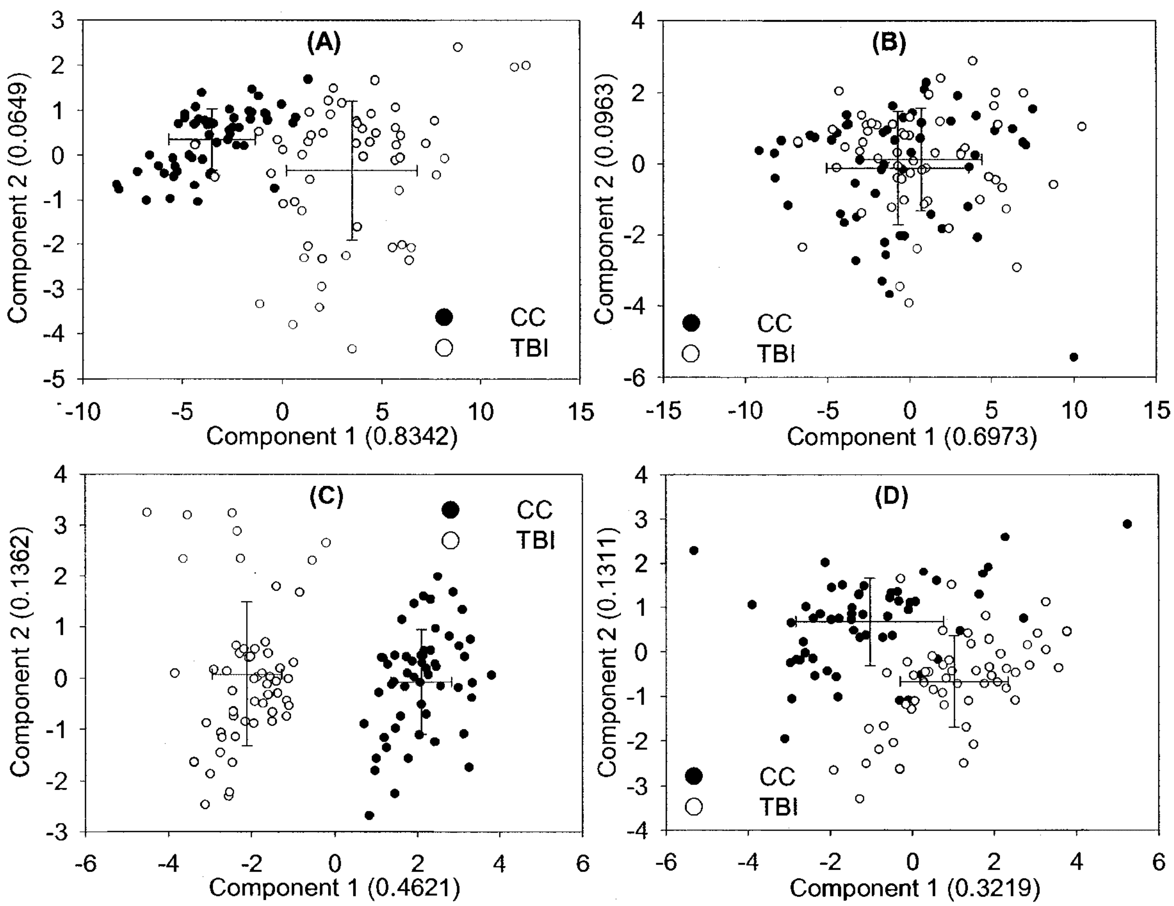

Fig. 2: Ordination biplots resulting from Principal Component Analysis (PCA) of 24 fatty acids found in TBI (white circles) and CC plots (black circles) in **(A)** St-Rémi and **(B)** Guelph, and 11 physico-chemical variables found in TBI and CC plots in **(C)** St-Rémi and of **(D)** Guelph. The proportion of the total variance explained by the first two principal components are shown in parentheses.

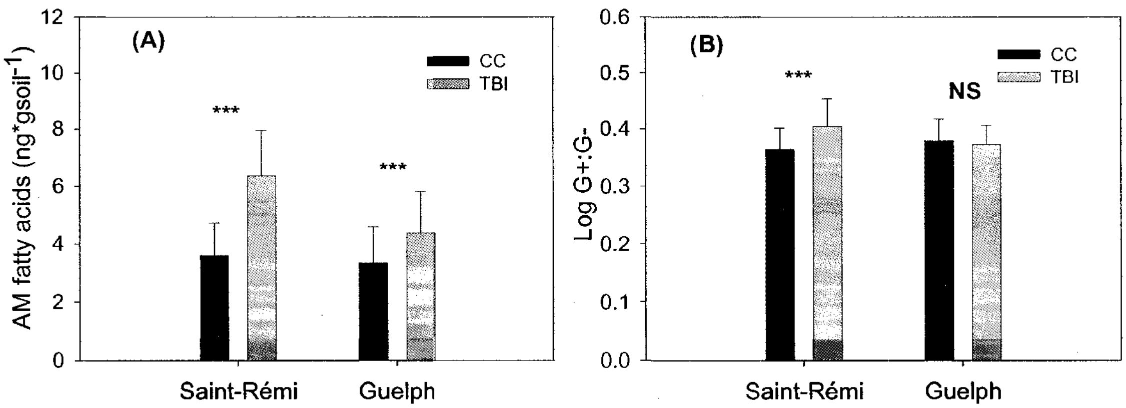

Fig. 3: Average (A) AM fatty acids and (B) log-transformed Gram positive to Gram negative fatty acid ratio (G+:G-) found in TBI (gray bars) and CC (black bars) plots in Saint-Rémi and Guelph. T-tests' significance levels are indicated by *($p<0.05$), **($p<0.01$), ***($p<0.001$) and NS ($p>0.05$, not significant)

1.4. Discussion

The combined results of PCA, PERMDISP, Levene and Moses tests on the PLFA data indicate that the spatial heterogeneity of soil microbial communities at the St-Rémi site is significantly higher in the TBI than in the CC system. We can only speculate as to why this was not so at the Guelph site. The most obvious differences between both sites are the types of crops that were grown previous to, and during, the summer of 2006, the tree species found on each TBI sites and the textural class of the soils. Further research should, therefore, strive to understand how these factors may interact with field-grown trees to control the spatial heterogeneity of soil microbial communities. For example, soybean is a crop that requires much less N fertilizer (30 kg N ha^{-1}) than corn (120-170 kg N ha^{-1}) (CRAAQ, 2003). The effect of high mineral fertilization rates may perhaps be greater than the effect of plant diversity on microbial communities. Hence, there may be a threshold fertilization rate over which plant effects are masked. Accordingly, Ruppel et al. (2007) found a higher prokaryotic phylogenetic diversity, and a higher diversity of substrate utilisation, in soils receiving low rates of mineral N fertilizers than in heavily fertilized soils. They hypothesized that mineral fertilization resulted in the selection of specialized microbial communities, hence in fewer functional groups. If this is so, then the possibility of reduced mineral fertilization in TBI systems due to the greater pools of soil organic matter derived from tree litter (Thevathasan and Gordon, 2004) could result in greater microbial diversity. Now concerning tree species, different plants are known to alter the microbial community in their soil environment (Nehl et al., 1997; Siciliano et al., 1998). While trees were more mature in the Guelph system, trees of the *Populus* genus as found in Saint-Rémi are known to produce more abundant superficial roots in the surface horizon than other species (Toky and Bisht, 1992; Puri et al., 1994). Combined with the smaller distance between tree rows on the Saint-Rémi TBI field, this could potentially explain the higher microbial heterogeneity found on that site. This is particularly important when considering that the effect of plants on soil microbial communities is mostly found in the rhizosphere (Baudoin et al., 2003; Renella et al., 2006). As for the possible effect of soil texture on microbial diversity, there is growing evidence that soil parent material may overshadow the effects of management practices (Yao et al., 2006; Lamarche et al., in press). For instance, Bossio et al. (1998) found that FAs associated to anaerobic bacteria were more present in agricultural soils with high clay content whereas FAs associated to aerobic bacteria and fungi were more likely to be found in sandier soils. This was the clearest determinant of PLFA profiles in a study that also tested the effect of changing fertilisation, management and season. We can surmise that a clay loam, such as found at the St-Rémi site, provides a wider range of soil particle sizes than a sandy loam, such as found at the Guelph site and, by implication, provides a wider array of microsites able to accommodate a wider range of bacterial niches. The textural class at the St-Rémi site is also apt to provide a better structured soil profile, again resulting in more diverse microsites for microbes. The higher clay content at the St-Rémi site is no doubt responsible for the higher organic matter content in TBI plots. Higher organic C provides more energy yielding substrates to the microbial community, and it has long been assumed that more energy cycling within a system allows more species to persist (Hutchinson, 1959). Higher organic C and a higher diversity of litter inputs in the TBI plots at St-Rémi are likely to increase catabolic diversity of microbial communities (Degens et al., 2000). Consequently, TBI as a means of enhancing microbial heterogeneity may be valid on heavier soils only.

Some of the results that did not concur with our hypotheses yielded interesting questions. First, knowing which soil characteristics control microbial communities would give us a hint on the mechanism through which trees of a TBI system may increase soil microfauna beta-diversity. However, regression procedures yielded no answers as to what soil physico-chemical properties determined PLFA profiles. We were consequently unable to say if the physico-chemical properties changing PLFA profiles were different between TBI and CC system. In addition, these soil environment data were at best only marginally more heterogeneous in TBI systems than in CC systems. It seems highly probable that the microbial heterogeneity was explained by soil characteristics that were not measured in this experiment. For example, root density is expected to vary more in a TBI system because of the presence of trees. It would thus be interesting to measure root density at each sampling point because, as discussed before, tree roots likely have an effect on microbial communities. Another unconfirmed hypothesis was the possibility of higher microbial stability in the TBI systems, although data seemed to suggest that a higher sampling effort might be required. It is likely that microbial biomass may not be the best ecological index to monitor in the fields we worked with. Griffiths et al. (2004) pointed out the unpredictability of diversity on ecological functions while giving examples where the removal or addition of specific species had evident impacts on particular functions. For instance, perhaps measuring soil nitrification after perturbation would have yielded interesting results.

In general, higher concentrations of FAs associated with AM fungi were observed in both TBI systems. Jeffries et al. (2003) have highlighted numerous studies demonstrating that AM mycorrhizae are important symbionts of plants and have been shown to decrease the frequency of plant diseases transmitted through soil pathogens, increase fertility and plant growth and improve physical characteristics of the soil. AM fungi have been the focus of an increasing amount of mycorrhizal studies (Klironomos and Kendrick, 1993; Kendrick, 2000) and it would be worthwhile to apply this research to agroforestry and to test whether certain trees act as a nursery of AM fungi inocula with benefits to annual crops. Plus, as mentioned in the introduction, the reduced tillage often associated with TBI systems could improve the viability of AM inocula in this type of land management (Kabir et al., 1997; Kabir et al., 1998). The

Saint-Rémi TBI system also had a significantly higher ratio of Gram positive bacteria in its soil. Gram positive organisms tend to have more capacity to sporulate (Doi, 1989) which gives them an advantage in adverse environmental conditions and they are also known for antibiotic production (many examples given by Martin et al. (2003)). They also include actinomycetes, a group of microorganisms known for their contribution to the formation of humic materials through the degradation of more complex substances like cellulose (Srinivasan et al., 1991). However, the G+:G- ratio was only marginally significantly higher in one TBI system and little can be said about the role of the TBI system in this.

The results of the study suggest that the spatial heterogeneity of soil microbial communities may increase when using TBI systems that include trees producing many superficial roots and/or are located on soils with high proportions of clay. Unfortunately, none of the measured soil physico-chemical properties could be linked to this heterogeneity and we could not observe a higher stability of TBI soil microbes. On the other hand, our data did reveal a more prominent presence of AM fungi in TBI systems. This, we believe, is a worthwhile lead for future research.

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CHAPITRE 2

AVANT-PROPOS

Nous nous tournons maintenant vers l'aspect « contribution à la santé environnementale » comme critère pour définir la qualité du sol. Comme on le sait, l'agriculture est une des sources significatives de polluants contribuant aux problèmes d'eutrophisations et d'algues bleues (Giani *et al.*, 2005). Toute nouvelle pratique agricole qui propose de diminuer la pollution agricole diffuse mérite donc d'être étudiée. Ici, nous nous sommes intéressés à la possibilité que les arbres d'une CI puissent réduire le lessivage du nitrate.

Cette expérience a été inspirée par le travail de Allen *et al.* (2004). Cette équipe a creusé des tranchées dans certaines parcelles agricoles pour enlever l'effet des racines de pacaniers dans une CI avec pacaniers et coton. L'absence des racines de pacaniers a eu comme résultat une hausse des concentrations en nitrates trouvées dans l'eau du sol sous la culture de coton. Il semblait donc que la présence des racines contribuait à une utilisation plus efficace des fertilisants en réduisant du même coup la pollution diffuse par les nitrates. Par contre, le système de Allen *et al.* (2004) était mature (arbres de 50 ans) et se trouvait en Floride où le climat diffère évidemment de ce que l'on trouve dans l'est du Canada. Nous avons donc répété l'expérience dans les conditions québécoises, où les systèmes de CI sont beaucoup plus jeunes (6 ans dans le cas présent) et où les extrêmes saisonniers sont plus grands.

Pour ce travail, j'ai pris en charge la construction du dispositif et du matériel requis pour l'échantillonnage appuyé par notre coordonnateur de laboratoire. J'ai aussi fait une partie de l'échantillonnage et des analyses de laboratoire avec l'aide de stagiaires. Les données hydrologiques ont été modélisées par le Dr. Paul Arp de l'Université du Nouveau-Brunswick. Je suis le rédacteur principal de cet article dont une version abrégée a été soumise aux actes du « International Symposiun on Soil and Plant Analysis 2007» et une version augmentée de nouveaux résultats sera soumise au « Canadian Journal of Soil Science ».

SIGNIFICANT REDUCTION OF SOIL NITRATE LEACHING IN A SIX-YEARS OLD TREE-BASED INTERCROPPING SYSTEM, IN SOUTHERN QUÉBEC.

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Abstract : We tested the hypothesis that tree roots of a tree-based intercropping (TBI) system in southern Québec act as a safety-net capturing nitrate leaching through the soil profile. Thirty-six lysimeters were installed along an alley of a TBI research plot near the town of Saint-Rémi (Québec). Tree roots on each side of the alley were trenched and isolated along the middle 18 water sampling devices. Water was sampled 11 times between May and November 2006. Daily water percolation, estimated with the ForHym hydrological model, was multiplied by estimated daily NO_3 concentrations to yield quantities of NO_3 leached per unit area. Conservative estimates indicate that the presence of tree roots decreased NO_3 leaching to the 60 cm depth of lysimeters by at least 5 kg NO_3*ha^{-1}, an 81% reduction, between May and October 2006. This suggests that TBI systems may significantly reduce soil nitrate leaching to water bodies around agricultural land. We propose repeating the experience on poorer and sandier soils which are currently common candidates for the implementation of TBI systems in Southern Québec.

Key words : agroforestry, lysimeters, nitrate leaching, safety-net hypothesis, tree-based intercropping

Short title : NO_3 leaching reduction in a Québec tree-based intercropping system.

2.1. Introduction

Nitrogen is acknowledged as the main plant growth limiting nutrient in most agricultural systems and is applied as a fertiliser to crops commonly grown in eastern North America in quantities often upwards of 100 $kg*ha^{-1}$ (Centre de Référence en Agriculture et Agroalimentaire du Québec (CRAAQ) 2003). However, research estimates of crop nitrogen use efficiency range between 30-50% (Raun and Johnson 1999). Consequently, nitrogen that is not absorbed in plant material is exported from the field through pathways such as volatilization, surface runoff or leaching to ground water leading to economic and environmental losses (Drury et al. 1996). Lately, the spread of noxious blue-green algae associated with fertilizer pollution in southern Québec lakes has been of particular interest (Giani et al. 2005).

We recently proposed the implementation of tree-based intercropping (TBI) systems in eastern Canada as means of improving soil quality (Rivest and Olivier 2007). Soil quality can be defined according to numerous criteria such as the role soil plays in maintaining unpolluted groundwater. TBI systems combine agricultural crops with rows of hardwood trees. It is possible that tree roots could capture leaching nitrates (NO_3) in what has been labelled "the safety-net hypothesis". Allen et al. (2004) confirmed the safety-net hypothesis in Florida with a cotton-pecan TBI system where the pecan trees were 50 years old. This system is obviously different from what can be found in eastern North America where TBI systems are still young and seasonal extremes are a reality. The present study is a root trenching experiment similar to that performed by Allen et al. (2004) where we tested the safety-net hypothesis with regards to NO_3 leaching in a 6 years old TBI system in southern Québec. While experiments have already shown the potential for soil NO_3 leaching reduction of *Salix* and *Populus* species (Vogeler et al. 2006), none have been performed in a northern climate TBI setting with trenches preserving the canopy. Because of the high water usage and fast growth found in the *Populus* genus (Pallardy and Kozlowski 1981), we hypothesized that the presence of young fast-growing hybrid poplars would be sufficient to reduce total NO_3 leaching in a TBI system.

2.2. Materials and methods

2.2.1. Study site and sampling material

The study site is located near the town of Saint-Rémi (45° 16' N, 73° 36' W), Québec, Canada. Mean annual temperature is 6.2 °C and mean annual precipitation is 978.9 mm of which 22% falls as snow (Environment Canada 2006). The TBI field was created in 2000 using alternating rows of hybrid poplars (*Populus trichocarpa x deltoides* TD-3230, *Populus nigra x maximowiczii* NM-3729, *Populus deltoids x nigra* DN-3308) which had an average diameter at breast height of 13.52 cm in 2006 and hardwood tree species (*Juglands nigra, Fraxinus americana*) which had an average height of 3,48 m in 2006 with 6 m spacings between rows (Rivest et al. 2005). Soybean (*Glycine max*) was grown between tree rows since 2004. The soil is a clay loam with 35% clay, 30% silt and 35% sand, a KCl measured pH of 6.05 and an organic matter content of 6.0%.

This study used lysimeters and trenches to determine the potential for tree roots in this TBI system to act as a safety-net against NO_3 leaching (Fig. 4). Lysimeters were built from 5.1 cm wide x 92 cm long PVC pipes ending with a high porosity ceramic cup obtained from Soil Moisture Equipment Corp. (Santa Barbara, CA, U.S.A.). In summer 2005, thirty-six lysimeters (numbered 1 to 36 from south to north) were installed at a depth of 60 cm and a distance of 150 cm from the stems of one row of hybrid poplars. The distance separating the lysimeters along the row varied between 4 m and 8 m. A 92 cm deep trench was dug at 1 m from the poplars' stems along lysimeters 10 to 27. The trench was lined with a double layer of polyethylene (0.15 cm thickness). A similar 60 cm deep trench was dug in the same fashion on the opposite side of the alley where hardwood trees were significantly smaller. Consequently, lysimeters 1 to 9 and 28 to 36 were installed in a zone where water seeped through soil presumably occupied by active tree roots (treatment "R") whereas lysimeters 10 to 27 collected water in a zone free of active tree roots (treatment "NR").

Three hundred and seventy-five $kg*ha^{-1}$ of 27-18-16 fertilizer were applied as prescribed for wheat production (CRAAQ 2003). However, this zone was left in fallow for 2006 because of unusually wet weather prohibiting intervention and was dominated by a dense cover of common weeds (mainly *Chenopodium album* and *Ambrosia alternifolia*) until the end of August. We assumed that these common agricultural weeds used soil resources at least as well as crops usually grown, and examples of this can be found in the literature (Andreasen et al. 2006, Lindquist et al. 2007).

Water was sampled on 11 dates between May 11th and November 8th 2006. Three drops of phenyl mercuric acetate (PMA) were added to each sample to prevent microbial alteration of NO_3 concentrations before the samples could be transported to the laboratory and frozen at minus 20°C. Samples were kept frozen until they were analysed colometrically for nitrate using a Technicon Auto-Analyzer (Pulse Instrumentations, Saskatoon, Canada).

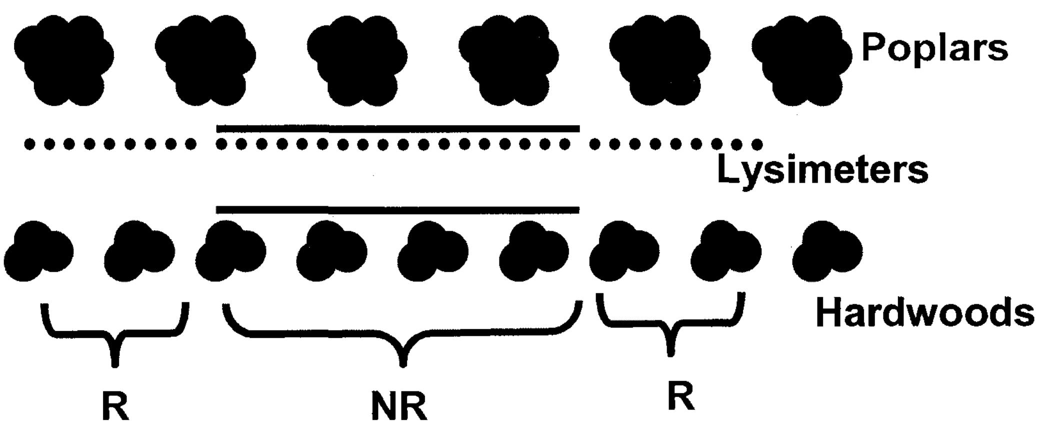

Fig. 4: Schematic overhead view of the study site showing the trenches (lines) in the midsection that were used to create a rootless zone for lysimeters (dots); R = roots treatment, NR = no roots treatment.

2.2.2. Data analysis

Water percolating through the soil profile throughout the season was estimated using the ForHym hydrological model (Balland et al. 2006). The ForHym model produces daily estimates of major water and heat flows in different ecosystem components from an input that includes weather, vegetation, aspect, soil and hydrological data. In our case, it produced two outputs: one accounting for and one ignoring the presence of trees. Consequently, the percolation estimates for soil around the lysimeters were different if the device was in the R or NR zone. For each lysimeter, NO_3 concentrations measured on each date were transformed into estimates of total NO_3 leached per hectare per sampling step. To do this, NO_3 concentrations found in one lysimeter at two consecutive dates were averaged and the result was taken as the average concentration for all dates between these two sampling dates (i.e. for this sampling step). Then, the daily estimates of water percolation (m^3*ha^{-1}) obtained with ForHym were multiplied by daily average NO_3 concentrations to yield a daily estimate of $g*NO_3*ha^{-1}$ leached. Those daily $g*NO_3*ha^{-1}$ were finally added up between the two sampling dates to yield estimated total $g*NO_3*ha^{-1}$ leached for this particular sampling step. These steps were then performed for all lysimeters and all sampling steps. This yielded a population of 18 R and 18 NR estimates of NO_3 leaching for each of the 10 sampling steps (i.e. 11 sampling dates = 10 sampling steps). A repeated measures ANOVA was used to test for treatment effects while accounting for within-lysimeter variability. Data was log-transformed prior to analyses. Since significant within-subjects effects of time and time x treatment were found (Table 3), each date was subsequently individually analysed with a T-test.

2.3. Results

There was a significant difference in the average NO_3*ha^{-1} leached for 8 of the 10 sampling steps (Fig. 5) and the NR treatment yielded consistently higher NO_3 leaching estimates. If the differences are summed for those 8 time periods, it appears that the presence of tree roots reduced NO_3 leaching to the studied depth by 17.7 $kg*ha^{-1}$. If we ignore the last sampling period (which yielded noticeably larger numbers), we still obtain a cumulative difference of

4.7 kg*ha^{-1}. This represents at least an 81% NO_3 leaching reduction when active tree roots were preserved.

Table 3: Repeated measures ANOVA testing the effect of poplar root presence, sampling date, and their interaction, on soil nitrate leaching (W. λ = Wilk's lambda).

Treatment	Source of variation	df	MS	F-value	Prob > F
Between subjects	Root presence (R)	1	95.56	35.10	.0001
	Error	34	2.72		
Within subjects (univariate test)	Time (T)	9	10.84	29.75	.0001
	T x R	9	6.74	18.50	.0001
	Error (time)	306	.36		
		df	W. λ	F-value	Prob > F
Within subjects (multivariate test)	T	9, 26	.0410	67.51	.0001
	T x R	9, 26	.1450	17.03	.0001

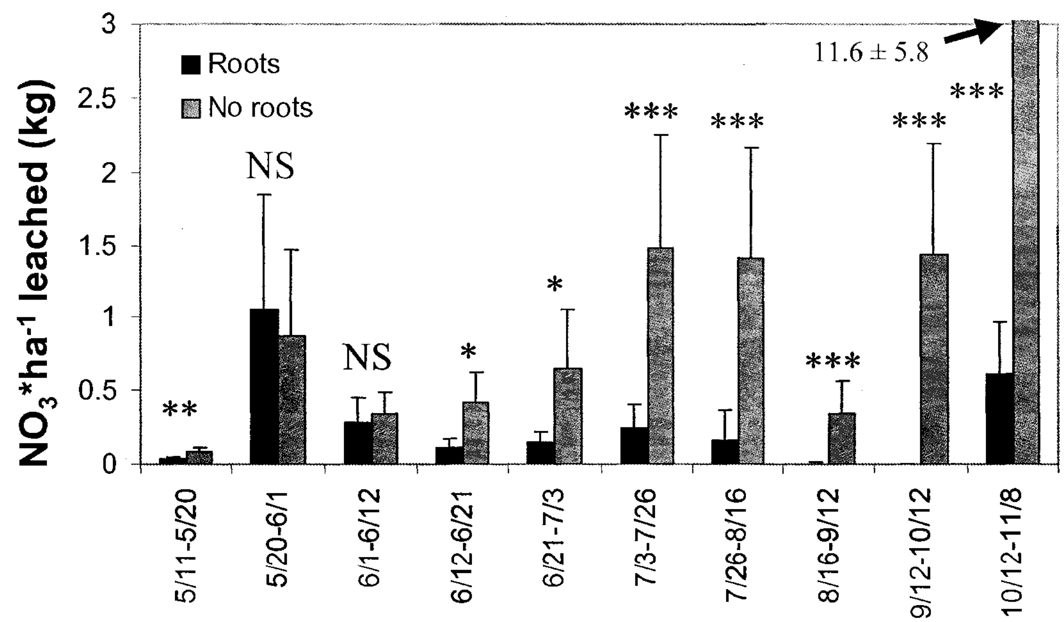

Fig. 5: Average NO_3*ha^{-1} leached per sampling step (n=18) in trench vs. non-trench plots. Error bars represent standard deviation. T-tests' significance levels are indicated by *(p<0.05), **(p<0.01), ***(p<0.001) and NS (p>0.05, not significant). Tests were performed on log-transformed data.

2.4. Discussion

By the end of the season, there was a significant reduction in the amount of NO_3 having reached the 60 cm depth when tree roots were kept intact. This 81% reduction compares with the results of Allen et al. (2004) who found seasonal cumulative reductions of 48% at 0.3 m depth and 71% at 0.9 m depth. In 2001, the average size of a Québec farm was 106 ha (Statistics Canada 2001). If such a farm producing wheat (i.e. same fertilisation rate as used here) adopted TBI practices, the more conservative figures of the present study estimate a reduction of 530 kg of NO_3 reaching 60 cm and threatening to enter groundwater between May and October. Yet two factors hint to the fact that actual NO_3 leaching reduction may be underestimated. First, this conservative figure does not include the mid-October to November time range. With this period included, NO_3 leaching reduction estimates for the same 106 ha field may go up to 1,800 kg. The numbers for this period may have been high because of the larger sampling step which was necessary because of logistical reasons. We recommend a higher sampling effort for fall leaching since results were promising. Second, because of the experimental design some of the lysimeters on the edges of the NR zone appeared to be affected by neighbouring trees from the R zone (Fig. 6). Consequently, some of the samples of the NR zone may have had lower NO_3 concentrations than they would have in a more isolated design where we could have further distanced the NR and R treatments.

The hydrological model has an important effect on the estimates of NO_3 leaching but its output seemed to corroborate with visual inspection of the field studied. Much drier soils were observed in the R zone for the time periods where ForHym estimated very little water leaching for that treatment. Even before any sample analyses were carried, the difference in soil humidity between the R and NR zones were evident. This may have implications regarding plant yields but that aspect could not be studied because of logistical reasons mentioned earlier. There is however evidence that light may be of greater importance when it comes to competition between common trees such as poplars and sugar maples (*Acer saccharum*) and common crops such as corn (*Zea mays*) and soybean (*Glycine max*) (Reynolds et al. 2007). The goal should be a balance between reasonable pollution abatement and acceptable plant yields. Choosing tree species with different water regimes or changing field stem density are options for fine-tuning the system. Another possible confounding factor is the N input from the dead trenched roots in the NR treatment, but part of the role of fertilisation in this study was to override this effect. Dead roots may also cause a flush of available C affecting N cycling. A study in southern Québec, however, demonstrated that any C flush created by trenching forest plots was not detectable less than one year after the treatment (Lavoie and Bradley 2003). Finally, while we realise that the placement of lysimeters and trenches in the TBI system is not statistically optimal, the current scarcity of such developed systems in eastern Canada and the destructive nature of the experiment inhibits larger ventures.

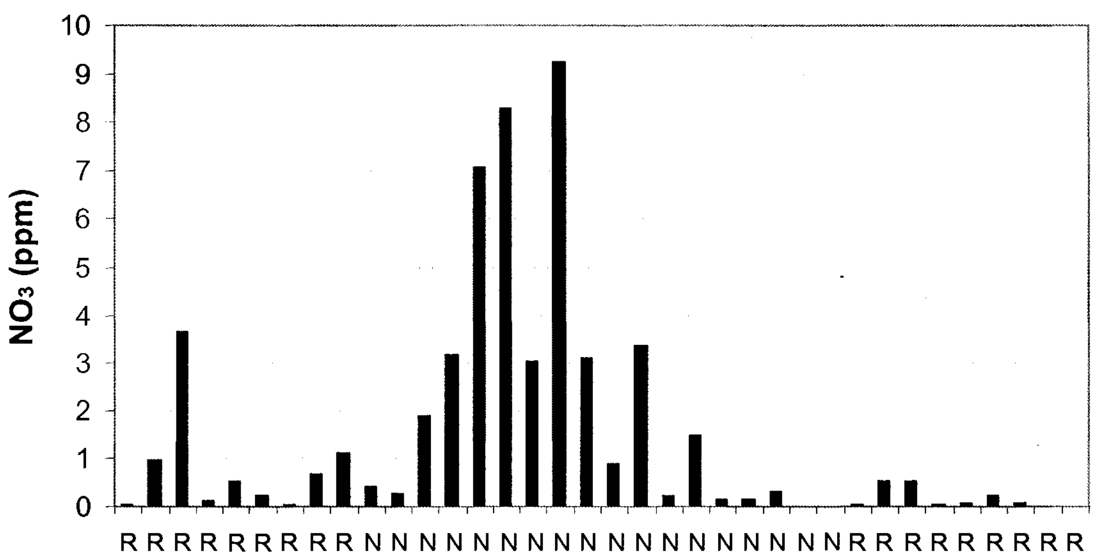

Fig. 6: Example of NO_3 concentrations found in the water sampled from the transect of 36 lysimeters on June 21st 2006. R = roots, N = no roots.

The results of this study suggest TBI systems may be considered as a way to significantly reduce NO_3 percolation in Québec agricultural lands. Still, there are additional research avenues that should be considered. For instance, the soil of the study site contained a significant portion of clay. It would be of interest to repeat the experiment on sandier soils as often found in marginal lands commonly proposed as suitable for TBI systems. In a Danish field experiment, with the same drainage and fertilisation rates, Simmelsgaard (1998) observed decreasing NO_3 leaching with increasing clay content. On the other hand, nitrogen mineralization and availability to plants can be higher in soils with finer textures (Reich et al. 1997) than those impoverished marginal soils suggested as TBI sites, although a certain level of drainage is required. These two possibly counterbalancing observations may affect NO_3 leaching and absorption in ways that warrant further investigation in TBI systems with sandier soils.

2.5. Acknowledgements

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CONCLUSION

Les travaux constituants ce mémoire ont atteint leurs objectifs en mettant en lumière deux façons par lesquelles les CI semblent pouvoir améliorer la qualité des sols agroforestiers au Québec. Premièrement, nous avons observé que le sol d'une CI pouvait contenir des communautés microbiennes plus diverses spatialement que celui d'une culture conventionnelle. Ce même travail a aussi soulevé d'autres hypothèses et avenues de recherche originales concernant l'origine de cette hausse en diversité et les conséquences possibles sur la stabilité des communautés microbiennes. Deuxièmement, nous avons pu observer que les racines d'arbres présentes dans le sol des CI semblent contribuer au captage des nitrates lessivant en profondeur. Ces bénéfices pour la diversité de la microfaune et la qualité de l'eau pourront être bonifiés par l'ajout de résultats provenant d'études sur la fertilité, les qualités physiques et les pathogènes du sol au sein d'un effort collectif pour réussir à décrire la qualité du sol dans les systèmes agroforestiers québécois. Il faut noter que les résultats présentés dans ce texte ont aussi mis en évidence l'importance du type de sol quant aux conséquences écologiques de l'utilisation des CI. Il faut envisager l'avenir de la recherche sur les CI en prévoyant des dispositifs expérimentaux situés sur une variété de conditions pédologiques.

Maintenant que nous avons montré que les CI peuvent affecter la communauté microbienne du sol, il est logique de poursuivre sur la lancée microbiologique. Par exemple, nous avons tenté d'expliquer la diversité microbienne par l'effet des plantes et des arbres, mais il serait aussi intéressant d'explorer l'avenue inverse; l'effet des microorganismes d'une CI sur les plantes. Nous sommes maintenant particulièrement intéressés par la conservation des mycorhizes arbusculaires dans les CI. La présence accrue de ces champignons pourrait être bénéfique pour les plantes agricoles tout en améliorant d'autres aspects de la qualité du sol. L'investigation du chapitre 2, quant à elle, pointe vers un élargissement de l'envergure des expériences sur la qualité de l'eau dans les CI. La pollution agricole diffuse est un sujet qui retient l'attention des décideurs, et exposer l'utilisation des CI comme une pratique agricole pouvant diminuer ce type de pollution pourrait faire découvrir ce système agronomique et sylvicole à une nouvelle tranche de la population.

Le sol n'est qu'une des multiples composantes des systèmes écologiques complexes que représentent les CI. Ces dernières ne pourront être adoptées à plus grande échelle au Québec que lorsqu'il sera clair pour les producteurs agricoles qu'elles sont économiquement viables. Ce but ne pourra être atteint que par la poursuite des recherches tentant de confirmer que les effets positifs de symbiose et de facilitation supplantent les effets négatifs de compétition et de logistique agricole. Dans un contexte où la qualité de l'eau et de l'air est au centre des discussions, la promotion du retour des arbres dans le paysage rural québécois va de soi. Il en est tout autrement pour la population de producteurs agricoles qui a longuement trimé pour enlever les arbres et souches qui occupaient autrefois ce même paysage. Il faudra répondre aux besoins des différents groupes pour qui la modification des pratiques conventionnelles résulte en des conséquences bien différentes.

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