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# EXPERIENCES FROM SOUTHERN QUEBEC PROVIDE ECOLOGICAL INSIGHTS FOR THE IMPLEMENTATION OF TREE-BASED INTERCROPPING SYSTEMS

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**Abstract:** Recent experiments in Quebec aimed to provide scientific arguments favouring the adoption of tree-based intercropping (TBI) systems in eastern Canada's rural landscape. The specific objectives of this research were to: (1) test whether a soybean intercrop between tree rows increases soil microbial biomass C, soil N fertility and tree growth compared to a harrowing treatment between tree rows; (2) determine the effects of the soybean intercrop on the diversity of arbuscular mycorrhizal fungi (assessed using SSU rRNA genes) compared to harrowing; and (3) determine whether TBI increases soil microbial beta-diversity (assessed using extractable phospholipid fatty acid (PLFA) profiles) compared to an adjacent conventional monocropping system. The TBI field was set in 2000 in southwestern Quebec using alternating rows of hybrid poplars (*Populus* spp.) and hardwood species spaced 8 m apart. Soybean (*Glycine max* L. (Merr.)) was grown between tree rows since 2004. During the 2005-2006 study period, soil microbial biomass C and N availability were higher in the soybean intercrop treatment or on par with those in the harrowing treatment. Hybrid poplar biomass increment in the soybean intercrop treatment was greater by 51 % to that in the harrowing treatment and diagnostic of leaf nutrient status indicated that hybrid poplars were positively affected by the increase in N supply provided through intercrop management. The arbuscular mycorrhizal fungal diversity, as expressed by the Shannon-Wiener indices, were 0.82 for the soybean and 0.70 for hybrid poplar under the soybean intercrop treatment, and 0.53 for hybrid poplar under the harrowing treatment. The neighboring trees and soybean plants hosted different arbuscular mycorrhizal fungal communities, suggesting that TBI systems may enhance arbuscular mycorrhizal fungal richness compared to monocultures. The soil microbial beta-diversity, based on a measure of dispersion among the PLFA profiles within each sampled grid, was higher in the TBI than in the conventional soybean monocropping system, suggesting that TBI systems could play an important ecological role in the conservation of microbial functions (e.g., nutrient cycling), even in the face of wide variations of environmental conditions and inputs.

**Key Words:** arbuscular mycorrhizal fungi, hybrid poplar, soil microbial beta-diversity, soil quality, soybean, tree growth

## INTRODUCTION

Tree-based intercropping systems (TBI), that combine widely spaced rows of trees and agricultural field crops, have shown great potential in terms of biodiversity conservation, carbon sequestration and water quality enhancement (Thevathasan et al. 2004). Interactions between trees and intercrops growing in close proximity may also include positive effects on soil quality and nutrient availability (Jose et al. 2007). These effects will depend on factors such as fertilization of the intercrop and mulching to prevent weed competition near tree rows. In this study, the effects of a soybean intercrop on indices of soil quality such as microbial biomass and inorganic N, as well as on hybrid poplar growth, were measured.

Because of their role in plant nutrient uptake and growth, arbuscular mycorrhizal fungi are of particular importance in agricultural and tree plantations systems. It has been pointed out that crop plants may facilitate mycorrhizal colonization and growth of recently planted tree seedlings (Enkhtuya and Vosatka 2005). In turn, the trees in TBI systems, as well as the adjacent herbaceous strip below them, could act as a permanent reservoir of arbuscular mycorrhizal fungal propagules (Ingleby et al. 2007), potentially benefiting future crop plants. In this study, molecular approaches were used to provide insights into the community diversity of arbuscular mycorrhizal fungi.

It is also known that the integration of trees into cropping systems may increase soil organic matter content due to the deposition of tree leaves and the turnover of their fine roots (Peichl et al. 2006), which may help to improve soil microbial activity. Differences in litter quality between trees and crops may also result in the development of distinct microbial communities, especially when compared to the rows of trees (Mungai et al. 2005). Soil microbial diversity is an important aspect of soil quality since it may ensure higher redundancy and stability of soil ecological functions. In this experiment, soil microbial beta-diversity was studied using extractable phospholipids fatty acids analysis.

The objective was to provide ecological insights on the interest of implementing TBI systems in southern Quebec, where they had not been tested yet.

## MATERIAL AND METHODS

### **Experimental site and field operations**

The experimental site was established near the town of St-Rémi (45°14' N, 73°40' W), in southern Quebec, Canada. Alternating rows of hybrid poplars (*P. nigra* x *P. maximowiczii*) and high-valued hardwood species (*Juglans nigra* L., *Fraxinus americana* L.), spaced 8 m apart, were planted in 2000. Twenty-cm cuttings of hybrid poplars were planted every 2 m along the row. Seedlings of hardwood species were planted every 3 m along the rows. In the winter of 2006, a thinning permitted the spacing between hybrid poplars in each row to be increased from 2 to 6 m.

Two treatments were introduced in June 2004: a TBI system comprising a soybean intercrop and a harrowed tree plantation system without intercrop. Each plot was bounded on each side with a row of hardwood species, with two alleys in the middle separated by one row of hybrid poplars.

Soybean (cv. S03-W4) was sown with a no-till planter on June 11, 2005 and July 3, 2006. Before sowing, the soil was amended with a N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O fertilizer (300 kg ha<sup>-1</sup> of 5-27-24 in 2005 and 275 kg ha<sup>-1</sup> of 9-24-21 in 2006). In the harrowing treatment, the herbaceous vegetation was harrowed (0-10 cm depth) on June 1 and July 14 in 2005, and on June 26 in 2006. Along each tree row, herbaceous vegetation was controlled by means of a 1.5 m-wide black polythene-film mulch.

### Soil sampling and analyses

For soil microbial biomass C and inorganic N, 16 soil cores (33 mm diameter, 0-15 cm depth) were collected in each of eight 288 m<sup>2</sup> (18 m x 16 m) plots on several dates along transects at 0 m (beneath the mulch), 2 m and 5 m from hybrid poplar rows (east side), and bulked into two composite samples per distance. Sampling dates were June 27, August 8, October 2, 2005, and July 30, August 29, September 21, 2006 for microbial biomass C, and July 20, September 6, 2005, and July 30, August 29, September 21, 2006 for inorganic N.

Microbial biomass C was determined by substrate-induced respirometry (SIR) (Anderson and Domsch 1978). Fresh subsamples (ca. 32 g dry mass equiv.) were placed into 500 ml plastic containers and amended with ground and sieved (65 μm) glucose (1000 μg C g<sup>-1</sup>) (Bradley and Fyles 1995). The amendments were applied as 250 mg mixtures with talc and dispersed throughout the soil subsamples using a spatula. Amended subsamples were transferred into 126 ml gas sampling jars and left uncovered for 100 min to reach optimum SIR rates. Subsamples were then flushed for 5 min with ambient air, sealed for 30 min, and headspace air was analyzed for CO<sub>2</sub> concentration using a GC. SIR rates were converted to microbial biomass C using equations derived by Anderson and Domsch (1978).

Inorganic N (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) concentrations were determined by extracting fresh soil subsamples (ca. 20 g) in 100 ml of 1 M KCl solution (1 h on a reciprocal shaker), filtering the extracts (Whatman No. 5), and analyzing the filtrates colorimetrically for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> using a Technicon autoanalyzer (Pulse Instrumentation, Saskatoon, SK, Canada).

For the study of the diversity of arbuscular mycorrhizal fungi, soil cores were collected from twelve 864 m<sup>2</sup> (54 m x 16 m) plots on July 20, 2005. Live roots were collected from each of the 192 samples. Details on the analysis procedure are provided in Chiffot et al. (2009).

For the study of the microbial beta-diversity, a rectangular grid of 56 sampling points, spaced 1 m apart, was established between tree rows. Soil cores (10 cm wide, 15 cm deep) were collected in late August 2006. Details on the analysis procedure are provided in Lacombe et al. (2009).

## **Hybrid poplar biomass increment and nitrogen response efficiency**

Hybrid poplar height and diameter at breast height (DBH) were measured at the end of each growing season (November) between 2004 and 2006. The aboveground biomass (stem + branches) of each tree was estimated following a generalized hybrid poplar biomass equation by Ben Brahim et al. (2000). In each plot, the total hybrid poplar biomass increment ( $\text{Mg DM ha}^{-1}$ ) was calculated as the increase in dry mass between November 2004 and November 2006. This value was scaled up assuming a stand density of 313 and 104 hybrid poplars  $\text{ha}^{-1}$  in 2005 and 2006, respectively.

The ratio of plant production to available N, referred as nitrogen response efficiency (NRE, Bridgham et al. 1995), was estimated in each plot, assuming that net N-mineralization (ammonification + nitrification) over a 28 d aerobic incubation constituted a good index of plant-available N. The amounts of mineralized N were averaged across the five incubation periods, converted to an areal basis using bulk density, then reported in  $\text{kg ha}^{-1}$  and multiplied by the number of growing-days, assuming a frost-free period of 161 days in 2005 and 158 days in 2006, respectively.

## **Statistical analyses**

Within each sampling date, the effects of treatment and distance from the hybrid poplar row on soil microbial C and inorganic N were tested by means of analysis of variance (ANOVA) for a randomized complete block, split-plot design with the treatment as the main plot, and distance as the subplot factor. The effects of treatments on hybrid poplar biomass increment and NRE were analyzed using one-way ANOVA. Significantly different means were separated using Tukey's multiple comparison test.

Arbuscular mycorrhizal fungal diversity was evaluated using the Shannon-Wiener index. Two-way ANOVA and Tukey's multiple comparison test were used to compare diversity among hosts (hybrid poplars and soybean) and distances from the hybrid poplar row (0.5, 2, 3.5 and 5 m). Univariate ANOVA tests following multivariate analyses of variance (MANOVA) were performed to assess the effect of soybean on the relative abundance of phylotypes associated with hybrid poplar roots.

Regarding soil microbial beta-diversity, the multivariate data set was analyzed with PERMDISP software (Anderson 2004), a non-parametric and multivariate permutational extension of Levene's test for equality of variances.

## **RESULTS**

### **Effects of a soybean intercrop on soil microbial biomass C, soil N fertility and tree growth**

During the 2005-2006 study period, soil microbial biomass ranged from 0.23 to 0.92  $\text{g C}_{\text{mic}} \text{kg}^{-1}$ . Across all distances from hybrid poplar trunks, soil microbial biomass C in the soybean intercrop treatment was on par with that in the harrowing treatment in all of the six sampling dates, except

for the last one in September 2006, when microbial biomass was 27 % higher in the soybean intercrop plots than in the harrowing plots ( $P = 0.04$ ).

During the same period of time, soil N availability was also higher in the soybean intercrop treatment or on par with that in the harrowing treatment. Soil inorganic N, that ranged from 3.5 to 38.4  $\mu\text{g mg}^{-1}$ , was higher, across all distances, in the soybean intercrop treatment than in the harrowing in two of the five sampling dates (July and August 2006,  $P = 0.001$  and  $P = 0.04$ , respectively).

Hybrid poplar biomass increment in the soybean intercrop treatment was greater by 51 % to that in the harrowing treatment (6.29 vs 4.17  $\text{Mg ha}^{-1}$ ,  $P = 0.01$ ). NRE in the TBI treatment was 47 % higher than that in the harrowing treatment (17.70 vs 12.05  $\text{kg dry mass kg}^{-1} \text{N}_{\text{mineralized}}$ ,  $P = 0.007$ ).

### **Effects of a soybean intercrop on the diversity of arbuscular mycorrhizal fungi**

The highest arbuscular mycorrhizal fungal diversity occurred under the soybean intercrop and the lowest under the hybrid poplars of the harrowing treatment. The arbuscular mycorrhizal fungal diversity, as expressed by the Shannon-Wiener indices, were 0.82 for soybean and 0.70 for hybrid poplar under the soybean intercrop treatment, and 0.53 for hybrid poplar under the harrowing treatment. However, differences between the hosts and between distances from the trunk were not statistically significant.

The arbuscular mycorrhizal fungal community structure was also studied for soybean and hybrid poplars under the TBI system, and for hybrid poplars under the harrowing treatment. Under the TBI system, nine phylotypes were associated with soybean roots and ten with hybrid poplar roots, among which seven were common to both plants. Eight phylotypes were found on hybrid poplar roots under the harrowing treatment.

Analysis of the composition of the arbuscular mycorrhizal fungal phylotypes by MANOVA showed a significant effect of the plantation system (soybean intercrop or harrowing) on the arbuscular mycorrhizal fungal community of hybrid poplar roots ( $F=4.81$ ,  $P=0.0276$ ).

### **Effects of a TBI system on soil microbial beta-diversity**

The soil microbial beta-diversity in the St-Rémi site, based on a measure of dispersion among the PLFA profiles within each sampled grid, was higher in the TBI system than in the conventional soybean monocropping system. According to the PERMDISP procedure, a significantly higher dispersion of PLFA profiles was observed in the TBI system than in the conventional soybean monocropping system.

## DISCUSSION

### **Effects of a soybean intercrop on soil microbial biomass C, soil N fertility and tree growth**

Soil microbial biomass tended to be higher in the soybean intercrop plots than in the harrowing plots at the end of the first growing season, and was higher in the soybean intercrop than in the harrowing treatment at the end of the second growing season. This could be due to a greater quantity and higher quality of plant litter. The litter inputs in the harrowing plots mainly came from hybrid poplar and some weeds while, in soybean intercrop plots, they came from both hybrid poplar and soybean residues. Since soybean litter is generally of higher lability than tree litter (Mungai and Motavalli 2006), soybean residues would result in higher nutrient turnover rates that would benefit hybrid poplar growth.

The increase in soil inorganic N in the soybean intercrop plots on some sampling dates is consistent with observations made by Wang et al. (2005) in subtropical TBI systems. Since the amount of fertilizer that was applied in the present study was relatively low (it was designed to deliver no more than 15 % of total crop requirement), the increase in soil  $\text{NO}_3^-$  in 2006 was due to the decomposition and mineralization of soybean residues that had been incorporated in June 2006. Thus, soybean intercrop would have the potential to improve soil nutrient supply for the trees.

It also seems that the soybean intercrop treatment may outperform the harrowing treatment in terms of hybrid poplar growth, since hybrid poplar biomass increment was greater in the soybean intercrop treatment. Diagnostic of leaf nutrient status indicated that hybrid poplars were positively affected by the increase in N supply provided through intercrop management.

### **Effects of a soybean intercrop on the diversity of arbuscular mycorrhizal fungi**

The results of the study show that the diversity of arbuscular mycorrhizal fungi was not statistically different from one host to another. However, co-occurring trees and crops showed differences in their respective arbuscular mycorrhizal fungal communities. Jansa et al. (2002) pointed out that the Shannon-Wiener index may not properly reflect the community structure, especially when the differences in abundance between the species are large, as was the case in the present study. Nevertheless, the diversity of arbuscular mycorrhizal fungal phylotypes tended to be higher in soybean roots than in hybrid poplar roots. This supports the idea that arbuscular mycorrhizal fungi would show host preference in the presence of co-occurring plant roots.

The results of the study also suggest that intercropping hybrid poplars with soybean positively affects the diversity of arbuscular mycorrhizal fungi colonizing tree roots as compared to the harrowing treatment. Although not statistically significant, the diversity of arbuscular mycorrhizal fungal phylotypes found on hybrid poplar roots tended to be higher under the TBI system than under the harrowing treatment. The possible role of co-occurring roots of soybean in facilitating arbuscular mycorrhizal fungal colonization of trees via ERM links (Enkhtuya and Vosatka 2005) should be considered.

## Effects of a TBI system on soil microbial beta-diversity

The higher heterogeneity that was observed in the TBI system as compared to the conventional soybean monocropping system suggests that TBI systems could play an important ecological role in the resistance of microbial functions (e.g. nutrient cycling) to a wide variation of environmental conditions and inputs.

## CONCLUSION

This study provided some evidence that TBI systems involving a soybean intercrop treatment, as compared to the harrowing treatment, could lead to increased soil quality by increasing microbial biomass and N fertility. It also showed that the soybean intercrop management significantly benefited hybrid poplar growth.

This study also suggests that a soybean intercrop could positively affect the arbuscular mycorrhizal fungal community structure of hybrid poplars, as compared to the harrowing treatment. The enhancement of soil microbial diversity could result in a greater conservation of microbial functions in the face of environmental stresses.

All these results suggest that TBI systems could be a valuable alternative to conventional tree plantation in terms of ecological functions and tree growth.

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