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Tree-based intercropping may reduce, while fertilizer nitrate may increase, soil methane emissions

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ABSTRACT

Tree-based intercropping (TBI) systems have shown some promise in mitigating greenhouse gas emissions, such as by sequestering carbon and decreasing soil nitrous oxide emissions. However, the effects of TBI on soil methane fluxes remain unknown. In a field study, we failed to show differences in soil CH₄ production between TBI and conventional mono-cropping (CM) systems. Within TBI plots, however, we found significantly lower CH₄ concentrations near the middle of the alleys than closer to tree rows. Soil CH₄ concentrations also decreased with soil depth, even dipping below mean global atmospheric concentrations. Laboratory assays revealed a higher CH₄ oxidation potential in soils collected from TBI plots compared to CM plots. These assays also revealed a decrease in CH₄ oxidation potential after soils were amended with nitrate. We conclude that TBI could potentially reduce soil CH₄ emissions whereas fertilizer nitrate may increase them.

Key words: Hardwood trees, hybrid poplar, mineral fertilizers, nitrate, soil depth, soil methane, and tree-based intercropping

INTRODUCTION

With growing awareness of global warming, agricultural land-management systems are increasingly scrutinized for their ability to reduce greenhouse gas (GHG) emissions to the atmosphere. Among these, tree-based intercropping (TBI) is an agroforestry system comprising widely spaced tree rows bordering annual alley crops. When compared to convention monocropping (CM) systems, TBI could mitigate GHG emissions in several ways. For example, Bambrick et al. (2010) reported a 12% increase in soil organic C (i.e. lower net CO₂ balance with the atmosphere) after 21 years of intercropping hybrid poplar (*Populus deltoides* (Bartr. ex Marsh.) × *P. nigra* L.) within a maize-soybean crop rotation system. This increase in soil organic C suggests a higher plant litter throughput within the system, as stable soil organic carbon originates primarily from microbial compounds produced during litter decomposition (Lützow et al. 2006; Mambelli et al. 2011; Cotrufo et al. 2013). For their part, Beaudette et al. (2010) found a 65% reduction in soil nitrous oxide (N₂O) emissions over two growing seasons, 4-5 years after planting rows of hybrid poplar, white and green ash (*Fraxinus americana* L. and *F. pennsylvanica* Marsh.) and red oak (*Quercus rubra* L.), with canola (*Brassica napus* L.) as the alley crop. The authors proposed that higher evapotranspiration rates of trees may have increased soil aeration during rainy periods thereby reducing anaerobic soil microsites where denitrification occurs.

Besides CO₂ and N₂O, agricultural soils may also produce methane (CH₄), another important greenhouse gas. CH₄ is about 28 times more efficient than CO₂ at trapping the earth's thermal radiation (Myhre et al. 2013), and atmospheric concentrations of this gas have steadily risen over the past 200 years (Cubasch et al. 2013). Studies have shown that a considerable amount of CH₄ may be emitted from soils that have been amended with livestock manure (Kim et al. 2014; Praeg et al. 2014). There is, therefore, an interest in agricultural practices that could reduce CH₄ emissions from manure amended soils. For example, management practices that increase native soil organic carbon could likewise stimulate soil methanotrophs (Gauthier et al., 2015), which could in turn reduce net CH₄ emissions. As TBI systems can increase soil C storage compared to CM systems (Bambrick et al. 2010), they could also potentially decrease soil CH₄ emissions.

In non-waterlogged soils, CH₄ emissions may occur over short intervals while remaining undetected for extended periods (Sommer and Fiedler 2002; Kernecker et al. 2015). Emissions

are caused by slight shifts in the equilibrium between gross CH₄ production and consumption rates, both of which can be orders of magnitude greater than net rates (Bradley et al. 2012). These shifts in equilibrium are, in turn, caused by a variety of environmental factors, notably the availability and concentration of various nutrients and electron acceptors (Gauthier et al. 2015). Accordingly, the application of inorganic fertilizers such as sulfate, nitrate or phosphate were shown to affect net soil CH₄ emissions in different ways (e.g. Pozdnyakov et al. 2011; Jugnia et al. 2012; Gauthier et al. 2015). There is a need, therefore, to test for possible interactive effects of cropping systems (i.e. TBI vs. CM) and inorganic fertilizers on soil CH₄ fluxes.

We report on a study that compared CH₄ fluxes at the soil surface, and at two soil depths, in horse manured TBI and CM plots. Since soil CH₄ emissions in the field may be difficult to detect, we also performed laboratory assays to assess the effects of TBI and soil depth on CH₄ oxidation potential. As an extension to these *in vitro* assays, we also tested the effects of various inorganic fertilizers on CH₄ oxidation potential.

METHODS AND MATERIALS

Experimental field site

The field study was conducted in 2012 on TBI and CM plots that had been established in 2004 on a farm near the town of St-Paulin (Québec, Canada; 46°27'N, 72°59'W). The region is characterized by a mean annual temperature of 4°C and a mean annual precipitation of 1113 mm (Environment Canada 2013). During 2012, average monthly air temperature was 12.1, 18.7, 21.0, 20.3, 14.3, and 9.1 °C respectively for May, June, July, August, September and October. The soil is classified as a Dystric Brunisol (Soil Classification Working Group 1998), with a sandy loam texture and a mean pH in water of 5.94.

The experiment was conducted as a complete randomized block design with two treatments (TBI vs. CM) replicated in three blocks. Each TBI plot (60 m × 24 m) consisted of one central hardwood row of alternating red oak (*Quercus rubra* L.) and black cherry (*Prunus serotina* Ehrhart) trees, bounded 12 m on either side by a row of fast-growing hybrid poplars (*P. deltoides* x *P. nigra*). These TBI plots were established in 2004, with hardwood seedlings planted at 3 m intervals along the rows and poplar cuttings planted at 2 m intervals. A 1 m strip on either side of each tree row had been covered by biodegradable plastic mulch at the time of planting, and was since left uncultivated. In 2012, poplar and hardwood rows were ca. 11 m and 4 m tall

respectively. The CM plots were identical and used the same crop rotation as the TBI plots, however no trees were planted in these plots.

Since 2004, horse manure was applied annually as the only soil amendment to all plots, whereas tree pruning residues were applied to TBI plots only. Over the course of eight years leading up to our study, crop rotations in the alleys consisted of oat (*Avena sativa* L.) and buckwheat (*Fagopyrum esculentum* Moench) in 2004-2005, buckwheat and canola in 2006, and buckwheat alone in 2007. These annual crops were followed by four years (2008–2012) of forage production (*Trifolium pratense* L. and *Phleum pratense* L.).

***In situ* CH₄ measurements**

Soil CH₄ measurements in TBI plots were made at 5 sampling points along a transect, which ran perpendicular to the tree rows. One sampling point was positioned < 1 m of the hardwood tree line, two points at 4 m on either side of this row, and two additional points at 2 m from each row of hybrid poplar. In the CM system (no trees), sampling points were located at the same distances from the plot edge as those used in the TBI plots.

At each sampling point, two 50 cm long gas access tubes, made of PVC tubing with a 0.5 cm internal diameter, were installed at 12.5 and 25 cm depth. Gas sampling was performed at both soil depths and at the soil surface, on days following a rainfall in July, August and September 2012. Gas access tubes were flushed with ambient air for 10 min with a plastic syringe, sealed with a septum, and soil atmosphere was sampled 1 h later using gas-tight syringes. Surface CH₄ emissions over 1 h were measured using closed-top PVC chambers (15 cm dia. x 22 cm height) equipped with septa. All gas samples (5 mL) were immediately injected into evacuated 3 mL Exetainer® vials (Labco Ltd., Lampeter, Ceredigion, UK) and transported to the laboratory where CH₄ concentrations were quantified using a CP-3800 gas chromatograph (Varian Canada, Mississauga, ON) equipped with a flame ionization detector.

***In vitro* CH₄ oxidation potential**

On 10 September 2012, bulk surface soil samples (0–20 cm depth) were collected next to each gas sampling location in each plot. These 30 samples were transported to the laboratory in coolers, sieved to pass a 2 mm mesh screen, and stored at 4 °C pending further analyses. Soil

subsamples were oven-dried to constant mass (48 h at 105 °C) to determine gravimetric moisture content. All soil samples were then adjusted to 30% moisture content.

In vitro CH₄ oxidation potential was measured on the sieved soil samples, after dividing these into four treatment groups. Thus, four soil subsamples (30 g oven-dry mass equivalent) from each sampling location were amended either with nitrate (0.25 mg g⁻¹ as KNO₃), phosphate (0.042 mg g⁻¹ as KH₂PO₄) or sulphate (0.086 mg g⁻¹ soil as K₂SO₄) salts, or were left unamended (controls). These nutrient treatments were roughly equal to field applications of 140 kg N ha⁻¹, 177 kg P₂O₅ ha⁻¹ and 175 kg K₂O ha⁻¹ respectively, assuming a crop rooting depth of 30 cm and an average soil bulk density of 1350 kg m⁻³. These rates compare well with prescribed fertilizer rates (CRAAQ 2010) for forage prairie crops growing on sandy loam soils in Quebec (i.e. 75-160 kg N ha⁻¹, 60-90 kg P₂O₅ ha⁻¹ and 150-300 kg K₂O ha⁻¹). The soil amendments were added as 0.5 g mixtures with talc, including the controls (0.5 g talc only). The amended soil subsamples were allowed to sit in ambient air for 30 min and then transferred into 500 mL mason jars. The jars were then sealed with gas-tight lids equipped with septa and the headspace was injected with 8 mL of a 500 ppm CH₄. This yielded an internal CH₄ concentration of ca. 18 ppm in each jar at the beginning of the incubations. After 15 min (t = 0 h), 1 mL of headspace gas was withdrawn from each jar and injected into the GC, as described above. The same procedure was repeated after 6, 24, 48 and 168 h. These incubations were performed at ambient room temperature (ca. 22 °C).

Statistical Analyses

When required, data were Box-Cox transformed prior to analysis (Box and Cox 1964) in order to improve the normality of their distributions.

A preliminary three-way ANOVA was used to test the main and interactive effects of management system, soil depth and sampling date. As this test revealed no significant differences in *in situ* CH₄ fluxes between sampling dates, the data were then pooled over sampling dates prior to further analyses. We next used two-way ANOVA to test the effects of management system and soil depth on *in situ* CH₄ fluxes, while pooling data across tree row distance (i.e. tree row distance is only relevant to TBI plots). We then analysed the principal and interactive effects of tree row distance and soil depth, using only the data collected from TBI plots. The significance level for both analyses was set to $\alpha = 0.05$. When treatment effects were declared significant, treatment means were separated using Student-Newman-Keuls and Bonferroni post-hoc tests.

Two way ANOVAs were used to test the interactive effects of cropping system and inorganic fertilizer amendments on the *in vitro* CH₄ oxidation potential (i.e. CH₄ oxidation after 168 h incubation). As no significant interactions were detected, three separate Repeated Measures ANOVA (RMANOVA) were performed on the *in vitro* CH₄ oxidation potential data, with each analysis emphasizing the importance of one particular factor as the between-subject (main treatment) effect, and time as the within-subjects factor. First, we pooled data across nutrient amendments and tested the effect of tree row distance in TBI plots only. Secondly, we pooled the data across cropping systems (TBI vs CM) and tree row distance (i.e. across sampling locations in the CM plots), and tested the effects of the four nutrient amendments. Thirdly, we pooled data across tree row distance and nutrient amendments and tested the effect of management system (TBI vs. CM) on CH₄ oxidation potential. All statistical analyses were performed using SPSS 11.01 (SPSS Inc., Chicago, IL.) software.

RESULTS AND DISCUSSION

We found no effect of management system, nor any interaction between management system and soil depth, on *in situ* CH₄ concentrations. In TBI plots, *in situ* soil CH₄ concentrations responded significantly (F -value = 4.80, P = 0.011) to tree row distance, although the differences between means were relatively small (Table 1). All the same, it is interesting that the lowest CH₄ concentrations occurred at the “middle of the alley” distance (i.e. 4 m from hardwoods and 8 m from poplars). Although it is not the Euclidian center of the alley, this sampling location is likely to experience an equal influence of hardwood and poplar rows, given their size difference. The plant litter mixture at this distance is thus likely to be the most diverse in terms of the relative contribution from each of the three component crops (poplars, hardwoods and forage plants) to the annual litter cohort. This might lead to a more diverse soil microbial community, higher soil nutrient cycling rates and thus stimulate CH₄ oxidizing bacteria. Also, this distance between rows is likely to receive the most light for the forage intercrop, hence where soil water uptake for photosynthesis is highest. Higher soil aeration might then lead to greater soil CH₄ oxidation at this mid-alley distance. Thus, our results suggest that tree diversity may increase soil methane oxidation potential.

From our field trials, we also noted that CH₄ concentrations gradually decreased (F -value = 301.80, $P < 0.001$) from the surface downwards (Table 1), down to concentrations lower than the average world atmospheric CH₄ concentration of 1.82 ppm (World Meteorological Organization 2013). In the context of global climate change, this finding implies that we should view soils not only as occasional CH₄ emitters, but also as possible net sinks of this greenhouse gas (e.g. Kirschke et al. 2013). Given that net CH₄ fluxes are controlled by the balance between two opposing processes, namely methanogenesis and methanotrophy (Gauthier et al. 2015), more work is required to determine how different land management systems concomitantly affect methanogenic and methanotrophic activity. For example, Bradley et al. (2012) used an isotope dilution approach to measure gross rates of CH₄ production and consumption in pasture soils, and determined that net CH₄ fluxes were in no way related to gross rates. A similar approach would be warranted for future research on tree-based intercropping systems.

TBI systems may affect soil CH₄ fluxes in the field, either by modifying environmental factors such as temperature and humidity, or by affecting microbial and chemical soil properties. Laboratory incubations, on the other hand, may be criticized for lacking realism and generality (Diamond 1986), but they do allow us to compare CH₄ oxidation potential at different soil depths and tree distances, based solely on microbial and chemical soil properties. In contrast with *in situ* measurements, we found no effect of tree row distance on *in vitro* CH₄ oxidation potential ($df = 2$, F -value = 0.19, $P = 0.83$), suggesting that the effect of tree row distance in the field is linked to differences in environmental conditions such as soil moisture or temperature. Results from the second RMANOVA analysis revealed a significant effect of soil amendment ($df = 3$, F -value = 9.67, $P < 0.001$), of time ($df = 4$, F -value = 550.93, $P < 0.001$) and of a time x soil amendment interaction ($df = 12$, F -value = 7.60, $P < 0.001$) on *in vitro* CH₄ oxidation. More specifically, CH₄ concentrations in the nitrate treatment decreased linearly over time, and at a slower pace than in the other amendments (Fig. 1.A). Thus, at the end of the incubation, only 47 % of the initial headspace CH₄ had been consumed in the nitrate treatment, compared to 84–87 % in the other three treatments. We conclude that the application of nitrate fertilizers at the St-Paulin site could potentially reduce the soil's capacity to oxidize CH₄. This outcome is especially relevant to other sites that were shown to increase soil CH₄ emissions after receiving annual manure applications (e.g. Radl et al. 2007). Also, future research should test whether forage mixtures that include

legume plants, such as the intercrop used at our study site, increases soil nitrate production with cascading effects on soil CH₄ oxidation.

The third RMANOVA analysis revealed that the presence of tree rows had a significant effect on *in vitro* CH₄ oxidation rates ($df = 1$, F -value = 3.12, $P = 0.043$). More specifically, greater quantities of CH₄ were consumed (i.e. oxidized) in soils collected in TBI plots compared to CM plots (Fig. 1.B). This may result from higher soil available C provided by above- and belowground tree litter, as well as pruning residues, stimulating soil heterotrophs and nutrient turnover. This, in turn, could stimulate CH₄ oxidizing bacteria. Furthermore, TBI systems result in higher nitrate use efficiency (Bergeron et al. 2011), which reduces fertilizer nitrate demand and improves soil CH₄ oxidation potential.

In summary, our results support our initial hypothesis that TBI comprises an interesting management option for potentially reducing soil CH₄ emissions. This conclusion is based, however, on *in vitro* soil incubations from a single site and extrapolation to other real world situations warrants caution. Our study also suggests a negative effect of NO₃⁻ additions on soil methane oxidation, in line with several other studies (e.g. Aronson and Helliker 2010). However, our fundamental knowledge of soil CH₄ flux response to nitrogen additions are still lacking, as different experiments continue to yield inconsistent results (e.g. Banger et al. 2012). In attempting to solve this conundrum, future research should focus on agricultural management systems, such as TBI, that have been shown to reduce N fertilizer addition (e.g. Bergeron et al. 2011).

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Figure caption

Figure 1. The effects of **(A)** inorganic nitrate, phosphate and sulphate amendments, and **(B)** cropping system (TBI vs. CM), on the rate of in vitro CH₄ consumption by surface (0-20 cm) soil samples over a 168 h laboratory incubation. Soil samples were first placed in Mason jars and headspace atmospheres were enriched to 18–20 ppm CH₄. Points have been slightly jittered right and left to avoid overlap of error bars.

Table 1. Mean ($\pm$ SE) *in situ* CH₄ concentrations (ppm) in TBI plots, at different depths and different tree-row distances, one hour after flushing buried access tubes and closed-top surface cylinders with ambient air. The “Middle of alley” distance refers to the approximate location (i.e. 8 m from poplars and 4 m from hardwoods) where the influence of each tree row is equal. Data are pooled across three sampling dates. Marginal means (in bold) followed by different lower case letters differ significantly ($P < 0.05$). There was no significant interaction between soil depth and tree row distance.

Depth (cm)	Tree row distance (m)			Mean
	2 m from poplars	“Middle of alley”	< 1m from hardwoods	
0	2.50 (0.07)	2.50 (0.07)	2.40 (0.07)	2.47 a (0.039)
12	1.96 (0.07)	1.62 (0.07)	1.81 (0.07)	1.79 b (0.038)
25	1.52 (0.07)	1.36 (0.07)	1.51 (0.07)	1.46 c (0.038)
Mean	1.99 a (0.04)	1.83 b (0.038)	1.91 a (0.039)