



Tree-based intercropping does not compromise canola (*Brassica napus* L.) seed oil yield and reduces soil nitrous oxide emissions

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ARTICLE INFO

Article history:

Received 18 February 2010

Received in revised form 15 June 2010

Accepted 23 June 2010

Available online 31 July 2010

Keywords:

Biofuel

Canola (*Brassica napus* L.)

N fertility

Seed oil yield

Soil N₂O emissions

Tree-based intercropping

ABSTRACT

Recent concerns over rising oil prices and greenhouse gas emissions have sparked an interest for the production of first generation biofuels on marginal agricultural land in Eastern Canada. Field trials were established to compare canola seed oil yield and soil nitrous oxide (N₂O) emissions in tree-based intercropping (TBI) and conventional monocropping (CM) systems. The 4–5 year-old TBI system comprised alternating rows of hybrid poplar and high-value hardwood species, with 8 m wide alleys. Each cropping system was planted with six canola cultivars, grown at four fertilizer N rates. Seed oil concentrations decreased linearly with fertilizer N, while seed oil yields increased either linearly or following a quadratic trend. An optimal fertilization rate was estimated at 80 kg N ha⁻¹. Seed oil concentrations were higher in the CM than in the TBI system, but the two systems did not differ significantly in terms of seed oil yield. N₂O emissions were three times higher in the CM than in the TBI system, probably as a result of higher soil moisture. The cultivar that produced the highest seed oil yield also produced significantly more N₂O, probably as a result of greater available C in the rhizosphere. Our results may be useful to future life cycle assessments for analyzing the net environmental impacts of producing and distributing fertilizer N to biofuel crops, and the choice of cropping system and canola cultivar that minimize N₂O emissions. In a first instance, we conclude that our model TBI system did not compromise canola seed oil yields, and substantially reduced soil N₂O emissions.

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1. Introduction

Rising oil prices and concerns about greenhouse gas emissions have sparked an interest in the production of biofuels as alternatives to fossil fuels. Biofuels represent a source of renewable energy with lower environmental impacts than fossil fuels, as they recycle carbon that was recently fixed from the atmosphere. Canola (*Brassica napus* L.) and related Brassicaceae species (e.g. *Brassica rapa* L. and *Brassica juncea* (L.) Czern) are already important oilseed crops, providing 12% of the world's comestible vegetable oil supply (United States Department of Agriculture, 2008). There is an opportunity, therefore, for major canola producing countries such as Canada, the U.S. and China, to increase their production of feedstock for first generation biofuels.

It is estimated that the global population is growing by 90 million people each year, thereby creating a growing demand for food. For this reason, the expansion of the biofuel industry should

not encroach on land currently producing food, or on land with a good agricultural potential. For example, the estimated 500 million hectares of abandoned agricultural land that is lying fallow worldwide, could be revitalized to grow crops such as canola for bio-energy (Cotula et al., 2008). There is a need, therefore, to test novel cropping systems that would entice would be producers to return fallow land into biofuel crop production.

Incentives to cultivate canola on abandoned land are possible through innovative ideas that provide landowners with market outlets for value-added production systems. Tree-based intercropping (TBI), which consists of widely spaced tree rows and annual alley crops, is one such system with potential economic and environmental benefits (Bradley et al., 2008). Within a TBI system, tree roots may absorb nutrients that are leached below the rooting zone of alley crops thereby increasing nutrient cycling efficiency and decreasing environmental impacts (Allen et al., 2004). When combining fast-growing short-rotation trees such as hybrid poplars (*Populus* spp.) and high-value hardwood trees such as black walnut (*Juglans nigra* L.), TBI systems are expected to supplement the landowners' income over that he would receive by conventional monocropping (CM) (Gordon, 2008). Within the context of present

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and future cap-and-trade carbon markets, TBI systems could also generate revenues because trees fix atmospheric CO₂ during their growth and tend to increase soil C sequestration (Peichl et al., 2006). It is uncertain, however, whether trees will interfere with or facilitate the growth of canola when it is grown as an alley crop. On the one hand, TBI systems have been shown to improve soil fertility (Thevathasan and Gordon, 2004), but trees also compete for water and cast shade on the intercrop, which could reduce yields. It is, therefore, necessary to compare canola seed oil concentrations and yields in TBI and CM systems.

Field trials should focus on the response of seed oil yield to N fertilizers. On the one hand, total seed yields are expected to increase with soil N fertility, but a concomitant increase in seed protein content could result in lower seed oil concentrations. A second concern is the impact N fertilizers may have on soil nitrous oxide (N₂O) emissions. Nitrous oxide is a potent greenhouse gas, approximately 300 times more effective (per mole) than CO₂ at trapping heat in the earth's atmosphere (Forster et al., 2007). The environmental benefits of producing bio-energy to reduce CO₂ emissions from fossil fuels could thus be offset if the associated agronomic practices increase soil N₂O emissions. These emissions may arise from chemoautotrophic nitrification, the process of oxidizing ammonia (NH₃) into NO₃⁻, or from heterotrophic denitrification, the process of reducing soil NO₃⁻ into N₂ gas. Both of these processes should respond positively to N fertilization, but differences in cropping practices or in rhizosphere dynamics among canola cultivars could conceivably affect net rates of N₂O emissions. For example, cropping systems and cultivars that improve fertilizer use efficiency and impede heterotrophic denitrification should result in lower N₂O emissions.

We report on canola field trials established on revitalized fallow land in the province of Québec, Canada. Three canola cultivars were grown in each of 2007 and 2008, on a pilot study site that included replicated TBI and CM systems, each cropped at four N fertility levels. We hypothesized that yields would be lower near the tree rows than in the center of the alleys because of competition for light and soil resources. We also hypothesized that seed oil concentration would decrease with increasing soil N fertility, and tested how this would affect seed oil yield. Lastly, we hypothesized that N₂O emissions would increase with N fertilization, and tested how cropping systems and canola cultivars affected these emissions. In order to gain insights into the factors controlling seed oil yield and N₂O emissions, we also measured soil moisture, soil microbial biomass, potential nitrification rates, as well as foliar traits.

2. Material and methods

2.1. Experimental design

Field trials were conducted at St-Édouard-de-Maskinongé, QC (46°20' N, 73°11' W) in 2007 and 2008. Soil characteristics are given in Table 1. The experimental design consisted of TBI and CM systems replicated in four blocks. The TBI systems consisted of rows of high-value hardwood species (*Fraxinus americana* L., *Fraxinus penn-*

Table 1
Average soil properties at the St-Edouard experimental site before fertilization in 2007.

Texture	Sandy loam
Organic matter (%)	4.59
Total C (mg/g)	26.65
Total N (mg/g)	1.99
C:N Ratio	13.39
Mehlich-extractible P (ppm)	1.20
Mehlich-extractible K (ppm)	4.37
pH _{water}	5.9

sylvanica Marshall and *Quercus rubra* L.) alternating with rows of fast-growing hybrid poplars (*Populus × canadensis* "Stormont" and *Populus × canadensis*), all planted in the spring of 2004. The tree rows were oriented along a South-West to North-East axis. Adjacent tree rows were separated by 8 m wide alleys in which canola was sown in each of the 2 study years. In 2007, the high-value hardwoods were about 1 m tall whereas the hybrid poplars were about 4 m tall, with a well-developed canopy. Alleys were disk-harrowed in mid-May, prior to sowing and fertilizing. Twelve treatments, consisting of three canola cultivars × four N fertility rates, were randomly assigned to 12 plots within each replicated cropping system. These 8 m long alley plots were established perpendicular to two adjacent tree rows, with hybrid poplars on the South-East side and slow-growing hardwoods on the North-West side of the alley. Given the space constraint of fitting twelve treatment plots into TBI and CM cropping systems of pre-established size, each treatment plot was 1 m wide. In order to reduce possible edge effects, adjacent plots were separated by 30 cm wide geo-textile strips. The plots in the CM systems were identical in size and orientation to those in the TBI systems, but excluded the tree rows.

The canola cultivars used in 2007 were Q2, Sentry and 46A65. These cultivars vary in seed yield and seed oil concentration. Q2 is a high seed yield, blackleg resistant cultivar (Stringam et al., 1999). Sentry is blackleg resistant, medium seed oil concentration cultivar (Rimmer et al., 1998). 46A65 is a Pioneer Hi-Bred, high seed yield, blackleg resistant cultivar (Canola Council of Canada, 2008). The canola cultivars used in 2008 were Polo, Topas and 04C204. Polo is a Danisco-bred, high oil concentration cultivar (Rahman et al., 2001). Topas is a Swedish spring canola cultivar marketed in 1982, created from parental lines (Bronowski × Gulle) × Hermes (Nordic Genetic Resource Center, 2009). 04C204 is a very high oil yield line (hereafter referred to as "cultivar") recently developed by the Plant Science Department, University of Manitoba.

Sowing and fertilization was done by hand in the 3rd week of May. Plant density was estimated at approximately 60 plants m⁻², below the recommended density of 80 plants m⁻² (Conseil des productions végétales du Québec, 1996). According to Angadi et al. (2003), yields are not significantly altered at this density because canola plants compensate with increased branching. The four fertilizer N rates were 0, 40, 80 and 120 kg N ha⁻¹ applied as urea by hand. All plots received a blanket application of 80 kg P₂O₅ ha⁻¹ and 80 kg K₂O ha⁻¹ according to provincial guidelines (CRAAQ, 2003), applied as triple superphosphate and potash. Boron and sulphur were also added in 2008, at 2 and 20 kg ha⁻¹ respectively.

Within TBI systems, the taller hybrid poplar rows were expected to create a gradient of light and soil conditions. For this reason, plots within TBI systems were sampled in "subplots" at 1, 4 and 7 m from the row of hybrid poplars. These three subplots, along with a single sampling location in the center of each adjacent CM plot, comprised a fourth experimental factor that we designated as the "plant environment". Thus, the full experimental design consisted of a full factorial array of three canola cultivars × four fertilizer N rates × four plant environments, replicated four times (N = 192).

2.2. N₂O flux measurements

In 2008, soil N₂O emissions were measured with closed-top cylindrical chambers (5.25 cm radius × 15 cm high) on four dates (July 8th, July 22rd, August 5th and August 22nd). The top 5 cm of each chamber was insulated with foam and equipped with a rubber septum. Cylinders were inserted 10 cm deep into the soil in each of the 192 treatment plots, and 8 ml of headspace air was sampled after 1 h. Preliminary tests performed in each block had revealed that N₂O accumulation rate from 30 min to 24 h followed a linear trend, and that hourly N₂O measurements were reliable estimates of daily N₂O flux. Soil temperature at 5 cm depth was monitored

for each sample. During the incubation, a 22-gauge needle was kept inserted in the septum in order to maintain equal gas pressure with the surrounding air while minimizing N_2O loss from the headspace (Hutchinson and Mosier, 1981). Gas samples were injected into 3.0 ml BD Vacutainer® Plus plastic serum tubes (Becton-Dickinson and Co., Franklin Lakes, NJ) and transported to the laboratory where N_2O concentrations were measured using a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a 80–100 mesh Hayesep® packed column, an electron capture detector (ECD), with ultra-high purity N_2 as carrier gas. N_2O measurements were corrected to a common temperature of 20 °C by assuming $Q_{10} = 2$, and extrapolated to a $ha^{-1} day^{-1}$ basis.

2.3. Soil sampling and analyses

A soil sample (0–10 cm) was collected from each of the 192 sampling locations twice in 2007 (July 3rd and August 11th), and following each N_2O measurement in 2008. These were transported to the laboratory where they were sieved (2 mm) and kept at 4 °C until analyzed. Soil water content was determined by weight loss after drying subsamples at 101 °C for 72 h.

Soil available C in each soil sample was inferred from microbial biomass (Bradley and Fyles, 1995) measured by substrate induced respiration (SIR) rates (Anderson and Domsch, 1978). Soil subsamples (20 g dry wt. equiv.) were placed in 500 ml plastic containers and amended with ground and sieved (65 μm) glucose (1000 $\mu g C g^{-1}$). Glucose was first mixed with talc (9:1 = talc:glucose), and 500 mg of the mixture was dispersed through each soil subsample using a handmixer with one beater. The subsamples were left uncovered for 100 min in order to reach optimum SIR rates (Anderson and Domsch, 1978). The headspace of each container was then flushed for 5 min with ambient air and sealed with lids equipped with rubber septa. Headspace air was sampled after 30 min using a needle and syringe, and CO_2 concentrations were detected with a CP-2002 P Micro-GC gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a thermal conductivity detector (TCD), using He as carrier gas. SIR rates were corrected to 20 °C by assuming $Q_{10} = 2$ and converted to microbial biomass with equations derived by Anderson and Domsch (1978).

Potential nitrification rates were measured by placing 25–30 g fresh subsamples in Mason jars, covering these with a polyethylene film to allow gas exchange and prevent desiccation, and incubating in the dark at ambient temperature for 30 days. Soil subsamples were then extracted in 100 ml of 1N KCl solution, stirred for 1 h on a rotary shaker, and the supernatants poured through Whatman No. 5 filter papers. Logistical constraints prevented us from analyzing all of the extracts, therefore samples were pooled across cultivars and N fertility levels to test only the effects of “plant environment” on potential nitrification. Pooled extracts were analyzed colorimetrically for NO_3^- using a Technicon Autoanalyser (Pulse Instrumentations Ltd., Saskatoon, Canada), with sulphanilamide color reagent and a Cu-coated Cd reduction column.

2.4. Physiological measurements

Photosynthetic rates at a constant CO_2 concentration of 400 ppm (A_{400}) were measured in each subplot once in 2007 during the flowering stage (July 21st to 23rd), and three times in 2008, at stem elongation (June 27th to 28th), early flowering (July 11th to 12th) and late flowering stages (July 30th to 31st), using a LI-COR 6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE). Measurements were made on a newly expanded leaf borne on the main shoot of a randomly chosen plant. In 2007, the leaf remained on the plant during the measurement, while in 2008 the leaf was clipped from the plant and placed in a water tube in order to rehydrate

prior to measurement. Hence, measurements made in 2007 represent actual leaf gas exchange rates, while those in 2008 represent potential leaf gas exchange rates after removing stomatal limitations (Wong et al., 1979). Each measurement was achieved under a photosynthetic photon flux density of 1000 $\mu mol m^{-2} s^{-1}$, and at a CO_2 concentration of 400 ppm. All leaves were then kept in total darkness during 48 h to deplete their sugar reserves (Garnier et al., 2001), scanned to determine their surface with WinFOLIA® software (Regent Instruments Inc., Quebec, Canada), dried at 30 °C for 2 days and weighed. Specific leaf area (SLA) was thus estimated for every leaf as the ratio of leaf surface area to dry mass.

2.5. Yield estimates

The 192 subplots were harvested by hand at the end of August of each year. Ten plants were randomly chosen, air-dried at 30 °C for 1 week, and threshed to extract the seeds. Seeds were weighed and sent to the Plant Biotechnology Institute – National Research Council (Saskatoon, Canada) in 2007, and to the Department of Plant Science – University of Manitoba (Winnipeg, Canada) in 2008, to be analyzed for seed oil concentration by near infrared spectroscopy. Seed oil yield in each subplot was estimated from seed weight of 10 plants, planting density and seed oil concentration.

2.6. Statistical analyses

Linear mixed-effects models were used to control the effects of sampling date and blocks (i.e. random variables) while testing the effects of cultivar, N fertility and plant environment, as well as their interactions, on all response variables. The models took into account the nested structure of the split-plot design. Orthogonal polynomials were used to test the statistical significance of linear and quadratic trends in yield as a function to N fertility. Tukey's HSD tests were used to reveal statistically different means in the other response variables. All tests were performed using the *lmer* package from R statistical software (2009) and used $\alpha = 0.05$ to designate statistical significance.

3. Results

3.1. Seed oil concentration and yield

In both years, seed oil concentration decreased linearly ($P < 0.01$) with fertilizer N application rate (Fig. 1a). Average seed oil concentration in 2007 (43.7%) was lower ($P < 0.01$) than in 2008 (49.1%). Seeds harvested at 1 m and 4 m from poplar rows in the TBI system had lower oil concentrations (42.7% and 48.5% for 2007 and 2008 respectively) than those grown in the CM system (46.6% and 50.9% for 2007 and 2008 respectively) (Fig. 1b and c).

Yields ranged from 0.7 to 1.3 $Mg ha^{-1}$ in 2007 and from 1.0 to 2.2 $Mg ha^{-1}$ in 2008. In 2007, seed oil yield increased linearly ($P < 0.01$) with fertilizer N application rate (Fig. 2a). Seed oil yield at 1 m from poplar rows was lower ($P < 0.01$) than at 7 m. In 2007, average seed oil yield in the TBI system was numerically higher (1.1 $Mg ha^{-1}$) than in the CM system (0.9 $Mg ha^{-1}$), but the difference was not statistically significant. In 2008, seed oil yield was affected by an interaction ($P = 0.02$) between N fertilization rate and plant environment (Fig. 2b). More specifically, a quadratic trend ($P = 0.02$) between yield and fertilizer N application rate was observed at 1 m ($P < 0.01$) and 7 m ($P = 0.02$) from the tree rows, whereas a linear trend was observed at 4 m and in the CM system. In 2008, average seed oil yield in the TBI system was lower (1.6 $Mg ha^{-1}$) than in the CM system (1.9 $Mg ha^{-1}$), but the difference between the two systems was only significant ($P < 0.01$) at the highest fertilizer N application rate. The two treatments that provided the highest yield (2.8 $Mg ha^{-1}$) were 04C204 fertilized with

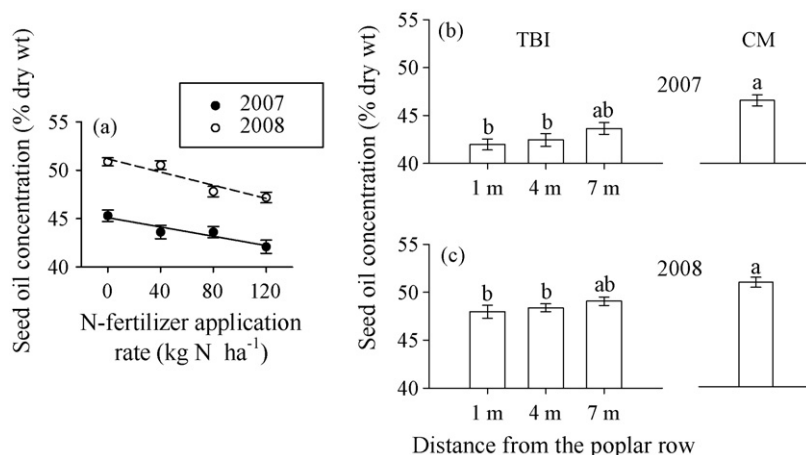


Fig. 1. Average canola seed oil concentrations in relation to (a) fertilizer N application rates and (b and c) plant environments. Different lower-case letters designate statistically significant ($P < 0.05$) means within each frame according to Tukey's HSD test. Error bars = 1 S.E.

80 kg N ha⁻¹, grown either in the CM system or at a 4 m distance from poplar rows in the TBI system.

In both years, cultivars had an effect on seed oil concentration and yield. In 2007, seed oil concentration was lower ($P < 0.01$) for Q2 (42.2%) than for 46A65 (44.0%) and Sentry (44.8%) (Fig. 3a). In 2008, seed oil concentration was higher ($P < 0.01$) for Polo (51.0%) than for Topas (47.7%) and 04C204 (48.5%) (Fig. 3b). In 2007, Sentry had higher ($P = 0.03$) seed oil yield (1.1 Mg ha⁻¹) than Q2 (1.0 Mg ha⁻¹) (Fig. 3c). In 2008, 04C204 had higher ($P = 0.04$) seed oil yield (1.9 Mg ha⁻¹) than Polo (1.6 Mg ha⁻¹) (Fig. 3d).

3.2. N₂O emissions

fertilizer N application rate had no effect ($P = 0.24$) on N₂O emissions in 2008. N₂O emissions were more than three times higher ($P < 0.01$) in the CM system (60 g N₂O-N ha⁻¹ day⁻¹) than in the TBI system (17 g N₂O-N ha⁻¹ day⁻¹) (Fig. 4a). N₂O emissions were more than twice as high ($P < 0.05$) with 04C204 (39 g N₂O-N ha⁻¹ day⁻¹) than with Polo (15 g N₂O-N ha⁻¹ day⁻¹) (Fig. 4b).

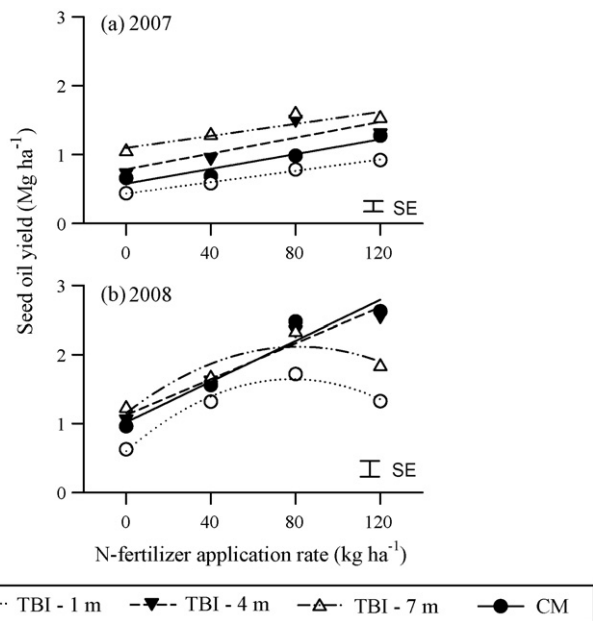


Fig. 2. Average canola seed oil yield in relation to fertilizer N application rates and plant environments. Lines and curves represent significant linear and quadratic trends. Error bars = 1 S.E.

3.3. Plant leaf traits

In 2007, photosynthetic rates were 15–17% lower ($P = 0.01$) at 1 m from poplar rows than in the other three plant environments (Table 2). In 2008, plant environment had no effect on photosynthetic rates of leaves that had been excised and re-hydrated prior to measurement. For both years, there was a significant N fertility × canola cultivar interaction controlling photosynthetic rates ($P = 0.03$ and $P = 0.02$ for 2007 and 2008 respectively) (Table 2).

In 2007, SLA was higher ($P = 0.03$) for Sentry than for 46A65 (Table 2). In 2008, SLA was higher ($P < 0.01$) for Polo than for Topas. In both years, SLA was higher ($P < 0.01$) in the TBI than in the CM system. In 2007, SLA was higher ($P < 0.02$) at 1 m than at 4 m from poplar rows.

3.4. Soil moisture, microbial biomass and potential nitrification

Average soil water content in 2008 was about 3% lower ($P < 0.01$) with Topas than with the two other cultivars (data not shown). In both years, soil water content was higher ($P < 0.01$) in the CM than in the TBI system (Table 3).

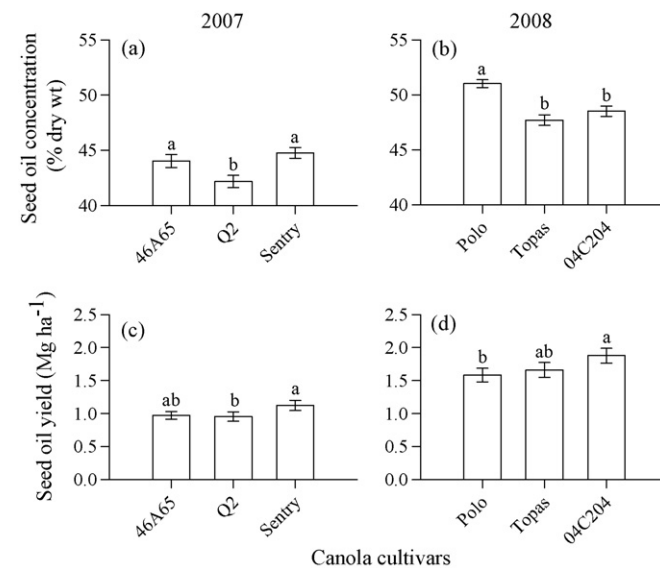


Fig. 3. Average seed oil concentration and yield of canola cultivars tested in 2007 and 2008. Different lower-case letters designate statistically significant ($P < 0.05$) means within each frame according to Tukey's HSD test. Error bars = 1 S.E.

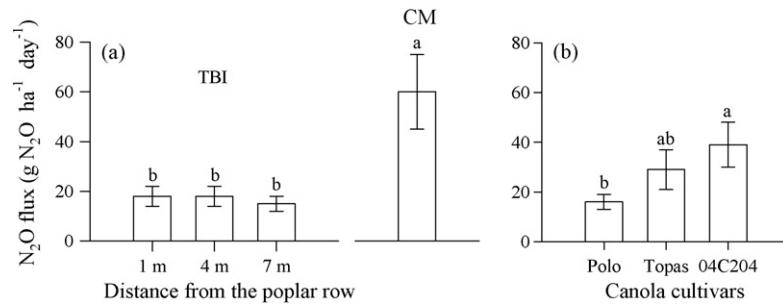


Fig. 4. Average soil nitrous oxide (N_2O) emissions in relation to (a) plant environments and (b) canola cultivars, over the 2008 growing season. Different lower-case letters designate statistically significant ($P < 0.05$) means within each frame according to Tukey's HSD test. Error bars = 1 S.E.

Table 2

Effects of canola cultivar and plant environment on photosynthetic rates (A400) and specific leaf area (SLA) of canola leaves grown in 2007 and 2008. Also displayed are significant interactions between fertilizer-N rate \times canola cultivar in controlling photosynthetic rates. Standard errors are shown in parentheses. Italicized lower-case letters represent statistically different ($\alpha = 0.05$) means, according to Tukey's HSD test.

			46A65	Q2	Sentry	TBI – 1 m	TBI – 4 m	TBI – 7 m	CM
A400 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	2007	0 kg ha ⁻¹	16.88a (0.96)	16.14a (1.20)	16.95a (1.04)	16.38b (0.72)	19.37a (0.61)	19.51a (0.57)	19.71a (0.47)
		40 kg ha ⁻¹	18.74a (0.92)	16.88a (0.73)	16.60a (1.12)				
		80 kg ha ⁻¹	20.82ab (0.82)	22.02a (0.69)	18.32b (1.16)				
		120 kg ha ⁻¹	20.00a (1.31)	21.91a (0.79)	18.84a (1.05)				
	2008	0 kg ha ⁻¹	8.33a (0.51)	9.86a (0.58)	9.88a (0.49)	11.06a (0.36)	10.66a (0.32)	10.77a (0.34)	11.02a (0.33)
		40 kg ha ⁻¹	9.82a (0.58)	10.81a (0.62)	10.34a (0.41)				
		80 kg ha ⁻¹	10.87b (0.58)	12.91a (0.53)	11.12b (0.52)				
		120 kg ha ⁻¹	11.41b (0.64)	13.76a (0.57)	11.31b (0.54)				
SLA ($\text{cm}^2 \text{ g}^{-1}$)	2007		267.11b (6.16)	281.49ab (6.68)	292.77a (7.52)	314.71a (8.07)	281.96b (5.36)	286.26ab (8.68)	238.88c (5.12)
	2008		347.6a (5.19)	329.5b (4.87)	340.3ab (5.01)	352.0a (5.86)	347.1a (5.66)	346.3a (6.09)	311.2b (5.08)

In 2008, there was a canola cultivar \times plant environment interaction ($P < 0.01$) controlling soil microbial biomass (Fig. 5). More specifically, microbial biomass in the CM system was higher with 04C204 and Topas than with Polo. Microbial biomass in the TBI system was generally higher ($P < 0.01$) with 04C204, but the significance of comparisons with the other two cultivars varied according to plant environment.

In both years, potential nitrification decreased with proximity to poplar rows, but a significant effect ($P = 0.03$) was observed only in

2008 between soils samples from the CM system and TBI subplots at 1 m distance from poplar rows (Table 3).

4. Discussion

The fact that the canola cultivars used in 2007 were not the same as those used in 2008 precludes all comparisons of seed oil concentration and yield between years. Our study was not meant, however, to be a rigorous canola cultivar trial, but rather a first esti-

Table 3

Effects of plant environment (TBI at 1, 4 and 7 m distance from poplar rows, and CM) on soil water content and potential nitrification rates. Different lower-case letters in italic represent statistically significant ($P < 0.05$) means within each year, according to Tukey's HSD test. Standard errors are shown in parentheses.

		TBI – 1 m	TBI – 4 m	TBI – 7 m	CM
Soil water content ($\text{g}_{\text{water}} \text{ g}_{\text{soil}}^{-1}$)	2007	0.146b (0.003)	0.148ab (0.003)	0.146b (0.003)	0.157a (0.004)
	2008	0.222b (0.002)	0.225b (0.002)	0.225b (0.002)	0.247a (0.003)
Potential nitrification ($\mu\text{g NO}_3^- \text{ N g}_{\text{soil}}^{-1} \text{ mo}^{-1}$)	2007	1.29a (0.53)	1.27a (0.36)	1.45a (0.37)	1.52a (0.54)
	2008	20.29b (1.27)	22.31ab (1.26)	22.96ab (1.62)	24.26a (1.61)

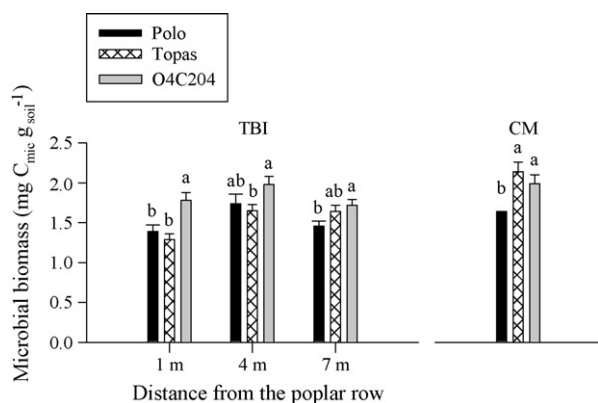


Fig. 5. Soil microbial biomass in relation to plant environments and canola cultivars, over the 2008 growing season. Different lower-case letters designate statistically significant ($P < 0.05$) means within each cluster according to Tukey's HSD test. Error bars = 1 S.E.

mate of potential seed oil yields on marginal fallow lands in Quebec, using different fertilizer N application rates and different cropping systems. The decrease in seed oil concentration with increasing fertilizer N application rate is likely the result of protein synthesis being favoured over oil synthesis when N becomes more abundant (Brennan et al., 2000). This decrease in seed oil concentration was more than compensated by an increase in total seed yield with increasing fertilizer N application rate, at least up to 80 kg N ha^{-1} . Results thus suggest that optimal yields can be attained on marginal agricultural land in Quebec with $80 \text{ kg fertilizer N ha}^{-1}$.

Compared to average seed oil yields found in other industrialized countries, the optimum yields that we obtained in 2008 rate as very high. For example, on its web site the U.S. Canola Association reports average winter canola seed yields of 23 mainland states ranging between 500 and 3800 kg ha^{-1} , with average seed oil concentrations generally lower than 45%. In fact, seed oil yields upwards of 2000 kg ha^{-1} , as we have found, have been reported in only a few other studies (Brandle and Mcvetty, 1988; Taylor et al., 1991; Karamanos et al., 2007). Our data thus suggest that the cultivars we used in 2008 may be more adapted to moist conditions such as those in Eastern Canada, than to the dryer prairie conditions where canola has traditionally been grown.

In the TBI system, a reduction in seed oil concentration and yield occurs in close proximity to poplar rows. Lower yields at 1 m distance from poplar rows could be due to light interception by the tree canopy. This is corroborated by the higher SLA observed at this distance in 2007, since SLA usually responds positively to reductions in light intensity (Jurik and Van, 2004). We did not observe significantly higher SLA at this distance in 2008, perhaps because of more daylight hours in 2007 (266 mm rainfall; 173 cooling degree days) compared to 2008 (369 mm rainfall; 154 cooling degree days), such that differences in SLA due to shading were more pronounced in the sunnier year. Hybrid poplar is a fast-growing tree crop with a high soil water demand, and this also could contribute to reduced yields at 1 m distance from poplar rows. This is corroborated by differences observed in A_{400} between years. More specifically, A_{400} at 1 m distance was significantly lower when measurements were made on non-excised leaves experiencing *in situ* soil water potential (i.e. 2007), but not when leaves were excised and re-hydrated prior to measurement (i.e. 2008). Finally, the potential nitrification data provide only weak support that lower yields at 1 m distance from poplar rows were due to lower soil N supply. Adequately testing for N deficiency would require a measurement of leaf N concentration which can be combined with A_{400} values to derive photosynthetic N use efficiency (Reich et al., 1989). Notwithstanding the lower yields observed at 1 m distance from poplar rows, our study shows that

average yields over the entire alley can be maintained when canola is intercropped with fast-growing hybrid poplar at 8 m intervals, at least for 5 years following the implementation of this cropping system.

In 2007, we did not expect higher seed oil concentration and yield with Sentry than with Q2, as these two cultivars had been respectively reported as being medium and high in seed oil (Rimmer et al., 1998; Stringam et al., 1999). The climate in Quebec is generally cooler and wetter than in central and western Canada where these cultivars have been tested, and this may have reordered the relative performance of the two cultivars. For example, Lafitte and Courtois (2002) found significant cultivar \times environment interactions controlling upland rice yields, with early maturing cultivars being favoured under drought. Results emphasize, therefore, the limit to which the relative performance of canola cultivars can be generalized beyond regional growing conditions.

Contrary to expectation, we did not find a relationship between fertilizer N application rate and soil N_2O emissions. It is possible that most of the fertilizer N had already been immobilized or lost (volatilized or leached) by the time N_2O measurements began, nearly two months following fertilizer application. On the other hand, we found a substantial three-fold increase of N_2O emissions in the CM system, when compared to the TBI system. N_2O production may arise from two biochemical pathways, chemoautotrophic nitrification that is more common under aerobic conditions, or heterotrophic denitrification that is more prevalent in oxygen depleted soils (Firestone and Davidson, 1989). Soil analyses in our study provide evidence that TBI could have decreased N_2O emissions by either one of these two pathways. For example, we found lower potential nitrification rates in the TBI system, possibly due to better fertilizer N utilization when alley crops and trees are intercropped (Thevathasan and Gordon, 2004). Lower potential nitrification in TBI systems could also reduce N_2O emissions by limiting the available substrate for heterotrophic denitrifiers. It is also possible that TBI systems reduced heterotrophic denitrification by reducing soil moisture (Xuejun et al., 2007). Given that hybrid poplar is a fast-growing species with extensive lateral roots, its high demand for soil water is likely met by foraging the solum well into the alley. This is corroborated by the lower soil moisture contents found at various distances in our TBI plots.

We did not expect to find an effect of cultivars on N_2O emissions. Given that nitrification and soil water content were not higher under cultivar O4C204, we hypothesize that the higher N_2O emissions with this cultivar was due to greater rates of rhizodeposition alleviating C limitation among heterotrophic denitrifying bacteria (Peng et al., 2007). This is corroborated by a higher microbial biomass, a reliable index of chronic low C-supply (Bradley and Fyles, 1995), under cultivar O4C204. The implication of this cultivar effect is important, given that the cultivar with the best seed oil yield is also the one with the highest rate of N_2O emission.

In summary, we have shown that seed oil yields of up to 2.8 Mg ha^{-1} can be obtained in either TBI or CM systems on marginal farmland in Southern Quebec. According to the 2009 Automotive Consumer Guide (HowStuffWorks Inc., 2009), a 4-cylinder mid-size car's average fuel consumption is 10.6 km l^{-1} , such that it would take approximately 0.67 ha of marginal land to fuel this mid-size car to run $20,000 \text{ km year}^{-1}$. These yields occur, however, under optimal fertilizer and cultivar conditions that are not necessarily environmentally sound. There is a need, therefore, to conduct life cycle assessments for analyzing the trade-offs between canola seed oil yields and environmental impacts brought on by the production and application of fertilizer N, or by the choice of cropping system and canola cultivar that substantially affect N_2O emissions. In a first instance, our study allows us to conclude that our model TBI system did not compromise canola seed oil yields,

and substantially reduced soil N₂O emissions compared to the CM system. Lower N₂O emissions may provide a further incentive for landowners to adopt TBI systems, based on current and proposed cap-and-trade programs that reward production systems that limit GHG emissions (e.g. European Commission, 2008).

Acknowledgments

The authors are grateful to Mrs. M. Arès for allowing us to perform this study on her farm. We thank the Plant Biotechnology Institute (Saskatoon) for analyzing seed oil concentrations in 2007. Drs. D. Rivest, A. Vanasse and B. Shipley provided useful agronomic and statistical advice. We thank undergraduate students M. Bergeron, J. Bradley, M. Lanoix, X. Francoeur, P. Grégoire, G. Joannis, H. Marie Johansen, A. Lamarche, J. Quirion, D. Robert, R. Roy and M. Thibeault, who provided technical assistance. The project was supported financially by the Natural Sciences and Engineering Research Council (NSERC) and the Green Crop Network (GCN).

References

- Allen, S.C., Jose, S., Nair, P.K.R., Brecke, B.J., Nkedi-Kizza, P., Ramsey, C.L., 2004. Safety-net role of tree roots: evidence from a pecan (*Carya illinoensis* K. Koch) – cotton (*Gossypium hirsutum* L.) alley cropping system in the southern United States. *Forest Ecol. Manage.* 192, 395–407.
- Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* 10, 215–221.
- Angadi, S.V., Cutforth, H.W., McConkey, B.G., Gan, Y., 2003. Yield adjustment by canola grown at different plant populations under semiarid conditions. *Crop Sci.* 43, 1358–1366.
- Bradley, R.L., Fyles, J.W., 1995. A kinetic parameter describing soil available carbon and its relationship to rate increase in C mineralization. *Soil Biol. Biochem.* 27, 167–172.
- Bradley, R.L., Olivier, A., Thevathasan, N.V., Whalen, J., 2008. Environmental and economic benefits of tree-based intercropping systems. *Policy Options* 29, 46–49.
- Brandle, J.E., McVetty, P.B.E., 1988. Genotype X environment interaction and stability analysis of seed yield of oilseed rape grown in Manitoba. *Can. J. Plant Sci.* 68, 381–388.
- Brennan, R.F., Mason, M.G., Walton, G.H., 2000. Effect of nitrogen fertilizer on the concentrations of oil and protein in canola (*Brassica napus*) seed. *J. Plant Nutr.* 23, 339–348.
- Canola Council of Canada, 2008. Prairie canola variety trials test results 2008. Available from: http://www.canola-council.org/uploads/pcvr/PCVT.Final.2008_web.pdf (accessed 10.02.10.).
- Conseil des productions végétales du Québec inc. – Comité Oléoprotéagineux, 1996. Oléoprotéagineuses – Canola. Available from: <http://www.agrireseau.qc.ca/grandescultures/documents/V9610.pdf> (accessed 10.02.10.).
- Cotula, L., Dyer, N., Vermeulen, S., 2008. Fuelling Exclusion? The Biofuels Boom and Poor People's Access to Land. International Institute for Environment and Development, London, U.K., 72 pp.
- CRAAQ (Ed.), 2003. Guide de référence en fertilisation, 1st ed. Centre de référence en agriculture et agroalimentaire du Québec (CRAAQ), Ste-Foy.
- European Commission, 2008. EU Action against climate change – The EU Emissions Trading Scheme. Available from: <http://ec.europa.eu/environment/climat/pdf/brochures/ets.en.pdf> (accessed 10.02.10.).
- Firestone, M.K., Davidson, E.A., 1989. Microbiological basis of NO and N₂O production and consumption in soil. In: Andreae, M.O., Schimel, D.S. (Eds.), *Exchange of Trace Gases Between Terrestrial Ecosystems and the Atmosphere*. John Wiley & Sons, New York, pp. 7–21.
- Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W., Haywood, J., Lean, J., Lowe, D.C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M., Van Dorland, R., 2007. Changes in atmospheric constituents and in radiative forcing. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, New York, pp. 129–234.
- Garnier, E., Shipley, B., Roumet, C., Laurent, G., 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct. Ecol.* 15, 688–695.
- Gordon, A.M., 2008. Agroforestry in the world: lessons for Canada. *Policy Options* 29, 50–53.
- HowStuffWorks Inc., 2009. Consumer Guide® Automotive. Available from: <http://consumerguideauto.howstuffworks.com/> (accessed 10.02.10.).
- Hutchinson, G.L., Mosier, A.R., 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Sci. Soc. Am. J.* 45, 311–316.
- Jurik, T.W., Van, K., 2004. Microenvironment of a corn–soybean–oat strip intercrop system. *Field Crops Res.* 90, 335–349.
- Karamanos, R.E., Goh, T.B., Flaten, D.N., 2007. Nitrogen and sulphur fertilizer management for growing canola on sulphur sufficient soils. *Can. J. Plant Sci.* 87, 201–210.
- Lafitte, H.R., Courtois, B., 2002. Interpreting cultivar × environment interactions for yield in upland rice: assigning value to drought-adaptive traits. *Crop Sci.* 42, 1409–1420.
- Nordic Genetic Resource Center (NordGen), 2009. Topas, on: Sesto. Accession number: NGB2695. Available from: <http://www.nordgen.org/index.php/skand/content/view/full/344> (accessed 10.02.10.).
- Peichl, M., Thevathasan, N., Gordon, A., Huss, J., Abohassan, R., 2006. Carbon sequestration potentials in temperate tree-based intercropping systems, southern Ontario, Canada. *Agroforest. Syst.* 66, 243–257.
- Peng, Y., Ma, Y., Wang, S., 2007. Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. *J. Environ. Sci.* 19, 284–289.
- R Development Core Team, 2009. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available from: <http://www.R-project.org>.
- Rahman, M.H., Joersbo, M., Poulsen, M.H., 2001. Development of yellow-seeded *Brassica napus* of double low quality. *Plant Breed.* 120, 473–478.
- Reich, P.B., Walters, M.B., Tabone, T.J., 1989. Response of *Ulmus americana* seedlings to varying nitrogen and water status. 2 Water and nitrogen use efficiency in photosynthesis. *Tree Physiol.* 5, 173–184.
- Rimmer, S.R., Scarth, R., McVetty, P.B.E., 1998. Sentry summer rape. *Can. J. Plant Sci.* 78, 615–616.
- Stringam, G.R., Degenhardt, D.F., Thiagarajah, M.R., Bansal, V.K., 1999. Q2 summer rape. *Can. J. Plant Sci.* 79, 597–598.
- Taylor, A.J., Smith, C.J., Wilson, I.B., 1991. Effect of irrigation and nitrogen-fertilizer on yield, oil content, nitrogen accumulation and water-use of canola (*Brassica napus* L.). *Fertil. Res.* 29, 249–260.
- Thevathasan, N.V., Gordon, A.M., 2004. Ecology of tree intercropping systems in the North temperate region: experiences from southern Ontario. *Can. Agroforest. Syst.* 61, 257–268.
- United States Department of Agriculture, 2008. World Agricultural Production. Available from: <http://www.fas.usda.gov/wap/circular/2008/08-12/productionfull12-08.pdf> (accessed 10.02.10.).
- Wong, S.C., Cowan, I.R., Farquhar, G.D., 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282, 424–426.
- Xuejun, J., Liu, X.J., Mosier, A.R., Halvorson, A.D., Reule, C.A., Zhang, F.S., 2007. Dinitrogen and N₂O emissions in arable soils: effect of tillage, N source and soil moisture. *Soil Biol. Biochem.* 39, 2362–2370.