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Impact of a Clover Cover Crop Combined With Organic or Mineral Fertilizer on Yield and Nitrogen Uptake of Canola

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Abstract

Factorial experiments were conducted at two locations in Quebec to measure the combined effects of cover crop (CC) (mix of red (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.)), fertilizer type (mineral (27-0-0) or organic (pig slurry)), and N rate (0, 50, 100, 150 kg nitrogen (N) ha⁻¹) on canola (*Brassica napus* L.) yield and N uptake in a split-plot design reproduced twice (2013-2014, 2014-2015) at each location. At Ckilling, the clover biomass contained 28 to 151 kg N ha⁻¹. Cover cropping did not change canola N uptake, but increased yield and had mineral fertilizer replacement value of 22 to 82 kg N ha⁻¹. Without N fertilizer application, the CC increased canola yields by 6 to 42%, the effect diminishing with increasing fertilizer N rate. Canola N uptake and yield were lower with pig slurry, compared to the mineral fertilizer. Maximum canola yield was reached at rates of 120 kg N ha⁻¹ and higher with the mineral fertilizer.

Therefore, clover CC may help reducing fertilizer application rate in canola without compromising yields.

Key words: *Brassica napus*, *Trifolium repens*, *Trifolium pratense*, Legume, Intercrop, Pig Slurry

Résumé

Une expérience factorielle a été réalisée à deux sites au Québec pour mesurer les effets d'un engrais vert (EV) (mélange de trèfles rouge (*Trifolium pratense* L.) et blanc (*Trifolium repens* L.)), du type de fertilisation (minérale (27-0-0) ou organique (lisier de porc)) et du niveau d'azote (N) (0, 50, 100 ou 150 kg N ha⁻¹) sur le prélèvement en azote et le rendement du canola (*Brassica napus* L.), selon un plan en tiroirs reproduit deux fois (2013-2014, 2014-2015) à chaque site. À l'enfouissement, la biomasse d'EV contenait entre 28 et 151 kg N ha⁻¹. L'EV n'a pas affecté le prélèvement en azote du canola, mais a augmenté son rendement pour un équivalent en azote minéral de 22 à 82 kg N ha⁻¹. Sans fertilisation, l'EV a augmenté le rendement du canola de 6 à 42 %, cet effet s'estompant avec des doses d'azote croissantes. Le lisier entraînait un prélèvement en azote et un rendement de canola moins élevés que l'azote minéral. Le rendement maximal du canola s'observait à 120 kg N ha⁻¹ et plus avec la fertilisation minérale. Le trèfle en EV pourrait aider à réduire les applications de fertilisants azotés au canola sans compromettre les rendements.

Mots-clés : *Brassica napus*, *Trifolium repens*, *Trifolium pratense*, Légumineuses, Intercalaire, Lisier de porc

INTRODUCTION

Canola (*Brassica napus* L.) is an oleo-protein cash crop developed in Canada in the 1970s, with an estimated value of \$19.3 billion CDN (Canola Council of Canada 2014). This crop, however, has high N requirements (Grant and Bailey 1993; Jackson 2000), with recommended N rates varying from 80 to 120 kg ha⁻¹ in Eastern Canada [Ontario Ministry of Agriculture, Food and Rural Affairs 2009; Centre de référence en agriculture et en agroalimentaire du Québec (CRAAQ) 2010].

In the Eastern Canadian province of Quebec, the hog industry is the second largest sector in agriculture, which produces more than 10 million m³ of slurry (Rochette et al. 2008). Used to fertilize crops, animal slurries can have variable efficiencies (CRAAQ 2013), mostly depending on application mode and timing, and weather conditions. In canola, pig slurry can be an efficient fertilizer (Mooleki et al. 2002). Sieling et al. (2014) reported similar canola yields with either mineral fertilizer or pig slurry, when application rate was based on mineral N content and corrected for ammonia volatilization following application.

There has been growing concern in the last decades about the impact of agriculture on the environment (Tilman et al. 1999), especially due to fertilizer application. For fertilizer N, pollution is generated through greenhouse gas emission by the Haber-Bosch industrial fixation process. Another type of environmental pollution is caused by both mineral and organic fertilizers, through the loss of N from the field by volatilization, leaching or denitrification, especially when N use efficiency is low (Jenkinson 2001; Cassman et al. 2002).

Cover crops (CC) are known to protect the soil against erosion, and to reduce the loss of applied N through leaching and denitrification (Reeves 1994). Moreover, legume CCs can provide symbiotically-fixed N to the soil, offering a potential replacement to Haber-Bosch N (Miguez and Bollero 2005; Liebman et al. 2012). Legume CCs can be intercropped in a cash crop and grown after its harvest. Cover crops can thus increase yields of the succeeding crop without sacrificing a cropping season. In Western Canada, Khakbazan et al. (2014) found that even though crop yields were greater following a whole-season legume green manure than following a legume CC, the difference was not sufficient to compensate for the loss of revenue during the green manure year.

In a meta-analysis, Tonitto et al. (2006) concluded that replacing conventional mineral fertilization by legume CC can be a viable practice. Averaged across studies, legume CCs achieved 90 % of crop yields obtained with the recommended rates of mineral fertilizers after a winter fallow. Some cash crop species such as sorghum produced similar yield with legume CC and inorganic fertilizers. Moreover, the increase in cash crop yield depended on CC species and climate, two parameters affecting symbiotic N fixation. In colder regions, or when using a less productive legume CC species, CC-derived N may be insufficient, and providing supplemental inorganic N may be necessary to achieve optimal yields (Tonitto et al. 2006).

According to Thorup-Kristensen et al. (2003), the best parameter to evaluate CC impact on cash crop yield is the fertilizer replacement value (FRV), which takes into account N as well as non-N related effects. It is calculated by constructing a yield response curve as a function of mineral N fertilizer rate for the cash crop, and then finding the N rate value

corresponding to the yield obtained with CC in the unfertilized (0 N) soil (Hesterman et al. 1992; Liebman et al. 2012). Examples of non-N related effects of CCs that have an impact on yields of the following cash crop include build-up of soil organic C and N (Angers et al. 1993; Destain et al. 2010) and improvement of soil structure (Abdallahi and N'Dayegamiye 2000).

Red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) can be successfully intercropped in a cereal crop without reducing its yield (Shrader 1966; Bergkvist 2003; Schipanski and Drinkwater 2011). These legumes are known for their high N-fixing capacity and their positive impact on crop yield in the following year (Vyn et al. 2000; Askegaard and Eriksen 2007; Bergkvist et al. 2011; Thilakarathna et al. 2015). They are preferred to other legume species because of the low price of seeds (Liebman et al. 2012). Intercropped red clover was estimated to have an FRV of 30 to 103 kg N ha⁻¹ in corn in the following year (Stute and Posner 1995a; Vyn et al. 2000), 97 kg N ha⁻¹ in potatoes and beets (Schröder et al. 1997), and 81 to 85 kg N ha⁻¹ in wheat (Garand et al. 2001). In a previous experiment in the province of Quebec, Verville (2014) found that a mixture of red and white clovers intercropped in barley contained 97 to 121 kg N ha⁻¹ in its above-ground biomass at time of termination in the fall, increasing spring wheat yields by 8 to 36% the following year.

To determine the optimal fertilization strategy combining a cover crop and supplemental fertilizations for canola, a study was conducted at four site-years in the province of Quebec, Canada. The objectives were to measure the impact of (i) a preceding clover CC and (ii) mineral or organic supplemental fertilization on canola N uptake and grain yield,

as well as to (iii) measure the FRV of the clover CC based on canola grain yield under a wet and cool climate.

MATERIALS AND METHOD

Description of Locations and Experimental Treatments

The experiment was conducted from 2013 to 2015 on four fields in the province of Quebec, Canada. Two of the fields were located in Normandin (NO), at the Agriculture and Agri-Food Canada research farm (48°49'57"N, 72°33'36"W), on a silty clay (Normandin series, orthic gleysol), and the other two were located in Saint-Augustin-de-Desmaures (SA), at the Laval University experimental farm (46°43'28"N, 71°30'15"W), on a sandy clay loam (Saint-Laurent series, orthic humic gleysol). Two-year cropping trials were established in two consecutive years (2013–2014 and 2014–2015) on different plots in adjacent fields at each location, for a total of four two-year trials. Mean annual air temperature is 0.8°C at NO, and 4.2°C at SA, whereas mean annual precipitations are 871 mm and 1190 mm, respectively (Environment Canada 2016). Selected soil characteristics are presented in Table 1. The monthly air temperature and precipitation during investigation are presented in Fig. 1.

The factorial experiment was arranged in a split-plot design. The main factor was the preceding crop (barley only or barley intercropped with clover CC), which was established in the first cropping year, and the sub-factor was fertilization, which compared two fertilizer types (mineral or organic fertilizer), each at four different N rates (0, 50, 100, and 150 kg N ha⁻¹); the fertilization sub-factor was applied to canola in the

second cropping year. Since the “Zero-N” plots did not receive any fertilizer, they were used as the control treatment for both the mineral and the organic fertilizer treatments, for a total of seven fertilization treatments. Experimental treatments were replicated four times in randomized blocks for a total of 56 plots per trial.

All trials were established in the spring, after a cereal crop that was tilled (moldboard plowing at 20 cm depth) in the previous fall. Main plots were established in May after a secondary tillage with field cultivator at a 5 cm depth. Barley (*Hordeum vulgare* L.) was sown 2.5 cm deep at 350 seeds m⁻² in barley-only plots, and at 245 seeds m⁻² in plots with the CC, using a Fiona seeder (Fiona Maskinfabrik A/S, Bogense, Denmark) with 12 cm row spacing at SA, and with a John Deere 452 seeder (John Deere, Moline, IL, USA) with 15 cm row spacing at NO. A mixture of red clover and white clover (var. Huia) was seeded the same day as barley, each at the recommended rate of 4 kg seed ha⁻¹ (Belzile et al. 2016), in half of the main plots with a Brillion seeder (Landoll corporation, Marysville, KS, USA). All plots were fertilized with fertilizer N, phosphorus (P), and potassium (K), based on pre-plant soil analyses and recommended rates for barley (CRAAQ 2010). In June, MCPB/MCPA (Tropotox Plus[®], 1594 g a.i. ha⁻¹/106 g a.i. ha⁻¹) was used to control weeds in all plots, as well as Thifensulfuron methyl/Tribenuron methyl (Refine[®] SG, 10 g a.i. ha⁻¹/5 g a.i. ha⁻¹) in barley-only plots at SA, while MCPB/MCPA (Clovitox Plus[®], 1125 g a.i. ha⁻¹/75 g a.i. ha⁻¹) was used at NO in all plots. Barley was harvested in August. Clover was chemically terminated with Glyphosate (Touchdown Total[®], 2750 g a.i. ha⁻¹) prior to fall tillage at NO, while no herbicide was used at SA. Plots were moldboard plowed (20 cm depth) between October 23 and 28 at NO, and between October 6 and 16 at SA.

For the second year of each trial, soil was prepared with a field cultivator at a 5 cm depth in mid-May at SA and in mid-June at NO. Subplots were delineated and the different fertilization treatments were randomly allotted to each sub-plot (1.44 m x 5.5 m in size at NO, and 1.62 m x 6 m at SA). Rates of 50, 100 and 150 kg N ha⁻¹ (Ca-NH₄NO₃, 27-0-0) were applied in mineral fertilized plots, whereas pig slurry was applied to provide the same rates of “available” N, which was assumed to represent 80% of slurry total N content (CRAAQ 2003). For every 50 kg N ha⁻¹ increment, application rates were 9492 and 8003 kg slurry ha⁻¹ at SA and 9861 and 8471 kg slurry ha⁻¹ at NO for 2014 and 2015, respectively. Selected pig slurry characteristics are presented in Table 2.

Plots fertilized with pig slurry were assumed to receive sufficient amounts of P, K and sulfur (S) with the slurry. The other plots (0, 50, 100, and 150 N of fertilizer N) received 50 kg P₂O₅ ha⁻¹ as triple super phosphate (Ca(H₂PO₄)₂, 0-46-0), 50 kg K₂O ha⁻¹ as potassium chloride (KCl, 0-0-60), and 18 kg S ha⁻¹ as potassium sulfate (K₂SO₄, 0-0-51-18) at NO, and 20 kg P₂O₅ ha⁻¹, 20 kg K₂O ha⁻¹, and 20 kg S ha⁻¹ as manganese sulfate (MnSO₄, 0-0-0-32) at SA, according to soil tests and local recommendations for canola (CRAAQ 2010). Mineral fertilizers and pig slurry were surface-applied and incorporated (2 cm) within 1-3 h with a chain harrow at NO and a tine harrow at SA.

Canola (var. 45H29 at NO in 2014 and 2015, var. L150 at SA in 2014, and var. L140 at SA in 2015) was sown at a rate of 6 kg ha⁻¹, at 1-1.5 cm depth and with 18-cm row spacing, using a Wintersteiger plot seeder (Wintersteiger, Salt Lake City, UT). All plots were fertilized with boron (B) at 1 kg ha⁻¹ (Na₂B₈O₁₃, 7.41 L ha⁻¹ of a 135 g B L⁻¹ solution) by foliar application at the rosette growth stage. At NO, control of striped flea

beetle was done using carbaryl (350 g ha⁻¹ as Sevin[®] XLR at 750 mL ha⁻¹), and control of weeds was done with glyphosate (650 g ha⁻¹ as Touchdown Total[®] at 1.3 L ha⁻¹ in 2014 and 448 g ha⁻¹ as Roundup R/T 540 at 830 mL ha⁻¹ in 2015). No insecticides were used at SA, while glufosinate (500 g ha⁻¹ as Liberty 200SN at 2.5 L ha⁻¹) was used for weed control in both years. At NO, diquat (480 g ha⁻¹ as Reglone[®] desiccant at 2 L ha⁻¹) was applied to canola before harvest to accelerate dry down. Harvest was done with a Wintersteiger plot harvester in late-August to early-September at SA, and in late-September to early-October at NO.

Slurry, Plant, and Soil Sampling and Analyses

Pig slurry was obtained from a commercial hog operation, and analysed prior to application to determine total N by Kjeldahl acid digestion and colorimetry as detailed in Chantigny et al. (2007). Available N content was calculated and application rates determined as indicated above. Composite slurry samples were also taken during application, and analysed for total N and total P (acid hydrolysis), pH (direct reading with a glass electrode), and NO₃-N and NH₄-N (1 M KCl) as detailed in Chantigny et al. (2007). Slurry total C was determined by wet combustion with an automated analyser (model TOC-5050, Shimadzu Corp., Kyoto, Japan).

Clover above-ground biomass was measured prior to its incorporation in the fall. In each plot, plants were sampled in two 50 × 50 cm (SA) or 30 × 30 cm (NO) quadrats, dried, and weighed at 55°C for 72 hours for DM yield determination. Samples were then ground with a Wiley mill (model 4, Thomas Scientific, Philadelphia, PA, USA) to pass a 1-mm sieve for total C and N analyses by dry combustion with a CNS analyser (model Trumac,

LECO Corporation, St. Joseph, MI, USA). The N accumulation in clover biomass was determined by multiplying DM yield by the corresponding total N concentration.

Canola total above-ground biomass was measured at the 20% bloom stage. In each plot, plants were sampled on a 50 cm-long row, dried, weighed, and then ground as described above for clover. The ground material was analysed for N content by acid digestion with a mixture of sulfuric and selenious acids (Isaac and Johnson 1976) and colorimetry using a continuous flow injection analyser (QuikChem 8500, Lachat Instruments, Loveland, CO). The N content in canola above-ground biomass was determined by multiplying DM yield by the corresponding N concentration.

Canola grain yields were determined at harvest, after drying at 35°C for 24 h and being manually cleaned with a canola sieve set (No. 6.5 round hole and No. .038 slotted, Farmtronics Ltd., Regina, SK, Canada) at SA, and with a seed cleaner (Model Eclipse 324, A.T. Ferrel Company Inc., Bluffton, IN, USA) at NO. Yield was corrected at 90% DM content. Grain protein and moisture content were measured with a near-infra-red spectrometer (model DS2500, Foss Company, Hillerød, Denmark). Grain N content was estimated by dividing protein content by 6.25, and total grain N uptake was determined by multiplying grain N content by grain yield at 100% DM content.

In the canola year, soil samples were collected in each plot at seeding, at the 2-4 leaf stage, at the rosette stage, at the 20% bloom stage, at the end of flowering – beginning of green silique stage, and after harvest to determine soil mineral N content over time. Composite soil samples (0-20 cm) were taken using a 2-cm stainless steel corer. Bulk soil density (0-20 cm) was taken at the 2-4 leaf stage and after harvest in 2014, and at the

rosette stage in 2015, using the cylinder method as described by Hao et al. (2008). Soil mineral N concentration was determined within 48 h of sampling by shaking 25 g of field-moist soil for 60 min with 125 mL of 1M KCl, as described in Chantigny et al. (2007). The extracts were analysed for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ by continuous flow colorimetry (QuikChem 8500, Lachat Instruments, Loveland, CO). The soil mineral N content (kg ha^{-1}) was calculated as the sum of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations, corrected for moisture content, sampling depth, and bulk density.

Statistical and Regression Analyses

Statistical analyses were done using SAS University edition (©2012-2015, SAS Institute Inc., Cary, NC, USA). The homogeneity of variance was verified by the analysis of residuals, and data normality was tested with the UNIVARIATE procedure. The data did not need any transformation to achieve normality or homogeneity of variance. In order to compare mineral and organic fertilization with polynomial contrasts, we doubled the “0 N” treatment from the field design. The result was a $2 \times 2 \times 4$ three-way ANOVA for preceding crop (PREC), fertilizer type (TYPE), fertilizer rate (RATE), and their interactions, which was analysed with the MIXED procedure of SAS. Main effects and interactions were tested on above-ground N content of canola at the 20% bloom stage, as well as on grain yields. For each location, blocks were considered random effects, whereas year, PREC, TYPE, and RATE were considered fixed effects. Locations were analysed separately because of significant location $\times$ treatment interactions. However, a measure of variation between locations was made by using above-ground N content of canola at the 20% bloom stage as well as grain yields and total N content from

unfertilized plots without CC only. For these analyses, blocks were considered random effects while year and location were considered fixed effects. The same random and fixed effects were used to test for CC biomass, N concentration, total N contribution and C:N ratio variation between year and locations, since no treatment had been applied yet. Analysis of polynomial contrasts for linear, quadratic and cubic effects of RATE as well as PREC X RATE and TYPE X RATE interactions were conducted, only when these interactions were significant. Effects were considered significant at $P < 0.05$, while P values between 0.05 and 0.10 were considered to reflect trends. In accordance with the polynomial contrasts analysis and the means obtained with the MIXED procedure, linear or quadratic regression equations were obtained using the REG and RSREG procedures, respectively.

Statistical analyses revealed no interaction between year and treatments for above-ground N content at the 20% bloom stage. However, there was an interaction between year and treatments for grain yield at both locations. The ANOVA for above-ground N content at the 20% bloom stage was therefore done separately for each location, while canola grain yields were analysed separately by year and location.

The FRV of CC was estimated using quadratic regressions of canola yields as a function of N rate in plots receiving fertilizer N without CC. Canola yield in the unfertilized CC plots was used to determine FRV by finding the corresponding fertilizer N rate from the regression curve.

RESULTS AND DISCUSSION

Weather Data

Over the course of the experiment, temperatures were generally higher and precipitations greater at SA than in NO (Fig. 1). Precipitation was especially high during clover establishment in May 2013 at both locations, compared to 2014. In the years when canola was grown, the 2015 growing season appeared to be more humid than in 2014 at SA, with a difference of 44 mm in total precipitations from May to August.

Cover Crop Yield and Nitrogen Content

At time of CC termination, clover above-ground dry biomass was 4737 and 1442 kg ha⁻¹ greater at the warmer SA location compared to NO in 2013 and 2014 respectively (Table 3), probably because the weather in SA, at time of clover seeding as well as after barley harvest, favored clover growth. The low clover biomass at NO in 2013 can be attributed to barley lodging, which interfered with clover development. The N concentration of CC biomass was higher at NO (3.9% in 2013 and 3.6% in 2014) compared to SA (2.6% and 2.8%, respectively). The C:N ratios of the CC biomass were lower at NO (9.9 in 2013 and 11.1 in 2014) than at SA (14.1 and 15.4, respectively). Since all ratios were ≤ 15 , mineralization potential of the CC was assumed to be good for the four trials (Kuo and Jellum 2000; N'Dayegamiye and Tran 2001). The total N content of CC biomass that could potentially be mineralized varied from 28.3 to 150.5 kg N ha⁻¹ depending on years and locations (Table 3). This wide range was mainly caused by the large variation in biomass yield.

Effect of Cover Crops on Canola

Canola N content at the 20% bloom stage was similar with and without previous CC at both locations (Table 4; PREC effect). The effect of CC on canola became more obvious at harvest, and showed a significant interaction with fertilizer rate on grain yield (Table 5; Fig. 2). At SA, the CC effect on yields was the greatest in the unfertilized plots, with increases of 578 and 801 kg ha⁻¹ in 2014 and 2015, respectively, as compared to unfertilized plots without CC. This CC effect gradually decreased as N rate increased, and no significant CC effect was observed in plots fertilized with ≥ 100 kg N ha⁻¹ in 2014, and with 150 kg N ha⁻¹ in 2015. At NO, CC increased canola yields in the unfertilized plots by 288 and 184 kg ha⁻¹ in 2014 and 2015, respectively, but the application of 50 kg N ha⁻¹ was enough to mask this effect. This type of yield response where the CC has a significant impact on yields at 0 kg N ha⁻¹ and reduces or has no impact where additional N is supplied is typical of a N-related effect of CC (Liebman et al. 2012). The grain yields at NO in 2015, however, also showed evidence of a non-N related effect: at a 150 kg ha⁻¹ N rate, grain yield in plots with preceding CC were 321 kg ha⁻¹ lower than those in plots without preceding CC (Fig. 2). This finding was likely related to the occurrence of sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) observed on canola at the end of the flowering stage. Clover being susceptible to this pathogen, it could have acted as a host for the disease. It is however impossible to tell if sclerotinia occurred equally in canola receiving different fertilization treatments, since canola yields in CC plots could have been influenced both positively by CC and negatively by sclerotinia, thus making the two factors impossible to tell apart.

The introduction of a CC before canola caused a greater yield increase at SA than at NO. Besides the difference in CC biomass and N content between the two locations, this

specific difference in canola response could also be explained in part by differences in soil N supply capacity. Considering only the unfertilized plots without CC, in both years, grain yield was 708 to 904 kg ha⁻¹ greater at NO than at SA (Fig.2) and canola grain N content was 40 to 42 kg ha⁻¹ greater at NO than at SA (data not shown). These observations suggest that the soil N supply capacity, through mineralization of the soil N reserve, was greater at NO than at SA, where this mineralized N also has a higher leaching potential due to the coarser soil texture. This would explain why adding N, either through preceding CC and/or fertilization, elicited a greater response from canola at SA.

Effect of Fertilizer Type on Canola Yield and Nitrogen content

Canola N content at the 20% bloom stage was greater in plots receiving the mineral fertilizer compared to the plots with pig slurry (Table 4), regardless of application rate, indicating that N availability in pig slurry was not as high as expected. The coefficient of 0.8 used to calculate slurry N application rates were based on local recommendation (CRAAQ 2003). However, the C:N ratios of slurry applied during the experiment varied from 3.6 to 7.7 (Table 2) and was larger than the mean C:N ratio on which the recommendation is based (3.4; CRAAQ 2003). Therefore, N availability from the pig slurry used was likely overestimated. Canola N content at the 20% bloom stage further indicated that the availability of slurry N was overestimated by 13 to 37%, compared with canola N content with the mineral fertilizer. Therefore, at the 150 kg N ha⁻¹ application rate, the actual amounts of available N from slurry were 122 and 147 kg N ha⁻¹ at SA in 2014 and 2015, respectively, and 128 and 134 kg N ha⁻¹ at NO. A revised

version of local recommendations (CRAAQ 2013), now takes the slurry C:N ratio into account to modulate estimates of slurry N availability, and should be used in future research. The warm, sunny, and often windy weather conditions at time of N applications may also have contributed to lower pig slurry N availability by favouring ammonia volatilization (Smith et al. 2008).

Canola grain yields in plots receiving the mineral fertilizers were generally greater than in plots with pig slurry, the effect of fertilizer type being in interaction with N rate (Table 5, Fig. 3). At SA in 2014, yields were 443, 573, and 413 kg ha⁻¹ greater with the mineral fertilizers than pig slurry at N rates of 50, 100, and 150 kg ha⁻¹, respectively; in 2015, yields were 291 and 466 kg ha⁻¹ greater at 50 and 100 kg N ha⁻¹, respectively, but were not significantly different at 150 kg N ha⁻¹. At NO in 2014, compared to pig slurry, the mineral fertilization resulted in greater canola yields (388 kg ha⁻¹) at 50 kg N ha⁻¹, similar yields at 100 kg N ha⁻¹, and smaller yields (-327 kg ha⁻¹) at 150 kg N ha⁻¹; in 2015, yields were 423 and 445 kg ha⁻¹ greater with the mineral fertilizer than pig slurry at 50 and 100 kg N ha⁻¹, but similar yields were obtained at 150 kg N ha⁻¹. These differences in yield were consistent with the differences found in canola N content at the 20% bloom stage. Compared to the results of Lafond (2004), who found no difference in canola grain yield when fertilized with pig slurry rather than fertilizer N, our results seem to point at the lower-than-expected availability of pig slurry N. However, at higher N rates (100 to 150 kg N ha⁻¹), there is no difference in canola grain yield between fertilizer N and pig slurry fertilization and pig slurry gave higher canola grain yield at 150 kg N ha⁻¹ in 2014 at NO, suggesting that there were positive non-N effects of the pig slurry.

Further analysis of the fertilizer type $\times$ N rate interaction on canola yields revealed that the interaction was quadratic at both locations (Table 5). When calculating the quadratic regression curves of canola response to fertilization (Fig. 3), maximum yield values were obtained with the mineral fertilization. Based on these regression curves, maximum yields were achieved with 138 kg N ha^{-1} at SA in 2015, and with 120 kg N ha^{-1} at NO in 2014. The N rate for maximum yield at SA in 2014 and NO in 2015 could not be estimated as they were beyond the maximum N rate used in this study ($>150 \text{ kg N ha}^{-1}$).

Our results are in line with those obtained in Eastern Canada by Ma et al. (2015) on sandy to loamy soils, where highest canola yields were achieved with N rates between 105 and 175 kg N ha^{-1} applied as urea at seeding. In another study conducted by Lafond and Pageau (2008) at Normandin and Hébertville (located at about 100 km from Normandin) on a silt clay and a silty clay loam respectively, highest canola yields were obtained at N rates between 80 and 120 kg N ha^{-1} applied as calcium ammonium nitrate.

In contrast with mineral fertilization, convex curves were obtained for the response of canola yields to pig slurry (Fig. 3). This type of response did not allow calculating N rate for maximum yield, and does not correspond to the response of canola that is generally found in the literature, when N is the limiting factor for yields (Khakbazan et al. 2011). The convex shape of the curves suggests that N was not the only limiting factor at low slurry application rates. Those other limiting factors however tended to be alleviated at greater slurry rates by non-N effects, considering that yields were either not significantly different or higher with pig slurry at rates between 100 and 150 kg N ha^{-1} at NO in 2014 and 2015, and at SA in 2015. Canola is known to have high S needs (Janzen and Bettany

1984), and the soils at both locations were considered to be low in S for brassicas, based on local standards (CRAAQ 2010). While the plots with mineral fertilization received the same amount of mineral S, the plots with pig slurry received S as provided by the slurry, which could have been insufficient to achieve the same yields as with the mineral fertilization, especially at the lower application rates. This could also explain the marked response of canola yield to pig slurry at rates $\geq 100 \text{ kg N ha}^{-1}$, especially at NO in 2014 where yields were greater with pig slurry than mineral fertilization at 150 kg N ha^{-1} (Fig. 3). Even though it was not evaluated in the present study, greater S supply as slurry application rate increased likely improved N utilization by canola (Janzen and Bettany 1984). Other non-N effects, such as increased soil microbial activity caused by the application of an organic amendment (Goyal et al. 1999) could have had a positive impact on canola yields, as reported for corn and barley by Harris et al. (1994). These observed effects cannot be explained and should be taken into consideration in further research.

An interaction between preceding crop and fertilizer type on canola yields, independent of N rate, was observed at SA in 2015 (Table 5). In the barley plots without CC, the mineral fertilization resulted in yields significantly greater ($+377 \text{ kg ha}^{-1}$) than with pig slurry, whereas the difference found in plots with previous CC ($+87 \text{ kg ha}^{-1}$) was not significant. Considering that the difference in yield between the two fertilizer types were most likely due to different N availability, our results are consistent with those of Liebman et al. (2012) who found that the effect of N fertilization was stronger where no legume CC had been previously grown.

Soil Mineral Nitrogen Dynamics

At both locations, soil mineral N content was the highest at the beginning of the growing season (from seeding to the 2-4 leaf stage) and was generally proportional to N rate (e.g. Fig. 4-5; as the pattern was similar for both years, only results for 2014 are shown, for both locations). The content decreased to background levels ($<10 \text{ kg N ha}^{-1}$) between the rosette growth stage and harvest. The effect of fertilizer type on soil mineral N content was consistent with that observed in canola N content at the 20% bloom stage, the plots with mineral fertilization having higher mineral N levels compared to the plots fertilized with pig slurry. It was also expected that the CC plots would have higher mineral N levels, since grain yield data revealed N-related effects of CC. However, the contribution of the previous CC to early-season soil N concentrations was inconsistent across years and among N rates. At SA, the plots with previous CC had higher levels of mineral N at the 2-4 leaf stage of canola, this effect being significant only in 2014 ($P = 0.042$). At NO, the soil mineral N levels tended to be higher ($P = 0.080$ to 0.096) at the first two sampling dates in 2015. The effect of CC was more obvious in the unfertilized plots at time of canola seeding, when soil mineral N content was significantly higher with previous CC, in all trials except at NO in 2014. As discussed above, total N accumulated in the CC biomass was low for this specific trial, since clover growth in the preceding year had been hampered by barley lodging. Comparing results from the two locations, soil mineral N content averaged over the two trials appeared to be generally higher at NO than at SA (Fig. 4-5). In the unfertilized plots without previous CC, soil mineral N content was 16 kg ha^{-1} higher at NO compared to SA at time of canola seeding. This further supports the earlier discussion that canola N uptake and yields were generally

greater at NO because of a greater N supply capacity in the fine-textured soil at NO than in the coarser soil at SA.

Cover Crop Fertilizer Replacement Value

The FRVs (Fig. 6) were calculated based on canola yields rather than its N uptake at the 20% bloom stage in order to consider the whole growing season in the estimates. The response of canola to N fertilization were assumed to be quadratic (Malhi and Gill 2006; Khakbazan et al. 2011). Since the canola yields in the unfertilized plots with previous CC were significantly greater than without previous CC for the four trials, and since canola showed a positive response to the applied fertilizer, it was therefore possible to calculate the FRV of CC (Hesterman et al. 1992). The FRVs of the previous clover CC were 36.4 and 81.8 kg N ha⁻¹ at SA in 2014 and 2015, respectively, and 27.3 and 21.8 kg N ha⁻¹ at NO (Fig. 6). While the FRVs obtained at SA corresponded to the FRVs found in the literature for different crops, i.e. between 30 and 108 kg N ha⁻¹ (Stute and Posner 1995a; Schröder et al. 1997; Vyn et al. 2000), the FRVs obtained in NO were lower.

Even though the low C:N ratios of CC biomass were predictive of a net and rapid mineralization (Table 3), differences in FRVs between years were not in close agreement with the differences in CC biomass C:N ratios, or with the potential N contribution of CC at time of plowing (Table 3). At SA, despite the exceptionally high N content of the CC biomass in the 2013-14 trial, the corresponding FRV was 2.25 times lower than in the 2014-15 trial when the CC biomass had accumulated much less N (Table 3). Similarly, at NO, the biomass CC produced in the 2013-14 trial resulted in a higher FRV compared to the 2014-15 trial, despite the lower N content. Factors other than the C:N ratio or N

accumulated in the CC biomass must therefore be taken into account. The climatic conditions (e.g. warmth and timing of precipitation in the spring, extreme events), which vary from year to year, have a marked influence on the response of canola to fertilization. For instance, at SA, canola responded more strongly to applied fertilizer N in 2014 than in 2015, possibly because of higher precipitations leading to higher N losses during the 2015 growing season, causing the FRV to be much lower in 2014, even though canola yields in the unfertilized CC plots, which were used to calculate the FRV, were relatively similar between years (2622 and 2695 kg ha⁻¹ respectively). Quemada and Cabrera (1995) observed that N mineralization of CC residues happened very quickly in the first 16 days after residue application to soil. If mineralization happened before the seeding of canola in the next spring, the mineral N from the CC could have been lost during the non-growing season (Chantigny et al. 2002). Non-growing season N losses likely varied between years and locations in the present experiment, especially as temperatures during the non-growing season were contrasted between years at both locations (Fig. 1). These conflicting findings indicate that the amount of N present in the CC biomass at time of incorporation to soil alone was not a good predictor of FRV in the next year.

CONCLUSION

Intercropping clover in barley had no significant impact on canola N uptake in the next year, but it did increase canola yields. The greatest increases were found without fertilization, and this positive response to the clover CC was attenuated as N rates increased, indicating that clover CC contribution to canola yields was mainly due to N-related effects. Canola response to clover CC appeared to be greater in the sandy loam

soil (at SA), which had a lower N supply capacity and a higher leaching potential, than in the silty clay soil at NO. The FRV of the clover CC ranged from 21.8 to 81.8 kg N ha⁻¹.

There was no interaction between the CC and fertilizer type on canola N uptake or yields. However, canola N uptake and yield were lower when fertilized with pig slurry than mineral fertilizer at rates ≤ 100 kg available N ha⁻¹, indicating that pig slurry N was less efficient than expected based on local recommendations. However, at rates higher than 100 kg available N ha⁻¹ (closer to 150 kg N ha⁻¹ at NO) pig slurry resulted in yield similar to or greater than with the mineral fertilizer. We conclude that a significant reduction in fertilizer N can be achieved in canola by growing clover as an intercrop in previous barley, especially in coarser soil, and that success in the use of an organic fertilizer, such as pig slurry, must be based on adequate N availability estimates.

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For Review Only

Table 1. Selected soil characteristics (0-20 cm depth) for the two trials (2013-2014, 2014-2015) at Saint-Augustin-de-Desmaures (SA) and Normandin (NO) in Québec,

Location	Sand	Clay	P ^a	K ^a	B ^b	S ^a	N ^c	C ^c	pH _{water}	CEC ^d	Bulk density
Year	----- g·kg ⁻¹ -----								-	M _{eq} ·100 g ⁻¹	g·cm ⁻³
SA											
2013-2014	521	244	100	129	0.50	10.31	1.88	20.4	6.4	18.4	1.24
2014-2015	374	311	89	127	0.51	11.11	2.15	22.7	6.7	23.0	1.17
NO											
2013-2014	106	440	48	121	0.44	11.47	1.55	21.0	5.6	19.2	1.09
2014-2015	106	440	42	119	0.50	9.12	1.65	21.6	6.1	19.3	1.17

Canada.

^aMehlich-III^bHot water^cDry combustion^dCation exchange capacity

Table 2. Selected characteristics of pig slurry applied in spring 2014 and 2015 at Saint-Augustin-de-Desmaures (SA) and Normandin (NO).

Location	pH	DM ^a	C:N	Total N	NH ₄ ^b	P	S
Year	-	g·kg ⁻¹	-	-----	g·kg ⁻¹	-----	-----
SA							
2014	6.3	80.3	5.6	6.24	4.10	1.24	0.89
2015	7.4	70.6	3.8	7.51	4.58	1.07	0.57
NO							
2014	6.3	127.0	7.7	7.23	4.80	1.95	1.09
2015	7.4	68.7	3.6	7.25	4.62	1.07	0.54

^aDM : Dry matter

^bNO₃ concentration was negligible (0.01-0.05% of mineral N).

Table 3. Above-ground biomass, N content, and C:N ratio of the clover cover crop measured at termination in fall 2013 and 2014 at Saint-Augustin-de-Desmaures (SA) and Normandin (NO).

Location Year	Biomass ^a (SE) ^b kg ha ⁻¹	N content	C:N ratio
SA			
2013	5455 (244) <i>a</i>	150.5 (4.8) <i>a</i>	14.1 (0.2) <i>b</i>
2014	3412 (244) <i>b</i>	86.7 (4.8) <i>b</i>	15.4 (0.2) <i>a</i>
NO			
2013	718 (246) <i>d</i>	28.3 (4.9) <i>d</i>	9.9 (0.3) <i>d</i>
2014	1970 (244) <i>c</i>	70.7 (4.8) <i>c</i>	11.1 (0.2) <i>c</i>

Note: Means within a column not sharing a lower case italic letter differ significantly at the $P < 0.05$ level

^a100% dry matter

^bStandard error

Table 4. Treatment means and *P*-values for the effect of preceding crop (PREC), fertilizer type (TYPE), and N rate (RATE) on total above-ground N content in canola at the 20% bloom stage at Saint-Augustin-de-Desmaures (SA) and Normandin (NO). Means are averaged over the two years.

Total above-ground N uptake		
(kg ha ⁻¹)		
	SA	NO
Barley with clover CC	105.5	136.4
Barley only	95.4	119.5
Fertilizer N	108.0	137.9
Pig slurry	93.0	117.9
0	77.2	93.2
50	102.4	117.0
100	110.5	143.1
150	111.8	158.4
<i>P</i> -values		
PREC effect	0.173	0.100
TYPE effect	0.009	0.007
PREC*TYPE	0.117	0.908
RATE effect	<0.001	<0.001
PREC*RATE	0.825	0.852
TYPE*RATE	0.185	0.242
PREC*TYPE*RATE	0.660	0.967
RATE linear	<0.001	<0.001
RATE quadratic	0.038	0.560
RATE cubic	0.682	0.685

Note: Significant *P*-values are written in bold.

Table 5. Summary of analysis of variance for the effect of preceding crop (PREC), fertilizer type (TYPE), and N rate (RATE) on canola grain yield at Saint-Augustin-de-Desmaures (SA) and Normandin (NO)

	<i>P</i> values			
	SA		NO	
	2014	2015	2014	2015
PREC effect	0.114	0.073	0.678	0.169
TYPE effect	<0.001	<0.001	0.688	<0.001
PREC*TYPE	0.897	0.011	0.825	0.822
RATE effect	<0.001	<0.001	<0.001	<0.001
PREC*RATE	0.040	<0.001	0.005	<0.001
TYPE*RATE	0.025	0.032	<0.001	<0.001
PREC*TYPE*RATE	0.544	0.163	0.917	0.639
PREC*RATE linear	0.016	<0.001	<0.001	<0.001
PREC*RATE quadratic	0.306	0.064	0.992	0.845
PREC*RATE cubic	0.202	0.600	0.692	0.568
TYPE*RATE linear	0.027	0.165	0.002	0.356
TYPE*RATE quadratic	0.030	0.011	<0.001	<0.001
TYPE*RATE cubic	0.969	0.473	0.055	0.902

Note: Significant *P*-values are written in bold.

Fig. 1 Average daily air temperature and monthly precipitation for the experimental period at Normandin (NO) and Saint-Augustin-de-Desmaures (SA). The dotted curve represents 30-y average temperatures. Data obtained from the Environment Canada weather stations located at Roberval, located approximately 50 km south of Normandin, and at Jean-Lesage International Airport, located approximately 10 km northeast of Saint-Augustin-de-Desmaures.

Fig. 2 Canola grain yield response to N fertilizer, with (◆) and without (■) preceding cover crop. Yield was measured at harvest in fall 2014 and 2015 at Saint-Augustin-de-Desmaures (SA) and Normandin (NO). Vertical bars indicate standard error of the mean. Asterisks indicate a significant yield difference for a given N rate.

Fig. 3 Canola grain yield response to mineral (▲) and organic (●) N fertilization. Yield was measured at harvest in fall 2014 and 2015 at Saint-Augustin-de-Desmaures (SA) and Normandin (NO). Vertical bars indicate standard error of the mean. Asterisks indicate a significant yield difference for a given N rate.

Fig. 4 Temporal dynamics of soil mineral N content on the canola year, as influenced by preceding crop and fertilizer type for the different N fertilizer rates ($\diamond$ 0 kg N ha⁻¹; $\square$ 50 kg N ha⁻¹; $\triangle$ 100 kg N ha⁻¹; $\circ$ 150 kg N ha⁻¹); results for the 2014 growing season at Saint-Augustin-de-Desmaures (SA). Each point corresponds to a sampling time (at seeding, at the 2-4 leaf stage, at the rosette stage, at the 20% bloom stage, at the end of flowering – beginning of green silique stage, and after harvest). Vertical bars indicate standard error for each date and for each treatment combination.

Fig. 5 Temporal dynamics of soil mineral N content on the canola year, as influenced by preceding crop and fertilizer type for the different N fertilizer rates ($\diamond$ 0 kg N ha⁻¹; $\square$ 50 kg N ha⁻¹; $\triangle$ 100 kg N ha⁻¹; $\circ$ 150 kg N ha⁻¹); results for the 2014 growing season at Normandin (NO). Each point corresponds to a sampling time (at seeding, at the 2-4 leaf stage, at the rosette stage, at the 20% bloom stage, at the end of flowering – beginning of green silique stage, and after harvest). Vertical bars indicate standard error for each date and for each treatment combination.

Fig. 6 Canola yield response to fertilizer N and apparent fertilizer replacement value (FRV) of cover crops (CC) in non-fertilized plots, as measured at harvest in fall 2014 and 2015 at Saint-Augustin-de-Desmaures (SA) and Normandin (NO). Vertical bars indicate standard error.