Nitrogen Supply from Green Manure Enhanced with Increased Tillage Frequency: A Note

Leonardo X. León Castro and Joann K. Whalen*

ABSTRACT

Tillage practices influence the decomposition of green manure and could be adjusted to synchronize the N supply from residues with crop N demand. This note evaluated ion exchange membranes (IEM) as an in situ tool for monitoring ammonium (NH_4^+) and nitrate (NO_3^-) dynamics after pea (*Pisum sativum* L.)–oat (*Avena sativa* L.) green manure was incorporated by one, two, or four passes of a rototiller. Mineral N from IEM and in 2 M KCl soil extracts was related to the cumulative N assimilated by arugula (*Eruca sativa* L.) during a 6-wk period. Greater tillage intensity increased the IEM-NO₃⁻–N concentration on ion exchange membranes significantly, from 1.94 to 18.7 µg cm⁻² wk⁻¹, and the N supplied from green manure increased arugula N uptake significantly. The IEM-N were as reliable as the soil chemical extractant in evaluating the mineral N released from green manure under field conditions.

Core Ideas

- Ion exchange membranes were tested for in situ evaluation of soil mineral N.
- More tillage passes increased green manure decomposition and soil mineral N.
- Ion exchange membrane were a good indicator of arugula N uptake.
- Pea-oat green manure supplied up to 20% of the N required by arugula.

Published in Agron. J. 111:935–941 (2019) doi:10.2134/agronj2017.08.0471

Copyright © 2019 by the American Society of Agronomy 5585 Guilford Road, Madison, WI 53711 USA All rights reserved **G** REEN MANURE is a crop residue that releases N for subsequent cash crops such as vegetables, potato (*Solanum tuberosum* L.), cereals, and oilseeds (Thorup-Kristensen, 1993; Vyn et al., 2000; Entz et al., 2001; Sincik et al., 2008). On average, the N released from green manure provides 17% of the crop N requirements during the growing season (Gardner and Drinkwater, 2009) improve the N recovery use efficiency from green manure, it is important to know how much mineral N (NH_4^+ plus NO_3^- concentrations) will be released from the residues, when net N mineralization occurs after the plow-down event, and how much mineral N is acquired assimilated by the subsequent crop. This can be evaluated in laboratory- and greenhouse-based studies on plant N uptake, or through non-plant testing methods.

Methods to predict the amount of organic N mineralization after the incorporation of green manure residues often rely on analysis of mineral N in field soil cores through soil chemical extraction with 2 M KCl or 0.5 M K₂SO₄ solution. The disadvantage of soil chemical extraction is that removal of soil cores disrupts the sampling area, demands more time, and does not always reflects the nutrient release dynamic because cores are collected at discrete point in time. In contrast, IEM can be deployed in situ, their two-dimensional nature allows a better contact with the soil matrix and causes minimal soil disturbance, and act as a sink for NH₄⁺ and NO₃⁻ ions, similar to plant roots, while showing similar responses to the environmental conditions and edaphic factors that affect plant N uptake (Qian and Schoenau, 2002; Zebarth et al., 2009). This was by León Castro and Whalen (2016a), who showed that the concentration of IEM-NO₃⁻-N was sensitive to arugula N uptake and ambient variables (i.e., rainfall, soil temperature, and soil moisture) in a field planted with green manure, before and after the green manure plow-down occurred. The reliability of IEM as an indicator of N release can be validated by correlating mineral N concentration, IEM-NH₄⁺–N and IEM- NO₃⁻–N values during crop residue decomposition.

L.X. León Castro and J.K. Whalen, Dep. of Natural Resource Sciences, Macdonald Campus of McGill Univ., 21111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, H9X 3V9, Canada; L.X.L. Castro, ESPOL Polytechnic Univ. Escuela Superior Politécnica del Litoral, ESPOL, Facultad de Ciencias de la Vida, Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador; L.X.L. Castro, ESPOL Polytechnic Univ. Escuela Superior Politécnica del Litoral, ESPOL, Centro de Investigaciones Biotecnológicas del Ecuador, Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil, Ecuador. Received 15 Aug. 2017. Accepted 25 Oct. 2018. *Corresponding author (joann.whalen@mcgill.ca).

Abbreviations: IEM, ion exchange membranes; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

Tillage is a common practice on Canadian farms, more than 75% of agricultural land cultivated with plow and harrow. Although soil conservation practices like reduced tillage and direct seeding are gaining in popularity, particularly in semiarid regions of Canada (Statistics Canada, 2012), the reality is that most farmland is tilled, especially in organic production systems. The main reason to use tillage is to incorporate organic residues such as compost and crop residues into the soil and mechanically fragment the residue into smaller particles, thereby accelerating their colonization by microorganisms, decomposition of polymeric compounds contained in the residue, and ions/nutrient release (Whalen, 2014). Termination of a green manure crop is traditionally accomplished through a plow-down event, which ends the crop growth, reduces the physical size of the aboveground and belowground residues, and brings the residue into contact with soil decomposers (fauna and microorganisms) that are responsible for nutrient recycling. Termination of green manure with tillage stimulates N mineralization and results in at least 50% increase in soil mineral N concentration, compared to no tillage/mulch systems of green manure termination (Groffman et al., 1987; Drinkwater et al., 2000; Halde and Entz, 2014). Reduction in the size of crop residue may be accomplished with tillage equipment, by mowing/chopping the aboveground biomass, or by the action of soil macrofauna in agroecosystems with low intensity, infrequent, or no tillage. Residues with smaller physical size are more exposed to microbial attack than intact residues due to the greater surface area for microbial colonization (Angers and Recous, 1997). In a study with wheat (Triticum aestivum L.) straw residue, Ambus and Jensen (1997) reported net N mineralization of 10.4% from residue <3 mm diam. and 8.6% from residue size of 25 mm, after 60 d. In the same study, the authors reported more N mineralized $(3.3 \text{ mg N kg}^{-1})$ from barley (Horeum vulgare L.) residues having a size <3 mm than 25 mm (2.7 mg N kg⁻¹). They concluded that the contact between fine crop residues and soil stimulated the N mineralization rate. Despite the advantages of tillage to increase disruption of the Ap profile might detrimental to soil biota, therefore, study is needed to optimize intensive tillage (i.e., more passes of the tillage equipment) as a method to incorporate green manure.

This note describes the use of IEM to monitor soil mineral N dynamics, prior to and during the 6 wk period following the plow-down of pea–oat green manure, using three levels of tillage intensity (one, two, or four passes of a rototiller). In situ soil mineral N on IEM was compared with the mineral N concentration in 2 M KCl extracts, and related to the N uptake of arugula, a short-season brassica crop. The relationship between tillage intensity and N supply from green manure was determined.

MATERIALS AND METHODS

The experiment was conducted from June to August 2015 at the Horticultural Research Centre on the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada (45°24' N, 73°56' W). The precipitation, soil temperature, and moisture were monitored every 30 min, with an automated weather station (Watch Dog 2000 Series, Spectrum Technologies Inc, Aurora, IL). Soil sensors (SM 100, Series, Spectrum Technologies Inc, Aurora, IL) were installed at 0.15-m depth to monitor soil temperature and soil moisture continuously during the study period (6 wk). Weather conditions during the experimental period were similar to long-term averages for June to August in the area (Environment Canada, 2016), with soil temperatures from 21 to 25°C, soil moisture between 42 and 266 g kg⁻¹, and precipitation of 2.7 to 86.3 mm per week (Table 1). The soil was a Saint Bernard Ioam (Canadian Agriculture Service Coordinating Committee, 1998) classified as a coarse-loamy, isotic, frigid Typic Haplorthod containing 400 g sand kg⁻¹ and 230 g clay kg⁻¹ with pH of 7.7 and 27 g organic C kg⁻¹ in the topsoil (0–0.2-m depth). Soil nutrient concentrations determined by Mehlich-3 extraction were 344 mg P kg⁻¹, 313 mg K kg⁻¹, 4371 mg Ca kg⁻¹, 643 mg Mg kg⁻¹, and 786 mg Al kg⁻¹. The field selected for this study was previously cultivated with pepper (*Capsicum annuum* L. 'Bell Boy') and used for tomato (*Solanum lycopersicum* 'Better Boy') production, 2 yr before this study began.

The experimental design was a randomized complete block with factorial treatment structure. Factor 1 was green manure (green manure and with green manure) and Factor 2 was the number of tillage passes (one, two, or four passes). Accordingly, the field site (17 by 25 m) had six experimental plots (3 by 3 m) in four blocks, for a total of 24 plots. Blocks were separated by a 1 m alley. Each experimental plot was divided into six sub-experimental units (1 by 1.5 m) for independent weekly sampling during the 6 wk study. The timeline for field activities is given in Table 2.

On 15 June 2015, the soil was cultivated and a green manure mixture of field pea and oat was broadcast at the rates of 90 and 83 kg ha⁻¹, respectively. Control plots without green manure were cultivated, but not planted. Since the cultivation stimulated weed seed germination, the control plots were kept weed-free by mowing the surface every 7 d with a string trimmer (Husqvarna 22cc, Stockholm, Sweden). On 19 July 2015, aboveground biomass in 1 m² quadrats of plots was equivalent to 3.6 t dry matter ha⁻¹. At this time, the field pea was at the vegetative stage, fourth node, leaf fully unfolded, more than one pair of leaflets (Knott, 1987), and oat was at Feekes growth stage 5, tillering, leaf sheaths strongly erected (Large, 1954). The green manure biomass had a C/N ratio of 15 and contained 400 g cellulose kg⁻¹, 270 g hemicellulose kg⁻¹, and 55 g of acid unhydrolyzable fiber kg⁻¹, based on the method of Van Soest et al. (1991). Tillage treatment began 6 wk after the green manure was planted, on 20 July 2015. A rototiller (Troy-Bilt ProLine CRT, Valley City, OH) set at a depth of 0.15 m was used to cultivate all plots. Control and green manure plots were subjected to one, two, or four passes over a 4-d period.

On 27 July 2015, arugula was sown by hand in all plots with spacing of 0.10 m between plants and 0.15 m between rows, giving a population of 200 plants per plot. Every week for the next 4 wk, arugula was sampled by removing nine plants (cut at ground level) at random from each plot. Plant material was weighed, oven-dried at 60°C for 24 h, ground to pass a 1 mm screen, and analyzed for total N with a Carlo Erba NC Soils analyzer (Milan, Italy). Arugula N uptake (g N plot⁻¹) was calculated by multiplying the g nutrient kg⁻¹ by the plant biomass (kg plant⁻¹) and the number of plants per plot. The proportion of arugula N uptake in arugula from green manure and soil was calculated according to Sharma and Behera (2009):

Relative N contribution from green manure (%) = [(Total N uptake – N uptake without green manure)/total N uptake] × 100

Table I. Weekly precipitation (total, in mm), average soil temperature and average soil moisture measured at 0.15-m soil depth during green manure plow-down and arugula growth periods (20 July–30 Aug. 2015) at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada.

Climatic parameter	Week I	Week 2	Week 3	Week 4	Week 5	Week 6
Precipitation, mm	86	20	26	3	49	10
Soil temperature, °C	21	22	24	22	22	25
Soil moisture, g kg ⁻¹	270	120	70	40	100	90

Relative N contribution from soil (%) = 100 - Relative N contribution from green manure (%)

Ion exchange membranes (Ionics CR67-HMR (cation) and AR204-SZRA (anion), Durpro, Candiac, QC, Canada) were used to quantify the IEM-NH4⁺-N and IEM-NO3⁻-N concentrations in soil solution, an indicator of mineral N available to arugula roots under the same environmental conditions (Ziadi et al., 2000; Qian and Schoenau, 2002; León Castro and Whalen, 2016b). Membrane sheets were cut into 0.02 m wide \times 0.05 m tall strips and stored in distilled water to avoid desiccation. Prior to use, the strips were saturated by shaking for 1 h in 1 M NaCl and kept in this solution until they were installed in the field. After green manure incorporation, one anion strip and one cation strip were buried in the planted row, 0.05 m from the closest arugula plant, in each plot. Each strip was placed in a random location along the planted row and no location was used more than once during the study. A slot was made with a garden trowel, the IEM strip was placed vertically in contact with the soil at 0.10-m to 0.15-m depth below the soil surface, and the slot was filled with soil by hand. One week later, the strip was retrieved, rinsed in the field with distilled water to remove soil particles, and placed in centrifuge tubes containing 25 mL of 1 M KCl to extract NH_4^+ and NO_3^- ions. In total, 144 IEM-NH₄⁺-N and 144 IEM-NO₃⁻-N samples were collected during the 6 wk study.

Soil samples were collected starting in the sixth week of the green manure growth period and continued weekly for the next 6 wk, including 1 wk after arugula harvest. Soil samples (0-0.15-m depth) were collected with a garden trowel to obtain one composite sample (from five locations) of approximately 1000 g field-moist soil from each plot. The composite sample was sieved (<10 mm mesh) to remove rocks and large crop residues, placed in Zip-lock bags and transported on ice to the laboratory. Half of the sample was sieved (<2 mm) and stored at -20° C, then thawed (20°C for 48 h) for soil mineral N and microbial biomass determination.

Soil mineral N (NH₄⁺–N and NO₃⁻–N) was extracted (1:10 ratio of field-moist soil: 2 M KCl), then the NH₄⁺ and NO₃⁻ concentrations in soil extracts and IEM extracts (described above) were measured by the modified indophenol blue method (Sims et al., 1995) at 650 nm on a microplate reader (μ Quant,

Biotek, Winooski, VT). Microbial biomass C and N (MBC and MBN, respectively) concentrations were determined by the chloroform fumigation-direct extraction method (Voroney et al., 2008). The MBC concentration was the difference in dissolved organic C of 0.5 mol L⁻¹ K₂SO₄ extracts (1:4 soil/solution) collected before and after fumigation, assayed on Sievers Innovox TOC analyzer (GE Analytical Instrument, Boulder, CO) and corrected for extraction efficiency with a $k_{\rm EC}$ value of 0.45. The difference in total extractable N of 0.5 mol L^{-1} K₂SO₄ extracts before and after fumigation, corrected for extraction efficiency ($k_{\rm FN} = 0.54$), gave the MBN concentration (Joergensen and Mueller, 1996). Crop residue size was determined using the rest of the composite soil sample by the wet-sieving method of Cambardella and Elliott (1993). Briefly, about 300 g of fieldmoist soil was placed on a nest of sieves with mesh sizes of 2 and 4 mm. Sieves were immersed repeatedly in water for 5 min at a rate of 20 immersions min⁻¹. Residues retained on the 2-mm sieve were dried (60°C for 24 h) and weighed.

Data was analyzed using SAS Version 9.3 software (SAS Institute, 2012). Two-way ANOVA was performed to examine the effects of green manure, tillage passes, and the green manure × tillage interaction on arugula N uptake and residue mass collected during the 6 wk. Mean comparison tests were done with a Tukey's test at the 95% confidence level. One-way ANOVA was performed to determine the effect of tillage treatment (one, two, and four passes) on net IEM-NH₄⁺-N, IEM-NO₃⁻-N, MBC, and MBN concentrations (net values were calculated as the difference between green manure-amended and control plots for each tillage treatment). Relationships between IEM-NO₃⁻-N, KCl-extractable NO₃⁻-N, and arugula N uptake were determined with Pearson's correlation coefficients.

RESULTS AND DISCUSSION

Soil mineral N concentration increased after the green manure plow-down, and then declined during the arugula growth period. The net change in $\rm NH_4^+$ pools, expressed as the difference between green manure-amended and control plots, reflected a decline in IEM-NH4⁺-N concentration from 0.10 to 0.01 μ g NH4⁺-N cm⁻² wk⁻¹, while the KCl-extractable NH4⁺-N concentration declined from 1.00 to -0.01 in control plots (Fig. 1A and 2A). This trend suggests a pulse of NH4⁺

Table 2. Summary of field activities during the study period (June–August 2015) at the Macdonald Campus of McGill University, Ste-Annede-Bellevue, QC, Canada.

Practice	Start date	Duration	End date
Soil cultivation, green manure broadcasted and growth period	l 5 June	5 wk	19 July
Green manure incorporation and plant decomposition	20 July	l wk	26 July
Soil sampling	20 July	6 wk	24 Aug.
$IEM-NH_4^+$ and $IEM-NO_3^-$ sampling	20 July	6 wk	24 Aug.
Arugula plant sampling	27 July	4 wk	17 Aug.
Precipitation, soil temperature, and moisture monitoring	I 5 June	l2 wk	30 Aug.



Fig. I. Net change in (A) IEM-NH₄⁺–N and (B) IEM-NO₃⁻–N concentrations, calculated as the difference between green manure and control plots, as affected by one, two, or four tillage passes at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada. Measurements were taken weekly, for 6 wk, after the tillage passes. Values are the mean (n = 4) and error bars represent standard error. Significant differences between the number of tillage passes is indicated with an asterisk (P < 0.05).

release from decomposing green manure that dimished with time, as confirmed by the significant (p = 0.001) effect of tillage passes × sampling time on the IEM-NH₄⁺–N concentration. Another explanation could be that NH₄⁺ uptake by arugula exceeded the NH₄⁺ production rate, which would explain the low soil NH₄⁺ concentration during the arugula growing period (Weeks 2 to 5 of the study).

In contrast, there was a net gain in the NO_3^- pools, also expressed as the difference between green manure-amended and control plots. On average, the IEM-NO₃⁻-N concentration increased from 7.9 to 18.7 $\mu g\,NO_3^{-}-N\,cm^{-2}\,wk^{-1}$ and the KCl-extractable NO_3^{-} -N concentration reached up to 17.4 mg $NO_3^{-}-N$ kg⁻¹ by Week 5 of the study (Fig. 1B and 2B). Compared to the pre-tillage levels, the green manure incorporation increased soil mineral N by 35% and even tillage of bare soil (control plots) resulted in 15% higher soil mineral N, due to mineralization of soil organic matter. A similar pattern of NH_4^+ and NO_3^- production and consumption was reported by Sarrantonio and Scott (1988) in the 6 wk after hairy vetch (Vicia villosa Roth) was incorporated by disc harrow, resulting in a 55% increase in soil mineral N due to green manure plow-down, which is much greater than the 22% increase in soil mineral N in no-till plots where green manure was terminated and left on the surface. Compared to plots with one tillage pass, those with



Fig. 2. Net change in soil mineral N in (A) KCI-extractable NH_4^+ -N and (B) KCI-extractable NO_3^- -N concentrations, calculated as the difference between green manure and control plots, as affected by one, two, or four tillage passes at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada. Measurements were taken weekly, for 6 wk, after the tillage passes. Values are the mean (n = 4) and error bars represent standard error. Significant differences between the number of tillage passes is shown with an asterisk (P < 0.05).

two or four tillage passes had higher IEM-NO₃⁻–N concentrations from 1 to 6 wk (p = 0.001; Fig. 1B), while four tillage passes resulted in greater KCl-extractable NO₃⁻–N than one tillage pass at 4 and 5 wk (Fig. 2B). This is similar to Lupwayi et al. (2006), who reported that the KCl-extractable NO₃⁻ concentration peaked 5 wk after the plow-down of pea green manure.

The IEM-NO₃⁻-N concentration reflects the cumulative NO₃⁻ adsorbed on ion exchange membranes during a period of time (7 d in this study) whereas KCl-extractable NO₃⁻-N concentrations are discrete measurements, however these NO₃⁻ values were correlated (r > 0.63, Table 3), suggesting that IEM reflected the temporal dynamics of NO₃⁻ following green manure plow-down.

Greater production of IEM-NO₃⁻–N with more tillage passes implies that tillage stimulates the activity of ammonia oxidizers and nitrifiers. Reasons why tillage may increase the IEM-NO₃⁻–N concentration include: (i) tillage reduces the

Table 3. Pearson correlation coefficients (*r*) between in situ measurements of ion exchange membrane (IEM)- NO_3^--N and soil NO_3^--N concentrations (n = 4 per wk), and between IEM- NO_3^--N and the N uptake by arugula (n = 9 per wk) in field plots with green manure and tillage treatments at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada.

	We	ek I	Wee	ek 2	Wee	ek 3	Wee	ek 4	Wee	ek 5	We	ek 6
-	IEM-N	0 ₃ –N†	IEM-N	O ₃ –N	IEM-N	O ₃ –N	IEM-N	O ₃ –N	IEM-N	O ₃ –N	IEM-N	O ₃ –N
Parameter	r	Р	r	Р	r	Р	r	Р	r	Р	r	Р
Soil NO ₃ –N‡	0.63	0.17	0.92**	<0.01	0.86*	0.02	0.90**	0.01	0.89**	0.01	0.95**	<0.01
Arugula N uptake	-	_	-	-	0.60	0.20	0.79*	0.05	0.7 9 *	0.05	0.76	0.07

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

+ IEM- NO₃⁻-N = NO₃⁻-N adsorbed on ion exchange membrane.

 \pm Soil NO₃⁻-N = NO₃⁻-N concentration by KCI-extraction of soil.

Table 4. Mass of residue (g dry matter kg⁻¹ soil) collected on a 2- to 4-mm sieve in control (no green manure) and green manure (peaoat mixture) field plots with one, two, or four tillage passes at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada. Measurements were taken weekly, for 6 wk, after the tillage passes.

— g dry mat 8 (0.25)a 5 (0.50)ab	ter kg ⁻¹ <u> </u>	1.3 (0.25)a	nd‡
8 (0.25)a 5 (0.50)ab	3.3 (0.19)b 1.9 (0.18)a	1.3 (0.25)a	nd‡
5 (0.50)ab	1.9 (0.18)a	00 (014)	
		0.0 (0.16)a	nd
0 (0.25)b	3.8 (0.37)b	nd	nd
0 (0.48)b	8.2 (0.58)c	I 3.3 (0.52)b	13.1 (0.09)a
l (0.25)c	8.6 (0.07)c	I 3.6 (0.49)b	23.0 (0.46)b
4 (0.26)c	8.6 (0.47)c	20.1 (0.47)c	25.9 (0.81)c
) (0.25)b) (0.48)b (0.25)c 4 (0.26)c	0 (0.25)b 3.8 (0.37)b 0 (0.48)b 8.2 (0.58)c (0.25)c 8.6 (0.07)c 4 (0.26)c 8.6 (0.47)c	0 (0.25)b 3.8 (0.37)b nd 0 (0.48)b 8.2 (0.58)c 13.3 (0.52)b 1 (0.25)c 8.6 (0.07)c 13.6 (0.49)b 4 (0.26)c 8.6 (0.47)c 20.1 (0.47)c

+ Average values (n = 4), with standard error in parenthesis, within the same column followed by the same letter do not differ significantly (P < 0.05, Tukey test).

‡ nd, Not detectable.

size of green manure residues making them more susceptible to decomposition, N mineralization and nitrification; (ii) tillage disrupts soil aggregates exposing and accelerating the turnover of native soil organic matter to decomposition, N mineralization and nitrification (Six et al., 1999); and (iii) tillage creates a better seedbed for plant roots, supports root growth and exudation of plant-based substrates that "prime" the microorganisms involved in soil N cycling (Clarholm, 1985; Cheng, 2009).

Tillage fragmented and reduced the size of green manure residues (p < 0.001, Table 4) while increasing microbial biomass from 7.58 to 20.81 mg MBC kg⁻¹ with one pass, and from 8.73 to 30.86 mg MBC kg⁻¹ with four passes (P = 0.03, Fig. 3). Throughout the experiment, the MBC and MBN concentrations were greater in plots with two or four tillage passes than one tillage pass (Fig. 3). Smaller residue size provides greater surface area for microbial colonization and decomposition, as confirmed by Bending and Turner (1999), who found more microbial biomass associated with fine residues (2 mm) than coarse residues (40 mm) of potato shoots (C/N ratio = 10). Delays in the decomposition of coarse residues are attributed primarily to physical limitation of surfaces for microbial colonization (Whalen et al., 2014), although soil conditions such as pH and moisture can also slow the biochemical breakdown of plantbased polymers (Swift et al., 1979). We conclude that the tillageinduced reduction in residue size (Table 4) was responsible for the higher IEM-NH₄⁺–N and IEM-NO₃⁻–N concentrations measured in green manure plots with more tillage passes.

Contribution of Green Manure to Arugula Nitrogen Uptake

Arugula N uptake was positively correlated with higher IEM-NO₃⁻–N concentrations (r > 0.60, p = 0.05, Table 3)

resulting from green manure input and tillage. After 4 wk, there was up to 30% more arugula N uptake with green manure than no green manure, and more arugula N uptake occurred in the green manure plots with two or four tillage passes than one tillage pass (p < 0.05, Table 5). Differences in arugula N uptake between control and green manure plots were noted in Week 3, probably due to higher N demand during this vegetative growth stage (Omirou et al., 2012).

The N uptake was between 45.8 to 78.8 g N kg⁻¹ dry matter in 3-wk-old plants, and there were no visual symptoms of N deficiency in any treatment, suggesting that the soil N supply was sufficient to meet the arugula N requirements. These values exceeded the critical N value of 30 g N kg⁻¹ for 3-wk-old brassica seedlings reported by Chen et al. (2004), including turnip (*Brassica campestris*) and Chinese cabbage (*Brassica rapa* subsp. *chinensis*), which is further evidence that arugula growth was not N limited.

The relative contribution of green manure to the arugula N requirements was 5 to 9% in plots with one tillage pass, from 5 to 17% with two tillage passes and between 7 and 20% with four tillage passes (Fig. 4). Although recently incorporated green manure supplies a relatively small percentage of the arugula N requirements, regardless of the number of tillage passes, the findings are consistent with previous studies. For instance, Seo et al. (2006) reported 15% N recovered by maize (*Zea mays* L.) as catch crop when hairy vetch was used as green manure. Likewise, Sharma and Behera (2009) reported that cowpea [*Vigna unguiculata* (L.) Walp.] and *Sesbania aculeata* (Willd.) Pers. green manures contributed 22 and 30% of N recovery in maize, respectively.

CONCLUSION

This note demonstrates that increasing tillage passes reduced the size of green manure residues and stimulated nitrification,



Fig. 3. Net change in (A) microbial biomass carbon (MBC) and (B) microbial biomass nitrogen (MBN) concentrations, calculated as the difference between green manure and control plots, as affected by one, two, or four tillage passes at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada. Measurements were taken weekly, for 6 wk, after the tillage passes. Values are the mean (n = 4) and error bars represent standard error. Significant differences between the number of tillage passes is shown with an asterisk (P < 0.05).

the size of the microbial biomass, and arugula N uptake. Two passes for the plow-down of pea/oat green manure supported better growth and N uptake by arugula than one pass, and is preferable to four passes, which is more costly in terms of labor requirements and might impact soil quality negatively. The pea–oat green manure supplied up to 30% of the N required by arugula and will likely continue to mineralize, given the increase in the mass residue retained on 2 to 4-mm sieves in the green manure plots from Week 1 to 6 of the study. The work presented here supports the use of IEMs to evaluate soil mineral



Fig. 4. Relative contribution of green manure to the N uptake of arugula grown in field plots at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada. Plots were cultivated with one, two, or four tillage passes to incorporate the green manure, a mixture of pea and oat. Control plots without green manure were cultivated with the same number of tillage passes.

N dynamics on a weekly basis. To increase N recovery from the spring-seeded green manure residue, it is recommended to plant a second cash crop, a fall-seeded cover crop or winter cereal crop.

ACKNOWLEDGMENTS

The authors thank the Technical Editor and anonymous reviewers for helpful comments that improved the quality of this work. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) through grant number 2383823-10. Leonardo León Castro was supported by a scholarship from the Secretary of Higher Education, Science, Technology and Innovation (SENESCYT), Ecuador.

REFERENCES

- Ambus, P., and E.S. Jensen. 1997. Nitrogen mineralization and denitrification as influenced by crop residue particle size. Plant Soil 197:261– 270. doi:10.1023/A:1004276631914
- Angers, D., and S. Recous. 1997. Decomposition of wheat straw and rye residues as affected by particle size. Plant Soil 189:197–203. doi:10.1023/A:1004207219678
- Bending, D.G., and K.M. Turner. 1999. Interaction of biochemical quality and particle size of crop residues and its effect on the microbial biomass and nitrogen dynamics following incorporation into soil. Biol. Fertil. Soils 29:319–327. doi:10.1007/s003740050559
- Cambardella, C.A., and E.T. Elliott. 1993. Methods for physical separation and characterization of soil organic matter fractions. Geoderma 56:449–457. doi:10.1016/0016-7061(93)90126-6

Table 5. Nitrogen uptake (g N plot⁻¹) by arugula in control (no green manure) and green manure (pea–oat mixture) field plots with one, two, or four tillage passes at the Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada.

Green manure/Number of tillage passes	Week I	Week 2	Week 3	Week 4			
	g N plot ⁻¹						
Control plots							
1	32.6 (7.35)†a	47.7 (14.7)a	48.6 (4.25)a	66.6 (10.06)a			
2	30.7 (2.55)a	35.4 (5.51)a	45.8 (1.57)a	61.7 (5.94)a			
4	31.8 (4.82)a	40.5 (4.34)a	49.2 (3.50)a	66.5 (6.84)a			
Green manure plots							
1	34.2 (5.75)a	47.0 (8.90)a	62.3 (10.31)b	67.0 (5.61)a			
2	33.0 (4.19)a	45.2 (8.40)a	61.9 (1.61)b	83.3 (7.71)b			
4	50.2 (3.67)b	62.8 (7.35)b	78.8 (9.94)c	94.6 (7.74)c			

† Average values (n = 4), with standard error in parenthesis, within the same column followed by the same letter do not differ significantly (P < 0.05, Tukey test).

- Canadian Agriculture Service Coordinating Committee. 1998. The Canadian system of soil classification, 3rd ed. Agric. and Agri-Food Canada Publ. 1646. NRC Research Press, Ottawa, ON, Canada.
- Chen, B.M., Z.H. Wang, S.X. Li, G.X. Wang, H.X. Song, and X.N. Wang. 2004. Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. Plant Sci. 167:635–643. doi:10.1016/j. plantsci.2004.05.015
- Cheng, W. 2009. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biol. Biochem. 41:1795–1801. doi:10.1016/j.soilbio.2008.04.018
- Clarholm, M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol. Biochem. 17:181–187. doi:10.1016/0038-0717(85)90113-0
- Drinkwater, L.E., R.R. Janke, and L. Rossoni-Longnecker. 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. Plant Soil 227:99–113. doi:10.1023/A:1026569715168
- Entz, M.H., R. Guilford, and R. Gulden. 2001. Crop yield and soil nutrient status on 14 organic farms in the eastern portion of the northern Great Plains. Can. J. Plant Sci. 81:351–354. doi:10.4141/P00-089
- Environment Canada. 2016. Canadian climate normals and averages. Environ. Canada, Ste Anne de Bellevue. http://climate.weather. gc.ca/climate_normals/results_1981_2010_e.html?stnID=5248& lang=&dCode=&dispBack=0&StationName=&SearchType=Co ntains&province=&provBut=Go&month1=12&month2=12&su bmit=View (accessed 15 Sept. 2016).
- Gardner, J.B., and L.E. Drinkwater. 2009. The fate of nitrogen in grain cropping systems: A meta-analysis of ¹⁵N field experiments. Ecol. Appl. 19(8):2167–2184. doi:10.1890/08-1122.1
- Groffman, P.M., P.F. Hendrix, and D.A. Crossley. 1987. Nitrogen dynamics in conventional and no-tillage agroecosystems with inorganic fertilizer or legume nitrogen inputs. Plant Soil 97:315–332. doi:10.1007/BF02383222
- Halde, C., and M.H. Entz. 2014. Flax (*Linum usitatissimum* L.) production system performance under organic rotational no-till and two organic tilled systems in a cool subhumid continental climate. Soil Tillage Res. 143:145–154. doi:10.1016/j.still.2014.06.009
- Joergensen, R.G., and T. Mueller. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the $k_{\rm EN}$ value. Soil Biol. Biochem. 28:33–37. doi:10.1016/0038-0717(95)00101-8
- Knott, C.M. 1987. A key for stages of development of the pea (*Pisum sativum*). Ann. Appl. Biol. 111:233–245. doi:10.1111/j.1744-7348.1987. tb01450.x
- Large, E.C. 1954. Growth stages in cereals illustration of the Feekes scale. Plant Pathol. 3:128–129. doi:10.1111/j.1365-3059.1954.tb00716.x
- León Castro, L., and J.K. Whalen. 2016a. Ion exchange membranes as an indicator of soil mineral nitrogen availability and nitrogen uptake by arugula (*Eruca sativa* L.) in soils amended with green manure. Biol. Agric. Hortic. 32:206–220. doi:10.1080/01448765.2016.1163293
- León Castro, L., and J.K. Whalen. 2016b. Ion exchange membranes are sensitive indicators of ammonium and nitrate released from green manures with low C/N ratios. Eur. J. Soil Biol. 77:4–8. doi:10.1016/j. ejsobi.2016.09.001
- Lupwayi, N.Z., G.W. Clayton, J.T. O'Donovan, K.N. Harker, T.K. Turkington, and Y.K. Soon. 2006. Nitrogen release during decomposition of crop residues under conventional and zero tillage. Can. J. Soil Sci. 86:11–19. doi:10.4141/S05-015
- Omirou, M., C. Papastefanou, D. Katsarou, I. Papastylianou, H.C. Passam, C. Ehaliotis, and K.K. Papadopoulou. 2012. Relationships between nitrogen, dry matter accumulation and glucosinolates in *Eruca sativa* Mills. The applicability of the critical NO₃–N levels approach. Plant Soil 354:347–358. doi:10.1007/s11104-011-1071-9

- Qian, P., and J.J. Schoenau. 2002. Practical applications of ion exchange resins in agricultural and environmental soil research. Can. J. Soil Sci. 82:9–21. doi:10.4141/S00-091
- Sarrantonio, M., and T.W.Scott. 1988. Tillage effects on availability of nitrogen to corn following a winter green manure crop. Soil Sci. Soc. Am. J. 52:1661–1668. doi:10.2136/sssaj1988.03615995005200060029x
- SAS Institute. 2012. SAS 9.4 for Windows. SAS Inst., Cary, NC.
- Seo, J.H., J.J. Meisinger, and H.J. Lee. 2006. Recovery of nitrogen-15-labeled hairy vetch and fertilizer applied to corn. Agron. J. 98:245– 254. doi:10.2134/agronj2005.0013
- Sharma, A.R., and U.K. Behera. 2009. Nitrogen contribution through Sesbania green manure and dual-purpose legumes in maize-wheat cropping system: Agronomic and economic considerations. Plant Soil 325:289–304. doi:10.1007/s11104-009-9979-z
- Sims, G.K., T.R. Ellsworth, and R.L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Commun. Soil Sci. Plant Anal. 26:303–316. doi:10.1080/00103629509369298
- Sincik, M., Z.M. Turan, and A.T. Göksoy. 2008. Responses of potato (Solanum tuberosum L.) to green manure cover crops and nitrogen fertilization rates. Am. J. Potato Res. 85:150–158. doi:10.1007/ s12230-008-9011-9
- Six, J., E.T. Elliott, and K. Paustian. 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63:1350–1358. doi:10.2136/sssaj1999.6351350x
- Statistics Canada. 2012. Over one-third of dairy cows were reported in Quebec. Statistics Canada Catalogue no. 95-640-X. Ottawa. http:// www.statcan.gc.ca/pub/95-640-x/2011001/p1/prov/prov-24-eng. htm (accessed 5 May 2016).
- Swift, M.J., O.W. Heal, and J.M. Anderson. 1979. Decomposition in terrestrial ecosystems (Vol. 5). Univ. of California Press, Berkeley.
- Thorup-Kristensen, K. 1993. Effect of nitrogen catch crops on the nitrogen nutrition of a succeeding crop: I. Effects through mineralization and pre-emptive competition. Acta Agric. Scand., Sect. B, Soil Plant Sci. 43:74–81. doi:10.1080/09064719309411222
- Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/ jds.S0022-0302(91)78551-2
- Voroney, R.P., P.C. Brookes, and R.P. Beyeart. 2008. Soil microbial biomass C, N, P and S. In: M.R. Carter and E.G. Gregorich, editors, Soil sampling and methods of analysis. 2nd ed. CRC Press, Boca Raton, FL. p. 637–651.
- Vyn, T.J., J.G. Faber, K.J. Janovicek, and E.G. Beauchamp. 2000. Cover crop effects on nitrogen availability to corn following wheat. Agron. J. 92:915–924. doi:10.2134/agronj2000.925915x
- Whalen, J.K. 2014. Managing soil biota-mediated decomposition and nutrient mineralization in sustainable agroecosystems. J. Adv. Agric. Vol. 2014, Article ID 384604. doi:10.1155/2014/384604
- Whalen, J.K., S. Gul, V. Poirier, S.F. Yanni, M.J. Simpson, J.S. Clemente et al. 2014. Transforming plant carbon into soil carbon: Process-level controls on carbon sequestration. Can. J. Plant Sci. 94:1065–1073. doi:10.4141/cjps2013-145
- Zebarth, B.J., C.F. Dury, N. Tremlay, and A.N. Cambouris. 2009. Opportunities for improved fertilizer nitrogen management in production of arable crops in eastern Canada: A review. Can. J. Soil Sci. 89:113– 132. doi:10.4141/CJSS07102
- Ziadi, N., R. Simard, G. Allard, and G. Parent. 2000. Yield response of forage grasses to N fertilizer as related to spring soil nitrate sorbed on anionic exchange membranes (AEMs). Can. J. Soil Sci. 80:203–212. doi:10.4141/S99-048