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Ion exchange membranes are sensitive indicators of ammonium and nitrate released from green manures with low C/N ratios

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ABSTRACT

Green manure is a valuable nitrogen (N) source, but must undergo decomposition, mineralization and nitrification to release mineral N (NH_4-N and NO_3-N). The pattern of soil mineral N released from green manure is related to its biochemical composition (e.g., C/N ratio) and soil texture. The objective of this study was to investigate the use of ion exchange membranes (IEMs) as a sensitive indicator of N mineralization and nitrification from green manure (mixture of pea and oat residues) having C/N = 8 and C/N = 12, mixed into sandy clay loam and sandy loam soils. The green manures decomposed rapidly and released mineral N that was captured by IEMs inserted in the soil during a 6-wk greenhouse incubation. Net NH_4 and NO_3 produced after 6-wk was greater in soil mixed with green manure having C/N = 8, and in the sandy clay loam mixed with green manure having C/N = 12, than unamended control soil. The IEM- NH_4-N concentration peaked one week after the green manure incorporation, with 0.13 μg IEM-NH_4-N cm^{-2} wk^{-1} in the sandy clay loam and 0.19 μg IEM- NH_4-N cm^{-2} wk^{-1} in the sandy loam soil, and declined thereafter. While the IEM- NO_3-N increased steadily in the sandy clay loam during the 6-wk incubation, there was a plateau (green manure C/N = 8) or decline (green manure C/N = 12) in IEM-NO_3-N concentration in the sandy loam soil. Net N mineralization and nitrification rates corresponded to the temporal fluctuations in mineral N detected with IEMs, confirming that this tool is a sensitive indicator of NH_4-N and NO_3-N dynamics in soils amended with green manures having low C/N ratios.

1. Introduction

When a green manure crop is terminated and incorporated into the soil, it undergoes decomposition, mineralization and nitrification to release plant-available N (mineral N; NH_4-N plus NO_3-N) for the subsequent crop. Chemical composition of green manure, particularly the C/N ratio, is a good predictor of its N fertilizer value [1–3] because residue C/N ratio is correlated significantly (r = 0.88) to soil NH_4-N and NO_3-N concentrations [4]. Residues from green manure with C/N ratios <25 are expected to release mineral N in the first weeks after its incorporation, based on the pattern of N release from crop residues with C/N ratios from 16 to 9 [5,6]. Soil texture is another factor that modulates mineral N release from green manure residues due to the physical occlusion of partially-decomposed residues within aggregates and the binding of soluble products of decomposition (e.g., carbohydrates and proteins) onto clay surfaces [7], both of which may impede the microbially-mediated processes of N mineralization and nitrification. Consequently, green manure-amended soils with higher clay content are expected to release less mineral N than those with low clay content.

The pattern of N release in green manure-amended soils can be evaluated by temporal sampling and analysis of the soil mineral N concentration, but these are static measurements that must be taken at short time-steps to reflect the dynamics of this plant-available N pool [8]. Ion exchange membranes (IEMs) are an alternative and practical *in situ* method, as the continuous adsorption of ions on IEMs from soil solution reflects the temporal pattern of N release in green manure-amended soils and mimics ion capture/interception by roots [9–12]. We propose that IEMs are good indicators of the short-term dynamics of NH_4-N and NO_3-N produced through N mineralization and nitrification of substrates released from decomposing green manure.

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The objective of this study was to evaluate IEMs as a sensitive indicator of NH_4-N and NO_3-N dynamics in two soils (sandy clay loam and sandy loam) amended with green manure residues having low C/N ratios (C/N = 8 and C/N = 12).

2. Materials and methods

The experiment was conducted in an unheated greenhouse using field soils from an organic vegetable farm in Les Cedres, Quebec, Canada (45° 20′N, 74° 8′ W). Soils were classified as Gleyed humo–ferric Podzols [13] having sandy clay loam texture (485 g sand kg^{-1} and 301 g clay kg^{-1}, pH 7.7 and 18 g organic C kg^{-1}) and sandy loam texture (650 g sand kg^{-1}, 112 g clay kg^{-1}, pH 6.7 and 17 g organic C kg^{-1}). Field soils (0–15 cm depth) were collected with a shovel, passed through a <10 mm sieve to remove rocks and large plant residues, transported in sealed plastic bins and stored in a 4 °C walk-in refrigerator until the experiment began. In the greenhouse prior start the experiment, soils were sieved through a <3 mm mesh screen to eliminate residues from previous years. Green manures were a mixture of field peas (*Pisum sativum* L.) and oats (*Avena sativa* L.) grown in pots in the greenhouse for 5 wks. When harvested, field peas were in the vegetative stage, fourth node, leaf fully unfolded, more than one pair of leaflets [14], and oats were at Feekes growth stage 5, tillering, leaf sheaths strongly erected [15]. Plant residues were rinsed to remove dust and dried (60 °C for 24 h). A subsample was ground to pass a <1 mm mesh sieve and analyzed for the total C and N content on a Flash EA 1112 series C/N analyzer (Thermo Finnigan, Mississauga, Ontario, Canada). The field peas had a C/N ratio = 7, whereas oats had a C/N ratio = 10. Mixing equal quantities of residues from these crops generated a residue with C/N = 8. Adding proportional amounts (33% field pea, 33% oats and 33% maize fiber from field-grown maize leaves collected at the VT growth stage having a C/N ratio = 17) produced a residue with C/N = 12. The dried residue mixtures were uniformly chopped with pruning shears and fragments retained on sieves of 2 mm–4 mm were used in the experiment.

Soil-residue mixtures were placed in plastic plant pot (10.5 cm diam. by 13 cm depth) with three drainage holes (0.5 cm diam.). Each experimental unit contained 1 kg (dry weight basis) of field-moist soil mixed with (a) 0.6 g kg^{-1} of green manure C/N = 8, (b) 0.6 g kg^{-1} of green manure C/N = 12 or (c) no green manure. The residue addition rate was based on a green manure input of about 3.6 t ha^{-1}, which is similar to the 3.7 t ha^{-1} of oat and pea reported by McKenzie and Spaner [16]. After adding and completely homogenizing the soil-residue mixture, soils were gently compressed to 1.36 g cm^{-3} for sandy clay loam and 1.54 g cm^{-3} for sandy loam, the bulk density of these soils under field conditions. Pots were arranged in a complete randomized design with four replications of each soil-residue mixture (6 factorial treatments representing 2 soils × 3 residue types) and each sampling date (once a week for six weeks) to allow for destructive sampling, for a total of 144 pots. The experiment simulated residue decomposition and N mineralization under field conditions in May–June, when green manure is generally terminated and incorporated in this region. The daily air temperature in the unheated greenhouse was, on average, 15.2 °C (SD ± 1.86) with temperature increasing from a minimum of 13 °C to maximum of 22 °C during the study (monitored every 2–3 d with an indoor tube thermometer, Taylor Precision Products, Oak Brook, Illinois, USA). These values are similar to the air temperature (13–18 °C) during these months, based on the 30 year average weather data from 1980 to 2010 [17]. Pots receiving the green manure treatment and the unamended control pots were subjected to this fluctuating temperature regime during the study. Pots were freely drained and irrigated every 2–3 d to maintain moisture content at 11–15 g water 100 g^{-1} soil (Field Scout TDR 100 system, Spectrum Technologies Inc, Aurora, Illinois, USA), corresponding to about 40–50% water-filled pore space. This is an optimal moisture level for decomposition in sandy soils [18] that should not induce denitrification, which is negligible below 80% water-filled pore space [19].

Cation and anion exchange membranes (Ionics CR67-HMR and AR204-SZRA, GE Water & Process Technologies, Trevose, Pennsylvania, USA) were used to monitor NH_4-N and NO_3-N concentrations in soil solution on a weekly basis. Ion exchange membrane strips of 2.8 × 5.5 cm were stored in deionized water until 24 h prior to use, when they were saturated by shaking with 1 M NaCl solution for 1 h and then placed in distilled water. Each pot had one cation and one anion exchange membrane buried to a depth of 10 cm. After 1 wk, the IEM was retrieved and replaced with a fresh IEM that was inserted into a new pot. After retrieval, IEMs were rinsed with deionized water to remove attached soil particles, then placed in a single conical screw cap tube with 25 ml of 1 M KCl. After extraction (shaking the tubes for 1 h in an orbital shaker, filtering through Whatman no. 5 filter paper), the concentration of IEM- NH_4-N and IEM- NO_3-N was determined by the modified indophenol blue method [20] at 650 nm on a microplate reader (μQuant, Biotek, Winooski, Vermont, USA). The IEM- NH_4-N and IEM- NO_3-N concentrations were reported as μg cm^{-2} wk^{-1} [8].

Soil from the beginning (wk 0) and end (wk 6) of the incubation was analyzed for mineral N, microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) concentrations. Mineral N (NH_4-N and NO_3-N) extracted with 2 M K_2SO_4 (1:4 soil:solution) was analyzed with the modified indophenol blue technique [20]. MBN and MBC concentrations were determined by the chloroform fumigation-direct extraction method with 0.5 M K_2SO_4 as the extractant followed by persulfate digestion [21]. The MBN concentration was [(total extractable N after fumigation-total extractable N before fumigation)/k_{EN}] where k_{EN} is the extraction coefficient 0.54. The concentration of MBC was determined by subtracting extractable C (determined by a Sievers Innovox TOC analyzer, GE Analytical Instrument, Boulder, Colorado, USA) before and after soil fumigation with ethanol-free chloroform and adjusted by 0.45, the efficiency of extraction of microbial biomass [21]. After 6-wk, soils were sieved through 1–2 mm and 3–4 mm sieves to collect the undecomposed green manure residues that were either undecomposed or not associated with organo-mineral complexes using the wet-sieving method. These residues were gently washed and weighed.

Data were normally distributed and had homogeneous variance, confirmed by the Shapiro-Wilk and Levene's tests, respectively. For each soil type, the effect of green manure C/N ratio on IEM- NH_4-N and IEM- NO_3-N concentration, corrected by subtracting the background IEM- NH_4-N and IEM- NO_3-N levels in the no green manure treatment, was determined using repeated measures (in time) one-way ANOVA. Post-hoc comparisons between C/N ratios (between-subject effects) and of C/N ratio x sampling time (within-subject effects) were evaluated with least significant difference (LSD) test. The effect of green manure C/N ratio on the mass of undecomposed residues, net N mineralization, net nitrification, MBN and MBC concentrations after 6-wk was analyzed using one-way ANOVA. When the main effects were significant ($p < 0.05$), treatment means were compared with LSD test. A paired sample *t*-test was conducted to compare the green manure residues remaining after 6-wk in the two soil types. Analyses were performed with SAS Version 9.3 software (SAS Institute Inc., Cary, NC, USA).