Nitrogen availability and early corn growth on plowed and no-till soils amended with different types of cover crops

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Abstract

Heavy application of nitrogen (N) fertilizers to agroecosystems contributes to water pollution and emission of nitrous oxide (N₂O), an atmospheric constituent implicated in the greenhouse effect and stratospheric ozone depletion. The incorporation of cover crops biomass into agricultural soil is a practice that could provide a portion of the N needed by growing crops, and thereby could help reduce N fertilizer input and N loss from agroecosystems. Although the cover crop composition and the tillage management history of receiving soils could affect the pattern of mineral N release and its fates, these questions have not been addressed in previous research. A greenhouse experiment (85 d) was conducted to investigate the effects of two cover crops, hairy vetch (*Vicia villosa*, C/N: 11) and rye (*Secale cereale*, C/N: 82) on mineral N flux, N₂O emission, and corn growth in soils under no-till (NT, 11 years) and plow-till (PT). Overall, tillage practice had only a marginal influence on N dynamics. Although NO₃⁻ flux was enhanced by both cover crops, the NO₃⁻ released was more efficiently utilized in the rye treatment. Corn growth and N uptake were significantly greater ($P < 0.02$; 1.5 times) with rye compared to the vetch treatment. Conversely, N₂O emission was significantly ($P < 0.03$) higher with vetch than rye addition, with cumulative emission (across PT and NT) averaging 2.45 kg N ha⁻¹ with vetch and 1.28 kg N ha⁻¹ with rye addition. Positive relationships were found between NO₃⁻ flux and N₂O emission, with the slopes of the regression lines suggesting a greater propensity of NO₃⁻ released from vetch to be converted into N₂O. By leading to less N₂O emission and better corn N uptake, the study results indicate that rye is a better cover crop option than vetch, regardless of tillage practice.

Keywords: Tillage practices, cover crop, nitrogen management, nitrous oxide

1. Introduction

In modern agriculture, application of nitrogen (N) fertilizer is needed to maintain crop productivity, but this activity has well-documented impacts on water quality and greenhouse gases (GHG) emission including nitrous oxide (N_2O), an atmospheric constituent implicated in global climate warming and stratospheric ozone depletion (Smith *et al.*, 2007). Although only a small portion (less than 3%) of applied N fertilizer is typically emitted as nitrous oxide (N_2O), these emissions can be considerable on national and global scales given the heavy reliance of modern agriculture on synthetic N fertilizer (Robertson *et al.*, 2000). It has been estimated that agriculture contributes 58% of the total anthropogenic N_2O emission (Smith *et al.*, 2007).

Since N_2O emission tends to be positively correlated with N fertilizer application rates (Millar *et al.*, 2010), reduction in N fertilizer input might be an effective way of mitigating N_2O emission, but this strategy could result in decreased crop yields, and thus not likely to be acceptable to farmers (Millar *et al.*, 2010). There are some alternative practices which could help mitigate N_2O emission without reducing crop yields, such as optimization of fertilizer source in relation to tillage practices (Venterea *et al.*, 2011), application of slow-release fertilizers (Halvorson *et al.*, 2010), and changing the depth of fertilizer placement (Omonode *et al.*, 2011). The effectiveness of these practices in reducing N_2O emission varies depending on local conditions and other factors. Work by Millar *et al.*, (2010) showed a non-linear exponential increase in N_2O emission when fertilizer is applied in excess of crop N demand for optimum production. Therefore, downward adjustments of N fertilizer application rate are needed in order to reduce agricultural N_2O emission. However, reduction in the rate of synthetic N fertilizer application must consider other strategies to providing growing crops with the amount of mineral N they need for optimum yield.

Cover crop could be a natural source of mineral N and provides an alternative management strategy to reduce synthetic N addition and N_2O emission from agroecosystems. In a cover-crop system, cereal and/or legume crops are grown during the dormant season for the purpose of scavenging residual nutrients. At the end of its life cycle, the cover crop is killed and plant nutrients are progressively released from the decomposing cover crop biomass, and become available to the succeeding cash crop. The rate of mineral N release and its fate depend, among other factors, on tillage practices and cover crop composition.

Cover crop residue returned to agricultural soils provides organic substrates to fuel microbial activity, and therefore can affect N cycling processes. Through its influence on soil microbial biomass composition and activity, tillage practices can influence cover crop biomass decomposition, the dynamics (mineralization/immobilization) of soil mineral N, and ultimately N availability to growing crops. Past studies have generally documented higher mineral N content, greater rate of organic matter mineralization, and increased propensity for N leaching under plowed-till (PT) (Tracy *et al.*, 1990), whereas long-term no-till (NT) has also been shown to result in enhanced N cycling efficiency and net N immobilization (Baggs *et al.*, 2003; Hayatsu *et al.*, 2008). These differences in N cycling are thought to be the result of higher soil moisture, lower oxygen and soil temperature in NT compared to PT soils. Bavin *et al.*, (2009) evaluated the impact of tillage and winter rye (*Secale cereale*) on N_2O emission, and noted that emission was mainly affected by N application rate, with limited difference between PT and reduced tillage (RT). Due to their effect on soil biological properties, tillage practices could influence the timing and the amount of N released from decomposing cover crop biomass.

In addition to tillage practices, the chemical composition of cover crops must also be considered when assessing their impact on gaseous emissions. The carbon/nitrogen (C/N) ratio and lignin content of plant residue are important variables that could determine N mineralization kinetics in soil ecosystems. The linkage between cover crop mineralization and N₂O emission has been shown to be dependent on the C/N ratio and the lignin:N ratio of cover crop residue, with negative correlations generally reported between these parameters and N₂O emission (Huang *et al.*, 2004; Millar and Baggs, 2004). Huang *et al.*, (2004) studied the effect of five crop residues with different C/N ratios (range: 8 to 118) on N mineralization, and measured the highest N₂O emission with rapeseed (lowest C/N ratio) and the lowest emission with sugarcane (highest C/N ratio). It has also been shown that at C/N ratios in the 20-30 range, the net N immobilization and N mineralization tend to be of similar magnitude (Tracy *et al.*, 1990; Hayatsu *et al.*, 2008; Partey *et al.*, 2014), suggesting that cover crop with C/N ratios < 20 can lead to net N mineralization whereas biomass with C/N ratios > 30 would most likely result in N immobilization. These laboratory data were later supported by an empirical model that predicted less N₂O emission from crop residues with high C/N ratios (Mu *et al.*, 2009).

As suggested by the literature reviewed above, in order to implement cover crop as a farming practice, one needs to account for both the cover crop composition (C/N ratio) and the tillage management history of receiving agricultural soils. In other words, the interaction of cover crop type (grass vs legume) and tillage management could determine N cycling direction (immobilization/mineralization) and mineral N availability. It is of great importance to understand the timing of mineral N release following termination of cover crop in agroecosystems, as this could lead to better synchronization between N availability and N demand by the subsequent crop (e.g. corn).

To investigate these questions, a greenhouse experiment was conducted using soils under plow-till (PT) and no-till (NT) amended with either a cereal or a legume cover crop (different C/N ratios), and the fate of N from cover crop was investigated. The following fates were considered for cover crop N: gaseous emission (N₂O), N mineralization, and N assimilation by vegetation. Plant uptake was taken as a measure of N-use efficiency, and thus considered as the most desirable outcome. It is hypothesized that the legume cover crop (low C/N ratio) is more suitable for the NT soil, whereas cereal cover crop (with high C/N ratio) is preferable for the PT. The overall goal of this study was to identify the best cover crops for fields under NT and PT.

2. Materials and Methods

2.1. Greenhouse experiment design

This experiment was conducted in a greenhouse using surface soil (0-25 cm) collected in October 2014 from agricultural fields at Starkey Farms in Hendricks county, Indiana (39°51' 49"N, 86°21'31"W). Management practices at these fields included conventional tillage (plow-till, PT) and no-till (NT, 11 years). Chemical properties of the PT soil were: pH=6.41, total C=13.3 ± 2.3 g C kg⁻¹ soil, and total N=1.9 ± 0.6 g N kg⁻¹ soil. Chemical properties of NT soil were: pH=7.42, total C=18.5 ± 2.3 g C kg⁻¹ soil, and total N=1.7 ± 0.2 g N kg⁻¹ soil. Soils at the sampling sites were intergraded between the silt-loam Crosby (aeric Epiaqualf) and the loamy Brookston (typic Argiaqualf) series. At the bottom of each pot (H: 37 cm; diam: 49.7 cm; area: 1940 cm²) a layer (5 cm) of acid-washed pea gravel, on top of which was placed a soil block extracted from either the NT or the PT field. Soil was extracted with care taken to minimize disturbance and preserve soil structure. Pots were transported to the greenhouse, irrigated daily for 7 days before cover crop addition.

The experiment involved soil from 2 tillage practices (NT, PT) and 2 cover crops (rye and hairy vetch). For each tillage, a control (with no cover crop added) was included. Each treatment was run in triplicate. Thus, a total of 18 pots was used in the experiment to accommodate the experimental factors. At the beginning of the experiment, each pot was amended with a solution of potassium nitrate (KNO_3) and dipotassium phosphate (K_2HPO_4) to provide an equivalent 20 kg N ha^{-1} and 10 kg P ha^{-1} . A second application of 40 kg N ha^{-1} as ammonium nitrate (NH_4NO_3) was made 60 days after the experiment started as the pool of mineral N was depleted and the corn plants began to show signs of nutrient deficiency.

2.2. Cover crops collection and corn planting

The cover crops selected for this study are among the most commonly-used in US Midwest agricultural fields (<http://mccc.msu.edu/species/>), and included hairy vetch (*Vicia villosa*) (C: 42%; N: 3.8%) and rye (*Secale cereale*) (C: 82%; N: 1%). Rye and vetch biomass (shoots and roots) was air and oven-dried (65°C), and shredded into small pieces (1–3 cm). An amount of 10 g of cover crop biomass was added to each pot ($\sim 500 \text{ kg dry matter ha}^{-1}$, equivalent to 19 and 5 kg N ha^{-1} as vetch and rye biomass, respectively). No cover crop was added to the control pots. The cover crop biomass was spread on the soil surface and mixed slightly with the first cm of soil to simulate the termination of a cover crop by roller/crimper. The next day, 3 corn seeds (*Zea mays* var. *dwarf*) were planted in each pot. Following germination, two plants were removed leaving the most vigorous corn seedling in each pot. Pots were irrigated once a week to keep the gravimetric soil moisture between 25–35%. The temperature in the greenhouse was maintained between $18\text{--}23^\circ\text{C}$, and light was provided for 12 h each day during the experiment. Corn height was measured on day 60, and at the end of the experiment (day 85). At that time, corn

biomass was harvested, shoots and roots were separated, washed, oven-dried (65°C until constant weight) and the dry weight recorded to determine standing crop biomass. Subsamples of leaf/root biomass were pulverized using a rolling grinder, and the fine powder was stored for C and N analysis.

2.3. Soil mineral nitrogen monitoring

Soil mineral nitrogen (NH_4^+ and NO_3^-) was monitored on a weekly basis using cation and anion PRSTM-probes obtained from Western Ag Innovations Inc (Saskatoon, Canada). Mineral N absorbed by the PRS-probes simulates the amount of mineral N intercepted by plant roots. One anion and one cation PRS-probe was labeled and inserted in each pot ($\sim 10 \text{ cm}$ depth) within 1–2 cm of corn roots. Every week, PRS-probes were removed from the pots and immediately transported to the laboratory for elution. PRS-probes were washed with deionized (DI) water to remove soil particles from the membranes, and eluted with 17.5 mL of HCl (0.5 N) in a zip lock bag. After 1 h, the eluate from each bag was transferred to a 20 mL clean plastic vial and stored frozen until analyzed. PRS-probes were cleaned in (1 h soaking and shaking in 0.5 N HCl) and regenerated (soaking in 0.5 N NaHCO_3). Regenerated PRS-probes were rinsed with deionized water and returned to the pots the same day they were removed. During the experiment, three anion and cation PRS-probes were kept in a sealed bag containing deionized water as blanks in a refrigerator. PRS-probe eluates were analyzed for NH_4^+ and NO_3^- . Weekly mineral N flux (F , $\mu\text{g N cm}^{-2}$) was computed as follows:

$$F = (C) \left(\frac{V}{S} \right)$$

where C is the mineral N (NH_4^+ and NO_3^-) concentration in the eluate ($\mu\text{g N mL}^{-1}$), V is the volume of eluent per probe (17.5 mL), and S is the surface area of each probe (17.5 cm^2).

Soil samples (0-5 and 5-10 cm) were collected from each pot (on day 60) to determine soil mineral N content. Soil samples were sieved (2 mm sieve), and a subsample (10 g) extracted with 30 mL of KCl (1 M) solution. Soil extracts were analyzed for NH_4^+ and NO_3^- .

2.4. Nitrous oxide fluxes monitoring

During the experiment, N_2O flux was measured weekly using the static chamber method. Static chambers consisted of PVC cylinders (H: 15 cm; diameter: 10 cm) were inserted ~5 cm into each pot, leaving a headspace of about 10 cm. During sampling, chambers were closed with PVC lids secured to the base with bungee cords and clamps. The lid was fitted with a gasket at its underside edge to make an air-tight seal, and with a butyl rubber septa at its center to form a sampling port. Chamber headspace was sampled 0, 45 and 90 min after closure, and air samples were stored in evacuated 10 mL glass vials. Concentration of N_2O was measured by gas chromatography (electron detector capture, ECD). Daily fluxes of N_2O (F) ($\mu\text{g N}_2\text{O cm}^{-2} \text{ hour}^{-1}$) were computed as:

$$F = \frac{dC}{dt} \frac{V}{A} k$$

where dC/dt is the N_2O concentration change in chamber headspace ($\mu\text{g N}_2\text{O-N m}^{-3} \text{ min}^{-1}$), V is the chamber volume (m^3), A is the area of soil circumscribed by chamber (m^2), and k is the time conversion factor ($1440 \text{ min day}^{-1}$) (Fisher et al., 2014).

2.5. Analytical methods

Soil pH was measured using a soil suspension (1:2 soil to water) and an Accumet-25 pH/ion meter.

To determine total C and N in soil and plant samples, an aliquot of each crushed soil/plant sample that passed through a 150- μm sieve was weighed (soil samples 7-9 mg, plant samples 2-3 mg) into a tin capsule, and analyzed for C and N contents by dry combustion (960 °C) using a Vario-Cube analyzer (Elementar Americas, New Jersey, USA). Total N in the standing corn biomass was computed as:

$$\text{Corn N uptake (kg N ha}^{-1}\text{)} = \frac{\text{mass of N in shoot and root (g)}}{\text{pot surface area (cm}^2\text{)}} \times \frac{1 \text{ (kg)}}{10^3 \text{ (g)}} \times \frac{10^6 \text{ (cm}^2\text{)}}{1 \text{ (ha)}}$$

Air samples from the static chambers were analyzed using a CP-3800 gas chromatograph (Varian Inc, Palo Alto, CA) interfaced with a Combipal headspace auto-sampler (CTC Analytics) and equipped with an electron capture detector (Jacinthe et al., 2015). The stationary phase consisted of a pre-column (L: 0.3 m; i.d.: 2 mm) and an analytical column (L: 1.8 m; i.d.: 2 mm) filled with Porapak Q (80-100 mesh).

Operating conditions of the gas chromatograph were as follows: carrier gas (UHP N_2 at 60 ml min^{-1}), oven temperature (90 °C), detector temperature (300 °C). The gas chromatograph was calibrated using certified N_2O standards (0.1, 0.5 and $1 \mu\text{L N}_2\text{O L}^{-1}$) obtained from Matheson Tri-Gas. Eluates from the PRS-probes and KCl soil extracts were analyzed for NH_4^+ and NO_3^- using US EPA method 350.2 and 353.1, respectively on a photometric analyzer (Aquakem 20, EST Analytical, Fairfield, OH, USA).

2.6. Statistical analysis

Data were first tested for normality using the normality test available in SigmaPlot ver. 13 (Systat, San Jose, CA). Since most of the data were not normally distributed, Kruskal-Wallis test was used to determine the significance of the experimental factors (tillage practice and cover crop) on N_2O and

mineral nitrogen (NH_4^+ and NO_3^-) fluxes and corn growth. The Kruskal-Wallis test was followed by Mann-Whitney pairwise test when a significant difference was detected. Unless otherwise noted, statistical significance was determined at $\alpha=0.05$. Statistical tests were conducted using PAST software (ver. 2.17c) downloaded from <http://nhm2.uio.no/norlex/past/download.html> (University of Oslo).

Figure 1. Weekly nitrate flux in plowed-till (PT) (a, c), and no-till (NT) (b, d) soils; control and amended with rye or vetch. Results are reported for the first 60 days of the experiment (a, b), and for the period between day 60 and harvest on day 85 (c, d). An application of $40 kg N ha^{-1}$ as ammonium nitrate was made on day 60, when corn plants began to show signs of N deficiency. Error bars represent SD from a mean of three replicates.

3. Results

3.1. Soil mineral nitrogen flux

Ammonium concentration in the PRS-probes eluates were near zero in most cases. Therefore, no further description of this mineral N species is provided. However, NO_3^- concentrations were detected and measured in the samples taken by the anion PRS-probes during the experiment. Results of NO_3^- flux are presented in two phases; phase 1 for the first 60 days of the experiment, and phase 2 for the period between day 60 and 85 (Figure 1).

During phase 1, similar pattern in NO_3^- flux was observed with both PT and NT soils (Figures 1a and 1b), i.e. high NO_3^- flux during the first two weeks and then

gradual decrease in all treatments. This gradual decrease was generally most visible in PT soil (Figure 1a). A few short-lived spikes in NO_3^- flux was observed during the first 2 weeks of the experiment, especially in the vetch-treated NT soils (Figure 1b). Although higher NO_3^- fluxes were recorded in the cover crop amendment than in the controls during the first two or three weeks of the experiment, difference in NO_3^- flux between controls and cover crop treatments was not significant ($P > 0.05$) different during that period (Figures. 1a and 1b). However, from week three to the end of phase 1, cover crop amended treatments showed significantly higher NO_3^- flux than controls in both PT and NT soils (Figure 1). No significant difference ($P = 0.12$) was observed between the two types of cover crops with regard to NO_3^- flux (Table 1).

Table 1. Average weekly nitrate flux in plowed-till (PT) and no-till (NT) soils non-amended (control) and amended with either rye or vetch as cover crop. Results are reported for the first 60 days of the experiment (phase 1), and for the period between day 60 and harvest on day 85 (phase 2). An application of 40 kg N ha⁻¹ as ammonium nitrate was made on day 60, when corn plants began to show signs of N deficiency. For each tillage practice, values in a column are significantly different ($P < 0.05$) if followed by different letters.

Tillage	Cover crop	Average nitrate flux ($\mu\text{g NO}_3^- \text{ cm}^{-2}$)	
		Phase 1	Phase 2
PT	Control	15.74b	29.53b
	Rye	23.58a	46.92a
	Vetch	18.62a	52.37a
NT	Control	16.65b	29.16b
	Rye	23.79a	45.83a
	Vetch	25.21a	45.99a

As stated previously, an application of mineral N fertilizer was made on day 60 to alleviate symptoms of N deficiency. Not surprisingly, a marked increase in NO_3^- flux was observed the week following fertilizer application, and the N flux gradually decreased over time (Figures 1c, 1d). Again, during that period (phase 2), NO_3^- flux was significantly ($P < 0.05$) higher in the cover crop treatments compared to controls, but the cover crop treatments were not significantly different from each other ($P = 0.09$). Although NO_3^- flux was generally higher in NT than PT soil, no significant difference ($P = 0.10$) between the tillage practices was detected (Table 1). Averaged across tillage practices, NO_3^- flux was 2 times higher with cover crop application compared to controls (Table 1). Although difference was not always significant, NO_3^- flux was generally higher in the pots treated with rye than in those treated with vetch (Figure 1, Table 1).

Measurements of NO_3^- concentration made on day 60 showed that most of the NO_3^- pool was in the first 5 cm soil layer (Figure 2). This depth distribution suggests limited NO_3^- leaching and accumulation at depth during the experiment. Irrespective of tillage practice, NO_3^- concentration in the 0-5 cm layer was significantly higher ($P < 0.05$) under cover crop treatments compared to controls (Figure 2). In almost all cases, NO_3^- concentration was significantly higher ($P < 0.05$) with rye amendment compared to vetch treatment (Figure 2).

The hairy vetch biomass was almost completely decomposed during the first 60 days of the experiment (phase 1) with only a few stems left on the soil surface. However, rye residue was still observable and persisted to the end of the experiment (day 85).

Figure 2. Nitrate concentration in plowed-till (PT) (a) and no-till (NT) (b) soils; control and amended with rye or vetch. Soil samples were taken from different depth (0-5 cm and 5-10 cm) on the day 60 and before the second fertilizer application event. Error bars represent SD from a mean of three replicates.

3.2. Nitrous oxide flux

During the first phase of the experiment (days 0-60), N_2O fluxes were the highest in the first two (PT soil, Figure 3a) and three (NT soil, Figure 3b) weeks, and then gradually decreased over time in all treatments. Regardless of tillage, N_2O flux was higher in the cover crop treatments compared to controls. Between the two cover crops, the highest N_2O fluxes (up to $23 \mu\text{g N}_2\text{O cm}^{-2} \text{h}^{-1}$) were observed in the vetch

treatments (Figures. 3a and 3b), but significant difference ($P < 0.05$) between the cover crops was mostly limited to the first two weeks. Following N fertilizer application on day 60 (phase 2), N_2O fluxes increased again (Figures. 3c and 3d), and most noticeably in the rye-amended PT soil (Figure 3c). This burst in N_2O emission was very short-lived, lasting only one week before N_2O fluxes gradually stabilized at around $< 1 \mu\text{g N}_2\text{O cm}^{-2} \text{h}^{-1}$ in all treatments.

Figure 3. Nitrous oxide flux in plowed-till (PT) (a, c), and no-till (NT) (b, d) soils; control and amended with rye or vetch. Results are reported for the first 60 days of the experiment (a, b), and for the period between day 60 and harvest on day 85 (c, d). An application of 40 kg N ha^{-1} as ammonium nitrate was made on day 60, when corn plants began to show signs of N deficiency. Error bars represent SD from a mean of three replicates.

Cumulative N_2O emission (Table 2) during the experiment was significantly ($P < 0.05$) higher in the vetch treatment compared to rye and control. This trend was observed regardless of tillage practice, although a borderline tillage $\times$ cover crop interaction was noted during phase 1. In the absence of cover crop addition, cumulative N_2O averaged 0.85 and 0.57 kg N ha^{-1} respectively in the PT and NT soil, with only a marginal increase from phase 1 to

phase 2 (Table 2). In the NT soil, the vetch treatment resulted in a cumulative N_2O emission of 2.54 and 2.88 kg N ha^{-1} during phase 1 and phase 2, respectively (Table 2). The rye treatment resulted in slightly lower emission averaging 1.15 and 1.46 kg N ha^{-1} for these respective periods. A similar trend was observed in the PT, with cumulative emission on average 2 times higher with vetch than with rye addition (Table 2).

Table 2. Cumulative nitrous oxide emission in plowed-till (PT) and no-till (NT) soils non-amended (control) and amended with either rye or vetch as cover crop. Results are reported for the first 60 days of the experiment (phase 1), and for the period between day 60 and harvest on day 85 (phase 2). An application of 40 kg N ha^{-1} as ammonium nitrate was made on day 60, when corn plants began to show signs of N deficiency. For each tillage practice, values in a column are significantly different ($P < 0.05$) if followed by different letters.

Tillage	Cover crop	Cumulative nitrous oxide emission ($\mu\text{g N}_2\text{O cm}^{-2}$) [†]	
		Phase 1	Phase 2
PT	Control	12.07b	14.52c
	Rye	13.39b	25.94b
	Vetch	32.32a	36.44a
NT	Control	7.48c	10.55c
	Rye	18.04b	22.99b
	Vetch	39.88a	45.22a

[†]Multiply by 0.06 to convert to $\text{kg N}_2\text{O-N ha}^{-1}$.

As stated above, the vetch treatment resulted in both higher NO_3^- flux and higher N_2O flux than rye. It follows that strong positive relationships ($r^2 > 0.66$) were found between these variables with the PT soil (Figure 4). It should be noted that only a weak trend between

these variables was observed with the NT soil. The slope of the relationships was nearly 3 times higher in the vetch compared to the rye treatment, indicating a greater propensity of NO_3^- release from vetch to be converted into N_2O (Figure 4).

Figure 4. Relationships between nitrate flux and nitrous oxide emission in plowed-till (PT) soil; (a) control, (b) rye-amended, and (c) vetch-amended, during the first 60 days of the experiment.

3.3. Corn shoot and root biomass

Regardless of tillage practice, corn grown in the rye-amended pots had significantly ($P < 0.02$) greater biomass (height as well) than those in the control and vetch-treated pots (Table 3). The shoot/root ratios (Table 3) were also much greater with the rye treatments compared to vetch and control. Corn height and shoot/root ratios were not significantly affected by tillage, although under rye amendment corn biomass was generally greater in PT than in NT. In both PT and NT soils, corn biomass was in the order rye > vetch > control (Table 3).

Although N content and C/N ratio of corn tissues (shoot and root) were generally similar regardless of treatment, the amount of N in the standing biomass was markedly higher in corn plants grown under cover crop treatments (Table 4). Results of total N uptake by corn plants in both PT and NT soils were in the order: rye > vetch > control (Table 4). This trend is consistent with the observation that corn growth was significantly better with rye amendment compared to vetch (Table 3). N uptake was generally higher in corn grown in NT than PT soil (Table 4).

4. Discussion

4.1. Decomposition rate of cover crop biomass

Two months after the experiment began, the hairy vetch biomass was almost completely decomposed (>90 %), whereas a good portion of the rye residue remained on the soil surface in the experimental pots. This observation is similar to those reported in several previous studies (Zougmoré *et al.*, 2006; Nagumo and Nakamura 2013) in which only 5% of hairy vetch residue typically remained at the end of a corn season. These results indicate the rapid mineralization and early release of mineral N from hairy vetch (C/N:

Table 4. Amount of N in the biomass of corn grown for 85 days in pots (1 plant per pot) filled with plowed-till (PT) and no-till (NT) soils non-amended (control) and amended with either rye or vetch as cover crop. Carbon content of corn shoot and root averaged 448.1 ± 18.6 and 411.8 ± 14.4 g C kg⁻¹, respectively. For each tillage practice, values in a column are significantly different ($P < 0.05$) if followed by different letters.

Tillage	Cover crop	Shoot			Root			Amount of N in standing corn biomass	
		N content (g N g ⁻¹ dm) [†]	Biomass (g dm)	Mass of N in shoot (g)	N content (g N g ⁻¹ dm)	Biomass (g dm)	Mass of N in root (g)	(g N pot ⁻¹)	(kg N ha ⁻¹) [‡]
PT	Control	0.10	16.73	1.71c	0.08	2.84	0.22b	1.93	99.48c
	Rye	0.08	65.47	5.34a	0.08	3.92	0.31a	5.65	291.24a
	Vetch	0.08	37.88	2.94b	0.07	4.79	0.35a	3.29	169.59b
NT	Control	0.08	9.43	0.73c	0.08	2.09	0.17b	0.9	46.39c
	Rye	0.13	47.59	6.40a	0.07	3.24	0.22b	6.62	341.24a
	Vetch	0.11	43.88	4.73b	0.09	5.54	0.47a	5.2	268.06b

[†] dm: dry matter

[‡] Amount of N in standing biomass expressed on an area basis using pot surface area (1940 cm²)

11), and from the biomass of other leguminous cover crops that have low C/N ratios. This rapid decomposition of vetch may have led to the high NO₃⁻ fluxes measured at the beginning of the experiment (Figure 1 and Table 1). This enhancement in NO₃⁻ release was likely due to the sudden exposure of soil microbes to a large supply of C and N-rich substrates, including easily decomposable non-protein N and soluble protein N. Lee *et al.*, (2002) noted that vetch lost 72-81% of its initial weight one month after application, leading to the conclusion that hairy vetch could be a substitute N starter fertilizer but not a sustainable N source due to its fast decomposition. Results of several past studies (Lee *et al.*, 2002; Ruffo and Bollero 2003; Muhammad *et al.*, 2011) suggested that, due to its higher N concentration, the leaf portion of crop residue decomposes faster than the stem and root portions. Although these factors may have played a role in the observed physical loss of cover crop mass, this cannot be accounted for in the present study because the cover crop applied was a mixture of stems and leaves. Further,

under field conditions, cover crop roots remain buried in the soils as they undergo decomposition; therefore, the N-supplying capacity of hairy vetch (and other legume cover crops) may last longer than the results of the present study would suggest.

4.2. Mineral nitrogen release: Relative significance of tillage practice and cover crop composition

As noted previously, NH₄⁺ flux was negligible in almost all treatments. That was an unexpected result that could be due to the rapid nitrification of the NH₄⁺ evolved from ammonification of organic N. A study by Sainju *et al.*, (2007), in which NH₄⁺ fertilizer was applied, reported similar results; significant accumulation of NH₄⁺ was only detected in treatments receiving N fertilizer in the 120-130 kg N ha⁻¹ application rates, 2-fold greater than the amount applied in the present experiment.

The observed enhancement in NO₃⁻ release with cover crop addition (Figure 1; Table 1) is in accord with

the findings of Frimpong *et al.*, (2011) who reported increased NO_3^- release following soil incorporation of maize residue. Similar to the temporal pattern observed in the present study, pulses in soil NO_3^- were short-lived, generally lasting two weeks or less (Frimpong *et al.*, 2011). In other words, following cover crop addition, N mineralization occurred rapidly due to the large amount of readily mineralizable C and N supplied by the fresh biomass, but the process decreased substantially over time as mineralized N was presumably consumed by growing corn plants and soil microorganisms.

Due to their chemical composition and generally low fiber content (Lee *et al.*, 2002), the biomass of legume plants should decompose faster than that of cereals. Observations consistent with that expectation were made in this experiment, as nearly all hairy vetch biomass was decomposed in about 7 weeks. In contrast, the rye biomass lasted much longer. These results suggest that N contained in hairy vetch biomass may have been released very rapidly, before the corn seedlings could fully utilize the available N. In contrast, decomposition and mineral N release with rye were more gradual and better synchronized with corn plants development. Similar patterns were reported in a study by Ruffo and Bollero (2003) in which hairy vetch and rye decomposition and NO_3^- release were compared. These authors (Ruffo and Bollero, 2003) concluded that rye was a more suitable material than vetch with regard to N supply to plants and soil conservation. In a decomposition study involving these same plant materials, Kuo and Sainju (1998) noted that rye, as a cover crop, could increase the crossover time of net N mineralization (i.e. the time when the amount of net N mineralized in the residue-amended soil is equal to that of control). Therefore, with rye addition the release of mineral N is more sustained, and its timing is better synchronized with crop N needs in the early growth phase. Vidal and Lopez (2005) noted that, due

to the slow N release, rye could be a more appropriate cover crop to prevent NO_3^- leaching and water pollution. Therefore, from a soil fertility standpoint, rye can be proposed as a better cover crop option than vetch.

A central hypothesis of this study was that tillage management history could dictate the performance of different types of cover crops applied to agricultural soils. Specifically, it was hypothesized that legume cover crops (low C/N ratio) would be more suitable for NT soils, and that cereal cover crops (high C/N ratio) would be preferable for soils under conventional tillage (PT). The study results did not, however, support that hypothesis. In this study, tillage management (PT and NT) did not significantly affect NO_3^- flux (Figure 1; Table 1) and had a marginal effect on N_2O emission (Table 2). These results are in accord with the findings of Dalal *et al.*, (2011) who reported no significant effect of tillage practices (NT and PT) on mineral N release from agricultural soils in which crop residue was retained. However, in another study (Sainju *et al.*, 2007), involving vetch and rye as cover crops in NT and PT soils, higher NO_3^- concentration was measured in PT soil with vetch incorporation than in the other treatments. Further, higher rate of N leaching was also reported in the vetch compared to the rye treatment (Sainju *et al.*, 2007). Several authors have therefore proposed a mixture of rye and vetch for either NT or PT system (Ruffo and Bollero, 2003; Vidal and Lopez, 2005; Sainju *et al.*, 2007). Results of the present study concur with that suggestion.

Both rye and vetch treatments resulted in NO_3^- fluxes and soil NO_3^- pool 1.5 to 2-fold higher than controls (Table 1). These results demonstrated that cover crops can be a source of N for growing crops, and therefore inclusion of cover crops in farming systems could result in a reduction of N fertilizer applied to agricultural soils. That should prove economically and environmentally beneficial given the deleterious ecological impacts of increased reactive N in natural systems.

Although cover crop can be advocated as a viable N management strategy (Lee *et al.*, 2002; Sainju *et al.*, 2007), it is important to recognize that the practice can, for most crops, only provide a portion of the needed N. As stated previously, N fertilizer had to be applied around day 60 in this experiment when corn growth was visibly impaired by a lack of mineral N.

4.3. Nitrous oxide emission: Relative significance of tillage practice and cover crop composition

In accord with several past studies (Muhammad *et al.*, 2011; Mitchell *et al.*, 2013), N_2O fluxes were highest in the 2-3 weeks following fertilizer application, and higher in the cover crop treatment than controls (Figure 3). Largely a product of denitrification in soils, the intensity of N_2O production is often controlled by the availability of NO_3^- and organic C to support the activity of heterotrophic denitrifiers. It has been suggested that increased N_2O emission in cover crop-amended soils could be linked to increased availability of labile C to denitrifiers as a result of cover crop application (Mitchell *et al.*, 2013). Although the cover crops did not significantly differ with regard to N_2O emission, vetch (low C/N ratio) addition resulted in higher N_2O flux compared to rye (high C/N ratio) (Figure 3). A similar negative trend between cover crop C/N ratio and N_2O flux was reported by Huang *et al.*, (2004). This result is also in accord with the finding of Zschornack *et al.*, (2011) who, in an investigation of N_2O emission from soils treated with the legume species serradella and ryegrass, measured higher emission with the legume cover crop. These results are also in agreement with those of others (Constantinides and Fownes, 1994; Millar and Baggs, 2004), and the general negative relationship between N_2O emission and C/N ratio of crop residue (Huang *et al.*, 2004). Additionally, results of the present study showed strong positive relationships between N_2O

emission and NO_3^- flux, as a measure of mineral N availability (Figure 4). The slope of the relationship was much steeper (3 times) in vetch-amended (Figure 4c) soil compared to rye (Figure 4b). This is an important and novel observation, and it underscores the propensity of NO_3^- evolved in the vetch treatment to undergo denitrification. That could be due to greater biodegradability of vetch-derived organic C, as well as poor synchronization between crop needs and the timing of NO_3^- release from decomposing vetch biomass. When plant N uptake is negligible, the conversion of NO_3^- to N_2O becomes the most favored fate. Finally, it is conceivable that the composition of the soil denitrifying population was differently affected by each type of cover crop, and that ultimately may have resulted in variable enhancement in N_2O production. To assess the merit of that contention, laboratory-scale investigations must be conducted to examine shifts in the size and diversity of the denitrifying community in soils treated with cover crops of varying composition. Cumulative N_2O emission was not significantly affected by tillage practices, but was strongly related to the type of cover crop. Several studies have reported statistically significant interactions between cover crop and tillage practice, with higher N_2O emission generally observed with reduced tillage than with PT under similar cover crop treatment (Bavin *et al.*, 2009; Abdalla *et al.*, 2012). During phase 2 of the present study, a borderline interaction between these factors was detected (Table 2). However, the most striking observation is the consistently lower (2 times on average) N_2O emission with rye compared to vetch. The lower N_2O emission with rye (and other biomass with higher C/N ratios) could be linked to N immobilization (Zschornack *et al.*, 2011; Partey *et al.*, 2014) as well as more efficient use of N by plants. As long as this N immobilization does not result in a complete exhaustion of the mineral N pool (thus impeding plant growth), this should be viewed as beneficial because

it leads to the retention of N which otherwise could be lost from the soil system as N gases. These results have significant management implications and support the view that, rye adoption as a cover crop, could help mitigate N₂O emission from agricultural soils.

4.4. Effects of cover crop addition on corn growth

Cover crop addition resulted in significant improvement in corn growth and N uptake (Tables 3 and 4). Nearly all parameters related to corn growth were 4-5 times greater in the cover crop treatments compared to control (Table 3). Similar results have previously been reported (Lee *et al.*, 2002; Nagumo and Nakamura, 2013). The enhancement in corn growth with cover crops application can be ascribed to greater N availability to growing plants. Providing strong support for that interpretation is the consistently greater biomass production and the higher N uptake (2-5 times) measured in cover crop-treated corn than in controls (Tables 3 and 4). These results confirm the positive effects of cover crops on corn growth, and these beneficial effects were more prominent in the rye than in the vetch treatments. Improved corn growth with rye amendment could be due to the greater resistance of rye to decomposition (compared to vetch) allowing the release of N to occur over a period of time that is longer and better matches with plant N needs. As noted above, vetch addition also resulted in increased N uptake but much less so than with rye. As the data presented in Figure 4 suggest, instead of being incorporated into corn biomass, a non-negligible portion of vetch-derived N is denitrified resulting in higher cumulative N₂O loss.

During the experiment, the amount of N added as cover crop biomass was < 20 kg N ha⁻¹, and that amendment was the only difference between the control and the treatments. Inspection of the data presented in Table 4 showed that the difference in N uptake

between treatments and controls is 6-8 times greater than the amount of N added in the cover crop. This observation suggests that the impact of cover crop on plant N nutrition could far outweigh the amount of N contained in the cover crop biomass. Through its effect on soil microbes and enzyme activity, cover crop addition could stimulate the conversion of native soil N into available N for plant uptake. This view would be consistent with the concept of added nitrogen interaction (ANI) that was suggested by Jenkinson *et al.*, (1985). Future studies, using ¹⁵N-labelled cover crop biomass, should be conducted to examine this mechanism and underlying processes.

5. Conclusions

The present study was undertaken with the overall objective of identifying the type of cover crop most suitable to agricultural soils under different tillage practices. In comparison to controls (without cover crop), application of cover crop biomass resulted in enhanced NO₃⁻ flux, higher corn N uptake and N₂O emission, but the extent of these enhancements was largely dependent on the cover crop composition and not on tillage practice. While no significant effect of tillage practices was detected, the increase in NO₃⁻ and N₂O fluxes was significantly higher in soil treated with vetch than with rye. On the other hand, corn growth was better and N incorporation into corn biomass was significantly (P<0.02) greater with rye amendment compared to vetch addition. Overall, these results indicate that, regardless of tillage practices, rye is a better cover crop option than vetch for enhancing soil fertility and N nutrition of corn.

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