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Non-Legume Cover Crops Can Increase Non-Growing Season Nitrous Oxide Emissions

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Cover crops retain post-harvest nutrients but how they impact non-growing season nitrous oxide (N_2O) emissions is unclear. Therefore, we quantified how cover crop type (fall rye [*Secale cereale* L.] or oilseed radish [*Raphanus sativus* L.]) and fertilizer source (compost or inorganic fertilizer) affected N_2O emissions, soil water-extractable organic C (WEOC) and nitrate (NO_3) dynamics over two non-growing seasons. A treatment with no fertilizer or cover crop was also included. Weekly, N_2O fluxes were determined using vented static chambers; soil WEOC and NO_3–N concentrations were measured monthly. Each non-growing season, mean N_2O fluxes were 74 to 450% greater in the winter (21 December–20 March) than spring (21 March–20 June) or fall (22 September–20 December). In winter 2014–2015, oilseed radish increased the mean N_2O flux by 39 and 323% compared with fall rye and no cover crop, respectively, while the mean N_2O fluxes were strongly correlated to the pre-winter (16 Dec. 2014) NO_3 concentrations (r = 0.96; P < 0.001), indicating NO_3 levels < 6 mg NO_3–N kg^{-1} limited N_2O fluxes. In 2014–2015, fall rye and oilseed radish had 76 and 154% greater cumulative N_2O emissions than amended soils with no cover crop, respectively. Across both winters, an exponential model explained 67% of variability between the pre-winter WEOC to NO_3 ratio and N_2O fluxes, indicating that organic C and NO_3 controlled over-winter N_2O fluxes. Non-legume cover crops increased non-growing season N_2O emissions, suggesting that cover crops concentrate denitrification substrates in root-associated soil to enhance N_2O fluxes.

Abbreviations: FRC, fall rye with compost; FRF, fall rye with inorganic fertilizer; MDL, minimum detection limit; ORC, oilseed radish with compost; ORF, oilseed radish with inorganic fertilizer; NCC, no cover crop with compost; NCF, no cover crop with inorganic fertilizer; CON, non-amended soil with no cover crop; WEOC, water-extractable organic carbon.

Core Ideas

- **Nitrous oxide emissions were greater in winter than spring or fall.**
- **Tillage radish increased over-winter N_2O fluxes.**
- **Non-legume cover crops increased N_2O fluxes under apparent NO_3 limiting conditions.**

Post-harvest seeding of cover crops reduces the risk of wind erosion and nutrient loss through leaching and runoff during the non-growing season, but how cover crops affect C and N transformations during this time is poorly understood. Although soil microbial activity slows during the non-growing season, this period is particularly prone to N_2O emissions in temperate regions (Wagner-Riddle and Thurtell, 1998; Dörsch et al., 2004; Ellert and Janzen, 2008; Hao, 2015). Whether cover crops reduce N_2O emissions during the non-growing season by assimilating ammonium (NH_4) and NO_3 is uncertain. In part, this is because cover crops release labile C and N through root exudates and rhizodeposition during their growth phase and freeze–thaw cycles, which can stimulate microbial activity and increase N_2O emissions (Petersen et al., 2011; Gul and Whalen, 2013; Mitchell et al., 2013). This may counter the crop N uptake and explain why there is no clear consensus on how non-legume cover crops effect N_2O emissions (Basche et al., 2014). A better understanding of N_2O emissions and the substrates that drive N_2O production during the non-growing season could improve cover

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crop management practices for optimized environmental and agronomic performance.

There are three primary biotic pathways that emit N_2O, denitrification, nitrifier-denitrification and nitrification (Gregorich et al., 2015). In the non-growing season, thaw events are associated with the greatest N_2O fluxes and denitrification is considered the dominant N_2O production pathway (Wagner-Riddle et al., 2008; Risk et al., 2014). Denitrification occurs when O_2 availability is low, which is most affected by increasing soil water content (Linn and Doran, 1984; Wallenstein et al., 2006; Kool et al., 2011), and to a lesser extent when labile C stimulates heterotrophic microbial activity, creating O_2 depleted microsites (Parkin, 1987; Butterbach-Bahl et al., 2013). Denitrification may be enhanced in the non-growing season as freezing and thawing alters water availability, which directly controls O_2 diffusion, and stimulates microbial activity by increasing substrate solubility (Skogland et al., 1988). Cover crops could reduce N_2O emissions because they deplete the NO_3 pool (Beauchamp, 1997; Liebig et al., 2015), the principal substrate for denitrification. Yet, there is limited information on the effect of cover crop management on N_2O emissions during repeated freezing and thawing during the non-growing season.

Lethbridge, AB is characterized by its semiarid climate, with short cool summers, and cold winters punctuated by highly variable temperature fluctuations with minimal snowfall accumulation. Throughout the non-growing season in Lethbridge, thawing events can be induced by sporadic 'chinook' winds carrying warm, dry air, increasing soil temperature and moisture to create suitable conditions for bursts of microbial activity (Chang et al., 1998). For example, during a chinook event, air temperature can increase 30°C and deplete snowpack from 15- to 3-cm, thawing the near soil-surface (McGinn, 2010). This leads to a soil surface that is exposed to repeated freezing, thawing, and drying (Bullock et al., 1999). About 39 to 90% of annual N_2O emissions occur during the non-growing season (Teepe et al., 2000; Jayasundara et al., 2007; Yanai et al., 2011; Abalos et al., 2016), mostly through the winter to spring thaw period in temperate regions (Nyborg et al., 1997; Wagner-Riddle and Thurtell, 1998; Dörsch et al., 2004; Ellert and Janzen, 2008). In cover cropped soils, decomposing root and aboveground tissues may supply organic C and NO_3 for N_2O production (Basche et al., 2014), exacerbating N_2O emissions during freeze-thaw events (Mørkved et al., 2006). Typically, WEOC is considered a surrogate of the C available for microbes (Appel and Mengel, 1993; Kalbitz et al., 2000; Chantigny, 2003; Zsolnay, 2003). Whether cover crops could detectably increase WEOC through root exudation or lysed root tissues during freeze–thaw cycles is uncertain, but could be related to cover crop characteristics.

Fall rye and oilseed radish are two contrasting cover crops. Fall rye is a perennial, with an extensive fibrous root system, while oilseed radish is an annual with a large taproot. How these contrasting root traits may affect non-growing season N_2O emissions is not clear because multiple environmental and edaphic factors interact with crops to affect N_2O fluxes (Rochette et al., 2004). While it is expected that soil NO_3 would be assimilated by the cover crops leading to reduced soil NO_3 concentrations and thus N_2O fluxes over the non-growing season, the winter to spring thaw period is typically when significant N_2O emissions occur in this region (Chang et al., 1998; Ellert and Janzen, 2008; Hao, 2015). In this period decomposing tissues and living roots are expected to supply labile C and N, retain moisture, and possibly create microsites depleted in O_2, thereby favoring N_2O production during the winter to spring thaw period.

The objective of this study was to quantify how cover crop type (fall rye or oilseed radish) and fertilizer source (compost or inorganic fertilizer) affected N_2O emissions and WEOC and NO_3 dynamics over two non-growing seasons in Lethbridge, AB, an area characterized by frequent snowmelt events with limited snowfall accumulation and a soil surface prone to freezing, thawing, and drying. We hypothesized that cover crops would increase N_2O emissions during the non-growing season in organically and inorganically fertilized soils, by increasing WEOC availability, and that the more extensive root system of fall rye would induce greater N_2O emission than oilseed radish. As N_2O emissions during the winter have been shown to be significant in this region, we also assessed whether the pre-winter NO_3, WEOC or the WEOC to NO_3 ratio was correlated to over-winter N_2O emissions. The rationale was, that for cover cropped soil in semiarid regions, a good time to make these measurements would be immediately before winter, and that the concentration of NO_3 and WEOC at this time is critical because N_2O may accumulate in soil solution near 0°C, as N_2O is two-fold more soluble in water at 0°C than 20°C (Weiss and Price, 1980). Thus, the NO_3 and WEOC at this time would represent the N_2O emission potential, representing what could enter the water soluble pool and later be released with a thaw as temperatures increase and the solubility of N_2O decreases (Goodroad and Keeney, 1984; Risk et al., 2013), and the N_2O that could be emitted by facultative anaerobes during thaw events as minimal leaching and no plant N uptake would occur over-winter.

MATERIALS AND METHODS

Study Site

A 2-yr field study was conducted at the Agriculture and Agri-Food Canada Lethbridge Research and Development Centre near Lethbridge, AB (49°38′N, 112°48′W; altitude: 929 m), with mean annual air temperature of 5.9°C and mean annual precipitation of 380 mm (1981 to 2010). The soil is calcareous with clay loam texture and is classified as an Orthic Dark Brown Chernozem (Soil Classification Working Group, 1998). Two adjacent field sites were selected and cropped to conventionally managed dryland spring wheat (*Triticum aestivum* L. 'AC Lillian') each growing season. The study began after the spring wheat harvest in August 2013 and was repeated on the adjacent field site in August 2014. Each field site was divided into four 37.2 m by 10 m blocks with 10-m buffers between blocks. There was a 2-m buffer between plots within each block and a 10-m headland buffer around the field sites. Seven experimental treat-

ments were randomly assigned to 3.6 m by 10 m plots within each block for a total of 28 experimental units. The treatments were fall rye 'AC Remington' with compost (FRC), fall rye with inorganic fertilizer (FRF), oilseed radish 'Tillage Radish' with compost (ORC), oilseed radish with inorganic fertilizer (ORF), no cover crop with compost (NCC), no cover crop with inorganic fertilizer (NCF), and a non-amended soil with no cover crop was included as a control (CON).

The compost and inorganic fertilizer were broadcasted on all plots 27 and 28 Aug. 2013 and 15 Aug. 2014. In 2013–2014, the compost (683 g dry matter kg^{-1}) contained 171 g total C kg^{-1} and 15.4 g total N kg^{-1} as determined by dry combustion (NA 1500 Series 2, Carlo Erba Instruments), and 30.2 mg NH_4–N kg^{-1} and 1140 mg NO_3–N kg^{-1} as determined by 2 mol L^{-1} KCl extraction (1:10 compost/KCl ratio). The NH_4–N and NO_3–N concentrations were determined with an automated colorimeter (Astoria-Pacific 305D Detector, Astoria-Pacific Inc.). In 2014–2015, the compost (672 g dry matter kg^{-1}) contained 173 g total C kg^{-1}, 16.0 g total N kg^{-1}, 20.1 mg NH_4–N kg^{-1}, and 607 mg NO_3–N kg^{-1}, which were determined as described above. The inorganic fertilizer-N source was ammonium nitrate and was applied at 45 kg N ha^{-1} each year. The compost was composted beef cattle feedlot manure and was applied at 100 kg total N ha^{-1} each year, this supplied 8 and 4 kg inorganic-N ha^{-1} in 2013 and 2014, respectively, 97% as NO_3–N each year. The inorganically fertilized plots also received 10 kg P ha^{-1} as triple superphosphate. After the compost and inorganic fertilizer were broadcasted, the plots were disked to incorporate compost and fertilizer to ~10-cm depth.

The cover crops were seeded on 30 Aug. 2013 and 15 Aug. 2014 at 7.8 kg seed ha^{-1} for oilseed radish and 90 kg seed ha^{-1} for fall rye using a custom Fabro double-disk forage seeder at 18-cm row spacing. In 2013, oilseed radish seed was not treated with insecticide and seedlings were severely damaged by flea beetle (*Phyllotreta cruciferae* [Goeze]), which necessitated replanting with a liquid seed treatment of Helix [Thiamethoxam] (Syngenta Group Co.) on 16 Sept. 2013 as described above. Each year after seeding, the plots were irrigated as needed until 16 Sept. 2013 and 23 Sept. 2014 to aid cover crop establishment. Daily mean air temperature, rainfall, snowfall, soil temperature and volumetric unfrozen moisture content (5-cm depth) were downloaded from a weather station <20 m from the field site.

Gas Sampling and Analysis

Gas samples were collected from vented static chambers (Chang et al., 1998). After seeding the cover crops, one chamber (30-cm diam. by 15-cm height) base was inserted 5 cm into the soil in each plot for the entire non-growing season. When necessary, the snow height and density were measured to estimate the chamber volume occupied by snow to correct the air volume of each chamber. Fluxes were measured weekly, between 0830 and 1130 h, by attaching the chamber covers to the base. At 0, 15, 30, and 60 min after the chamber was covered, 10 mL of headspace gas was drawn through a septum using a 10-mL polypropylene syringe, injected into an evacuated 5.9 mL Exetainer (Labco Ltd.), and analyzed for N_2O-N concentration with a gas chromatograph (Varian 3800, Varian Instruments) equipped with an electron capture detector.

For each chamber, the N_2O fluxes were calculated by fitting a second-order polynomial equation to the four successive gas sampling points versus time. The linear coefficient was assumed to represent the N_2O flux at time 0, before the flux gradient was affected by the increasing gas concentration in the chamber. When a poor fit of the nonlinear model was evident, we used a linear model (Pedersen et al., 2010; Chadwick et al., 2014). For example, if the coefficient of determination for the model was <0.85, all data were visually inspected to determine whether a nonlinear or linear model was the best fit, or if the linear coefficient of the nonlinear model was negative (the line of best fit was concave up, resulting in a negative linear coefficient) the response was assumed to be linear. For the linear model fitted to the N_2O concentration data versus time, the slope was used to calculate N_2O flux. The minimum detection limit (MDL) for the N_2O flux was calculated (Parkin et al., 2012; Johnson and Barbour, 2016) using a known concentration of N_2O (0.322 μL L^{-1}). Twelve samples were analyzed by gas chromatography and their mean concentration (0.400 μL L^{-1}), standard deviation (0.007 μL L^{-1}), and coefficient of variation (0.018) calculated. The linear MDL N_2O flux was 0.1 μg N m^{-2} h^{-1} and the quadratic MDL N_2O flux was 2.1 μg N m^{-2} h^{-1}. Gas fluxes were expressed in g N_2O-N ha^{-1} d^{-1}. For each plot, cumulative non-growing season N_2O-N emissions were calculated by summing the products of the measurement interval (i.e., days between two consecutive measurements) and the mean flux for that interval (i.e., arithmetic mean of the fluxes at the start and end of the interval). For each non-growing season the mean gas fluxes were grouped by calendar-based season into fall (22 September–20 December), winter (21 December–20 March) and spring (21 March–20 June). Although fluxes were calculated as N_2O-N, hereafter, fluxes will be referred to as N_2O.

Soil Sampling and Analysis

Soil samples were collected monthly from the 0- to 7.5-cm depth each non-growing season from October to May (except April each year). The soils were then analyzed for NO_3–N concentration by 2 mol L^{-1} KCl extraction (Keeney and Nelson, 1982), and WEOC by water extraction. The 2 mol L^{-1} KCl-extractable NO_3–N concentrations were determined with an automated colorimeter (Astoria-Pacific 305D Detector, Astoria-Pacific Inc.). The WEOC was determined by water extraction adapted from Chantigny et al. (1999). Briefly, 40 mL deionized water was added to 20 g of soil, shaken for 30 min, centrifuged and passed through a 0.45-μm nylon filter. The WEOC concentrations were determined by measuring non-purgeable organic C in the water extracts with a Shimadzu total organic C analyzer TOC-V_{CSH} (Shimadzu Scientific Instruments).

Statistical Analysis

All statistical analyses were computed with SAS 9.3 software (SAS Institute, Inc., 2011). Each non-growing season was analyzed separately. Residuals were checked for normality using the UNIVARIATE procedure. The N_2O emissions were log transformed and then back transformed for presentation in figures and tables. Repeated measures ANOVA was conducted with the MIXED procedure to assess the response of the seasonal mean N_2O fluxes and WEOC and NO_3 concentrations to cover crop type (fall rye, oilseed radish, no cover crop) and fertilizer source (compost, inorganic fertilizer) as a 3 × 2 factorial. Block was a random effect, cover crop type and fertilizer source were fixed effects and time was a repeated measure. For means comparisons, differences among least square means were tested at $\alpha = 0.05$. The WEOC and NO_3 concentrations were not calculated based on season because the sampling frequency was monthly with no sample collection in April each year. This meant that only one or two sampling events would be included in the calculation of seasonal means, which was considered inadequate for representing a seasonal mean. The CORR, REG, and NLIN procedures were used to relate WEOC, NO_3, and the WEOC to NO_3 ratio to N_2O fluxes.

RESULTS AND DISCUSSION

Nitrous Oxide Emissions

In 2013–2014, the N_2O fluxes could not be determined for the first five gas sampling dates (2, 8, 15, 22, and 29 Oct. 2013) because the electron capture detector malfunctioned. Peak N_2O fluxes corresponded with thawing events throughout the non-growing seasons (Fig. 1). Across the non-growing seasons, the greatest N_2O flux occurred on 13 Mar. 2014, which corresponded with a thawing event and a surge of unfrozen water content, whereby soil temperatures increased from −7.8 to −0.1°C in 13 d (5 cm depth). In 2014–2015, decreasing N_2O fluxes between 3 October and 12 December coincided with decreasing soil temperature (Fig. 2). In Winter 2014–2015, the peak N_2O fluxes occurred on 13 and 29 Jan. 2015, which corresponded with a thawing event and a surge of unfrozen soil water content.

As the freeze temperature modulates the potential N_2O flux at thawing (Risk et al., 2013), the amplitude of the temperature change from frozen to thawed (Wertz et al., 2016), the 'transition effect' (Butterbach-Bahl et al., 2013), controls the magnitude of the N_2O flux during a thaw event. Furthermore, the solubility of N_2O is inversely related to temperature, water at 0°C will contain about twice the N_2O as water at 20°C (Weiss and Price, 1980). This may partly contribute to soils near 0°C emitting unexpectedly large quantities of N_2O, as diffusion gradients between the dissolved pool of N_2O and the atmosphere may drive degassing of N_2O during warming events (Goodroad and Keeney, 1984; Risk et al., 2013). However, N_2O fluxes persisted even when soil temperatures did not rise above the freezing point. This is consistent with findings that significant N_2O was emitted from soil incubated statically at −1°C and amended with red clover residue and NO_3 (Wertz et al., 2013), suggesting the mechanism may be inhibition of N_2O reductases (Holtan-Hartwig et al., 2002). Whether N_2O reductase inhibition may contribute to increased N_2O fluxes during winter was not investigated in our field study, and warrants further investigation.

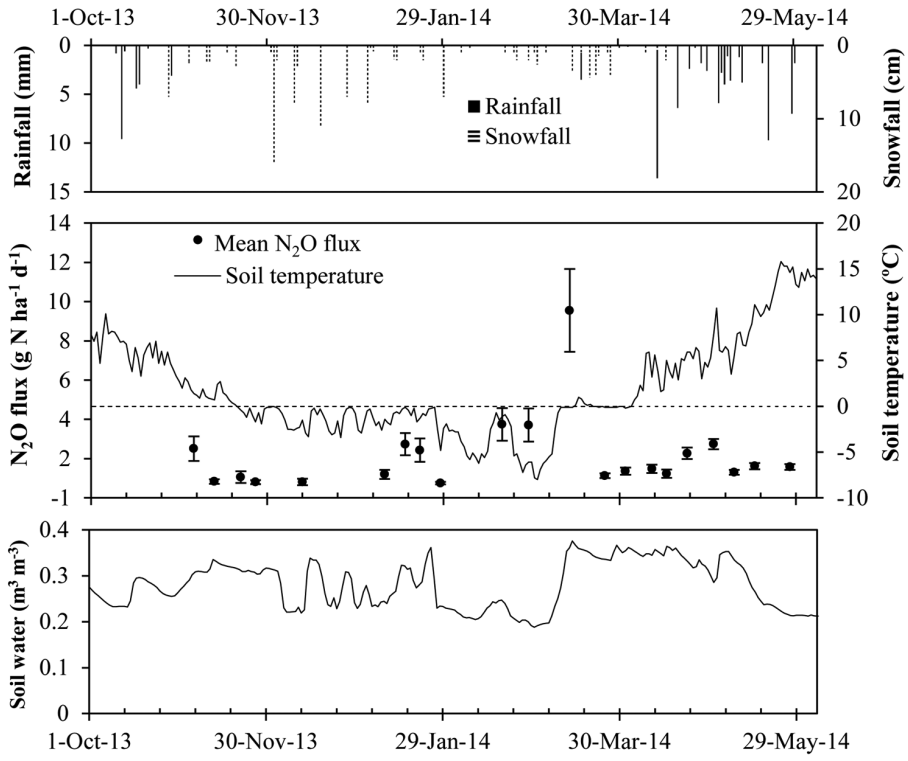

Fig. 1. Daily rainfall (mm), snowfall (cm), mean daily soil temperature (5 cm depth), mean daily N_2O flux (average across all treatments) and mean daily volumetric soil water content (m^3 m^{-3}) over the 2013–2014 non-growing season. Error bars represent ± standard error of the mean. The dotted line indicates 0°C.

The mean N_2O flux followed the order: winter > spring > fall in 2013–2014, and winter > fall > spring in 2014–2015 (Fig. 1 and 2). This is consistent with other studies from southern Alberta, Canada, which showed peak N_2O fluxes during winter (Hao, 2015) or the late-winter to spring period (Chang et al., 1998; Hao et al., 2001; Ellert and Janzen, 2008) during the non-growing season. Typically, N_2O fluxes are greatest during the spring snowmelt in temperate regions (Wagner-Riddle and Thurtell, 1998; Johnson et al., 2012; Risk et al., 2013; Congreves et al., 2016). However, our study location typically does not accumulate much snow over-winter. Frequent snowmelt events occur during the non-growing season during sporadic 'chinook' wind events that carry warm dry air over the region. For example, 30-yr climate data shows that in December, January, February, and March about 50% of days have <1 cm

of snow depth (Government of Canada, 2016). Thus, the surface soil is poorly insulated from air temperature fluctuations and is prone to freeze–thaw cycles during the winter. As freeze–thaw cycles are considered the principal mechanism affecting the physical release of N_2O from below the thawing surface layer and from the increased biological activity at the onset of thaw (Goodroad and Keeney, 1984; Chang and Hao, 2001; Risk et al., 2013), these cycles exert significant control over the magnitude of N_2O fluxes in the non-growing season in southern Alberta.

For 2013–2014, there were no significant differences in mean N_2O fluxes caused by cover crop type or fertilizer source in fall, winter or spring, or the cumulative N_2O emission (Table 1). In winter 2014–2015, the mean N_2O flux was significantly affected by cover crop type (Table 1). Oilseed radish significantly increased N_2O emission by 39 and 323% compared with fall rye and amended soils with no cover crop, respectively (Table 1). In 2014–2015, the cumulative N_2O emitted was significantly affected by cover crop type (Table 1, Fig. 3). Oilseed radish and fall rye and led to 154 and 76% more cumulative N_2O emissions over the non-growing season than amended soil with no cover crop, respectively ($P < 0.05$).

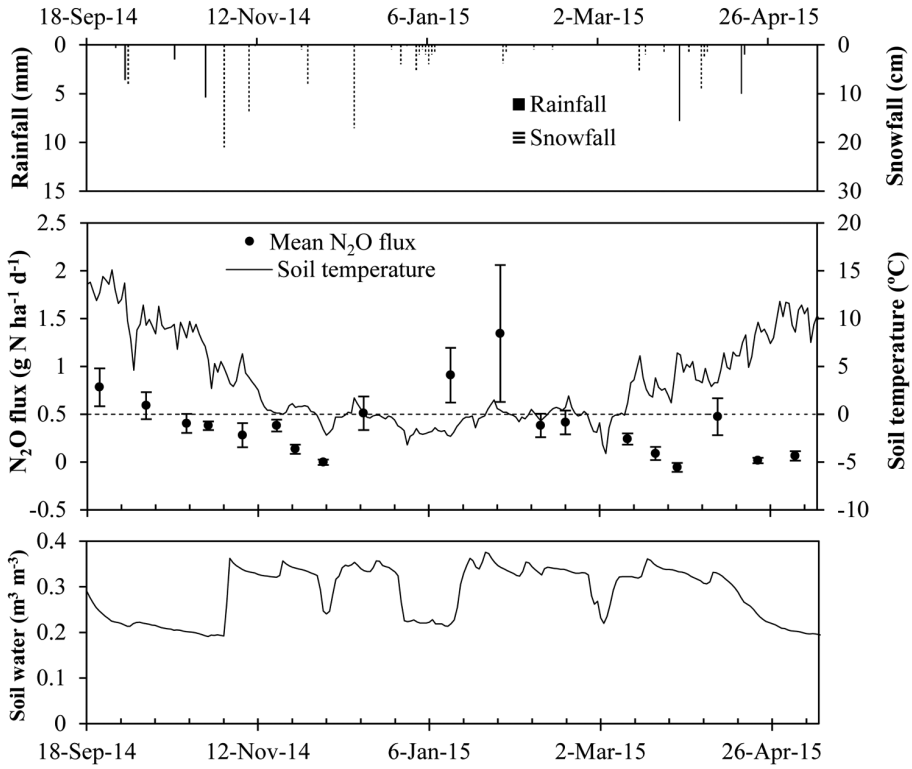

Fig. 2. Daily rainfall (mm), snowfall (cm), mean daily soil temperature (5 cm depth), mean daily N_2O flux (average across all treatments) and mean daily volumetric soil water content (m^3 m^{-3}) over the 2014–2015 non-growing season. Error bars represent ± standard error of the mean. The dotted line indicates 0°C.

The cumulative N_2O emissions over the two non-growing seasons were relatively small, ranging from 0.2 to 0.7 kg N_2O-N ha^{-1} in 2013–2014 and 0.1 to 0.2 kg N_2O-N ha^{-1} in 2014–2015. This represents a relatively insignificant N loss pathway during this period of time. Our cumulative N_2O emissions were near the lower range of the 0.4 to 3.5, 0.2 to 3.5, and 0.1 to 1.5 kg N_2O-N ha^{-1} between November and May over three consecutive years at a nearby field site (Ellert and Janzen, 2008). Year to year differences in cumulative N_2O emissions were quite obvious in our study, indicating environmental conditions strongly interact with site-specific soil properties to control the magnitude of seasonal N_2O fluxes (Rochette et al., 2004; Pelster et al., 2012; Butterbach-Bahl et al., 2013).

Water Extractable Organic Carbon and Nitrate Dynamics

In 2013–2014, WEOC was significantly affected by cover crop type and fertilizer source (Fig. 4). There was significantly more WEOC in soils planted with fall rye than oilseed radish or amended soil with no cover crop, and there was a trend to-

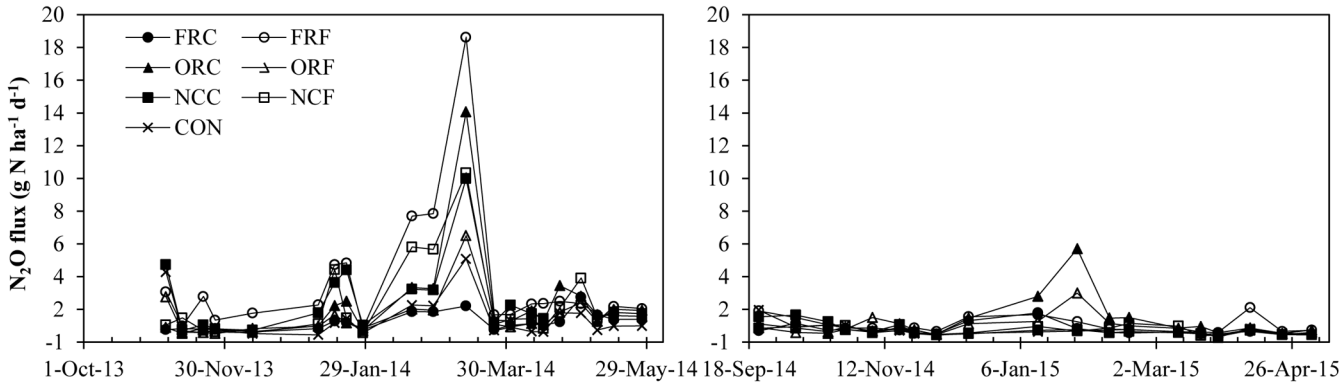

Fig. 3. The N_2O flux in response to cover crop type and fertilizer source in the 2013–2014 and 2014–2015 non-growing seasons. FRC, fall rye with compost; FRF, fall rye with inorganic fertilizer; ORC, oilseed radish with compost; ORF, oilseed radish with inorganic fertilizer; NCC, no cover crop with compost; NCF, no cover crop with inorganic fertilizer; CON, non-amended soil with no cover crop.

Table 1. Mean seasonal N_2O fluxes and cumulative emissions in response to cover crop type and fertilizer source and their interaction over the 2013–2014 and 2014–2015 non-growing seasons.

Parameter	Fall† N_2O flux	Winter N_2O flux	Spring N_2O flux	Cumulative N_2O emission
2013–2014		g N ha^{-1} d^{-1}		g N ha^{-1}
Cover crop				
Oilseed radish (*Raphanus sativus* L.)	0.41‡	2.52	1.20	312
Fall rye (*Secale cereale* L.)	0.88	3.56	1.21	419
No cover crop	0.75	3.55	1.31	404
Fertilizer source				
Compost	0.54	2.57	1.22	313
Inorganic	0.82	3.85	1.26	444
Source of variation		ANOVA (*P*-values)		
Cover crop (CC)	0.713	0.757	0.909	0.630
Fertilizer source (FS)	0.497	0.496	0.942	0.660
CC × FS	0.173	0.289	0.411	0.327
2014–2015				
Cover crop				
Oilseed radish	0.36	1.10a	0.13	173a
Fall rye	0.49	0.79b	0.28	120a
No cover crop	0.36	0.26b	-0.02	68b
Fertilizer source				
Compost	0.37	0.86	0.10	123
Inorganic	0.43	0.58	0.16	117
Source of variation		ANOVA (*P*-values)		
Cover crop (CC)	0.559	0.014	0.425	0.011
Fertilizer source (FS)	0.642	0.905	0.316	0.748
CC × FS	0.139	0.305	0.399	0.164

†Fall, 22 September–20 December; Winter, 21 December–20 March; Spring, 21 March–20 June.

‡Mean values within a column under a cover crop or fertilizer source subheading with the same letter or no letter are not significantly different at the $P < 0.05$ probability level.

ward soil planted with oilseed radish to have lower WEOC than amended soil with no cover crop. Compost-amended soil had significantly more WEOC than inorganically fertilized soil. In the 2014–2015 non-growing season, the WEOC concentration was not significantly affected by cover crop type or fertilizer source (Fig. 4). Our results suggest that fall rye can increase the soil WEOC concentration and that the difference can be detected using water extraction.

In the 2013–2014 non-growing season, the soil NO_3 concentration was significantly affected by cover crop type and fertilizer source (Fig. 4). There was significantly more soil NO_3 in oilseed radish planted soil than soil planted with fall rye or amended soil with no cover crop; fall rye significantly depleted soil NO_3 compared with the amended soil with no cover crop. There was significantly more soil NO_3 with inorganic fertilizer than compost (Fig. 5). In the 2014–2015 non-growing season, the soil NO_3 concentrations were significantly affected by cover crop type (Fig. 4).

Similar to 2013–2014, fall rye led to significantly lower soil NO_3 concentrations than oilseed radish and amended soils with no cover crop ($P = 0.055$), while soil planted with oilseed radish had higher NO_3 levels than amended soil with no cover crop ($P < 0.05$). Soil planted with oilseed radish had the greatest NO_3 concentrations and significantly greater over-winter N_2O emissions than soil planted with fall rye and amended soil with no cover crop. Although biomass accumulation in fall rye was greater than oilseed radish by November 2013 (1.5 vs. 0.5 Mg ha^{-1}), oilseed radish produced similar biomass to fall rye by November 2014 (both 1.2 Mg ha^{-1}), but fall rye still reduced NO_3 levels more than oilseed radish (Thomas et al., 2016). The greater soil NO_3 with oilseed radish appeared to boost over-winter N_2O emissions, in 2014–2015. This was in contrast to our hypothesis that fall rye would lead to the greatest N_2O emissions because of its extensive fibrous root system. As oilseed radish winter kills, it may have decomposed more quickly than fall rye root tissues, supplying more labile C and N, which could have stimulated over-winter N_2O emissions (Petersen et al., 2011; Mitchell et al., 2013). Consistent with our result, fodder radish increased over-winter N_2O fluxes compared with bare soil in Denmark, where air temperatures reached about −6°C. However, few studies have quantified the response of non-growing season N_2O fluxes as a function of cover crop type.

Cover crops may significantly decrease bulk soil NO_3 concentrations during the non-growing season via mass flow and subsequently decrease the N_2O emitted, but counter balance by increasing N_2O emitted from the rhizosphere, to have no net or a net positive effect on N_2O emissions compared with amended soils without cover crops. This is consistent with fall rye decreasing soil NO_3 levels but not reducing N_2O emissions during the non-growing season in Iowa (Parkin and Kaspar, 2006), where

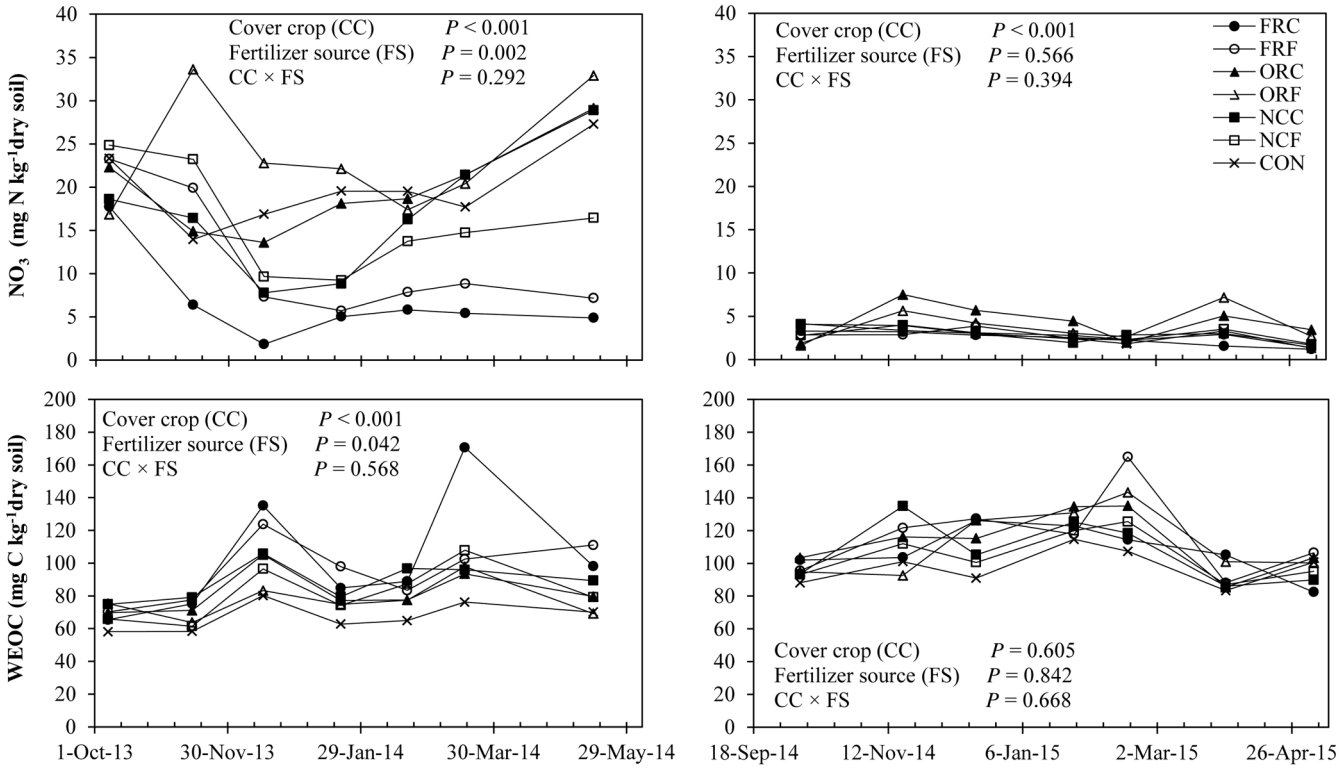

Fig. 4. Soil nitrate (NO_3–N) and water-extractable organic carbon (WEOC) in the 0- to 7.5-cm depth in response to cover crop type and fertilizer source in the 2013–2014 and 2014–2015 non-growing seasons. Analysis of variance results are shown for the main effects and their interaction. FRC, fall rye with compost; FRF, fall rye with inorganic fertilizer; ORC, oilseed radish with compost; ORF, oilseed radish with inorganic fertilizer; NCC, no cover crop with compost; NCF, no cover crop with inorganic fertilizer; CON, non-amended soil with no cover crop.

the winter air temperature reached about −20°C, similar to temperature lows in southern Alberta. In 2013–2014, the reduced soil NO_3 concentrations in the amended soils planted with fall rye did not significantly lower N_2O emissions compared with soils planted with oilseed radish despite their greater soil NO_3 concentrations. This may imply that the reduced soil NO_3 concentration lowered N_2O emissions from the bulk soil, but were counter balanced by increased N_2O emissions from the decomposing tissues in the root zone. As denitrifying activity was shown to have highly heterogeneous distributions with hot spots associated with particulate organic C (Parkin, 1987), the fall rye root zone would provide ideal conditions for denitrifying activity during the over-winter decomposition of roots, especially with repeated freezing and thawing. This may explain why the N_2O flux was not significantly affected by the fall rye cover crop, even though significant reductions in NO_3 levels were evident in the 2013–2014 non-growing season. More research is required to test how bulk soil and rhizosphere soil N_2O fluxes are affected under NO_3–limiting conditions.

In 2014–2015, the mean N_2O flux decreased by a factor of 3.9 compared with 2013–2014, the mean soil NO_3 concentration decreased by a factor of 5.6, while the mean WEOC concentration increased by a factor of 1.2 over the same time period. Although the year to year decrease in N_2O fluxes was not proportional to the reduced NO_3 levels, these findings suggest that the availability of NO_3 was more limiting than WEOC. Thus, the risk of N_2O emissions may be mitigated when soil NO_3 concentrations are low (Lemke et al., 1998; Wagner-Riddle and Thurtell, 1998; Burton et al., 2008; Chantigny et al., 2010). Other studies have reported that low available C appeared to limit N_2O emissions (Lemke et al., 1998; Rochette et al., 2004; Gillam et al., 2008; Chantigny et al., 2010; Pelster et al., 2012), but this depends on soil characteristics, climate and cropping system (Rochette et al., 2004), with no clear distinction between C and NO_3 limitation. Overall, the NO_3 concentration at any given time may not be strongly correlated to the instantaneous N_2O flux, but over the course of a growing season, or non-growing seasons in our case, the mean NO_3 level or intensity was an important control on the potential magnitude of N_2O fluxes (Burton et al., 2008; Chantigny et al., 2010).

Nitrous Oxide Fluxes were Similar with Compost and Inorganic Fertilizer

Seasonal N_2O emissions responded more strongly to cover crops in 2014–2015 than 2013–2014. As noted above, a major difference between the two non-growing seasons was the soil NO_3 concentration in 2013–2014 being 5.6 times greater than the 2014–2015 NO_3 levels, whereas the WEOC concentration remained relatively consistent between non-growing seasons. This may imply that N_2O emissions are more responsive to cover crops under NO_3 limitation. Overall, our result suggests that cover crop type and background soil NO_3 levels probably influence N_2O emissions more than the fertilizer source, when the compost or inorganic fertilizer is applied at low rates to aid cover crop establishment. Typically most studies have compared inorganically fertilized and manure-amended soils (Rochette et

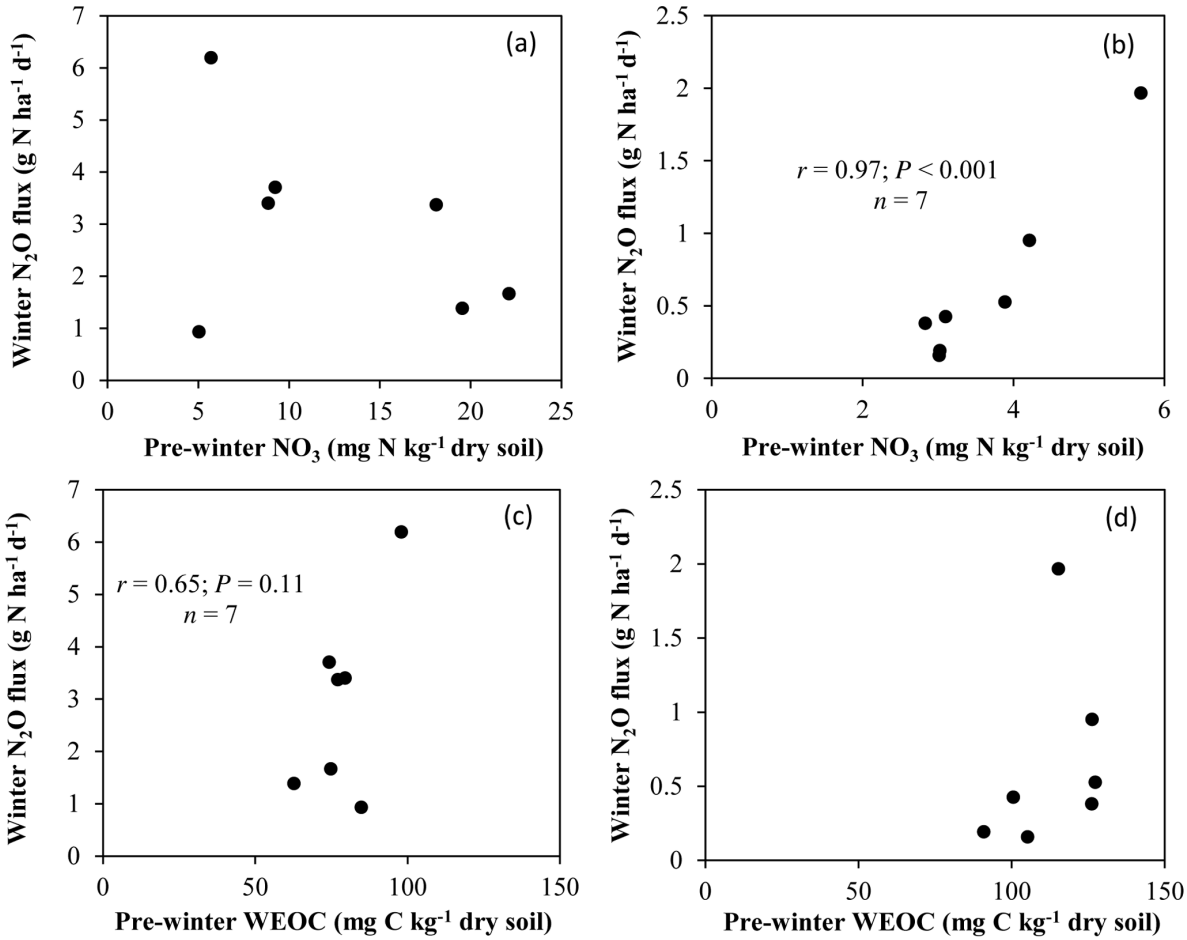

Fig. 5. The functional linear relationships between mean winter N_2O fluxes and the pre-winter concentrations of NO_3 in (a) 2013–2014 and (b) 2014–2015 and pre-winter concentrations of WEOC in (c) 2013–2014 and (d) 2014–2015. Pre-winter measurements were made 12 Dec. 2013 for 2013–2014 and 16 Dec. 2014 for 2014–2015 at the 0- to 7.5-cm depth. Each data point represents a treatment mean. Correlation coefficients (*r*) are shown in (b) and (c).

al., 2004; Ellert and Janzen, 2008; Chantigny et al., 2010; Pelster et al., 2012), whereas N_2O emissions from compost-amended soils represents an apparent research gap. Although it is difficult to precisely compare a compost-amended soil to an inorganically fertilized soil, our data suggests that applying inorganic-N fertilizer at 45 kg N ha^{-1} and compost at 100 kg total N ha^{-1} led to comparable N_2O responses.

Water Extractable Organic Carbon, Nitrate, and Over-winter Nitrous Oxide Fluxes

In 2014–2015, when soil NO_3 levels were substantially lower than 2013–2014, the pre-winter NO_3 concentrations measured 16 Dec. 2014 were strongly correlated with the mean winter N_2O fluxes (r = 0.97; P < 0.001; n = 7; Fig. 5b). This provides evidence that the N_2O flux was limited by NO_3 and that denitrification in the surface soil was an important source of over-winter N_2O production (Tenuta and Sparling, 2011). This is consistent with other studies that have indicated de novo N_2O production was a large N_2O source over the non-growing season in temperate regions (Wagner-Riddle et al., 2008; Németh et al., 2014; Risk et al., 2014; Congreves et al., 2016). In Winter 2013–2014, there was no apparent correlation between pre-winter NO_3 levels and the mean winter N_2O fluxes (Fig. 5a); indicating NO_3 was not limiting N_2O fluxes. However, there was a positive correlation between WEOC and N_2O flux (r = 0.65; P = 0.112; n = 7; Fig. 5c), indicating that WEOC may have limited N_2O fluxes over the winter in 2013–2014. This is consistent with other studies that have shown that N_2O production was limited by NO_3 in low NO_3 soils and mineralizable C in high NO_3 soils (Weier et al., 1993; Gillam et al., 2008; Mitchell et al., 2013).

In the presence of readily available NO_3, facultative anaerobes may avoid using N_2O as a terminal electron acceptor because their preference follows the order O_2 > NO_3 > N_2O (Firestone and Davidson, 1989). The result is increased N_2O emissions under high NO_3 availability (Gillam et al., 2008) because microbes preferentially select NO_3 as a terminal electron acceptor under O_2 limiting conditions (Cho et al., 1997). When NO_3 is not limiting facultative anaerobic activity, the availability of the energy source (electron donor–organic C) may limit denitrification. This is consistent with laboratory incubations that found N_2O emissions were controlled by the supply of electron acceptors (O_2, NO_3, N_2O) relative to the demand created by the availability of electron donors (Gillam et al., 2008).

Across both non-growing seasons, there was an apparent alternating relationship between WEOC limitation and NO_3 limitation controlling the over-winter N_2O fluxes. Therefore,

we related the WEOC to NO_3 ratio with the mean over-winter N_2O fluxes. Across years, 67% of the variability among 13 of 14 mean over-winter N_2O fluxes could be explained by a simple nonlinear exponential model (Fig. 6). This relationship suggests that over a larger scale the over-winter N_2O fluxes responded as a function of the WEOC to NO_3 ratio. This may imply that when soil NO_3 concentrations are not limiting N_2O fluxes, strategies to increase the organic C concentration could reduce N_2O emissions (Congreves et al., 2016), but its effectiveness may depend on the background NO_3 level (Senbayram et al., 2012). Whether this relationship suggests that immobilization or the balance between the supply of electron donors and terminal electron acceptors is the primary mechanism is not clear. However, due to low CO_2 emissions during the over-winter period (data not shown) it reduces the likelihood that microbial activity was sufficient to immobilize N and limit N_2O production. It is more likely that this ratio implies that the potential to emit N_2O is a function of the supply of electron donors controlling the demand for terminal electron acceptors (Gillam et al., 2008). A low WEOC to NO_3 ratio infers that NO_3 is more abundant relative to WEOC, thus facultative anaerobes would be more likely to preferentially use NO_3 rather than N_2O as a terminal electron acceptor (Cho et al., 1997), thereby increasing the N_2O to N_2 mole ratio for denitrification. At high WEOC to NO_3 ratio the electron donor source (WEOC–organic C) is more abundant than the preferred electron acceptor source (NO_3), thus facultative anaerobes would be more likely to use N_2O as a terminal electron acceptor to maintain anaerobic respiration, thereby reducing the N_2O to N_2 mole ratio of denitrification.

When NO_3 concentrations were greater than about 6 mg N kg^{-1}, NO_3 did not appear to limit over-winter N_2O production. This is consistent with other studies that found NO_3 levels greater than 5 to 10 mg N kg^{-1} did not limit N_2O production (Ryden, 1983; Gillam et al., 2008). It appeared over-winter soil conditions favored the use of NO_3 by facultative anaerobes as a terminal electron acceptor. However, we cannot dismiss the physical release of N_2O from the soil solution and soil layers beneath the thawed surface layer during the over-winter period, which represented 22% of spring thaw N_2O emission in a temperate climate (Risk et al., 2014). Nevertheless, the major source of N_2O production during these freeze-thaw transitions appears to be of biotic origin (Chang and Hao, 2001; Wagner-Riddle et al., 2008; Risk et al., 2014; Wertz et al., 2016).

CONCLUSIONS

The mean N_2O flux was greatest in winter than spring or fall in both non-growing seasons. Across the two non-growing seasons, the peak N_2O flux corresponded with a thawing event, whereby the soil temperature increased from −7.8 to −0.1°C in 13 d (5 cm depth). Oilseed radish and fall rye only significantly increased cumulative non-growing season N_2O emissions when NO_3 was apparently limiting the N_2O fluxes. The proposed mechanism occurs under NO_3 limiting conditions, whereby non-legume cover crops increased the connectivity between

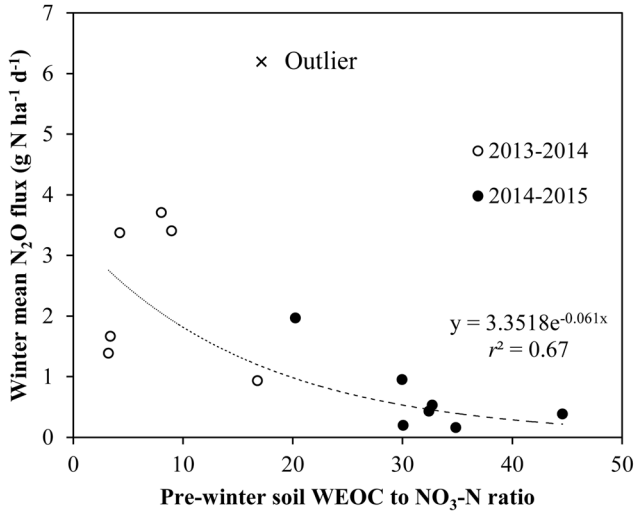

Fig. 6. The functional nonlinear relationship between the mean winter N_2O flux and the pre-winter WEOC to NO_3 ratio across two non-growing seasons fitted to an exponential function (dotted line) with the coefficient of determination (r^2). Pre-winter measurements were made 12 Dec. 2013 in 2013–2014 and 16 Dec. 2014 in 2014–2015 at the 0- to 7.5-cm depth. Each data point represents a treatment mean.

denitrification substrates (Organic C and NO_3) and microbes in the root-associated soil, resulting in greater N_2O fluxes than amended soils with no cover crop. Even when fall rye depleted NO_3 compared with oilseed radish, there was no corresponding reduction in N_2O flux. This suggests that cover crops with extensive root systems like fall rye may counter the NO_3 depletion with increased N_2O emissions from the root-associated soil, an area where denitrification substrates are densely concentrated and sensitive to surface soil freeze–thaw cycles.

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