ABSTRACT

Title: NITROUS OXIDE EMISSIONS IN COVER CROP-

                                    BASED CORN PRODUCTION SYSTEMS

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Nitrous oxide (N_2O) is a potent greenhouse gas; the majority of N_2O emissions are the result of agricultural management, particularly the application of N fertilizers to soils. The relationship of N_2O emissions to varying sources of N (manures, mineral fertilizers, and cover crops) has not been well-evaluated. Here we discussed a novel methodology for estimating precipitation-induced pulses of N_2O using flux measurements; results indicated that short-term intensive time-series sampling methods can adequately describe the magnitude of these pulses. We also evaluated the annual N_2O emissions from corn-cover crop (*Zea mays;* cereal rye [*Secale cereale*], hairy vetch [*Vicia villosa*], or biculture) production systems when fertilized with multiple rates of subsurface banded poultry litter, as compared with tillage incorporation or mineral fertilizer. N_2O emissions increased exponentially with total N rate; tillage decreased emissions following cover crops with legume components, while the effect of mineral fertilizer was mixed across cover crops.

NITROUS OXIDE EMISSIONS IN COVER CROP-BASED
CORN PRODUCTION SYSTEMS

By

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Chapter 1 – Agricultural contributions to nitrous oxide emissions

Rationale

Human activities have greatly increased the atmospheric concentration of trace gases associated with radiative forcing relative to pre-industrial levels, in particular carbon dioxide (CO_2, 43%), methane (CH_4, 125%), and nitrous oxide (N_2O, 22%) (Lashof & Ahuja, 1990; IPCC, 2014). The vast majority of current greenhouse gas emissions are from industrial sectors such as power generation and transportation; although agriculture currently only accounts for 10% of US global warming potential annually, emissions associated with agriculture are steadily rising (US EIA, 2011).

Agricultural contributors to GHG emissions are primarily the result of soil management (e.g., tillage, fertilizer amendments, and cropping system), livestock enteric fermentation, manure management, and rice cultivation (US EPA, 2013). Enteric fermentation and rice cultivation are associated with fluxes of CH_4, while soil and manure management are the major drivers of N_2O flux, the focus of this thesis. Application of N to soils alone accounts for 64% of US emissions of N_2O.

Demand for high crop yields for an ever-growing human population has resulted in the continued intensification of agricultural production, including the increased use of N fertilizers (Searchinger *et al.*, 2008; Snyder *et al.*, 2009; Erisman *et al.*, 2013). As global incomes rise, so does the demand for grain-fed animal products and ethanol feedstock: corn acreage in the US for 2013 was the highest since 1936, with 39.4 million hectares planted (Fang *et al.*, 2002; NASS-USDA, 2014). Grain production is the largest user of fertilizers globally, and in the US, corn alone accounted for 43% of N applied over the last 50 years (USDA-ERS, 2013). As grain production is not forecast to decline,

N_2O emissions will continually increase unless techniques are developed to mitigate emissions without negatively impacting yield (Foley *et al.*, 2011).

Microbial Basis

Soil emissions of N_2O are primarily produced through two microbial pathways, nitrification and denitrification. Both pathways use inorganic N substrates, either from mineral fertilizers or the mineralization of soil organic matter (SOM). Simplified models for these pathways are shown in Fig. 1.1, where mineralization to ammonium (NH_4^+) and dissolution of nitrate (NO_3^-) are represented by the top and bottom pathways on the left of the diagram.
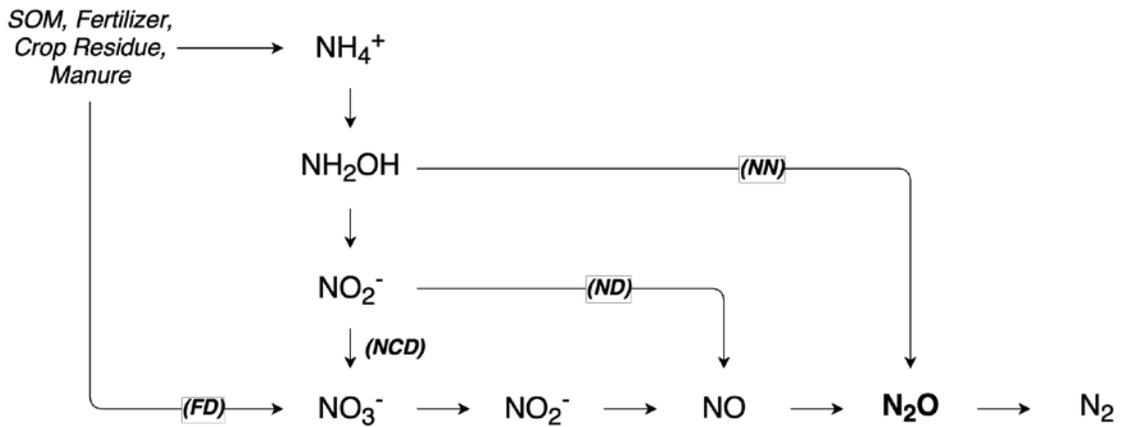
Figure 1.1. A summary of the pathways of N_2O production, adapted from Kool *et al.* (2011). NN = nitrifier nitrification, ND = nitrifier denitrification, NCD = nitrification-coupled denitrification, and FD = fertilizer denitrification.

Nitrification (represented by the column of reactions, proceeding downward) is the aerobic oxidation of NH_4^+ to NO_3^-. N_2O is an alternate product of this pathway (*NN*): as O_2 partial pressure decreases , N_2O production is favored relative to nitrite (NO_2^-) production (Goreau *et al.*, 1980). Nitrifying organisms may also produce N_2O via a nitric

oxide (NO) intermediate *(ND)*, and N_2O is also favored at low partial pressures of O_2 in this pathway (Anderson & Levine, 1986). Denitrification *(NCD, FD)* is a series of anaerobic dissimilatory reduction steps from NO_3^- to dinitrogen gas (N_2), represented by the horizontal reactions along the bottom of the figure. In each step, denitrifying organisms oxidize organic C from SOM, using nitrogen oxides as electron acceptors; N_2O is an intermediate product in this pathway. N_2O fluxes due to denitrification are generally an order of magnitude greater than those due to nitrification under agricultural conditions (Bateman & Baggs, 2005).

The production of N_2O in soils has been described by the hole-in-the-pipe model (Firestone & Davidson, 1989). Overall nitrification and denitrification rates are represented by a leaky "pipe", and the loss of N_2O to the atmosphere is the "hole". In non-flooded agricultural soils, the size of the pipe is driven by organic carbon and inorganic nitrogen availability, presence of anaerobic microsites, and temperature. The size of the hole is increased by low C:N ratios, the presence of O_2 at low partial pressures, and low pH (Firestone *et al.*, 1980).

Production of N_2O during both nitrification and denitrification is associated with low O_2 concentration within the soil pore. As the rate of diffusion of O_2 in water is much lower than in air, water filled pore space (WFPS) is often used as a surrogate measure of anoxic conditions (Linn & Doran, 1984; Dobbie *et al.*, 1999). In field trials of pasture and row crops, Smith et al. (1998) demonstrated that N_2O emissions from soil display a sigmoidal response, where very low to negligible emissions occur below 60% WFPS. Above this threshold, N_2O emissions increase rapidly up to 90% WFPS; beyond this point increasing moisture may decrease emissions as denitrification completes to N_2.

Since soil texture strongly influences soil moisture dynamics, the denitrification threshold can vary. For very high clay soils the onset threshold may be as low as 40% WFPS (Del Grosso *et al.*, 2000). Field-based monitoring of N_2O emissions has focused on taking measurements at the onset of denitrification triggered by rain or irrigation (Jambert *et al.*, 1997). The largest N_2O fluxes are most often seen at the onset of these water content thresholds; as pores remain saturated over the course of days or weeks, NO_3^- is consumed and fluxes decrease, following first-order kinetics (Reddy *et al.*, 1978). This is coincident with evapotranspiration or drainage; however, fluxes may be observed during prolonged periods of high soil moisture when associated with freezing and thawing (Mørkved *et al.*, 2006).

As with most soil microorganisms, the activity of nitrifiers and denitrifiers increases exponentially with temperature in the range of 5-40°C (Smith *et al.*, 1998). The freeze-thaw response of N_2O production is a shorter pulse of biological activity upon thawing, and fluxes decrease after the initial peak. This has been hypothesized to be caused by the release of previously inaccessible organic C and N substrates due to physical binding with soil colloids or immobilization in microbial biomass (Skogland *et al.*, 1988; Christensen & Christensen, 1991; Schimel & Clein, 1996). The expansion of water crystals during the freezing process can disturb clay-organic matter complexes and lyse microbial and plant cells. Release of N_2O due to hard soil freezing has only been observed in very cold climates, such as Canada, the upper midwestern US and northern Europe, but insufficient work has been done in more temperate regions that may experience incomplete freezing (Christensen & Tiedje, 1990; Koponen & Martikainen, 2004).

Agronomic Context

Both the "pipe" and "hole" regulating factors for N_2O production—N substrate concentration, C substrate concentration, pore moisture, temperature, and pH—are affected directly or indirectly through a number of agricultural management strategies, including fertility rate, source, timing, and placement; residue management; and cover crop use. The use of manures and cover crops provides both N and C substrates in various proportions, compared with mineral fertilizers that provide only inorganic forms of N. N sources also affect soil acidity, generally increasing pH with manures and decreasing pH with mineral fertilizers; N_2O emissions increase as pH decreases (Fox & Hoffman, 1981; Whalen *et al.*, 2000; Sainju *et al.*, 2005). Surface residues increase soil temperatures during winter, which may insulate against freezing, but decrease soil temperatures during spring and summer, which may decrease microbial activity (Bussière & Cellier, 1994). Likewise, decomposing stover and other surface residues increase soil moisture during summer and fall, but living cover crops may decrease soil moisture in fall, winter, and spring (Krueger *et al.*, 2011). Of these factors, N management has been identified as the largest factor affecting N_2O production (Millar *et al.*, 2010).

To achieve high grain yields, large amounts of N must be mineralized from organic matter or provided in the form of mineral fertilizers, but reactive N in the soil has potential to contribute to N_2O flux (Velthof & Oenema, 1995). It is proposed that N application rate affects both the "pipe"—overall denitrification rate—and the "hole"—$N_2O:N_2$ relative abundance—in the Firestone model (Zebarth *et al.*, 2008). Overall denitrification is a function of nitrate availability, but relative production of N_2O may be

a consequence of low $C:NO_3^-$ ratio, if complete denitrification to N_2 is limited by labile C (Luo *et al.*, 1999; Del Grosso *et al.*, 2000).

The ratio between N_2O produced and fertilizer N application rate is referred to as the emission factor (EF) and varies based on climate, soil texture, and management techniques, usually from 0-10% of N applied, but often assumed to be 1%, although this default value is accompanied by an uncertainty range from 0.3-3% (Dobbie & Smith, 2003; IPCC, 2007). These EFs are aggregated by land use modelers and policymakers for budgeting greenhouse gases at larger scales (Lesschen *et al.*, 2011; Skiba *et al.*, 2012).Lesschen *et al.*, 2011)

If EF is assumed to be a static percentage, then it implies a positive linear relationship in which N_2O emissions increase in proportion to any fertilizer application rate (Shcherbak *et al.*, 2014). However, reported EFs in the literature are not normally-distributed, but rather right skewed, such that EFs increase with N application rate (Fig. 1.2). Like many other biological phenomena, N_2O production appears to be log-normally distributed (Limpert *et al.*, 2001). Thus it is important to characterize emissions across a range of fertilizer rates within a given agricultural system, rather than extrapolating from a single rate.
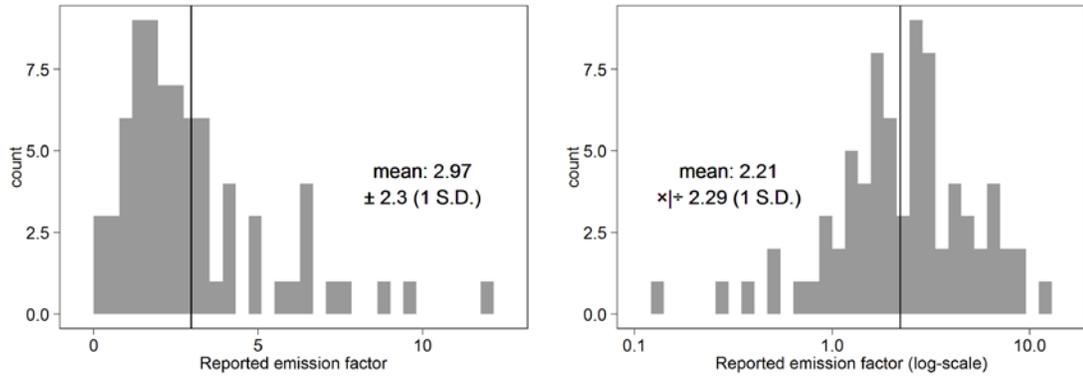
Figure 1.2. Histograms of reported emission factors (left, untransformed; right, natural log transformed) from selected agricultural studies in the central and eastern US, adapted from Cavigelli & Parkin (2012). EFs are lognormally distributed (Shapiro-Wilk, W=0.85, p<0.001 before transformation; W=0.97, p=0.08 after transformation).

Plant-soil N synchrony is a major focus of sustainable agricultural research to maximize nitrogen use efficiency (NUE)—that is, limiting N leaching to groundwater, surface erosion, weed uptake, and gaseous losses. Best management practices include using split applications of fertilizer, timing those applications to crop phenology, and placing fertilizer in the crop root zone. However, only limited work has been conducted to assess whether these fertilizer techniques can mitigate N_2O emissions (Cavigelli & Parkin, 2012). In a study on high-yield corn systems, 4 split applications of fertilizer increased N_2O emissions in continuous corn in 1 year but not in 2 other years, while split applications did not affect N_2O emissions in corn-soybean rotations; however, the split application systems used higher rates of N fertilizer, 70-120 kg N ha^{-1} greater (Adviento-Borbe *et al.*, 2007). Placement of mineral fertilizer in the crop root zone spatially concentrates N, which could have the potential to create sites of high denitrification rates,

but N_2O emissions were not affected when compared with conventional tillage or surface broadcasting (Halvorson *et al.*, 2008; Nash, 2010; Smith *et al.*, 2011).

This thesis will explore 1) the temporal dynamics of nitrous oxide flux and 2) the cumulative effects of cover crop and fertility management on nitrous oxide emissions in an agricultural setting. The following two chapters address these topics through field-scale sampling of N_2O fluxes over differing time scales (1 week vs. 1 year) within an agronomic trial; the trial was initiated to investigate high residue, no-till management of corn following cover crops and to compare a novel method of applying poultry litter (subsurface banding) with conventional fertility practices. These management factors created a wide range of soil nutrient and water balances, which both directly and indirectly affected soil N_2O emissions outcomes.

Chapter 2 – A novel method of estimating nitrous oxide emissions from flux measurements

Abstract

Precipitation and irrigation induce pulses of N_2O emissions in agricultural soils, but the magnitude, duration, and timing of these pulses remain uncertain. This uncertainty makes it difficult to accurately extrapolate emissions from unmeasured time periods using static chambers sampled manually. Therefore, we developed a protocol to predict N_2O emissions using a model derived from data collected daily for 7 d following wetting events. Within a cover crop-based corn (*Zea mays* L.) production system in Beltsville, MD, we conducted the 7 d time series during four time periods representing a range of corn growth stages in both 2013 and 2014. Treatments included mixtures and monocultures of grass and legume cover crops that were fertilized with pelletized poultry litter or UAN, 9-276 kg N ha^{-1}. Most gas flux time series did not exhibit expected exponential decay over time (82%); therefore, cumulative emissions were calculated using trapezoidal integration over 7 days following the wetting event. We observed a wide range of fluxes (-9.33 to 2940 g N_2O-N ha^{-1} d^{-1}) and cumulative 7-d emissions (-7.40 to 9080 g N_2O-N ha^{-1} week^{-1}). Cumulative 7-d emissions were well-correlated with single point gas fluxes on the second day following a wetting event using a generalized linear mixed model (*ln*[emissions]=0.809·*ln*[flux]+2.47). Soil chemical covariates prior to or following a wetting event were weakly associated with induced cumulative emissions. The ratio of dissolved organic carbon to total inorganic nitrogen was negatively correlated with cumulative emissions (R^2=0.23-0.29), while nitrate was positively correlated with cumulative emissions (R^2=0.23-0.33). Our model is an

innovative approach that is calibrated using site-specific time series data, which may then be used to estimate short-term N_2O emissions following wetting events using only a single flux measurement.

Introduction

Demand for high crop yields has resulted in the continued intensification of agricultural production, particularly the increased use of N fertilizers (Searchinger *et al.*, 2008; Snyder *et al.*, 2009; Erisman *et al.*, 2013). Application of N to soils accounts for 64% of US emissions of N_2O, a potent greenhouse gas with 298 times the global warming potential of CO_2 (US EIA, 2011). Attempts to mitigate N_2O production rely on research to estimate the emissions associated with various agricultural management regimes. However, a lack of uniformity in greenhouse gas sampling methodologies constrains our ability to compare results among studies, in particular the frequency of sampling and interpolation of fluxes during unsampled periods (Rochette & Eriksen-Hamel, 2008).

Fluxes of N_2O are not uniformly distributed throughout the year; they are higher during periods of high soil temperature, moisture, and N concentration (Dobbie *et al.*, 1999). However, within those periods fluxes remain highly variable due to high temporal variability of O_2, denitrification substrates (i.e. OM and nitrate), and denitrifier enzyme status within the soil (Parsons *et al.*, 1991; Dendooven *et al.*, 1996). As a result, temporal variability of N_2O production remains an area of active research (Smith & Dobbie, 2001; Flessa *et al.*, 2002; Parkin, 2008).

Since manual static chamber methods require substantial commitment of resources–e.g. labor and analytical equipment–they often result in researchers reducing replication in space or reducing treatment factor levels (Chadwick *et al.*, 2014). Increasing sampling efficiency, then, could alleviate some of these challenges associated with manual sampling.

Due to these limitations, many researchers have chosen to monitor N_2O either for short periods during the growing season, or on regular intervals throughout the year, up to three times per week (Flessa *et al.*, 1995; Mosier *et al.*, 2005; Ma *et al.*, 2010; Roy *et al.*, 2014). However, protocols that sample during the growing season only have limited utility in evaluating the impact of management on an annual basis. A comparison of simulated methods that targeted regimes of weekly versus daily sampling showed errors in cumulative N_2O loss calculations of up to 50% for the longer intervals over the course of 4 weeks (Kroon *et al.*, 2008). For year-round protocols, techniques applied to near-continuously sampled data have shown that fixed time-interval sampling can underestimate or overestimate annual emissions by 20% for short intervals ($\leq$4 d) and up to 80% for long intervals ($\leq$14 d) (Parkin, 2008). This effect is influenced by relative timing of N_2O fluxes and sampling events. If scheduled sampling events fall immediately prior to or following soil wetting or fertilization, emissions are likely to be underestimated or overestimated, respectively.

Other approaches to increasing sampling efficiency increase sampling intensity when there is potential for large magnitude fluxes to occur while reducing sampling intensity when low emissions are expected. Zaman et al. (2008) used this approach by using high sampling intensity after fertilization and less frequent sampling later in the

season. This approach focuses on N inputs as the main driver of fluxes, but does not address the variability associated with changing soil moisture. While a near-continuous automated gas sampling apparatus provides the highest temporal resolution of data, manual sampling protocols have been shown to accurately measure individual N_2O fluxes if conducted more intensely following both fertilization and wetting events for up to one week (Smith & Dobbie, 2001; Flessa *et al.*, 2002). This stratified approach requires daily sampling following these events, with decreased sampling during periods of lower soil water and nutrient availability (Parkin & Kaspar, 2004; Parkin, 2008).

The temporal dynamics of N_2O fluxes during unsampled periods have not been well-defined; most studies use linear interpolation to calculate daily fluxes between sampling dates (Lemke *et al.*, 1999; Smith & Dobbie, 2001; Gagnon *et al.*, 2011; Omonode *et al.*, 2011; Venterea *et al.*, 2011). The assumption of linear changes in fluxes between sampling dates is likely not accurate because denitrification and associated N_2O production exhibit first-order kinetics, with peak N_2O production some hours after a wetting event, followed by exponential decrease in fluxes over several days (Reddy *et al.*, 1978; Murray *et al.*, 1989). One approach to address the bias introduced with linear interpolation is to sample daily after an inducing event until fluxes return to a baseline (Cui *et al.*, 2012). The disadvantage to this method is the large numbers of samples required; return to baseline may take up to 14 days after each rainfall and fertilization event.

An alternative approach has been proposed: rather than integrating discontinuous sampling events throughout the year, annual emissions can be better estimated by characterizing discrete pulses using intensive event-based sampling schemes (Cavigelli,

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2010; Cavigelli *et al.*, 2014). If pulses follow a pattern over the days following a wetting

event, models could be developed and used to predict emissions during non-sampled

days, reducing the resources required for sampling, thus allowing increased replication or

numbers of treatment levels.

In addition to improving temporal N_2O sampling patterns, soil chemical

covariates have also been proposed as a way to predict N_2O emissions. Nitrate (NO_3^-) and

nitrite (NO_2^-) intensity (a time-weighted average of soil concentration over the growing

season) have received particular attention; both of these indices are positively correlated

with N_2O emissions (Zebarth *et al.*, 2012; Maharjan & Venterea, 2013). Laboratory

studies have observed that total denitrification increases with increasing soil C,

particularly dissolved organic C (DOC), but that N_2O was relatively less abundant at

higher soil C rates (Burford & Bremner, 1975; Weier *et al.*, 1993). A laboratory study

found that treatments that increased soil DOC concentrations also increased rates of total

denitrification (Senbayram *et al.*, 2012). However, NO_3^-, NO_2^-, and DOC concentrations

can change rapidly within the soil due to microbial activity and plant uptake, and NO_2^-

can be especially difficult to measure due to low concentrations under typical field

conditions (Binnerup & Sørensen, 1992). Therefore, timing of soil sampling potentially

presents similar challenges as those discussed above for N_2O.

The goal of this study was to develop an event-based method of sampling N_2O

emissions that allows the creation of a predictive temporal interpolation model. This

method was applied within a no-till corn system that was expected to generate a wide

range of flux values based on a range of fertility application rates, fertility sources, and

surface residues. We hypothesized that 1) N_2O fluxes would exhibit exponential decay

dynamics in the days following an inducing event (in particular, rainfall or irrigation);

2) N_2O emissions during the days following an inducing event could be predicted from a

single flux measurement; and 3) cumulative short-term emissions could be predicted by

measures of chemical substrates for denitrification: labile C and inorganic N in the soil

either before or after an inducing event.

Methods

We quantified the N_2O emissions induced by single wetting events in a field

experiment conducted in 2013 and 2014 at the Beltsville Agricultural Research Center

(BARC) in Maryland, USA. This experiment was designed to evaluate cover crop

mixtures and poultry litter application rate and placement method within a corn-soybean-

wheat rotation on the South Farm at the BARC (39.018 N, 76.943 W) (Poffenbarger *et

al.*, 2015). The experiment was conducted on an approximately 2.5 ha field; the following

year it was repeated in an adjacent field at the same study site. The soils at the site were

mapped as fine-loamy, mixed, mesic Typic Dystrudepts (Codorus series) (NRCS, 2013),

which are moderately well drained, with a silt loam surface texture. Average bulk density

in the upper 30 cm of the soil was 1.2 g cm^{-3}, and average soil organic matter was 1.3%.

The 20 year precipitation average at this site was 830 mm annually, with highest rainfall

events occurring during June and July (USDA-Agricultural Research Service, 2015). Due

to the site's history of manure use, soil P was high, 67 ppm by Mehlich-3; additional P

fertilizer was not applied. Potassium was applied prior to experiment initiation at 67 kg-K

ha^{-1} as K_2SO_4.

The experimental design was a full-factorial strip-plot with three replicates. The main effects were cover crop species and fertility management. Cover crop treatments were applied in the fall prior to each experiment year, and the fertility treatments were applied at corn planting and sidedress during the spring and summer of the experiment year. Each experimental unit was 6 m $\times$ 9 m.

The cover crop treatment levels consist of cereal rye (*Secale cereale* L., "Aroostook") or hairy vetch (*Vicia villosa* Roth., "Groff") planted in monoculture, 168 kg seed ha^{-1} or 34 kg seed ha^{-1}, respectively, or in a mixture, 100 kg seed ha^{-1} cereal rye and 14 kg seed ha^{-1} hairy vetch. Additionally there was a bare-ground control treatment with no cover crop that was maintained plant-free using glyphosate at 1.6 kg ae ha^{-1}, applied in fall after cover crop planting, and in spring after the germination of cool-season annual weeds.

Table 2.1. Fertility treatments used in this study including application method, source (pelletized poultry litter or urea-ammonium nitrate), timing, and rate of plant available nitrogen (PAN).

Treatment	Source	Planting	Sidedress
		— kg N PAN ha^{-1} —	
Subsurface-banded low	PL	9	67
Subsurface-banded moderate	PL	9	135
Subsurface-banded high	PL	9	267
Subsurface-banded zero control[1]	PL	9	0
Mineral fertilizer, surface broadcast	UAN	30	120
Tilled, surface broadcast	PL	67	0

[1]Subsurface-banded zero control received operations by the banding equipment at sidedress, but no poultry litter was applied.

To capture a wide range of N_2O flux magnitudes, we sampled six fertility treatments across each cover crop treatment (Table 2.1). Treatments were applied at corn

planting in late May and sidedressed in late June when corn was at growth stages V5-V6. The fertility treatment levels include three rates of subsurface-banded pelletized poultry litter (PL), a PL broadcast tillage treatment, and both a no-fertilizer and mineral fertilizer controls. All fertilizer factor levels, except for the mineral fertilizer treatment, received a starter fertilizer application of PL at corn planting. The mineral fertilizer was urea-ammonium nitrate solution (UAN) surface-applied on alternating corn rows, split between planting (Turbo TeeJet 0.04 nozzles) and sidedress (Quick TeeJet drop nozzles, Wheaton, IL). The tilled-broadcast treatment represents typical management of PL in tillage-based crop production. Poultry litter was analyzed for total N and ammonium (NH_4^+- N) content to calculate gross application rates (Preusch *et al.*, 2002).

Gas sampling was conducted during four periods of the corn growing season based on phenology: following planting (emergence, VE), following sidedress (V6-V7), at early grain fill (R2-R3), and at crop maturity (blacklayer, R6) (Table 2.2). These periods were chosen to represent a range of expected soil mineral N concentrations based on the balance between N mineralization rates from fertilizer and PL and corn and microbial N uptake (Hanway, 1962; Sims, 1987; Benjamin *et al.*, 1997). Each sampling period was initiated when rainfall in excess of 12 mm occurred; however, precipitation during the growing season was highly variable. If rain did not achieve this threshold during the targeted growth stage, 20-40 mm of irrigation water were applied prior to the sampling period to ensure sufficient soil wetting to induce substantial N_2O fluxes (Table 2.2).

Table 2.2. Dates of initiation for time series of gas and soil sampling in 2013 and 2014, including source of soil moisture.

Year	Corn growth stage	Date of initiation	Source of moisture
2013	VE	June 3	Rain
	V6	June 30	Irrigation
	R2	July 29	Irrigation
	R6	August 30	Rain
2014	VE	June 12	Rain
	V7	July 14	Rain
	R3	August 12	Irrigation
	R6	September 26	Rain

After each of these precipitation or irrigation events, gases were sampled from all treatment levels daily for 7 d. Throughout, we refer to each 7 d period as a time series. To measure N_2O fluxes, a non-flow-through, non-steady-state chamber system was installed, using established guidelines for optimum design, with one chamber per plot (Parkin & Venterea, 2010; Rochette, 2011). The chambers consist of semi-permanently installed aluminum frames and a removable stainless steel lid. The open rectangular frames (72 cm by 41 cm) were pressed into the soil surface using a tamper, penetrating approximately 4 cm deep. In plots with cover crop mulch, surface residues were first cut with handheld electric shears in a 72 cm by 41 cm rectangle to allow the frame to penetrate the soil. During a sampling event, a channel gutter on the outer edge of each frame is filled with water and the lid is placed into the channel, sealing a volume of gas under the lid. The lid was fitted with a sampling port and an exhaust port. The sampling port was sealed with a rubber septum. The exhaust port was fitted with approximately 40 cm of 1 mm inner diameter copper tubing. The exhaust port permits pressure equalization with minimal diffusion of exterior atmosphere into the static volume.

Flux of N_2O has been shown to reach its daily mean between 8AM and 12PM, as fluxes vary diurnally with soil and air temperature, both of which reach their daily means during this period (Alves *et al.*, 2012). Gas sampling events were scheduled to occur during this time window. During a gas sampling event, each replicate was sampled simultaneously by one of three individuals. Frames were sampled in a pattern such that all six fertility treatments within each cover crop treatment could be sampled simultaneously. After placing the lid onto the frame, a 10 mL sample of gas was drawn from the chamber through the sampling port with a plastic syringe and a 22-gauge needle at 0, 7, 14, and 21 min. The diffusion of N_2O from the soil was assumed to be constant over this 21 min period, so the change in concentration within the chamber is calculated linearly; this assumption was supported by our samples in this study (Davidson *et al.*, 2002).

Gas samples were injected into 12 mL glass vials (Labco, Lampeter, UK) fitted with rubber septa that had been previously flushed with N_2 gas. Vials were stored at ambient temperature in the lab and analyzed within 6 weeks after collection (an experiment showed that these vials could be stored at least 10 weeks without detectable diffusion through the rubber septa; Davis et al. unpublished data). Samples were analyzed on a Varian GC450 gas chromatograph (Santa Clara, CA) equipped with an electron capture detector (ECD). All samples from a sampling event were analyzed in a single run on the GC. Standard curves were constructed in duplicate with standard gases from a tank (Airgas Specialty Gases, 2.30 ppm (Riverton, NJ) or 48.3 ppm (Chicago, IL) N_2O, balance N_2) to generate 15 known concentrations.

At each wetting event, soil samples were taken concurrently with gas samples 12 days prior to and 1, 2, and 3 d after the wetting event. Samples were taken in each plot within 2 m of the frame and in the same corn inter-row, using a 2.5-cm diameter stainless steel probe. Samples were taken to a depth of 15 cm, which approximates the application depth of the PL in subsurface-banded treatments. Four cores per plot were combined in the field to capture spatial variability of parameters. The cores were taken uniformly across the inter-row, including the corn row and the band of PL or UAN. Samples were transported in a dark cooler on ice and stored at 4°C until processing the same day. Soils were passed through a 6.3 mm sieve prior to air-drying. A 10 g subsample of field moist soil was weighed, oven dried at 105°C for 48 h, and re-weighed to calculate gravimetric water content.

Air-dried soils were ground to pass a 2-mm sieve; one 3 g subsample was extracted with 30 mL 1 M KCl and one 3 g subsample was extracted with 30 mL distilled de-ionized water (60 min on a reciprocating shaker at 200 rpm for both procedures). Extracts were filtered using vacuum manifolds and 0.45 µm polyethersulfone filters (Pall Corporation, Port Washington, NY). Filtrate of the KCl extraction was analyzed for inorganic N (IN, NO_3^--N and NH_4^+-N) using a SEAL Auto-Analyzer 3 (Mequon, WI), and filtrate of the water extraction was analyzed for DOC using a Tekmar Phoenix TOC Instrument (Jones & Willett, 2006). The ratio of DOC:IN was calculated for each sample. All calculations were based on the oven-dry equivalent mass of soil used in each analytic procedure.

The exponential decay hypothesis was tested by fitting a least-squares regression to each treatment level at each wetting event, pooling the three field replicates. The

cumulative emissions for each 7 d sampling event were calculated for each chamber separately using trapezoidal integration of the daily fluxes (Flessa *et al.*, 1995). Seven-day cumulative emissions were fit using generalized linear mixed models (GLMM). Three models were fit using predictors of 1) applied treatments, corn growth stage, and site-year; 2) Day 1 fluxes and site-year, and 3) Day 2 fluxes and site-year. GLMMs were used due to the non-normality of the residuals; the appropriate error family was chosen by maximizing the likelihood function of the Box-Cox power transformation, using the *MASS* package of R (Venables & Ripley, 2002; Dag *et al.*, 2013; Stroup, 2015). Variance decomposition of these models was used to identify the most appropriate predictive variables, using the *lme4* package of R (Grömping, 2007; Bates *et al.*, 2015). Emissions outcomes were linked to soil DOC and IN using ordinary least-squares regression on data transformed using the Box-Cox procedure. In all models, the Box-Cox procedure indicated that maximum likelihoods were located at a power parameter of 0, representing natural-log transformations of the response variable and of continuous predictors.

Results

Emissions from 7 d observations

Daily N_2O fluxes were highly variable at each growth stage, ranging from -9.33 to 2940 g N_2O-N ha^{-1} d^{-1}. Likewise, the cumulative pulse of emissions following a wetting event was highly variable, ranging from -7.40 to 9080 g N_2O-N ha^{-1} for 7 d periods.

The pattern of most observed fluxes after wetting events was not well-fit by theorized exponential decay models. Some sampling events (18%; n=157) showed decreasing N_2O flux with time (Fig. 2.1a), as expected, but over 75% showed no significant change in N_2O flux with time (Fig. 2.1b), and 6% exhibited increasing fluxes

over the 7 d period. These patterns were not consistent among replicates of the same treatment. The goodness-of-fit of these regressions ranged from $R^2 < 0.001$ to 0.786, with a median of $R^2 = 0.074$.
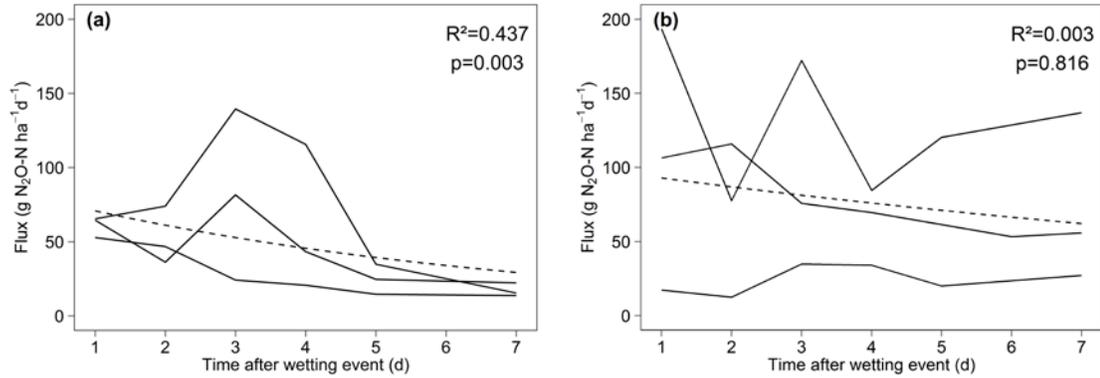
Figure 2.1. Representative N_2O flux time series following a rain event for two treatment combinations: (a) tillage-incorporated poultry litter, 67 kg PAN ha^{-1}, at corn growth stage VE in 2014, following bare ground; (b) subsurface-banded poultry litter, 144 kg PAN ha^{-1}, at corn growth stage R3 in 2014, following hairy vetch. In a given panel, the three solid lines represent three replicates of a treatment combination, and the dashed line is an exponential regression for the three replicates. In (a) fluxes decreased over time, $y = 91.2 \cdot e^{-0.230 \cdot x}$; in (b) fluxes did not significantly change with time, $y = 65.2 \cdot e^{-0.023 \cdot x}$.

Since the exponential decay hypothesis was not supported by these results, we used an alternate method to describe the observed dynamics. First, the magnitude of the 7 d pulse of N_2O associated with a given wetting event was calculated for each plot. This cumulative pulse was then predicted by several possible models, using either known treatment factors or observed covariates. All analyses that follow were conducted on log-transformed data, and estimates are presented back-transformed. There was a single time series (one replicate of 2013, V6, cereal rye, subsurface-banded zero control) for which the cumulative 7 d emission was negative; it was discarded during log-transformation.

We first fit a model to quantify the within-condition variability in our 7 d emissions estimates, using the following factors: cover crop, fertility, corn growth stage, and their interactions, and the year of the experiment, along with a replicate error term. This model explained 81.9% of the variance in cumulative 7 d emissions (Table 2.3; AIC: 1187.6, BIC: 1748.3). The strongest predictor of emissions was the fertility treatment applied, accounting for 34.6% of the variance, with growth stage accounting for an additional 22.2%. This model was a strong predictor of N_2O emissions overall, but cannot be used to estimate emissions from unsampled events because time and seasonality were represented through the growth stage term, which is a categorical rather than a continuous predictor.

Table 2.3. Variance components (V.C.) of a generalized linear mixed model (GLMM) predicting cumulative 7 day N_2O emissions based on 3 models: Within-Condition Variability (WCV) (factors are cover crop, fertility, corn growth stage, and their interactions, and year); Day 1 (observed fluxes one day after a wetting event); and Day 2 (observed fluxes two days after a wetting event).

Model	Term	V.C.
WCV	Cover Crop	0.0407
	Fertility	0.3460
	Stage	0.2222
	Cover×Fertility	0.0074
	Cover×Stage	0.0482
	Fertility×Stage	0.1109
	Cover×Fertility×Stage	0.0116
	Year	0.0189
	Replicate Error	0.0131
	Residual Error	0.1810
Day 1	Day 1 Flux	0.7213
	Year	0.0056
	Replicate Error	0.0122
	Residual Error	0.2608
Day 2	Day 2 Flux	0.884
	Year	<0.001
	Replicate Error	<0.001
	Residual Error	0.116

Next we fit a model that used the observed flux on the first day (Day 1) following a wetting event as the predictor for the 7 d cumulative emissions, along with experiment year and a replicate error term. While this model explained less (73.9%) of the variance in cumulative 7 d emissions than the within-condition variability model, it was more parsimonious (Table 2.3; AIC: 1091.2, BIC: 1111.9). This model was consistent across space, with only 1.22% of variability being due to between-replicate errors, and also

consistent across years, with only 0.56% of variability being due to differences between years. This approach does not address negative or zero fluxes on Day 1; three data points were discarded during log-transformation.

Our third model used observed fluxes on the second day (Day 2) following the wetting event as the predictor for the 7 d cumulative flux, along with experiment year and a replicate error term. This model was both parsimonious and well-fit, with 88.4% of variance explained (Table 2.3; AIC: 835.9, BIC: 856.6). This model was more consistent across space and years than the Day 1 model, with <0.001% of variability being due to between-replicate errors and the Year term. While this approach also does not address non-positive fluxes, none were observed on Day 2.

There was a good fit between single-day observed fluxes with cumulative emissions for the majority of the range of values; however, for both the Day 1 and Day 2 models, fluxes less than 1 g N_2O-N ha^{-1} d^{-1} were not accurate predictors (Fig. 2.2a,b). The Day 2 Flux model was superior to the others based on the selection criteria of minimizing residual error, AIC, and BIC.
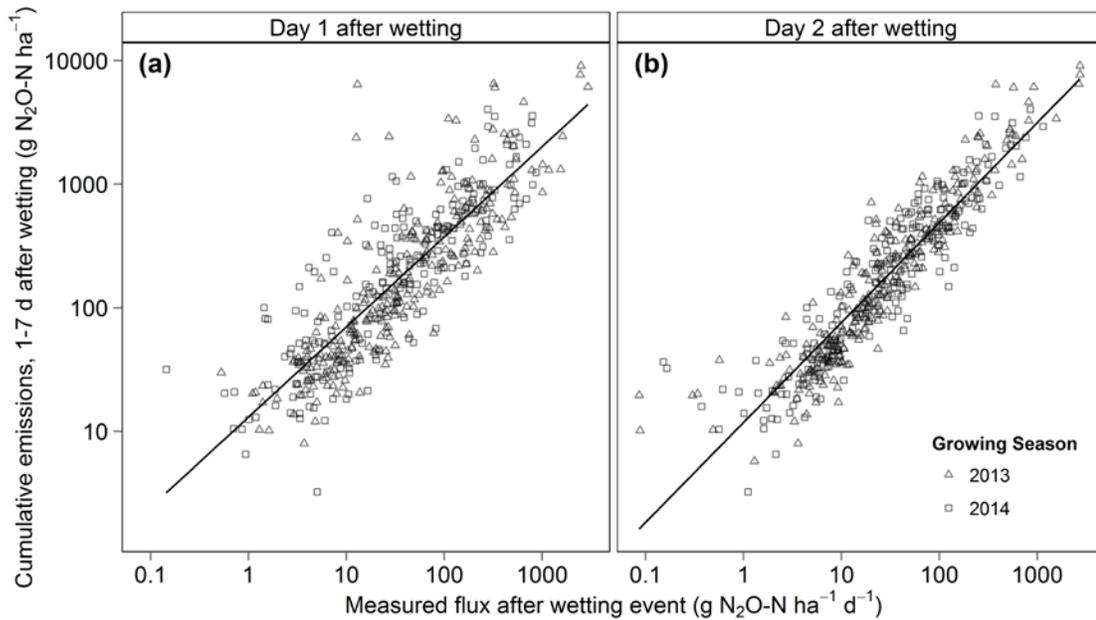
Figure 2.2. Cumulative 7 d N_2O emissions with respect to the observed flux on either Day 1 (a) or Day 2 (b) from individual plots (n=467; 470). Both x and y axes are log-scaled. The superimposed lines represent the fit of the generalized linear mixed models (GLMMs) discussed in Table 2.3; Day 1: ln y = 0.730·ln x + 2.574, Day 2: ln y = 0.809·ln x + 2.47.

The flux on Day 2 is necessarily a numeric component of the area-under-the-curve estimates, and so it is expected that there will be autocorrelation. However, the model coefficient estimates were not significantly different ($F_{1,410}$=0.015, p=0.90) when emissions were calculated omitting Day 2 data from the 7 d estimate.

Soil chemical covariates

Contrary to our hypothesis, soil nutrient status was not a robust predictor of N_2O emissions. DOC concentration ranged from 100.2 to 1325 mg DOC-C kg^{-1} dry soil; soil

DOC was a poor predictor of emissions, with $R^2 < 0.11$ for each sampling time (data not shown). There was a weak negative correlation between DOC:IN ratio prior to a wetting event and 7-d N_2O emissions (Figure 2.3). The DOC:IN ratios at 1 d, 2 d, and 3 d after the wetting event exhibited similar weak negative correlations ($R^2 = 0.23$, 0.25, and 0.29 respectively).
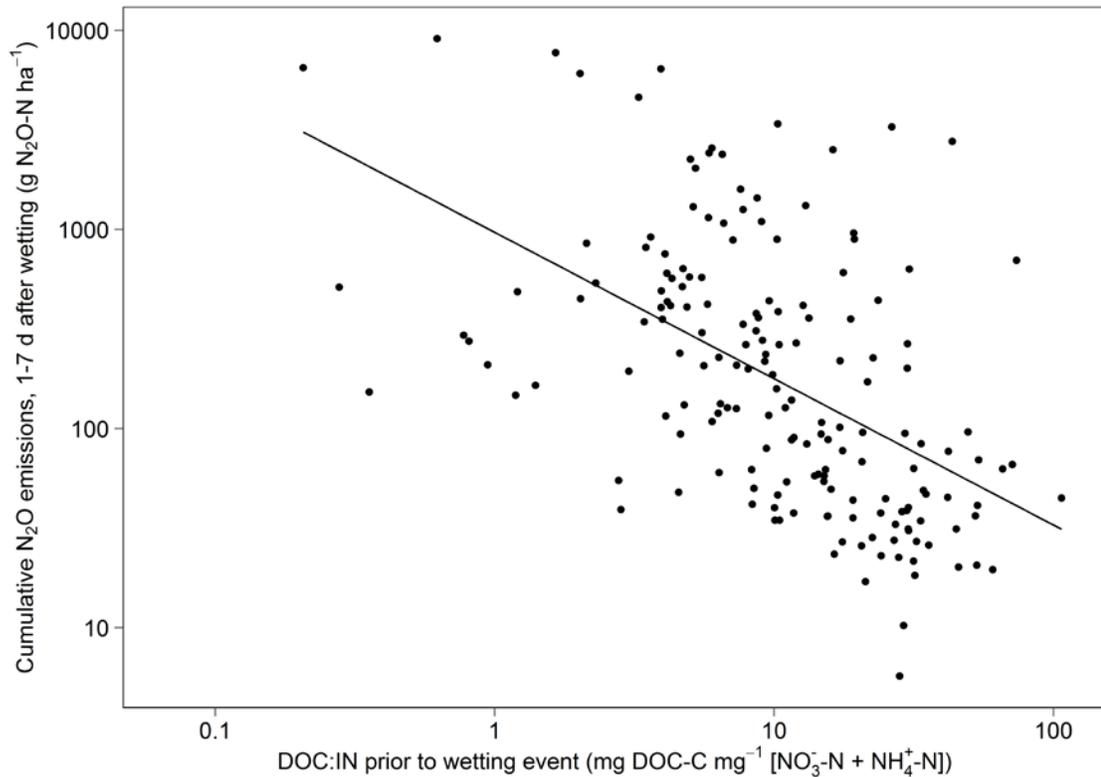
Figure 2.3. Cumulative 7 d N_2O emissions with respect to the ratio of dissolved organic carbon (DOC) in a water extract to total inorganic nitrogen (IN) in a 1 M KCl extract from a soil sample taken in dry conditions prior to the wetting event. Both x and y axes are log-scaled. The superimposed line is an ordinary least-squares regression on the transformed data, $ln\ y = -0.74 \cdot ln\ x + 6.88$, $R^2 = 0.28$.

Soil NO_3^--N concentration was weakly positively correlated with cumulative emissions when measured in moist soil 2 d after a wetting event (Fig. 2.4). When measured prior to the wetting event, 1 d after, or 3 d after, similar weak positive relationships were seen (R^2=0.28, 0.23, and 0.28 respectively).
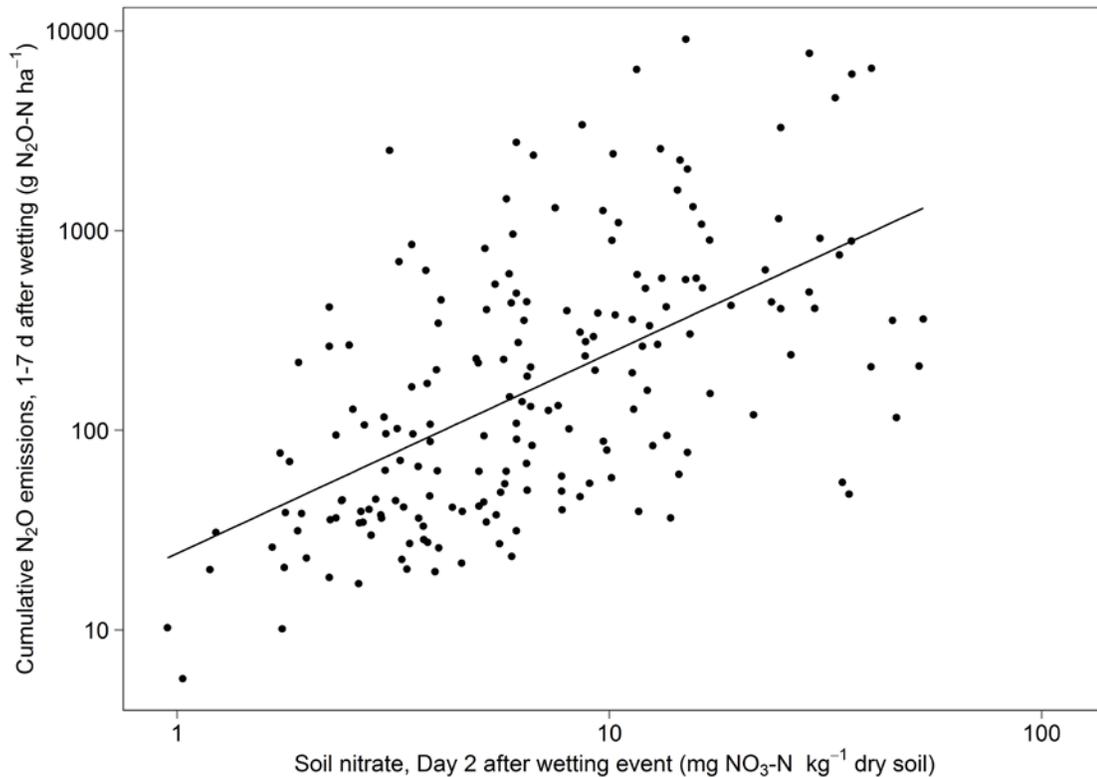
Figure 2.4. Cumulative 7 d N_2O emissions with respect to the soil concentration of nitrate-N from a 1 M KCl extract from a soil sample taken 2 d following the wetting event. Both x and y axes are log-scaled. The superimposed line is an ordinary least-squares regression on the transformed data, ln y = $1.00{\cdot}ln$ x+3.19, R^2=0.33.

Discussion

Exponential decay models are supported by microbiological theory, but were not consistent with the patterns of our flux data following wetting events. Exponential

regressions on N_2O flux over the course of the 7 d time series resulted in over 75% non-significant coefficients; the variability within each replicate among days and among replicates was too high for such models to be useful. Therefore we used trapezoidal integration to calculate cumulative 7 d emissions.

We found a strong relationship between short-term N_2O emissions (7 days) and single time-point flux measurements across a wide range of emissions magnitudes using data from an experiment that included a range of fertility management practices, cover crop residues, and corn growth stages. Our model with Day 2 N_2O flux covariate explained 88.4% of the variability in the emissions data. The Day 1 flux was also predictive of 7 d emissions, but was less well-fit, explaining only 73.9% of the variability in the data.

Our single time-point models could be used to estimate annual emissions by collecting a single flux measurement for each unmodeled wetting event (rainfall or irrigation ≥5 mm) throughout the year. To establish our predictive model, we used a total of 28 flux measurements each year per treatment level, in 4 time series of 7 days each. Estimation of annual emissions would then require only one additional flux measurement for each unmodeled wetting event throughout the year. In 2013 and 2014 at our site, the numbers of wetting events were 20 and 28 respectively; thus, we collected samples on 48 or 56 dates in 2013 and 2014, respectively. This is substantially fewer than the ≥110 flux measurements used by Cui, *et al*. (2012) who were also using a sampling scheme designed to improve temporal variability estimates.

We did not validate our Day 2 model and therefore we cannot evaluate if our emission estimates are generally robust for other 7-day emission periods. The year term

28

in the Day 2 model was not significant, explaining less than 0.001% of the variance. This

suggests that the method is robust across years and that a single year of data should be

adequate for application of this emission estimate protocol.

The goodness-of-fit of our Day 2 model was relatively poor at fluxes below

1 g N_2O-N ha^{-1} d^{-1}; however, this limitation has little impact given that total annual N_2O

emissions from cropping systems in the central and eastern US are in the range of ~400 to

19,300 g N_2O-N ha^{-1} y^{-1} (Cavigelli & Parkin, 2012).

We observed weak or no correlations between N_2O emissions and soil chemical

analyses. Our results differ from those of a recent study that showed strong correlation

between soil DOC and N_2O emissions (Senbayram *et al.*, 2012). These researchers

observed, over 5 d in a pot study, that N_2O emissions were greatest, 18.9 and

22.5 mg N_2O-N kg^{-1} soil, in treatments with initial DOC of ~500 mg DOC-C kg^{-1} soil.

The lowest emissions, 14.3 and 17.9 N_2O-N kg^{-1} soil, were from treatments with extreme

DOC concentrations, ~50 and ~1100 mg DOC-C kg^{-1} soil (Senbayram *et al.*, 2012). We

found a similar range of DOC concentrations (100.2 to 1325 mg DOC-C kg^{-1} dry soil) in

our experiment. A possible explanation for a lack of relationship between DOC and N_2O

emissions in our study is that a substantial carbon source in our experiment was poultry

litter, which also provided a range of N inputs, which could have confounded DOC

results, while Senbayram et al. held N inputs constant in their study.

The weakly positive relationship we observed between soil NO_3^--N and N_2O

fluxes (Fig. 2.4) was similar to those previously seen between soil total inorganic N and

N_2O fluxes, with correlation coefficients of approximately 0.25-0.35 (Cui *et al.*, 2012). A

shortcoming of our methodology was the use of air-dried soil samples for chemical

analysis, an approach selected due practical limitations on processing and analyzing field-moist soils. Other researchers have indicated that moist samples must be used in order to be able to measure NO_2^-, an intermediate in the production of N_2O that rapidly oxidizes upon drying (Burns *et al.*, 1995; Van Cleemput & Samater, 1995; Venterea, 2007).

Conclusions

We found that short-term N_2O emissions following a rain or irrigation event may be estimated from single point flux measurements. The implementation of this protocol would require collection of flux time series data spaced throughout the season at a given site and modeling the relationship between single day flux measurements and cumulative short-term emissions. In addition, studies should include a wide range of fertilizer amendment rates to ensure model fit over a wide range of N_2O fluxes. More work is needed to determine the optimal number and temporal spacing of time series to derive accurate emission estimates.

The relationship we observed between the Day 2 flux and the induced pulse of emissions was consistent between field replicates and years on a single soil type. The relationship was also consistent across a wide range of residue and fertility management methods within a corn growing season across two years. One limitation to this method is that it is dependent on having a large range of N_2O fluxes as a response surface in order for the dataset to be robustly described by a GLMM —fertilizer inputs in our study induced N_2O fluxes across four orders of magnitude. Our design pooled our imposed treatments to generate this response surface, and thus we were unable to separate the effects of fertility rate, fertility source, or cover crop species. While we had good model

fit with these factors pooled, more work is needed to improve this protocol to test its effectiveness across sites, under other rates and sources of fertility, and following different cover crops. Also, we only sampled following wetting events; other events that induce high N_2O emissions, such as freeze-thaw and fertilizer application, may not have the same relationship with single-day fluxes. This protocol has the potential to estimate N_2O emissions following individual soil wetting events by sampling on a single day, which may allow for increased accuracy in the estimation of annual emissions.

Chapter 3 – Nitrous oxide emissions increase exponentially with N rate in cover-crop based corn production

Abstract

Subsurface banding of manure can reduce losses of nitrogen through volatilization in the form of NH_3, but the effect on soil N_2O emissions has not been established. Cover crops are also promoted for their nitrogen management, but their impact on N_2O emissions has been inconsistent and not well-evaluated across a range of fertility management regimes. To assess the interactive effects of subsurface banded poultry litter (PL) and cover crops, we measured annual N_2O emissions for three years in a field trial of corn (*Zea mays* L.) in Beltsville, MD, following either winter fallow or mulched cover crops (cereal rye [*Secale cereale* L.], hairy vetch [*Vicia villosa* Roth.], and a biculture mixture of both species), with four rates of subsurface banded PL (9-276 kg PAN ha^{-1}) and selected contrasts with urea-ammonium nitrate (UAN, 150 kg N ha^{-1}) or incorporated PL (67 kg PAN ha^{-1}). N_2O emissions increased exponentially with total N input (subsurface banded PL + cover crop residue). This relationship differed by cover crop treatment: the model intercept ranged from 0.336-1.389 kg N_2O-N ha^{-1} (cereal rye < bare ground = mixture < hairy vetch; p=0.01, 0.29, 0.03), and the exponential coefficient ranged from 0.00293-0.00586 kg N_2O-N kg^{-1} N (hairy vetch < cereal rye = bare ground = mixture; p=0.02, p>0.10). Subsurface banding of PL increased N_2O emissions relative to tillage-incorporation at equivalent rates following hairy vetch or cover crop mixture (by 76% or 60%; p<0.001), but we did not detect an effect following cereal rye or bare ground. Subsurface banded PL decreased emissions relative to UAN at equivalent rates following the cover crop mixture, but increased emissions following bare ground (by 34% or 45%;

p=0.002). Our results suggest that reducing agricultural N_2O emissions can be achieved by reducing total N rates, including both applied N and cover crop N. In particular, reducing manure or fertilizer N following a cereal rye:hairy vetch mixture cover crop did not increase N_2O emissions, while a hairy vetch monoculture did increase N_2O emissions. However, further work is needed to evaluate application methods as an N_2O mitigation strategy.

Introduction

Managing agricultural N inputs to minimize reactive N and maximize crop uptake may serve to mitigate soil N_2O emissions, a major greenhouse gas equivalent to 298 times the global warming potential of carbon dioxide (CO_2) on a mass basis over a 100 year timescale (IPCC, 2014). While there are many management strategies employed to increase nitrogen use efficiency (NUE) by crops, past N_2O research has focused primarily on reducing N fertilization rates, as this is the primary source of soil mineral N in agricultural systems (Velthof *et al.*, 1996). However, the amount of fertilizer used represents a trade-off between grain yield potential and environmental impacts (Roberts, 2007). To minimize impact on crop yield, more attention should be placed on use efficiency and reducing the period of time with abundant reactive nitrogen.

Policymaking guidelines for N_2O emissions accounting assume a linear relationship with increasing N fertilizer application rate (Gregorich *et al.*, 2005; IPCC, 2007). However, field experiments in corn have shown an exponential increase in N_2O emissions across a gradient of N application rates (McSwiney & Robertson, 2005; Hoben *et al.*, 2011). Meta-analyses have supported this exponential relationship, and linearly scaled estimates overestimate N_2O emissions compared to empirical observations in most

of the world at low to moderate N rates (Velthof *et al.*, 1996; Van Groenigen *et al.*, 2010; Shcherbak *et al.*, 2014). Most work in this area has focused on mineral fertilizers, although the exponential response has also been observed using liquid swine manure (Jarecki *et al.*, 2009).

While fertilizer application rate is the major driver of soil reactive N, fertilizer source may also affect N_2O emissions due to differences in N mineralization rates, which influence soil inorganic N levels throughout the year and subsequent denitrification potential. Among common mineral fertilizers such as anhydrous ammonia, urea-ammonium nitrate (UAN), calcium ammonium nitrate (CAN), and potassium nitrate, some researchers have found no evidence of product type influencing rates of N_2O emissions, while others indicate that urea may reduce emissions compared to CAN (Stehfest & Bouwman, 2006; Harty *et al.*, 2015). Slow-release mineral fertilizers improve N release synchrony with crop uptake and have been shown to reduce N_2O emissions; but the cost of these products is higher than conventional mineral fertilizers (Halvorson *et al.*, 2008; Hyatt *et al.*, 2010; Venterea *et al.*, 2011; Bernardi *et al.*, 2014; Pereira *et al.*, 2015). An alternative to slow-release mineral fertilizers is the use of animal byproducts, which also have temporally extended N mineralization (Gioacchini *et al.*, 2006).

Manure is a significant component of U.S. corn production fertilizer use (12%) and is often considered an integral component of sustainable agricultural systems (MacDonald *et al.*, 2009; Powlson *et al.*, 2011; Chadwick *et al.*, 2015). Since manures supply C as well as N, it is expected that denitrification rates will be higher relative to mineral fertilizers. However, increased use of manure and other C-rich amendments, (e.g., biochar or compost), will also increase the $C:NO_3^-$ ratio, which decreases the

proportion of N_2O production relative to N_2 during denitrification (Kramer *et al.*, 2006; Taghizadeh-Toosi *et al.*, 2011). When comparing animal and green manures with mineral fertilizers, it is necessary to contrast these fertility sources on a plant-available N basis. Unlike with mineral fertilizers, plant-available N estimates for manures in the first year are difficult to determine because mineralization rate depends on factors including weather and soil type (Motavalli *et al.*, 1989; Muñoz *et al.*, 2004). In addition, manures and mineral fertilizers are sometimes applied with differing placement and timing in relation to the cash crop growth stage (Schröder, 2005). Over longer time scales, large annual applications of manure can create residual soil C and N pools, which may contribute to high N_2O emissions (Sommerfeldt *et al.*, 1988; Cavigelli & Parkin, 2012).

In addition to N application rate and source, depth of N placement in the soil can affect N_2O losses. Oxygen concentration decreases with depth in the soil profile, potentially increasing N_2O production, but deeper N placement may result in C or temperature limitations to N_2O production (Langeveld & Leffelaar, 2002). When comparing surface-broadcast and tillage-incorporated mineral fertilizer, emissions outcomes have been mixed. In a one-year study tillage decreased N_2O emissions at two sites, while in a separate three-year study tillage either increased, decreased, or had no effect on emissions, respectively, in each year (MacKenzie *et al.*, 1997; Gregorich *et al.*, 2008). Alternative placement methods include subsurface banding or knifing-in of urea or liquid UAN. Spatially concentrating N in a narrow band with these methods could have the potential to create zone of high denitrification, but N_2O emissions have not been found to be affected when compared with conventional tillage or surface broadcasting (Halvorson *et al.*, 2008; Nash, 2010; Smith *et al.*, 2011). Application methods that further

increase the heterogeneous distribution of N in the soil may mask the detection of treatment effects by increasing the variance in observed N_2O emissions.

In the case of animal manures, the common methods of application have been surface broadcasting in no-till or reduced-tillage systems and incorporation in conventionally tilled systems. However, concerns over ammonia volatilization, P losses in runoff, and erosion are leading to regulatory restrictions on surface broadcasting (Thompson and Meisinger, 2002; Eghbal and Gilley, 1999; Maryland Nutrient Management Manual, 2012). While incorporation of manure through tillage can address these concerns, tillage increases N mineralization rates through increased soil-manure contact, which in turn can increase N_2O emissions relative to surface application or injection (Webb *et al.*, 2010). Liquid slurry injection has been the more common practice for subsurface placement of manure N, but agricultural engineers are working to develop tools for subsurface banding of dry manure solids (Pote *et al.*, 2009; Tewolde *et al.*, 2009). While injecting or banding animal manures results in minimal disturbance to the soil surface and residues, higher moisture below the surface relative to surface broadcasting may lead to greater mineralization and subsequent nitrification, which has the potential to increase N_2O emissions (Rice & Smith, 1982). Evaluation of these systems with respect to N_2O emissions has produced mixed results. In one study, subsurface banded poultry litter (PL) acted as a net sink of N_2O, rather than a source (Nyakatawa *et al.*, 2011). Another study showed that injected liquid swine manure resulted in N_2O emissions that were higher than broadcast application in one year and lower the next (Sistani *et al.*, 2010). More research is needed to test how subsurface

banding dry manure solids influences N_2O emissions relative to surface application and tillage incorporation of dry manure solids, and application of mineral fertilizer sources.

Compared with mineral fertilizers and animal manures, cover crops have received less evaluation regarding their impact on N_2O emissions (Basche *et al.*, 2014). Living cover crops decrease soil moisture and available N during winter and spring, which reduces the potential for N_2O emissions (Shipley *et al.*, 1992). Cover crops contribute C to the soil profile through rhizodeposition while growing and decomposition of residues after termination. In addition, leguminous cover crops contribute biologically-fixed N (Wagner & Zapata, 1982), which can replace a portion of N otherwise applied as manure or mineral fertilizer. However, the C and N mineralization rates from these residues depend in part on how they are managed, and the temporal dynamics of available C and N from decomposing residues may have positive or negative effects on N_2O production. Incorporation through tillage typically leads to rapid decomposition, while techniques that preserve surface residues, such as reduced tillage, usually result in much slower residue decomposition (Poffenbarger *et al.*, 2012). The presence of surface residues increases moisture at the soil surface and in the crop rhizosphere, subsequently increasing N_2O emissions (Baggs *et al.*, 2003). Tillage in general also affects pore structure and connectivity, while increasing surface evaporation, both factors that decrease N_2O production (Rice & Smith, 1982). Cereal rye has been shown to reduce N_2O emissions in the northern and midwestern US (Parkin *et al.*, 2006; Jarecki *et al.*, 2009; Dietzel *et al.*, 2011). In contrast, annual ryegrass as a winter cover crop was observed to increase emissions relative to bare ground (Smith *et al.*, 2011). Leguminous cover crops have been shown to increase total denitrification and N_2O production when incorporated but have

not been tested in no-till applications (Aulakh *et al.*, 1991; Adviento-Borbe *et al.*, 2010). Additionally, grass-legume cover crop mixtures have been evaluated to maximize N fixation and biomass accumulation (Ranells & Wagger, 1996, 1997), but the impact of these bicultures on N_2O emissions has not been evaluated.

Improved NUE of a crop results from better synchrony between soil N availability and crop demand. In the case of corn, this is commonly attempted through split fertilizer applications. Best management practice for corn involves a split application at planting (starter fertilizer), and at sidedress, growth stage V5-V8 (Jayasundara *et al.*, 2007). Only recently have animal manures been evaluated for use in a sidedress application (Spargo *et al.*, 2011). Little work has been done to assess how mineral fertilizer application timing affects N_2O emissions and none in the case of animal manures.

To better understand the effects of N rate, source, and placement and their interactions with cover crops on N_2O emissions, we designed a field experiment in which corn was grown with and without surface residues under a range of fertility management. We investigated grass, legume, and grass-legume mixture cover crops; a range of suboptimal to excess fertilization rates of subsurface banded PL; and specific contrasts with tillage incorporated PL or surface applied mineral fertilizer. We hypothesized that 1) N_2O emissions will increase with N application rate non-linearly; 2) leguminous cover crops will contribute more to emissions than grass cover crops or bare ground, and this effect will be most pronounced at higher N application rates; 3) incorporation of PL will increase emissions relative to subsurface banding as tillage increases surface contact between PL and the soil; and 4) mineral fertilizer will increase emissions when applied at

an equivalent plant available nitrogen rate of PL due to the immediate availability of fertilizer N.

Methods

To determine the influence of cover crop composition and fertility management on nitrous oxide emissions a field experiment was initiated each fall in 2011, 2012, and 2013 at the USDA-ARS Beltsville Agricultural Research Center (BARC) (Beltsville, MD). The cover crop mixture/PL management trial was embedded within a conventional corn-soybean-wheat rotation on the South Farm at the BARC (39°01'04.8"N 76°56'34.8"W). The experiment was conducted in an approximately 2.5 ha field, and repeated in an adjacent fields each subsequent year. The trial was conducted within the corn phase of the rotation. Gas sampling was conducted for up to 14 months in each field, beginning prior to the break of cover crop dormancy in late winter of the experiment year and concluding with the topdress of fall-planted wheat in the spring of the following year. Treatments were applied in a strip-plot experimental design with three replicates.

The soils at the site had a silt loam surface texture and were mapped as fine-loamy, mixed, mesic Typic Dystrudepts (Codorus series) (NRCS, 2013), which are naturally moderately well-drained. Bulk density at cover crop termination was 1.2 g cm^{-3}, and soil organic matter was 1.3%. Annual precipitation averages 830 mm, with the highest rainfall in June and July.

The cover crop treatment levels consisted of cereal rye (*Secale cereal* L., 'Aroostook') and hairy vetch (*Vicia villosa* Roth., 'Groff') planted in replacement proportions of 100:0, 60:40, and 0:100, based on percentages of total recommended seeding rates (Table 3.1). Additionally, there was a bare ground control treatment

maintained weed-free using glyphosate (N-(phosphonomethyl)glycine, Durango DMA,

Dow AgroSciences, Indianapolis, IN) at 1.6 kg ae ha^{-1} as needed.

Table 3.1. Seeding rates and management of cover crop treatments at initiation of the experiment year in early October.

Treatment	Proportion	Cereal rye	Hairy vetch	Weed management
	— %:% —	—— seed kg ha^{-1} ——		
Cereal rye	100:0	168.0	0	n/a
Cereal rye:hairy vetch mixture	60:40	100.4	13.6	n/a
Hairy vetch	0:100	0`	34.0	n/a
Bare ground	n/a	0	0	1.6 kg ae ha^{-1} glyphosate

We tested six fertility factor levels across each cover crop treatment (Table 3.2).

Fertility treatments were applied in late May at corn planting and in late June at sidedress

(corn growth stage V5-V8). The fertility treatment levels included three rates of

subsurface-banded pelletized PL, a PL broadcast tillage treatment, and both a no-fertilizer

and mineral fertilizer controls (Table 2). All fertilizer factor levels, except for the mineral

fertilizer treatment, received a starter fertilizer application of pelletized PL at corn

planting. The mineral fertilizer, urea-ammonium nitrate (UAN), was applied on

alternating corn rows, split between planting and sidedress using Turbo TeeJet 0.04

nozzles (planting) and Quick TeeJet drop nozzles (sidedress) (TeeJet, Wheaton, IL). The

tilled-broadcast treatment represented typical management of PL in tillage-based crop

production. Fertilization rates were selected on the basis of either adequate N, adequate P,

or excess N and P (University of Maryland Cooperative Extension, 2009). Poultry litter

was analyzed for total N content to calculate gross application rates (Preusch *et al.*, 2002).

Table 3.2. Source, application method, timing, and rate of fertility treatment levels.

Treatment	Source	Planting	Sidedress
		— kg N PAN ha^{-1} —	
Subsurface banded low[1]	PL	9	67
Subsurface banded moderate[2,5]	PL	9	135
Subsurface banded high[5]	PL	9	267
Subsurface banded zero control[3]	PL	9	0
Mineral fertilizer, surface broadcast[2,4]	UAN	30	120
Tilled, surface broadcast[1,5]	PL	67	0

[1]These rates were based on recommendations for P fertilization.
[2]These rates were based on recommendations for N fertilization.
[3]This treatment received operations by the banding equipment at sidedress, but no PL was applied.
[4]This treatment was only applied to the cereal rye:hairy vetch mixture in 2013 and to all cover crops in 2014, but was not applied in 2012.
[5]This treatment was not applied to the bare ground cover crop in 2012, but was applied in 2013 and 2014.

The cash crop prior to the initiation of the experiment was a fall-planted wheat crop followed by a soybean cover crop. The soybean cover crop was planted to reduce spatial variability of areas of high and low soil inorganic N levels. Soybeans were plowed and disked in late August. Due to the site history of agricultural management, soil P was high at 67 ppm by Mehlich-3; additional P fertilizer was not applied. Potassium was applied prior to cover crop planting at 67.3 kg-K ha^{-1} as K_2SO_4. The seedbed was then disked again followed by a field cultivator and cultimulcher prior to seeding cover crops.

Cover crops were planted in early October using a no-till grain drill on a 19 cm row spacing in 3.05 m wide strips. Three adjacent strips were planted, for a total of 9.2 m per cover crop. To achieve precision of seeding rates, each species was drilled separately. Cover crops were planted in rows in a north-south orientation. Cover crop termination,

cash crop planting, and fertilizer and herbicide applications were conducted in May in an east-west orientation. Each fertility treatment was 6.1 m (eight corn rows) wide to accommodate destructive plant sampling throughout the growing season. Thus each experimental unit was 6.1 m by 9.2 m.

Cover crops were terminated with a roller-crimper when hairy vetch reached 50% flowering (Mischler *et al.*, 2010). Residue within a 0.5 m^2 quadrat was clipped, dried, and subsampled for analysis of C and N content by total combustion (LECO CHN Analyzer, St. Joseph, MI). Corn was no-till drilled at 76 cm row spacing through the mulch using a no-till planter. The cover crops in the tilled plots were terminated and incorporated one week earlier than termination for the no-till treatments to allow for seedbed preparation and a common planting date between all treatments. The tilled plots were mowed, disked, and broadcast with PL, which was incorporated by full-inversion tillage. In the subsurface banded plots, starter PL was applied at the same time as corn planting. In the mineral fertilizer plots, the starter application of UAN was dribbled on immediately after corn planting. As needed, glyphosate was applied to all plots for weed control, with the same rate and formulation used to maintain the bare ground treatment.

The subsurface bander was a Dawn 6000 Universal Fertilizer Applicator (Dawn Equipment, Sycamore, IL) fitted with a gravity-fed hopper (Clampco, Wadsworth, OH) for PL, with continuous-speed paddles for consistent flow rate. The applicator consisted of coulters that cut a trench in the inter-row about 10 cm deep and 3 cm wide. Each coulter was regulated by a leading wheel to control depth and was independently articulated to allow for uneven soil surfaces and the presence of coarse fragments. A metal chute fed PL to the trench, and a wheel closed the trench. The system is gravity fed

at a continuous flow, so PL application rate is dependent on tractor speed, which was calibrated and maintained the full length of a replicate block. The resulting pocket of PL was completely covered with soil and uniformly packed, approximately 3 cm by 3 cm in cross-section. The cover crop mulch was sliced through by the coulter, but otherwise undisturbed.

Sidedress fertilizer was applied in late June. The subsurface bander passed all subsurface PL treatments, including the zero-rate control. The mineral fertilizer plots received dribbled UAN in the same alternating rows as at planting. Tilled, broadcast PL plots received no operations at sidedress.

In October, corn was harvested with a combine (Almaco, Nevada, IA); grain yield was estimated at 15.5% moisture from 7.6 m each of two neighboring corn rows. Remaining corn stover and cover crop residues were chopped using a turbotill. Wheat was then no-till drilled across the entire field.

To measure N_2O fluxes, a non-flow-through, non-steady-state chamber system was installed using established guidelines for optimum design (Parkin & Venterea, 2010; Rochette, 2011). The chambers consisted of semi-permanently installed aluminum frames and a removable stainless steel lid. The open rectangular frames were 72 by 41 cm and pressed into the soil surface using a tamper, penetrating approximately 4 cm deep. Surface residue was cut with handheld electric clippers evenly with the frame border prior to installation. The frames featured a channel gutter welded to the outer edge, which was filled with water to seal a static gas volume under the lid. The lid was stainless steel with a sampling port and an exhaust port. The sampling port was sealed with a rubber septum. The exhaust port was fitted with approximately 40 cm of 1 mm inner diameter

copper tubing. The exhaust port permitted pressure equalization with minimal diffusion of exterior atmosphere into the static volume. Frames were removed and re-installed within one day of any equipment operations that disturbed the soil, including planting, sidedress, and post-harvest tillage.

Initial installation into the cover crop during late winter captured several rows of rye or vetch, and the plants continued to grow inside the frame. A single frame was installed into each cover crop treatment approximately 1 m from the edge of the plot, to minimize plant disturbance and soil compaction associated with sampling. When cover crops were too tall to be enclosed by the lid, they were compressed into the chamber for the duration of the sampling event.

After corn planting, frames were re-installed between the corn rows. Frames captured a representative area of cover crop biomass as well as the full width of the inter-row space, including the fertility treatment band. One frame was installed in each PL plot, avoiding rows with tire compaction and areas of poor crop stand. The mineral fertilizer treatment received UAN on alternate rows. The fertilized row coincided with the tire track, leaving the unfertilized row undisturbed. Equipment compaction is known to affect N_2O production, so in 2013 and 2014, a frame was placed in the fertilized row and an additional frame was placed in the adjacent row (Ball *et al.*, 1999). The fertility status of the subsurface banded plots did not differ prior to sidedress, so only one frame was installed per cover crop treatment for all the subsurface banded treatments. The tilled-broadcast plots each had a single frame.

Following sidedress operations, frames were re-installed, positioned 50-100 cm beyond the original disturbed placement site, away from the plot edge. These frames

remained in place until corn harvest. Following corn harvest and wheat planting, frames

were re-installed an additional 50-100 cm beyond the original placement, away from the

plot edge. Frames remained aligned with the corn row to capture the residual effects of

the fertility placement, and two rows of wheat were captured.

Nitrous oxide emissions are associated with periods of high soil moisture in

agricultural settings, so monitoring was stratified around rain events and irrigation (Smith

& Dobbie, 2001). Sampling was triggered by a 5 mm rainfall event, which was estimated

to be sufficient precipitation to raise volumetric water content (VWC) at 10 cm soil depth

above 20%. When soil moisture dropped below 20% VWC, N_2O production was

assumed to be small, based on six years of gas collection at a nearby agricultural site

(Cavigelli, unpublished data). Gases were also sampled following soil disturbance events

(fertilization and tillage) for two days.

Nitrous oxide production shows a pattern of diurnal variation since it is affected

by both soil and air temperatures; daily mean fluxes occur between 8AM and 12PM

(Alves *et al.*, 2012). To avoid bias, gases were sampled during this period and each

replicate was sampled by one individual, with all replicates being sampled

simultaneously. Chambers were arranged in clusters that could be accessed by walking

within a short period of time; gas production inside the frame is assumed to be constant

for intervals less than 30 minutes, so that concentrations change linearly with time

(Davidson *et al.*, 2002). Each cluster contained all the levels of the fertility treatments

across one cover crop, so that each of seven chambers could be sampled simultaneously.

The lid was placed onto the frame to seal the chamber, and samples were withdrawn

using a 10 mL syringe with a 22-gauge needle at 0, 7, 14, and 21 minutes, and then

immediately injected into storage vials. Air temperature within the frame was recorded after removing the lid, which was used to adjust the concentration of gases sampled using the ideal gas law, since pressure remains constant with the ambient atmosphere.

Gas samples were stored in N_2-flushed 12 mL glass vials (Labco, Lampeter, UK) with rubber septa. Vials were stored at ambient temperature in the lab and analyzed within six weeks after collection. A separate study showed that gas concentrations in vials stored this way did not change over 10 weeks (Davis et al., unpublished data). Samples were analyzed on a Varian GC450 gas chromatograph (Agilent Technologies, Santa Clara, CA) using an electron capture detector (ECD), with peaks calculated for N_2O and O_2. All samples from a single sampling event were analyzed in a single run on the ECD. Standard curves were constructed in duplicate with standard gases from a tank (Airgas Specialty Gases, 2.30 ppm (Riverton, NJ) or 48.3 ppm (Chicago, IL) N_2O, balance N_2) to generate 15 known concentrations. Standard curves were not constructed for O_2, but readings that were substantially (>50%) lower or higher than the mean for each run indicated either a blank sample (due to human error) or atmosphere infiltration (due to septum leakage) respectively.

Gases were sampled more frequently during the summer (assumed to be the more biologically active time periods) than fall, winter, and spring (periods that generally have low soil inorganic N or low soil temperatures). Following a rain or irrigation event, samples were taken daily for two days from May to August, and one day from September to April. During the winter, soils remained above 20% VWC winter due to lower evapotranspiration than precipitation. However, biological activity was assumed to be low during this period, so gases were only sampled monthly and following hard-freeze

disturbance events when soil temperature at 10 cm soil depth was < -0.5°C (two-day

sampling following thawing after hard-freezes).

Individual flux measurements on the second day after a wetting event were used

in a model (Table 3.3) to estimate emissions associated with the pulse of biological

activity following each wetting event for each plot, as developed in Chapter 2. These

fitted values were then summed for the entire year to estimate total annual N_2O

emissions; periods between these induced pulses were assumed to have negligible N_2O

fluxes. There was a small subset of events for which second-day fluxes were not

measured; these were omitted from cumulative totals. Emission factors were calculated

from applied N rate and total annual emissions after subtracting emissions from

unfertilized bare ground plots (IPCC, 2006).

Table 3.3. Coefficients and standard errors for the Day 2 model used to estimate the

induced pulse of emissions from the flux on the second day following a wetting event,

$ln(g\ N_2O\text{-}N\ ha^{-1}) = \beta_1 \cdot ln(g\ N_2O\text{-}N\ ha^{-1}\ d^{-1}) + \beta_0.$

	Estimate	s.e.
slope, β_1	0.809	0.0159
intercept, β_0	2.47	0.0633

To meet residual assumptions of generalized linear mixed models (GLMM), the

Box-Cox procedure in the *MASS* package of R was used to identify the power parameter

that maximized the likelihood function for annual emissions (Venables & Ripley, 2002;

Dag *et al.*, 2013). Emissions from each cover crop treatment were linked to N input rate

by a GLMM with N application rate, cover crop residue N rate, tillage, and N source as

fixed effects and field replicate crossed with fertility strip and cover crop strip repeated

by year as random effects. To evaluate these potential terms in the model, we used an information-theoretic approach (Burnham & Anderson, 2002). In addition to AIC and BIC, there is ongoing debate about appropriate measures of explained variance, or R^2 analogues, for GLMMs (Kramer, 2005; Gelman & Pardoe, 2006; Orelien & Edwards, 2008). Therefore, we used three such methods of model selection: the coefficient of determination between a model's fixed effects and observations (R^2_β), the marginal information gain of each term relative to a null model based on the likelihood ratio (R^2_{LR}), and the proportion of the model's total variance not associated with the residuals (Ω^2) (Magee, 1990; Xu, 2003; Edwards *et al.*, 2008).

Means separations of slopes and intercepts of the final model were computed using the *lsmeans* package of R (Milliken & Johnson, 2009; Lenth, 2016). Emissions from equivalent N rates but differing application method or N source were compared using contrasts; standard errors and p-values were estimated using least-squares means with Kenward-Roger approximated degrees of freedom (Gelman & Hill, 2006; Gelman *et al.*, 2012; Halekoh & Højsgaard, 2014). Throughout, $\alpha=0.05$ was used to test significance.

Results

N contribution by cover crop residue

Aboveground hairy vetch residue contained the greatest amounts of N each year, ranging from 67.8 to 256 kg N ha^{-1}, while the cereal rye:hairy vetch mixture contained a smaller but substantial amount of N, 45.1 to 190 kg N ha^{-1} (Table 3.4). The monoculture hairy vetch residues had C:N ratios that ranged from 11.4 to 22.4 and the monoculture cereal rye residues had C:N ratios that ranged from 63.0 to 137.8, while the C:N ratio of

the combined residues in the cereal rye:hairy vetch mixture ranged from 21.9 to 57.0, intermediate to the monocultures, While not fixed from the atmosphere, the cereal rye scavenged 20.6 to 106.9 kg N ha^{-1} from the soil over winter.

Cereal rye had the greatest aboveground biomass in 2012 and 2013, ranging from 2.9 to 16.7 Mg ha^{-1}. The cereal rye:hairy vetch mixture had greater mean biomass in 2014; the cereal rye:hairy vetch mixture residues in each plot ranged from 3.06 to 14.5 Mg ha^{-1} across all three years. Hairy vetch produced the least biomass, 2.4 to 6.4 Mg ha^{-1}. For all cover crops, biomass was lower in 2014 than 2012 and 2013.

Table 3.4. Mean N content, C:N ratio, and biomass contributed by three cover crop treatments in three experiment years with respective standard deviations (s.d.); cereal rye, hairy vetch, and the cereal rye:hairy vetch mixture, terminated in the spring of 2012, 2013, and 2014. The bare ground treatment is not shown here; winter annual weed biomass in all plots was less than 0.036% of total biomass.

	Year	N content		C:N ratio		Biomass	
		— kg N ha^{-1} —		— kg C kg^{-1} N —		— Mg ha^{-1} —	
		mean	s.d.	mean	s.d.	mean	s.d.
Cereal Rye	2012	55.9	24.0	91.1	24.3	10.30	3.26
	2013	61.7	14.0	78.5	9.4	10.23	1.52
	2014	30.0	4.7	65.3	17.8	4.19	0.67
Hairy Vetch	2012	162.6	30.7	17.2	2.3	5.79	0.37
	2013	141.9	36.0	16.7	2.8	5.03	0.68
	2014	102.2	20.8	16.3	2.0	3.65	0.74
Mixture	2012	136.3	27.8	29.0	6.3	8.26	1.02
	2013	104.6	31.3	42.6	8.1	9.29	2.12
	2014	87.2	25.6	26.5	3.4	4.89	1.21

Seasonal distribution of fluxes

N_2O fluxes were not uniformly distributed throughout the year (Fig. 3.1). Larger fluxes generally occurred during the biologically active summer growing season,

particularly following tillage, cover crop termination, and corn fertilization. There were also substantial fluxes (>100 g N_2O-N ha^{-1} d^{-1}) following field preparations for the subsequent wheat crop, as well as following hard soil freezing (soil temperature at 10 cm soil depth < -0.5°C) and thawing in February and March of both 2014 and 2015; no hard soil freezes were observed in 2013. For many plots, the highest flux of the year was observed immediately following either primary tillage and corn planting (2012: 32% of plots, 2013: 15%, 2014: 49%) or sidedress N application (14%, 47%, 24%)

The lowest observed fluxes were negative, where very small rates of uptake N_2O diffused back into the soil from the atmosphere, with extrema of -12.2, -9.3, and -4.4 g N_2O-N ha^{-1} d^{-1} in 2012, 2013, and 2014 respectively. In contrast, the positive fluxes were much larger, with maxima of 27,300; 2936; and 2460 g N_2O-N ha^{-1} d^{-1} in each respective year. The extreme high values in 2012 were observed on the day following sidedress fertilization; there were eight fluxes observed from the highest rate of subsurface banded PL that exceeded 4500 g N_2O-N ha^{-1} d^{-1}; these values are unusually high for the mid-Atlantic region (Djurickovic, 2010). Due to these extreme values, Figure 1a-b shows data from that year split into two scales.

Cumulative annual emissions were calculated by summing pulses predicted from a model which estimates 7-day emissions from each flux measurement on day 2 following a rain, tillage, or fertilization event, as described in Chapter 2 (Cavigelli *et al.*, 2014). The resulting N_2O emissions estimates ranged from 1.07 to 23.50 kg N_2O-N ha^{-1} y^{-1}, and were right skewed, γ_1=1.25. Annual N_2O emissions reported in the literature for the central and eastern US have a similar range, 0.48 to 18.6 kg N_2O-N ha^{-1} y^{-1} (Cavigelli & Parkin, 2012).
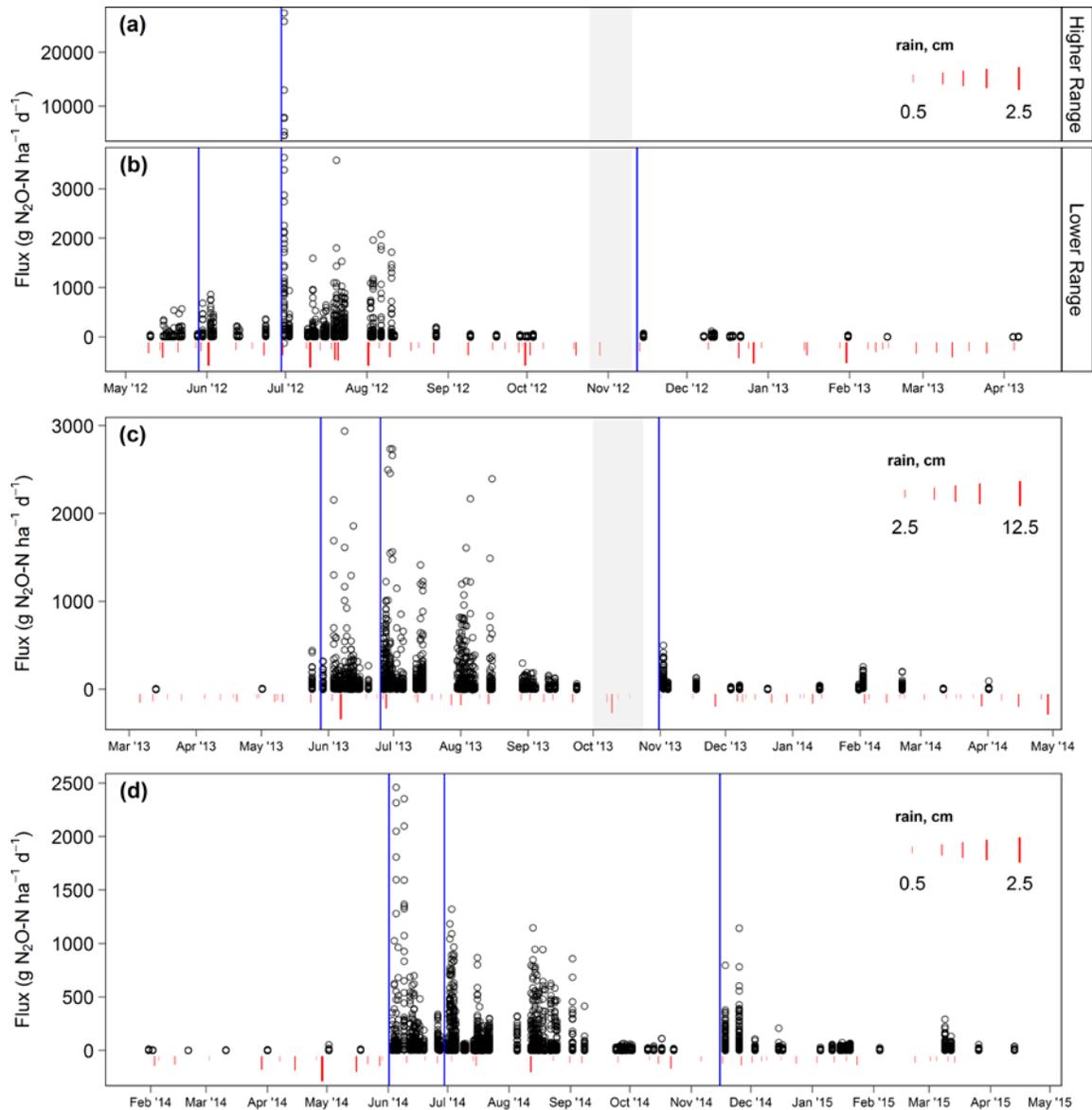
Figure 3.1. Nitrous oxide fluxes across all fertility and cover crop treatments are shown as black circles for experiment years 2012 (a,b), 2013 (c), and 2014 (d). Note that the y-axes differ for each year, and that there are two scales shown for 2012 (a,b) due to the wide range of values. The grey boxes in 2012 and 2013 indicate periods where sampling was not permitted due to Hurricane Sandy and the US federal government shutdown, respectively. The red bars below the figure indicate the size and timing of each rainfall or irrigation event. The vertical blue lines, from left to right, indicate the dates of

corn planting (and tillage for the incorporated PL treatments), sidedress N application, and turbotillage/wheat planting.

Cumulative annual emissions and N rate

Cumulative annual emissions exhibited a positive relationship with N application rate (Fig. 3.2b). To test homogeneity of variances, cumulative annual emissions were first modeled linearly using N application rate as a fixed effect with random effect terms crossing field replicate with fertility strip and field replicate with cover crop strip, repeated by year (Eq. 3.1). The standard deviation of the residuals of the untransformed data exhibited a positive correlation with fitted values, $p<0.001$ (Supplemental Fig. S.1), and the Box-Cox procedure indicated maximum log-likelihood of the model was reached in a 95% confidence interval $-0.03<\lambda<0.31$. The log-link is represented by $\lambda=0$ and was used; the log-linked data had no association between residuals and fitted values, $p=0.56$ (Eq. 3.2a, Supplemental Fig. S.2). We therefore concluded that the relationship between N_2O emissions and N application rate was exponential, with log-normally distributed error terms (Eq. 3.2b).

$$\text{Emissions} = \beta_1 \cdot (\text{N rate}) + \beta_0 + \varepsilon_{\mathcal{N}} \qquad \text{Eq. 3.1}$$

$$ln(\text{Emissions}) = \hat{\beta}_1 \cdot (\text{N rate}) + \hat{\beta}_0 + \varepsilon_{\mathcal{N}} \qquad \text{Eq. 3.2a}$$

$$\text{Emissions} = e^{\hat{\beta}_1 \cdot (\text{N rate})} \cdot e^{\hat{\beta}_0} \cdot \varepsilon_{\mathcal{LN}} \qquad \text{Eq. 3.2b}$$

To investigate the effects of cover crop residue N, cover crop species, tillage, and applied N source, we added additional fixed terms stepwise, while maintaining the same random terms. Explained-variance estimates are presented for four possible models in Table 5, along with traditional selection criteria. Each added variable lowered the AIC

and BIC; we chose to use the full model, which includes all the hypothesized terms as recommended by Bolker *et al*. (2009). We then tested each effect at selected levels.

Table 3.5. The Akaike information criterion, Bayesian information criterion, Edwards' R^2_β, Magee's R^2_{LR}, and Xu's Ω^2 for four generalized linear mixed models predicting cumulative annual N_2O emissions as a function of the N application rate (N_A), stepwise adding each additional fixed effect: N contributed by cover crop residue (N_R), cover crop species (Species), and the contrasts of subsurface banded PL with either application of UAN (Source) or tillage-incorporated PL (Till). The error structures for all four models are uniform: field replicate crossed with fertility strip and field replicate crossed with cover crop strip, repeated by year.

	AIC	BIC	R^2_β	R^2_{LR}	Ω^2
N_A	263.2	282.6	0.538	0.466	0.538
N_A+N_R	210.1	229.4	0.682	0.598	0.681
$(N_A+N_R)\times$Species	181.2	226.3	0.638	0.595	0.884
$(N_A+N_R+$Source$+$Till$)\times$Species	144.5	215.5	0.712	0.662	0.869

The full model explained the most variance in fixed effects (71.2%) and had the lowest AIC (144.5). Applied N rate alone explained the largest amount of variance (53.8%), with residue N and N application methods each explaining an additional 14.4% and 3.0% respectively.

The sum of applied N from subsurface banded PL and residue N content generates a continuous response predictor for N_2O emissions (Fig. 3.2a). The slope of this model represents the exponential response rate of N_2O emissions to each unit of N input (Fig. 3.2b). Hairy vetch had the lowest response rate and all others had a higher response

rate ($p \le 0.02$) (Table 3.6). The bare ground, cereal rye, and cereal rye:hairy vetch mixture did not differ significantly in slope from one another, $p > 0.10$. The intercept coefficient (the independent effect of cover crop species in the model) was significantly different across cover crops; it was highest for hairy vetch treatments ($p \le 0.01$), with cereal rye having the lowest emissions at zero N application rate ($p \le 0.03$). The intercepts for the bare ground treatment and cereal rye:hairy vetch mixture were significantly different from and intermediate to the monoculture cover crop treatments, but were not different between the two, $p = 0.29$.

Table 3.6. Coefficients and standard errors (s.e.) of the estimates for a log-normal generalized linear mixed model predicting N_2O emissions as a function of subsurface banded poultry litter N application rate, $ln(\text{kg } N_2\text{O-N ha}^{-1} \text{ y}^{-1}) = \beta_1 \cdot (\text{kg N ha}^{-1}) + \beta_0$. The random effects are field replicate crossed with fertility strip and field replicate crossed with cover crop strip, repeated by year. Estimates that do not share a letter within each column differ significantly at $\alpha = 0.05$.

	Slope, β_1	s.e.		Intercept, β_0	s.e.	
Bare ground	0.00490	0.00061	a	0.929	0.161	b
Cereal rye	0.00586	0.00046	a	0.336	0.167	c
Cereal rye:hairy vetch mixture	0.00470	0.00054	a	0.742	0.197	b
Hairy vetch	0.00293	0.00051	b	1.389	0.193	a
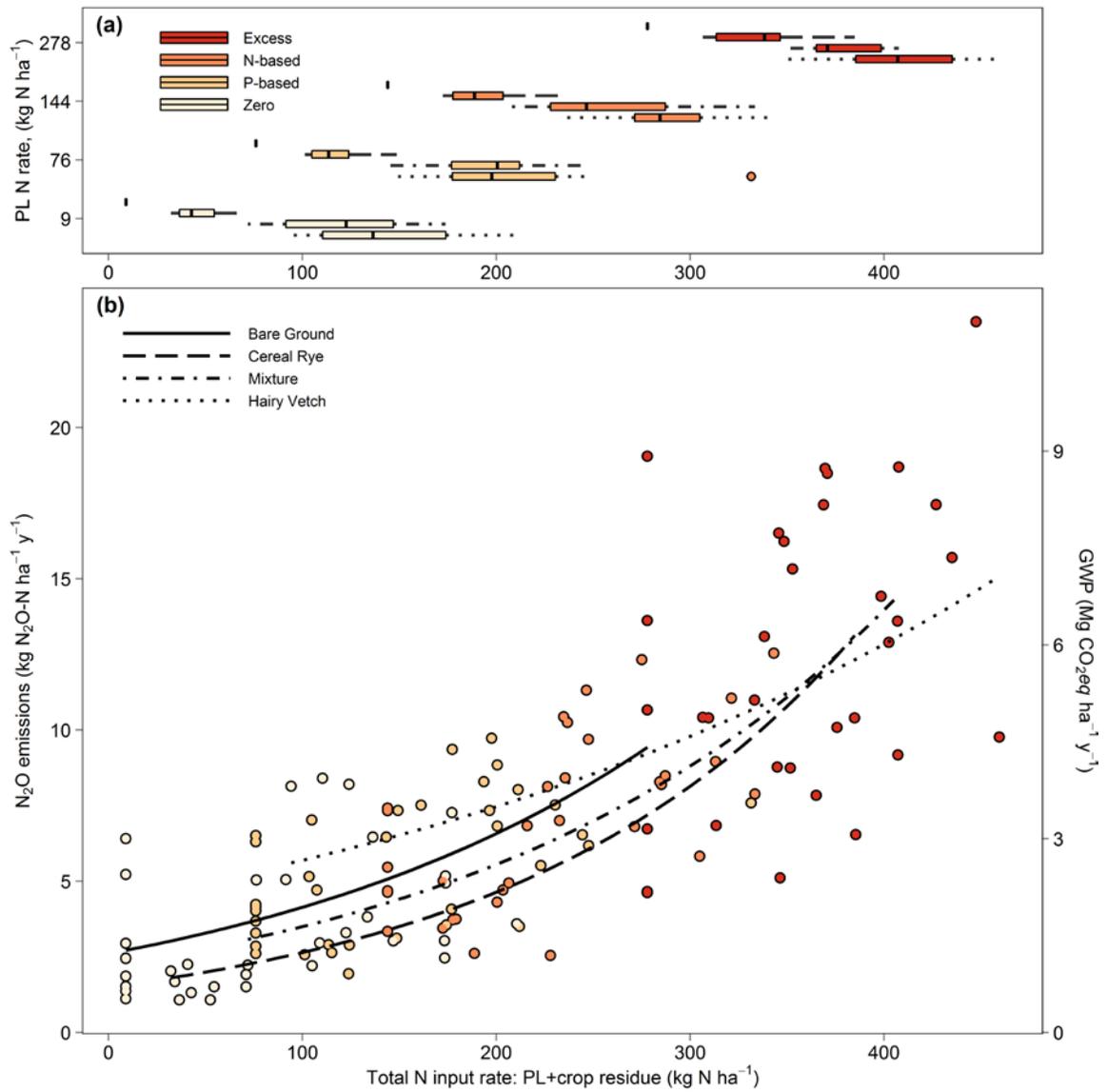
Figure 3.2. Four rates of subsurface banded poultry litter (PL) with the respective

distributions of total N (applied N + N contained in aboveground cover crop residue) for

each cover crop (a). The exponential relationship of nitrous oxide emissions to total N

rate (b). Data in both panels are from three experiment years, 2012, 2013, and 2014; x-

axis in both panels is uniform, the total N rate. In (a), boxes represent the 1st and 3rd

quartiles of each distribution, center hashes represent the median, and whiskers represent

all observations within 1.5 times the interquartile range; 1 outlier is shown as a circle

(hairy vetch, P-based rate). In (b), circles represent N_2O emissions from each plot;

regression lines indicate the fit from a GLMM with the coefficients presented in Table 6; the fixed effects are applied PL N, cover crop residue N, and cover crop species; the random effects are field replicate crossed with fertility strip and field replicate crossed with cover crop strip, repeated by year. In both panels, colors indicate the SSB PL application rate, from lightest to darkest: zero control (9 kg N ha^{-1}, starter only), P-based recommendation (76 kg N ha^{-1}), N-based recommendation (144 kg N ha^{-1}), and excess nutrients (278 kg N ha^{-1}). Separate whiskers (a) and regression lines (b) are shown for each cover crop treatment: bare ground (solid), cereal rye (dash), a cereal rye:hairy vetch mixture (dash-dot), and hairy vetch (dotted). The right-hand y-axis (b) is the global warming potential of N$_2$O emissions in 100 year CO$_2$ equivalents.

Using the IPCC Tier 1 methodology, our emission factors (EF) were correlated with N application rate after accounting for background emissions from unfertilized plots: the lowest EF was observed at the P-based rate and the highest EF was observed at the excess nutrient rate for all cover crops (IPCC, 2006). The lowest mean EFs by cover crop were observed in the cereal rye and cereal rye:hairy vetch mixture; 1.05% and 1.94%, respectively, of N applied was emitted as N$_2$O-N; the mean EFs for hairy vetch and bare ground were higher, 2.39% and 2.12%. All of these values exceed the default EF of 1%, but are within the IPCC "uncertainty range" of 0.3%-3.0%. Approximating the Tier 2 regional methodology would allow EF to vary with N rate (Millar *et al.*, 2010). These adjusted mean EFs would range from 0.04% to 4.6%.

Effects of tillage and N source

Tillage incorporation of PL at the P-based rate did not affect emissions in bare ground or cereal rye plots relative to subsurface banding (Fig. 3.3). However, subsurface

banding significantly increased emissions over tillage-incorporation in plots with either hairy vetch or the cereal rye:hairy vetch mixture , by 3.06 and 1.82 kg N_2O-N ha^{-1} y^{-1} (p<0.001). Note that tillage was confounded with timing of fertilization in this experimental design, so these effects were not distinguished.
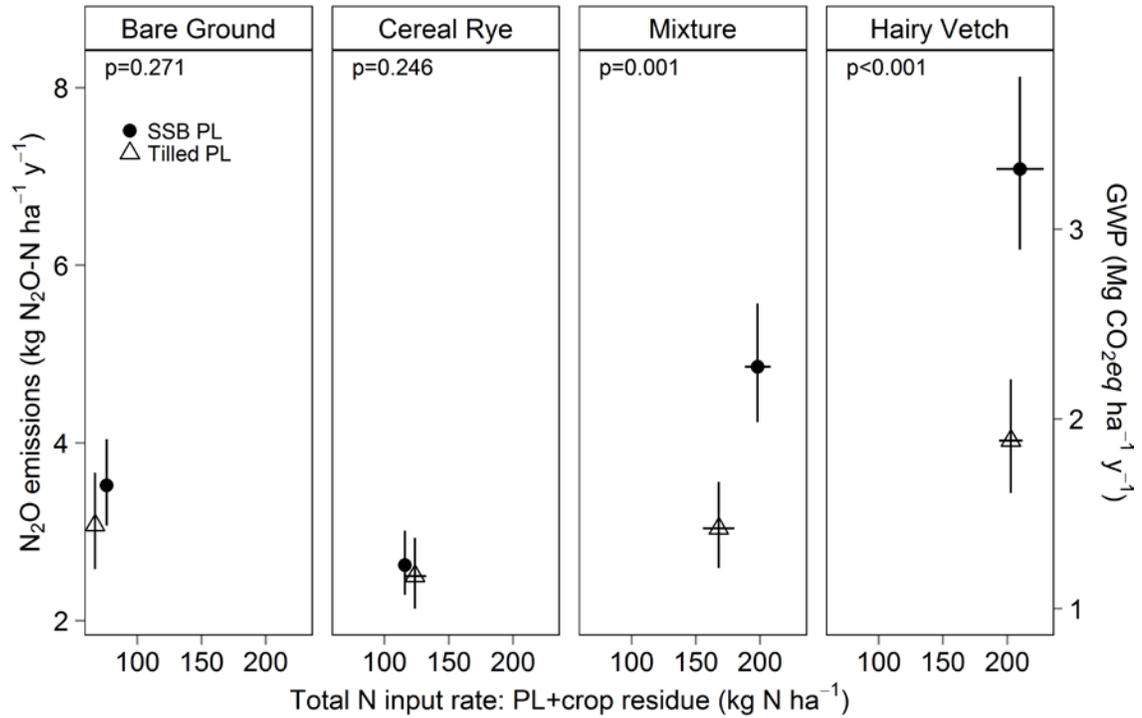
Figure 3.3. N_2O emissions as affected by poultry litter (PL) application method and timing (tillage-incorporated at planting, open triangles, or subsurface-banded at sidedress, closed circles) for the P-based application rate (67, 76 kg N ha^{-1}) under four cover crops, pooled across three experiment years; the Bare Ground treatment was only measured in 2013 and 2014. Vertical bars are one standard error from the mean, and the annotation is the p-value for the contrast between the N_2O emissions for the pairs (n=66). Horizontal error bars are one standard error from the mean N input rate; cover crop residue N did not differ significantly for any pair of fertility treatments (p>0.37). The

second y-axis is the 100 year global warming potential of N_2O emissions in CO_2 equivalents.

N source did not affect N_2O emissions at the N-based fertility rate in the cereal rye or hairy vetch plots (Fig. 3.4). UAN decreased emissions relative to SSB PL in the bare ground treatment by 1.66 kg N_2O-N ha^{-1} (p=0.047). However, UAN increased emissions over SSB PL by 3.71 kg N_2O-N ha^{-1} (p=0.002) in the cereal rye:hairy vetch mixture. Note that N source was confounded by application method in this experimental design, but the timing of application was the same between the surface-applied UAN and the subsurface banded PL, split between planting and sidedress.
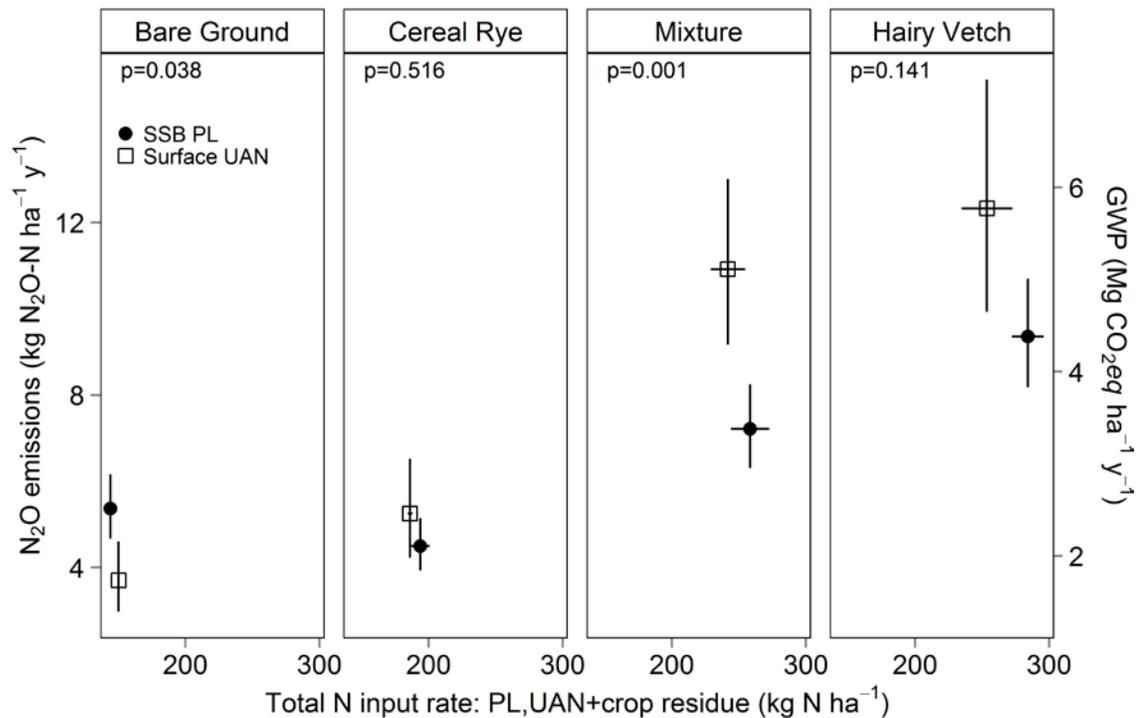
Figure 3.4. N_2O emissions as affected by N source, poultry litter (PL, closed circles) or urea-ammonium nitrate (UAN, open squares), at the N-based application rate (144, 150 kg N ha^{-1}) under four cover crops. UAN was not applied to all cover crops in all years; data shown are for all cover crops during the 2014 experiment year, and under

the cereal rye:hairy vetch mixture during the 2013 and 2014 experiment years. Vertical

bars are one standard error from the mean, and the annotation is the p-value for the

contrast between the N_2O emissions for the pairs (n=30). Horizontal error bars are one

standard error from the mean N input rate; cover crop residue N did not differ

significantly for any pair of fertility treatments (p>0.95). The second y-axis is the

100 year global warming potential of N_2O emissions in CO_2 equivalents.

Individual plot grain yields ranged from 3.45 to 16.24 Mg ha^{-1}, with a median of

12.75 and a mean of 11.86. Almost all of the treatment combinations resulted in yields

higher than the local average. The mean corn yields in Prince George's County and

bordering counties were 8.07 Mg ha^{-1} for the years of the experiment (NASS-USDA,

2014); only the incorporated PL (at the P-based rate) and unfertilized cereal rye plots did

not produce those mean yields, 7.37 and 5.92 Mg ha^{-1}, respectively. Given the high yields

in most treatments, it is unlikely that high N_2O emissions could be attributed to high

residual soil N concentrations that resulted from poor corn growth.

Discussion

Reductions in N inputs exponentially decreased N_2O emissions in our study. This

concurs with previous work which has treated N rate as the main predictor for N_2O

emissions, but there has been some debate about the relationship between applied N rate

and N_2O emissions (Millar *et al.*, 2010). Some researchers have observed linearly

increasing emissions with increasing N rate, but these were often limited by the number

of levels of applied N evaluated (MacKenzie *et al.*, 1997; Mosier *et al.*, 2006; Dusenbury

et al., 2008; Halvorson *et al.*, 2008). Others have observed an exponential relationship,

particularly in studies with between four and nine levels of N rate (McSwiney &

Robertson, 2005; Jarecki *et al.*, 2009; Ma *et al.*, 2010; Hoben *et al.*, 2011). Meta-analyses

across many N rates have found that exponential and similar non-linear models best fit

aggregated data, but that differences between exponential and linear models are often

small relative to the overall effect of N rate on emissions (Velthof *et al.*, 1996; Bouwman

et al., 2002; Van Groenigen *et al.*, 2010; Philibert *et al.*, 2012; Shcherbak *et al.*, 2014).

One hypothesis discussed in the literature for these contrasting findings is that N_2O

emissions may show a sigmoidal response across a much wider range of N rates; across

regions, agronomically appropriate fertilization rates fall within different phases of such

an S-shaped curve, but there has not yet been sufficient data to test this relationship (Kim

et al., 2013). The exponential slopes in our study ranged from 0.0029-0.0059 kg^{-1} N ha,

which are low but within the range of reported values of 0.0025-0.0175 kg^{-1} N ha. A

possible reason for the relatively low N response rates is that we observed high

background N_2O emissions (emissions in unfertilized plots with no cover crop) at 1.11-

6.41 kg N_2O-N ha^{-1}, compared to reported values of 0.05-2.6 kg N_2O-N ha^{-1}. The long

history of applying animal manure and growing cover crops at our sites resulted in

relatively high soil fertility even in plots unfertilized in this experiment.

The greatest disadvantage to a linear approach to modeling the relationship

between N_2O emissions to N applied is that heteroscedasticity increases confidence

intervals across all levels when compared to our exponential model, making means

separation tests less sensitive (Long & Ervin, 2000). Using a linear model, we would not

have found differences in the slopes among any of the cover crops, and confidence

intervals for the intercepts would have been 23-63% larger. A linear model generally

overestimated emissions in the middle of the N rate range and underestimated emissions at the tails relative to our exponential model. At the P- and N-based application rates, emissions would be overestimated by 0.1-29.5%, and underestimated at the zero control and excess nutrient rates by 0.2-8.5%. When evaluating management techniques, these increased errors could mask detection of potential mitigation strategies.

At all N input rates, the use of a cereal rye monoculture cover crop decreased N_2O emissions relative to Bare Ground (>1 kg N_2O-N ha^{-1} y^{-1}, 0.5 Mg CO_2eq ha^{-1} y^{-1}); however, N_2O emissions under the cereal rye:hairy vetch mixture were not significantly different from bare ground (Fig. 3.2). The high C:N ratio of the cereal rye residue likely resulted in microbial immobilization of reactive N, which could make N unavailable to both crops and denitrifying organisms. When considering only applied N from PL or UAN, cereal rye monoculture reduced N_2O emissions relative to bare ground at the zero, P-, and N-based target rates, but not the excess nutrient rate (Supplemental Fig. S.3). The hairy vetch monoculture contributed 67.8-256.6 kg N ha^{-1}. The low C:N ratio of the hairy vetch residue results in rapid mineralization (Poffenbarger *et al.*, 2015). At all application rates, this residue N resulted in higher emissions than the bare ground treatment (>3 kg N_2O-N ha^{-1} y^{-1}, 1.4 Mg CO_2eq ha^{-1} y^{-1}). However, the rate response of N_2O emissions to N input under the hairy vetch monoculture was lower than the other cover crop treatments; this could be evidence supporting the sigmoidal relationship hypothesized by Kim *et al.* (2013).

When determining application rates in this experiment, we did not account for the amount of N added by biological nitrogen fixation in the legume-containing cover crops, nor the amount scavenged from soils over winter by the cereal rye, and these sources of

N should be considered when developing a nutrient budget in production settings. In addition, management of cereal rye:hairy vetch mixtures (early planting date and late termination date) can substantially increase the accumulated biomass and total fixed N; in this experiment, we followed these practices, resulting in substantial surface residues at termination with a wide range of C:N ratios (Clark *et al.*, 1994). The very low C:N ratio of the hairy vetch residues resulted in rapid mineralization, while the very high C:N ratio of the cereal rye residues may have led to net immobilization of N (Poffenbarger *et al.*, 2015). The interaction of cover crop nutrient input with applied N should be further investigated as it relates to N_2O outcomes.

We found that tillage, when compared with no-till subsurface banding, decreased emissions under hairy vetch and the cereal rye:hairy vetch mixture by as much as 3.08 kg N_2O-N ha^{-1} y^{-1}, but we did not detect an effect of tillage in bare ground or cereal rye treatments (Fig. 3.3). In a prior study without cover crops that tested tillage relative to banding of mineral fertilizer, tillage had no effect compared to shallow N banding (2 cm depth), but deeper N banding showed that conventional tillage increased N_2O emissions over zone-tillage or no-till, to 4.81 kg N_2O-N ha^{-1} y^{-1} from 2.98 and 3.71, respectively (Drury & Reynolds, 2006). This deeper treatment was similar to the placement of subsurface banded PL in our experiment, but the effect was in the opposite direction from what we found. Tillage generally induces two opposing effects on N_2O production potential: increased soil-N substrate contact, which increases total denitrification, and increased soil drying, which decreases total denitrification. The drying effect of tillage may have been higher on hairy vetch cover crops than the cereal rye, as no-till hairy vetch residue forms a dense mulch that may retain soil moisture longer. This effect is

most pronounced early in the season, as the high-N vetch residue decomposes quickly (Teasdale & Mohler, 1993). Future work on alternative N placement methods should attempt to quantify soil-N contact and surface drying rates to resolve these mixed findings.

There was not a uniform effect of mineral fertilizer compared to PL in our experiment (Fig. 3.4). Differences were observed in only two of the cover crop treatments, and the direction of the effect was not the same; surface-applied UAN decreased N_2O emissions over SSB PL for bare ground by 1.66 kg N_2O-N ha^{-1} y^{-1}, while UAN increased emissions under the cereal rye:hairy vetch mixture by 3.70 kg N_2O-N ha^{-1} y^{-1}. We tested UAN at only the N-based rate, so we cannot conclude that these effects would hold at other rates. Previously, a comparison of injected dairy manure slurry versus mineral ammonium and nitrate fertilizers found N_2O emissions from the manure to be one to two orders of magnitude smaller (Velthof *et al.*, 1996). When used at very high N rates (>450 kg N ha^{-1}), manures may reduce N_2O emissions relative to mineral fertilizers by 90%, but such rates are associated with P loading and nitrate leaching (Ball *et al.*, 2004). In contrast, other researchers have found mixed results or no differences in N_2O emissions between mineral fertilizer and manure over 1-3 year time scales (Hernandez-Ramirez *et al.*, 2009; Adviento-Borbe *et al.*, 2010; Sistani *et al.*, 2010).

Nitrous oxide emissions have shown stronger response than grain yields to increasing N rate; it is imperative to minimize N inputs to economically optimum N rates (Cui *et al.*, 2013). The nonlinear relationship between N rate and N_2O emissions observed here and in other studies (Shcherbak *et al.*, 2014) is a cause for concern as N use

increases worldwide. Global use of N fertilizer could be reduced by 28% without decreasing overall yields, in particular in North America, China, and Western Europe; grain yields in other parts of the world could be improved by irrigation or improving soil water retention, rather than intensified nutrient inputs (Good & Beatty, 2011; Mueller *et al.*, 2012). Water management is a feature of cover crop adoption; cover crops may lower soil moisture in spring, but can retain moisture and improve infiltration during the rest of the cash crop growing season (Ward *et al.*, 2012; Liang *et al.*, 2014; Wells *et al.*, 2014).

Management techniques such as application method or N source affected N_2O emissions in some cover crop treatments, but reducing overall N inputs (including fixed N from legume sources) is the most critical factor in mitigating emissions. When compared to bare ground, we found that cereal rye and cereal rye:hairy vetch mixture cover crops decreased emissions at all but the highest rates of N fertilization, whereas the hairy vetch increased emissions at all rates. The cereal rye:hairy vetch mixture produced lower N_2O emissions than hairy vetch monoculture at all rates despite containing similar amounts of N; the cereal rye residue counteracts the increased N input from the hairy vetch residue. The nature of this interaction could be biological, chemical, or physical, and further work is needed to optimize management of cover crop mixtures in this respect.

Supplementary Material
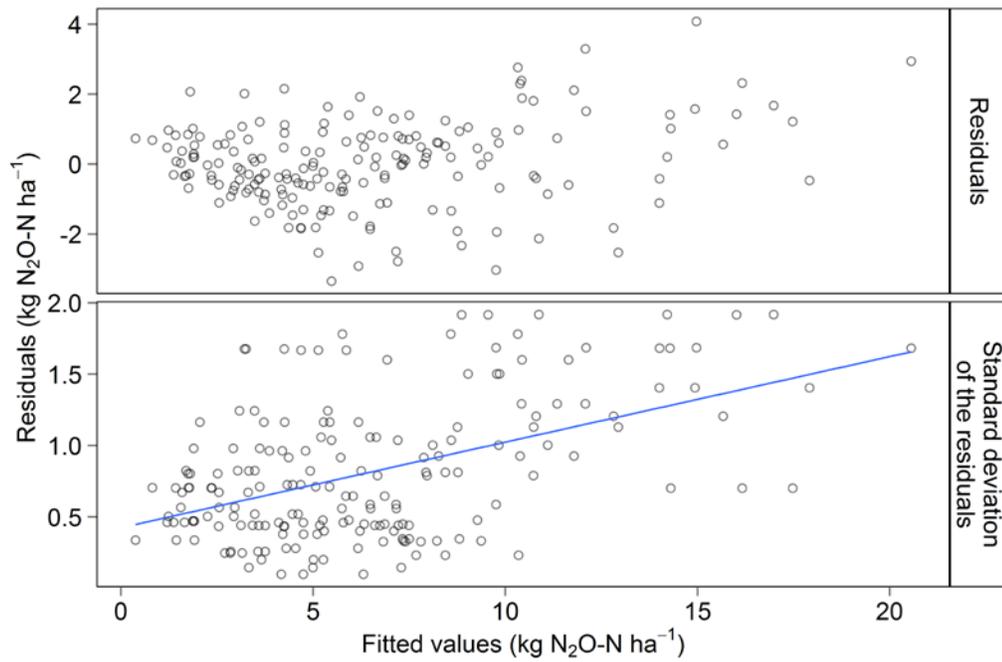
Figure S.1. The relationship of residuals to fitted values from an untransformed generalized linear mixed model of the relationship of N_2O emissions to applied N rate, Eq. 3.1. Regression line indicates the positive correlation between the standard deviation of the residuals and the fitted values, $y=0.086{\cdot}x+0.253$, $R^2=0.32$, $p<0.001$.
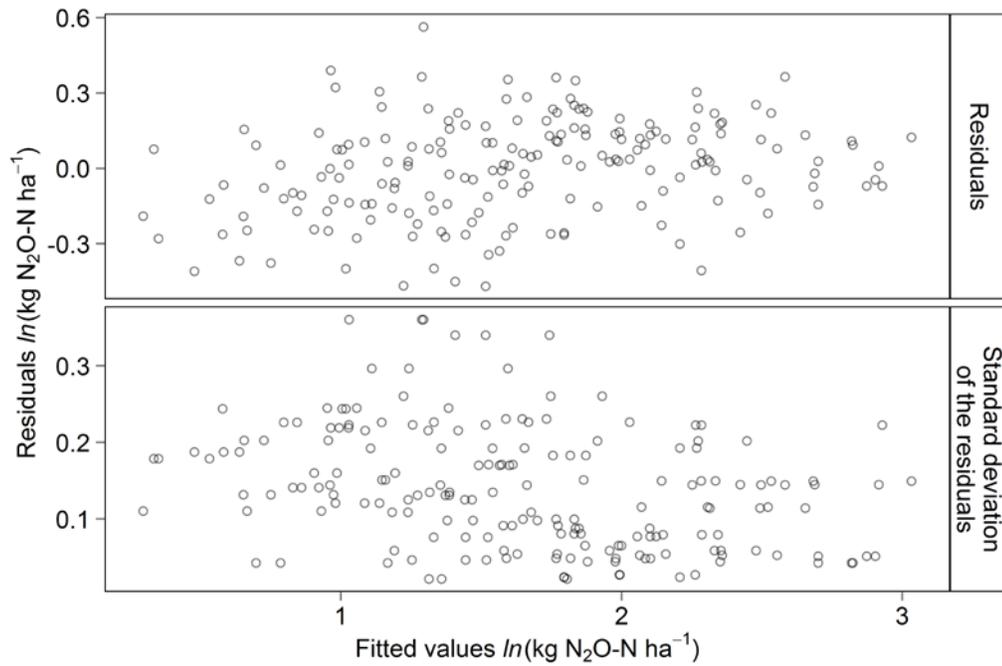
Figure S.2. The relationship of residuals to fitted values from a log-linked generalized linear mixed model of the relationship of N_2O emissions to applied N rate, Eq. 3.2a,b. There was no correlation between the standard deviation of the residual and the fitted values, $R^2=0.02$, $p=0.56$.
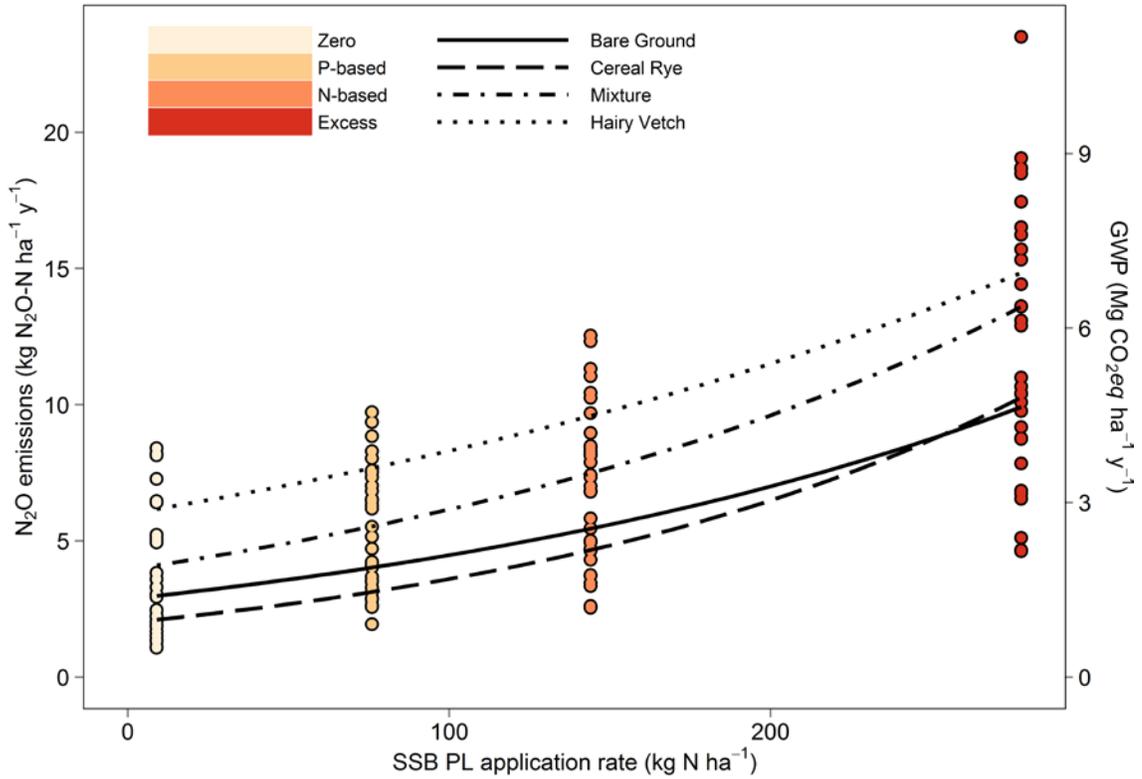
Figure S.3. The exponential relationship of N_2O emissions to subsurface banded poultry litter application rate for three years, 2012-2014. Regression lines represent a log-linked generalized linear mixed model with N application rate and cover crop species as fixed effects, with random effects for field replicate crossed with fertility strip and field replicate crossed with cover crop strip, repeated by year. Colors indicate the four rates of SSB PL, from lightest to darkest: zero control (9 kg N ha^{-1}, starter only), P-based recommendation (76 kg N ha^{-1}), N-based recommendation (144 kg N ha^{-1}), and excess nutrients (278 kg N ha^{-1}).

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