

Long-term nitrous oxide fluxes in annual and perennial agricultural and unmanaged ecosystems in the upper Midwest USA

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Abstract

Differences in soil nitrous oxide (N₂O) fluxes among ecosystems are often difficult to evaluate and predict due to high spatial and temporal variabilities and few direct experimental comparisons. For 20 years, we measured N₂O fluxes in 11 ecosystems in southwest Michigan USA: four annual grain crops (corn–soybean–wheat rotations) managed with conventional, no-till, reduced input, or biologically based/organic inputs; three perennial crops (alfalfa, poplar, and conifers); and four unmanaged ecosystems of different successional age including mature forest. Average N₂O emissions were higher from annual grain and N-fixing cropping systems than from nonleguminous perennial cropping systems and were low across unmanaged ecosystems. Among annual cropping systems full-rotation fluxes were indistinguishable from one another but rotation phase mattered. For example, those systems with cover crops and reduced fertilizer N emitted more N₂O during the corn and soybean phases, but during the wheat phase fluxes were ~40% lower. Likewise, no-till did not differ from conventional tillage over the entire rotation but reduced emissions ~20% in the wheat phase and increased emissions 30–80% in the corn and soybean phases. Greenhouse gas intensity for the annual crops (flux per unit yield) was lowest for soybeans produced under conventional management, while for the 11 other crop × management combinations intensities were similar to one another. Among the fertilized systems, emissions ranged from 0.30 to 1.33 kg N₂O-N ha⁻¹ yr⁻¹ and were best predicted by IPCC Tier 1 and ΔEF emission factor approaches. Annual cumulative fluxes from perennial systems were best explained by soil NO₃⁻ pools ($r^2 = 0.72$) but not so for annual crops, where management differences overrode simple correlations. Daily soil N₂O emissions were poorly predicted by any measured variables. Overall, long-term measurements reveal lower fluxes in nonlegume perennial vegetation and, for conservatively fertilized annual crops, the overriding influence of rotation phase on annual fluxes.

Keywords: corn, cover crops, crop type, forest, nitrogen fertilizer, no-till, rotation phase, soybean, succession, wheat

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Introduction

Nitrous oxide (N₂O) is an important greenhouse gas (GHG) that also depletes stratospheric ozone (Portmann *et al.*, 2012), and agricultural soils represent the largest single source of anthropogenic N₂O emitted to the atmosphere (Fowler *et al.*, 2009). Understanding the controls and dynamics of N₂O emissions from agricultural soils is thus important for developing mitigation strategies for agriculture and for predicting future climate impacts (Robertson & Vitousek, 2009; Smith, 2012; Gelfand & Robertson, 2015a; Paustian *et al.*, 2016). And understanding the dynamics of N₂O emissions from both managed and unmanaged soils is important for

predicting and closing the global N₂O budget, currently unbalanced (Syakila & Kroeze, 2011).

Nitrogen inputs are a major regulator of soil N₂O emissions: Globally, terrestrial ecosystems with low N inputs tend to have low emissions, and in general, N₂O emissions increase with increasing soil N availability (e.g., Matson & Vitousek, 1987; Groffman *et al.*, 2000; Robertson *et al.*, 2000; Bouwman *et al.*, 2002; Butterbach-Bahl *et al.*, 2002), especially in response to fertilizer (Hoben *et al.*, 2011; Shcherbak *et al.*, 2014). While the effect of N added to agricultural soils on N₂O emissions depends on a number of factors, including crop type, tillage, and fertilizer formulation, placement, and timing (Venterea *et al.*, 2012), the amount of N added is usually the most important factor (Millar *et al.*, 2010) and is the metric used to construct most national N₂O inventories (De Klein *et al.*, 2006).

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Two major factors further affect N₂O emissions: soil disturbance (e.g., tillage in agricultural systems) and life-history traits of dominant plants (e.g., perennality, N fixation, seasonal phenologies, and deciduousness). For example, a recent meta-analysis (van Kessel *et al.*, 2013) shows that tillage effects vary but N₂O emissions often increase during the first 10 years of no-till (NT) management in nonmesic climates and decline thereafter. Plant functional type and species affect soil N₂O emissions particularly through their effects on soil N availability. Perennial plants, for example, are active for a greater portion of growing seasons and might thus be expected to leave less N in soil for microbial conversion to N₂O (Oates *et al.*, 2016). In contrast, legumes fix atmospheric N₂ that eventually becomes soil inorganic N available to microbial N₂O producers, and thus, legume-based systems might be expected to emit N₂O at rates similar to those of fertilized nonlegume systems (Robertson *et al.*, 2000; cf. Schwenke *et al.*, 2015). And unmanaged successional ecosystems with their greater diversity of plant species, rooting depths, and N uptake strategies might be expected to capture soil inorganic N more efficiently than monocultures whether fertilized or not, and thus emit less N₂O (Robertson *et al.*, 2000). Few studies, however, have made such direct comparisons (Hooper *et al.*, 2005).

Despite a solid conceptual understanding of the mechanisms controlling soil N transformations (e.g., Robertson & Groffman, 2015) and the development of various biogeochemical, physical, and mixed models of soil N₂O production (Wang & Chen, 2012), our general ability to predict N₂O emissions accurately at the subannual scale is extremely limited (Conen *et al.*, 2000; Blagodatsky & Smith, 2012). While cumulative annual emissions correlate well with ecosystem-scale variables such as annual rainfall (Groffman *et al.*, 2000) and N availability (Li *et al.*, 2011), daily and other short-term emissions remain poorly predictable by combinations of environmental attributes such as coincident measures of soil inorganic N, moisture, and temperature, and thus are poorly predicted by process-level models.

Here we use 20 years of measured soil N₂O emissions in eleven very different replicated ecosystems on the same soils subjected to the same climate in order to examine specific questions regarding long-term N₂O fluxes. Our systems range from intensively managed row crops to unmanaged, late successional deciduous forests and provide the opportunity to assess the effects of different agricultural and land management practices on long-term soil N₂O emissions. We couple these fluxes to coincident measures of soil N availability,

temperature, and water content, together with periodic estimates of N mineralization, to investigate:

1. How long-term patterns of N₂O production are influenced by management intensity, plant life-history traits, and successional age;
2. How, within annual grain-based cropping systems, (i) long-term emissions are related to crop management (conventional vs. no-till vs. reduced input vs. biological/organic management), (ii) different crop types (corn vs. soybean vs. wheat) contribute differentially to N₂O emissions within a rotation, and (iii) cover crops influence N₂O emissions;
3. How well different combinations of environmental attributes can explain variability in daily and cumulative annual N₂O fluxes; and
4. The ability of current global greenhouse gas inventory metrics to reliably estimate long-term N₂O emissions.

Materials and methods

Site description

Measurements were taken continuously from 1991 to 2011 (except 1995 for all systems and 1991, 1992, and 1993 for forested systems) in the Main Cropping System Experiment (MCSE) of the Kellogg Biological Station (KBS) Long-term Ecological Research (LTER) site (www.lter.kbs.msu.edu). KBS is located at 42°24' N, 85°24' W and 288 m asl in southwest Michigan, USA, in the northeastern portion of the U.S. Corn Belt. The mean annual air temperature at KBS is 10.1 °C, ranging from a monthly mean of -9.4 °C in January to 28.9 °C in July. Rainfall averages 1027 mm yr⁻¹, evenly distributed seasonally; potential evapotranspiration exceeds precipitation for about four months of the year. Loam soils are well-drained Typic Hapludalfs developed on glacial outwash with soil carbon contents (Syswerda *et al.*, 2011) that vary from 1% (long-term conventional tillage) to 2.5% (mature deciduous forest). More details are available in Robertson & Hamilton (2015).

System types and management

We compared eleven ecosystems: four annual cropping systems, three perennial systems, and four unmanaged successional communities. The four annual systems are corn (*Zea mays* L.)–soybean (*Glycine max* L.)–winter wheat (*Triticum aestivum* L.) rotations managed differently: (i) with conventional chemical inputs and tillage, managed according to prevailing practices in the region (CT), (ii) with conventional chemical inputs and no-tillage (NT), (iii) with reduced synthetic chemical inputs (RI), and (iv) with no chemical inputs, that is, biologically based (BIO). The BIO system is USDA-certified organic but receives no manure or compost, and both the RI and BIO systems include winter cover crops: red clover (*Trifolium pratense* L.) interseeded in the spring of the wheat phase

of the rotation prior to corn, and cereal rye (*Secale cereale*) planted following corn harvest prior to soybeans. Red clover is a legume that provides biologically fixed N and rye is an N-scavenging annual grass. The RI system received, on average, one-third of the synthetic chemicals applied in the CT and NT systems, including N fertilizer (details below). Weed control in the RI and BIO systems was provided by mechanical cultivation, which was supplemented in the RI system with herbicide as described below. None of the four cropping systems received compost or manure. Annual crop yields for this period (Robertson *et al.*, 2014) are similar to or exceed Michigan and average US yields (Robertson & Hamilton, 2015).

The three perennial cropping systems were alfalfa (AA; *Medicago sativa* L.), hybrid poplar clones (POP; *Populus × canadensis* Moench 'Eugenei' [*Populus deltoides* × *P. nigra*], also known as *Populus × euramericana* 'Eugenei'), and a coniferous forest plantation (CF; comprised mainly of red (*Pinus resinosa*) and white (*P. strobus*) pine; sampled between 1993 and 2011).

The four successional systems were (i) an early successional community of herbaceous perennial vegetation (SUC) that was abandoned from agriculture in 1989 and periodically burned after 1996 to exclude trees, (ii) a never-tilled mown grassland with herbaceous vegetation mown every fall (NTG), (iii) a mid-successional community of herbaceous and woody perennial vegetation (SF; sampled between 1993 and 2011), and (iv) a deciduous forest (DF; sampled between 1993 and 2011). Species compositions of all systems are provided in Robertson & Hamilton (2015).

The experimental systems were replicated three (CF, SF, and DF), four (NTG), or six (all others) times. Replicate plots for all systems were 10⁴ m², except for NTG (450 m²), DF (~3800 m²), SF (~5990 m²), and CF (~1550 m²).

All farming operations were performed using commercial-sized equipment as per local practice. The CT and NT systems received 137 ± 20 kg N fertilizer ha⁻¹ yr⁻¹ during the corn phase and 77 ± 17 kg N ha⁻¹ yr⁻¹ during the wheat phase of the rotation, and the soybean phase sometimes received minor N fertilizer inputs as part of P, K, and herbicide applications, which were applied as needed. Both fertilizer and herbicide applications were conservative and followed Michigan State University (MSU) extension recommendations (Warncke *et al.*, 2009). The RI system was managed following organic agricultural practices, with the exception that this treatment received N fertilizer and herbicides at a rate equivalent to ~one-third that of the CT system: The RI system received 30 ± 3 kg N fertilizer ha⁻¹ yr⁻¹ during the corn phase and 40 ± 13 kg N ha⁻¹ yr⁻¹ during the wheat phase of the rotation and herbicides (at full strength) were banded only onto rows rather than broadcast. Herbicides were the only pesticides required in the CT, NT, and RI systems. The BIO system received no synthetic chemicals.

Fertilization dates were different for the different cropping systems and crops; in the CT and NT systems, corn was fertilized once on ~June 15 in 1991 and was thereafter managed by split fertilizer applications of ~30 kg N ha⁻¹ at planting and the remainder was side-dressed at V6 on June 28 (±9 days). Crops in the RI system were fertilized

on May 13 (±7 days), while those of the BIO system were never fertilized. Wheat was fertilized on April 19 (±7 days), except for one additional fall application on October 8 in 2003 in the CT and NT systems, but not the RI system. N fertilizer was added as ammonium nitrate and urea ammonium nitrate (UAN) injected ~10 cm into soil between crop rows at planting and as side-dressing (Millar & Robertson, 2015).

Across all study years, corn was planted at a rate of ~74 × 10³ seeds ha⁻¹ on May 10 (±6 days) and was harvested on October 25 (±13 days); soybean was planted at a rate of ~450 × 10³ seeds ha⁻¹ on May 25 (±6 days) and was harvested on October 11 (±7 days); and wheat was planted in the fall on October 22 (±9 days), immediately after soybean harvest, at a rate of ~4.5 × 10⁶ seeds ha⁻¹ and was harvested on July 16 (±6 days) of the next calendar year. Cover crops in the RI and BIO systems were planted differently during different phases of the rotations. During the wheat phase prior to corn, red clover was frost-seeded in March, germinated but became light-suppressed by wheat until wheat harvest in July, lay dormant over winter, and was killed by tillage the following spring prior to planting corn. During the corn phase (prior to soybean), two different cover crops were used until 2008: Red clover was planted into the corn in mid-June, and rye was planted into the clover after the corn harvest. After 2008, only rye was planted. Clover and rye were tilled into the soil one to two weeks before planting the soybean crop. No cover crop was planted into the soybean crop prior to the wheat crop because wheat was planted within 7–10 days of soybean harvest. Between 1991 and 1993, the CT and NT systems followed a corn–soybean rotation, while the RI and BIO systems followed a corn–soybean–wheat rotation. Beginning with the 1993 corn crop, all of the annual cropping systems followed the same three-year corn–soybean–wheat rotation.

The AA system was replanted on a 5-year cycle with grain break years and was harvested 3–4 times per growing season between May and October (Robertson & Hamilton, 2015). Lime and mineral fertilizers (phosphorus, potassium, and boron) were applied periodically as recommended by soil tests at the following rates annualized over the 20-year study period: lime ~160 kg ha⁻¹ yr⁻¹ as CaCO₃ or CaMg(CO₃)₂, phosphorus ~3 kg P ha⁻¹ yr⁻¹ as monoammonium phosphate or triple superphosphate, potassium ~73 kg K ha⁻¹ yr⁻¹ as K₂O, and boron ~1 < kg ha⁻¹ yr⁻¹.

The POP system was planted in 1989 with *Populus × euramericana* 'Eugenei' as 15-cm stem cuttings on a 1 × 2 m row spacing and fertilized once with 124 kg N ha⁻¹ as ammonium nitrate. The POP system was managed for an 8- to 10-year rotation cycle and was harvested for the first time in December 2000 and then was allowed to re-sprout (coppice) without fertilizer application. It was harvested again in winter 2008 and then replanted in May 2009 using *Populus nigra* × *Populus maximowiczii* clones on 1.5 × 2.4 m row spacing. The CF system was planted in 1965 and not yet harvested. The NTG system was mowed annually and the SUC system was burned in April to prevent trees encroachment; all other successional systems were unmanaged.

N₂O emissions and soil moisture, temperature, and N availability

Emissions of N₂O were measured biweekly between 1991 and 2011 using the manual chamber method (Holland *et al.*, 1999) in four replicate plots of the CT, NT, BIO, RI, AA, POP, SUC, and NTG systems and in all three replicate plots of the CF, SF, and DF systems. For the POP system, we used results of measurements between 1991 and January 2008 only, as poplars were harvested in January 2008. The results for the POP system thus encompass a full 2-harvest crop rotation as described above. Sampling in all ecosystems started after soil thaw usually in March and continued until soil freezing usually in December. Sampling frozen soil under snow was discontinued after an initial winter sampling shortly after the start of the experiment yielded negligible fluxes. All ecosystems were sampled on the same day; the CT, NT, BIO, RI, AA, POP, SUC, and NTG systems were sampled between 08:30 and 11:30 hours; CF, SF, and DF ecosystems were sampled between 13:00 and 16:00.

For gas sampling, manual open-bottom chambers (29 cm × 29 cm × 14 cm high) constructed of opaque polycarbonate sheeting and fitted with rubber septa were placed on aluminum bases (28 cm × 28 cm × 10 cm high) with a surrounding channel that was filled with water to provide a gas-tight seal. The aluminum bases were permanently installed ~3 cm into the soil and removed only for farming activities and winter. Base heights above the soil surface (to determine chamber volume) were measured for each chamber at each sampling. Chamber volume was ~12 L.

Gas fluxes were determined from four gas samples taken at 15-min sampling intervals over a one-hour period. Chamber gas samples were collected in a headspace-flushed syringe, stored in 5.9-mL Exetainer vials (Labco Limited, Lampeter, UK) overpressurized to avoid contamination and detect leakage, and transported to the laboratory for N₂O analysis. N₂O was analyzed with a gas chromatograph (Hewlett-Packard 5890 series, CA, USA, until 2008 and 7890A Agilent Technologies Inc., CA, USA, thereafter) equipped with a ⁶³Ni electron capture detector (350 °C), a Poropak Q column (1.8 m, 80/100 mesh) at 80 °C, and a carrier gas of argon/methane (90/10).

Nitrous oxide flux was calculated as the change in headspace N₂O concentration over the chamber closure period (ppbv min⁻¹) using linear least squares approximation (all fluxes were screened for potential nonlinearity) and corrected for temperature and pressure using the air temperature and pressure measured at 10:00 a.m. on the MCSE site, 3 m above ground (<http://lter.kbs.msu.edu/datatables/167>). Soil N₂O fluxes were further extrapolated from hourly to daily values, assuming that measured values are representative for the day of measurement.

During gas sampling at each site, soil temperature was measured by inserting a soil thermometer to 10 cm soil depth. Gravimetric soil moisture to 25 cm depth was measured from 2-cm-diameter soil cores taken near chambers. On different dates, soil cores were also removed from five random locations in each plot biweekly between April and September of each year, extracted with 2 M KCl in a 1 : 10 soil:extractant ratio, and analyzed for ammonium (NH₄⁺) and nitrate (NO₃⁻)

using an Alpkem 3550 continuous flow analyzer (OI Analytical, College Station, TX, USA; for details, see <http://lter.kbs.msu.edu/protocols>).

Nitrogen mineralization and NO₃⁻ production potentials were measured once per year in July. In brief, soil samples were incubated in buried polyethylene bags *in situ* for 28 days and NH₄⁺ and NO₃⁻ contents measured as described above both before and after incubation. The difference in soil NO₃⁻ content after vs. before incubation represents the NO₃⁻ production (nitrification) potential, and change in total inorganic N represents net N mineralization (Robertson *et al.*, 1999).

Aboveground productivity and harvestable biomass

Annual net primary productivity (aboveground, ANPP) in the CT, NT, BIO, RI, AA, SUC, and NTG systems was measured by hand-harvesting aboveground biomass from five 0.5 × 2 m quadrats (except 0.5 × 0.5 m quadrats in the NTG system) in each replicate plot at peak biomass. For agricultural crops, ANPP was measured at peak biomass before harvest, which occurred in mid-October for corn, late September for soybean, and mid-July for wheat. The ANPP of cover crops was measured in spring just before their incorporation into soil, and the ANPP of successional systems was measured at peak biomass in early to mid-August. In the POP, CF, and DF systems, ANPP was estimated using site-specific allometric equations based on diameter at breast height (<http://lter.kbs.msu.edu/protocols/111>).

Plant carbon (C) and N (g g⁻¹) contents were determined from subsamples of oven-dried biomass weighed into tin capsules and measured using an elemental analyzer (Carlo Erba NA1500 Series II CN Analyzer, Carlo Erba Instruments, Milan, Italy). The harvestable biomass (yield) of agricultural crops was determined by machine harvest of six replicate plots, and according to standard farming practices converted to standard agronomic moisture levels (soybean and wheat at 13% moisture, corn at 15.5% moisture), and reported here on a dry mass basis.

Data analysis

Data used for analyses were acquired for 20 continuous years from 1991 to 2011, except for 1995 and the POP system. For the POP system, we used data acquired between years 1991 and January 2008, which encompasses a full rotational cycle: planting and coppicing with two harvests. Crop year was defined as the time between planting the main crop and planting the next main crop in the rotation (Syswerda *et al.*, 2012): 380 days for corn, 150 days for soybean, and 565 days for winter wheat (hereafter 'crop year'). Off-season was defined as the time between harvesting the main crop and planting the next main crop in the rotation. Perennial and successional systems were analyzed on a calendar year basis.

Cumulative N₂O emissions were estimated using a linear interpolation between the successive sampling dates for each replicate. The N₂O flux from the last sampling date before winter to the first sampling date after winter was assumed to be zero due to mostly frozen soils (Gelfand *et al.*, 2013, but see

Ruan and Robertson, *in review*). Average daily soil N₂O fluxes were calculated by dividing the cumulative emissions over a specific crop year by the number of days in that crop year.

We estimated N₂O emission intensity for annual crops:

$$\text{N}_2\text{O Emission Intensity (kg N Mg}^{-1}\text{)} = \sum F_{\text{N}_2\text{O}} \times \text{Yield}^{-1} \quad (1)$$

where $\sum F_{\text{N}_2\text{O}}$ is the cumulative emissions of N₂O-N (kg ha⁻¹ crop yr⁻¹) for a given main crop and *Yield* is the harvested dry biomass (Mg) for that year.

We compared our measured soil N₂O emission factors (EFs) with factors estimated for our annual cropping systems using the IPCC Tier 1 methodology (De Klein *et al.*, 2006), van Groenigen *et al.*'s (2010) surplus-N method, and Shcherbak *et al.*'s (2014) ΔEF method. In order to compare emission factors, we used calendar year rather than crop year emissions from the studied systems. Calendar year emissions were calculated similarly to crop year emissions, using linear interpolation between the sampling dates, but instead of crop year we used calendar as for the perennial systems. IPCC Tier 1 emission factors were calculated as the percentage of total N input emitted as N₂O-N. Total N input is defined as the sum of fertilizer N, N fixed by legumes, crop residue N, and N mineralized from soil organic matter due to management or land-use change (De Klein *et al.*, 2006).

$$\text{N}_2\text{O}_{\text{IPCC}} \text{ (kg N ha}^{-1}\text{ yr}^{-1}\text{)} = \frac{(F_{\text{SN}} + F_{\text{ON}} + F_{\text{CR}} + F_{\text{SOM}})}{\times \text{EF}_{\text{IPCC}}} \quad (2)$$

where N₂O_{IPCC} is direct soil emission from N inputs, F_{SN} is synthetic N fertilizer inputs, F_{ON} is organic N inputs (e.g., manure, compost, and N-fixing crops), F_{CR} is N in cover crop or crop residues, and F_{SOM} is new N mineralized from soil organic matter change. EF_{IPCC} is the IPCC Tier 1 emission factor of 0.01 (1%). Nitrogen inputs from incorporated cover crops were estimated as the total N content of cover crop biomass at tillage. N fixation by soybean was estimated using 58% of the N that was measured in the grain biomass and 38% of the total N content in the vegetative biomass (Gelfand & Robertson, 2015b). We assumed N mineralization from soil organic change was nil; while there is evidence of additional soil organic matter (carbon) loss due to climate change at our site (Senthilkumar *et al.*, 2009), the absolute amount is still uncertain (Kravchenko & Robertson, 2011; Syswerda *et al.*, 2011) and in any case very small.

Surplus emission factors, N₂O_{surplus} (kg N ha⁻¹ yr⁻¹), were determined from the difference between N input and aboveground N uptake using formulas presented in van Groenigen *et al.* (2010):

$$\text{N}_2\text{O}_{\text{surplus}} \text{ (kg N ha}^{-1}\text{ yr}^{-1}\text{)} = 1.435 + 0.081 \times e^{0.0443 \times (\text{N}_{\text{in}} - \text{N}_{\text{out}})} \quad (3)$$

where N_{in} is the total N input to the crop (kg N ha⁻¹ crop yr⁻¹), calculated as the sum of the applied fertilizer, N fixation, N content of the aboveground and belowground residues, and N content of the cover crops (when applicable). N_{out} is the N content of the aboveground biomass at peak productivity, here estimated as the total N in aboveground biomass (grain plus residue).

We also estimated emission factors based on ΔEF for specific crops (Shcherbak *et al.*, 2014):

$$\text{N}_2\text{O emissions (kg N ha}^{-1}\text{ yr}^{-1}\text{) for N fixers} = 1.677 + (3.06 + 0.1800 \times \text{N}_{\text{in}}) \times \text{N}_{\text{in}} \quad (4)$$

and

$$\text{N}_2\text{O emissions (kg N ha}^{-1}\text{ yr}^{-1}\text{) for upland grain crops} = 1.218 + (6.49 + 0.0187 \times \text{N}_{\text{in}}) \times \text{N}_{\text{in}} \quad (5)$$

where N_{in} is N input into the system as above.

Indirect N₂O emissions due to nitrate (NO₃⁻) leaching (N₂O_{LEACH}) were calculated as percent of leached NO₃⁻:

$$\text{N}_2\text{O} - \text{N}_{\text{LEACH}} \text{ (kg N ha}^{-1}\text{ yr}^{-1}\text{)} = F_{\text{NO}_3^- - \text{N}} \times \text{EF}_{\text{NO}_3^- - \text{N}} \quad (6)$$

where $F_{\text{NO}_3^-}$ is NO₃⁻ leached from annual cropping systems (kg NO₃⁻ - N ha⁻¹ yr⁻¹) from Syswerda *et al.* (2012) and $\text{EF}_{\text{NO}_3^-}$ is the IPCC emission factor for leached N of 0.0075 (0.75%).

Descriptive statistics of the daily N₂O emissions were calculated for every experimental plot based on all available data and included the coefficient of variation (CV) and mean-to-median ratio. CV was used as a quantitative measure of temporal variability, while mean-to-median ratio was used as a quantitative measure of the lack of normality: mean-to-median ratios >1 point to positive skewness. Comparisons among the ecosystems in terms of these characteristics were conducted using ANOVA via a mixed model approach (Milliken & Johnson, 2009). The statistical model for these comparisons consisted of a fixed effect of ecosystem and a random effect of block.

For cumulative N₂O emissions, data comparisons were conducted using a statistical model with fixed effects of ecosystem, year, and ecosystem-by-year interactions, and random effects of block and block-by-treatment interactions. The latter interaction was used as an error term for testing significance of the ecosystem effect. Analyses were performed on log-transformed data because the data were highly positively skewed. Data analysis was conducted using the PROC MIXED procedure of SAS (SAS Institute, Chicago, IL USA).

Multiple comparisons among the ecosystems were conducted when the main effect of ecosystem was found to be statistically significant at either 0.05 or 0.1 level. We refer to the differences among the treatments with *P*-values <0.05 as statistically significant differences and to the differences with *P*-values between 0.05 and 0.1 as statistically significant trends. Agricultural field experiments are known for very high variability of their observations. High variability commonly results from a large number of unidentified and/or unquantifiable sources of influences to which the experimental plots and plants are subjected. Thus, we use a somewhat higher probability of type I error levels, for example, 0.1, in conducting planned comparisons among the treatments, consistent with generally accepted practice (Ott & Longnecker, 2008).

Associations between cumulative soil N₂O emissions and soil N variables (NO₃⁻ production potential and extractable inorganic N pools) were addressed using best fit regression analysis based on *P* and *R*² values. Multilinear regression analyses also were used to find best prediction factors for daily N₂O emissions.

Data availability

Data used in this study were extracted from the KBS LTER data catalog (<http://lter.kbs.msu.edu/datatables>). Extracted data have been separately archived in Gelfand *et al.* (2016), doi:10.5061/dryad.bb095.

Results

We present our results in a hierarchical order from daily to annual scales to show how different scales are each affected by environmental and management factors. We further compare indirect N₂O emissions estimates from our annual crop systems.

Daily N₂O emissions

In every system, daily N₂O emissions were non-normally distributed, with high coefficients of variation, mean-to-median ratios (Fig. 1), and skewness and kurtosis values (Table S1). Further, daily fluxes were highly variable in all systems, with coefficients of variation ranging between 1.5 ± 0.2 and 3.6 ± 1.1 (Fig. 1a, c). Fluxes also were highly positively skewed with average mean-to-median ratios ranging between 1.8 ± 0.3 and 3.8 ± 0.3 , with highest ratios for the corn phase of the annual crops (3.0 ± 0.3 to 3.8 ± 0.3 ; Fig. 1b, d). Multi-linear regression analysis for average daily N₂O

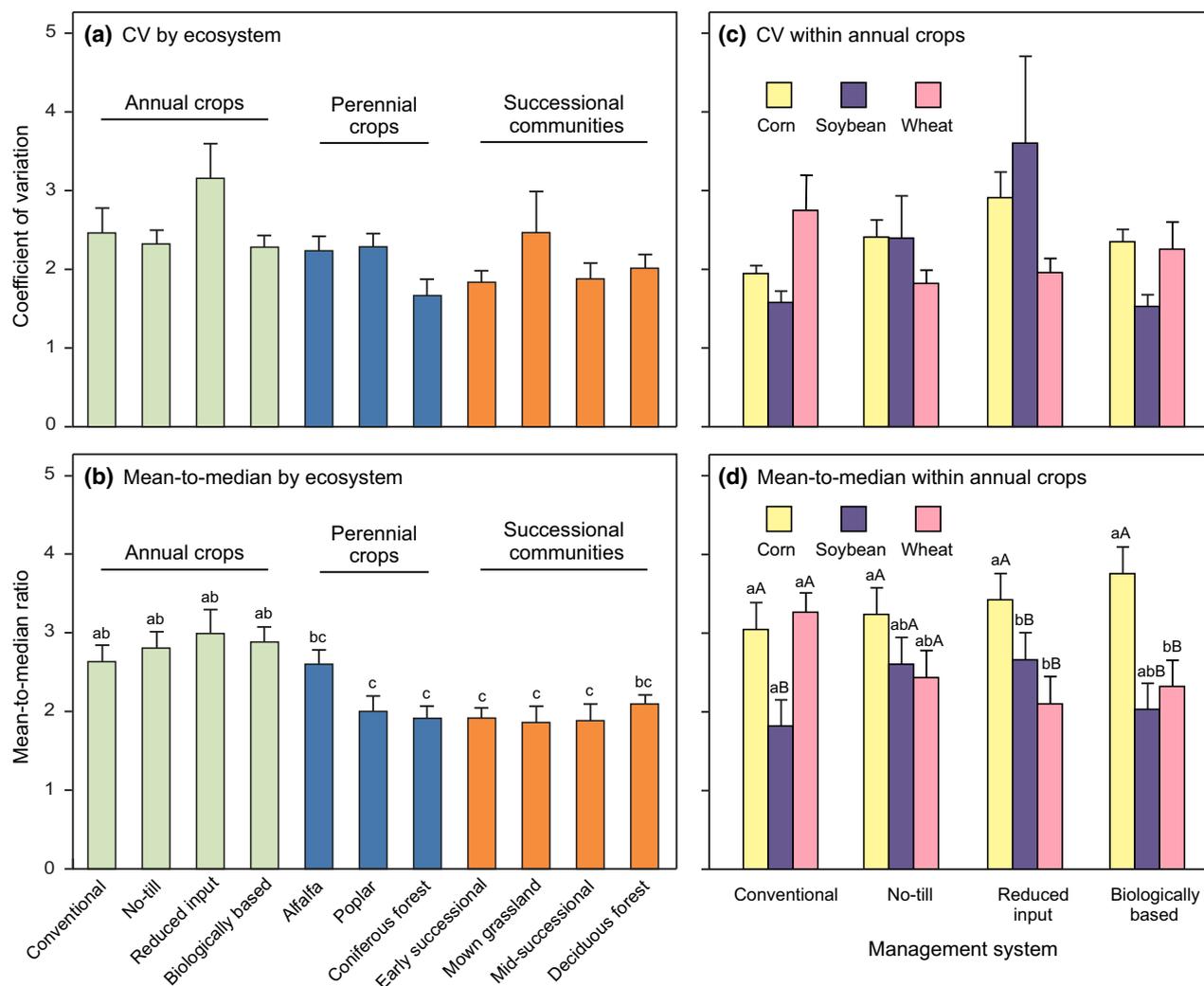


Fig. 1 Coefficient of variation (panels a and c) and mean-to-median ratio (panels b and d) for daily average soil N₂O emissions ($\text{g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$; $n = 4$ replicate plots for conventional (CT), no-till (NT), reduced input (RI), biologically based (BIO), alfalfa (AA), poplar (POP), early successional (SUC), and mown grassland (NTG) systems and $n = 3$ for coniferous forest (CF), mid-successional (SF), and deciduous forest (DF) systems). Significant differences among systems are represented by different lowercase letters. Upper case letters signify differences among annual crops within the same management ($P < 0.05$ for panel b; $P < 0.1$ for panel d). Where letters are absent (panels a and c), there were no significant differences.

emissions with soil nitrate concentrations, soil moisture, and temperature (Table S2) explained overall no more than 20% of emissions variability using best first and second degree models. Daily N₂O emissions, therefore, were not used for further analyses and are presented in supplemental materials (Figs S1 and S2).

Cumulative N₂O emissions

Among all ecosystems, average annual N₂O emissions ranged from 0.14 ± 0.01 kg N₂O-N ha⁻¹ in the POP system to 2.5 ± 0.2 in the NT system (Fig. 2a). In the annual cropping systems, across all management practices the soybean phase of the rotation contributed the least to overall N₂O fluxes (13–24% of total fluxes; calculated from Fig. 2b) with the majority of emissions during the growing season (Table 1). The corn and wheat phase contributions differed by management: Under CT management, corn contributed 31% of total CT system fluxes vs. wheat's 56%; under NT, the respective percentages were 38% and 40%; and with cover crops (RI and BIO), the respective percentages were ~46% of total RI and BIO fluxes for corn and ~31% for wheat (Fig. 2b). The majority of emissions during the corn phase of all annual systems was during the growing season, while during the wheat phase of all annual systems the growing season contribution was ~50% (Table 1). Additional losses of 5–15% or up to ~0.2 kg N₂O-N ha⁻¹ yr⁻¹ for the CT system during the winter thaws (Ruan & Robertson, *in review*) were unaccounted for in the present study.

Soil N availability and N₂O emissions

Cumulative annual (crop year for annual crops, see *Methods* section) N₂O emissions were correlated with NO₃⁻ production potentials (Fig. 3a; $R^2 = 0.454$, $P = 0.0016$) and, for systems with perennial vegetation, with soil extractable NO₃⁻ pools (Fig. 3b), but not with soil extractable NH₄⁺ pools (Fig. 3c). The correlation between N₂O emissions and extractable NO₃⁻ pools within perennial systems exhibited a strong linear relationship (Fig. 3b; $R^2 = 0.717$, $P = 0.016$). The inclusion of annual cropping systems, however, substantially negated this relationship (Fig. 3b). Overall, in systems where NH₄⁺ pools were greater than NO₃⁻ pools (i.e., grass- and tree-dominated successional ecosystems where the ratio of NH₄⁺-N to NO₃⁻-N was >2; Fig. 3d), cumulative emissions were lower and less variable than in systems where the ratio was lower (e.g., annual crop CT and NT rotations; Fig. 3d).

Emission intensities and emission factors

Emission intensities (Eqn 1) were similar across annual cropping systems with somewhat higher intensities in

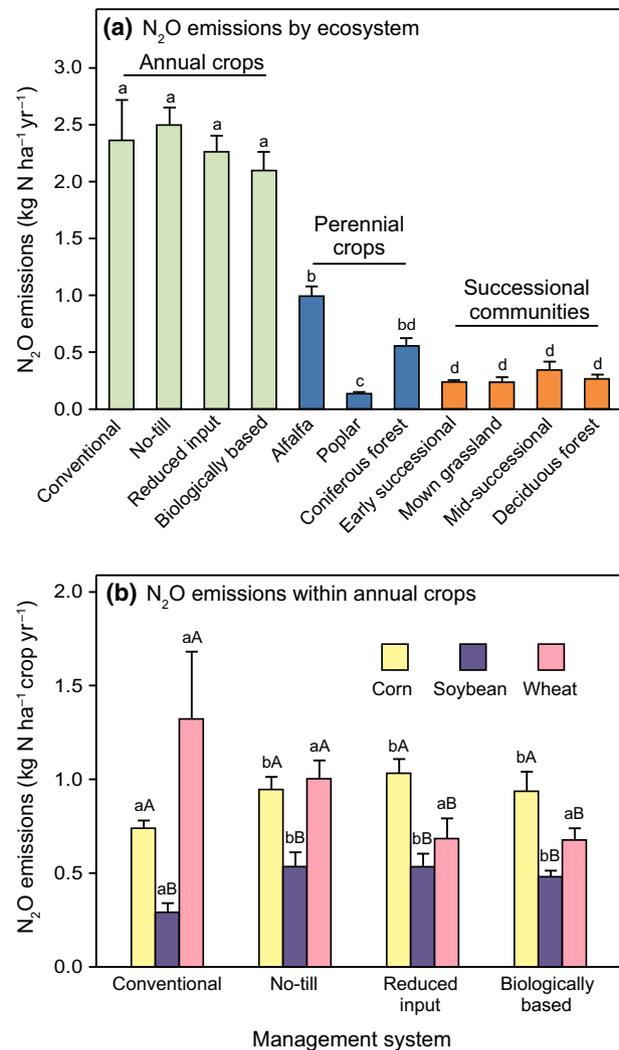


Fig. 2 Average cumulative annual N₂O emissions between 1991 and 2011 (mean \pm standard error) from annual and perennial systems. Panel (a) by system (all rotation phases for annual crops); panel (b) by rotation phase (on crop year basis) for annual crops. For means and error terms $n = 4$ replicate plots for conventional, no-till, reduced input, biologically based, alfalfa, poplar, early successional, and mown grassland systems and $n = 3$ for coniferous forest, mid-successional, and deciduous forest systems. Significant differences among systems are represented by different lowercase letters (e.g., corn under CT is different from corn under NT, RI, and BIO management). Uppercase letters signify differences ($P < 0.1$) among annual crops within the same management system.

the BIO system as compared to other systems (Table 2). Within systems, corn in the CT and NT systems had a lower intensity compared with its intensities in systems with cover crops in the rotation: 0.102 ± 0.021 and 0.103 ± 0.017 kg N₂O-N Mg⁻¹ harvested grain vs. 0.120 ± 0.046 and 0.165 ± 0.041 kg N₂O-N Mg⁻¹ harvested grain in RI and BIO systems, respectively (Table 2). The emission intensity of the soybean phase

Table 1 Percent of total cumulative annual N₂O emissions from annual cropping systems emitted during in-season (crop present) and off-season (crop absent) portions of the crop year. Mean (\pm standard error; $n = 4$ replicate blocks)

Annual system	Corn year*		Soybean year*		Wheat year*	
	In-season	Off-season	In-season	Off-season	In-season	Off-season
Average duration (days)	170	210	142	12	265	302
Conventional (CT, %)	91	9	100	0	67	33
No-till (NT, %)	90	10	100	0	56	44
Reduced input (RI, %)	93	7	100	0	46	54
Biologically based (BIO, %)	89	11	97	3	46	54

*Crop year as defined in Methods section. The discrepancy between the sum of 'in-season' and 'off-season' and average cumulative annual emissions is due to differences in linear interpolation and total interpolated days.

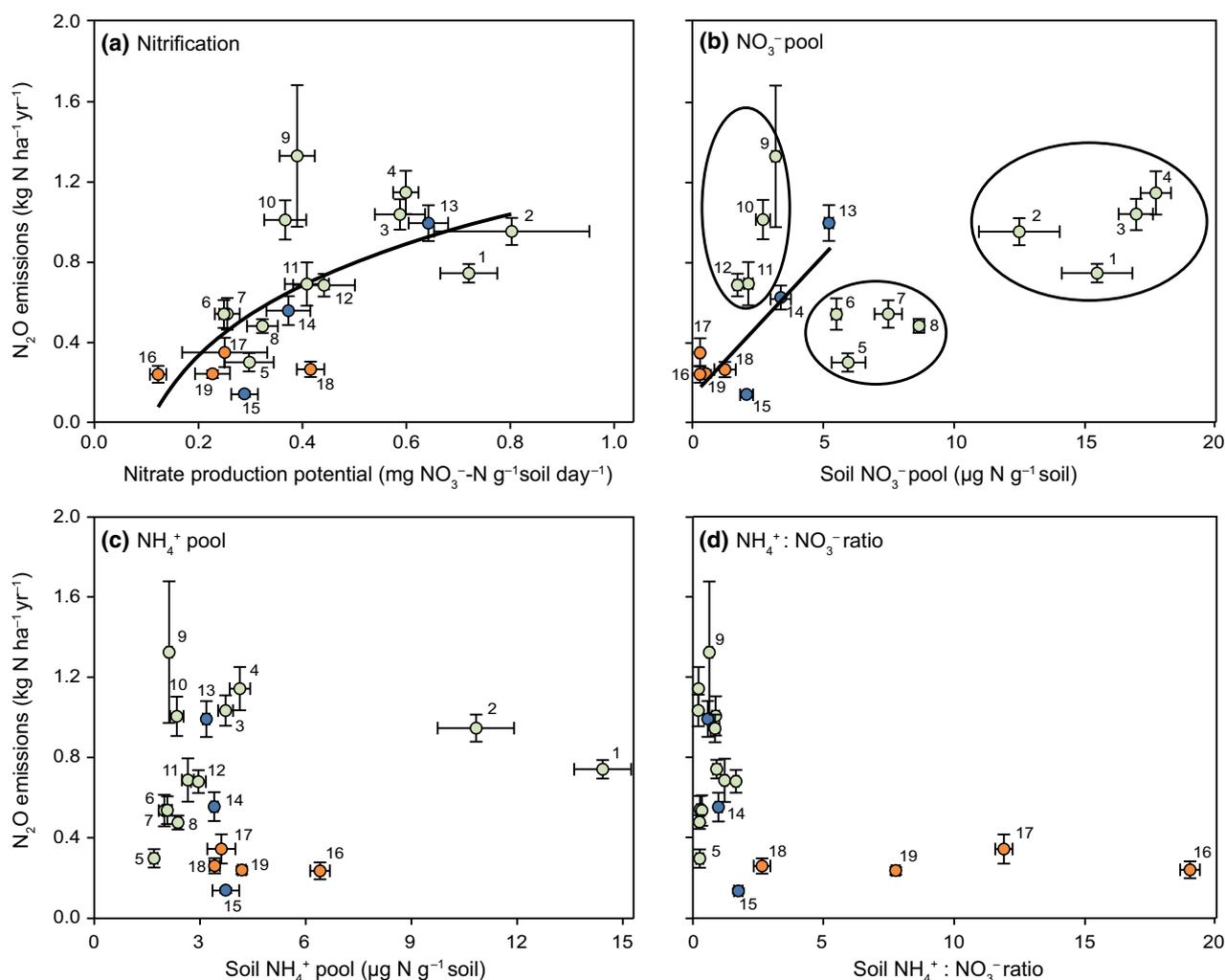


Fig. 3 Relationships between cumulative annual N₂O emissions (crop year for annual crops) and (a) nitrification (NO₃⁻ production potential; mg NO₃⁻-N g⁻¹ soil day⁻¹), (b) extractable soil NO₃⁻ pool (μg N g⁻¹ soil), (c) extractable soil NH₄⁺ pool (μg N g⁻¹ soil), and (d) ratio between extractable soil pools of NH₄⁺ and NO₃⁻. All systems are numbered between 1 and 19: 1 – CT corn, 2 – NT corn, 3 – RI corn, 4 – BIO corn, 5 – CT soybean, 6 – NT soybean, 7 – RI soybean, 8 – BIO soybean, 9 – CT wheat, 10 – NT wheat, 11 – RI wheat, 12 – BIO wheat, 13 – AA, 14 – CF, 15 – POP, 16 – NTG, 17 – SF, 18 – DF, and 19 – ES. Green symbols represent perennial herbaceous vegetation (DF, ES, and NTG), blue symbols represent perennial vegetation (CF, AA, and POP), and brown symbols represent annual systems (CT, NT, RI, and BIO). Correlation between N₂O emissions and nitrification (a) is logarithmic $F = y_0 + a \times \ln(\text{abs}(x))$; $a = 0.49 \pm 0.13$, $y_0 = 1.15 \pm 0.14$; $R^2 = 0.454$, $P = 0.0016$. Correlation between NO₃⁻ pool and soil N₂O emissions in perennial systems (b) is linear $F = y_0 + ax$; $a = 0.14 \pm 0.04$, $y_0 = 0.14 \pm 0.01$; $R^2 = 0.7165$, $P = 0.016$. Circles on panel (b) identify the three rotation phases (corn, soybean, wheat) of the annual cropping systems.

Table 2 Emission intensities (cumulative N₂O fluxes divided by agronomic yield, Equation 1) for the harvested cropping systems; lower intensities are better from a climate change mitigation perspective

Crop/management	Conventional (CT)	No-till (NT)	Reduced input (RI)	Biologically based (BIO)
	kg N ₂ O-N Mg ⁻¹ yield*			
Overall‡	0.213 (0.165–0.280)	0.202 (0.178–0.224)	0.209 (0.191–0.236)	0.263 (0.223–0.288)
By crop†				
Corn	0.102 (0.021) ^A	0.103 (0.017)	0.120 (0.046)	0.165 (0.041)
Soybean	0.083 (0.029) ^{Aa}	0.130 (0.050) ^b	0.156 (0.044) ^b	0.146 (0.024) ^b
Wheat	0.229 (0.106) ^B	0.165 (0.062)	0.168 (0.028)	0.230 (0.053)

*Yield measured as grain in corn, soybean, and wheat. Data were analyzed as a two-way factorial with treatments and crops and the interaction between them as fixed factors. Experimental blocks and plot effects were treated as random factors with plots used as an error term for testing treatment effects. Years and the interaction between years and treatments were treated as random effects. The residuals were checked for normality and homogeneity of variances. As both normality and homogeneous variance assumptions were violated, the data were log-transformed for the analysis. The table reports back-transformed values of the means and standard errors from the raw data.

†Values are means (± standard error, $n = 4$ replicate blocks). Lowercase letters indicate significant differences within rows across columns ($P < 0.1$). Uppercase letters indicate significant differences within columns across rows ($P < 0.1$).

‡Overall values (top row only) are means with 95% confidence intervals in parentheses, $n = 4$ replicate blocks.

in the CT system was lower than that of corn under CT management, and was significantly lower than emission intensities of soybean in all other systems (Table 2). Wheat emission intensities were not significantly different across different management systems, but in the CT system wheat emission intensities were significantly higher than emission intensities of other crops under the same management (Table 2).

Among different indirect methods for N₂O emission factor estimation, the IPCC (Tier 1, including emissions due to NO₃⁻ leaching) and ΔEF methods estimated closer to measured emissions than the surplus approach (Table 3, Fig. S4). IPCC-, ΔEF-, and surplus-based emission factors, calculated from measured N inputs and outputs, were higher than measured emission factors during the corn phase of all annual systems (Table 3) except the BIO system. For the soybean phase, measured emissions were much lower than surplus approach estimates, but similar to ΔEF- and IPCC-based estimates (Table 3). For the wheat phase, measured emissions were also similar between IPCC- and ΔEF-based estimates in annual systems (Table 3). The surplus approach, however, largely overestimated soil N₂O fluxes irrespective of management (Fig. S4).

Discussion

Long-term patterns of N₂O production

All studied systems exhibited very high variability of daily N₂O fluxes, typical of many other systems (reviewed by Henault *et al.*, 2012), and stems from both temporal and spatial variabilities. Variability was especially high in the annual systems, likely due to

Table 3 Nitrous oxide emissions from the annual cropping systems (kg N₂O-N ha⁻¹ per calendar year, not crop year): measured emissions (means ± standard error, $n = 4$ replicate blocks) and direct emissions estimated by IPCC Tier 1 [indirect emissions in brackets], ΔEF, and surplus methodologies

System	Measured*	Estimated		
		IPCC‡	ΔEF§	Surplus†
	kg N ₂ O-N ha ⁻¹ yr ⁻¹			
Conventional (CT)				
Corn	0.74 (0.04)	1.71 [0.25]	1.35	1.93
Soybean	0.39 (0.04)	0.39 [0.04]	0.33	2.14
Wheat	1.25 (0.36)	1.21 [0.17]	0.88	1.67
No-till (NT)				
Corn	0.91 (0.06)	1.64 [0.17]	1.36	1.95
Soybean	0.71 (0.07)	0.40 [0.03]	0.37	2.20
Wheat	0.92 (0.10)	1.20 [0.11]	0.93	1.77
Reduced input (RI)				
Corn	1.06 (0.08)	1.44 [0.1]	1.21	2.03
Soybean	0.62 (0.07)	0.48 [0.03]	0.50	1.93
Wheat	0.61 (0.12)	0.68 [0.05]	0.48	2.11
Biologically based (BIO)				
Corn	1.08 (0.10)	1.01 [0.08]	0.77	2.08
Soybean	0.62 (0.04)	0.40 [0.03]	0.36	2.02
Wheat	0.62 (0.05)	0.28 [0.04]	0.17	2.25

*Annual, not crop year, basis.

†Calculated according to van Groenigen *et al.* (2010).

‡Calculated according to IPCC Tier 1 emission factor (De Klein *et al.*, 2006): 1% of all of inputs; additional indirect emissions [in brackets] are based on leaching losses.

§Calculated according to Shcherbak *et al.* (2014).

postfertilization emissions from those fertilized (CT, NT, and RI) and post-tillage emissions from those cover-cropped (RI and BIO).

We found overall higher emissions from more intensively managed systems and from N-fixing crops. Emissions decreased with increasing system complexity and perennality except for coniferous tree plantations. Cumulative N₂O emissions measured in our fertilized annual systems (Fig. 2) were generally lower than those measured in other studies (e.g., Parkin & Kaspar, 2006; Rees *et al.*, 2013), probably due to our conservative use of N fertilizer – we fertilize based on N response trials at the site and university recommendations (Millar & Robertson, 2015), but perhaps also due to our regularized sampling regime that misses some event-based fluxes (Barton *et al.*, 2015). The less managed (i.e., mown never-tilled grassland and deciduous forest) and seminatural (conifer plantation and successional) ecosystems exhibited fluxes similar to those reported elsewhere (Stehfest & Bouwman, 2006).

The relatively high N₂O fluxes in our coniferous tree plantations (CF) are surprising. While high fluxes in the annual cropping systems and alfalfa can be readily explained by high soil N availability from N fertilizer inputs and N₂ fixation (Robertson *et al.*, 2000), the high fluxes observed in CF are less easily explained and may be related to a number of other factors. First, the conifer canopy structure may affect stemflow and N delivery to soil, which may result in the development of high concentration gradients of N and available C between trees and therefore serve to concentrate resources for denitrification into emissions ‘hot spots’ (Robertson *et al.*, 1988; Butterbach-Bachl *et al.*, 2002a). Second, conifers prefer NH₄⁺ as their N source (Kronzucker *et al.*, 1997), which may lower competition between plants and denitrifies for available NO₃⁻ and thereby favor denitrification (Fig. 3d). Finally, high N₂O emissions in CF may result from a lower soil pH, which can cause a higher N₂O/N₂ ratio during denitrification (Jangid *et al.*, 2011; Robertson & Groffman, 2015).

Our lowest N₂O fluxes were measured in the grass-dominated and deciduous forest ecosystems irrespective of successional stage and are most likely related to plant cover: Perennial vegetation together with the low N inputs suppress soil N availability (Fig. 3b), leading to lower emissions as has been shown for other perennial systems (Butterbach-Bahl *et al.*, 2013; Oates *et al.*, 2016).

Long-term emissions related to crop management and different crop types

Full-rotation cumulative emissions for annual crops were similar among different management systems, as was the case for the first decade of emissions at this site (Robertson *et al.*, 2000). However, cumulative emissions for individual crops within rotations (rotation phases)

did significantly differ, as noted below, and as well interacted with tillage, underscoring the importance of rotations as well as N fertilizer and tillage management for regulating N₂O emissions.

Soil tillage. We found no overall full-rotation differences due to tillage during the 20 years of our study, and no shifts in emissions trends between the first and second decades. These results are similar to expectations derived from van Kessel *et al.*'s (2013) no-till meta-analysis, which predicts no general differences in N₂O emissions between NT and CT systems in humid cropping systems. We did, however, find rotation phase differences: 24% lower NT emissions for the wheat (only) phase of the rotation that over the full rotation was offset by 28 and 81% increases for the corn and soybean phases, respectively. Thus, our results support a tillage effect that interacts with rotation phase – lower emissions for NT wheat but significantly higher emissions for NT corn and soybean. Because each rotation phase is managed differently depending on specific crop needs, these tillage differences are not necessarily a species effect but rather a cropping systems effect, with specific factors that contribute to the differences – including species – not fully extractable from this analysis.

Annual crop type. Although there were no cumulative N₂O flux differences among any of the annual cropping systems, we found substantial differences among individual crops (rotation phases) within systems (Fig. 2b). For example, regardless of management system, soybean years exhibited lower annual cumulative fluxes than did corn or wheat years (Fig. 2b). Mostly this is due to the shorter crop year for soybeans (~5 months) in our rotation rather than to lower average daily soybean fluxes. In fact, average daily fluxes for soybeans were similar or higher than daily fluxes in wheat (Fig. S2). But because the wheat phase in our rotation is planted within days of soybean harvest there is no overwinter fallow period as would be present if a summer annual like corn were to follow soybean (Table 1). Considering only the May–October growing season, however, the corn crop had higher cumulative fluxes than soybean and wheat crops, except for the CT system where wheat had the highest in-season emissions (Table 1).

Within specific crops, there were also significant management effects on N₂O fluxes. For example, approximately two times more N₂O were emitted from the wheat phase of the CT system than from the wheat phase of the RI and BIO systems (Fig. 2b). This may be a cover crop effect: In the RI and BIO systems, continuous soil cover is provided by cover crops that are frost-

seeded into the wheat the prior winter; after wheat is harvested in July, germinated but light-suppressed cover crops are released to grow rapidly, at the same time scavenging residual N from the soil, thereby likely suppressing N₂O production. Cover crops may also suppress N₂O fluxes during winter and early spring thaws (Wagner-Riddle & Thurtell, 1998), although more winter-time measurements in well-drained soils are needed to generalize (Basche *et al.*, 2014).

That the corn phase of the CT system emitted less N₂O than did the NT, RI, and BIO systems (Fig. 2b) may be related to substantially higher nitrate leaching from CT systems (Syswerda *et al.*, 2012), leaving less for microbial N₂O production. This may also explain the lower N₂O emissions in CT soybeans as compared to soybeans in the other management systems (Fig. 2b). Although indirect emissions calculated as per IPCC (De Klein *et al.*, 2006) from leached nitrate would add between 30 and 250 g N₂O-N ha⁻¹ crop yr⁻¹ to our measured emissions (Table 2), the overall trend among management systems would remain unchanged.

Environmental attributes associated with daily and cumulative annual fluxes

Our analysis of the relationship between cumulative N₂O fluxes and N availability (Fig. 3) supports earlier findings of a positive correlation (e.g., Matson & Vitousek, 1987; Davidson *et al.*, 2000; Robertson *et al.*, 2000). However, the relationship for nitrification (microbial nitrate supply) differs from that for nitrate pools, which additionally include the influence of added fertilizer N. Using nitrification potentials as an N availability index, we show that in ecosystems with higher NO₃⁻ production levels, the association between N₂O and NO₃⁻ production becomes logarithmic (Fig. 3a). Our logarithmic pattern suggests that at some high rate of NO₃⁻ production (in Fig. 3a ~0.4 μg NO₃⁻-N g⁻¹ soil day⁻¹), increasing amounts of additional NO₃⁻ from nitrifiers have a proportionately less effect on N₂O flux. This implies a possible N₂O production saturation threshold similar to that found by Bowden *et al.* (2000), who explained a forest soil threshold by lack of another resource such as carbon. Davidson *et al.* (2000) found a similar pattern for tropical forest soils as did Schelde *et al.* (2012) for arable lands in Denmark.

NO₃⁻ pool size predicts cumulative N₂O emissions (Fig. 3b) only for systems with perennial vegetation. The linear relationship here is similar to those for a broad variety of natural ecosystems (Matson & Vitousek, 1987; Groffman *et al.*, 2000). That inclusion of the annual cropping systems eliminates the relationship suggests an overriding effect of management systems and in particular the influence of specific crops

(rotation phases): All three crops (corn, soybean, and wheat) group separately despite substantial management system differences (Fig. 3b).

Higher emissions from the wheat portions of rotations as compared to the soybean portions despite lower soil N availability (Fig. 3b) represent how management overrides controls on soil N₂O emissions by soil extractable NO₃⁻ pool.

The lack of a significant correlation between the soil NH₄⁺ pool and soil N₂O emissions in our study, together with the significant correlation with the soil NO₃⁻ pool and nitrification potentials (NO₃⁻ production), suggests that denitrification is the dominant process of N₂O production in these soils, and is in agreement with Ostrom *et al.* (2010), who at the same site used isotopologue patterns to identify denitrification as a major N₂O source. This interpretation is also consistent with the bimodal relationship we found between annual N₂O emissions and the ratio of soil NH₄⁺ to NO₃⁻ (Fig. 3d), which suggests that emissions are lower when the soil inorganic N pool is dominated by NH₄⁺, and higher when NO₃⁻ dominates, implying that NO₃⁻ pool size rather than total extractable inorganic N is a better predictor of soil N₂O emissions.

Very little of the variation in daily N₂O fluxes can be explained by our environmental measurements. Statistical empirical models derived from our 20 years of data for soil N, moisture, and temperature can explain only 20% of variability in daily N₂O fluxes (Table S1). Such low explanatory power may be due to (i) high variability in N₂O fluxes across time and space (Groffman *et al.*, 2009), (ii) other environmental attributes that affect N₂O production but were not measured (e.g., carbon availability for denitrification; Hill & Cardaci, 2004), and (iii) lagged responses that may not be apparent without very frequent measurements (Barton *et al.*, 2015).

Emission intensities and general methods for estimating N₂O emissions

Emission intensities provide information for reducing the relative agricultural climate impact through intensification (Snyder *et al.*, 2009). Lower intensities or yield-scaled flux values (flux per unit yield), which can be achieved by higher yields or lower fluxes or both, are more climate-friendly. Directly measured emission intensities (Eqn 1) were surprisingly similar for all annual cropping systems and crops (Table 2). Across systems, only for soybeans did intensities differ: Emission intensities for CT soybeans were lower than for NT, RI, and BIO soybeans. Within systems, only in CT did crop phases differ: CT wheat had higher emission intensities than did soybeans or corn. As for cumulative

fluxes above, these patterns speak to the importance of rotations as well as N fertilizer and tillage management for regulating N₂O emission intensities.

A comparison of our direct measurements of N₂O in annual crops with generalized estimates based on IPCC Tier 1 accounting methods (De Klein *et al.*, 2006), the N surplus-based approach (van Groenigen *et al.*, 2010), and the Δ EF approach (Shcherbak *et al.*, 2014) showed varying levels of agreement (Table 3; Fig. S4). IPCC Tier 1-based estimates were within 50% of measured values, ranging from a 50% underestimate for cumulative average emissions from BIO wheat to a 130% overestimate of emissions from CT corn. Over all managements and rotation phases, the IPCC Tier 1 approach provided an 11% overestimate.

The Δ EF-based approach provided estimates that were within 80% of measured values, ranging from a 70% underestimate for BIO wheat to an 80% overestimate for CT corn. Over all managements and rotation phases, the Δ EF-based approach provided an 11% underestimate.

The surplus-based approach overestimated measured fluxes for all rotation phases and management systems by 180% on average, ranging from a 30% overestimate for CT wheat to a 450% overestimate for CT soybean.

Among different crop phases, the IPCC Tier 1 and Δ EF approaches both overestimated corn phase emissions by 62% and 29%, respectively; underestimated soybean emissions by 25% and 31%; and underestimated wheat emissions by 4% and 31%. The surplus-based approach overestimated corn, soybean, and wheat phases by 115%, 274%, and 159%, respectively.

Good agreement between empirical and IPCC Tier 1 and Δ EF emission estimates ($\pm 11\%$ averaged across all management systems and crop phases) is likely due to N availability being a major driving factor in both estimation methods. That both methods reasonably estimated emissions over all management systems and crop phases when considered together suggests broad applicability of both approaches. However, this applicability applies in this case only to crops fertilized at conservative rates based on site-specific N fertilizer rate trials; as shown in other analyses (e.g., Hoben *et al.*, 2011; Shcherbak *et al.*, 2014), the Δ EF approach better estimates N₂O fluxes for crops fertilized in excess of crop need.

Management implications

There are a number of generalizations and insights relevant to broad patterns of N₂O emissions that can be gleaned from this long-term analysis. First is that N₂O fluxes are, in general, substantially lower in nonlegume

perennial systems than in perennial systems, regardless of management intensity. For example, fluxes in intensively managed hybrid poplar systems were as low as those in unmanaged successional systems and substantially lower than those in annual crops and alfalfa.

Second, and more uniquely, rotation phase (crop type) appears to matter greatly. Within rotations, and often interacting with management system, emissions from corn differed from soybean differed from wheat. But differences occurred only within particular management systems – in some systems, emissions were indifferent to crop phase. Understanding the factors that underlie these interactions may provide the opportunity to better design cropping systems – including the addition and order of rotation phases – to reduce N₂O fluxes from annual crops.

Third, across all ecosystems N availability was the best predictor of N₂O fluxes only for systems with perennial vegetation – for annual systems, management effects overrode the simple effects of N availability based on average soil nitrate pool sizes. Likely this is due to management effects associated with specific crops: Crop phases, not management systems, grouped remarkably close together in a regression of N₂O flux \times N availability (Fig. 3b).

Fourth, no-till does not significantly affect N₂O emissions overall, but does interact with rotation phase. Relative to conventional tillage, no-till increased emissions during corn and soybean years and decreased emissions during wheat years. This too has implications for rotation complexity insofar as most US Midwest crops are either continuous corn, corn–soybean, or (less commonly) corn–soybean–wheat rotations.

Fifth, cover-cropped systems also exhibited no overall emission differences from conventional systems, but again mainly due to lower emissions during wheat years' offsetting somewhat higher emissions during corn and soybean years.

Sixth, emission intensities were remarkably similar among all annual cropping systems with the exception of two management \times crop phase interactions. This underscores the importance of matching N fertilizer rates to crop need: More liberal fertilizer use would have increased emission intensities; that emission intensities were similar for fertilized (CT, NT) and non-fertilized (BIO) crops suggests the potential for very efficient fertilizer use.

Seventh, of general estimators of annual crop emissions, both IPCC Tier 1 and Δ EF approaches provided reasonable estimates of overall emissions, on average either 11% over (IPCC Tier 1) or 11% under (Δ EF) measured emissions. This suggests broad applicability, although estimates were not so robust for individual management systems \times crop phase combinations.

However, in this case applicability applies mainly and perhaps only to crops fertilized at conservative, recommended rates; in a recent meta-analysis, Shcherbak *et al.* (2014) showed that the ΔEF approach better estimates N_2O flux for crops fertilized in excess of crop need.

Finally, that simple environmental factors were poor predictors of N_2O emissions even within this same climate \times soil type experiment suggests the need for a deeper understanding of the underlying factors that drive variable N_2O fluxes. Within annual crops, the importance of crop phases suggests that these factors are related to C and N management differences not yet well understood, and as well to plant–microbe interactions that affect microbial N_2O producers and the microhabitat and community they inhabit.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The distributional parameters of daily soil N₂O fluxes.

Table S2. Multi-linear regression analysis for average daily N₂O emissions with soil nitrate concentrations, soil moisture and temperature.

Fig. S1. Daily average soil N₂O emissions for specific crop years.

Fig. S2. Growing season average daily soil N₂O emissions by crop in annual cropping systems.

Fig. S3. Time series of soil N₂O emissions from annual cropping systems between years 1991 and 2011.

Fig. S4. Comparison between measured N₂O emissions and those estimated by IPCC Tier 1, EF, and Surplus approaches.

Fig. S5. Correlation between annual (calendar year) and crop year (as defined in the Methods section) cumulative N₂O emissions.