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Nitrification is a minor source of nitrous oxide (N_2O) in an agricultural landscape and declines with increasing management intensity

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Abstract

The long-term contribution of nitrification to nitrous oxide (N_2O) emissions from terrestrial ecosystems is poorly known and thus poorly constrained in biogeochemical models. Here, using Bayesian inference to couple 25 years of in situ N_2O flux measurements with site-specific Michaelis–Menten kinetics of nitrification-derived N_2O, we test the relative importance of nitrification-derived N_2O across six cropped and unmanaged ecosystems along a management intensity gradient in the U.S. Midwest. We found that the maximum potential contribution from nitrification to in situ N_2O fluxes was 13%–17% in a conventionally fertilized annual cropping system, 27%–42% in a low-input cover-cropped annual cropping system, and 52%–63% in perennial systems including a late successional deciduous forest. Actual values are likely to be <10% of these values because of low N_2O yields in cultured nitrifiers (typically 0.04%–8% of NH_3 oxidized) and competing sinks for available NH_4^+ in situ. Most nitrification-derived N_2O was produced by ammonia-oxidizing bacteria rather than archaea, who appeared responsible for no more than 30% of nitrification-derived N_2O production in all but one ecosystem. Although the proportion of nitrification-derived N_2O production was lowest in annual cropping systems, these ecosystems nevertheless produced more nitrification-derived N_2O (higher V_{max}) than perennial and successional ecosystems. We conclude that nitrification is minor relative to other sources of N_2O in all ecosystems examined.

KEYWORDS
agriculture, ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), forest, greenhouse gas, nitrification, row crop, soil nitrogen

1 | INTRODUCTION

Nitrous oxide (N_2O) is a potent greenhouse gas with a 100-year global warming potential ~300 times higher than CO_2, and has the third largest radiative forcing among the biogenic greenhouse gases (Myhre et al., 2013). N_2O also depletes stratospheric ozone (Revell et al., 2012). Globally, soils are the dominant sources of both anthropogenic and natural emissions of N_2O, with 1.7–4.8 Tg N_2O-N $year^{-1}$ emitted by agricultural soils and 3.3–9.0 Tg N_2O-N $year^{-1}$ from soils under natural vegetation (Ciais et al., 2013).

Ammonia (NH_3) oxidation, the rate-limiting step of nitrification, is performed in soil mainly by aerobic ammonia-oxidizing bacteria

(AOB) and archaea (AOA), and releases N_2O during conversion of NH_3 to nitrite (NO_2^-) and nitrate (NO_3^-). Although the recently discovered complete ammonia oxidizers (comammox bacteria) can also produce N_2O abiotically (Kits et al., 2019), only AOB and AOA are known for potentially significant contributions to global fluxes (Stein, 2020). Denitrification, performed in soil mainly by heterotrophic bacteria, releases N_2O during the stepwise reduction of NO_3^- to N_2O and thence dinitrogen (N_2) when soils are anaerobic (Robertson & Groffman, 2021). Additionally, under hypoxic conditions, AOB that encode nitric oxide reductase (NorB) can reduce NO_2^- to N_2O via NO through the nitrifier denitrification pathway (Stein, 2019). Nitrification and denitrification, including nitrifier denitrification, occur in most soils, and understanding the relative contributions of each is important for informing future N_2O mitigation potentials and strategies, and as well for constraining uncertainties in biogeochemical models of N_2O emissions.

Partitioning N_2O emission pathways between nitrification and denitrification in situ have proved historically challenging. Both aerobic and anaerobic microsites occur within the same soil volume such that nitrification and denitrification often occur simultaneously (Kuenen & Robertson, 1994; Smith, 1980). In general, three types of approaches have been used to attribute N_2O emission sources: specific inhibitors, stable isotope enrichment, and isotopomer analysis. Specific inhibitors have mainly been used in short-term laboratory incubations, where acetylene (C_2H_2) can be used to selectively inhibit NH_3 oxidation at 10 Pa and N_2O reduction at 10 kPa (Robertson & Tiedje, 1987), and 1-octyne can be used to selectively inhibit AOB ammonia monooxygenase (AMO; Taylor et al., 2013, 2015). Isotope enrichment approaches typically use either $^{15}N\text{-}NH_4^+$ or $^{15}N\text{-}NO_3^-$ to differentiate nitrification and denitrification-derived N_2O in short-term laboratory experiments (Stevens et al., 1997). Isotopomers of N_2O reflect the differential intramolecular distribution (site preference, SP) of ^{15}N at α and β positions of the N_2O molecule ($N^{\beta}\text{-}N^{\alpha}\text{-}O$) and have been used to differentiate N_2O sources in both the laboratory (Sutka et al., 2006) and field (Buchen et al., 2018; Opdyke et al., 2009; Ostrom et al., 2010).

Though helpful for identifying biochemical pathways, the use and interpretation of inhibitors and isotope enrichment approaches in situ suffer from the difficulty of achieving homogeneous distributions of added compounds in intact soils with their heterogeneously distributed microsites (Groffman et al., 2006). Artifacts of C_2H_2 use include further concerns of microbial C_2H_2 consumption (Terry & Duxbury, 1985; Topp & Germon, 1986), and as well heterotrophic nitrifiers are resistant to C_2H_2 (Hynes & Knowles, 1982; Schimel et al., 1984). ^{15}N enrichment adds additional N to soils, potentially leading to overestimated rates of nitrification and denitrification especially in non-agricultural soils (Baggs, 2008). The isotopomer approaches can be confounded by the overlap of SP values among different microbial processes. For example, N_2O from fungal denitrification has an SP of 37‰, which is also within the range of nitrification (hydroxylamine oxidation; Sutka et al., 2008). An additional limitation of all three techniques is their short-term nature in light of highly dynamic soil processes known to exhibit substantial temporal variation (Boone et al., 1999) with known effects on N_2O emissions.

An alternative method for assessing the maximum potential importance of nitrification versus other N_2O generating processes in soil is to combine soil-specific kinetics of nitrification-derived N_2O with long-term field N_2O flux measurements. Nitrification kinetics measure a soil's existing potential to nitrify NH_4^+ to N_2O and NO_3^- under conditions unconstrained by resource limitations (Norton & Stark, 2011; Stark & Firestone, 1996), thus allowing maximum potentials for nitrification-derived N_2O emissions to be estimated. Such potentials, if stable in time, might then be combined with field-based measurements of N_2O fluxes to allow calculation of the likely maximum percentage of nitrification-derived N_2O in relation to all other N_2O sources.

Here we combine measured site-specific nitrification kinetics for N_2O production with over 25 years of field-based N_2O fluxes to estimate the maximum potential contribution of nitrification to N_2O emissions along a long-term management intensity gradient in the upper U.S. Midwest. Our replicated ecosystems range from intensively managed annual cropping systems to an unmanaged late successional deciduous forest. We first use short-term laboratory incubations to build Michaelis–Menten kinetics models of $N_2O\text{-}NH_4^+$ relationships, and show them to be seasonally stable. Then we predict the potential maximum nitrification-derived N_2O of each ecosystem by assuming that all microbially available (soil solution phase) NH_4^+ can be oxidized into N_2O. Finally, we use a Bayesian approach to calculate the maximum relative importance of nitrification for N_2O emissions from each ecosystem based on long-term field-based N_2O fluxes.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted in the Main Cropping System Experiment (MCSE) of the Kellogg Biological Station (KBS) LTER site located in southwest Michigan (42° 24'N, 85° 23'W). The MCSE was established in 1988 and includes, on the same soil series, ecosystems that form a management intensity gradient: annual cropping systems, perennial cropping systems, and unmanaged systems at different stages of ecological succession (Robertson & Hamilton, 2015). Most of the ecosystems are replicated in blocks as 1 ha (90 × 110 m) plots. KBS features a temperate climate with an average of 1005 mm annual precipitation distributed evenly throughout the year and a 10.1°C mean annual temperature (30-year mean from 1981). Soils are well-drained Alfisol loams (co-mingled Kalamazoo and Oshtemo series Typic Hapludalfs), formed from glacial till and outwash with some intermixed loess (Crum & Collins, 1995; Luehmann et al., 2016). Average sand and clay contents in surface soils are 43% and 17%, respectively (Robertson & Hamilton, 2015).

We studied two annual cropping systems: conventionally managed (Conventional) and biologically managed (Biologically-based) corn–soybean–winter wheat rotations; a hybrid poplar system (Poplar); and three successional systems of different ecological age: an early successional system (Early successional), a never-tilled annually mown grassland system (Grassland), and a late successional

deciduous forest (Deciduous forest). The Biologically-based system is certified organic but receives no compost or manure. The two annual cropping systems and the Poplar and Early successional systems are replicated in each of six randomized blocks; four were selected for this study. The Grassland system is replicated four times and the Deciduous forest system is replicated three times.

The Conventional agricultural system received standard rates of N fertilizer: 137 ± 20 kg N ha^{-1} $year^{-1}$ for corn and 77 ± 17 kg N ha^{-1} $year^{-1}$ for wheat (Gelfand et al., 2016). Soybeans received <5 kg N ha^{-1} $year^{-1}$. Nitrogen fertilizer was mostly applied as urea-ammonium nitrate (28-0-0). The Biologically-based agricultural system received no N fertilizer; instead, winter cover crops included the legume red clover (*Trifolium pratense* L.) following wheat prior to corn, and annual rye grass (*Lolium multiflorum* L.) following corn prior to soybean. Red clover was frost-seeded into wheat in March, lay dormant over winter, and was terminated just prior to planting corn the following spring. Over this period, it fixes ~35–53 kg N ha^{-1} (Snapp et al., 2017). Both red clover and ryegrass scavenge soil N otherwise leached or denitrified. Tillage for both systems included chisel plowing to a depth of 15–18 cm followed by secondary tillage. Herbicides were used to suppress weeds in the Conventional system and additional tillage provided weed control in the Biologically-based system.

The Poplar system was planted in 1989 to *Populus* × *canadensis* Moench "Eugenei." Fertilizer was applied as 123 kg N ha^{-1} ammonium nitrate in the establishment year and the first harvest was in 1999. After the second harvest in 2008 and one fallow year, *Populus nigra* × *P. maximowiczii* "NM6" was planted in 2009. Fertilizer was then applied once in 2011 at 157 kg N ha^{-1} as ammonium nitrate.

The Early successional system was abandoned from agriculture in 1989 and has been burned every spring since 1997 to exclude woody plants. Canada goldenrod (*Solidago canadensis* L.), Kentucky bluegrass (*Poa pratensis* L.), arrow leaved aster (*Aster sagittifolius*), and timothy grass (*Phleum pratense* L.) were dominants at the time of this study (https://lter.kbs.msu.edu/datatables/237). The Grassland system was established on a cleared woodlot ca. 1959 and has never been plowed, but likely received manure in the 1960s. Grass is mown annually to inhibit woody species. Current dominants include smooth brome grass (*Bromus inermis* Leyss.), Canada goldenrod (*Solidago canadensis* L.), tall oatgrass (*Arrhenatherum elatius* L.), blackberry (*Rubus allegheniensis* Porter), sassafras (*Sassafras albidum*), and Kentucky bluegrass (*P. pratensis* L.). The late successional Deciduous forest is unmanaged and has never been cleared or plowed. Overstory dominant species include red oak (*Quercus rubra* L.), pignut hickory (*Carya glabra* Mill.), white oak (*Q. alba* L.), and sugar maple (*Acer saccharum* Marsh.).

2.2 | Soil sampling

Soils were sampled seasonally for testing nitrification-derived N_2O potentials, once for nitrification-derived N_2O kinetics, and once for solution-phase NH_4^+ partitioning. For nitrification-derived N_2O potentials, soils from all systems but the Grassland were sampled in summer (late June 2016), winter (early December 2016), and spring (early May 2017). Grassland soils were sampled when determining the kinetics of nitrification-derived N_2O, for which samples were collected in 2017 from all systems from early fall (late September) to early winter (early December), after having first established no seasonal patterns for nitrification-derived N_2O potentials. For determining solution-phase NH_4^+ partitioning, soil samples were collected in summer (late June) 2019 in all systems. For all experiments, five random samples were taken at either 0–15 cm (N_2O potentials and N_2O kinetics experiments) or 0–25 cm (solution-phase NH_4^+ partitioning) depths and composited by field replicate. Soils were passed through a 4 mm mesh immediately and sieved soils were stored at 4°C before analysis within 4 days.

2.3 | Nitrification potentials

To evaluate potentials for nitrification-derived N_2O, 5 g of freshly sieved soil was placed into a 155 ml Wheaton bottle amended with 50 ml deionized water containing 10 mM NH_4Cl to maximize nitrification-derived N_2O emissions (Figure 1). We used 1-octyne, a recently developed and tested chemical inhibitor of AOB AMO to distinguish relative contributions from AOA and AOB (Taylor et al., 2013, 2015). We used a gradient of octyne concentrations ranging from 0 to 10 μM aqueous concentration (C_{aq}) to test for optimal inhibition and we found 4 μM C_{aq} sufficient to inhibit AOB in all soils (Liang et al., 2020), which is in agreement with previous studies (Taylor et al., 2013). Capped bottles with or without 4 μM C_{aq} octyne were immediately placed on a shaker table and shaken for 24 h at a constant speed of 200 rpm at room temperature (25°C). This method inhibits denitrification-derived N_2O as soil slurries are continuously aerated by high-speed shaking.

Samples for N_2O were taken at 2 and 24 h and N_2O emission rates were calculated based on N_2O accumulations over 22 h. Slurry pH was buffered naturally as no apparent pH change was detected during the incubation. Emissions of N_2O in the presence of octyne are attributed to AOA. Emissions of N_2O from AOB are calculated as the difference between N_2O without octyne (total nitrification-derived N_2O) minus N_2O from AOA. Although comammox could also contribute to N_2O emissions, recent evidence suggests that comammox plays only a very minor role in soil nitrification (Kits et al., 2019; Robertson & Groffman, 2021; Wang et al., 2020). N_2O samples were stored overpressurized in 6 ml N_2-flushed glass vials (Exetainers, Labco Ltd). N_2O was measured with a gas chromatograph (Agilent 7890A) coupled to an autosampler (Gerstel MPS2XL) and equipped with a ^{63}Ni electron detector at 350°C and a Porapak Q column (1.8 m, 80/100 mesh) at 80°C (https://lter.kbs.msu.edu/protocols/159).

2.4 | Nitrification kinetics

We placed 5 g of freshly sieved soil from each ecosystem into a 155 ml Wheaton bottle. We then added $(NH_4)_2SO_4$ to make eight

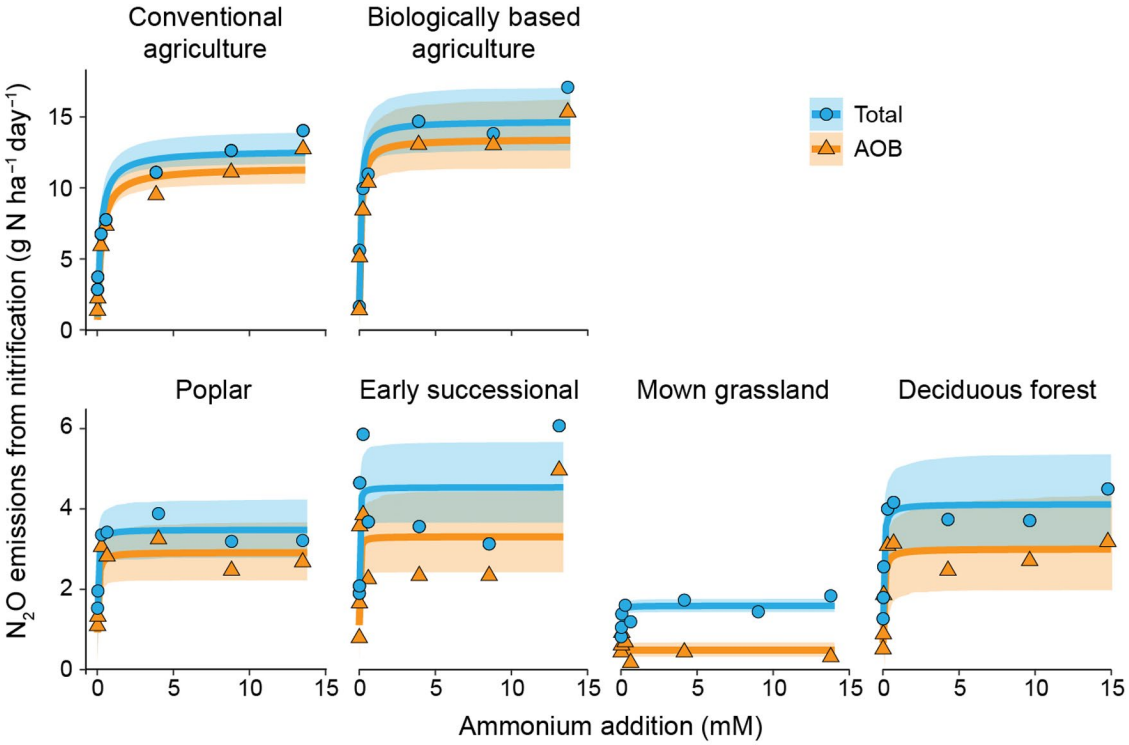

FIGURE 1 The kinetics of nitrification-derived N_2O in soils from different systems varying in management intensities. Michaelis–Menten models were fit to total nitrification-derived N_2O emissions (blue lines) and AOB-derived N_2O emissions (orange lines). Blue circles and orange triangles are the mean N_2O emissions from total and AOB-derived nitrification at each ammonium addition, respectively. Note *y*-axis scale differs by system. Shaded bands represent 95% confidence intervals. Ammonium additions ranged from 0.05 and 15 mM for Poplar and annual cropping systems because N_2O accumulation at 0.01 mM could not be reliably estimated. For all other systems, ammonium additions ranged from 0.01 to 15 mM. AOB, ammonia-oxidizing bacteria; N_2O, nitrous oxide

different NH_4^+ concentrations ranging from 0.01 to 15.0 mM (0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 15 mM NH_4^+) with a final liquid volume of 50 ml. Bottles were capped and placed on a shaker table at a constant speed of 200 rpm at room temperature (25°C) and shaken for 24 h. Initial N_2O samples were taken after 2 h, and we then added either 2.8 ml of octyne stock gas (see Taylor et al., 2013, for octyne stock gas preparation) to create 4 μM C_{aq} concentrations or 2.8 ml of air without octyne. Another set of N_2O samples were taken at 24 h. Nitrification kinetics were based on measured NH_4^+ concentrations, and included both added NH_4^+ as well as NH_4^+ produced from net N mineralization during the incubation. NH_4^+ concentrations were measured by a Lachat QuikChem 8500 flow injection analyzer (Hach).

Kinetics of nitrification-derived N_2O emissions were fit to Michaelis–Menten models using the equation:

$$V = \frac{V_{max} S}{K_m + S} \quad (1)$$

where V is the N_2O emission rate from nitrification, V_{max} is the maximum N_2O emission rate from nitrification under conditions of unlimited substrate (NH_4^+), S is the NH_4^+ concentration, and K_m is the half-saturation constant that represents the NH_4^+ concentration when the N_2O emission rate from nitrification is ½ V_{max}. V_{max} reflects the maximum capacity of a soil to oxidize NH_4^+ and produce nitrification-derived N_2O, and K_m reflects the NH_4^+ affinity of soil AMO.

In addition, because nitrification can be inhibited at very high NH_4^+ concentrations (Suwa, 1994), we also fitted data with Haldane models when appropriate (Koper et al., 2010; Stark & Firestone, 1996):

$$V = \frac{V_{max} S}{K_m + S + S^2/K_i} \quad (2)$$

The Haldane model introduces a third parameter K_i that reflects the maximum NH_4^+ concentration at which nitrification-derived N_2O emissions rates are ½ V_{max}. We performed an Akaike's information criterion (AIC)-based model comparison, followed by an *F*-test to determine model superiority between Michaelis–Menten and Haldane kinetics (Table 1).

2.5 | In situ N_2O flux, soil NH_4^+, and soil bulk density

We used 25 years of in situ N_2O flux data (from 1991 to 2016) to calculate the relative contribution of nitrification to N_2O emissions within each system, except for the Grassland and Deciduous forest systems for which N_2O fluxes were measured from 1992 to 2016 and

1993 to 2016, respectively. Most of these data have been previously published (Gelfand et al., 2016; Robertson et al., 2000). From 1991 to 2012, emissions were sampled every 2 weeks from March/April to November/December with the static chamber method (Holland et al., 1999). Additional winter samples were taken monthly starting from 2013. Square chambers (29 × 29 × 14 cm high) were placed on aluminum bases (28 × 28 × 10 cm high) semi-permanently installed about 3 cm into soil. Gas samples were taken at approximately 20-min intervals during a 1-h sampling period. Volume-based N_2O fluxes were calculated by linearly regressing headspace N_2O concentrations over time (μg N_2O-N L^{-1} min^{-1}), which was then further converted to area-based N_2O fluxes by accounting for the volume of gas in the chamber and soil surface area covered by the chamber (g N_2O-N ha^{-1} day^{-1}; Kahmark et al., 2020). The few headspace fluxes that exhibited nonlinearity were not used in the analysis.

Soil cores for inorganic N determinations were taken approximately biweekly after the soils thawed in the spring, usually in March or April, and discontinued before soils froze, usually in November. Soils were sampled to 25 cm depth from 1989 to 2016 except from 1993 to 2016 for the Deciduous forest system. Soil was sieved through a 4 mm sieve and 10 g of fresh soil were extracted with 100 ml 1 M KCl to determine NH_4^+ concentrations. Soil bulk density (0–10 cm depth) was measured in 2013 when collecting deep core soil samples to a depth of 1 m with a hydraulic probe. Soil was sieved through a 4 mm sieve and then oven-dried at 60°C for 48 h. When present, the weight of gravel (>4 mm) was recorded separately and then discarded. The gravel-free bulk density was calculated as the dry mass of the soil (without gravel) divided by the volume of the core.

2.6 | Microbially available (solution phase) soil NH_4^+

We partitioned long-term KCl extracted soil NH_4^+ pools into sorbed-phase ($srNH_4^+$) and solution-phase ($slNH_4^+$) pools by performing an NH_4^+ sorption capacity assay modified from Venterea et al. (2015). We assume only $slNH_4^+$ is available to soil nitrifiers. Briefly, for each ecosystem, we added 10 g of sieved fresh soils into 100 ml of water containing an NH_4^+ gradient ranging from 0 to 50 mg NH_4^+-N L^{-1} (0, 5, 10, 20, 30, 40, and 50 mg NH_4^+-N L^{-1} generated by $(NH_4)_2SO_4$ addition). Mixtures were shaken on an orbital shaker table at a constant speed of 100 rpm at room temperature (25°C) for 18 h. We centrifuged 10 ml aliquots at 10,000 g at room temperature (25°C) for 15 min. NH_4^+-N was then analyzed by flow injection analysis as above after filtering aliquots through a 1 mm glass fiber filter. We calculated $srNH_4^+$ as the difference between added NH_4^+ ($addNH_4^+$) and the $slNH_4^+$ (measured as above) accounting for soil NH_4^+ contents ($soilNH_4^+$):

$$soilNH_4^+ = NH_{4\,KCl}^+ - NH_{40}^+ \quad (3)$$

$$srNH_4^+ = addNH_4^+ - slNH_4^+ + soilNH_4^+ \left(\text{when } addNH_4^+ > 0\right) \quad (4)$$

$$srNH_4^+ = soilNH_4^+ \left(\text{when } addNH_4^+ = 0\right) \quad (5)$$

where $NH_{4\,KCl}^+$ is the 1 M KCl extractable NH_4^+ concentrations and NH_{40}^+ is the water extractable NH_4^+ concentrations at 0 NH_4^+-N L^{-1} addition. The relationship between $srNH_4^+$ (mg N kg^{-1}) and $slNH_4^+$ (mM) is usually described by a Langmuir model:

$$srNH_4^+ = \frac{\mu \times slNH_4^+}{K + slNH_4^+} \quad (6)$$

where μ (mg N kg^{-1}) is the maximum NH_4^+ content adsorbed by soil and K (mM) is the NH_4^+ concentration in solution phase at which $srNH_4^+$ is ½ μ. We modeled and plotted $srNH_4^+$ against $slNH_4^+$ (Figure S1), which allows one to convert total KCl-based soil NH_4^+ values into $slNH_4^+$ for every NH_4^+ soil measurement taken between 1989 and 2016.

TABLE 1 Comparisons between Michaelis–Menten and Haldane kinetics models for total or AOB-derived N_2O emissions from nitrification

Ecosystem[a]	Nitrification	AIC[b] (Michaelis–Menten)	AIC[b] (Haldane)	*F*-value[c]	*p*-value[c]
Poplar	Total	111	113	0.188	0.668
	AOB	105	106	0.488	0.491
Early successional	Total	143	144	0.134	0.718
	AOB	130	131	1.13	0.298
Grassland	Total	27.9	28.1	1.70	0.202
	AOB	30.2	30.6	1.50	0.233
Deciduous forest	Total	109	111	0.001	0.980
	AOB	106	108	0.049	0.827

Abbreviations: AIC, Akaike information criterion; AOB, ammonia-oxidizing bacteria; N_2O, nitrous oxide.

[a]Data from Conventional and Biologically-based systems were not fit to Haldane models because no signs of inhibition of nitrification-derived N_2O were found.

[b]Models with lower AIC were considered superior.

[c]Models were also compared based on *F*-test. A *p*-value > .05 supports the minimal model as the adequate model.

2.7 | Statistical analysis

2.7.1 | ANOVA for seasonal nitrification-derived N_2O

We converted gravimetric N_2O emissions from the nitrification potential experiment into areal N_2O emissions based on soil depth (15 cm) and bulk density:

$$N_2O_{area} = N_2O_{mass} \times DP \times \frac{BD}{10} \quad (7)$$

where N_2O_{area} is expressed as g N_2O-N ha^{-1} day^{-1} and N_2O_{mass} is expressed as ng N_2O-N g^{-1} dry soil day^{-1}, DP is the soil depth in cm, and BD (0–10 cm depth) is the bulk density expressed as g cm^{-3}.

Potentials for nitrification-derived N_2O were analyzed with PROC GLIMMIX of SAS 9.4 (SAS Institute). The statistical model included 5 ecosystem types ×3 seasons × 2 sources of nitrification-derived N_2O, and the interaction among them was considered fixed factors. Field replicates nested within ecosystem types and the interaction between field replicates and seasons nested within ecosystem types were considered random factors. Analysis of variance (ANOVA) was performed by considering ecosystem types as a whole plot factor and season and sources of nitrification-derived N_2O as subplot and sub-subplot factors. Homogeneity of variance assumptions was checked by Levene's test and normality of residuals was visually inspected. No violations of assumptions were detected. Pairwise comparisons among different ecosystems were conducted and we refer to $p < .05$ (two-sided) as significantly different throughout the paper.

2.7.2 | Model comparisons and kinetic parameters

Total or AOB-derived N_2O emissions from nitrification were fit to both Michaelis–Menten and Haldane kinetics models. We first used the "nls" function in R (version 3.5.0; R Core Team, 2020) to obtain AIC values for each kinetics model. Then we conducted an *F*-test to further determine model superiority using the "anova" function. Models with lower AIC were considered superior, and a *p*-value > .05 supports the minimal model (Michaelis–Menten) as the adequate model (Table 1). Once the appropriate kinetics model (Michaelis–Menten) was selected, V_{max} and K_m for total and AOB-derived N_2O emissions from nitrification for each ecosystem were estimated by the "nls" function (Table 3).

TABLE 2 AIC of field-based nitrous oxide fluxes from different ecosystems fitted with different distributions

	Distribution			
Ecosystem	Log-normal	Gamma	Weibull	Normal
Conventional agriculture	4602	5038	4915	7922
Biologically-based agriculture	5030	5489	5344	8629
Poplar	2303	2881	2659	6378
Early successional	2591	2804	2808	4392
Grassland	1733	1872	1865	3106
Deciduous forest	2452	2690	2648	4687

Abbreviation: AIC, Akaike information criterion.

2.7.3 | Distribution for field N_2O fluxes

In situ N_2O fluxes typically show a highly skewed distribution with a long tail of high values, which makes constraining the range of the mean fluxes challenging (Cowan et al., 2017). N_2O emissions can be assumed proportional to the product of the interactions of multiple biological and environmental variables such as population sizes and activities of soil nitrifiers and denitrifiers, soil moisture, soil temperature, soil inorganic N contents, and soil oxygen status. Thus, we consider multiplicative processes to influence N_2O emissions, which follow log-normal distributions (Limpert et al., 2001):

$$F_{N_2O} \sim lognorm\left(\bar{x}, s^2\right) \quad (8)$$

where $\bar{x}$ and s are the mean and standard deviation of log-transformed N_2O emissions, respectively.

The mean of a log-normal distribution (without log-transformation) is usually described as follows:

$$\mu = exp\left(\bar{x} + \frac{s^2}{2}\right) \quad (9)$$

TABLE 3 Michaelis–Menten kinetic parameters of total or AOB-derived N_2O emissions from nitrification. V_{max} represents maximum nitrification-derived N_2O emissions (g N_2O-N ha^{-1} day^{-1}) and K_m represents half saturation constant (mM). Numbers within the parentheses represent standard errors

Ecosystem	Nitrification	V_{max}	K_m
Conventional agriculture	Total	12.7 (0.6)	0.20 (0.06)
	AOB	11.4 (0.6)	0.24 (0.06)
Biologically-based agriculture	Total	15.1 (1.2)	0.079 (0.042)
	AOB	13.8 (1.3)	0.088 (0.056)
Poplar	Total	3.48 (0.40)	0.025 (0.019)
	AOB	2.92 (0.36)	0.033 (0.026)
Early successional	Total	4.54 (0.52)	0.009 (0.008)
	AOB	3.31 (0.47)	0.012 (0.011)
Grassland	Total	1.59 (0.08)	0.012 (0.004)
	AOB	0.49 (0.09)	0.002 (0.002)[a]
Deciduous forest	Total	4.12 (0.61)	0.031 (0.026)
	AOB	3.01 (0.58)	0.042 (0.045)

Abbreviations: AOB, ammonia-oxidizing bacteria; N_2O, nitrous oxide.
[a]K_m value was estimated by constraining estimate >0.

Here, we estimated log-normal means of N_2O fluxes using a Bayesian approach by evaluating the parameters in Equation (9). We chose vague prior probability distributions to reduce their impact on the inference.

Although fitting log-normal distributions for N_2O fluxes makes biological and theoretical sense, there are other distributions that describe continuous positive data with large variances well. Thus, we also fit N_2O data with other candidate distributions including Gamma and Weibull distributions using the "fitdistrplus" package for R (Delignette-Muller & Dutang, 2015; Table 2).

2.7.4 | Estimation of contributions from nitrification

Similar to N_2O emissions from nitrification potentials, before fitting Michaelis–Menten models we converted gravimetric N_2O emissions from each nitrification kinetics experiment into areal N_2O emissions using Equation (7) based on soil depth (15 cm) and bulk density. We then used the "nls" function in R (version 3.5.0; R Core Team, 2020) to estimate V_{max} and K_m and their associated standard errors, which were then specified as prior information when we conducted a Markov Chain Monte Carlo simulation to sample posterior parameter distributions with the "jagsUI" package (Kellner, 2017) for R. We ran three chains of 15,000 iterations with 2000 burn-in iterations with a thinning rate of three, which yielded 13,002 total samples for posterior distribution.

Based on the Michaelis–Menten model, we developed for each ecosystem, long-term solution-phase NH_4^+ data were applied to predict maximum potential N_2O emissions from nitrification. The potential maximum contribution of nitrification to total N_2O was estimated with the mean of the predicted nitrification-derived N_2O divided by the log-normal mean of field N_2O measurements for Conventional, Biologically-based, Poplar, Grassland, and Deciduous forest systems. Because the contribution from nitrification cannot be >100%, we constrained our analysis with contributions ranging between 0 and 1. Overall, over 96% of the posterior distributions for contributions from total nitrification and over 99% of the posterior distributions for contributions from AOB-derived nitrification were included.

3 | RESULTS

3.1 | Seasonal N_2O emissions from nitrification potential

Across all seasons examined, soils from the Conventional and Biologically-based annual cropping systems had the highest nitrification-derived N_2O potentials (Figure 2), ranging from 17.6 to 24.8 and from 13.1 to 24.6 g N_2O-N ha^{-1} day^{-1}, respectively. In comparison, Deciduous forest soils exhibited the lowest total and AOB-derived N_2O potentials: 2.39 ± 0.67 (standard error of the mean)

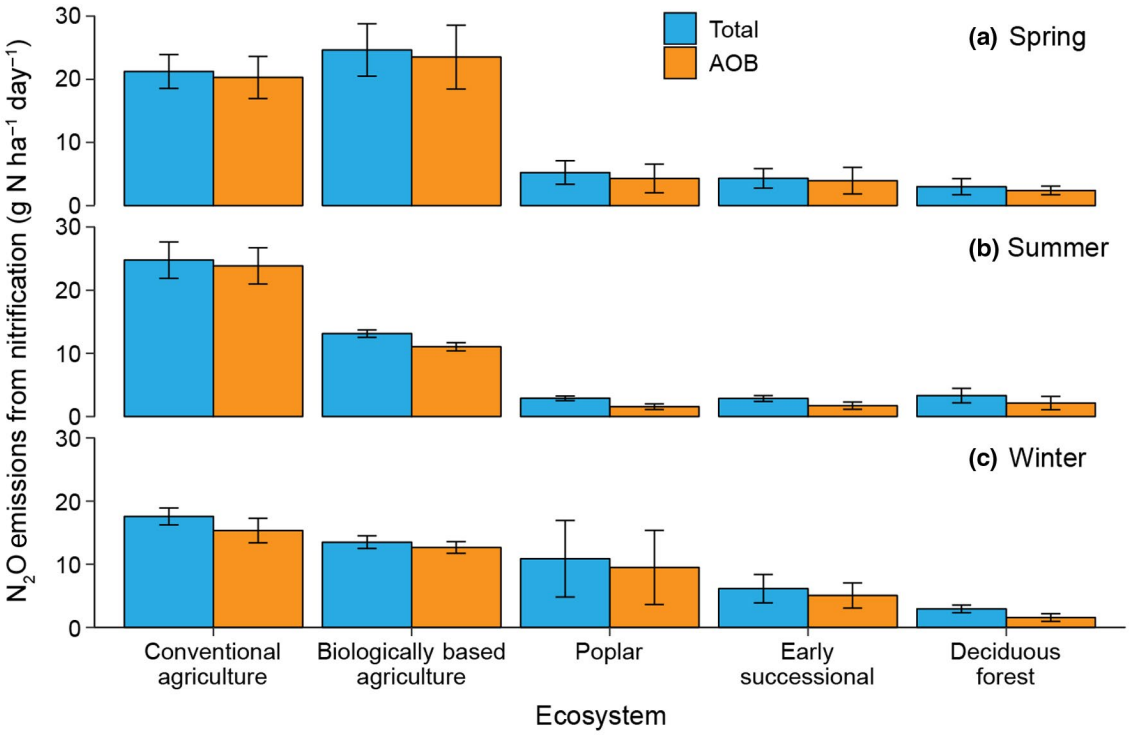

FIGURE 2 Seasonal potential N_2O production from nitrification (total or AOB-derived) across a management intensity gradient. Bars represent standard errors (for total, $n = 4$ except deciduous forest $n = 3$; for AOB, $n = 3$–4 except deciduous forest $n = 2$–3). No significant differences among seasons were detected ($p = .30$). AOB, ammonia-oxidizing bacteria; N_2O, nitrous oxide

and 2.98 ± 1.28 g N_2O-N ha^{-1} day^{-1}, respectively, for spring, and 1.56 ± 0.60 and 2.93 ± 0.60 g N_2O-N ha^{-1} day^{-1} for winter. Although seasonal nitrification-derived N_2O potentials from the Conventional and Biologically-based systems were significantly higher than from the Early successional or Deciduous forest ($p < .05$) systems, the differences between the two agricultural systems were not significant ($p > .30$) for two out of three seasons. Similarly, N_2O potentials via nitrification were generally indistinguishable among Poplar, Early successional, and Deciduous forest systems ($p > .15$) in any given season.

No significant overall seasonal differences of nitrification-derived N_2O potentials were observed ($p = .30$, Figure 2). There were also no significant interaction effects between sources of N_2O and seasons ($p = .76$) nor interactions among ecosystem types, sources of N_2O, and seasons ($p = .73$).

3.2 | Kinetics of nitrification-derived N_2O

Michaelis–Menten models fit nitrification-derived N_2O data well (Figure 1; Table 1). The Conventional and Biologically-based cropping systems exhibited the highest values of V_{max} (Table 3), ranging from 12.7 to 15.1 g N_2O-N ha^{-1} day^{-1} for total nitrification-derived N_2O, and 11.4 to 13.8 g N_2O-N ha^{-1} day^{-1} for AOB-derived N_2O. The Grassland system had the lowest V_{max}, 1.59 ± 0.08 N_2O-N ha^{-1} day^{-1} and 0.49 ± 0.09 g N_2O-N ha^{-1} day^{-1} for total and AOB-derived N_2O, respectively, followed by Poplar but with a V_{max} 2–6 times higher than the Grassland system. V_{max} for Early successional and Deciduous forest systems were similar, ranging from 3.01 to 3.31 and 4.12 to 4.54 g N_2O-N ha^{-1} day^{-1} for AOB and total nitrification-derived N_2O, respectively.

K_m values indicate how quickly NH_4^+ saturates nitrification-derived N_2O (Table 3). The Conventional agricultural system had the highest K_m for both total and AOB-derived N_2O, reaching 0.20 ± 0.06 and 0.24 ± 0.06 mM NH_4^+, respectively, which was about 2.5 times higher than the Biologically-based system, and 5–20 times higher than for all other systems.

3.3 | The relative importance of AOA and AOB for nitrification-derived N_2O

Based on the posterior distributions of V_{max}, we found that compared to AOA, AOB were the major contributors to nitrification-derived N_2O in most soils, accounting for more than 70% of total nitrification-derived N_2O (Figure 3) in all but the Grassland system, where the contribution from AOB averaged only 32 ± 4%. In addition, there was a decreasing trend of AOB's contribution to N_2O along the management gradient: about 90% of the nitrification-derived N_2O was from AOB in row crop systems, whereas in the Early successional and Deciduous forest systems, AOB's contribution decreased to about 70% of total N_2O. Concomitantly,

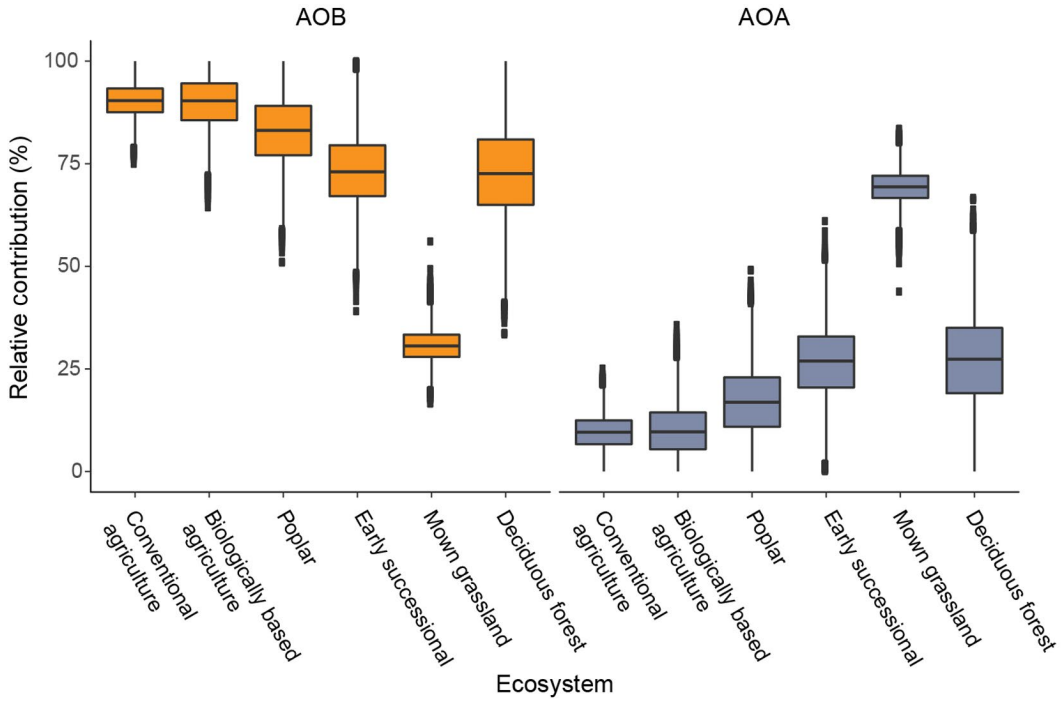

FIGURE 3 Relative contributions of AOA and AOB to nitrification-derived N_2O emissions in systems that differ in management intensities. Contributions from AOB (%, orange) were calculated with posterior distributions of V_{max} derived from Michaelis–Menten models for AOB and total nitrification-derived N_2O kinetics. Contributions from AOA (%, blue) were calculated as 1 − AOB (%). The upper, mid, and lower lines of each boxplot indicate 25th, median, and 75th percentiles, respectively. The upper and lower whiskers indicate 1.5 × interquartile range. AOA, ammonia-oxidizing archae; AOB, ammonia-oxidizing bacteria; N_2O, nitrous oxide

the contribution of AOA to nitrification-derived N_2O generally increased from the intensively managed row crop to unmanaged Grassland and Deciduous forest.

3.4 | Contribution of nitrification to long-term N_2O emissions

Among all ecosystems, row crop systems appear to have the lowest maximum potential N_2O contributed from nitrification. The percentage of 25th–75th posterior intervals from nitrification, assuming all soil NH_4^+ is available only to nitrifiers, ranged between 13.1% and 16.7% for the Conventional agricultural system and 27.4%–41.6% for the Biologically-based system (Figure 4a). For the Poplar and Grassland systems, a maximum potential of 52.0% and 54.8% of field-based N_2O fluxes can be attributed to nitrification. The Deciduous forest system was associated with the highest maximum potential contribution from nitrification, with the percentage of 25th–75th posterior intervals ranging between 51.2% and 76.9% for total nitrification-derived N_2O and 27.2%–49.6% for AOB-derived N_2O (Figure 4a,b). For all ecosystems, the median maximum potential contributions of AOB to N_2O were below 40%, ranging from 11.4% to 36.4% (Figure 4b).

4 | DISCUSSION

Soils from different ecosystems showed distinct patterns of Michaelis–Menten kinetics for nitrification-derived N_2O emissions, with highest and lowest V_{max} and K_m observed in the row crop and the Grassland ecosystems, respectively. Combining kinetic parameters with 25 years of in situ N_2O flux and solution-phase in situ soil NH_4^+ measurements suggests that nitrification is a minor source of N_2O in these ecosystems. Results also show AOB rather than AOA are the dominant source of nitrification-derived N_2O in all ecosystems but the mown grassland.

4.1 | Seasonal nitrification-derived N_2O emissions from AOA and AOB

Seasonal nitrification-derived N_2O potentials from AOB were 5–26 times higher than from AOA in Conventional and Biologically-based systems (Figure S2), suggesting a greater capacity of AOB for emitting nitrification-derived N_2O from agricultural soils. Wang et al. (2016) have also reported the dominance of AOB over AOA for N_2O produced in soils amended with inorganic ammonium fertilizer, although their study was conducted in static microcosms rather than in microcosms on shaker tables, so results could have been confounded by nitrifier denitrification since hypoxic conditions can develop in soil aggregates during static incubations (Lu et al., 2018; Stein, 2019).

Taken together, results suggest that low soil ammonium, in unfertilized systems derived primarily from soil organic matter mineralization, promotes a greater relative contribution of AOA to nitrification-derived N_2O as also found by Hink et al. (2018). Additionally, nitrifier community compositions in unfertilized systems could be very different from row crop systems, which,

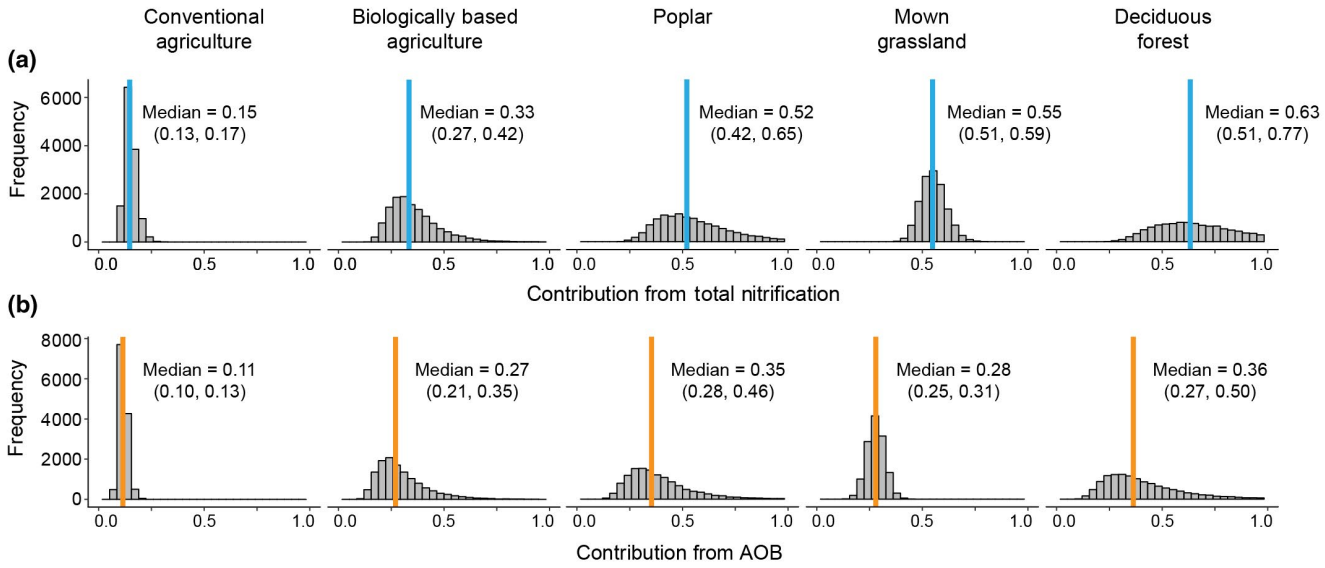

FIGURE 4 Contribution of nitrification to N_2O production. Maximum relative contributions of (a) total nitrification and (b) AOB-derived nitrification to long-term field N_2O emissions in systems that differ in management intensities assuming all solution-phase in situ ammonium is oxidized and no nitrification-derived N_2O is reduced. Field-based N_2O fluxes were estimated assuming log-normal distributions. Vertical lines indicate the median contribution for each system. Values in parentheses indicate the 25th–75th posterior intervals, respectively. Note that the Early successional system is not included as 95% of the posterior nitrification-derived N_2O was higher than the field fluxes. AOB, ammonia-oxidizing bacteria; N_2O, nitrous oxide

in turn, could affect relative N_2O production. Upon fertilization, nitrifier community composition appears to favor AOB and in particular *Nitrosospira* spp., with no similar consistent changes in AOA yet identified (Bertagnolli et al., 2016; Kong et al., 2019; Phillips et al., 2000; Wu et al., 2011; Xue et al., 2016). Soil *Nitrosospira* spp. have been shown to positively respond to urea and as well are associated with increased N_2O emissions (Cassman et al., 2019).

The absence of seasonal effects suggests that the composition and capacity for soil nitrifiers to produce N_2O remain reasonably constant throughout any given year. These findings are consistent with a year-round metagenomic study reporting remarkably stable nitrifier community composition and abundance in a US Midwest agricultural soil (Orellana et al., 2018). Similarly, both abundance and community structure of *amoA* genes of AOA and AOB have been shown to be stable across seasons in two acid forest soils (Qin et al., 2019). Thus, it seems reasonable to conclude that long-term management practices in our ecosystems have selected soil nitrifier populations that are adapted to seasonal environmental fluctuations such as soil temperature (Séneca et al., 2020).

4.2 | The responses of N_2O kinetics to management intensities

The Conventional and Biologically-based agricultural systems were associated with the highest values for V_{max} and K_m, suggesting a greater capacity of row crop soils to emit nitrification-derived N_2O than soils from our other systems. Notably, the Biologically-based system had a similar V_{max} but lower K_m compared with the Conventional system. This difference may be because in the Biologically-based system, the slower-paced release of NH_4^+ from decomposing cover crop and other residues has selected nitrifier communities with high NH_4^+ affinities (Hink et al., 2017, 2018) and less tolerance for high NH_4^+ input as compared to nitrifiers from the Conventional system. The low V_{max} and K_m in Early successional, Grassland, and Deciduous forest systems may reflect their histories of no fertilizer inputs, resulting in a low capacity to produce nitrification-derived N_2O even under substrate-unlimited conditions.

Existing studies of nitrification kinetics have mainly focused on the effects of NH_4^+ on $NO_2^- + NO_3^-$ accumulation. Koper et al. (2010) reported that the V_{max} of soils receiving ammonium sulfate at 200 kg N per hectare for 6 years was about twice higher than the V_{max} of non-fertilized soils, but no significant differences in K_m were detected. It is possible that substrate affinity responds to fertilizer more slowly than maximum nitrification rate. In addition, although V_{max} and K_m of AOB and total nitrification could be boosted significantly within a month of fertilization, they can also decline rapidly within 3 months of fertilizer application (Ouyang et al., 2017). Together, these results suggest that long-term management practices shaped differences in V_{max} and K_m responses among ecosystems varying in management intensity.

4.3 | Contribution of AOA and AOB to V_{max} along the management intensity gradient

We used a Bayesian approach to calculate the relative contributions of AOA versus AOB to nitrification-derived N_2O based on posterior distributions of V_{max} for each ecosystem, which is different from the traditional method of separating AOA from AOB based on 1 mM NH_4^+ addition (Lu et al., 2015; Ouyang et al., 2016; Taylor et al., 2010). As noted earlier, 1 mM NH_4^+ additions did not always yield the highest N_2O emissions in our systems (Figure 1), especially for agricultural soils. Thus, partitioning sources of nitrification-derived N_2O with V_{max} derived from substrate kinetics aligns with the concept of nitrification potential assays, which reflect the maximum nitrification-derived N_2O from nitrifier communities (Norton & Stark, 2011).

The declining importance of AOB to N_2O production along the management intensity gradient likely reflects different strategies of soil nitrifiers' responding to different agronomic practices. First, the Conventional system constantly receives high N inputs, which favor AOB activity or population size in agricultural soils (Habteselassie et al., 2013; Jia & Conrad, 2009; Shen et al., 2008; Taylor et al., 2010, 2013). In contrast, AOA's contribution is more important in systems where the major NH_4^+ source is via decomposition of soil organic matter. Thus, the speed of NH_4^+ supply to soil seems important for shaping the dynamics of AOA versus AOB N_2O-generating activities. Indeed, Hink et al. (2018) observed that AOA dominated nitrification-derived N_2O in incubated soils receiving slow-release fertilizer instead of free urea.

A second major difference between row crop and unfertilized systems is the history of tillage. Both the Conventional and Biologically-based systems have been either moldboard or chisel-plowed since well before 1988. In contrast, the Early successional and Poplar systems have been untilled since 1989 and the Deciduous forest and Grassland systems have never been tilled. Tillage accelerates soil organic matter turnover, which results in more pulse-like releases of NH_4^+ in soil compared with non-tilled systems. As a result, AOB likely also outcompetes AOA following tillage-induced pulses of NH_4^+.

The dominance of AOA for nitrification-derived N_2O in the Grassland system seems anomalous and might be attributed to differential inhibition of AOB versus AOA induced by root-released nitrification inhibitors known to occur in at least one grass species. While we have no direct evidence of inhibitors produced by grasses in our study sites, in a 3-year field study, Subbarao et al. (2009) showed that brachialactone, a root exudate isolated from the forage grass *Brachiaria* sp., inhibited 90% of in situ NH_4^+ oxidation and over 90% of cumulative N_2O emissions in a tropical pasture. Moreover, the inhibition seemed to be specific to AOB rather than AOA. Historically, among all of our ecosystems, the Grassland system has always had the highest monthly soil NH_4^+ concentrations and exhibited the lowest relative nitrification potentials (Millar & Robertson, 2015). Since root exudates of *Bromus* spp., a dominant species in the Grassland system, have been reported to significantly inhibit

nitrification in vitro in both AOB culture and whole soils (O'Sullivan et al., 2017), we suspect AOB inhibition in the Grassland system.

4.4 | Long-term contribution of nitrification to in situ N_2O fluxes

Seasonally stable nitrification-derived N_2O fluxes allow us to apply kinetics models to predict potential maximum N_2O emissions from nitrification and, subsequently, the theoretical maximum relative contribution of nitrification to field-based N_2O emissions assuming nitrifiers has exclusive access to solution-phase NH_4^+. Since the kinetics results are based on aerobic incubations of shaken soil slurries that eliminate both N_2O reduction and N_2O from nitrifier denitrification (Wrage et al., 2001; Wrage-Mönnig et al., 2018), N_2O rates can be considered nitrifier nitrification rather than nitrifier denitrification, and when applied to historical solution-phase in situ NH_4^+ pools, reveal maximum potential nitrification-derived N_2O in situ.

An important consideration in whole-soil kinetic assays is that they ignore the likelihood that some taxa will be nitrifying at rates lower than their maximum possible as nitrifiers exhibit significant phylogenetic and physiological diversity (Hazard et al., 2021). That said, whole-community incubations under laboratory conditions that favor nitrification in general, allow us to identify the maximum likely rates of whole-soil nitrification, were such conditions possible in the field. So though our controlled laboratory conditions might be suboptimal for some taxa, the assay overall seems a reasonable, conservative proxy for obtaining maximum whole-community nitrification rates under different substrate conditions.

The finding that total nitrification contributed a theoretical maximum of 13%–17% of field-based N_2O fluxes in the Conventional agricultural system suggests that nitrification is unlikely to be a significant source of N_2O in long-fertilized systems. That a theoretical maximum of only 27%–42% of field-based fluxes were nitrification-derived in the Biologically-based system suggests that nitrification is likewise unlikely to be a dominant N_2O source in even unfertilized annual cropping systems. Using N_2O SP analysis, Opdyke et al. (2009) and Zou et al. (2014) reported a small role for nitrification in N_2O produced by agricultural soils (including ours), although these studies were short-term snapshots. Similarly, AOB-derived nitrification is unlikely to be the major process leading to N_2O production in any of our ecosystems regardless of management. These results are also consistent with Buchen et al. (2018), who also used SP in situ to suggest that >80% of N_2O can be attributed to denitrification (whether heterotrophic or nitrifier-derived) in managed grasslands.

Since our Michaelis–Menten models were necessarily developed under laboratory conditions that favored nitrification, the calculated contributions of nitrification to N_2O reflect maximum in situ potentials that assume all solution-phase NH_4^+ is available exclusively to nitrifiers and no nitrification-derived N_2O is further denitrified to N_2. Neither of these assumptions are realistic in situ. Soils are rarely completely aerobic, and even if in situ nitrification emitted N_2O equivalent to the amount from shaken soil slurries, some of the N_2O will be captured by denitrifiers and reduced to N_2 before being emitted to the atmosphere (Decock & Six, 2013; Lewicka-Szczebak et al., 2017; Shcherbak & Robertson, 2019).

Malhi and McGill (1982) estimated that the daily maximum NH_4^+-N oxidation rate is <10% of available NH_4^+-N (100 μg N g^{-1}) based on laboratory incubations. Prosser et al. (2020) reported pure culture N_2O yields for AOB and AOA to be only 0.1%–8% and 0.04%–0.3%, respectively, although a greater diversity of nitrifiers in situ (Amann et al., 1995) will reflect a wider range. Hence, our assumption of 100% of daily NH_4^+ is oxidized and consequently eligible for transformation to N_2O is undoubtedly an overestimate by a factor of 10 to 100 or more. That said, our conclusion of nitrification being a minor source of N_2O in these ecosystems is conservative by nature. Actual contributions of nitrification to measured N_2O fluxes in situ are likely to be only 0.1%–10% of the potential maximum rates we identify.

By way of example, the least-constrained nitrifier contribution to N_2O fluxes was measured in Early successional and Deciduous forest soils where 51%–77% of total N_2O fluxes might potentially derive from nitrification in the Deciduous forest system (Figure 4a), and over 95% of the predicted nitrification-derived N_2O was higher than the field fluxes in the Early successional system. But here, perhaps especially, the extrapolation assumptions seem severe. The Early successional and Deciduous forest soils have high concentrations of macroaggregates (2000–8000 μm; Grandy & Robertson, 2007) and thus a larger volume fraction of anoxic centers (Schlüter et al., 2018), which contribute to high measured denitrification rates (Robertson & Tiedje, 1984). So even in our systems with the greatest percentage of N_2O contributed by nitrifiers based on Michaelis–Menten kinetics, actual results will be but a fraction.

Overall, we conclude that nitrification is a minor source of N_2O emissions in all of the systems examined. This finding has significant implications for biogeochemical N_2O flux models that assume a significant fraction of emissions are nitrifier derived (e.g. Parton et al., 2001). Our findings further suggest that taxa-specific N_2O mitigation might better target processes other than nitrification, except insofar as nitrification makes nitrate available to denitrifiers.

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CONFLICT OF INTEREST

The authors declare no conflict of financial interests.

DATA AVAILABILITY STATEMENT

KBS MCSE long-term field N_2O flux measurements can be accessed at https://lter.kbs.msu.edu/datatables/28; long-term in situ soil NH_4^+-N concentrations are available at https://lter.kbs.msu.edu/datatables/55. Soil bulk densities of deep core soil samples are available from https://lter.kbs.msu.edu/datatables/308. Species composition of Early successional and Deciduous forest systems is available from https://lter.kbs.msu.edu/datatables/237 and https://lter.kbs.msu.edu/datatables/238. All other data used in this study are available at datadryad.org (https://doi.org/10.5061/dryad.37pvmcvkz).

Code availability: The code for estimating log-normal mean of in situ N_2O fluxes and the maximum contribution of nitrification to total N_2O are shown in Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplementary information for

Nitrification is a minor source of nitrous oxide (N_2O) in agricultural landscapes and declines with increasing management intensity

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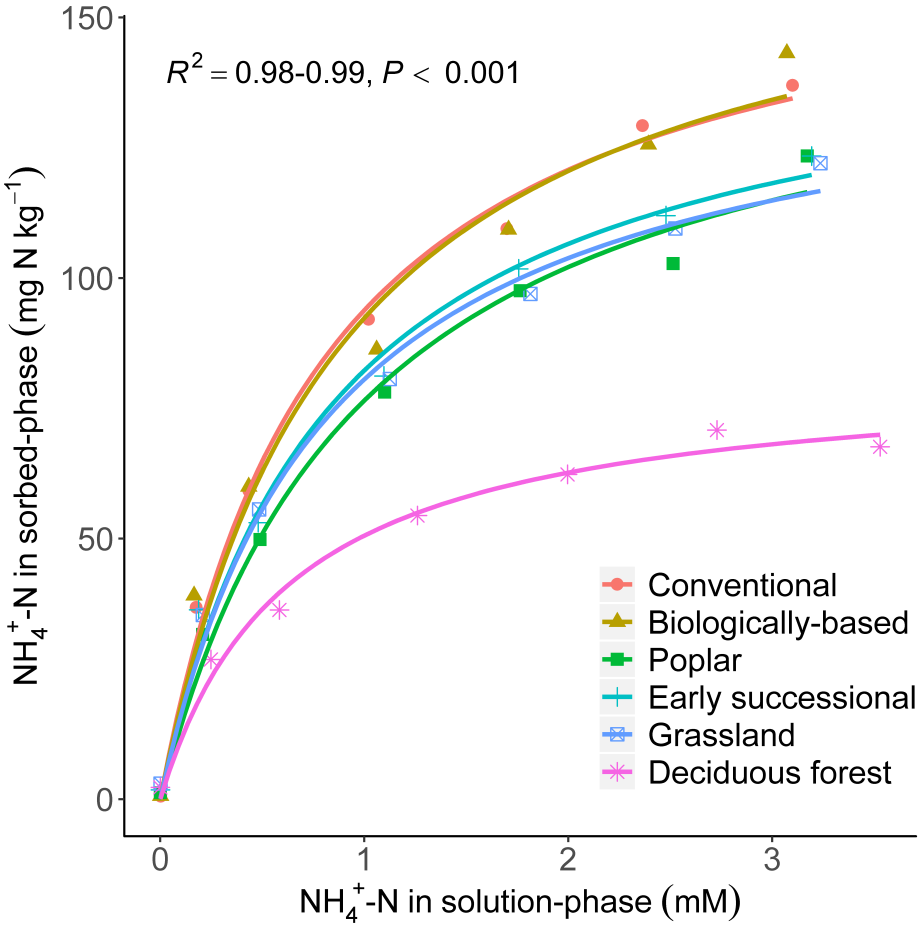
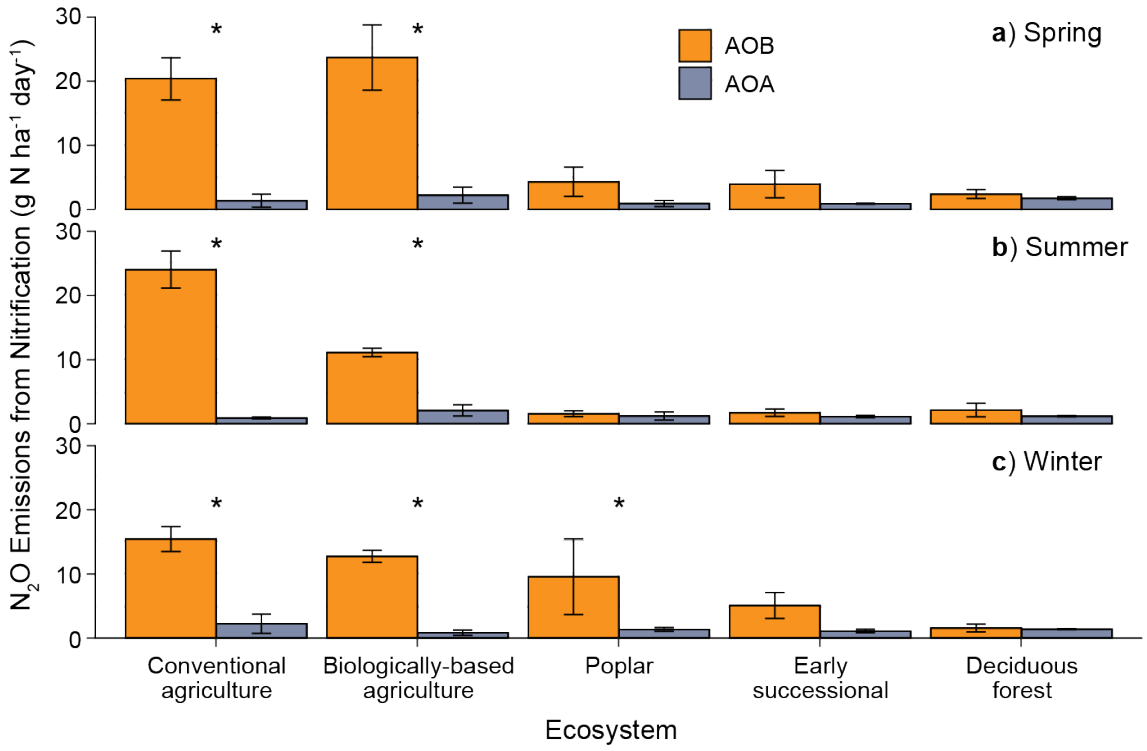
Contains:

Supplementary Figures 1-2

R scripts for estimating the log-normal mean and the maximum contribution of nitrification to *in situ* N_2O fluxes



Supplementary Figure 1. The relationship between NH_4^+-N in solution-phase (mM) vs. NH_4^+-N in sorbed-phase (based on dry soil mass). Langmuir models were used to fit relationships of different ecosystems varying in management intensities.



Supplementary Figure 2. Seasonal N_2O potentials from nitrification (AOB or AOA-derived) across a management intensity gradient. Bars represent standard errors (n = 3-4 except deciduous forest n = 2-3). No significant differences among seasons were detected ($P = 0.28$). "*" indicates significant differences between AOA and AOB-derived N_2O potentials.

R scripts for estimating the log-normal mean and the maximum contribution of nitrification to *in situ* N_2O fluxes

```
library(jagsUI)
library(fitdistrplus)

#First, estimate parameters for log-normal distribution
# file_name1 is the positive long-term in situ N2O fluxes
N2O_ln<- fitdist(file_name1$N2O,"lnorm")

#Second, estimate parameters for Michaelis-Menten equations
# file_name2 and file_name3 are the total and AOB-derived N2O from nitrification
P1_total_parm<-nls(n2o~ a*amo/(b + amo),data= file_name2,start =  list(a=10,b=.5),
                algorithm = "port", trace = F, na.action = na.omit, model=T,
                control = nls.control(maxiter = 1000, warnOnly = F))
P1_aob_parm<-nls(n2o~ a*amo/(b + amo),data= file_name3,start = list(a=10,b=.5),
               algorithm = "port", trace = F, na.action = na.omit, model=T,
               control = nls.control(maxiter = 1000, warnOnly = F))


#Third, estimate the maximum contribution of nitrification to total N2O fluxes
sink("your_file_name.txt")
cat("
   model {
   # Likelihood-N2O
   for( i in 1 : Q ) {
   y[i] ~ dlnorm( muOfLogY, 1/sigmaOfLogY^2)
   }

   # Likelihood-nitrification-total
   for (k in 1:M) {
   mu[k] <- a1*ammonia[k]/(b1+ammonia[k])
   n[k] ~ dnorm(mu[k],tau0)
   n.p[k] ~ dnorm(mu[k],tau0)
   }

   # Likelihood-nitrification-aob
   for (j in 1:N) {
   c[j] <- a2*ammonia1[j]/(b2+ammonia1[j])
   n1[j] ~dnorm(c[j],tauc)
   n1.p[j] ~dnorm(c[j],tauc)
   }

   # Priors
   sigmaOfLogY ~ dunif( 0.001*sdOfLogY , 1000*sdOfLogY)
   muOfLogY ~ dnorm(meanOfLogY, 1/(10*sdOfLogY)^2)
```

```
    a1 ~ dnorm (mean1,tau1)
    b1 ~ dnorm (mean2,tau2)
    a2 ~ dnorm (mean3,tau3)
    b2 ~ dnorm (mean4,tau4)
    tau0 <- 1/(sigma0*sigma0)
    tauc <- 1/(sigmac*sigmac)
    tau1 <- 1/(sigma1*sigma1)
    tau2 <- 1/(sigma2*sigma2)
    tau3 <- 1/(sigma3*sigma3)
    tau4 <- 1/(sigma4*sigma4)
    sigma0 ~ dunif(0,5)
    sigmac ~ dunif(0,5)

    # Derived quantities
    muOfY <- exp(muOfLogY+sigmaOfLogY^2/2)
    for (m in 1:P) {
    nitri_total[m]<- a1*x[m]/(b1+x[m])
    nitri_aob[m]<- a2*x[m]/(b2+x[m])}
    total_avg <- mean(nitri_total[])
    aob_avg <- mean(nitri_aob[])
    con_total1 <- total_avg/muOfY
    con_aob1 <- aob_avg/muOfY
    major_aob <- a2/a1
    }
    ",fill=TRUE)
sink()

#jags data
jags.data <- list(y = file_name1$N2O,
                  Q = length(file_name1$N2O),
                  ammonia= file_name2$amo,
                  ammonia1= file_name3$amo,
                  n = file_name2$n2o,
                  n1 = file_name3$n2o,
                  M = nrow(file_name2),
                  N = nrow(file_name3),
                  x = file_name4$Ammonia,
                 #file_name4 is the in situ long-term solution-phase NH4+ concentrations
                  P = nrow(file_name4),
                  meanOfLogY = N2O_ln$estimate[1],
                  sdOfLogY = N2O_ln$estimate[2],
                  mean1=coef(summary(P1_total_parm))[1,1],
                  mean2=coef(summary(P1_total_parm))[2,1],
                  mean3=coef(summary(P1_aob_parm))[1,1],
                  mean4=coef(summary(P1_aob_parm))[2,1],
```

```
            sigma1=coef(summary(P1_total_parm))[1,2],
            sigma2=coef(summary(P1_total_parm))[2,2],
            sigma3=coef(summary(P1_aob_parm))[1,2],
            sigma4=coef(summary(P1_aob_parm))[2,2])

# Initial values
inits <- function() list(a1=runif(1, 8, 10),
                  b1=runif(1, 0.1, 0.3),
                  a2=runif(1, 8, 10),
                  b2=runif(1, 0.1, 0.3),
                  sigmaOfLogY=1)

# Parameters monitored
params <- c("a1", "b1","a2", "b2","total_avg","aob_avg","muOfY","con_total1",
        "con_aob1","major_aob")

# MCMC settings
ni <- 15000
nt <- 3
nb <- 2000
nc <- 3
max_contribution <- jags(jags.data, inits, params, "your_file_name.txt ", n.chains = nc,
          n.thin = nt, n.iter = ni, n.burnin = nb)
```