INTRODUCTION

Nitrous oxide (N\textsubscript{2}O) is a potent greenhouse gas with a 100-year global warming potential ~300 times higher than CO\textsubscript{2} and has the third largest radiative forcing among the biogenic greenhouse gases (Myhre et al., 2013). N\textsubscript{2}O also depletes stratospheric ozone (Revell et al., 2012). Globally, soils are the dominant sources of both anthropogenic and natural emissions of N\textsubscript{2}O, with 1.7–4.8 Tg N\textsubscript{2}O-N year\textsuperscript{-1} emitted by agricultural soils and 3.3–9.0 Tg N\textsubscript{2}O-N year\textsuperscript{-1} from soils under natural vegetation (Ciais et al., 2013).

Ammonia (NH\textsubscript{3}) oxidation, the rate-limiting step of nitrification, is performed in soil mainly by aerobic ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). Nitrification is the conversion of NH\textsubscript{3} to nitrite (NO\textsubscript{2}−) and nitrate (NO\textsubscript{3}−) by AOB, and NH\textsubscript{3} is the major sink for available NH\textsubscript{4}\textsuperscript{+} in situ. Most nitrification-derived N\textsubscript{2}O was produced by AOB rather than AOA, who appeared responsible for no more than 30% of nitrification-derived N\textsubscript{2}O production in all but one ecosystem. Although the proportion of nitrification-derived N\textsubscript{2}O production was lowest in annual cropping systems, these ecosystems nevertheless produced more nitrification-derived N\textsubscript{2}O (higher V\textsubscript{max}) than perennial and successional ecosystems. We conclude that nitrification is minor relative to other sources of N\textsubscript{2}O in all ecosystems examined.
(AOB) and archaea (AOA), and releases N₂O during conversion of NH₃ to nitrite (NO₂⁻) and nitrate (NO₃⁻). Although the recently discovered complete ammonia oxidizers (comammox bacteria) can also produce N₂O 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 N₂O during the stepwise reduction of NO₃⁻ to N₂O and thence dinitrogen (N₂) when soils are anaerobic (Robertson & Groffman, 2021). Additionally, under hypoxic conditions, AOB that encode nitric oxide reductase (NorB) can reduce NO₂⁻ to N₂O 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 N₂O mitigation potentials and strategies, and as well for constraining uncertainties in biogeochemical models of N₂O emissions.

Partitioning N₂O 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 N₂O emission sources: specific inhibitors, stable isotope enrichment, and isotopomer analysis. Specific inhibitors have mainly been used in short-term laboratory incubations, where acetylene (C₂H₂) can be used to selectively inhibit NH₃ oxidation at 10 Pa and N₂O 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 ¹⁵N-NH₄⁺ or ¹⁵N-NO₃⁻ to differentiate nitrification and denitrification-derived N₂O in short-term laboratory experiments (Stevens et al., 1997). Isotopomers of N₂O reflect the differential intramolecular distribution (site preference, SP) of ¹⁵N at α and β positions of the N₂O molecule (N²⁻-O) and have been used to differentiate N₂O 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 C₂H₂ use include further concerns of microbial C₂H₂ consumption (Terry & Duxbury, 1985; Topp & Germon, 1986), and as well heterotrophic nitrifiers are resistant to C₂H₂ (Hynes & Knowles, 1982; Schmeltzer et al., 1984). ¹⁵N 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, N₂O 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 N₂O emissions.

An alternative method for assessing the maximum potential importance of nitrification versus other N₂O generating processes in soil is to combine soil-specific kinetics of nitrification-derived N₂O with long-term field N₂O flux measurements. Nitrification kinetics measure a soil’s existing potential to nitrify NH₄⁺ to N₂O and NO₃⁻ under conditions unconstrained by resource limitations (Norton & Stark, 2011; Stark & Firestone, 1996), thus allowing maximum potentials for nitrification-derived N₂O emissions to be estimated. Such potentials, if stable in time, might then be combined with field-based measurements of N₂O fluxes to allow calculation of the likely maximum percentage of nitrification-derived N₂O in relation to all other N₂O sources.

Here we combine measured site-specific nitrification kinetics for N₂O production with over 25 years of field-based N₂O fluxes to estimate the maximum potential contribution of nitrification to N₂O 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 N₂O-NH₄⁺ relationships, and show them to be seasonally stable. Then we predict the potential maximum nitrification-derived N₂O of each ecosystem by assuming that all microbially available (soil solution phase) NH₄⁺ can be oxidized into N₂O. Finally, we use a Bayesian approach to calculate the maximum relative importance of nitrification for N₂O emissions from each ecosystem based on long-term field-based N₂O 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 x 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: $137 \pm 20$ kg N ha$^{-1}$ year$^{-1}$ for corn and $77 \pm 17$ 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$^{-1}$ over winter, and was terminated just prior to planting corn. Then applied once in 2011 at 157 kg N ha$^{-1}$.

After the second harvest in 2008 and one fallow year, ammonium nitrate in the establishment year and the first harvest was used to suppress weeds in the Conventional system and additional tillage was used to a depth of 15–18 cm followed by secondary tillage. Herbicides were also 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 “Eugene.” 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 dominant in the early part of the study. 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$_2$O potentials, once for nitrification-derived N$_2$O kinetics, and once for solution-phase NH$_4^+$ partitioning. For nitrification-derived N$_2$O 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$_2$O, 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$_2$O 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$_2$O potentials and N$_2$O 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$_2$O, 5 g of freshly sieved soil was placed into a 155 ml Wheaton bottle amended with 50 ml deionized water containing 10 mM NH$_4$Cl to maximize nitrification-derived N$_2$O 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 $\mu$M aqueous concentration ($C_{aq}$) to test for optimal inhibition and we found 4 $\mu$M $C_{aq}$ 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 $\mu$M $C_{aq}$ 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$_2$O as soil slurries are continuously aerated by high-speed shaking.

Samples for N$_2$O were taken at 2 and 24 h and N$_2$O emission rates were calculated based on N$_2$O accumulations over 22 h. Slurry pH was buffered naturally as no apparent pH change was detected during the incubation. Emissions of N$_2$O in the presence of octyne are attributed to AOA. Emissions of N$_2$O from AOB are calculated as the difference between N$_2$O without octyne (total nitrification-derived N$_2$O) minus N$_2$O from AOA. Although comammox could also contribute to N$_2$O 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$_2$O samples were stored overpressurized in 6 ml N$_2$-flushed glass vials (Exetainers, Labco Ltd). N$_2$O 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$_4$)$_2$SO$_4$ to make eight
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 \(\mu\)M \(C_{aq}\) 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:

\[
V = \frac{V_{max}S}{K_m + S}
\]

(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 \(\frac{1}{2} V_{max}\). \(V_{max}\) 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):

\[
V = \frac{V_{max}S}{K_m + S + S^2/K_i}
\]

(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 \(\frac{1}{2} V_{max}\). 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 N₂O fluxes were calculated by linearly regressing headspace N₂O concentrations over time (µg N₂O-N L⁻¹ min⁻¹), which was then further converted to area-based N₂O fluxes by accounting for the volume of gas in the chamber and soil surface area covered by the chamber (g N₂O-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 NH₄⁺ concentrations. Soil bulk density (0–10 cm depth) was measured in 2013 when collecting deep core soil samples to a depth of about 3 cm into soil. Gas samples were taken at approximately biweekly after the soils thawed in the spring, usually in March or April, and discontinued before soils froze, usually in November. Soils were subjected to long-term KCl extraction to determine NH₄⁺ concentrations. Soil bulk density (0–10 cm depth) was measured in 2013 when collecting deep core soil samples to a depth of about 3 cm into soil. Gas samples were taken at approximately biweekly after the soils thawed in the spring, usually in March or April, and discontinued before soils froze, usually in November.

2.6 | Microbiologically available (solution phase) soil NH₄⁺

We partitioned long-term KCl extracted soil NH₄⁺ pools into sorbed-phase (srNH₄⁺) and solution-phase (slNH₄⁺) pools by performing an NH₄⁺ sorption capacity assay modified from Ventera et al. (2015). We assume only slNH₄⁺ is available to soil nitrifiers. Briefly, for each ecosystem, we added 10 g of sieved fresh soils into 100 ml of water containing an NH₄⁺ gradient ranging from 0 to 50 mg NH₄⁺-N L⁻¹ (0, 5, 10, 20, 30, 40, and 50 mg NH₄⁺-N L⁻¹ generated by (NH₄)₂SO₄ 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. NH₄⁺-N was then analyzed by flow injection analysis as above after filtering aliquots through a 1 mm glass fiber filter. We calculated srNH₄⁺ as the difference between added NH₄⁺ (addNH₄⁺) and the slNH₄⁺ (measured as above) accounting for soil NH₄⁺ contents (soilNH₄⁺):

\[
\text{soilNH}_4^+ = \text{NH}_4^+_{\text{KCl}} - \text{NH}_4^+_{\text{sl}}
\]

srNH₄⁺ = addNH₄⁺ - slNH₄⁺ + soilNH₄⁺ (when addNH₄⁺ > 0)  

srNH₄⁺ = soilNH₄⁺ (when addNH₄⁺ = 0)  

where NH₄⁺_{KCl} is the 1 M KCl extractable NH₄⁺ concentrations and NH₄⁺_{sl} is the water extractable NH₄⁺ concentrations at 0 NH₄⁺-N L⁻¹ addition. The relationship between srNH₄⁺ (mg N kg⁻¹) and slNH₄⁺ (mM) is usually described by a Langmuir model:

\[
\text{srNH}_4^+ = \frac{\mu \times \text{slNH}_4^+}{K + \text{slNH}_4^+}
\]

where \( \mu \) (mg N kg⁻¹) is the maximum NH₄⁺ content adsorbed by soil and \( K \) (mM) is the NH₄⁺ concentration in solution phase at which srNH₄⁺ is \( \frac{1}{2} \mu \). We modeled and plotted srNH₄⁺ against slNH₄⁺ (Figure S1), which allows one to convert total KCl-based soil NH₄⁺ values into slNH₄⁺ for every NH₄⁺ soil measurement taken between 1989 and 2016.

TABLE 1 Comparisons between Michaelis–Menten and Haldane kinetics models for total or AOB-derived N₂O emissions from nitrification

<table>
<thead>
<tr>
<th>Ecosystem*</th>
<th>Nitrification</th>
<th>AICb (Michaelis–Menten)</th>
<th>AICb (Haldane)</th>
<th>F-valuec</th>
<th>p-valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poplar</td>
<td>Total</td>
<td>111</td>
<td>113</td>
<td>0.188</td>
<td>0.668</td>
</tr>
<tr>
<td></td>
<td>AOB</td>
<td>105</td>
<td>106</td>
<td>0.488</td>
<td>0.491</td>
</tr>
<tr>
<td>Early successional</td>
<td>Total</td>
<td>143</td>
<td>144</td>
<td>0.134</td>
<td>0.718</td>
</tr>
<tr>
<td></td>
<td>AOB</td>
<td>130</td>
<td>131</td>
<td>1.13</td>
<td>0.298</td>
</tr>
<tr>
<td>Grassland</td>
<td>Total</td>
<td>27.9</td>
<td>28.1</td>
<td>1.70</td>
<td>0.202</td>
</tr>
<tr>
<td></td>
<td>AOB</td>
<td>30.2</td>
<td>30.6</td>
<td>1.50</td>
<td>0.233</td>
</tr>
<tr>
<td>Deciduous forest</td>
<td>Total</td>
<td>109</td>
<td>111</td>
<td>0.001</td>
<td>0.980</td>
</tr>
<tr>
<td></td>
<td>AOB</td>
<td>106</td>
<td>108</td>
<td>0.049</td>
<td>0.827</td>
</tr>
</tbody>
</table>

Abbreviations: AIC, Akaike information criterion; AOB, ammonia-oxidizing bacteria; N₂O, nitrous oxide.

*Data from Conventional and Biologically-based systems were not fit to Haldane models because no signs of inhibition of nitrification-derived N₂O were found.

Models with lower AIC were considered superior.

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\textsubscript{2}O

We converted gravimetric N\textsubscript{2}O emissions from the nitrification potential experiment into areal N\textsubscript{2}O emissions based on soil depth (15 cm) and bulk density:

\[
N\textsubscript{2}O\textsubscript{area} = \frac{N\textsubscript{2}O\textsubscript{mass} \times DP \times BD}{10}
\]  

(7)

where \(N\textsubscript{2}O\textsubscript{area}\) is expressed as \(g N\textsubscript{2}O-N ha^{-1} day^{-1}\) and \(N\textsubscript{2}O\textsubscript{mass}\) is expressed as \(ng N\textsubscript{2}O-N g^{-1} dry soil day^{-1}\). \(DP\) 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\textsubscript{2}O were analyzed with PROC GLIMMIX of SAS 9.4 (SAS Institute). The statistical model included 5 ecosystem types \(\times\)3 seasons \(\times\) 2 sources of nitrification-derived N\textsubscript{2}O, 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\textsubscript{2}O as subplot and sub-subplot factors. Homogeneity of variance assumptions were 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\textsubscript{2}O 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, \(V_{\text{max}}\) and \(K_m\) for total and AOB-derived N\textsubscript{2}O emissions from nitrification for each ecosystem were estimated by the "nls" function (Table 3).

2.7.3 | Distribution for field N\textsubscript{2}O fluxes

In situ N\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O emissions, which follow log-normal distributions (Limpert et al., 2001):

\[
F_{\text{N}_2\text{O}} \sim \text{lognorm}(\bar{x}, \sigma^2)
\]

(8)

where \(\bar{x}\) and \(\sigma\) are the mean and standard deviation of log-transformed N\textsubscript{2}O emissions, respectively.

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

\[
\mu = \exp(\bar{x} + \frac{\sigma^2}{2})
\]

(9)

### Table 2 | AIC of field-based nitrous oxide fluxes from different ecosystems fitted with different distributions

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

Abbreviation: AIC, Akaike information criterion.

### Table 3 | Michaelis–Menten kinetic parameters of total or AOB-derived N\textsubscript{2}O emissions from nitrification. \(V_{\text{max}}\) represents maximum nitrification-derived N\textsubscript{2}O emissions (g N\textsubscript{2}O-N ha\(^{-1}\) day\(^{-1}\)) and \(K_m\) represents half saturation constant (mM). Numbers within the parentheses represent standard errors

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

Abbreviations: AOB, ammonia-oxidizing bacteria; N\textsubscript{2}O, nitrous oxide.

*\(K_m\) value was estimated by constraining estimate >0.
Here, we estimated log-normal means of \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) fluxes makes biological and theoretical sense, there are other distributions that describe continuous positive data with large variances well. Thus, we also fit \( \text{N}_2\text{O} \) data with other candidate distributions including Gamma and Weibull distributions using the “fitdistplus” package for R (Delignette-Muller & Dutang, 2015; Table 2).

2.7.4 | Estimation of contributions from nitrification

Similar to \( \text{N}_2\text{O} \) emissions from nitrification potentials, before fitting Michaelis–Menten models we converted gravimetric \( \text{N}_2\text{O} \) emissions from each nitrification kinetics experiment into areal \( \text{N}_2\text{O} \) 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 \( V_{\text{max}} \) 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.

3 | RESULTS

3.1 | Seasonal \( \text{N}_2\text{O} \) emissions from nitrification potential

Across all seasons examined, soils from the Conventional and Biologically-based annual cropping systems had the highest nitrification-derived \( \text{N}_2\text{O} \) potentials (Figure 2), ranging from 17.6 to 24.8 and from 13.1 to 24.6 g \( \text{N}_2\text{O}-\text{N} \) ha\(^{-1}\) day\(^{-1}\), respectively. In comparison, Deciduous forest soils exhibited the lowest total and AOB-derived \( \text{N}_2\text{O} \) potentials: 2.39 ± 0.67 (standard error of the mean). Based on the Michaelis–Menten model, we developed for each ecosystem, long-term solution-phase \( \text{NH}_4^+ \) data were applied to predict maximum potential \( \text{N}_2\text{O} \) emissions from nitrification. The potential maximum contribution of nitrification to total \( \text{N}_2\text{O} \) was estimated with the mean of the predicted nitrification-derived \( \text{N}_2\text{O} \) divided by the log-normal mean of field \( \text{N}_2\text{O} \) 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.

![Figure 2](image-url) Seasonal potential \( \text{N}_2\text{O} \) 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; \( \text{N}_2\text{O} \), nitrous oxide.
and 2.98 ± 1.28 g N₂O-N ha⁻¹ day⁻¹, respectively, for spring, and 1.56 ± 0.60 and 2.93 ± 0.60 g N₂O-N ha⁻¹ day⁻¹ for winter. Although seasonal nitrification-derived N₂O 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, N₂O 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 N₂O potentials were observed (p = .30, Figure 2). There were also no significant interaction effects between sources of N₂O and seasons (p > .30) nor interactions among ecosystem types, sources of N₂O, and seasons (p = .73).

3.2 | Kinetics of nitrification-derived N₂O

Michaelis–Menten models fit nitrification-derived N₂O data well (Figure 1; Table 1). The Conventional and Biologically-based cropping systems exhibited the highest values of Vₘₐₓ (Table 3), ranging from 12.7 to 15.1 g N₂O-N ha⁻¹ day⁻¹ for total nitrification-derived N₂O, and 11.4 to 13.8 g N₂O-N ha⁻¹ day⁻¹ for AOB-derived N₂O. The Grassland system had the lowest Vₘₐₓ, 1.59 ± 0.08 N₂O-N ha⁻¹ day⁻¹ and 0.49 ± 0.09 g N₂O-N ha⁻¹ day⁻¹ for total and AOB-derived N₂O, respectively, followed by Poplar but with a Vₘₐₓ 2–6 times higher than the Grassland system. Vₘₐₓ for Early successional and Deciduous forest systems were similar, ranging from 3.01 to 3.31 and 4.12 to 4.54 g N₂O-N ha⁻¹ day⁻¹ for AOB and total nitrification-derived N₂O, respectively.

Kₘ values indicate how quickly NH₄⁺ saturates nitrification-derived N₂O (Table 3). The Conventional agricultural system had the highest Kₘ for both total and AOB-derived N₂O, reaching 0.20 ± 0.06 and 0.24 ± 0.06 mM NH₄⁺, 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 N₂O

Based on the posterior distributions of Vₘₐₓ, we found that compared to AOA, AOB were the major contributors to nitrification-derived N₂O in most soils, accounting for more than 70% of total nitrification-derived N₂O (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 N₂O along the management gradient: about 90% of the nitrification-derived N₂O 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 N₂O. Concomitantly,
the contribution of AOA to nitrification-derived \( \text{N}_2\text{O} \) generally increased from the intensively managed row crop to unmanaged Grassland and Deciduous forest.

### 3.4 Contribution of nitrification to long-term \( \text{N}_2\text{O} \) emissions

Among all ecosystems, row crop systems appear to have the lowest maximum potential \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) and 27.2%–49.6% for AOB-derived \( \text{N}_2\text{O} \) (Figure 4a,b). For all ecosystems, the median maximum potential contributions of AOB to \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) emissions, with highest and lowest \( V_{\text{max}} \) and \( K_m \) observed in the row crop and the Grassland ecosystems, respectively. Combining kinetic parameters with 25 years of in situ \( \text{N}_2\text{O} \) flux and solution-phase in situ soil NH\(^4\) measurements suggests that nitrification is a minor source of \( \text{N}_2\text{O} \) in these ecosystems. Results also show AOB rather than AOA are the dominant source of nitrification-derived \( \text{N}_2\text{O} \) in all ecosystems but the mown grassland.

#### 4.1 Seasonal nitrification-derived \( \text{N}_2\text{O} \) emissions from AOA and AOB

Seasonal nitrification-derived \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) from agricultural soils. Wang et al. (2016) have also reported the dominance of AOB over AOA for \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O} \) as also found by Hink et al. (2018). Additionally, nitrifier community compositions in unfertilized systems could be very different from row crop systems, which,
in turn, could affect relative N\textsubscript{2}O production. Upon fertilization, nitrifier community composition appears to favor AOB and in particular *Nitrosospora* spp., with no similar consistent changes in AOA yet identified (Bertagnoli et al., 2016; Kong et al., 2019; Phillips et al., 2000; Wu et al., 2011; Xue et al., 2016). Soil *Nitrosospora* spp. have been shown to positively respond to fertilizer and as well are associated with increased N\textsubscript{2}O emissions (Cassman et al., 2019).

The absence of seasonal effects suggests that the composition and capacity for soil nitrifiers to produce N\textsubscript{2}O 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 (Sénéca et al., 2020).

### 4.2 The responses of N\textsubscript{2}O kinetics to management intensities

The Conventional and Biologically-based agricultural systems were associated with the highest values for \( V_{\text{max}} \) and \( K_m \), suggesting a greater capacity of row crop soils to emit nitrification-derived N\textsubscript{2}O than soils from our other systems. Notably, the Biologically-based system had a similar \( V_{\text{max}} \) but lower \( K_m \) compared with the Conventional system. This difference may be because in the Biologically-based system, the slower-paced release of \( \text{NH}_4^+ \) from decomposing cover crop and other residues has selected nitrifier communities with high \( \text{NH}_4^+ \) affinities (Hink et al., 2017, 2018) and less tolerance for high \( \text{NH}_4^+ \) input as compared to nitrifiers from the Conventional system. The low \( V_{\text{max}} \) 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\textsubscript{2}O even under substrate-unlimited conditions.

Existing studies of nitrification kinetics have mainly focused on the effects of \( \text{NH}_4^+ \) on NO\textsubscript{3}\textsuperscript{-} + NO\textsubscript{2}\textsuperscript{-} accumulation. Koper et al. (2010) reported that the \( V_{\text{max}} \) of soils receiving ammonium sulfate at 200 kg N per hectare for 6 years was about twice higher than the \( V_{\text{max}} \) 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 \( V_{\text{max}} \) and \( K_m \) of AOB and total nitrification could beboosted 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 \( V_{\text{max}} \) and \( K_m \) responses among ecosystems varying in management intensity.

### 4.3 Contribution of AOA and AOB to \( V_{\text{max}} \) along the management intensity gradient

We used a Bayesian approach to calculate the relative contributions of AOA versus AOB to nitrification-derived N\textsubscript{2}O based on posterior distributions of \( V_{\text{max}} \) for each ecosystem, which is different from the traditional method of separating AOA from AOB based on 1 mM\text{NH}_4\textsuperscript{+} addition (Lu et al., 2015; Ouyang et al., 2016; Taylor et al., 2010). As noted earlier, 1 mM \( \text{NH}_4\textsuperscript{+} \) additions did not always yield the highest N\textsubscript{2}O emissions in our systems (Figure 1), especially for agricultural soils. Thus, partitioning sources of nitrification-derived N\textsubscript{2}O with \( V_{\text{max}} \) derived from substrate kinetics aligns with the concept of nitrification potential assays, which reflect the maximum nitrification-derived N\textsubscript{2}O from nitrifier communities (Norton & Stark, 2011).

The declining importance of AOB to N\textsubscript{2}O 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 (Habteslassie 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 \( \text{NH}_4^+ \) source is via decomposition of soil organic matter. Thus, the speed of \( \text{NH}_4^+ \) supply to soil seems important for shaping the dynamics of AOA versus AOB N\textsubscript{2}O-generating activities. Indeed, Hink et al. (2018) observed that AOA dominated nitrification-derived N\textsubscript{2}O 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 \( \text{NH}_4^+ \) in soil compared with non-tilled systems. As a result, AOB likely also outcompetes AOA following tillage-induced pulses of \( \text{NH}_4^+ \).

The dominance of AOA for nitrification-derived N\textsubscript{2}O 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 \( \text{NH}_4^+ \) oxidation and over 90% of cumulative N\textsubscript{2}O 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 \( \text{NH}_4^+ \) 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 N₂O fluxes

Seasonally stable nitrification-derived N₂O fluxes allow us to apply kinetics models to predict potential maximum N₂O emissions from nitrification and, subsequently, the theoretical maximum relative contribution of nitrification to field-based N₂O emissions assuming nitrifiers has exclusive access to solution-phase NH₄⁺. Since the kinetics results are based on aerobic incubations of shaken soil slurries that eliminate both N₂O reduction and N₂O from nitrifier denitrification (Wrage et al., 2001; Wrage-Mönnig et al., 2018), N₂O rates can be considered nitrifier nitrification rather than nitrifier denitrification, and when applied to historical solution-phase in situ NH₄⁺ pools, reveal maximum potential nitrification-derived N₂O 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 N₂O fluxes in the Conventional agricultural system suggests that nitrification is unlikely to be a significant source of N₂O 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 N₂O source in even unfertilized annual cropping systems. Using N₂O SP analysis, Opdyke et al. (2009) and Zou et al. (2014) reported a small role for nitrification in N₂O 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 N₂O 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 N₂O 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 N₂O reflect maximum in situ potentials that assume all solution-phase NH₄⁺ is available exclusively to nitrifiers and no nitrification-derived N₂O is further denitrified to N₂. Neither of these assumptions are realistic in situ. Soils are rarely completely aerobic, and even if in situ nitrification emitted N₂O equivalent to the amount from shaken soil slurries, some of the N₂O will be captured by denitrifiers and reduced to N₂ before being emitted to the atmosphere (Decock & Six, 2013; Lewicka-Szczechak et al., 2017; Shcherbak & Robertson, 2019).

Malhi and McGill (1982) estimated that the daily maximum NH₄⁺-N oxidation rate is <10% of available NH₄⁺-N (100 µg N g⁻¹) based on laboratory incubations. Prosser et al. (2020) reported pure culture N₂O 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 NH₄⁺ is oxidized and consequently eligible for transformation to N₂O is undoubtedly an overestimate by a factor of 10 to 100 or more. That said, our conclusion of nitrification being a minor source of N₂O in these ecosystems is conservative by nature. Actual contributions of nitrification to measured N₂O 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 N₂O fluxes was measured in Early successional and Deciduous forest soils where 51%–77% of total N₂O fluxes might potentially derive from nitrification in the Deciduous forest system (Figure 4a), and over 95% of the predicted nitrification-derived N₂O 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 (Schüttler et al., 2018), which contribute to high measured denitrification rates (Robertson & Tiedje, 1984). So even in our systems with the greatest percentage of N₂O 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 N₂O emissions in all of the systems examined. This finding has significant implications for biogeochemical N₂O 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 N₂O 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 N₂O flux measurements can be accessed at https://lter.kbs.msu.edu/databales/28; long-term in situ soil NH₄⁺ -N concentrations are available at https://lter.kbs.msu.edu/datables/55. Soil bulk densities of deep core soil samples are available from https://lter.kbs.msu.edu/databales/308. Species composition of Early successional and Deciduous forest systems is available from https://lter.kbs.msu.edu/databales/237 and https://lter.kbs.msu.edu/databales/238. All other data used in this study are available at datadryad.org (https://doi.org/10.5061/dryad.37pvmcvk2).

Code availability: The code for estimating log-normal mean of in situ N₂O fluxes and the maximum contribution of nitrification to total N₂O are shown in Supporting Information.

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REFERENCES


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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 (N₂O) 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 N₂O 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$_2$O 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$_2$O potentials.
R scripts for estimating the log-normal mean and the maximum contribution of nitrification to *in situ* N$_2$O fluxes

library(jagsUI)
library(fitdistrplus)

# First, estimate parameters for log-normal distribution
# file_name1 is the positive long-term *in situ* N$_2$O 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 N$_2$O 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 N$_2$O 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)
")
\[ a_1 \sim \text{dnorm}(\text{mean1}, \text{tau1}) \]
\[ b_1 \sim \text{dnorm}(\text{mean2}, \text{tau2}) \]
\[ a_2 \sim \text{dnorm}(\text{mean3}, \text{tau3}) \]
\[ b_2 \sim \text{dnorm}(\text{mean4}, \text{tau4}) \]
\[ \text{tau0} \leftarrow \frac{1}{(\sigma_0 \times \sigma_0)} \]
\[ \text{tauc} \leftarrow \frac{1}{(\text{sigmac} \times \text{sigmac})} \]
\[ \text{tau1} \leftarrow \frac{1}{(\text{sigma1} \times \text{sigma1})} \]
\[ \text{tau2} \leftarrow \frac{1}{(\text{sigma2} \times \text{sigma2})} \]
\[ \text{tau3} \leftarrow \frac{1}{(\text{sigma3} \times \text{sigma3})} \]
\[ \text{tau4} \leftarrow \frac{1}{(\text{sigma4} \times \text{sigma4})} \]
\[ \text{sigma0} \sim \text{dunif}(0, 5) \]
\[ \text{sigmac} \sim \text{dunif}(0, 5) \]

# Derived quantities
\[
\text{muOfY} \leftarrow \exp(\text{muOfLogY} + \text{sigmaOfLogY}^2/2)
\]
for (m in 1:P) {
    \[
    \text{nitri\_total}[m] \leftarrow a_1 \times [m] / (b_1 + x[m])
    \]
    \[
    \text{nitri\_aob}[m] \leftarrow a_2 \times [m] / (b_2 + x[m])
    \]
    \[
    \text{total\_avg} \leftarrow \text{mean}((\text{nitri\_total[]}])
    \]
    \[
    \text{aob\_avg} \leftarrow \text{mean}((\text{nitri\_aob[]})
    \]
    \[
    \text{con\_total1} \leftarrow \text{total\_avg} / \text{muOfY}
    \]
    \[
    \text{con\_aob1} \leftarrow \text{aob\_avg} / \text{muOfY}
    \]
    \[
    \text{major\_aob} \leftarrow a_2 / a_1
    \]
}

"", 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],  
mean2 = coef(summary(P1_total_parm))[,2],  
mean3 = coef(summary(P1_aob_parm))[,1],  
mean4 = coef(summary(P1_aob_parm))[,2],}
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)