

ORIGINAL RESEARCH ARTICLE

Agrosystems

Nitric and nitrous oxide fluxes from intensifying crop agriculture in the seasonally dry tropical Amazon–Cerrado border region

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Abstract

Rapid expansion and intensification of crop agriculture in the tropics may accelerate ecosystem losses of reactive nitrogen (N). We quantified emissions of nitric oxide (NO) and nitrous oxide (N₂O) in forest, single-cropped soybean [*Glycine max* (L.) Merr.], and N-fertilized double-cropped soybean–maize (*Zea mays* L.) at three N fertilizer levels within the largest area of recent cropland expansion on earth, in the Amazon and Cerrado biomes in Brazil. The NO emissions were 2.1 kg N ha⁻¹ yr⁻¹ in forest, 0.6 kg N ha⁻¹ yr⁻¹ in soybean, and 1.3 kg N ha⁻¹ yr⁻¹ in soybean–maize. The N₂O fluxes were <1.1 kg N ha⁻¹ yr⁻¹ across all land uses. As fertilization levels increased from 80 to 160 kg N ha⁻¹ yr⁻¹ in soybean–maize double-cropped sites, NO emissions increased from 0.6 to 6.7 kg N ha⁻¹ mo⁻¹ in the month immediately after fertilization, but N₂O emissions only increased from 0.6 to 1.8 kg N ha⁻¹ mo⁻¹. These results indicate that NO emissions do not increase when forests are converted to croplands under current fertilization levels, and that NO will respond more strongly than N₂O fluxes to increases in fertilizer applications. Our findings suggest that if N fertilization rates in the region were increased, NO fluxes could increase rapidly.

1 | INTRODUCTION

Expanding croplands and increasing fertilizer use in tropical regions can be a growing source of reactive nitrogen (N) emissions to the atmosphere. A global frontier for tropical agricultural intensification is the Amazon and Cerrado biomes in Brazil, where large-scale commodity cropping has propelled Brazil to become the world's leading exporter and the second largest producer of soybeans [*Glycine max* (L.) Merr.], and the world's second largest exporter and third largest producer of maize (*Zea mays* L.) by 2018 (FAO, 1997). Approximately 12% of tropical forest in the Amazon biome has been cleared (MapBiomass, 2019), and about half of the Cerrado (savanna) biome has been converted to agriculture (Klink &

Machado, 2005; Sano et al., 2010). Much of the tropical forest, often initially cleared for pasture in the region in the 1980s (Gibbs et al., 2010), was converted to large-scale mechanized soybean and other crops (Morton et al., 2006) in the early 2000s. Another shift soon followed in the state of Mato Grosso, Brazil, where there was a sixfold increase from single cropping of soybean to double cropping of soybean and maize between 2001 and 2011 (Spera et al., 2014). Whereas soybean fixes atmospheric N₂ through a symbiotic association with bacteria, maize requires N fertilization, and therefore maize cropping introduced large-scale N fertilization to the region. This is important because N losses from fertilizer can cause environmental problems such as smog and ozone production from nitric oxide (NO) (Fowler et al., 2013) and

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greenhouse warming from nitrous oxide (N_2O) (Ciais et al., 2013).

Future increases in population, per-capita food consumption, and global trade will continue to drive agricultural intensification and N fertilizer use over large regions of remaining tropical forests and savannas (Laurance et al., 2014). Given that current use of N fertilizers in many tropical croplands is low, raising N fertilization rates could greatly increase crop yields (Vitousek et al., 2009). At the same time, high applications of N fertilizers associated with commodity crops could increase N losses and negative environmental impacts. The relationships between N fertilizer use and fluxes of NO and N_2O are fairly well established in temperate agroecosystems, but unintended N losses from fertilizer are poorly understood in tropical croplands. A recent meta-analysis identified only 11 studies with NO measurements from fertilized agricultural systems in the tropics (Huddell et al., 2020). Although across the global tropics increased fertilizer use results in larger emissions of N_2O and NO, these emissions are highly variable from site to site (Huddell et al., 2020). Although N_2O emissions from croplands in tropical and subtropical Brazil at current levels of N fertilization have been found to be smaller than responses to similar levels of fertilization of temperate croplands (Meurer et al., 2016), NO emissions may be higher (Huddell et al., 2020).

Although the soybean–maize double-cropping system has expanded rapidly in recent years, climate change is shortening the duration of the rainy season and threatening the double-cropping system in the region (Costa et al., 2019). Irrigation is a way to overcome this challenge, as it can maintain or increase food production in this region (Costa et al., 2019; Lathuillière et al., 2018; Sentelhas et al., 2015) while sparing intact forest and savanna for conservation. If irrigation were introduced, current N rates for maize of $80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the region (Hampf et al., 2020) may rise closer to a rate of $160 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, which is comparable with N fertilization in maize in the U.S. Midwest (USDA, 2015) and has maximized yields in well-watered conditions and similar environmental conditions in Thailand (Moser et al., 2006). At present, there are few measurements of N_2O and no known NO measurements from the enormous region in the southern Amazon where soybean–maize double cropping continues to expand rapidly. Previous studies on land use change in Amazonia have found that land use affects NO and N_2O emissions (Davidson et al., 2001; Verchot et al., 1999), and the hole-in-the-pipe model would predict that NO emissions may exceed N_2O emissions in well-aerated soils (Davidson et al., 2000). We hypothesized that NO losses may be larger than N_2O losses in the southern Amazon because of the seasonally dry climate and well-drained soils, and that both NO and N_2O losses should increase with fertilization as in other regions, but that the rate of NO losses with fertilization may exceed N_2O losses.

Core Ideas

- Nitric oxide fluxes were measured across three land uses in the southern Amazon.
- Nitric oxide fluxes from forest were significantly higher than in soybean fields.
- Increases in nitrogen fertilizer caused large nitric oxide fluxes in soybean–maize.
- Nitrous oxide fluxes across all land uses were $<1.1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$.

In this study, we quantified N oxide fluxes from single-cropped soybean and double-cropped soybean–maize cropland in an area of the southern Brazilian Amazon in Mato Grosso that has recently undergone rapid cropland expansion and intensification. To establish background emission levels, we also quantified fluxes from adjacent native tropical forest. We addressed two questions with field measurements:

1. How do N_2O and NO fluxes vary among forest, soybean cropland, and double-cropped soybean–maize cropland?
2. How do N_2O and NO fluxes respond to increased N fertilizer applications in double-cropped soybean–maize cropland?

Measurements from this region will help predict how cropland expansion and intensification in similar lowland tropical forest and savanna regions could affect regional and global N oxide budgets.

2 | MATERIALS AND METHODS

2.1 | Description of the field site

This study took place at Tanguro Ranch (Figure 1), a commercial soybean and soybean–maize farm with remnant intact forest in the Cerrado–Amazon transition region in eastern Mato Grosso, Brazil. The climate, topography, soils, and cropping systems at Tanguro are similar to those in the expanding soybean and soybean–maize cropland industry in the Cerrado–Amazon region. Approximately half of the farm's 80,000 ha were cleared in the early 1980s for cattle pasture and converted to soybean between 2003 and 2008. Since 2008, parts of the ranch have been converted to double cropping with soybean and maize in a single growing season (Neill et al., 2013). The site lies on the Brazilian Shield on Precambrian gneisses of the Xingu Complex, at 320–390 m asl. The dominant soils are Oxisols (Haplustox); these soils are deep ($>10 \text{ m}$), acidic (pH of native forest soil = 3.9), and have a sandy clay texture (Figueira et al., 2016). Mean annual temperature and rainfall are $27 \text{ }^\circ\text{C}$ and 1,770 mm (Neill et al., 2013), and there is a

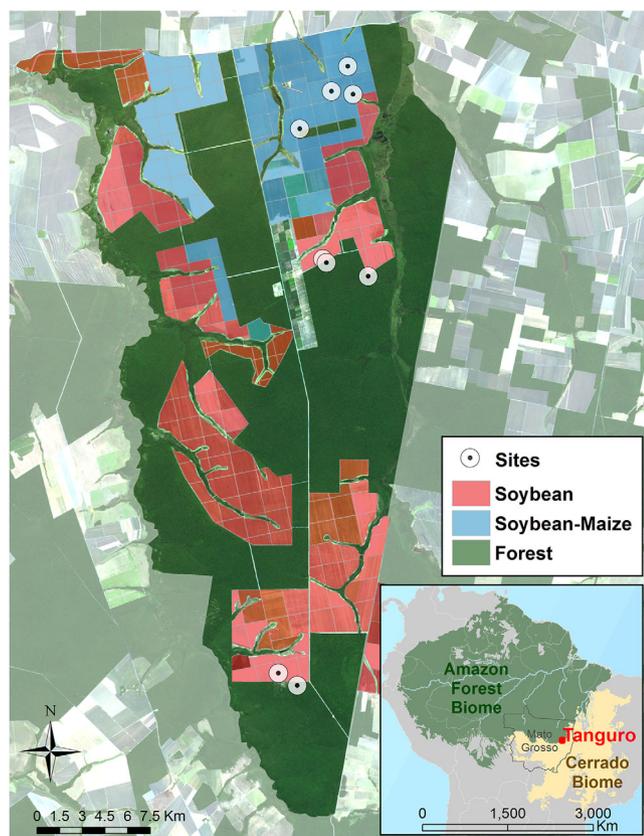


FIGURE 1 | Map of the study area

strong dry season from May to September in which total precipitation for the 5-mo period averages 80 mm (Figueira et al., 2016). The native, primary forest is seasonally dry but largely evergreen and has never been logged. See Ivanauskas et al. (2004) for a detailed description of this forest type.

2.2 | Study design and sampling description

We conducted two studies that measured NO and N₂O fluxes: (a) a land use comparison, and (b) a fertilizer manipulation. For the land use comparison, we measured NO and N₂O fluxes during 1 yr from the intact forest, single-cropped soybean, and double-cropped soybean–maize, which make up the three dominant land uses at Tanguro. Single-cropped soybeans received no N fertilizer. Double-cropped soybean and maize received 80 kg N ha⁻¹ during maize cropping.

We established three sites each in forest, soybean fields, and soybean–maize fields. These were separated by distances of 3–28 km within each treatment. Within each site, we sampled five soil chamber replicates for NO fluxes. We sampled almost daily for 1 wk in the soybean–maize double-cropped plots after the fertilization period, when a pulse of fluxes was expected, and then weekly for the rest of the first month. For the rest of the NO measurements (soybean–maize after the

first month, and all the soybean and forest sites), we took five replicate NO measurements at each of the three sites approximately every 2 mo throughout the year. The sampling scheme for N₂O was slightly different. For the first 2 wk after fertilization in soybean–maize plots, we sampled five replicate chambers for N₂O. For the rest of the N₂O measurements (1 mo after fertilization in soybean–maize and throughout the year for the forest and soybean sites), we sampled three replicate chambers monthly.

For the fertilization manipulation, we measured NO and N₂O fluxes before and after a single dose of urea fertilizer (which is standard management practice at Tanguro) at three different application rates—80 (the current application rate), 120, and 160 kg N ha⁻¹ yr⁻¹—applied to the maize crop in the middle of the rainy season in February 2017, after the soybean crop had been harvested. The plots were established within three soybean–maize double-cropped fields (between 3 and 4 km apart from one another). We had to stagger the fertilization starting dates because it was only logistically possible to measure two sites in a day, but we fertilized our smaller plots at the same time as the ranch fertilized each large field. Because we conducted the experiment in working crop fields, there was no zero-fertilizer treatment. These fertilization levels were comparable with N fertilization in more intensified systems and mirrored a previous study at Tanguro that focused on the relationship between N₂O fluxes and N fertilization (Jankowski et al., 2018). Based on that study’s findings of low and not highly variable N₂O fluxes during the dry season and during soybean cropping, we concentrated more replicates of N₂O measurements in the period after fertilization, when we expected larger fluxes. For N₂O, we sampled five replicate chambers at each soybean–maize double-cropped site and fertilization treatment every day possible for the first week, once in the second week, and then decreased to three replicates per site and treatment measured weekly for the rest of the month. We sampled NO concurrently with N₂O measurements, and there were always five replicate NO chamber measurements. All the chambers in the fertilization experiment were sampled on the same day, mostly during the hotter part of the day (10:00 a.m.–3:00 p.m.) because of the time required to travel to these remote field sites.

We measured soil–atmosphere N₂O fluxes with static chambers (Jankowski et al., 2018; Venterea et al., 2005). Rectangular stainless steel (20-gauge) chamber bottoms were made by cutting the bottom of stainless-steel restaurant “steamtable” pans (53 cm long × 32 cm wide × 12 cm deep). We modified the tops of the pans by adding sampling ports (with rubber septa), vent tubes (0.64-cm diam., 20 cm long, stainless steel; near the bottom edge to allow pressure to equilibrate within the chamber), rubber weather stripping (ethylene propylene diene tetrapolymer, to seal the bottom edges), and reflective aluminum insulation wrap (around the top to prevent large temperature fluctuations). We sealed the

chamber tops to the bases using six binder clips around the flanges and collected 30-ml gas samples with plastic syringes at 0, 15, 30, and 45 min. We injected these samples into pre-evacuated 20-ml glass vials fit with thick Geo-Microbial Technologies rubber septa. The chambers were large enough to straddle the distance between the maize crop rows; we carefully placed them to try to capture within- and between-crop row variation in maize and soybean. Because these measurements were taken in a working ranch, the chamber bases had to be removed and reinstalled at each site visit and were not placed in exactly the same location each time.

The first 800 N₂O samples were transported by bus to Piri-cicaba, São Paulo, for analysis by gas chromatography on a Shimadzu greenhouse gas analyzer model GC-2014 with an electron capture device (ECD) and calibrated with analytical-grade standards (Scott Specialty Gases). The 1,350 remaining samples were flown to New York and analyzed by ECD on an SRI 8610C gas chromatograph and calibrated with analytical grade standards by Tech Air. We cross-checked standards between the two instruments. Carbon dioxide was measured simultaneously by a flame ionization detector.

To measure NO fluxes we used a flow-through static chamber where air from the chamber was connected to a field-portable N₂O analyzer (2B Tech model 410, detection range: 2–2,000 nl L⁻¹; precision: ±1.5 nl L⁻¹; measurement rate: 0.1 Hz) as in Eberwein et al. (2020) and Oikawa et al. (2015) and then recirculated to the chamber to measure soil-atmosphere NO fluxes. We modified one of the same steel chambers to create a flow-through chamber attached to the analyzer that measured NO concentrations continuously in the chamber by ozone consumption by NO using ultraviolet absorbance at a flow rate of ~1 L min⁻¹ and then returned sampled air to the chamber. We connected the NO monitor to the chamber with 0.6-cm polytetrafluoroethylene-lined tubing and mixed air inside the chamber with a computer fan. We used the same chamber bases that were installed for N₂O measurements and attached the modified NO chamber top. The calibration was checked once per week during frequent sampling periods by pumping a known concentration of NO using a NO calibration source (2B Tech model 208) to the 2B tech model 410 NO analyzer. Our analyzer did not require adjustment throughout the field campaign, as expected given that the calibration on the 2B Tech model 410 is much more stable than traditional chemiluminescent monitors. The portability and stability of the calibration of this NO monitor makes it suitable for environments with higher NO fluxes (with ambient conditions at or above 2 nl L⁻¹ NO) and working in remote locations.

At the same time that we sampled trace gases, we measured soil extractable nitrate (NO₃⁻) and ammonium (NH₄⁺), gravimetric soil moisture, and chamber air temperature at each sampling point in both experiments. We sampled soil from the

top 5 cm of soil with a hand trowel, homogenized the sample, added it to 2 M KCl, hand shook the samples, and then extracted them for 24 h. Simultaneously, we saved a subset of the soil sample to determine gravimetric water content of the soil by drying it at 105 °C to a constant weight. We used the soil gravimetric water content, bulk density, and particle density to calculate soil water filled pore space. The 2 M KCl extractions were filtered and analyzed for NH₄⁺ and NO₃⁻ on a SmartChem 170 discrete analyzer (Westco Scientific Instruments). To measure precipitation, we set out rain gauges at each soybean–maize double-cropped site for the first 7 d of the fertilizer addition measurements. For the rest of the year, we relied on precipitation measurements from the station headquarters at Tanguro, between about 2–30 km from our sites.

2.3 | Flux calculations

We calculated N₂O fluxes using linear regressions on the rate of the concentration data at 0, 15, 30, and 45 min. We chose linear regressions rather than nonlinear regressions to calculate the chamber fluxes because linear models are less sensitive to noise in the data and preferable for comparing across treatments (Levy et al., 2011; Venterea et al., 2009), because the linear method is recommended for fluxes below 22 g N m⁻² h⁻¹ (Parkin & Venterea, 2010), and because our preliminary analyses showed no clear evidence of saturation of the N₂O concentrations over time.

We used the CO₂ concentrations in the static chambers to check for evidence of air leaks during the incubations because high CO₂ fluxes enabled us to detect concentrations fluctuations caused by leaks. We filtered out from further analysis all chambers that had $R^2 < .60$ for CO₂ fluxes and chambers that had observations from fewer than three time points. For chambers with $.60 < R^2 < .80$ for CO₂ concentrations, we removed visual time point outliers ($n = 40$ of 2,151 total) based on the CO₂ concentrations. After removing those points, the minimum R^2 was .73 and the median R^2 was .98. The remaining N₂O chambers with R^2 fits below .60 ($n = 34$) generally had small positive or small negative flux rates; only six of those chambers fell above the median flux rate. Generally, higher N₂O flux estimates had higher R^2 fits than the smaller fluxes.

We calculated flux estimates in mass per unit area per time (mg N₂O–N m⁻² h⁻¹) assuming ideal gas relations by using the linear fluxes in µl L⁻¹ N₂O h⁻¹, individual chamber volume estimates from six base depth measurements, chamber area, and individual chamber temperature measurements. Nitric oxide fluxes were calculated from the slope of a linear regression as in (Oikawa et al., 2015) on the rate of concentration change between 1 and 4 min after the chamber was closed. We imputed zeroes for NO concentrations that fell below the detection limit for individual chamber fluxes.

2.4 | Models and statistical analyses

All statistical analyses were conducted using the R statistical programming language version 3.5.0 (R Core Team, 2018). We tested the relationships between N oxide fluxes, treatment, and date for the individual flux measurements over time using an ANOVA on linear mixed effects models in the following models:

$$\text{response} = \beta_0 + \gamma_i + \beta_1 \times \text{treatment} + \beta_2 \times \text{day} + \varepsilon \quad (1)$$

where response is the N oxide flux (NO or N₂O) in mg N m⁻² h⁻¹ or the soil N pool (NO₃⁻ or NH₄⁺) in mg N kg soil⁻¹; β₀ is the fixed-effect intercept; γ_i is the random variation in the intercept for each site *i*; β₁ is the coefficient of treatment (either the N fertilizer levels or the land use type); β₂ is the coefficient of the day (the number of days since fertilization for the fertilization study, or the date for the land use study); and ε is unexplained residual variation. We ran identical models for NO and N₂O fluxes and soil NO₃⁻ and NH₄⁺ pools. We used ANOVA and least square means post-hoc comparisons (with Tukey *P* value adjustments) tests on model fit with treatment and date as fixed effects and site as a random effect to compare the effects of treatment and date for the individual flux measurements from both studies (Equation 1).

We used the same approach to test the relationships between cumulative N oxide fluxes and treatment using linear mixed effects models in the following models:

$$\text{NO or N}_2\text{O} = \beta_0 + \gamma_i + \beta_1 \times \text{treatment} + \varepsilon \quad (2)$$

where NO or N₂O is the N oxide flux in kg N ha⁻¹ yr⁻¹; β₀ is the fixed-effect intercept; γ_i is the random variation in the intercept for each site *i*; β₁ is the coefficient of treatment (either the N fertilizer levels or the land use type); and ε is unexplained residual variation. We ran identical models for NO and N₂O fluxes.

We explored the relationships between N oxide fluxes and soil N and moisture content using linear mixed effects models with the lme4, emmeans, and lmerTest packages in R (Bates et al., 2018; Kuznetsova et al., 2017; Lenth et al., 2019):

$$\text{NO or N}_2\text{O} = \beta_0 + \gamma_i + \beta_1 \times \text{SWFPS} + \beta_2 \times \text{soil N} + \beta_3 \times (\text{SWFPS} \times \text{soil N}) + \varepsilon \quad (3)$$

where NO or N₂O is the N oxide flux in mg N m⁻² h⁻¹; β₀ is the fixed-effect intercept; γ_i is the random variation in the intercept for each site *i*; β₁ is the coefficient of soil water-filled pore space (SWFPS, a measure of soil moisture %); β₂ is the coefficient of soil N (the sum of soil NO₃⁻ and NH₄⁺ in mg N kg soil⁻¹); β₃ is the coefficient of the interaction between SWFPS and soil N; and ε is unexplained residual variation. We standardized SWFPS and soil N by subtracting the mean and dividing by the standard deviation of each variable.

To calculate cumulative monthly or annual fluxes, we bootstrapped the data to assemble five flux measurements (within each site, treatment, and sampling date) for each unique sampling event. The chambers were moved from one day to another, so replicates were not linked across sampling dates. For unique sampling events with five replicates, no observations were repeated, but some observations were repeated in cases with fewer than five replicates. We used trapezoidal integration from the *pracma* package on the bootstrapped data to calculate cumulative fluxes for both NO and N₂O (Borchers, 2018). This method sums the area under trace gas measurement points across sampling points throughout the year by day of the year, assuming that the hourly flux rates we measured were mean daily rates. To roughly scale up the monthly flux rates for the medium and high fertilization treatments to annual estimates for soybean–maize double-cropping, we added the values for the first month after fertilization for each treatment to the N fluxes total from the rest of the year (excluding the first month after fertilization) measured in the low fertilization treatment. This rough scaling is intended to provide an approximate comparison with the annual flux estimates measured in the low N treatment, and likely an underestimate of the annual flux rate of the medium and high N treatments, which we did not have enough resources to measure throughout the year.

We calculated cumulative NO fluxes in two ways due to uncertainty about distinguishing between true NO uptake into soil and apparent NO uptake that may have been an artifact of the closed chamber method. Some chambers with negative NO flux calculations appeared to demonstrate true N uptake, where concentrations well above the detection limit of the instrument steadily declined. However, other chambers with negative NO flux measurements appeared to be artifacts of the closed chamber measurements, where concentrations rose slightly (to about 8 nl L⁻¹ NO) after closing the chamber and then quickly declined to below the detection limit (2 nl L⁻¹ NO). In the main text, we imputed zeroes for chambers with negative flux estimates, assuming that measured negative fluxes were an artifact of the method. In the supplement, we report cumulative NO fluxes with the negative chamber fluxes included (assuming the measured uptake is real; Supplemental Table S9). There were no significant differences in the results calculated using either method.

3 | RESULTS

3.1 | NO fluxes, N₂O fluxes and soil N from the land use comparison

Fluxes of both NO and N₂O in the land use comparison followed similar seasonal patterns and varied significantly with time but not among treatments (Figures 2a and 2b, Supplemental Table S1). Nitric oxide and N₂O fluxes were low

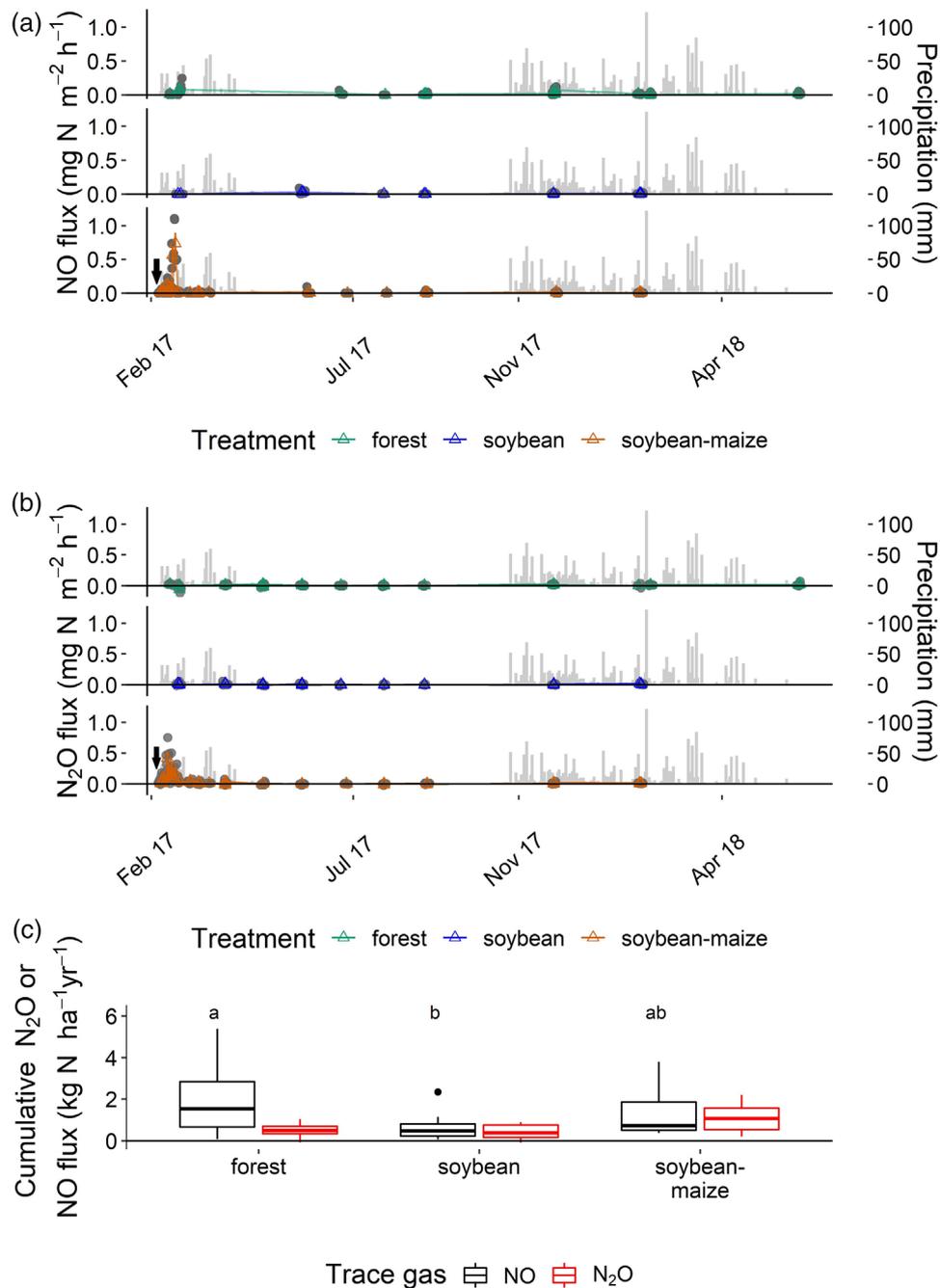


FIGURE 2 N_2O and NO fluxes by different land uses. Forest, soybean, and soybean–maize double-cropped are shown (a, b) through time and (c) summed over the year. Precipitation from the main gauge station throughout the study period is also shown as light gray bars (a, b). The dark gray points are individual observations, and the colored triangles with error bars are the means and standard errors across replicates and sites (a, b). The arrows (a, b) indicate approximately when fertilizer was applied to maize seedlings in February 2017. Box plots show the cumulative annual fluxes across replicates and sites; the outer edges of the box are the first and third quartiles and the middle line is the median (c). The soybean–maize sites received the standard $80\text{-kg N ha}^{-1} \text{yr}^{-1}$ fertilizer treatment, whereas the soybean and forest received no fertilizer. Significant differences among treatments in the cumulative NO estimates are noted with different letters in Panel c (there were no significant differences among treatments in cumulative N_2O fluxes)

during most of the year in all land uses, especially in the dry months, but they increased near the onset of the rainy season and after fertilization in the cropping period (Figures 2a and 2b). Fluxes of NO and N_2O peaked immediately after fertilization for the soybean–maize double-cropped treatment,

but the period of elevated fluxes was brief (Figures 2a and 2b).

Cumulative annual NO fluxes in soybean were significantly lower than fluxes in forest (Figure 2c, Supplemental Table S2), and sites within the treatment varied, as captured

by the random effects in our models (Supplemental Figure S1, Equation 2). Despite the peak in both N oxide fluxes when soybean–maize sites were fertilized with the current practice of 80 kg N ha⁻¹, annual fluxes from soybean–maize sites tended to be less than those from the forest, but the difference was not significant (Figure 2c, Supplemental Table S2). Cumulative annual N₂O fluxes were slightly but insignificantly higher in soybean–maize, fertilized croplands, compared with the forest (Figure 2c, Supplemental Table S2). The statistically insignificant 0.5-kg N ha⁻¹ increase in annual N₂O fluxes between the forest and soybean–maize croplands was approximately equal to the cumulative one month pulse for the 80-kg N ha⁻¹ yr⁻¹ treatment (Figure 3b, Supplemental Table S2), suggesting that the N fertilization pulse that we captured in the fertilization experiment led to a small but insignificant rise annual N₂O fluxes (Supplemental Tables S2 and S3, Figure 2c).

Soil extractable NH₄⁺ concentrations in the forest varied with time but were significantly higher than in both cropping systems (Figure 4a, Supplemental Tables S4 and S5). Soil extractable NO₃⁻ concentrations varied with time but not across land uses (Figure 4b, Supplemental Table S4).

3.2 | NO fluxes, N₂O fluxes and soil N from the fertilization manipulation

Nitric oxide and N₂O fluxes both increased after N fertilizer application. Fluxes from the 160-kg N ha⁻¹ yr⁻¹ dose were greater than those from the 80-kg N ha⁻¹ yr⁻¹ treatment, with much of the difference explained by different durations of large emissions (Figures 3a and 3b, Supplemental Tables S1 and S6). Cumulative NO fluxes significantly increased from low and medium fertilization to the high fertilization treatment, rising to >6 kg N ha⁻¹ mo⁻¹ in the first month after fertilization (Figure 3d, Supplemental Table S3). Cumulative N₂O fluxes in the first month after fertilization also increased significantly at each step from low to medium and high fertilization treatments, though the difference between high and low treatments was only ~1.2 kg N ha⁻¹ mo⁻¹ (Figure 3d, Supplemental Table S3). Both NO and N₂O fluxes in the first month after fertilization at the 80 kg N ha⁻¹ yr⁻¹ currently used at Tanguro Ranch were relatively low (0.6 kg N ha⁻¹ mo⁻¹; Figure 3d, Supplemental Table S3). Precipitation in the first week after fertilization appeared to be higher at Site 3 (Figure 3c), which corresponded to higher measured fluxes and significant random effects for the N₂O (but not NO) model on fertilization treatment and days since fertilization (Supplemental Table S1).

Soil extractable NH₄⁺ concentrations varied significantly with time, but not among fertilization treatments (Figure 4c, Supplemental Table S4). Soil extractable NO₃⁻ concentration in the high N treatment was significantly higher than in the

low N treatment (Figure 4d, Supplemental Tables S4 and S7). Soil extractable NO₃⁻ concentration varied significantly with time and increased towards the end of the month after fertilization, especially in the high N treatment (Figure 4d, Supplemental Table S4). Soil water-filled pore space ranged from 4 to 93%, but both the mean and median were 46%, indicating that the soils were generally well oxygenated (Supplemental Table S8).

3.3 | Results from models on NO and N₂O fluxes, soil nutrients, and soil moisture

Neither soil N (the sum of soil NH₄⁺ and NO₃⁻) nor soil water-filled pore space alone were significant predictors of NO or N₂O fluxes (Supplemental Table S8). However, the coefficient estimate for the interaction of soil N and soil water-filled pore space was positive and significant in the case of NO ($P = .01$) and was positive and marginally significant for N₂O ($P = .06$) (Supplemental Table S8).

4 | DISCUSSION

4.1 | Overall findings and comparison to previous work

Both NO and N₂O fluxes in soybean–maize cropping at the current N fertilization level were relatively small and were similar to native, intact forest (Figure 2c). Nitric oxide fluxes from soybean croplands (0.6 kg N ha⁻¹ yr⁻¹) were significantly lower than from forest fluxes (2.1 kg N ha⁻¹ yr⁻¹) (Figure 2c, Supplemental Table S2), but similar to another study from soybean cropland in the Brazilian Cerrado that found NO fluxes of 0.5 kg NO–N ha⁻¹ yr⁻¹ (Cruvinel et al., 2011; Supplemental Table S2). The NO fluxes from the forest in our measurements (2.1 kg N ha⁻¹ yr⁻¹, Supplemental Table S2) were similar to other NO fluxes measured from mature forests across the Amazon (between 1.4 and 1.7 kg N ha⁻¹ yr⁻¹; Davidson et al., 2001; Neill et al., 2005; Verchot et al., 1999), indicating that the forest has relatively high rates of nitrification and or denitrification that allow for large NO losses. Our N₂O measurements in croplands were also similar to previous estimates (Jankowski et al., 2018). The annual N₂O fluxes we measured for soybean–maize (1.1 kg N ha⁻¹ yr⁻¹) and soybean (0.4 kg N ha⁻¹ yr⁻¹) were not significantly different from the forest (0.5 kg N ha⁻¹ yr⁻¹; Figure 2c, Supplemental Table S2). The N₂O fluxes we measured and previous estimates from croplands in the biome were small, likely because of the soil type and seasonal dry climate. Our forest measurements were low compared with a review across Amazon forest sites, with mean annual precipitation rates between 1,770 and 2,539 mm

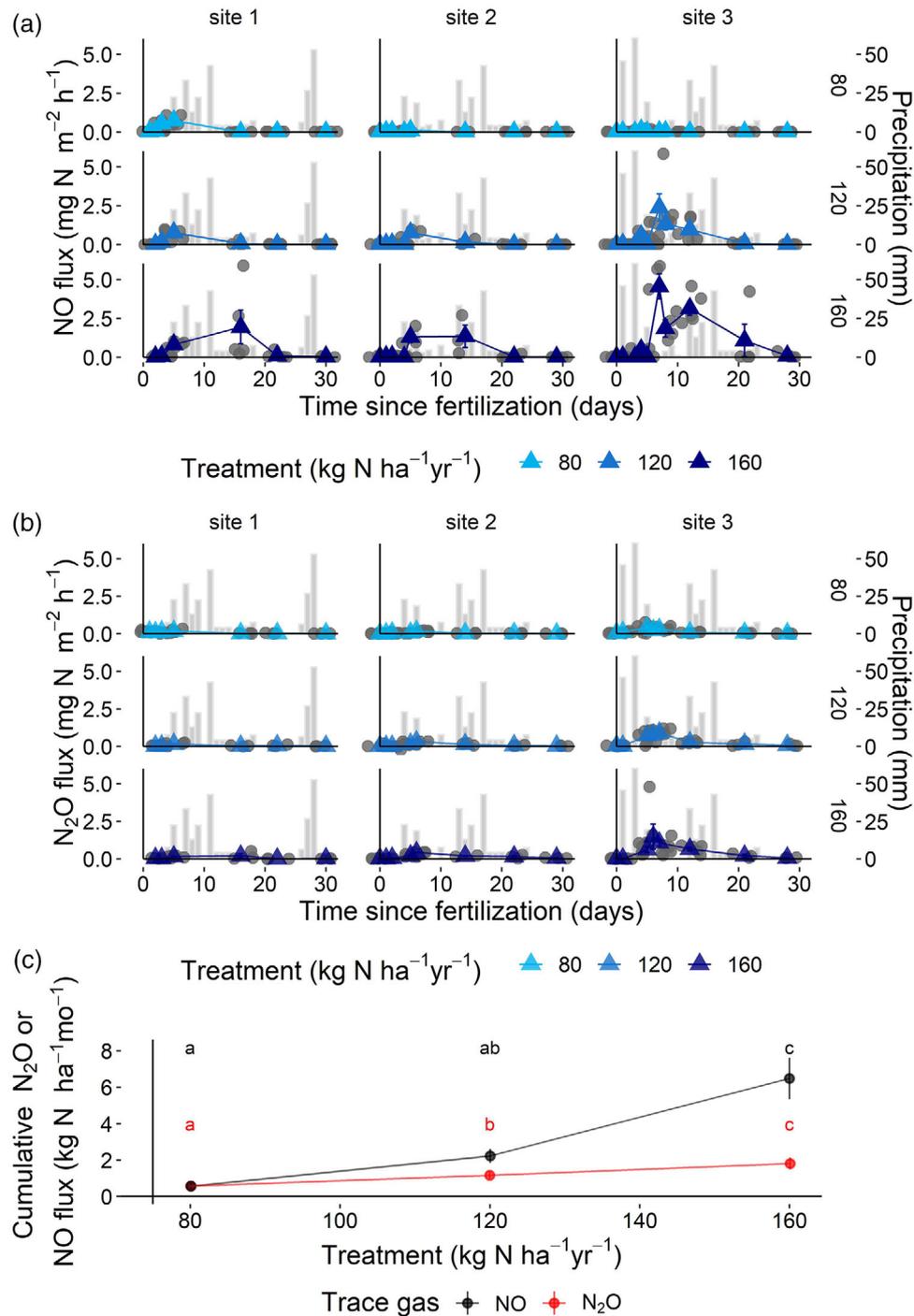


FIGURE 3 NO and N_2O fluxes at different fertilization levels in soybean–maize double-cropped sites. The (a) NO and (b) N_2O fluxes are shown by the time since fertilization in three soybean–maize double-cropped sites (1, 2, and 3) in the panels from left to right, and fertilization treatments (80, 120, and 160 kg N ha^{-1}) in the panels from top to bottom. (c) Precipitation data from the first 7 d come directly from rain gauges at each site, but the data from the rest of the days are from the main station. The NO and N_2O fluxes are also shown as cumulative 30-d fluxes averaged across sites in Panel d; note that the units are per month (the first month after fertilization), not per year, as in Panel c. The gray points are individual observations, and the colored triangles with error bars are means and standard errors across replicates within each site in Panels a and b, and across replicates and sites in Panel c. Significant differences among treatments in the cumulative NO or N_2O estimates are noted with different letters in Panel c

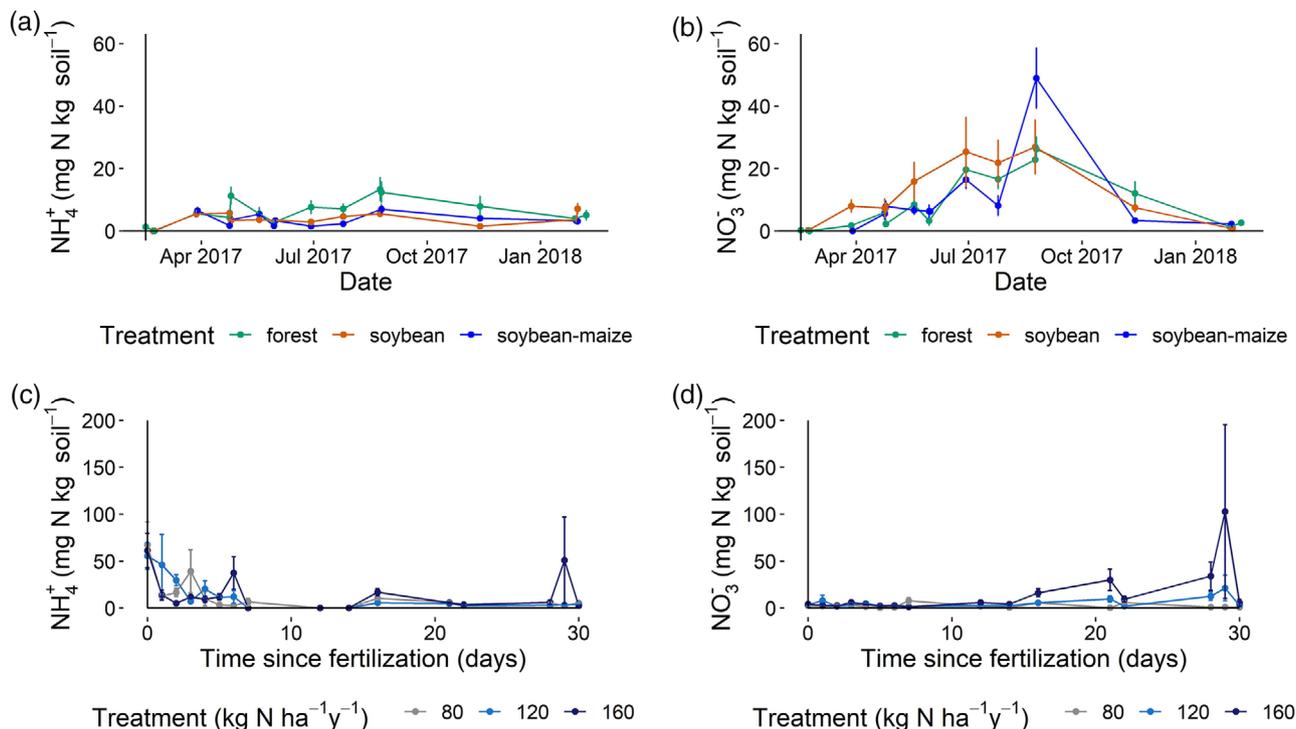


FIGURE 4 Soil (a, c) NH_4^+ and (b, d) NO_3^- across (a, b) land uses and (c, d) fertilization treatments. As in Figure 3, the soybean–maize land use in Panels a and b received the standard $80 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ of fertilizer, whereas the soybean and forest land use received no fertilizer. The points and bars are means and standard errors across replicates

with a median of $2.4 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$, but this was consistent with our forest sites lying at the low end of that precipitation range (Meurer et al., 2016).

Our N_2O measurements summed over the first month after fertilization increased more with N application rate than the annual N_2O estimates in a previous study at Tanguro ranch (Jankowski et al., 2018), likely because of wetter conditions and high measurements of soil water-filled pore space in our measurement period. At the medium N treatment ($120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), we measured $1.15 \text{ kg N}_2\text{O-N ha}^{-1} \text{ mo}^{-1}$, which would roughly scale to $1.68 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ based on our annual estimates (Figure 2c), compared with the previous estimate of $0.38 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$; and at the high N treatment ($160 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), we measured $1.81 \text{ kg N}_2\text{O-N ha}^{-1} \text{ mo}^{-1}$, which would roughly scale to $2.34 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ based on our annual estimates (Figure 2c), compared with a previous estimate of $0.58 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ (Supplemental Table S3) (Jankowski et al., 2018). We do not know exactly how our monthly estimates would translate to annual fluxes because we did not continue to measure the medium and high N treatments beyond the first month since fertilization, but it is likely that annual N_2O fluxes would be higher unless significant N_2O uptake occurred in the rest of the year. Nitrous oxide emission factors (N_2O fluxes for each treatment minus the N_2O fluxes from unfertilized plot, divided by fertilizer inputs) from Jankowski et al. (2018) varied from 0.1 to 0.2% of fertilization, much lower than the Intergovernmental Panel on

Climate Change (IPCC) emission factor estimate of 1% (De Klein et al., 2006). If we estimate emission factors from our cumulative annual estimates using the zero-fertilizer annual N_2O estimate from Jankowski et al. (2018), our emission factors estimates were 1.0% for the low N treatment, 1.2% for the medium N treatment, and 1.4% for the high treatment. These emission factors demonstrated that N_2O losses from this system can be higher than previously thought and reach the IPCC emission factor of 1% in wetter conditions.

4.2 | Important mechanisms underlying N oxide fluxes

Fertilized croplands and dry ecosystems like savannas and seasonally dry forests are important sources of NO globally (Davidson & Kinglerlee, 1997; Stehfest & Bouwman, 2006). Dry, well-oxygenated soils favor nitrification and more NO loss because NO can easily escape from the soil, whereas in more saturated, anaerobic soils, NO often gets reduced to N_2O before diffusing from the soil to atmosphere (Davidson et al., 2000). Despite a buildup of soil NO_3^- (Figure 4d), which often accumulates in tropical forests and pastures (Davidson et al., 2000; Keller & Reiners, 1994; Verchot et al., 1999), N_2O losses were low.

Clayey, well-structured Oxisol soils hold plant-available water well and also drain well because of the microaggregates

in the clay, and form a sandy-like soil texture structure (Sanchez, 2019). The forest maintains enough moisture for nitrification and some NO loss to occur year-round (Figure 2a); however, the soils across all land uses at this site drain very well (Scheffler et al., 2011) and seem not to reach the anaerobic conditions required for large N₂O fluxes. Meurer et al. (2016) speculated that the generally low responses of N₂O fluxes to fertilization across crop types in Brazil may be because these stable microaggregates of clay create oxygenated soils. We think that oxic conditions in the soil (mean soil water-filled pore space of 46%, Supplemental Table S8) from stable microaggregates and rapid nitrification in the soils at our site promoted higher NO than N₂O losses. Consistent with the previous study at Tanguro (Jankowski et al., 2018), we found high soil extractable NH₄⁺ concentrations after fertilization generally diminished, whereas NO₃⁻ concentrations increased (Figures 4c and 4d), indicating high nitrification.

5 | SUMMARY AND CONCLUDING THOUGHTS

We found that sites that were previously converted to croplands did not have higher NO fluxes compared with intact forests sites, that N₂O fluxes were generally low, and that NO responded more strongly than N₂O fluxes to increases in fertilizer applications. At the current fertilization level of 80 kg N ha⁻¹ yr⁻¹, NO and N₂O fluxes from croplands were similar to those from intact forests fluxes in the region, and NO fluxes from single-cropped soybean were even smaller than fluxes from forest.

However, doubling fertilizer levels from 80 to 160 kg N ha⁻¹ yr⁻¹ in soybean–maize double-cropped systems increased NO fluxes by an order of magnitude, from 0.6 to 6.7 kg N ha⁻¹ mo⁻¹, which would roughly scale to 1.1 and 7.4 kg N ha⁻¹ yr⁻¹, much higher than most observations from a NO fluxes at a 160-kg N ha⁻¹ yr⁻¹ fertilization rate that were typically <2 kg N ha⁻¹ yr⁻¹ at the same fertilization rate in a global meta-analysis (Huddell et al., 2020). Nitric oxide fluxes of 7.4 kg N ha⁻¹ yr⁻¹ exceeded the expected value for NO fluxes at a 160-kg N ha⁻¹ yr⁻¹ fertilization rate, but well within the range of observations from a global meta-analysis on fertilized croplands (Huddell et al., 2020). In the high fertilization treatment, our hourly NO flux measurements were among the highest ever measured in agricultural systems, comparable with the record highs reported in Matson (1998) and Oikawa et al. (2015). A recent analysis of NO emissions from croplands in California found that, at a mean of 19.8 (± 27.3 SD) kg N ha⁻¹ yr⁻¹, agricultural ecosystems are a dominant and overlooked source of NO in the state, with a significant effect on the regional N budget (Almaraz et al., 2018). If N fertilization rates in the region increased in conjunction with more intensification, our findings suggest NO has potential

to become a relatively large N loss in this system and that NO fluxes would increase rapidly and potentially degrade regional air quality.

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AUTHOR CONTRIBUTIONS

Alexandra Huddell: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Visualization; Writing-original draft; Writing-review & editing. Christopher Neill: Conceptualization; Data curation; Formal analysis; Funding acquisition; Supervision; Writing-original draft; Writing-review & editing. Leonardo Maracahipes-Santos: Investigation; Project administration; Writing-review & editing. Carlos Eduardo Pellegrino Cerri: Investigation; Writing-review & editing. Duncan Menge: Conceptualization; Formal analysis; Resources; Supervision; Writing-original draft; Writing-review & editing.

CODE AVAILABILITY

The code used to analyze data in this study can be downloaded at <https://doi.org/10.5281/zenodo.4609272>.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA STATEMENT

The data collected and analyzed in this study can be downloaded at <https://doi.org/10.5281/zenodo.4609272>.

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