Nitrous oxide and nitric oxide fluxes differ from tea plantation and tropical forest soils after nitrogen addition

Galina Y. Toteva[1,2], David Reay[2], Matthew R. Jones[1],
Nicholas Cowan[1], Ajinkya Deshpande[1], Buddhika Weerakoon[3],
Sarath Nissanka[3] and Julia Drewer[1]*

[1]UK Centre for Ecology & Hydrology, Penicuik, United Kingdom, [2]School of GeoSciences, The
University of Edinburgh, Edinburgh, United Kingdom, [3]Faculty of Agriculture, University of Peradeniya,
Peradeniya, Sri Lanka

South Asia is experiencing a rapid increase in nitrogen (N) pollution which is predicted to continue in the future. One of the possible implications is an increase in gaseous reactive N losses from soil, notably in the form of nitrous oxide (N_2O) and nitric oxide (NO). Current knowledge of N_2O and NO dynamics in forest ecosystems is not sufficient to understand and mitigate the impacts on climate and air quality. In order to improve the understanding of emissions from two major land uses in Sri Lanka, we investigated the emission potential for N_2O and NO fluxes measured by absorption spectroscopy and chemiluminescence, respectively, in response to three different N addition levels (the equivalent of 0, 40 and 100 kg N ha^{-1} yr.$^{-1}$ deposition in the form of NH_4^+) from soils of two typical land uses in Sri Lanka: a secondary montane tropical forest and a tea plantation using soil laboratory incubations of repacked soil cores. We observed an increase in NO fluxes which was directly proportional to the amount of N applied in line with initial expectations (maximum flux ranging from 6−8 ng NO-N g^{-1} d^{-1} and from 16−68 ng NO-N g^{-1} d^{-1} in forest and tea plantation soils, respectively). However, fluxes of N_2O did not show a clear response to N addition, the highest treatment (100 N) did not result in the highest fluxes. Moreover, fluxes of N_2O were higher following the addition of a source of carbon (in the form of glucose) across treatment levels and both land uses (maximum flux of 2−34 ng N_2O-N g^{-1} d^{-1} in forest and 808−3,939 ng N_2O-N g^{-1} d^{-1} in tea plantation soils). Both N_2O and NO fluxes were higher from tea plantation soils compared to forest soils irrespective of treatment level, thus highlighting the importance of land use and land management for gaseous reactive N fluxes and therefore N dynamics.

KEYWORDS

greenhouse gases, nitrogen deposition, ammonium, tropics, land use, Sri Lanka

1 Introduction

Reactive nitrogen (Nr) entering ecosystems has increased dramatically over the past few decades (Erisman et al., 2011; Malik et al., 2022). Galloway et al. (2021) estimated that Nr created by anthropogenic activities (primarily the Haber-Bosch process) has increased from <60 Tg N yr.$^{-1}$ in 1961 to 226 Tg N yr.$^{-1}$ in 2020. South Asia has been identified as a major source of Nr pollution, where both the absolute quantities and the growth rates in emissions

of several Nr compounds (ammonia – NH_3, nitrous oxide – N_2O, nitric oxide – NO and nitrogen dioxide – NO_2) were higher than global averages for the period 1970 to 2015 (Yang et al., 2022). The amount of Nr fertilizer added to agricultural land increased particularly sharply in South Asia (Begho et al., 2022). For instance, there was a 370 and 3,932% increase in total nitrogen (N) inputs in Sri Lanka and in India, respectively, in 2013 relative to 1961 (Raza et al., 2018). This is consistent with Deshpande et al. (2024) who modeled atmospheric NH_3 concentrations over Sri Lanka using the EMEP-WRF (European Monitoring and Evaluation Program – Weather Research and Forecast) model (Ge et al., 2021) and found high concentrations in the central and southwest parts of the country (approximately 10 to 30 $\mu g\,m^{-3}$). The increase in NH_3 emissions and hence atmospheric concentrations can in turn lead to increased deposition to agricultural and natural ecosystems (Galloway et al., 2004; Liu et al., 2022). Although a number of N policies have been implemented in recent years (Kanter et al., 2020; Yang et al., 2022), NH_3 pollution is predicted to further increase in the future (Lassaletta et al., 2016).

The United Nations Environment Program (UNEP) Frontiers Report identified nitrogen pollution as one of the five emerging threats facing our planet (UNEP, 2019a). Atmospheric deposition acts as a major source of Nr to natural ecosystems which are not subject to managed fertilization (Bobbink et al., 2010). This can have several impacts such as soil acidification (Tian and Niu, 2015) and eutrophication (Sutton et al., 1993; de Vries, 2021), as well as changes to the vegetation communities (Bobbink et al., 2010; Sheppard et al., 2014; Levy et al., 2019), microbial populations (Li et al., 2021) and greenhouse gas (GHG) dynamics (Leeson et al., 2017; Song et al., 2020). The impact of nitrogen deposition on GHG dynamics is not fully understood in the context of natural tropical ecosystems such as forests and may present an underestimated emission source that could contribute to climate change.

One potential but highly uncertain implication of increased N deposition is the increase of gaseous Nr losses, namely N_2O and NO. Nitrous oxide is a potent GHG whose global warming potential is 273 times that of carbon dioxide on a 100-year timescale (IPCC, 2023). Elevated N input has been reported to increase the emissions of N_2O from agricultural fields (Reay et al., 2012). However, the impacts on forests are more variable and less well understood (van Lent et al., 2015). Tropical forest soils present favorable conditions for high N_2O emissions as high temperatures and soil moisture content have been demonstrated to stimulate microbial and enzymatic activity which results in the production and release of N_2O (Davidson et al., 2000; Malhi and Wright, 2004; Braker et al., 2010). In a meta-analysis based on 46 studies across various biomes, it was found that N_2O emissions were 55% higher on average as a result of increased precipitation levels, irrespective of seasonality, biome type and treatment method (Li et al., 2020).

In addition to N_2O, gaseous losses of Nr can also occur in the form of NO (Butterbach-Bahl et al., 2011; van Groenigen et al., 2015). Concentrations of NO play an important role in atmospheric chemistry through its effects on the production and destruction of ozone (O_3) (Pilegaard, 2013). This in turn affects the oxidation capacity and the radiation budget of the atmosphere (Jacob, 1999; Ehhalt et al., 2015; Abrol and Adhya, 2017). Similarly to N_2O fluxes, NO fluxes from soils have been proposed to be higher in response to increased Nr availability (Firestone and Davidson, 1989; Venterea

et al., 2003; Pilegaard et al., 2006; van Lent et al., 2015). Data on NO are relatively rare, especially from tropical regions, compared to other trace gases both from laboratory and field studies (van Lent et al., 2015). Nitrous oxide and nitric oxide share microbial production pathways and therefore studying them together can provide useful insights into the Nr dynamics in an ecosystem.

Tropical regions, including South Asia, have been undergoing dramatic changes to land use in the past century (Li et al., 2023). For instance, forest cover in Sri Lanka decreased from >80% around 1880 to 28% in 2020 predominantly for agriculture, timber extraction, settlements and tea plantations (Geekiyanage and Vithanage, 2015; Pushpakumari, 2021). The latter have been proposed to be a hotspot for N_2O emissions (Yu et al., 2023). Wang et al. (2020) estimated annual N_2O emissions from tea plantations globally to be 17.1 $kg\,N\,ha^{-1}\,yr.^{-1}$ on average (or 8,008 $kg\,CO_2eq\,ha^{-1}\,yr.^{-1}$) compared to 62–3,757 $kg\,CO_2eq\,ha^{-1}$ emitted from cereal croplands. Tea plantations often receive high levels of N fertilizer (typically >500 $kg\,N\,ha^{-1}\,yr.^{-1}$) (Wang et al., 2020) and soils are often more acidic compared to natural ecosystems (Cheng et al., 2015). Both factors can contribute to higher N_2O emissions relative to other land uses. When investigating the impacts of increased N inputs in tropical areas, it is important to consider different land uses due to the dynamic land use changes in those regions.

Little is known on how future increases in N deposition might affect N_2O and NO fluxes from tropical forest soils. Most N studies in tropical regions have been carried out in South America, and South Asian forests remain severely under-represented in global datasets. Sri Lanka was selected as a case study due to the relatively high Nr concentrations in the area and rapidly changing N policy (Yang et al., 2022). For example, the country was a host and a pioneer in signing the Colombo Declaration on Sustainable Nitrogen Management which calls for halving N waste by 2030 (UNEP, 2019b). Later, in 2021, Sri Lanka became the first country to officially ban the use of all synthetic fertilizers; a policy which was reversed a few months later (Weerahewa et al., 2021). The dynamic N policy context in Sri Lanka, high levels of N pollution, high forest cover and absence of previous measurements indicates a global scientific knowledge gap that needs investigation.

In this study, we measured fluxes of N_2O and NO from soil cores from a secondary montane tropical forest in Sri Lanka and compared these with soil cores from a local tea plantation which represents a typical land use in the country. To our knowledge, N_2O and NO fluxes have not previously been measured from Sri Lankan forest soils despite their potentially large contributions to national emissions. The objectives of the current study are to (1) evaluate the effects of increasing Nr availability (in the form of NH_4^+) on N_2O and NO fluxes from Sri Lanka soils and (2) to compare the response of two typical land uses (a secondary tropical forest and a tea plantation).

2 Materials and methods

2.1 Site description

Soil samples were collected from a secondary montane tropical forest (6°58′10.73″ N, 80°35′25.39″ E) and an organic tea plantation (6°58′15.1″N 80°35′37.6″E), both in Queensberry Estate, Kandy region, Central province, Sri Lanka (Supplementary Figure S1). These

were located approximately 500 m apart and as such share similar climatic conditions and soil parent material (Mapa, 2020). The elevation is approximately 1,650 m. The climate in the area is classed as tropical monsoon climate (Am) according to the Koppen Climate Classification (Beck et al., 2018) and falls within the Intermediate climatic zone in Sri Lanka (Mapa, 2020). The average annual temperature is approximately 21°C and the annual precipitation is around 2,800 mm per year (Climate-data.org, 2023). The soil type in the forest site was predominantly red laterites.

The main land use in the area is tea plantations. The tea plantation study site has been under organic management since 2020, implying that no synthetic N fertilizer has been applied to the soils since then. Consequently, there is a larger range of understory species compared to other tea plantations in the area, such as *Achyranthes aspera, Bidens pilosa, Cleome aspera, Oxalis debilis, Scoparia dulcis*. The tea (*Camellia sinensis*) was planted in rows and occupied a total area of two hectares. The predominant soil type is red yellow podzoles.

The forest site is a secondary montane tropical forest. The previous land use was a tea plantation which was allowed to naturally regenerate 80 years ago. At present, the vegetation community composition resembles that of a primary forest, including tree species such as *Syzygium aqueum, Litsea gardneri, Cinnamomum ovalifolium, and Symplocus cordifolia*. Average atmospheric NH_3 concentrations in the forest site ranged from 0.03 to 1.02 $\mu g\,m^{-3}$ for the period April 2022 to January 2023. This equates to monthly deposition rates to all canopy layers combined of −0.30 to 0.22 kg ha^{-1} month^{-1} (Deshpande et al., 2024).

2.2 Field collection methodology and soil pre-treatment

Soil samples were collected in March 2022 from the top 10 cm of soil from both sites. Samples were collected from nine and twenty distinct locations within the forest and tea plantation sites, respectively. Each sample was collected from an area of approximately 10 × 10 cm to ensure that different sampling points contributed similar amount to the final homogenized bulk sample.

All samples were air-dried at approximately 25°C in Sri Lanka prior to shipping to the UK Centre for Ecology and Hydrology, Edinburgh, United Kingdom. The soil was sieved through a 2 mm steel sieve to preserve soil macro aggregate structures (Wilpiszeski et al., 2019). Samples were then homogenized to reduce variability and increase representability. For the laboratory incubations 800 g of soil were placed into Perspex chamber bottoms (d = 19 cm, h = 10) and repacked to approximately field bulk density (ranging between 1.0 and 1.2 g cm^{-3}). At the beginning of each experiment, repacked soil cores were rewetted using deionized water to reach 100% water filled pore space (WFPS). Nitrogen treatments were applied five days later in order to allow for any 'Birch effect' impacts and resulting artifacts in GHG emissions (Birch, 1964) to subside.

2.3 Experimental treatments

We compared the N_2O and NO fluxes of tea plantation versus forest soils from Sri Lanka in response to different treatment levels of ammonium (NH_4^+) aqueous solution. We applied Nr in the form of

NH_4^+ since NH_3 and NH_4^+ act as major sources of Nr to natural ecosystems through atmospheric N deposition (Erisman and De Vries, 2000). Each treatment had three replicate incubated cores for trace gas measurements and one destructive core for measurements of other properties (Supplementary Figure S2). Soil was incubated for 40 days in total. There were two N applications: on days 5 and 27 after the start of the experiment. The amount of N applied to each core (800 g of soil on average) to simulate N deposition of 0, 40 and 100 kg N ha^{-1} yr.$^{-1}$ was 0 g (0 N), 8.5 g (40 N) and 21.3 g (100 N) per core, respectively. The amount of N added during the first and the second applications was identical. The concentration of NH_4^+ in the solution was 1.1 g N L^{-1}. Soil moisture was kept constant for 11 days after the first N application and for 9 days after the second application. Soils were then left to dry for approximately one week in both cases. A solution of 1% glucose was applied to all cores together with the second N application as a source of additional carbon (Sanchez-Martín et al., 2008). All soils were kept at constant temperature of 20°C throughout the experiment which is representative of the temperature at both sites.

The NH_4^+ stock solution was prepared by bubbling NH_3 volatized from chicken manure through water (Cowan et al., 2024). Concentrations of NH_4^+ and NO_3^- of each solution were confirmed prior to application using a Seal AQ2 analyser (Seal Analytical Inc., Wisconsin, USA). The application solutions were prepared on the day of application by adding deionized water to a stock solution to reach a target concentration which simulates N deposition at the required levels. Deionized water was selected over rainwater to minimize the introduction of Nr compounds beyond the known amount in the application solution.

The 1% glucose solution was prepared by adding 20 g analytical grade glucose (Sigma Aldrich) to 1 L deionized water (Sanchez-Martín et al., 2008). This is the equivalent of 6.6 g of sugar per soil core (800 g on average).

2.4 N_2O and NO flux measurements

Fluxes of N_2O and NO were measured every day during the first week following the treatment applications and approximately every second day thereafter for a total of 40 days.

Concentrations of N_2O were measured using the dynamic chamber method described in Cowan et al. (2014). The experimental setup consisted of a quantum cascade laser (QCL) (Tildas-FD, Aerodyne Research Inc., Billerica, MA, USA), a thermocube unit used to maintain a constant temperature (Thermocube, SS cooling systems, New York, USA), and a pump used to circulate the air (SH-110, Dry Scroll Vacuum Pump, Agilent Technologies, Lexington, MA, USA). This formed a closed loop, non-steady state flow through system. The components of the system were connected via Synflex tubing which is inert in relation to N_2O (Nemitz et al., 2018).

A Perspex chamber (diameter = 19 cm, height = 20 cm) was attached to a cylindrical Perspex bottom (diameter = 19 cm, height = 10 cm) which contained the 800 g of soil. The containers were sealed via bulldog clips and a neoprene seal for the duration of each measurement. The flow rate of air through the system was approximately 3.5 L min^{-1} on average. The total headspace of the chamber plus soil-containing bottom part was 0.01 m^3 on average. Concentrations of N_2O were recorded continuously at a frequency

of 1 Hz (once every second) for 5 min producing 300 data points on average for each measurement. Twenty seconds from the beginning and end of each measurement were removed to account for the lag time that it takes for the gas sample to travel through the closed loop system and reach the detector. Flux of N_2O was calculated using Equation 1, where F is gas flux from the soil ($nmol\,m^{-2}\,s^{-1}$), dC/dt is the rate of change in concentration with time in $nmol\,mol^{-1}\,s^{-1}$, ρ is the density of air in $mol\,m^{-3}$, V is the volume of the chamber in m^3 and A is the ground area enclosed by the chamber in m^2.

$$F = \frac{dC}{dt_0} \cdot \frac{\rho V}{A} \qquad (1)$$

Concentrations of NO were measured separately from N_2O using a $NO\text{-}NO_2$ ultra-sensitive chemiluminescence analyser (Model T200UP, Enviro Technology Services plc, UK) as part of a gas flow-through system (Drewer et al., 2015). A pump was used to circulate the air through the system at a flow rate of approximately $1\,L\,min^{-1}$. This formed an open loop system with a vent to the lab, unlike the QCL setup which was a closed loop. NO concentrations were measured for 20 to 30 min, depending on how quickly the system reached equilibrium. Equilibrium concentrations were recorded for two to four minutes.

Concentrations of O_3 were monitored using a 49C O_3 analyser (Thermo Environmental Instruments Inc., USA) to ensure that they were below 5 ppb and thus the probability of chemical reactions occurring in the system was low (Seinfeld and Pandis, 2016). O_3 data were not included in further analysis.

Fluxes of NO were calculated using equilibrium concentrations unlike N_2O flux calculations that utilize changes in concentrations over time (Schindlbacher et al., 2004) (Equation 2). F is NO-N flux expressed in $\mu g\,m^{-2}\,h^{-1}$, M is atomic weight ($N = 14.008\,g\,mol^{-1}$), Q is the mass flow rate of air through the chamber ($1\,L\,min^{-1}$ on average), V_m is the standard gaseous molar volume ($24.055 * 10^{-3}\,m^3\,mol^{-1}$), C_{eq} is the mixing ratio of NO at equilibrium, C_0 is the mixing ratio of NO from an empty chamber (blank), and A is the soil surface area of the soil core ($0.03\,m^2$).

$$F = \left(C_{eq} - C_0\right) * \frac{M * Q * 10^6}{V_m * A * 10^9} * 60 \qquad (2)$$

Empty chambers (blanks) were measured daily, and these blank fluxes were subtracted from the measured fluxes of the soil cores (Drewer et al., 2015).

Fluxes of N_2O and NO were then converted from $nmol\,m^{-2}\,s^{-1}$ and $\mu g\,m^{-2}\,h^{-1}$, respectively, to $ng\,N\,g^{-1}$ dry soil d^{-1} to facilitate the comparison among different Nr forms.

2.5 Soil properties

There was one equivalent and separate "destructive" core for each treatment so that physicochemical soil properties could be measured without interfering with the gas measurements. "Destructive" cores were treated in the same way as the "gas" cores. Soil subsamples were

taken from the destructive cores to measure NO_3^-, NH_4^+, soil pH, and total carbon and total nitrogen contents.

Subsamples of 20 g of soil were collected from each destructive core on the same day relative to Nr application when the gas measurements took place. 15 g of soil were extracted in 50 mL of 1 M KCl solution to obtain mineral N. The samples were placed on an orbital shaker (SSL1 orbital shaker, Stuart) at 100 rpm for 60 min. The extracts were filtered through Whatman No 40 ashless filter paper (pore size = 8 μm). The concentrations of KCl-extractable NO_3^- and NH_4^+, were measured using a discrete multi chemistry analyser (Seal AQ 2, Seal Analytical Inc., Wisconsin, USA). Soil samples were corrected for moisture content by measuring the gravimetric content of separate samples (dried at 105°C for three days).

In an earlier experiment, soil extractions were carried out with three replicates from each core of 6 g of soil and 20 mL of 1 M KCl solution. Preliminary chemical analysis suggested that the variability in NH_4^+ and NO_3^- concentrations was small among the replicates due to the soil being homogenized before packing the cores. Consequently, only one sample per core of 15 g was extracted in 50 mL KCl for this study. This decreased the number of samples three times and allowed us to obtain a better representation of the changes in Nr over the duration of the experiment.

The amount of N in the form of NH_4^+ and NO_3^- is expressed per gram of dry soil (Equation 3). N refers to the mass of NH_4-N or NO_3-N expressed in mg N per g of dry soil; c is the concentration of NH_4^+ or NO_3^- in $mg\,L^{-1}$; v is the volume of KCl solution used during soil extractions in L; m is the mass of dry soil in g. We subtracted the water content on the day of measurement and corrected for the amount of water in air-dried samples to calculate the dry mass of each subsample. The latter was calculated after drying a 15 g air-dried subsample at 105°C until constant weight.

$$N = \frac{c * v}{m} \qquad (3)$$

Destructive soil samples were also collected from the gas cores on the last day of the experiment and/or the day before the second N application. This was done to (1) compare if the amount of Nr in the gas cores was similar to their counterpart destructive cores; (2) check the variability in Nr among the triplicates of the same treatment. Values were consistent and hence data are not presented here.

Total C and total N in soil samples were measured at the beginning and at the end of each experiment using an elemental analyser (Flash SMART, Thermo Fisher Scientific). Approximately 4 g of soil were taken from each destructive core, dried at 105°C and milled using a ball mill (MM200 ball mill, Retsch). Approximately 2 mg of soil sample were used for further elemental analysis.

Soil pH was measured using a pH meter (MP 200, Mettler Toledo GmbH, Schwerzenbach, Switzerland) where 10 g of soil from the destructive cores were mixed with 20 mL of deionized water (soil to water ratio of 1:2). The pH was measured 60 min later. A 2-point calibration was performed with calibration solutions of pH 4 and 7 at the beginning of each measurement day.

Bulk density was measured by collecting field samples using a metal cup (diameter = 6 cm, height = 4 cm). Samples were oven-dried at 105°C for three days or until constant weight was reached.

2.6 Data analysis

All data were inspected, cleaned, transformed, visualized and statistically analyzed using R software version 4.2.2 (R Core Team, 2022).

We used a series of two-sample Welch t-tests to check if land use and N dose had a significant impact on cumulative N_2O and NO fluxes. These were fitted using the "t.test" function from the stats package in R. Linear regression was performed between the gaseous N forms (N_2O and NO) as a response variable and the mineral N forms measured from the soils (NH_4^+ and NO_3^-) as predictor variables both in normal and in log space. Linear models were fitted using the "lm" function from the stats package. None of the relationships were significant (p-value > 0.05) and these data are not presented further.

Percentage gaseous losses in the form of N_2O and NO were calculated relative to the amount of N applied to the soils plus the amount of the inorganic N present in the soil on the day before the N application (in line with emission factor calculations). N losses were calculated relative to inorganic N as the latter represented only a small fraction of total N present in the soil (ranging between 0.5 and 0.7% at the beginning of the experiment). The first and second N applications were treated as two separate events. Cumulative fluxes of N_2O and NO were also calculated separately for the two application periods via linear interpolation.

3 Results

3.1 N_2O and NO fluxes

There was a short-lived, two-day burst of N_2O fluxes at the beginning of the experiment in both forest and tea plantation soil types (Figure 1). This was most likely the response of the air-dried soil to rewetting. This is the well documented Birch effect (Birch, 1964) and hence it will not be considered in further discussions here.

Fluxes of N_2O from the forest soils did not increase in response to the first N application across the three treatment levels. Fluxes remained at background levels and close to 0 for 18 days after N application. The maximum value across the three treatments during the first application period ranged from 1 to 2 ng N_2O-N g^{-1} d^{-1}. There was a small increase in N_2O fluxes on the day of the second application (N + glucose), followed by a peak the day after (maximum value ranging from 16 to 34 ng N_2O-N g^{-1} d^{-1}). The control treatment (0 N) had the highest peak of N_2O fluxes after the second application, yet it was also associated with the highest uncertainty (34 ± 39 ng N_2O-N g^{-1} d^{-1}) (Figure 1). The control treatment cores (0 N) were associated with the highest cumulative fluxes (164 ng N_2O-N g^{-1}), followed by the medium treatment (137 ng N_2O-N g^{-1}). The highest N treatment had the lowest cumulative fluxes from forest soils (116 ng N_2O-N g^{-1}) (Table 1). Cumulative forest fluxes of N_2O from the 40 N and 100 N treatments were not significantly different from the control (t-test, df = 3, p-value = 0.37 and 0.42 for 40 N and 100 N, respectively).

Fluxes observed from tea plantation soils were two orders of magnitude higher than their forest counterparts in response to the same level of N addition. This difference was statically significant (t-test, df = 8, p-value = 0.04). The tea plantation soils exhibited a response to the first N addition, albeit smaller compared to the second application (N + glucose). The maximum value of the N_2O peak

ranged from 80 ng N_2O-N g^{-1} d^{-1} (100 N treatment) to 808 ng N_2O-N g^{-1} d^{-1} (40 N treatment) during the first application period. The highest N_2O peak occurred two days after the second application with little response on the day of the application (Figure 1). The peak value during the second application period ranged from 669 ng N_2O-N g^{-1} d^{-1} (100 N treatment) to 3,939 ng N_2O-N g^{-1} d^{-1} (40 N treatment). The cumulative N_2O fluxes from the tea plantation soils were the highest from the medium treatment (40 N) at approximately 10,102 ng N_2O-N g^{-1} d^{-1}. The control cores (0 N) still exhibited larger cumulative fluxes relative to the highest treatment (9,416 compared to 3,140 ng N_2O-N g^{-1} d^{-1}) (Table 1). Similarly to forest soils, N_2O fluxes from the tea plantation soils did not differ significantly between the control and the two treatment levels (t-test, df = 3, p-value = 0.94 and 0.54 for 40 N and 100 N, respectively).

Similar to N_2O, NO fluxes from tea plantation soils were also significantly higher than their forest counterparts, although the difference in the flux magnitude was less pronounced (Figure 2) (t-test, df = 8, p-value = 0.02). All cores across treatments and vegetation types exhibited the Birch effect. The NO peak did not appear rapidly after applications, in contrast to N_2O fluxes. Instead, the peak fluxes occurred around day 23 of the experiment in both the forest and tea plantation soils. The maximum NO peak during the first application period ranged from 6 ng NO-N g^{-1} d^{-1} (0 N) to 8 ng NO-N g^{-1} d^{-1} (100 N) for forest soils and from 16 ng NO-N g^{-1} d^{-1} (0 N) to 68 ng NO-N g^{-1} d^{-1} (100 N) for tea plantation soils. Both the peaks and the cumulative NO fluxes were proportional to the treatment levels across the two land uses (Figure 2; Table 1). The differences among the three treatment levels were more pronounced in the tea compared to the forest soils, especially during the first application period. The highest NO flux (68 ng NO-N g^{-1} d^{-1}) occurred in response to the highest treatment level (100 N). However, the only significant difference in NO fluxes was between the control and the 100 N treatment in tea plantation soils (t-test, df = 3, p-value <0.01).

3.2 Gaseous N losses

Fluxes of N_2O as a percentage of N application were higher during the second application period (with glucose) compared to the first (Table 2). This was particularly pronounced in the tea plantation soils, 40 N treatment, where almost half (48.6%) of the N applied was re-emitted in the form of N_2O. However, if we consider the amount of mineral N that is already present in the soil, the percentage losses become more similar. This was the case for both the forest and the tea plantation soils. The percentage loss of N_2O was comparable among treatments within the same land use (forest soils percentage loss ranging from 0.01 to 0.05%; tea plantation soils percentage loss ranging from 4.04 to 5.52%). One exception was the 100 N tea plantation soils which exhibited a notably lower percentage loss (0.51 and 1.06% for the first and second applications, respectively).

Percentage loss of NO fluxes was higher during the first compared to the second application period across treatments and for both land uses (Table 2). All the percentage losses from the forest soils were low and similar across treatments (approximately 0.1%). The percentage loss of NO from tea plantations was higher compared to forest soils (ranging from 0.29% for 0 N to 0.61% for 100 N for the first application). It increased slightly at higher N treatments, especially during the first application period.
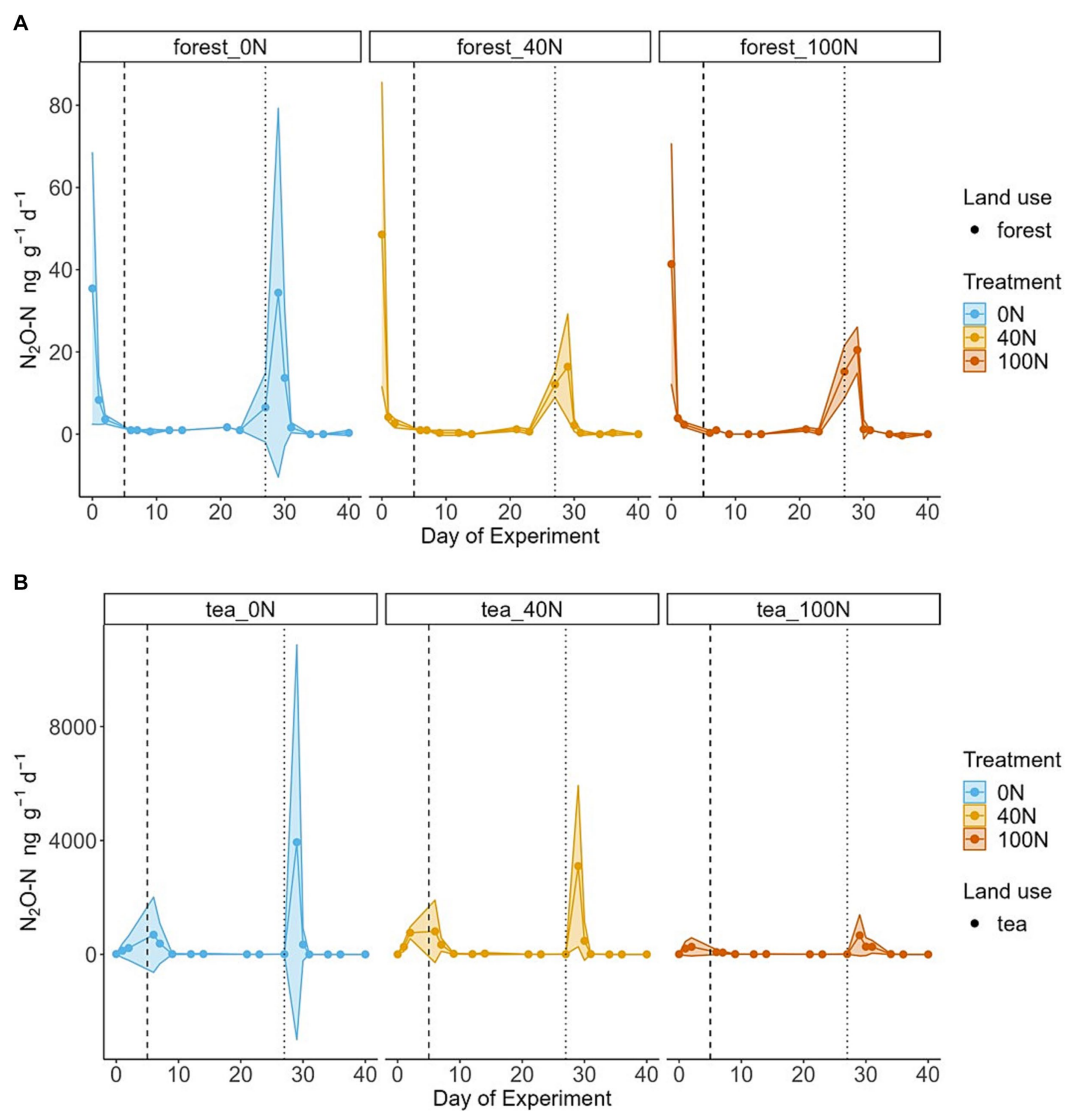
FIGURE 1
Temporal changes in N_2O fluxes from forest **(A)** and tea **(B)** soils. The 95% confidence intervals (C.I.) based on triplicates are presented as shaded areas. Blue, orange and red refer to simulated N deposition of 0, 40 and 100 kg N ha^{-1} yr.$^{-1}$, respectively. The vertical dashed line corresponds to the first N application event, and the vertical dotted line marks the second application of N together with glucose. Please note the different scales on the y axes between the forest soils (top panel) and the tea plantation soils (bottom panel).

TABLE 1 Cumulative N_2O and NO fluxes from forest and tea plantation soils at three levels of N addition (the equivalent of 0, 40 and 100 kg N ha^{-1} yr.$^{-1}$).

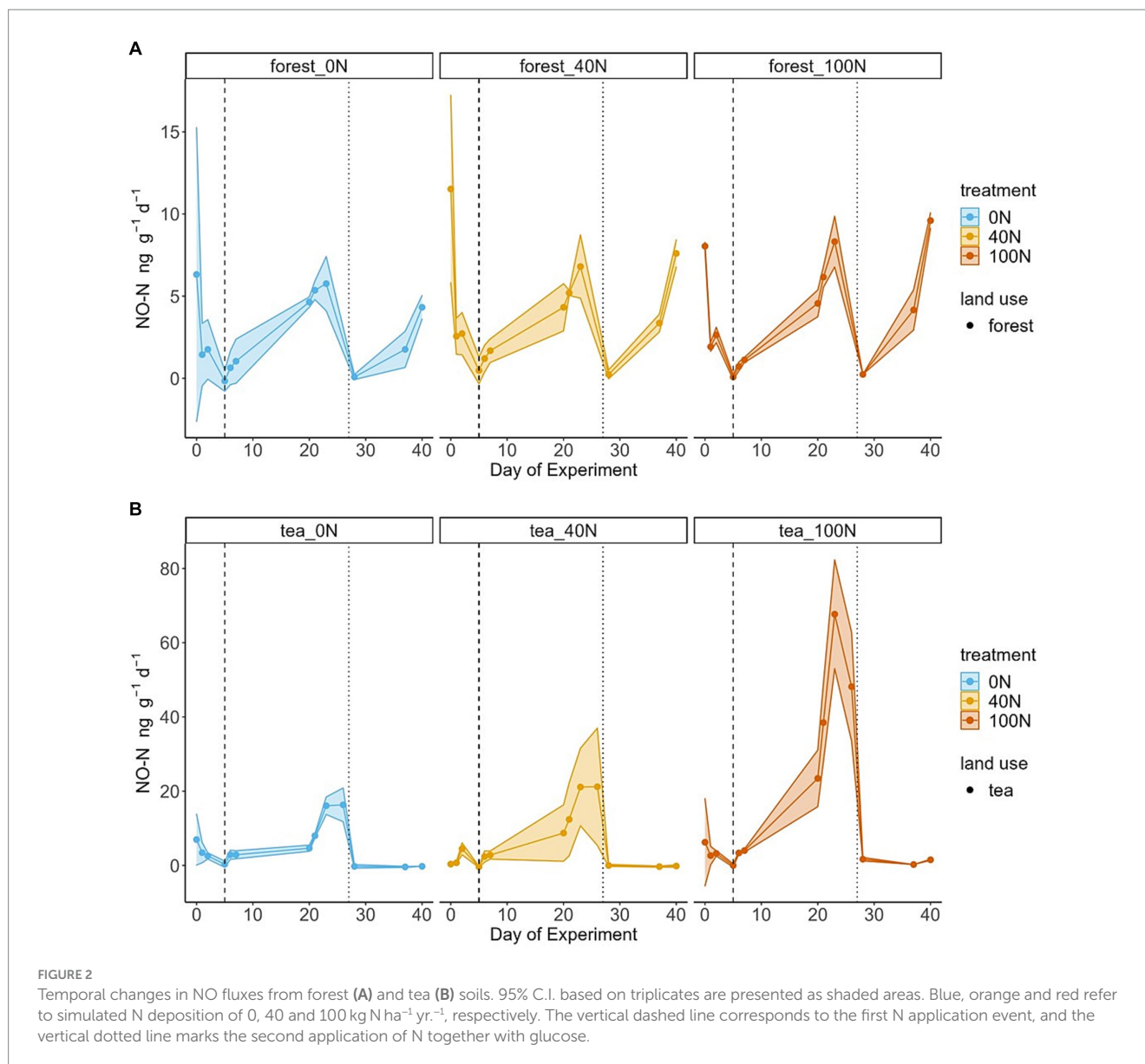| Land use	Treatment	Time, days	Cumulative Flux, N_2O-N, ng g^{-1}	Cumulative Flux, NO-N, ng g^{-1}
Forest	0 N	40	164.1	92.7
Forest	40 N	40	136.7	125.7
Forest	100 N	40	116.0	129.4
Tea	0 N	40	9415.8	156.9
Tea	40 N	40	10102.0	209.5
Tea	100 N	40	3139.9	558.6

Fluxes were calculated at the end of the experiment, at day 40.

3.3 Soil properties

Tea plantation soils were more acidic relative to forest soils (pH of 5.1 ± 0.3 compared to 5.6 ± 0.3, respectively). These were also more compacted than forest soils (bulk density of 1 ± 0.02 g cm^{-3} and 0.89 ± 0.02 g cm^{-3}, respectively) (Supplementary Table S1).

Volumetric water content (VWC) was controlled and monitored during the experiment and followed an identical pattern for all cores. Initially, water was added to the repacked soil cores to reach 100% WFPS, resulting in approximately 35.8 to 38.6% VWC. Soil moisture was maintained constant for 11 and 9 days after the first and second N applications, respectively. The cores were then allowed to dry for 11 and 7 days, respectively.

Forest soils had higher concentrations of total soil carbon both at the beginning and at the end of the experiment relative to the tea

FIGURE 2
Temporal changes in NO fluxes from forest **(A)** and tea **(B)** soils. 95% C.I. based on triplicates are presented as shaded areas. Blue, orange and red refer to simulated N deposition of 0, 40 and 100 kg N ha⁻¹ yr.⁻¹, respectively. The vertical dashed line corresponds to the first N application event, and the vertical dotted line marks the second application of N together with glucose.

plantation soils. Total soil C at the start of the experiment ranged from 4.9 to 5.0% in forest soils and between 3.8 and 4.0% in tea plantation soils. Similarly, forest C concentrations were also higher than that in the tea plantation at the end of the experiment (5.2 to 5.3% compared to 3.8 to 4.1%). There was relatively little change in C in the medium and high forest treatments, except for the glucose application event on day 32, when C content increased to 5.2 and 5.3%, respectively. However, the control forest soils (0 N) showed evidence of C consumption around day 24 when C concentrations dropped to 3.7%, which were then replenished by the application of glucose (to 6.5%). Carbon concentrations in the tea soils were similar at the beginning and end of the experiment (around 4%) across the three treatments.

The amount of mineral N in the form of NO_3^- and NH_4^+ varied over the course of the experiment and differed between land use types (Figure 3). In the forest soils, NH_4^+ concentrations were relatively low and consistent among the three cores at the start of the experiment and before any treatment application (range between 21.2 and 22.1 μg NH_4-N g⁻¹). Concentrations increased in response to the N

application. We detected two peaks: on day 17 (range between 158.9 and 196.7 μg NH_4-N g⁻¹) and on day 31 (range between 192.0 and 235.0 μg NH_4-N g⁻¹). The second peak was higher in all three treatments and occurred one day before the second application. The decrease in NH_4^+ concentrations around day 21 (range between 138.6 and 218.8 μg NH_4-N g⁻¹) could correspond to the slight increase in NO_3^- concentrations around day 31 in the forest soils (range between 4.6 and 20.0 μg NO_3-N g⁻¹). NO_3^- concentrations were generally low and close to 0 in all three forest soil cores except for an increase around day 31. In this case, the magnitude of the concentration increase was proportional to the treatment level.

Tea plantation soils showed different mineral N dynamics compared to forest soils. Nitrate concentrations in tea plantation soils were notably higher than NH_4^+ concentrations. For instance, the NO_3^- peak in the 100 N treatment was 245.6 μg NO_3-N g⁻¹ (day 31) compared to an NH_4 peak of 9.8 μg NH_4-N g⁻¹ (day 7). Nitrate concentrations continued to increase after the first N application and reached a peak (range between 113.4 and 262.1 μg NO_3-N g⁻¹)

TABLE 2 Percentage N loss in the form of N₂O and NO as a proportion of experimental N application (1) and the sum of experimental N application plus initial mineral N stock.

	Forest			Tea		
	0 N	40 N	100 N	0 N	40 N	100 N
1. Losses as % of N addition						
N_2O cumulative (day 23) as % of application 1	NA	0.1	0.0	NA	14.7	1.1
N_2O cumulative (day 40) as % of application 2	NA	0.5	0.4	NA	48.6	7.0
NO cumulative (day 26) as % of application 1	NA	0.6	0.2	NA	1.3	1.3
NO cumulative (day 40) as % of application 2	NA	0.5	0.2	NA	0.0	0.0
2. Losses as % of N addition + initial stock (N mineral)						
N_2O cumulative (day 23) as % of application 1 + stock on day 3	0.04	0.02	0.01	4.84	4.04	0.51
N_2O cumulative (day 40) as % of application 2; separate cumulative + stock on day 26	0.03	0.03	0.05	5.52	4.08	1.06
NO cumulative (day 26) as % of application 1 + stock on day 3	0.14	0.12	0.08	0.29	0.34	0.61
NO cumulative (day 40) as % of application 2; separate cumulative + stock on day 26	0.01	0.03	0.02	0.00	0.00	0.01

We define initial mineral N stock as the amount of mineral N present in the soil on the day before N application. Cumulative fluxes of N_2O and NO were calculated separately for application periods 1 and 2. 0, 40, and 100 N correspond to treatment levels of 0, 40 and 100 kg N ha^{-1} yr.$^{-1}$, respectively.

one day before the second N application. These decreased rapidly after the second application and remained low after day 35 (<4 μg NO_3-N g^{-1}) and during the rest of the experiment. In contrast, NH_4^+ concentrations from tea plantation soils, showed some increase after the first N application, yet notably lower compared to the forest soils. The highest NH_4^+ value occurred during the first application period, around day 7, and ranged from 27.2 to 97.6 μg NH_4-N g^{-1}. These concentrations then gradually decreased over the course of the experiment (between 5.5 and 25.2 μg NH_4-N g^{-1} on day 21) and remained low (<10 μg NH_4-N g^{-1}) even after the second application.

4 Discussion

4.1 Why did higher N addition result in higher NO fluxes but not N₂O fluxes?

We observed higher NO fluxes in response to higher treatment levels (both peak and cumulative fluxes) in line with initial expectations. This pattern was present in both land use types, but particularly pronounced in the tea plantation soils. The well-established Hole in the Pipe (HIP) model proposed by Firestone and Davidson (1989) suggests that the more Nr is available in a system, the higher the amount of gaseous Nr losses in the form of NO and N_2O

will be. However, fluxes of N_2O demonstrated the opposite pattern where the highest N treatment (100 N) had the lowest cumulative fluxes in both land use types.

Fluxes of N_2O were higher than NO fluxes across treatments and for both soil types. This is in line with previous findings and might suggest that gaseous Nr losses (i.e., those other than N_2) occur predominantly as N_2O rather than NO from these soils (Hall et al., 2018; Drewer et al., 2020). We observed relatively small N_2O fluxes from most cores following the first N application (<1 ng N_2O-N g^{-1} h^{-1} in forest and < 33.7 ng N_2O-N g^{-1} h^{-1} in tea plantation). This went contrary to initial expectations. Fluxes of N_2O only increased in response to the second application which included both N and C. In a laboratory incubation experiment using Malaysian soils from a logged forest and two oil palm plantations, Drewer et al. (2020) found that both N_2O and NO fluxes were higher after rewetting than after N addition in the form of NH_4NO_3. One of their proposed explanations was that microbial activity could be limited by carbon availability based on the abundance of gene transcripts in the soil (Drewer et al., 2020). The latter hypothesis is consistent with our findings where the larger N_2O peak occurred in response to the addition of both N and C as opposed to nitrogen alone. Garcia-Montiel et al. (2003) also reported increased N_2O fluxes following the addition of C in the form of glucose using soils from a humid tropical forest in Brazil. Similarly, Figueiredo et al. (2016) suggested that the amount of organic matter in riparian and upland boreal soils affects both N dynamics in general,
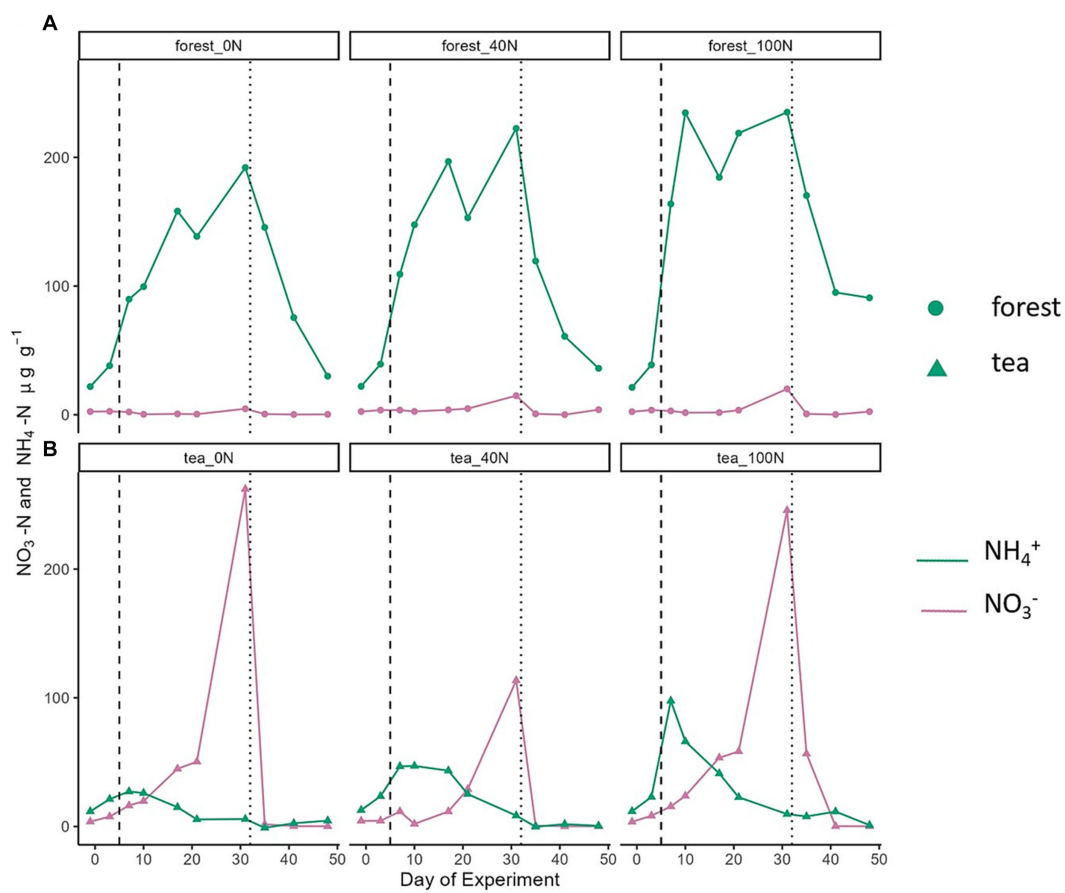
FIGURE 3
Temporal changes in NO$_3^-$ (pink) and NH$_4^+$ (green) concentrations from forest **(A)** and tea plantation **(B)** soils. The vertical dashed line corresponds to the first N application event, and the vertical dotted line marks the second application of N together with glucose.

and N$_2$O production in particular. Fluxes of N$_2$O were reported to be three to five times larger in soils rich in organic matter in that study. One potential explanation is an increase in the Dissimilatory Nitrate Reduction to Ammonium (DNRA) in response to higher C levels which has previously been reported in a meta-analysis of different terrestrial ecosystems, including forests (Cheng et al., 2022).

In the context of our study, it should be noted that glucose (a monosaccharide) is less complex than other organic carbon compounds present in soils and hence is more easily available to microbes. While this could introduce bias to our results, it can still be indicative of a carbon limitation in the microbial community. Morley and Baggs (2010) demonstrated that the form of C compounds plays an important role in the response of the microbial community to N addition as well as in the associated N$_2$O emissions. These experiments were performed in temperate agricultural soils, where microbial communities are subjected to different environmental conditions compared to microbial communities in tropical regions. While it might be difficult to predict whether C form has a stimulating or suppressing effect on microbial diversity and function, we would still expect to observe a differentiated response.

The opposite pattern has also been reported, where increasing amount of organic carbon in soils was suggested to reduce N$_2$O

emissions from agricultural soils (Neal et al., 2023). The authors proposed that adding organic C to soils could improve connectivity among soil pores thus increasing soil aeration and consequently decreasing N$_2$O emissions. While this theory might apply to some ecosystems, it assumes that only a small proportion of N$_2$O fluxes were produced as a result of nitrification (an aerobic process). However, based on the NO$_3^-$ concentrations that we observed in our study and the experimental addition of NH$_4^+$ solution, nitrification could be responsible for a large fraction of the N$_2$O fluxes, especially in the tea plantation soils. Furthermore, Liyanage et al. (2020) cautioned against applying large amounts of C alongside high N levels in tropical regions as these might result in high N$_2$O emissions. Their results were based on maize and soybean trials in Sri Lanka. Although their experimental N doses (the equivalent of 0, 100, 220, 300 kg N ha^{-1} yr.$^{-1}$) were not representative of natural ecosystems, the report of microbial activity in tropical soils potentially being limited by C is in line with both the current and previous studies (Garcia-Montiel et al., 2003).

There was an increase in NO$_3^-$ concentration up until the day before the second (N + C) application followed by a sharp decrease in NO$_3^-$ to nearly 0 µg g^{-1}. This pattern was observed in all cores, although it was more pronounced in tea plantation soils. The sharp decrease in NO$_3^-$ concentrations following the C addition likely

suggested that denitrification took place. This is also consistent with the observed peak in N_2O fluxes after the second application. While N_2O fluxes were present in all cores ($n = 18$), it is possible that much of the N was re-emitted in the form of N_2. This would suggest complete denitrification and is consistent with the relatively low NO_3^- concentrations that we observed. Almaraz et al. (2023) proposed that "excess" N might be re-emitted as N_2 rather than as N oxides, especially in wet tropical soils where O_2 concentrations tend to be low. Morley et al. (2014) suggested that this process of complete denitrification might also be modulated by C availability. However, N_2 production remains a less well understood process compared to N_2O and NO fluxes (Galloway et al., 2004; Lehnert et al., 2021) and tends to be reported less often (as it is difficult to measure given very high background concentrations).

The potential carbon limitation on the microbial community still did not explain why the 100 N treatment resulted in the lowest cumulative N_2O fluxes, as we observed the same pattern after the N + C addition. Our laboratory study represented a closed system. We hypothesize that Nr could only exit the system in the form of gaseous emissions as neither leaching, nor plant uptake could occur within the incubation cores. Similarly, mineral N concentrations at the end of the experiment were low, particularly in tea plantation soils ($<5 \mu g g^{-1}$), thus suggesting that N did not accumulate in the soils. Another uniqueness of our study is that N availability was increased by adding NH_4^+ solution rather than the commonly used NH_4NO_3 or another N form. While NH_4NO_3 is often utilized in N addition studies due to convenience and ease of comparison to past research, it is less representative of atmospheric deposition to natural ecosystems (Erisman and De Vries, 2000). N form has been reported to affect the diversity and composition of microbial communities (Liu et al., 2021; Ding et al., 2023). It has been suggested that NH_4^+ plays an important role in shaping bacterial but not fungal communities based on a study of temperate forest soil (Ding et al., 2023). This is an important consideration given that bacteria tend to dominate over fungi in biomes with high mean annual temperature and net primary productivity, such as tropical regions (Yu et al., 2022). However, specific data on the microbial community at our study sites were not available.

As ammonia oxidizers use carbon dioxide for biomass synthesis, previous studies have reported enhanced nitrification-based N_2O production after ammonium based fertilization (Hink et al., 2017, 2018). Hence, ammonia oxidation could also have contributed to the second N_2O emission pulse after N fertilization in our case. However, since the nitrogen source was sufficiently applied, we suspect that the depleted organic carbon might be the limiting factor affecting the denitrification-related N_2O production as previously reported by Drewer et al. (2020) who also identified denitrifiers and archaeal ammonia oxidizers as the main contributors to N_2O emissions, albeit with varying activity levels. The relative contribution of denitrification vs. ammonia oxidation was difficult to estimate in their study despite extensive microbial analysis. Based on the mineral N dynamics in our study, the nitrification or ammonia oxidation processes in the tea plantation soils likely contributed less than in forest soils.

The carbon and nitrogen cycles are closely coupled in ecosystems (Zaehle, 2013) and hence it is likely that a C limitation might modulate the response of the microbial community to increased N availability.

This emphasizes the fact that N availability is not the only factor that affects N_2O production. While our results support a potential carbon limitation, this is not sufficient to establish a causal relationship based on the current data alone.

4.2 Why were gaseous Nr losses higher from tea plantation than from forest soils?

The N_2O emission potential from the tea plantation soils was two orders of magnitude higher compared to the forest soils. Fluxes of NO were also higher from the tea plantation soils although the difference was less pronounced. This is consistent with a meta-analysis on the effects of land use on N fluxes which proposed that fertilized systems often emit higher N_2O fluxes compared to natural ecosystems (van Lent et al., 2015). Since we performed a laboratory experiment, our results more accurately depict the emission potential of soils rather than absolute field emissions. In a similar laboratory incubation experiment of tropical soils, Drewer et al. (2020) reported higher N_2O and NO emission potential from logged forest soils compared to an oil palm plantation (15.6 and 11.5 ng N_2O-N g^{-1} h^{-1} and 6.0 and 1.6 ng NO-N g^{-1} h^{-1}, respectively). In their study, a riparian reserve forest showed the lowest emission potential of all. This study highlighted that some forest soils have the potential to be a significant source of N_2O emission, on the same order of magnitude as plantations, depending on the environmental conditions and disturbances. We also observed lower emission potential from a natural ecosystem compared to a plantation, with the caveat that these observations could change in the future depending on environmental conditions.

A major difference that we observed between the tea plantation and forest sites was opposing trends in NO_3^- and NH_4^+ dynamics. Forest soils exhibited high NH_4^+ and low NO_3^- concentrations, whereas tea plantation soils contrastingly showed high NO_3^- and low NH_4^+ concentrations. In a meta-analysis of 104 studies from tropical areas, it was reported that plantation soils tend to have higher NO_3^- concentrations compared to natural ecosystems (van Lent et al., 2015). This is likely due to nitrification taking place in the soils. This hypothesis is consistent with our observations, as NH_4^+ concentrations from the tea plantation soils decreased following the first N application. Simultaneously, NO_3^- concentrations increased, thus suggesting nitrification. In contrast, the change in NO_3^- concentrations from the forest soils was less pronounced and concentrations were close to $0 \mu g g^{-1}$ for the majority of the experiment. While NO_3^- concentrations in the forest soils did not change notably, there was an increase in NO fluxes around the time of the decrease in NH_4^+ concentrations (approximately day 20). This suggests that nitrification took place in both land use types, however it is likely that most of the NH_4^+ was emitted as NO (and possibly N_2) rather than remaining in the soil as NO_3^- in the case of the forest soils.

In general, tropical forests have been suggested to be rich in N and plant growth tends to be limited by P and not N availability (Vitousek, 1984). Consequently, tropical forests are considered to exhibit an open N cycle, meaning that the rate of cycling and loss of N from the system is high compared to systems with N limitation, where N is cycled more efficiently (Vitousek, 1984). This can also result in higher gaseous Nr losses from the system (Aber, 1992). However, Wolf et al. (2011) demonstrated that this is not always the case, particularly in the context

of tropical montane forests. They found that N availability decreased alongside an elevation gradient (1,000 to 3,000 m) in Ecuador, suggesting a potential N limitation in the system. The paradigm of high N availability in tropical forests was further contested by Gallarotti et al. (2021) study using a δ15N isotopic approach in the Congo basin. While tropical soils are still widely considered to be poor in P, primarily due to leaching, N might be the limiting nutrient especially in montane forests (Dalling et al., 2016). This could explain why we observed smaller gaseous Nr losses from montane forest soils compared to tea plantation soils. This is further supported by the fact that tea plantations are likely to be saturated in N due to high fertilizer application (typically >500 kg N ha^{-1} yr.$^{-1}$) (Wang et al., 2020). Moreover, tea plantations have previously been proposed to be hotspots of N_2O emissions (Wang et al., 2020; Yu et al., 2023). Even though our tea plantation study site has been under organic cultivation for the past three years, the soil is still likely to exhibit memory effects due to past management practices (Targulian and Bronnikova, 2019).

4.3 Implications

Our findings raise the question of what might happen in the future if forest soils continue to be exposed to elevated amounts of atmospheric N deposition. Could those ecosystems become N saturated, similar to plantations and so result in significant increases in gaseous Nr emissions? And if this is the case, when will saturation occur? In the meantime, there are international initiatives for increasing the amount of carbon stored in soils as a potential climate change mitigation measure, such as "4 per 1,000" (The International "4 per 1000" Initiative, 2015). Several studies have raised concerns over that as increasing soil carbon could incur a penalty in terms of increased N_2O emissions (Lugato et al., 2018; Guenet et al., 2021). This is in line with our findings which suggested a potential C limitation on the microbial community.

It is important to acknowledge that N_2O and NO production in soils can occur because of several different microbial processes, each of which is controlled by different environmental conditions (Baggs, 2011; Butterbach-Bahl et al., 2013). This complexity is often not captured in emission inventories, which are one of the most commonly used tools for estimating GHG emissions on regional and global scales. Instead, inventories rely on activity data multiplied by an emission factor (IPCC, 2019). This can pose several challenges, especially in the case of N_2O emissions from tropical soils. Namely: (1) emission factors are often built upon relationships observed in temperate regions and as such might not always apply to tropical soils; (2) limited activity data which only account for N inputs, (3) do not capture change in environmental conditions such as changes to N deposition, nutrient availability and climate-change-induced changes to temperature and precipitation; (4) a default value is typically used for all natural ecosystems, regardless of vegetation type and other relevant biome-specific conditions.

We recommend that future studies focus on better understanding the mechanisms that underlie N_2O and NO production in tropical soils, including the role of nutrient limitations and the coupling of the C and N cycles. Long-term field scale experiments would be particularly useful for that purpose. Ideally, application of default (tier 1) emission factors in such

systems should be replaced by tier 2 and 3 approaches that better account for the dynamically changing environmental conditions and take advantage of the increasing amount of environmental data available.

5 Conclusion

Our findings highlighted the importance of land use and land management in modulating N_2O and NO fluxes from tropical soils. Ecosystems that have previously been exposed to high levels of Nr, such as tea plantations, were more likely to exhibit higher gaseous Nr losses compared to natural ecosystems, when exposed to the same controlled conditions and N addition. This was particularly pronounced after adding a source of carbon to the soil cores under investigation. Future land use strategies should carefully consider the balance between managing land for agricultural productivity and carbon storage versus any potential penalties in terms of non-CO_2 greenhouse gases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2024.1335775/full#supplementary-material

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