

Dear Editor and reviewers:

We wish to thank you all for your constructive comments in this round of review. Your comments provided valuable insights to refine its contents and analysis. We have studied these comments carefully and have made corresponding corrections that we hope will meet with your approval.

All line numbers cited in this response letter correspond to the revised manuscript with full continuous line numbers and visible track-change revisions. The original comments from the editor and reviewers are shown below in black. Each is followed by our response (in blue) and the corresponding revisions in the manuscript (in black italics).

RC1: Anonymous Referee #2

The manuscript entitled “Measurement report: Soil reactive nitrogen gas emissions from the Tibetan Plateau” by Deng et al. presents a comprehensive investigation of soil reactive nitrogen (Nr) gas emissions from Tibetan Plateau soils. The study combines soil microbial functional gene data, soil physicochemical properties, and Nr gas emissions measured during wetting–drying cycles in laboratory dynamic chamber experiments. These datasets are then integrated into a parametric model to (i) characterise differences in Nr emission patterns across land - use types and (ii) estimate the contribution of Tibetan Plateau soils to the missing daytime HONO source via a simple upscaling approach.

The manuscript is concise, well-structured, and generally clearly written. The authors manage to harmonise a complex set of data—ranging from microbial functional genes to dynamic chamber flux measurements—into a coherent picture of soil Nr emissions and their underlying mechanisms. Their conclusions align well with the last decade of soil HONO research and provide (albeit statistical but) mechanistic insight into the contribution of microbial processes and chemical transformations of soil N to reactive N gas emissions.

The upscaling approach is necessarily simplified and comes with limitations, but the systematic consideration of multiple land - use types is a clear strength of this work and significantly enhances its value. While I think the fumigation experiment could be more prominently showcased in the main text (see Comment 2 below), I also understand the authors’ concern that this could dilute the main narrative.

Overall, I find the study robust and well-presented, thus I recommend publication after minor revision.

Below, I list a few major and several minor comments that I hope will help to further strengthen the manuscript.

Re: We would like to thank the reviewer for the supportive and constructive comments. Please find our point-by-point responses below on how we improve our manuscript based on your comments.

Major comments

1. Optimum flux vs. integrated flux patterns (Fig. 3)

The comparison between optimum flux and integrated flux across land use types is particularly interesting. In several cases, these two metrics appear to show similar patterns across land uses, while the behavior of HONO and NO₂ differs more markedly.

- I encourage the authors to explicitly discuss these similarities and differences in the Results and/or Discussion sections, in connection with Fig. 3.
- For example, under which land use types do optimum and integrated fluxes track each other closely? Where do they diverge? How do these patterns differ? and what might this imply about the temporal dynamics of production and consumption processes?

Re: Thank you very much for highlighting this interesting point of comparison. We fully agree with your view that the similarities and differences between optimal flux and integrated emissions can reveal the temporal dynamics of reactive nitrogen gases across different land-use types. Optimal flux reflects the instantaneous release potential under the combined influence of specific meteorological and nutrient-related drivers, whilst cumulative emissions reflect the final net effect of these processes over time, after offsetting sink processes. Relying solely on instantaneous fluxes (such as peak emissions) to assess the climate impact of reactive nitrogen from cropland or forests may be seriously misleading, and only by taking time-integrated source-sink balances into account can true understanding of the comprehensive environmental effects of different land-use types. In accordance with your suggestion, we systematically incorporate the analysis of the temporal dynamics of optimal fluxes and integrated emissions, as compared in Fig. 3, into lines 329–333 of the revised manuscript to enhance the paper’s scientific interpretation of flux dynamics over time. *“Optimal fluxes and integrated emissions of HONO, NO and NO₂ showed high consistency and convergence across cropland soils. Cropland soils consistently remained the primary source of*

HONO, NO and NO₂ emissions (42.1 ± 28.1 , 49.2 ± 31.8 and $19.9 \pm 3.6 \text{ ng N m}^{-2} \text{ s}^{-1}$, respectively), whether in terms of instantaneous peaks or cumulative emissions. The findings suggest that the emission mechanisms in cropland ecosystems for HONO, NO, and NO₂ are relatively continuous over time and highly stable (Fig. 3)."

2. Fumigation experiment in the main text

The fumigation experiment provides important insight into microbial N balance and potential changes in atmospheric N input that altered microbial activity across different land uses.

- At the moment, this appears mainly in the Supplementary Information and is only briefly referenced.
- I recommend including at least one key figure or summary panel from the fumigation experiment in the main text (e.g., a figure showing how fumigation affects HONO/NO emissions or relevant N processes across land-use types), even if this information is not directly used in the upscaling.
- A concise description of the main fumigation results would help readers better appreciate the mechanistic link between microbial dynamics and Nr emissions and would make this valuable part of the work more visible.

Re: We sincerely thank the reviewer for the valuable suggestion to integrate the fumigation experiment more prominently into the main text to strengthen the mechanistic links. Following this recommendation, we have concisely summarized the effects of high- and low-concentration NO and NO_x + O₃ fumigation on microbial functional genes, physicochemical properties, and Nr emissions across forest, grassland, and cropland soils, and presented them as a consolidated panel (Fig. 5). The detailed raw data and other supplementary analyses from the fumigation experiment remain available in the Supplementary Information for interested readers to consult in depth. Please see lines 385-388 in the revised manuscript.

"Figure 5: Conceptual diagram linking the effects of fumigation with high and low concentrations of NO and NO_x+O₃ on soil microbial functional genes, physicochemical properties, and Nr fluxes across forest, grassland, and cropland soils. Red bold indicate statistically significant increases, while blue bold indicate statistically significant decreases."

	High vs Low NO fumigation				High vs Low NO _x +O ₃ fumigation		
	Forest	Cropland	Grassland		Forest	Cropland	Grassland
Fumigation				Fumigation			
Soil responses	● NO₂⁻ ↓ ● DOC ↓ ● pH ↓ ● amoA_AOA ↑ ● amoA_AOB ↑ ● nxrA ↑/ nxrB ↑ ● nirS ↑/nirK ↑	● NO₂⁻ ↓ ● DOC ↑ ● pH ↓ ● amoA_AOA ↓ ● amoA_AOB ↑ ● nxrA ↓/ nxrB ↓ ● nirS ↓/nirK ↑	● NO₂⁻ ↓ ● DOC ↓ ● pH ↑ ● amoA_AOA ↑ ● amoA_AOB ↓ ● nxrA ↓/ nxrB ↑ ● nirS ↑/nirK ↓	Soil responses	● NO₂⁻ ↓ ● DOC ↑ ● pH ↑ ● amoA_AOA ↓ ● amoA_AOB ↑ ● nxrA ↓/ nxrB ↓ ● nirS ↑/nirK ↓	● NO₂⁻ ↓ ● DOC ↑ ● pH ↓ ● amoA_AOA ↑ ● amoA_AOB ↓ ● nxrA ↑/ nxrB ↑ ● nirS ↑/nirK ↓	● NO₂⁻ ↓ ● DOC ↓ ● pH ↑ ● amoA_AOA ↑ ● amoA_AOB ↑ ● nxrA ↑/ nxrB ↓ ● nirS ↓/nirK ↓
Flux effect	● HONO ↓ ● NO ↓ ● NO₂ ↓ ● NH₃ ↑	● HONO ↓ ● NO ↑ ● NO₂ ↑ ● NH₃ ↓	● HONO ↓ ● NO ↓ ● NO₂ ↑ ● NH₃ ↑	Flux effect	● HONO ↑ ● NO ↑ ● NO₂ ↑ ● NH₃ ↑	● HONO ↑ ● NO ↑ ● NO₂ ↑ ● NH₃ ↓	● HONO ↓ ● NO ↓ ● NO₂ ↑ ● NH₃ ↓

Minor comments

Line 52. Please clarify the phrase “nitrate leaching” as: “nitrate leaching to groundwater/aquifers”

Re: Done.

Lines 82–85. The description of the sampling is currently somewhat ambiguous. It appears that: larger composite samples were taken for the dynamic chamber experiments (three subsamples in 10m x 10m grid, sampled diagonally and homogenised? If so, how many kilograms approximately?), and smaller subsamples (e.g. 1–2 g) were used for microbial analyses.

Please clarify:

- Exactly how the soil samples were collected (e.g., “three subsamples per 10m x 10m plot, taken diagonally across the plot, from 0–5 cm depth, then homogenised to form one composite sample per plot”), and why the number of samples are different across land use types
- At what stage homogenisation occurred, and
- Whether the microbial subsamples (1–2 g) were taken from the same homogenised 0–5 cm composite used for the chamber experiments.

Re: The specific method for collecting soil samples was as follows: at each sampling point, a 10 m × 10 m square plot was delineated, and three subsamples were collected along the diagonal, then homogenized to form a composite sample (total weight ~1.5 kg). Subsequently, using a sterile sampling spoon, 1–2 g of soils were collected from the composite sample and placed into 2 mL sterile centrifuge tubes for subsequent microbiological analysis. Soil samples for microbial analysis were stored at –80°C, while those for chamber experiments were stored at –20°C. We have incorporated this information into the revised manuscript. Please refer to lines 83–86.

“At each sampling site, a 10 m × 10 m sample square was delineated, and three sub-samples of topsoil (0–5 cm) were collected along the diagonal, subsequently homogenized to form a composite sample (total weight ~1.5 kg). Subsequently, using a sterile sampling spoon, 1–2 g of soils were collected from the composite sample and placed into 2 mL sterilized centrifuge tubes for subsequent microbiological analysis.”

Different sample numbers across land-use types were determined by taking into account the area coverage of different land-use types in the region (forestland accounts for ~ 50% of the total area, while grassland accounts for ~ 20%) and literature-based hotspot emissions (cropland).

Line 94 – The phrase “measured by a continuous flow analyser” is too vague, since the analyser is an instrument, not a method. Please specify the analytical method used (e.g., colorimetric method for nitrate and ammonium, specific reagents/reactions, detection wavelengths), or at least provide a reference to a standard method.

Re: The extraction of soil inorganic nitrogen followed the procedure of Song et al. (2024), and the analytical measurements were consistent with the standard method GB/T 42487-2023. Ammonium nitrogen was determined based on the Berthelot indophenol blue reaction at 660 nm. For nitrate measurement, a copper-coated cadmium column was utilized to reduce NO_3^- to NO_2^- . Subsequently, both the original NO_2^- in the soil extract and the NO_2^- derived from NO_3^- reduction were quantified by the Griess-Ilosvay reaction, using sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride as reagents under acidic conditions, forming a diazo complex with absorbance recorded at 543 nm. We have revised the manuscript and incorporated this information on [lines 95-98](#).

“Soil inorganic nitrogen (NO_3^- , NO_2^- , NH_4^+) content was determined by mixing soil with deionized water (DIW) at a ratio of 1:5 (w/v) (Song et al., 2024), shaking and centrifuging, and then the extracted supernatant was analyzed by a continuous flow analyzer (Skalar San++ System, Skalar, Breda, Netherlands) (GB/T 42487-2023).”

Line 130 – Since a three-valve rotary system is employed to alternate between chambers, the current flux calculation equation may not fully account for the time interval between chamber switching. Please clarify whether any interpolation was used to estimate fluxes between switching events.

If fluxes were assumed constant over certain intervals, this assumption should be stated. Furthermore, the flux equation should be reformulated to correctly reflect the time-averaging or interpolation procedure inherent to the switching system.

Re: In this study, measurements for each chamber were conducted for 2 min, after which the system switched to the subsequent chamber using a multi-point switching valve. To mitigate potential uncertainties caused by chamber replacement, we exclusively used the corresponding average values calculated from the 30 s interval immediately prior to the switch. The fluxes were assumed constant over the intervals and the flux equation has been re-expressed, see Equation (1):

$$F_N = \frac{Q}{A} \times \frac{1}{V_m} \times (x_{out}(t) - x_{in}) \quad (1)$$

where F_N denotes the flux of Nr gases ($\text{ng N m}^{-2} \text{ s}^{-1}$); Q denotes the flow rate of the gases ($\text{m}^3 \text{ s}^{-1}$); A denotes the surface area of the soil (m^2); V_m denotes the molar volume of air ($\text{m}^3 \text{ mol}^{-1}$); t represents the 30 s before chamber switching; and χ_{out} and χ_{in} denote the headspace mixing ratios at the outlet and inlet of the chamber (ppb).

Line 148 – The choice of a 5-day fumigation period should be briefly justified:

Was this based on previous studies, preliminary tests, or methodological constraints? How sensitive do the authors expect their results to be to the duration of fumigation (e.g., would shorter or longer fumigation times yield qualitatively similar effects on the microbial community and Nr emissions)?

A short rationale here would help readers interpret the fumigation results and understand the scope of inference.

Re: Prior to the formal experiment, a preliminary test was performed on sample F3 by fumigating it with 50 ppb NO gas for 3, 5, and 7 days, respectively. Based on the evaluation of the measured fluxes, the 5-day fumigation period was ultimately selected. We have included this information in the revised manuscript. Please see lines 162-163 and Table S1.

“Based on the results from preliminary tests, we selected a fumigation period of 5 days (Table S1), during which the fumigant gas input was repeated every 24 h to ensure a stable concentration of fumigant gas in the incubation bottles.”

Table S1. Gas flux results for NO gas fumigation at 50 ppb for 3, 5, and 7 days (sample F3).

Fumigation duration	HONO	NO	NO ₂	NO _x	NH ₃
Average flux ($\text{ng N m}^{-2} \text{ s}^{-1}$)					

DAY3	2.28	6.88	1.93	8.39	589.76
DAY5	13.92	33.50	6.72	37.19	364.06
DAY7	9.99	63.29	5.15	62.95	396.20
Optimum flux (ng N m ⁻² s ⁻¹)					
DAY3	43.51	23.94	16.67	25.18	3046.20
DAY5	165.95	96.62	29.85	117.71	1645.00
DAY7	301.24	266.26	30.13	271.61	1965.78
Integrated emission (mg N m ⁻²)					
DAY3	0.22	0.56	0.16	0.65	47.23
DAY5	1.25	2.70	0.58	2.89	29.06
DAY7	0.85	5.12	0.43	4.91	31.72

Data availability – Zenodo repository

In the Zenodo repository, it would be helpful if file names were provided in English.

[Re: Done.](#)

RC2: Anonymous Referee #3

General Comment

Reactive nitrogen species play important roles in atmospheric chemistry; however, soil emissions of Nr remain poorly constrained. In this study, Deng et al. collected soil samples from different landscapes across the Tibetan Plateau. Emissions of HONO, NO_x, and NH₃ from these soils were measured, and their relationships with soil properties and microbial community distributions were investigated. In addition, the impacts of NO_x and O₃ fumigation were examined. Finally, the laboratory results were combined with a parameterized model to estimate annual emissions of these Nr species. Considering the importance of Nr sources for atmospheric chemistry over the Tibetan Plateau, this study provides valuable insights into the Nr budget on the Tibetan Plateau and its potential atmospheric impacts.

[Re: We would like to thank the reviewer for the supportive and constructive comments. Please refer to our responses below on how we improve our manuscript based on your comments.](#)

Major Comments

- Soil–atmosphere exchange of reactive nitrogen is typically bidirectional (Bao et al., 2022). However, by using dry Nr-free air as the carrier gas, only net emissions can be observed. In addition, surface water exchange strongly affects Nr emissions (Xue et al., 2024). Under dry-air conditions, surface water evaporation is likely maximized, which may enhance emissions compared with natural conditions. Related discussion is needed.

Re: We thank the reviewer for this rigorous and critical comment. We fully agree that using dry air as the carrier gas indeed fails to capture bidirectional exchange and might amplify emissions by enhancing evaporation. In accordance with your advice, we have incorporated this discussion into the revised manuscript. We emphasize that the standardized dry-air method was adopted to exclude atmospheric background interference and accurately gauge the soil's maximum net emission potential. Nevertheless, caution is required when applying these absolute fluxes to natural scenarios. The relevant discussion regarding moisture effects on Nr emissions has been added. Please see [Lines 317–321](#) and [Lines 343–347](#).

“Under natural conditions, soil–atmosphere exchange of reactive nitrogen is typically bidirectional (Bao et al., 2022). Here, we quantify net soil emissions and eliminate the masking effect of atmospheric Nr background by using dry Nr-free air as the carrier gas. For example, the observed HONO flux represents the net apparent flux, which integrates direct soil biogenic emission and additional HONO formation via heterogeneous uptake and conversion of atmospheric NO₂ on soil particle surfaces (Fan et al., 2025).

However, it should be noted that extrapolating the results of this study to actual field conditions has inherent limitations. Xue et al. (2024) showed that surface water exchange plays a critical role in mediating soil Nr emissions. Under the dry-air conditions employed in our experimental setup, the evaporation of surface water is likely maximized. This enhanced evaporation could potentially facilitate the escape of dissolved Nr gases from the soil matrix, thereby leading to an overestimation of the net fluxes compared to ambient conditions with higher relative humidity.”

- In the field, wet–dry cycles are regulated by water exchange at the soil–air interface and are influenced by environmental factors such as temperature and relative humidity. How does the current study account for these processes when extrapolating laboratory results to field conditions?

Re: We thank the reviewer for this insightful comment concerning the extrapolation of laboratory findings to field scenarios. We concur that the controlled environment in our laboratory experiments is inherently distinct from the complex natural conditions, especially regarding the wet-dry cycles driven by water exchange at the soil–atmosphere interface. In response to your suggestion, we have incorporated a dedicated discussion in our revised manuscript. We explicitly clarify that the standardized dry-air carrier gas method was adopted to eliminate background atmospheric interference and humidity, which allows us to accurately assess the maximum net emission potential of the soil. Nevertheless, we recognize that these constant dry conditions fail to fully replicate natural wet-dry cycles. Hence, we emphasize in the Discussion that, although our results are robust for evaluating relative emission intensities among diverse land-use types, direct extrapolation of absolute flux values to field environments warrants caution and requires consideration of the interplay among soil moisture dynamics, temperature, and relative humidity. We have included this detailed discussion regarding the laboratory-field discrepancy. Please refer to [Lines 343–351](#).

“However, it should be noted that extrapolating the results of this study to actual field conditions has inherent limitations. Xue et al. (2024) showed that surface water exchange plays a critical role in mediating soil Nr emissions. Under the dry-air conditions employed in our experimental setup, the evaporation of surface water is likely maximized. This enhanced evaporation could potentially facilitate the escape of dissolved Nr gases from the soil matrix, thereby leading to an overestimation of the net fluxes compared to ambient conditions with higher relative humidity. Therefore, our findings provide theoretical maximum emission potentials, and caution is advised when directly applying the absolute flux magnitudes obtained here to field assessments without considering local in situ environmental data (e.g., soil moisture, temperature). Future studies integrating in situ field measurements or environmentally controlled microcosm systems are essential to delineate the dynamic impacts of wet-dry cycles on Nr emissions.”

- Considerable NO₂ emissions were observed. However, neither nitrification nor denitrification mechanisms fully explain direct NO₂ emissions. This should be further discussed.

Re: We sincerely thank the reviewer for this insightful and critical comment. We fully agree that classical nitrification and denitrification pathways are insufficient to fully explain the direct NO₂ emissions observed in our study. To address this concern, we have expanded the discussion in the

revised manuscript. In this added section, we explicitly explore the alternative mechanisms driving direct NO₂ emissions. Please refer to [Lines 338–342](#).

“It is worth noting that the substantial NO₂ emissions observed in this study cannot be fully explained by classical microbial nitrification and denitrification mechanisms alone. Apart from biological pathways, direct NO₂ emissions are likely driven by several abiotic or non-classical biotic mechanisms. Chemodenitrification is recognized as a significant source of NO₂ in soils; specifically, under acidic conditions, the accumulated NO₂[−] in soil undergoes chemical decomposition to produce NO and NO₂ (Cai et al., 2012).”

■ The effects of NO/NO_x/O₃ exposure on Nr emissions were investigated, and impacts were observed. However, were these effects incorporated into the estimation of annual emissions?

Re: We thank the reviewer for this insightful question. We acknowledge that the current estimation of annual soil Nr emissions (Section 2.6) is not directly incorporating the enhanced/suppressed effects observed in the NO_x/O₃ fumigation experiments. The fumigation experiments (Section 2.3) were designed specifically to elucidate the mechanistic pathways by which atmospheric NO_x and O₃ impact soil Nr emissions across different land use types. The current annual emission model (Section 2.6) relies on statistical relationships established between soil Nr fluxes and key in-situ soil properties (including soil pH, inorganic nitrogen content, and the abundance of nitrogen-cycling functional genes). Since these measured soil properties represent the long-term integrated status of soil biogeochemistry, they inherently reflect the historical exposure to atmospheric deposition, but capturing the short-term dynamic chemical feedback of ambient NO_x/O₃ into a regional-scale statistical model is complex. Therefore, while not directly parameterized into the upscaling model, we qualitatively discussed these effects in Section 3.2 and concluded that they highlight the intricate relationships between atmospheric chemistry and soil biogeochemistry. Please see [lines 216-221](#) and [lines 437-441](#) in the revised manuscript.

“This model takes into account factors such as soil temperature, precipitation, and nitrogen fertilizer application (on farmland). It should be clarified that the current annual emission model does not directly incorporate the effects observed in the fumigation experiments. The upscaling model relies on statistical relationships established between soil Nr fluxes and key in-situ soil properties. While these long-term in-situ properties inherently reflect historical deposition, capturing the short-term chemical feedback of ambient NO_x/O₃ into a regional-scale model is

complex. Thus, these effects are qualitatively discussed in relation to the fumigation results (Figs. S5–S7).

It should be noted that the effects observed in the fumigation experiments (Figs. S5–S7) are discussed qualitatively but are not incorporated into the annual emission parameterization. The annual model relies on long-term in-situ soil properties, which makes it challenging to extrapolate short-term chemical feedback to a regional scale. Therefore, this qualitative discussion highlights the complex interplay between atmospheric chemistry and soil biogeochemistry.”

- The derived Nr emission fluxes are based solely on laboratory experiments. These estimates should be compared with published field measurements and/or top-down estimates to evaluate their representativeness and reliability.

Re: We appreciate the reviewer's emphasis on validation. We acknowledge that upscaling from laboratory results to regional emissions could cause large uncertainties. To constrain the uncertainties, we integrated both our measured data and published field measurement data from the Tibetan Plateau into the upscaling model (Table S4). We also compared our estimated annual grassland NO emissions ($0.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) with published field-based observations ($0.021 - 0.29 \text{ kg N ha}^{-1} \text{ yr}^{-1}$) from the Plateau (Gao et al., 2016; Holst et al., 2007; Lin et al., 2019; Yao et al., 2019; Zhang et al., 2018) and found excellent agreement (Table S4). Additionally, our HONO and NO_x emissions estimates were compared with recent regional bottom-up inventories (Li et al., 2026; Wu et al., 2022). We have included these validations in our revised manuscript. Please see [lines 460-462](#).

“To validate our estimates, the upscaling model integrated both measured and published field data from the Tibetan Plateau (Table S4). Our HONO and NO_x estimates are consistent with recent regional bottom-up inventories (Li et al., 2026; Wu et al., 2022) and provide useful constraints for GEOS-Chem simulations over the Third Pole (Wang et al., 2025).”

Minor Comments

- L62–64: This conclusion remains controversial. Please also consider the discussions and comments published after the paper appeared.

Re: We sincerely thank the reviewer for this critical and rigorous comment. During the revision process, we systematically searched for and carefully reviewed the relevant discussions and subsequent research studies published after the original paper (Labzovskii et al., 2024). We

acknowledge that this conclusion remains somewhat controversial within the academic community, particularly regarding the serious anomalies in NO₂ retrievals over lakes, which may arise from interference due to water absorption and could potentially bias the retrieval results. Accordingly, we have removed this content in the revised manuscript.

■ L77: It would be helpful to include a map showing all sampling sites.

Re: We sincerely thank the reviewer for this constructive suggestion. In response, we have now included a detailed map showing the geographical distribution of all sampling sites in the revised manuscript. This map is presented as Fig. S1, and it clearly illustrates the locations and land use types of the sampling points. We believe this addition significantly enhances the clarity of our experimental design and provides readers with a comprehensive understanding of the study area.

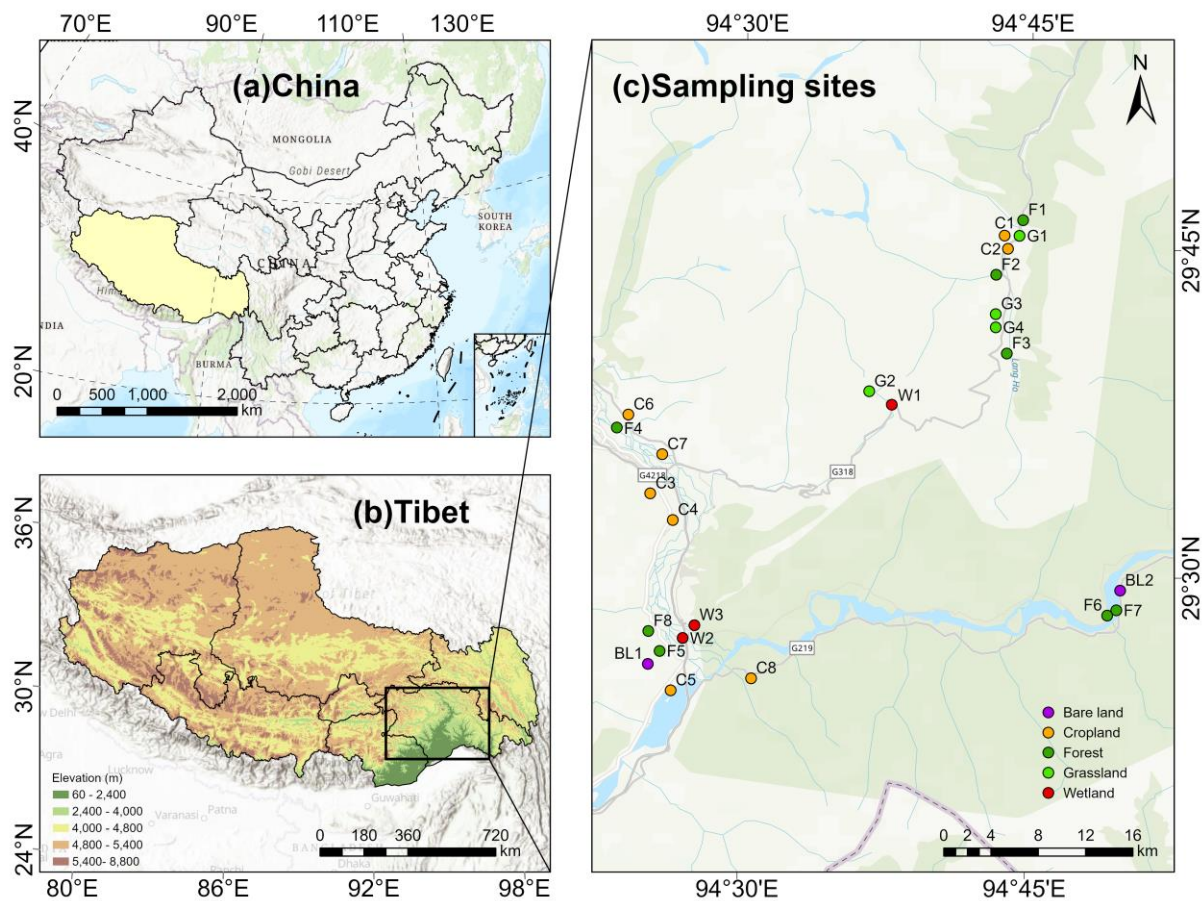


Fig. S1. Spatial distribution of the sampling sites in the study area.

- L137: Why do the first two treatments focus on NO, whereas the other two focus on NO_x + O₃? Since NO_x reacts rapidly with O₃, the actual concentrations inside the chamber may differ substantially from the initial values.

Re: We sincerely thank the reviewer for this insightful comment regarding our experimental design and the underlying chemical kinetics. We fully understand that NO_x and O₃ react rapidly, which may lead to discrepancies between the actual concentrations inside the chambers and the initial set values. The rationale behind our different experimental treatments is as follows: (1) The first two treatments (solely NO) were designed to isolate the direct effect of NO gas on soil nitrogen cycling, independent of its rapid oxidation process; (2) The other two treatments (NO_x + O₃) were designed to simulate the synergistic effects after photochemical aging, where NO is rapidly converted to NO₂ in the presence of O₃, thereby assessing the combined impacts of NO, NO₂, and strong oxidants on the soil system. We have included these information in our revised manuscript (lines 145-149).

“The first two treatments (solely NO) were designed to isolate the direct effect of NO gas on soil nitrogen cycling, independent of its rapid oxidation process, the other two treatments (NO_x + O₃) were designed to simulate the synergistic effects after photochemical aging, where NO is rapidly converted to NO₂ in the presence of O₃, thereby assessing the combined impacts of NO, NO₂, and strong oxidants on the soil system.”

- L217: BLH usually exhibits strong diurnal variation rather than remaining constant.

Re: We fully agree with the reviewer that the boundary layer height (BLH) exhibits strong diurnal variations. We acknowledge that assuming a constant BLH in our original manuscript was a simplification. However, we would like to clarify that the value of 182 m was not arbitrarily chosen. It was derived as the daily-averaged BLH for the Nam Co station during the study period based on the ERA5 reanalysis dataset. We intentionally used this daily-averaged BLH for the following reason: our primary focus is on the daytime atmospheric oxidation capacity driven by soil HONO photolysis. Since HONO photolysis is almost negligible at night due to the absence of solar radiation, the primary source contribution is determined during the daytime. While the daytime BLH is higher than the nightly BLH, using the daily-averaged BLH (182 m) in our first-order estimate provides a representative central baseline that integrates the overall atmospheric mixing conditions. We have included these information in our revised manuscript (lines 236-237)

“A constant BLH is a simplification. The 182 m daily-average from ERA5 for Nam Co was adopted to focus on daytime oxidation capacity driven by HONO photolysis.”

■ L363–364: Nr emissions were generally observed from soil based on results of this study.

Why did cropland and grassland soils act as sinks for HONO and NO?

Re: We sincerely thank the reviewer for raising this intriguing point regarding the contrast between natural and experimental conditions. We agree that while arable land and grassland soils are generally considered natural sources of HONO and NO, our fumigation experiments showed that their emissions were significantly suppressed and even acted as sinks under elevated NO and NO_x-O₃ levels. This contrast fundamentally reflects that the source-sink balance of soil reactive nitrogen is strongly regulated by atmospheric background concentrations. To address this, we have added a detailed discussion of the underlying mechanism in the revised manuscript (see [Lines 401-404](#)).

“Interestingly, while cropland and grassland soils typically serve as sources of HONO and NO under natural conditions, their fluxes were significantly suppressed under fumigation with elevated NO and NO_x + O₃. These results indicate that high concentrations of NO and NO_x + O₃ may lead to a decrease in the soil production rates of HONO and NO, thereby causing a reversal of the source-sink balance.”

RC3: Anonymous Referee #1

Summary. The manuscript by Deng et al. reports on fluxes of reactive nitrogen (Nr = NO, NO₂, and HONO) from soil samples collected from sites in the Tibetan Plateau. Fluxes were measured from sieved soil during microcosm experiments. In addition, the flux measurements are accompanied by measurements of soil bio- chemical-physical parameters in an effort to identify drivers of soil emissions. Lastly, the authors carried out experiments to study the impact of air pollution components on soil emissions by ‘fumigating’ soil samples with a mixture of NO_x and ozone prior to measuring soil Nr fluxes. The manuscript concludes by using a model to understand the impact of Nr emissions on air quality in the Tibetan Plateau region. Overall, the study provides useful observations of Nr fluxes and characterizes the microbial community in the various soils samples. For this reason, it would be useful to publish these observations and measurements. However, I have some serious concerns about the experimental approach, its environmental relevance, and the rationale behind some of the experiments. Thus, I recommend the authors address the following concerns prior to acceptance.

Re: We would like to thank the reviewer for the supportive and constructive comments. Please refer to our responses below on how we improve our manuscript based on your comments.

Major concerns.

The most serious concern I have has to do with how the samples were handled prior to measuring Nr fluxes. Following sampling, the authors report storing the soil at -20 degrees C and then thawed within a matter of hours/days prior to sieving and flux measurement. How can we be sure that this treatment provides a representative sample. Presumably, the freezing can lyse cells and release Nr into the soil matrix. While it is conceivable that the soil may have been exposed to such temperatures naturally during wintertime, in the real environment, soil has months of time to thaw and for microbial communities to change. Have the authors conducted samples on freshly collected soil that has not undergone this treatment to verify that their approach is valid? Without demonstrating this, we cannot trust that these measured Nr and mineralization and nitrification rates are representative of what is happening in nature. For that reason, it should be very clear that these Nr fluxes are observed, and the appropriate disclaimers should be made.

Re: We sincerely thank the reviewer for raising this critical and rigorous methodological concern. While we did not have the additional resources to conduct a parallel control experiment on freshly collected soils, we fully acknowledge the validity of your concern. The soil sample preservation protocol employed in this study (storage at -20 °C followed by thawing) may have exerted a non-negligible influence on the measured Nr fluxes. The freezing process can cause microbial cell lysis, releasing intracellular soluble organic nitrogen and ammonium into the soil matrix, thereby artificially increasing the soil inorganic nitrogen pool and potentially amplifying the estimated emission potential. Although freeze-thaw cycles naturally occur during winter in the field, these field processes involve extremely slow, gradual thawing and successive shifts in microbial community structure, which fundamentally differ from the rapid thawing performed under laboratory conditions. Therefore, the flux data obtained in this study should not be interpreted as absolute in-situ fluxes in the field, but rather as the maximum potential emissions under the specific stimulated conditions. The primary conclusions of this study (e.g., the relative differences among different land-use types and the regulatory effects of different fumigation treatments on flux trends) were drawn based on strictly parallel controlled experiments, and thus their scientific reliability remains robust at a comparative level. We recommend that future studies, when resources permit,

incorporate in-situ or rapid measurements of fresh field soils to obtain baseline emissions closer to natural reality. We have included these information in our revised manuscript (lines 133-136).

“The soil sample preservation protocol used in this study (storage at -20°C followed by thawing) may have amplified the potential for soil Nr emissions; therefore, we adopted the optimal flux (i.e., the highest value) under specific stimulating conditions, which represents the maximum potential emissions at the maximum water-holding capacity.”

The other concern I have is regarding the rationale behind the “fumigation” studies. In them, the authors exposed soil samples to NO_x and Ozone to see what kind of effect these gases have on Nr emissions. A convincing rationale for this experiment is not presented. Also, I don’t see how this experiment will yield any useful knowledge as the experiment seems to be flawed. For example, ozone will react extremely rapidly with soil organic matter and effectively be removed as soon as it is introduced into the chamber. If NO doesn’t react with ozone to form NO_2 in the chamber, then it may artificially affect the net direction of flux based on the soil compensation point. So, I do not see how results of the fumigation studies could be used to draw any meaningful conclusions.

Re: We greatly appreciate the reviewer's critical and insightful comments regarding the rationale and methodological flaws of our fumigation experiments. We fully understand the necessity of clarifying this design. In the real atmosphere, emitted NO is rapidly oxidized to NO_2 in the presence of O_3 ($\text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2$). We aimed to evaluate the synergistic effects of gaseous reactive nitrogen and strong oxidants on soil microbes and net fluxes. Even if a large portion of the NO and O_3 is transformed or dissipated by soil, the residual oxidants may still modify the soil's microscopic redox environment, leaving statistically significant differences in soil microbial communities (e.g., functional gene abundances of nitrification and denitrification) and soil physicochemical properties (DOC, pH, etc., see Fig. S8). This confirms that the experimental stimuli effectively drove internal soil responses. Although NO reacts rapidly with O_3 and O_3 is heterogeneously consumed by soil organic matter, under the same dynamic flow rate and identical fumigation system, the physical and chemical kinetic conditions for gas conversion and consumption are highly consistent across all treatment groups (including the 10 ppb and 50 ppb NO and $\text{NO}_x + \text{O}_3$ groups). Therefore, while the absolute concentrations do not equal the initial set values, the relative gradient (hierarchical order) of actual exposure intensities among different treatment groups remains valid. The conclusions regarding the "sensitivity differences of land-use types to high concentrations of NO and oxidants" is based on strictly parallel controlled

experiments, and their relative trends remain robust. We have included these information in our revised manuscript (lines 144-152).

“Four fumigation treatments were installed, i.e., 10 ppb NO, 50 ppb NO, 10 ppb NO_x + 30 ppb O₃, and 50 ppb NO_x + 100 ppb O₃. The first two treatments (solely NO) were designed to isolate the direct effect of NO gas on soil nitrogen cycling, independent of its rapid oxidation process, the other two treatments (NO_x + O₃) were designed to simulate the synergistic effects after photochemical aging, where NO is rapidly converted to NO₂ in the presence of O₃, thereby assessing the combined impacts of NO, NO₂, and strong oxidants on the soil system. Although NO rapidly reacts with O₃, and O₃ is concurrently consumed by reactions with soil organic matter, the physicochemical kinetic conditions governing gas conversion and consumption are consistent across all treatment groups under identical flow rates and fumigation regimes. Thus, the relative ranking of actual exposure intensities among the different treatment groups is still considered valid.”

Specific comments.

Line 86: I am not sure what “sampling via cold chain” is. Please clarify.

Re: We sincerely thank the reviewer for pointing out this ambiguity. We apologize for the lack of clarification. The "cold chain" we mentioned refers to the professional cold-chain logistics service provided by SF Express (Shunfeng). Specifically, immediately after sampling in the field, the fresh soil samples were sealed in sterile bags and handed over to the SF Express cold-chain service, which maintained a constant temperature at approximately 4 °C throughout the transportation process. The samples were delivered to our laboratory within 48 hours and immediately transferred to a −20 °C freezer for storage before subsequent analyses. This strictly controlled commercial cold-chain process is a widely adopted method in China for preserving biological and soil samples during long-distance transport, as it effectively minimizes microbial activity and prevents nitrogen transformation after sampling. We have added the relevant information in the revised manuscript. Please see Lines 86-90.

“The soil samples were transported back to the laboratory within 48 hours via SF Express’s standard cold-chain service (maintained at a constant temperature of 4 °C throughout the entire process). Upon arrival at the laboratory, soil samples for microbiological analyses were stored at −80°C, while soil samples for determining Nr gas emissions and physicochemical properties were stored at −20°C.”

Line 121: In addition to instrument limits of detection based on concentration, can the authors please provide limits of detection for fluxes. What is the minimum flux detectable for each gas?

Re: We thank the reviewer for this important methodological comment. In addition to the concentration-based instrument LODs, we have now calculated the minimum detectable fluxes for each target gas. Specifically, the minimum detectable fluxes were approximately $0.8 \text{ ng N m}^{-2} \text{ s}^{-1}$ for NO, $1.6 \text{ ng N m}^{-2} \text{ s}^{-1}$ for NO₂, $0.8 \text{ ng N m}^{-2} \text{ s}^{-1}$ for NH₃, and $1.2 \text{ ng N m}^{-2} \text{ s}^{-1}$ for HONO. We have included these information in our revised manuscript (lines 123-125).

“In addition to the concentration-based instrument LODs, we have now calculated the minimum detectable fluxes for each target gas. Specifically, the minimum detectable fluxes were approximately $0.8 \text{ ng N m}^{-2} \text{ s}^{-1}$ for NO, $1.6 \text{ ng N m}^{-2} \text{ s}^{-1}$ for NO₂, $0.8 \text{ ng N m}^{-2} \text{ s}^{-1}$ for NH₃, and $1.2 \text{ ng N m}^{-2} \text{ s}^{-1}$ for HONO.”

Line 300: replace “detected to emit of” with “found to emit”

Re: Done.

Line 348-9: The authors write, “This demonstrates that high NO emissions from cropland soils probably coincide tightly with Actinobacteriota abundances. Are the authors suggesting that the NO emissions are coming from the Actinobacteria. Please explain if so.

Re: We sincerely thank the reviewer for raising this critical point regarding the distinction between correlation and causation. When we describe the NO emissions as "coinciding tightly" with Actinobacteriota abundances, we are reporting a highly significant statistical correlation rather than a direct causal link. Nevertheless, given the well-established role of Actinobacteria in soil nitrogen cycling, we do consider this strong correlation to imply that Actinobacteria may act as key biological drivers or active contributors to NO emissions in cropland soils. However, statistical correlation alone is insufficient to prove direct causation. Therefore, to avoid overinterpretation, we have revised the wording to be more rigorous, as shown in Lines 381-383.

“High NO emissions from cropland soils showed a strong consistency with Actinobacteriota abundances. Therefore, it is suggested that Actinobacteriota may act as a significant microbial regulator for NO emissions in Tibetan cropland soils.”

Line 373-4: It was found that soil pH tended to increase after fumigation, especially in forest soils, with an average increase of ~ 0.53 . However, the authors do not explain what the increase in pH is due to. Has this ever been documented in the literature? How sure are the authors that the pH change is not due to some underlying process occurring over time that is independent of the fumigation procedure? The microbe communities will be in constant flux once removed from the environment and subjected to all the lab treatments, so it may not be surprising to see such changes. Do the authors have controls with which to compare soil changes (in pH but also for nitrification and mineralization rates)? If so, those should be included and discussed.

Re: We sincerely thank the reviewer for raising this critical question regarding whether the observed increase in soil pH was caused purely by time-dependent effects or by the incubation background. pH elevation can be primarily attributed to changes in redox states, H^+ consumption during the reduction of nitrogen intermediates, and shifts in microbial functional communities (Chen et al., 2023; Slessarev et al., 2016; Wu et al., 2026). We acknowledge that this study did not directly measure nitrification, mineralization, and denitrification rates. However, the pH increase is a direct consequence of a shifted balance between H^+ production and consumption within the soil. Under strong oxidative fumigation conditions, we observed an increase in the abundance of denitrifying genes (Fig. S8). Denitrification is a H^+ -consuming process that can directly elevate soil pH, particularly in forest soils, which typically exhibit lower initial buffering capacities. To ensure that the observed pH changes were genuinely induced by fumigation rather than incubation artifacts, we measured the baseline pH of the soils prior to the experiments. The initial values were 5.59 ± 0.16 for forest soils, 5.44 ± 0.18 for cropland soils, and 5.10 ± 0.05 for grassland soils. If the pH increase were simply a general phenomenon resulting from microbial succession during laboratory incubation, we would expect comparable magnitudes of pH increase across all different fumigation doses. Furthermore, the pH increase was most pronounced in forest soils ($+0.53$), whereas the shifts in cropland and grassland soils were much weaker. If these changes were merely a consequence of post-sampling disturbance over time, such stark differences among land-use types would be very difficult to explain. Therefore, we conclude that the observed pH changes were predominantly driven by the fumigation gases. We have included these information in our revised manuscript (lines 411-418).

“Forest (pH $\sim 5.59 \pm 0.16$), cropland (pH $\sim 5.44 \pm 0.18$) and grassland (pH $\sim 5.10 \pm 0.05$) soils were acidic before fumigation in the study. It was found that soil pH tended to increase after fumigation, especially in forest soils, with an average increase of ~ 0.53 (Fig. S8). The increase in

pH is primarily attributed to changes in the redox state, H^+ consumption during the reduction of nitrogen intermediates, and shifts in microbial functional communities (Chen et al., 2023; Slessarev et al., 2016; Wu et al., 2026). Under strong oxidative fumigation conditions, the abundance of denitrification genes increased (Fig. S8), which likely led to greater H^+ consumption, thereby raising the soil pH. Furthermore, the differential responses among soil types, rather than a uniform shift across all treatments, suggest that the pH increase was predominantly driven by the fumigation gases rather than merely by the duration of laboratory incubation.”

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