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Nitrifier controls on soil NO and N_2O emissions in three chaparral ecosystems under contrasting atmospheric N inputs

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ABSTRACT

High rates of atmospheric N deposition can increase ecosystem N availability and stimulate N losses from soils via nitric oxide (NO; an air pollutant at high concentrations) and nitrous oxide (N_2O; a strong greenhouse gas) emissions as predicted by N saturation theory. However, it remains unclear whether theories developed in mesic ecosystems apply to drylands, where plant N uptake and N availability are often decoupled. NO and N_2O are produced during the oxidation of ammonia (i.e., nitrification) by ammonia-oxidizing archaea (AOA) or ammonia-oxidizing bacteria (AOB). Because AOB may be favored in N-rich environments and may emit more NO and N_2O than AOA, high atmospheric N inputs may favor both NO and N_2O emissions. To assess whether atmospheric N deposition favors AOB- and AOA-derived N emissions, we selectively inhibited AOA and AOB and measured NO and N_2O from soils collected from three dryland sites exposed to relatively low (3.8 kg ha^{-1} = Low N) or high (11.8 kg ha^{-1} = High N-A; 15.6 kg ha^{-1} = High N–B) atmospheric N inputs. We found that while the High N–B deposition site had the lowest AOA:AOB ratio (2.3 ± 0.6), consistent with expectations, this site did not emit the most NO and N_2O. Rather, AOA emitted between 21 and 78% of the NO from our sites, with higher AOA-derived NO emissions from relatively coarse-textured soils in the Low N deposition site. In addition to nitrification, other processes also emitted NO and N_2O, especially in the High N-A site where non-nitrifier NO and N_2O emissions were ~2–4 × higher than the other sites, and where finer textured soils may favor denitrification. Interactions between soil texture and N availability, rather than shifts in nitrifier communities, likely determine whether atmospheric N deposition is retained in these dryland sites or reemitted to the atmosphere as NO or N_2O.

1. Introduction

Elevated rates of atmospheric nitrogen (N) deposition can enrich soils with N and lead to adverse ecosystem effects such as soil acidification, nutrient imbalances, shifts in species composition, and increased emission of trace gases like nitric oxide (NO; a regional air pollutant at high concentrations) and nitrous oxide (N_2O; a powerful greenhouse gas and destroyer of stratospheric ozone) (Fenn et al., 2006; Ravishankara et al., 2009; Bobbink et al., 2010; Yahdjian et al., 2011; Fowler et al., 2013; Tian et al., 2020; Sha et al., 2021). To help determine when atmospheric N inputs exceed the capacity of an ecosystem to retain N, the concept of N saturation was developed in mesic forests, where the negative impacts of N enrichment on ecosystems were first studied (Aber et al., 1989; Lovett and Goodale, 2011). N saturation theory predicts that as ecosystems become enriched with N, NO and N_2O emissions increase in proportion to the N available in excess (Aber et al., 1989). However, in mesic forests, moist soils keep N biogeochemical cycling coupled to ecosystem N sinks (e.g., plants and soil organic matter; Lovett and Goodale, 2011), whereas in drylands, predominantly dry conditions decouple N cycling from N sinks (Homyak et al., 2014; Osborne et al., 2022a). For example, when rainfall rewets dry soils, both biotic and abiotic processes are well known to favor large gaseous N losses before

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ecosystem N sinks activate (Homyak et al., 2014; Osborne et al., 2022b; Krichels et al., 2023), suggesting it remains unclear how N-saturation theory applies to dryland environments. Moreover, N pollution may affect N emissions by altering the relative abundance of microorganisms that produce oxidized forms of N during nitrification: ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) (Prosser et al., 2019). Given that AOB may emit more NO and N_2O during nitrification than AOA (Mushinski et al., 2019), understanding how AOA and AOB respond to excess N availability may help determine whether atmospheric N inputs are sequestered or reemitted as NO and N_2O to the atmosphere.

Ammonia oxidizing bacteria and archaea have distinct nitrification pathways that can affect how much N is emitted from soils to the atmosphere. Specifically, AOA may emit less NO than AOB during the oxidation of hydroxylamine (NH_2OH), the second step in the sequential oxidation of ammonia to nitrite (NO_2^-) via nitrification (Mushinski et al., 2019). During this step, AOA may require NO as a co-reactant to oxidize NH_2OH, while AOB do not, making NO more vulnerable to escape to the atmosphere when AOB nitrify (Kozlowski et al., 2016; Prosser et al., 2019). Moreover, AOB can enzymatically reduce NO to N_2O—there is no evidence for a similar NO reduction pathway in AOA—allowing for higher nitrifier-derived N_2O emissions in soils where AOB are more active than AOA (Hink et al., 2017; Prosser et al., 2019). While these mechanisms suggest that the AOB nitrification pathway may be "leakier" by emitting more NO and N_2O compared to AOA, both AOA and AOB can release NH_2OH and NO_2^- into the soil environment (Ermel et al., 2018), which can be enzymatically reduced to NO and N_2O via denitrification, or chemically converted to NO and N_2O via chemodenitrification (Firestone and Davidson, 1989; Zhu-Barker et al., 2015; Heil et al., 2016). Nonetheless, nitrification is an important process regulating N trace gas emissions from many drought-prone ecosystems (Homyak et al., 2014; Krichels et al., 2022), and whether the relatively leakier AOB dominate nitrification may influence how much N is lost from ecosystems.

Soil N enrichment may influence whether AOA or AOB dominates nitrification in dry soils. While both AOA and AOB oxidize NH_3 to NH_2OH, AOA may have higher affinity for NH_3 and higher tolerance to withstand drought stress, allowing them to nitrify in drought-stressed environments and recycle N more efficiently (Martens-Habbena et al., 2009; Delgado-Baquerizo et al., 2016; Prosser et al., 2019). In contrast, in N-rich soils, AOB may have more access to NH_3, potentially amplifying N trace gas emissions from the relatively leakier AOB nitrification pathway (Prosser et al., 2019; Mushinski et al., 2020). However, N enrichment can also acidify soils if there is enough water to leach base cations (Falkengren-Grerup, 1989; Püspök et al., 2022), which may favor AOA over AOB (Delgado-Baquerizo et al., 2016). Given the many ways in which N pollution may affect nitrifier communities, it remains unclear whether elevated rates of atmospheric N deposition affects NO and N_2O emissions in dryland ecosystems.

In addition to N pollution, drying–rewetting cycles can influence how much NO and N_2O is emitted from drylands. While desiccation can limit soil microbial activity (Moyano et al., 2013), rewetting dry soils can increase soil N availability and stimulate microbial processes that emit NO and N_2O into the atmosphere (Birch, 1958; Homyak et al., 2016; Leitner et al., 2017; Krichels et al., 2022). However, it is not clear whether differences between AOA and AOB nitrification affect rates of N gas efflux during these rewetting events; the contribution of AOA versus AOB to N trace gas emissions has primarily been tested in mesic forested and agricultural ecosystems where soils are relatively moist (Taylor et al., 2013; Thion and Prosser, 2014; Hink et al., 2018; Prosser et al., 2019; Mushinski et al., 2020). Given that dryland ecosystems experience frequent drying-rewetting cycles, with some exposed to among the highest rates of atmospheric N deposition in the world (Fenn et al., 2006; Sickman et al., 2019), we ask: can tradeoffs in AOB and AOA community composition in dryland soils exposed to high rates of atmospheric N deposition affect soil NO and N_2O emissions?

We tested two hypotheses to determine how nitrifier community composition controls N trace gas emissions from dryland ecosystems exposed to elevated N deposition: i) AOB produce more N trace gases than AOA do during rewetting events, and ii) higher soil N availability favors nitrification by AOB compared to AOA, increasing N trace gas emissions from soils exposed to relatively high rates of atmospheric N deposition. To test these hypotheses, we measured soil NO and N_2O emissions from soils collected from three remote chaparral sites exposed to a range of atmospheric N deposition rates in southern California (Table 1). To determine whether AOA or AOB controlled N emissions, we selectively inhibited either AOB nitrification or both AOA and AOB nitrification (Taylor et al., 2013; Mushinski et al., 2019). We also measured the abundance of *amoA* genes (which encode for the first step in nitrification) in AOA and AOB, soil pH, and inorganic N availability at each of the three sites.

2. Methods

2.1. Study site

We collected soils from three sites exposed to a range of atmospheric N deposition rates in southern California: a Low N deposition site and two high N deposition sites (hereafter, High N-A and High N–B; Table 1; EPA, 2021). Vegetation in all sites was dominated by chamise (*Adenostoma fasciculatum*). Soils from all sites were derived from granite parent material. Soils from the Low N site are fine sandy loams from the Sheephead series and are classified as shallow Entic Haploxerolls. Soils from the High N-A site are coarse loams from the Shepherdsaddle series and are classified as Ultic Haploxeralfs. Finally, soils from the High N–B site are fine sandy loams from the Trigo series and are classified as shallow Typic Xerorthents (Table S1; Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, 2019). The climate at all sites is Mediterranean with hot, dry summers and cool, wet winters. Annual precipitation ranges from 500 to 670 mm, and average monthly air temperatures range from 8 to 40 °C.

In the year leading up to the experiment, the High N–B deposition site had the highest ambient atmospheric concentrations of NO_x (NO_x = nitric oxide + nitrogen dioxide; 3.60 ± 1.51 ppm), while the High N-A deposition site had the highest ambient atmospheric concentrations of ammonia (NH_3; 2.98 ± 2.28 ppm), suggesting that they may receive different forms of N deposition (Fig. S1). The three sites also have slightly different soil textures: the High N-A site is more enriched in clay (38.3 ± 2.57 %) compared to the Low N (20.4 ± 2.92 %) or High N–B sites (15.8 ± 4.93 %; Table 1). Given that soil texture and the form of deposited N varied among the three remote sites, we cannot isolate the effects of N deposition and, instead, aim to: i) understand what controls gaseous N losses from dryland soils and ii) assess how NO and N_2O emissions derived from nitrifiers vary among remote sites that differ in atmospheric N inputs and soil properties.

Table 1
Soil physical and chemical properties from the Low N, High N-A, and High N–B deposition sites.

	pH	Clay (%)	Silt (%)	Latitude	Longitude	Modeled N dep (kg ha^{-1})
Low N	7.10 ± 0.14	20.4 ± 2.92	19.6 ± 2.46	33.379	−116.626	3.8
High N-A	6.34 ± 0.31	38.3 ± 2.57	13.8 ± 1.37	36.513	−118.807	11.8
High N–B	6.17 ± 0.31	15.8 ± 4.93	8.75 ± 2.09	34.203	−117.794	15.6

Atmospheric N deposition rates were estimated from the 2019 CMAQ model (EPA, 2021).

2.2. Experimental design

At each of the three sites, we collected dry soils (0–10 cm depth; A horizons) from underneath five *Adenostoma fasciculatum* shrubs across a ~50-m transect (each shrub was roughly 10 m apart) in September 2021. We collected soils in September because this is near the end of the dry season at our sites, allowing us to assess the contribution of AOA and AOB to N emissions after experimentally rewetting soils in the lab. Soils were transported back to the lab where they were sieved to 2 mm and stored at 4 °C until the experiment began (soils were refrigerated for less than one month). Soils were removed from the refrigerator two days before beginning the experiment and were incubated at lab temperature (~22 °C). Two soil samples were analyzed at a time. Each soil sample was split into three 50-g subsamples and transferred to canning jars (118 mL volume). The subsamples in each canning jar were exposed to one of three treatments: AOB inhibition, total nitrifier inhibition, or a control (Fig. S7). To inhibit NH_3 oxidation by autotrophic AOB, 1-octyne was added to one subsample to bring the headspace in the jar to 4 μmol L^{-1} (Taylor et al., 2013; Mushinski et al., 2019). The 1-octyne was prepared by adding 40 μL of liquid 1-octyne to a 125-mL bottle fitted with a butyl stopper, over-pressurizing the bottle with 100 mL of air, and, once the liquid 1-octyne evaporated, removing 2.7 mL of the bottle headspace to inject into the subsample. To inhibit NH_3 oxidation by all autotrophic nitrifiers, acetylene was added to one subsample to bring the jar headspace to 6 μmol L^{-1}. The acetylene was prepared by first bubbling acetylene through a sulfuric acid trap to remove impurities, diluting the purified acetylene 10-fold with air, and then injecting 0.28 mL into the subsample. The third jar was treated as a control and was incubated under ambient lab conditions. All jars were incubated for 24 h.

Following the 24-h incubation, NO and N_2O emissions were measured from each jar after experimentally wetting soils. To slow the growth of new AOB after wetting, the jar that was treated with 1-octyene was wet with a solution containing the antibiotic kanamycin at a concentration of 220 μg g^{-1} soil (Mushinski et al., 2019). To slow the growth of any new nitrifying bacteria or archaea, the jar that was treated with acetylene was wetted with a solution containing kanamycin (220 μg g^{-1} soil), the archaeal protein synthesis inhibitor fusidic acid (800 μg g^{-1} soil), and the nitrification inhibitor nitrapyrin (200 μg g^{-1} soil; (Taylor et al., 2013; Mushinski et al., 2019). The control jar was wetted with deionized water only. We note that the addition of bacterial and archaeal growth inhibitors could also limit the growth of non-nitrifying microorganisms that might affect NO and N_2O emissions. However, use of these inhibitors did not significantly affect soil CO_2 emissions (Figs. S5 and S6; $p = 0.06$), suggesting their effects on non-nitrifying organisms may be minor during our 46-h incubation. All jars were wetted with enough solution to reach 100% water-holding capacity, designed to simulate rapid rewetting events driving both microbial and abiotic processes and their potential to contribute to N trace gas emissions (Birch, 1958; Austin et al., 2004). NO and N_2O emissions were measured from six of the jars (representing two samples) every 2 h for 46 h after wetting. Net nitrification and net N mineralization rates were measured as the difference in NO_3^- (nitrification) or NO_3^- and NH_4^+ (N mineralization) between the start and end of the 46-h incubation. This process was repeated twice weekly until all 45 jars were analyzed (3 sites × 5 replicates × 3 treatments = 45 jars total).

The contribution of AOB to NO and N_2O emissions was determined by subtracting how much NO or N_2O was emitted from the AOB inhibition treatment (i.e., treating soils with 1-octyne and kanamycin) from how much NO or N_2O was emitted from control soils (wetted with water only). The contribution of AOA to NO and N_2O emissions was determined by subtracting how much NO or N_2O was emitted from the total nitrifier inhibition treatment (i.e., treating soils with acetylene, kanamycin, nitrapyrin, and fusidic acid) from how much NO or N_2O was emitted from the AOB inhibition treatment. Finally, NO or N_2O emissions from soils under the total nitrifier inhibition treatment were classified as "other processes", likely including denitrification, heterotrophic nitrification, and/or abiotic reactions. At the low acetylene concentrations used, neither N_2O production from denitrification nor N_2O reduction to N_2 should be inhibited (Smith et al., 1978). However, acetylene can oxidize NO to NO_2^- and NO_3^- (Bollmann and Conrad, 1997; Nadeem et al., 2013), potentially lowering N emissions from other processes.

2.3. NO and N_2O emissions

Immediately after wetting, six jars (2 samples × 3 treatments) were connected to a recirculating sample loop joining a multiplexer (LI-8150, LI-COR Biosciences), an infrared CO_2 gas analyzer (IRGA; LI-8100, LI-COR Biosciences, Lincoln, NE), and an N_2O laser analyzer (Model 914–0027, Los Gatos Research, Inc., Mountain View, CA). To measure N_2O emissions from each jar, air was recirculated through the closed sample loop at a rate of 1.5 L min^{-1}. Soil N_2O emissions were calculated as the linear change in N_2O concentrations over a 9-min period. Because the chemiluminescent NO_2 analyzer (LMA 3D; Unisearch Associates, Concord, ON, Canada) consumes NO, the NO_2 analyzer was not connected to the sample loop during this initial 9-min incubation. Rather, after 9-min, an automated 3-way solenoid valve (Parker Hannifin Corp., Series 11/25/26, #991-000539-006) activated so that the NO_2 analyzer pulled from the sample loop at a rate of 1.5 L min^{-1}. To replace the air that the NO_2 analyzer consumed from each jar (the air from the jars was vented out of the NO_2 analyzer into the lab), a second valve activated at the same time to allow zero air (Ultra Grade Zero Air, Airgas, Radnor, PA) to enter each jar at a rate of 1.5 L min^{-1}. The NO_2 analyzer used a CrO_3 converter to oxidize NO to NO_2; we did not detect NO_2 when CrO_3 was removed from the sample loop, suggesting our measurements were mostly NO. NO emissions were calculated using the following equation:

$$\text{NO flux} = \left([\text{NO}]_{\text{outlet}} - [\text{NO}]_{\text{inlet}}\right) \times \text{flow} \times \text{mass N} \div \text{soil wt.} \div \text{R} \div \text{temp} \quad \text{(EQ1)}$$

where $[NO]_{outlet}$ is the concentration of NO leaving the jar headspace (ppb), $[NO]_{inlet}$ is the concentration of NO entering the jar (assumed to be 0 ppb), flow is the flow rate of the sample loop (1.5 L min^{-1}), mass N is the molar mass of N in NO (14 g mol^{-1}), soil wt. is the mass of soil in the jar (g), R is the molar gas constant (0.0821 L atm K^{-1} mol^{-1}), and temp is the room air temperature (Hall et al., 2018). We measured NO concentrations for 10 min while zero air was flowing through the jar, allowing NO concentrations to reach equilibrium within the sample loop. NO fluxes were calculated using the average NO concentrations during the final 30 s of the 10-min incubation. After 10 min, the sample loop was exposed to ambient lab air for 1 min to purge zero air from the sample loop. After this 20-min incubation, the multiplexer connected the next jar to the sample loop, and this process was repeated, allowing us to measure each of the 6 jars once every ~2 h. The continuous stream of dry air decreased soil moisture throughout the 46-h incubation (Fig. S2).

We modified a publicly-available script to calculate N_2O and NO emissions (Andrews and Krichels, 2022). N_2O emissions were calculated as the change in N_2O concentrations over the last 7 min of the incubation when the NO analyzer was not connected to the sample loop. Emissions were considered 0 if the linear relationship between time and N_2O concentrations was not statistically significant ($p > 0.05$). NO emissions were calculated using EQ (1). Both the N_2O and the NO analyzer recorded trace gas concentrations every second. We used the trapezoidal integration (trapZ function in R; Borchers, 2022; R Core Team, 2023) to calculate cumulative N_2O and NO emissions over the 46-h period post-wetting (final units were ng N g dry $soil^{-1}$).

2.4. Net nitrification and net N mineralization rates

We measured soil extractable NO_3^- and NH_4^+ before wetting soils and

immediately after the 46-h incubation to calculate net nitrification and net N mineralization rates. Briefly, 3 g of soil (dry weight equivalent) were extracted in 30 mL 2M KCl for 1 h, filtered (Whatman 42 filter paper; 2.5 μm pore size), and the extracts were then frozen until analysis. Extracts were analyzed for NO_3^- (SEAL method EPA-126-A) and NH_4^+ (SEAL method EPA-129-A) using colorimetric assays in the Environmental Sciences Research Laboratory at UC Riverside (https://envisci.ucr.edu/research/environmental-sciences-research-laboratory-esrl). Net nitrification and net N mineralization rates were calculated as the difference in inorganic N (NO_3^- for nitrification and NO_3^- plus NH_4^+ for net N mineralization) before and after the incubation divided by the length of the incubation (~46 h). While nitrifiers may not mineralize N, nitrification can emit N trace gases or supply NO_3^- to denitrifiers, which can affect the calculation of net N mineralization rates. We also measured soil gravimetric water content by drying soil samples (~10 g) at 104 °C for 24 h. We estimated 100% soil water holding capacity (WHC) as the amount of water held by soils after saturating them with water and allowing them to drain in an air-tight container (to limit evaporation) for 8 h.

2.5. *amoA* gene quantification

A subsample (~5 g) of each soil sample ($n = 15$) was frozen (−20 °C) as soon as soils arrived in the lab. Within one month of freezing, DNA was extracted from 0.25 g of each subsample using a DNA extraction kit (Qiagen DNeasy PowerSoil Pro, Hilden, Germany) following the manufacturer's guidelines after an overnight incubation to enhance DNA extraction (700 μL CD1 + 100 μL ATL at 4 °C; Qiagen). Quantitative polymerase chain reaction (qPCR) was used to estimate the abundance of bacterial and archaeal *amoA* genes (Beman et al., 2008); the AmoA1F/amoA2R primer set was used for bacteria (Rotthauwe et al., 1997) and the Arch-amoAF/ArchamoAR primer set was used for archaea (Francis et al., 2005). Each qPCR was run in 10 μL reactions containing 5 μL master mix (Forget-Me-Not EvaGreen qPCR Master Mix, Biotium, Inc., Fremont, CA), 0.8 μL of 2 mM $MgCl_2$, 0.25 μL of 0.5 mg mL^{-1} bovine serum albumin, 0.125 μL of 0.25 μM forward and reverse primer, 2.5 μL H_2O, and 1.2 μL sample DNA. Bacterial *amoA* was amplified using the following protocol: 5 min at 95 °C, followed by 40 cycles of 45 s at 95 °C, 30 s at 56 °C and 60 s at 72 °C (CFX384 Touch Real-Time PCR Detection System, Bio-Rad, Hercules, CA). Archaeal *amoA* was amplified using the following protocol: 4 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 45 s at 53 °C and 60 s at 72 °C. The standard sequences were chosen from well-known archaeal (crenarchaeota genomic fragment 54d9) and bacterial (*amo*A gene of *Nitrosomonas europaea* ATCC, 19718) ammonia oxidizing microorganisms. Standard curves were prepared using serial dilutions for bacterial *amoA* (10^6 to 10^2 copies) and archaeal *amoA* (10^7 to 10^3 copies). The bacterial *amoA* standards had efficiencies of 83.5% ($R^2 = 0.998$) and archaeal *amoA* standards had efficiencies of 66.9% ($R^2 = 0.997$).

2.6. Atmospheric N deposition

We used the Community Multiscale Air Quality Modeling System (CMAQ) to estimate total N deposition rates at our three sites. We report data from measurement-model "fused" outputs that estimate total N deposition rates in 2019 (the most recent year from which modeled N deposition data were available) (EPA, 2021).

We also used passive atmospheric samplers to estimate the relative contribution of reduced N (ammonia; NH_3) and oxidized N ($NO + NO_2$; NO_x) to atmospheric N deposition at each site. Passive samplers (Ogawa pads; Ogawa USA, Pompano Beach, FL) chemically pretreated to absorb NO_x or NH_3 were installed ~1 m above the ground at each site (two samplers per site). The passive samplers were left in the field for approximately one month during the spring, winter, summer, and fall seasons (i.e., four one-month periods), followed by analysis for N content at the USDA Forest Service fire laboratory in Riverside, CA. Atmospheric ambient NO_x and NH_3 concentrations were then estimated according to manufacturer instructions (https://ogawausa.com/), from which we calculated the one-year average concentration for each species using the four seasonal measurements. While we did not estimate N deposition rates, atmospheric NO_x and NH_3 concentrations were used to compare which N species dominated N deposition at our three sites.

2.7. Statistical analyses

All statistics were conducted in R version 4.3.1 (R Core Team, 2023). We used two-way analysis of variance (ANOVA) to assess if cumulative NO emissions, cumulative N_2O emissions, or net N transformation rates differed between sites and in response to experimental treatments (i.e., selectively inhibiting AOB nitrification and total nitrification). Similarly, we used one-way ANOVA to assess if AOA abundance, AOB abundance, or initial soil N concentrations differed among sites. If the one-way ANOVA was significant ($p < 0.05$), we used Tukey-corrected multiple comparisons to assess which sites differed from one another. Model residuals were assessed for normality using the olsrr package in R (Hebbali, 2020) and log transformations were applied when residuals did not follow a normal distribution; this was only the case for *amoA* gene abundance from AOB.

3. Results

3.1. NO and N_2O emissions

Wetting dry soils without nitrification inhibitors increased NO emissions at our three sites, averaging 136 ± 21.5 ng N–NO g^{-1} (hereafter, ±standard error), but the emissions did not significantly differ among the sites (Fig. 1A; $F_{2,36} = 0.22$, $p = 0.80$). Adding nitrification inhibitors to soils produced a significant treatment effect ($F_{2,36} = 4.86$, $p = 0.014$), with this effect driven by the 26–72% reduction in NO emissions when both AOA and AOB nitrification were inhibited by acetylene (Fig. 1A). In contrast to using acetylene, treating soils with 1-octyne to exclusively inhibit AOB nitrification never altered NO emissions by more than 10% compared to control soils (Fig. 1A).

While soil N_2O emissions were of similar magnitude and variability to NO, they were higher in the High N-A deposition site (463 ± 188 ng $N–N_2O$ g^{-1} from control soils without inhibitors) than in either the Low (124 ± 70.6 ng $N–N_2O$ g^{-1}) or High N–B deposition sites (182 ± 90.5 ng $N–N_2O$ g^{-1}; $F_{2,36} = 10.5$, $p < 0.001$; Fig. 2). In contrast to NO emissions, using nitrification inhibitors did not affect N_2O emissions, either 1-octyne to inhibit AOB nitrification or acetylene to inhibit both AOA and AOB nitrification ($F_{2,36} = 0.15$, $p = 0.76$).

3.2. Soil inorganic N and net N transformation rates

Soils exposed to elevated N deposition rates had generally higher extractable NH_4^+ concentrations. NH_4^+ concentrations in dry soils prior to lab incubations were 1.5 × higher in the High N-A deposition site (3.32 ± 0.23 μg NH_4^+-N g^{-1}) than in the Low N deposition site (2.15 ± 0.26 μg NH_4^+-N g^{-1}; Fig. 3A), but the difference was only significant at $p = 0.07$. In contrast, NH_4^+ did not differ between the High N-A and High N–B deposition site (2.68 ± 0.47 μg NH_4^+-N g^{-1}; $p = 0.39$). Soil NO_3^- followed similar patterns to soil NH_4^+. Soil NO_3^- in dry soils differed among sites ($F_{2,12} = 5.7$, $p = 0.02$); NO_3^- was higher in the High N-A (1.52 ± 0.48 μg NO_3^--N g^{-1}) than in both the Low (0.20 ± 0.11 μg NO_3^--N g^{-1}; $p = 0.02$) and High N–B deposition sites (0.40 ± 0.15 μg NO_3^--N g^{-1}; $p = 0.05$; Fig. 3B), but NO_3^- did not differ between the Low and High N–B deposition sites ($p = 0.88$).

Consistent with having the highest soil NH_4^+ and NO_3^- concentrations, Net N mineralization rates were highest in the High N-A deposition site (0.60 ± 0.082 μ g N g^{-1} hr^{-1} in control soils without nitrification inhibitors; Fig. 4A) and responded differently to inhibitor treatments in each site (treatment by site interaction $F_{4,34} = 2.73$, $p =$

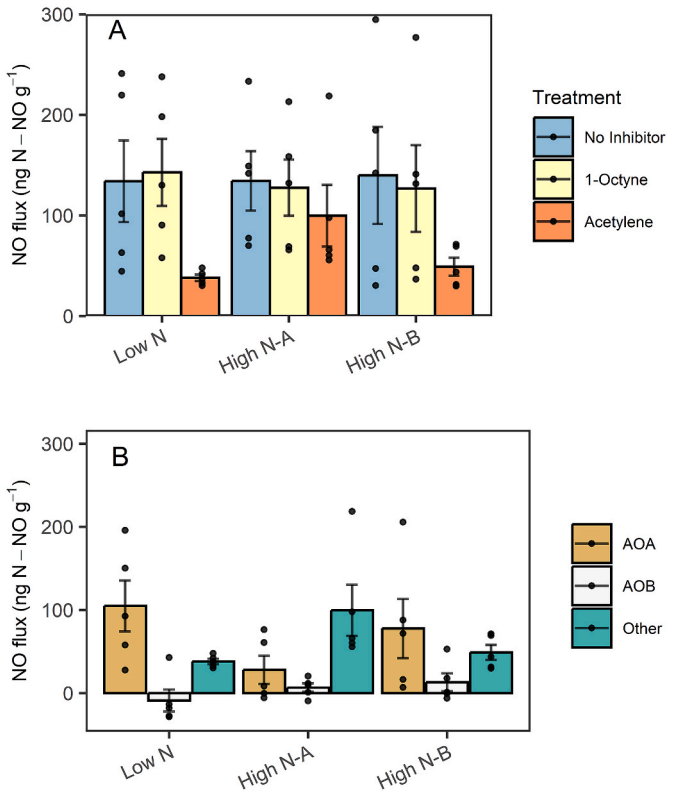

Fig. 1. Cumulative NO emissions (ng N–NO g^{-1} dry soil) over the course of incubating soils for ~48 h after wetting. The top panel (A) shows NO emissions after treating soils with only water, 1-octyne (AOB inhibitor), or acetylene (AOA and AOB inhibitor). The bottom panel (B) shows the contribution of AOA, AOB, and heterotrophs to NO emissions. AOB-derived NO emissions were determined by subtracting how much NO was emitted from the AOB inhibition treatment from how much NO was emitted from the control. AOA-derived NO emissions were determined by subtracting how much NO was emitted from the total nitrifier inhibition treatment from how much NO was emitted from the AOB inhibition treatment. Finally, heterotrophic NO emissions were assumed equal to the NO emitted under acetylene. Bars represent the mean, error bars represent one standard error of the mean, and dots represent individual observations (n = 5).

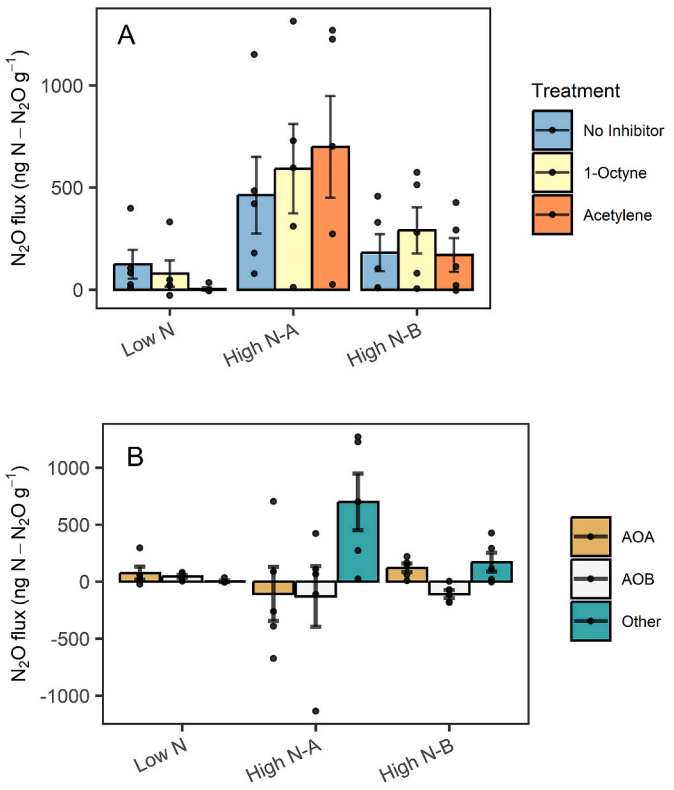

Fig. 2. Cumulative N_2O emissions (ng N–N_2O g^{-1} dry soil) over the course of incubating soils for ~48 h after wetting. The top panel (A) shows N_2O emissions after treating soils with only water, (AOB inhibitor), or acetylene (AOA and AOB inhibitor). The bottom panel (B) shows the contribution of AOA, AOB, and heterotrophs to NO emissions. AOB-derived N_2O emissions were determined by subtracting how much N_2O was emitted from the AOB inhibition treatment from how much N_2O was emitted from control soils. AOA-derived N_2O emissions were determined by subtracting how much N_2O was emitted from the total nitrifier inhibition treatment from how much N_2O was emitted from the AOB inhibition treatment. Finally, heterotrophic N_2O emissions were assumed equal to the N_2O emitted under acetylene. Bars represent the mean, error bars represent one standard error of the mean, and dots represent individual observations (n = 5).

0.045). Inhibiting AOB nitrification with 1-octyne decreased net N mineralization rates by 24% in soils from the High N-A deposition site (Fig. 4A). Net N mineralization rates were lower in the High N–B deposition site (0.12 ± 0.027 μ g N g^{-1} hr^{-1} in control soils) and decreased by 102% when AOA and AOB nitrification was inhibited with acetylene. Net N mineralization rates in soils from the Low N deposition site were close to zero in all treatments.

Net nitrification rates were highest in the Low N deposition site relative to other sites (0.081 ± 0.022 μ g N g^{-1} hr^{-1} in control soils; $F_{2,34} = 11.7$, $p < 0.001$; Fig. 4B) and did not consistently respond to the inhibition treatments at any of the sites ($F_{2,34} = 0.62$, p = 0.54). The lack of treatment effect likely stems from variable rates that were all close to zero in soils from the High N-A and High N–B deposition sites (Fig. 4B). In the Low N deposition site, inhibiting AOB nitrification with 1-octyne did not decrease nitrification rates, while inhibiting AOA and AOB nitrification with acetylene decreased nitrification rates by 66%.

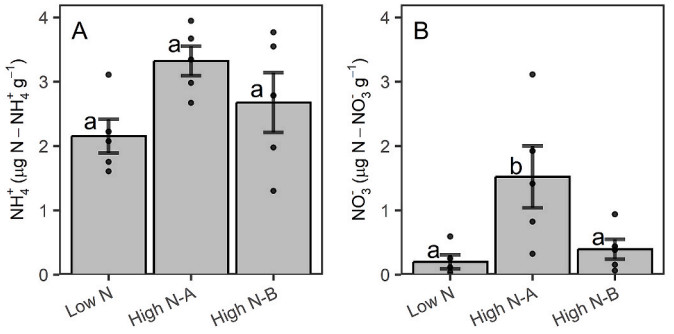

Fig. 3. (A) Soil extractable NH_4^+ and (B) NO_3^- concentrations in dry soils from our three sites. Bars represent the mean, error bars represent one standard error of the mean, and dots represent individual observations (n = 5). Lower case letters represent statistical significance (p < 0.05).

3.3. AOA and AOB amoA gene abundance

amoA gene abundance from AOA was higher in both the High N-A ($1.48 \times 10^6 \pm 3.20 \times 10^5$ gene copies g^{-1}) and Low N deposition sites ($1.27 \times 10^6 \pm 1.93 \times 10^5$ gene copies g^{-1}) than in the High N–B deposition site ($6.27 \times 10^5 \pm 2.35 \times 10^5$ gene copies g^{-1}; Fig. 5A), but this was only significant at p = 0.085 ($F_{2,12} = 3.05$). In contrast, *amoA* gene abundance from AOB did not differ among sites ($F_{2,12} = 1.27$, $p = 0.316$), averaging $2.3 \times 10^5 \pm 2.1 \times 10^4$ gene copies g^{-1} across all sites (Fig. 5B). The ratio of *amoA* genes in AOA relative to AOB was highest in the Low N deposition site (7.07 ± 1.25) and lowest in the High N–B deposition site (2.33 ± 0.57; $F_{2,12} = 6.00$, $p = 0.016$; Fig. 5C).

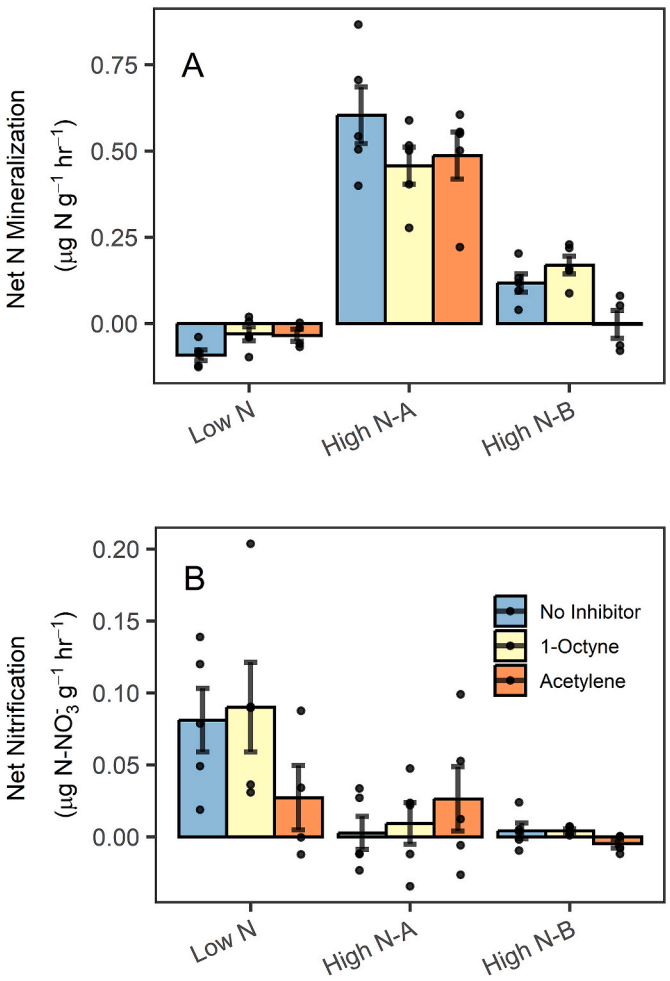

Fig. 4. Net rates of (A) N mineralization and (B) nitrification over the ~48-h incubations after wetting soils with water, 1-Octyne (AOB inhibitor), or Acetylene (AOA and AOB inhibitor). Bars represent the mean, error bars represent one standard error of the mean, and dots represent individual observations (n = 5).

4. Discussion

N saturation theory predicts that as N inputs exceed the capacity of ecosystems to assimilate N, N losses via gaseous pathways will increase in proportion to the N available in excess (Aber et al., 1989; Lovett and Goodale, 2011; Homyak et al., 2014). Here, we studied whether high rates of atmospheric N inputs can increase gaseous N losses by lowering the AOA:AOB ratio of nitrifiers, potentially altering nitrifier efficiency and, thereby, amplifying soil N trace gas emissions from drylands (Liu et al., 2017; Mushinski et al., 2019; Prosser et al., 2019). Our measurements suggest that while higher atmospheric N deposition rates are consistent with relatively low AOA:AOB ratios as hypothesized, this change in nitrifier community composition did not correspond with differences in NO and N_2O emissions among sites. Furthermore, NO emissions were primarily derived from AOA, even at the sites with the highest N inputs, in contrast to the hypothesis that AOB would dominate NO production with increasing atmospheric N inputs. While N deposition was not associated with higher nitrifier-derived NO emissions, the site exposed to the highest atmospheric concentrations of reduced N species (i.e., NH_3) had both the highest soil N concentrations and NO and N_2O emissions from non-nitrifying processes, consistent with predictions based on N saturation theory and with the assessment that this site may be N saturated (Michalski et al., 2004).

High rates of atmospheric N deposition were associated with low AOA:AOB ratios, consistent with the expectation that higher soil N availability would favor AOB over AOA (Thion and Prosser, 2014; Delgado-Baquerizo et al., 2016; Prosser et al., 2019; Mushinski et al., 2020). However, the relative abundance of AOA versus AOB nitrifiers did not explain differences in NO emissions among sites. In fact, AOB abundance and AOB-derived NO emissions were consistently low in all three sites (Fig. 1B; Fig. 5)—regardless of soil N availability (Fig. 3)—suggesting that chronic N enrichment does not always favor increased AOB-derived NO emissions in these drylands. Perhaps there were not enough AOB in these dry soils to produce appreciable amounts of NO, consistent with the fewer AOB we observed relative to temperate forests (Mushinski et al., 2019) and mesic agricultural systems (Hink et al., 2017), both of which found that AOB emitted more N than AOA. Thus, despite relatively high rates of atmospheric N deposition, relatively small AOB populations in our dryland sites may limit AOB-derived N trace gas emissions and their predictive power for estimating N losses via NO.

In contrast to AOB nitrification, AOA nitrification emitted between 21 and 78% of the total NO from all three sites (Fig. 1). Even though AOA were much more abundant than AOB in our sites (Fig. 5), it is still surprising AOA controlled NO emissions post-wetting, as they are not often associated with substantial NO production during nitrification (Mushinski et al., 2019). In these dryland sites, it is possible that AOA nitrification released nitrification intermediates (NO_2^- and/or NH_2OH) into the soil environment, producing NO via chemodenitrification or denitrification (Zhu-Barker et al., 2015; Heil et al., 2016). We also found that while NO emissions were similar from all three sites during lab incubations, NO production by AOA varied independent of atmospheric N deposition rates. AOA-derived NO emissions and AOA net nitrification rates were highest in the Low N deposition site, consistent with the hypothesis that AOA outcompete AOB in low-N environments. However, AOA-derived NO emissions were also elevated in the High N–B deposition site, where AOA abundance was low, suggesting that other factors

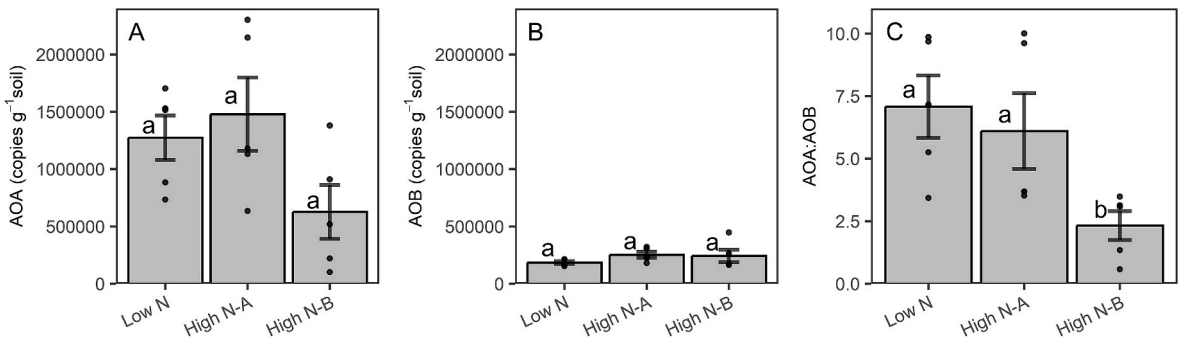

Fig. 5. Abundance of the *amoA* gene in (A) ammonia oxidizing archaea (AOA), (B) ammonia oxidizing bacteria (AOB), and (C) the ratio of *amoA* genes in AOA relative to AOB. Bars represent the mean, error bars represent one standard error of the mean, and dots represent individual observations (n = 5). Lower case letters represent statistical significance ($p < 0.05$).

in addition to N availability and AOA abundance determine how much NO is produced by AOA. For example, coarse-textured soils in the Low and High N–B deposition sites held less water throughout the lab incubation (Table 1; Fig. S2), suggesting that faster drying could have increased oxygen diffusion, allowing nitrification—an aerobic process—to emit more NO regardless of soil N availability. Conversely, finer soil texture in the High N-A deposition site drained water more slowly during the lab incubation (Fig. S2), potentially slowing oxygen diffusion (Lacroix et al., 2023) and, thereby, hindering nitrifier-derived NO emissions despite high AOA abundance. Such dynamics are consistent with studies that demonstrate soil edaphic properties, not only N availability and nitrifier communities, control N cycling in dryland ecosystems (Scholes et al., 1997; Osborne et al., 2022b; Ren et al., 2024).

Beyond the contribution of AOA and AOB to NO and N_2O emissions, other processes, such as heterotrophic nitrification, abiotic reactions, and/or denitrification, produced over 20% of the NO emitted from the Low and High N–B deposition sites, and roughly 74% of NO emitted from the High N-A deposition site. Of these processes, heterotrophic nitrification likely contributed the least given the NH_4^+-poor soils at our study sites that limit this process (Song et al., 2021). In the slightly more acidic soils found in the High N–B and High N-A deposition sites, it is possible lower pH may have promoted abiotic decomposition of nitrite to NO (Slessarev et al., 2021) or hydroxylamine to N_2O (Zhu-Barker et al., 2015). In addition to abiotic processes, the high NO_3^- concentrations in the High N-A site may have also stimulated denitrification, especially given the higher clay content in these soils that could have limited O_2 diffusion and favored anaerobic conditions at 100% WHC required for denitrification (Sexstone et al., 1985; Lacroix et al., 2023). In support of a dominant role by denitrification, the High N-A deposition site emitted the most N_2O, exceeding NO emission rates, and suggesting N_2O can potentially dominate gaseous N losses from drylands relative to NO (Eberwein et al., 2020; Krichels et al., 2022). We also note that the High N-A deposition site is exposed to more atmospheric NH_3 than NO_x, likely due to its proximity to agricultural land in the San Joaquin Valley (Li et al., 2016), and that we also measured the highest net N mineralization rates at this site (Fig. 4A). Because retention of NH_4^+ in clays is greater than for oxidized forms of N (Johnston and Tombácz, 2002), drylands exposed to NH_3 deposition may be more at risk of becoming saturated with N and favor N_2O emissions via denitrification and/or abiotic reactions when soils saturate with water during the wet season.

5. Conclusions

We found that the dryland soils exposed to high rates of atmospheric N deposition did not always emit more NO and N_2O to the atmosphere than soils from the low N deposition site, as N saturation theory would predict. While high rates of atmospheric N deposition were associated with lower soil AOA:AOB ratios, the relative abundance of AOA versus AOB did not predict N trace gas emissions, suggesting it is not a robust indicator of N saturation status. Rather, AOA-derived NO emissions were favored in coarse-textured soils, suggesting that edaphic variables must be considered when forecasting soil N dynamics as ecosystems saturate with N. Nevertheless, soil inorganic N was elevated in the site exposed to the highest concentrations of atmospheric NH_3 relative to NO_x (but not the site with the highest rates of total N deposition), corresponding to higher NO and N_2O emissions from denitrification and/or abiotic reactions. Interactions between soil texture, atmospheric deposition of NH_3 vs. NO_x, and soil N availability may, therefore, determine whether atmospheric N deposition is retained in dryland ecosystems or emitted as NO (an air pollutant at high concentrations) and N_2O (a strong greenhouse gas).

CRediT authorship contribution statement

Alexander H. Krichels: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Aral C. Greene:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Elizah Z. Stephens:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Sharon Zhao:** Writing – review & editing, Investigation, Data curation. **Joshua P. Schimel:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Emma L. Aronson:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Erin J. Hanan:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Peter M. Homyak:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Schimel serves on the editorial board for Soil Biology and Biochemistry but was not involved in handling this manusctipt. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data are publically available in the Dryad data repository: https://doi.org/10.5061/dryad.xpnvx0kp0.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2024.109482.

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