



# Large nitrogen oxide emission pulses from desert soils and associated microbiomes

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**Abstract** Nitrogen (N) trace gas emission pulses produced after wetting dry soils may be important pathways of ecosystem N loss. However, the rates and mechanisms controlling these emissions remain unclear. We tested whether changes in microbial community structure and increased rates of atmospheric N deposition could explain N emissions at two desert sites differing in atmospheric N deposition by ~ six fold. We measured peak NO<sub>x</sub> (sum of nitric oxide and nitrogen dioxide) emissions 12 h post-wetting. NO<sub>x</sub> emissions remained elevated over 24 h and increased after adding N. In contrast, we measured the highest nitrous oxide (N<sub>2</sub>O) emissions within only 15 min post-wetting. N<sub>2</sub>O emissions decreased within

12 h, were insensitive to adding N, and were among the highest reported globally. Microbial communities at the high N deposition site were less diverse with higher 16S nitrifier and bacterial *amoA* gene abundances relative to the low N deposition site, suggesting an increased capacity for nitrification. Nevertheless, N emissions were lower at the high N deposition site. While microbial communities changed after wetting, these changes were not correlated with N emissions. We conclude that desert soils can produce substantial NO<sub>x</sub> and N<sub>2</sub>O emission pulses, but that these emissions do not appear directly governed by changing microbial community characteristics or higher atmospheric N inputs. These findings highlight the importance of gaseous N loss pathways from dryland ecosystems that may contribute to sustained N limitation, with implications for atmospheric chemistry and Earth's climate.

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## Introduction

Soil emissions of nitrous oxide ( $\text{N}_2\text{O}$ ) and  $\text{NO}_x$  (the sum of nitric oxide  $[\text{NO}]$  and nitrogen dioxide  $[\text{NO}_2]$ ) are key components of ecosystem nitrogen (N) budgets; they are pathways for ecosystem N loss and contribute to N limitation (von Sperber et al. 2017). In drylands, theory and measurements suggest limited water and N availability constrain N emissions (Hartley and Schlesinger 2000; Meixner and Yang 2006; Hall et al. 2012; Oerter et al. 2018). However, wetting dry soils can increase metabolic activity (Jenerette and Chatterjee 2012) and produce large emission “pulses” of  $\text{NO}_x$  and  $\text{N}_2\text{O}$  over relatively short periods (i.e. hours to days; Hartley and Schlesinger 2000; Hall et al. 2008; Oikawa et al. 2015; Homyak et al. 2016; Soper et al. 2016). While short-lived, N emissions can be substantial and are hypothesized to represent  $\sim 77\%$  of the N inputs to a desert ecosystem (Peterjohn and Schlesinger 1990). Because some drylands experience among the highest rates of atmospheric N deposition in the world (up to  $\sim 71 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ; Fenn et al. 2008), soil N availability may be increasing with unknown consequences for N emissions. Determining how changes in N availability may control dryland N emissions is critical from a N mass balance perspective, but also because  $\text{NO}_x$  can adversely affect regional air quality (Crutzen 1979) and  $\text{N}_2\text{O}$  is a powerful greenhouse gas and primary driver of stratospheric ozone depletion (Conrad et al. 1996; Ravishankara et al. 2009).

Soil  $\text{NO}_x$  and  $\text{N}_2\text{O}$  emissions are primarily controlled by nitrification and denitrification, but it remains unclear what mechanisms control N emission pulses when dry soils wet up (Davidson et al. 2000). Ammonia ( $\text{NH}_3$ ) oxidation, the first and rate-limiting step of nitrification where  $\text{NH}_3$  is first oxidized to hydroxylamine ( $\text{NH}_2\text{OH}$ ) and then to nitrite ( $\text{NO}_2^-$ ), is primarily performed by a phylogenetically constrained group of obligate chemoautotrophic *Proteobacteria* (ammonia-oxidizing bacteria; AOB) and *Thaumarchaeota* (ammonia-oxidizing archaea; AOA) (Hayatsu et al. 2008). In contrast, denitrification is a phylogenetically broad facultative process carried out by a consortium of bacteria, archaea, and fungi (Schimel and Gullledge 1998) and generally correlates with microbial diversity metrics (Graham et al. 2016). This suggests that microbial community structure, including composition and diversity (Graham et al.

2016; Philippot et al. 2013; Cavigelli and Robertson 2000), as well as activity or dormancy (Blagodatskya and Kuzyakov 2013) can influence soil N emissions. Assessing changes in microbial community structure (composition and diversity) may help characterize N emission pulses and their potential response to increasing atmospheric N inputs (Hall et al. 2018).

One way to assess changes in microbial community structure is through DNA amplicon sequencing of universal genes such as the 16S rRNA gene for bacteria and archaea. This approach can capture broad-scale taxonomic changes in microbial community structure, including changes in richness and relative abundance of particular taxa of interest. Assessing functional gene abundance may also help characterize N emission pulses and their response to atmospheric N inputs. The *amoA* functional gene encodes the  $\alpha$ -subunit of the ammonia monooxygenase enzyme in AOA and AOB, and is used for molecular assessment of these chemoautotrophic taxa (Leininger et al. 2006). Atmospheric and experimental N inputs to soils generally increase *amoA* functional gene abundance, often favoring AOB over AOA (Carey et al. 2016). Since AOB produce significantly more  $\text{NO}$  (Mushinski et al. 2019) and  $\text{N}_2\text{O}$  (Liu et al. 2017) than AOA, soil N emissions may increase under chronic N inputs. However, long-term N enrichment in one desert ecosystem increased AOA over AOB with associated increases in  $\text{NH}_3$  oxidation (Marusenko et al. 2015). Soil microbial activity and composition also differ within hours to days after wetting dry soils, which may also affect N cycling (Placella et al. 2012; Št'oviček et al. 2017). How microbial community structure and functional gene abundance respond to both increasing N deposition and wetting may help explain the magnitude of N emission pulses.

Quantifying how chronic N inputs alter N emissions through changes in microbial community structure and function remains challenging, especially because increasing N availability may also influence N emissions through other mechanisms. For example, experimentally adding N often increases  $\text{N}_2\text{O}$  (Aronson and Allison 2016; Aronson et al. 2019; Gu et al. 2019) and  $\text{NO}$  emissions (Hartley and Schlesinger 2000; Vourlitis et al. 2015). However, adding N may also acidify soils (Vitousek et al. 1997) and increase plant biomass (Rao and Allen 2010), both of which can constrain the mechanisms producing N gases (Homyak et al. 2016; Li et al. 2018). To address some

of the uncertainties governing dryland N emissions following wetting and responses to atmospheric N deposition we ask: (1) What is the magnitude of  $\text{NO}_x$  and  $\text{N}_2\text{O}$  emission pulses after wetting dry desert soils? (2) Are the pulses related to changes in microbial community structure? And (3) do chronic atmospheric N inputs produce higher soil N emissions?

To answer these questions, we conducted field experiments in two desert sites 30 km apart but differing in atmospheric N deposition by  $\sim$  sixfold. At each site, we wetted dry soils with and without experimentally adding N, and measured  $\text{NO}_x$  and  $\text{N}_2\text{O}$  emission pulses, changes in microbial community composition and richness, and *amoA* gene copy numbers. We hypothesized that: (1) pulses of N trace gas emissions would be large; (2) chronic N deposition would reduce microbial species richness, increase the relative abundance of nitrifier communities, and favor AOB over AOA; and (3) emissions would be limited by N and increase with *amoA* gene copy numbers, nitrifier relative abundance, microbial diversity, and rates of atmospheric N deposition. Field tests of these hypotheses will help quantify N trace gas emission rates from desert soils, a key uncertainty in desert N budgets (Peterjohn and Schlesinger 1991), and inform how increasing rates of atmospheric N deposition in drylands may affect soil microbiomes and N emissions with implications for ecosystem function, air quality, and climate.

## Materials and methods

### Study sites

We selected two desert sites along a gradient of atmospheric N deposition generated by the Los Angeles, CA, USA metropolis. The Low N deposition site is located in Boyd Deep Canyon Desert Research Center (BDC), a part of the University of California Natural Reserve System (UCNRS) near Palm Desert, CA. The High N deposition site is located in Oasis de los Osos, another site in the UCNRS located at the north face of the San Jacinto Mountains. Both sites are located within the Colorado Desert, with hot summers and occasional precipitation occurring primarily during winter. Soils at the Low N site are classified as hyperthermic Typic Torriorthents; they are stony

sands mapped within the Carrizo series. Soils at the High N site are classified as thermic Typic Xeropsamments; they are gravelly loamy sands mapped within the Tujunga series. Creosote bush scrub (*Larrea tridentata*) is dominant at both sites with larger shrubs and interspaces between shrubs populated by more extensive annual herbaceous plants at the High N Deposition than at the Low N Deposition site.

Rates of atmospheric N deposition were higher at the High N ( $12.8 \text{ kg N ha}^{-1} \text{ y}^{-1}$  for throughfall and  $2.3 \text{ kg N ha}^{-1} \text{ y}^{-1}$  for bulk deposition; Supplementary Materials 1; Fenn and Poth 2004) than at the Low N deposition site ( $2.2 \text{ kg N ha}^{-1} \text{ y}^{-1}$  for throughfall and  $1.5 \text{ kg N ha}^{-1} \text{ y}^{-1}$  for bulk deposition; *t*-test  $p < 0.001$ ). At both sites, N deposition was higher under the canopy (throughfall) of *Larrea tridentata* than in the interspaces (bulk) between shrubs ( $p < 0.0001$ ) and was roughly composed of equal parts of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ;  $p > 0.05$ ). In contrast to N deposition between sites, total soil C and N was similar between the High and Low N deposition sites with no significant differences detected (Table 1;  $p > 0.05$ ). Soil inorganic N (sum of  $\text{NO}_2^-$  and  $\text{NO}_3^- + \text{NH}_4^+$ ) was not significantly different between the High ( $67.2 \mu\text{g g}^{-1}$  winter;  $34.5 \mu\text{g g}^{-1}$  summer) than at the Low ( $77.8 \mu\text{g g}^{-1}$  winter;  $12.1 \mu\text{g g}^{-1}$ ) N deposition site. These differences were observed in summer ( $p > 0.05$ , although sample size was limited at Low N deposition,  $n = 3$ ) when inorganic N was  $34.5 \mu\text{g g}^{-1}$  at the High and  $12.5 \mu\text{g g}^{-1}$  at the Low N deposition site. Nitrate was the dominant form of soil inorganic N at both sites during both winter and summer. Soil pH differed between the two sites ( $p = 0.004$ ) and was more acidic at the High (6.6 pH) than the Low N deposition site (8.0 pH).

### Experimental design

Field experiments were conducted in July 1–15, 2014, months before the onset of the wet season (Oct – Apr), and when daily peak air temperature exceeded  $40^\circ\text{C}$ . At both sites we established six replicate plots under the canopy of creosote bushes (*Larrea tridentata*) to capture N dynamics in soils known to be active “hot spots” of biogeochemical cycling. At each plot, four PVC collars (30 cm diameter by 15 cm height) were spaced  $\sim 20$  cm apart from each other and driven  $\sim$

**Table 1** Site differences (mean, standard deviation) for deposition, climate, and soil properties (0–10 cm depth)

|       | Variable                                                           | Low N deposition | High N deposition |
|-------|--------------------------------------------------------------------|------------------|-------------------|
| N Dep | Throughfall N Deposition ( $\text{kg N ha}^{-1} \text{ y}^{-1}$ )* | 2.2 (0.5)        | 12.7 (3.3)        |
| Clim  | Mean Ann. Max. T ( $^{\circ}\text{C}$ )                            | 41.1             | 40.6              |
|       | MAP (mm)                                                           | 145              | 139               |
| Soil  | Texture                                                            | Sand             | Loamy Sand        |
|       | Total C ( $\text{mg C g}^{-1}$ )                                   | 11.4 (5.8)       | 8.1 (3.4)         |
|       | Total N ( $\text{mg N g}^{-1}$ )                                   | 1.8 (0.5)        | 1.5 (0.3)         |
|       | Inorganic N ( $\mu\text{g g}^{-1}$ ) Winter                        | 77.8 (26.8)      | 67.2 (53.6)       |
|       | Inorganic N ( $\mu\text{g g}^{-1}$ ) Summer                        | 12.1 (9.8)       | 34.5 (28.1)       |
|       | pH*                                                                | 8.0 (0.3)        | 6.6 (0.3)         |

\*Denotes significant difference between Low and High N Deposition site ( $p < 0.05$ )

10 cm into the soil approximately two weeks before the start of our measurements. Two of the four collars were irrigated with deionized water to simulate a 2 cm rainfall event, a high intensity rain event but consistent with the range of event totals in the region. The remaining two collars were similarly irrigated but with an additional N enrichment (water + N;  $30 \text{ kg N ha}^{-1}$ , as  $\text{H}_4\text{NO}_3$ , equivalent to  $10.5 \text{ kg N ha}^{-1}$ ). One collar for each irrigation treatment was used exclusively for trace gas measurements, while the other collar was used to sample soils for microbial characterization ( $n = 6$  for both trace gases and microbial community analyses).

The experiments were initiated at approximately 7 am. Soil trace gas fluxes were measured before wetting (“Pre”), and then at 15 min, 12 h, and 24 h after adding water or water + N. Sampling periods were chosen based on studies showing rapid changes in both N trace gas emissions (Homyak et al. 2016) and microbial community structure (Placella et al. 2012). At each sampling time, soil temperature was recorded at 0–5 cm with a ProCheck Sensor equipped with GS3 probe (Decagon Device, Inc Pullman, WA) and soil moisture was measured from 0–10 cm with a handheld water content probe (HCS620 HydroSense, Campbell Scientific, Inc. Logan, CO) calibrated for our sites.

#### Microbial community assessments

At each trace gas sampling time, and also at 2 h and 48 h post-wetting, soils were cored (5 cm depth) from replicate collars adjacent to those used to measure N emissions after irrigating with water and water + N. The additional temporal soil samples were collected to

improve characterization of microbial community dynamics. In the field, soils were stored in sterile Whirlpak bags (Nasco Inc. Atkinson, WI, USA) and placed inside a cooler with dry ice and then transported to the laboratory where they were frozen at  $-20^{\circ}\text{C}$  until extraction. We extracted microbial DNA, which was sequenced for the 16S rRNA gene on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the UCR Genomics Core Facility (Supplementary Material 2; Klindworth et al. 2013). With the same soil extracts, we used Quantitative Polymerase Chain Reaction (qPCR) to quantify the amount of bacterial and archaeal *amoA* (Supplementary Material 2; Rotthauwe et al. 1997; Beman et al. 2008).

#### Trace gas fluxes

Soil collars were fitted with a custom-made PVC chamber lid with a mixing fan mounted on the inside and reflective tape covering the outside of the chamber (Davidson et al. 1990; Oikawa et al. 2015). A 30 cm diameter by 15 cm tall collar was used for N trace gas measurements. Air was pulled from the chamber top at approximately  $1 \text{ L min}^{-1}$  and routed sequentially to analyzers to measure concentrations of  $\text{N}_2\text{O}$  and NO.  $\text{N}_2\text{O}$  concentrations were measured with cavity-enhanced laser absorption spectroscopy with a tunable diode laser (Model 908–0014, Los Gatos Research, Inc. Mountain View, CA, USA). The outflow of the  $\text{N}_2\text{O}$  analyzer was routed to a NO monitor (Model 410 and Model 401, 2B Technologies, Boulder CO), where the quantitative depletion of ozone was measured using UV absorbance. This system also uses a molybdenum converter, which converts all nitrogen

dioxide ( $\text{NO}_2$ ) to nitric oxide ( $\text{NO}$ ), before sending sample air to the  $\text{NO}$  monitor and therefore measurements are expressed as  $\text{NO}_x$  flux ( $\text{NO} + \text{NO}_2$ ). Soil N fluxes were calculated using the rate of increase in  $\text{NO}_x$  or  $\text{N}_2\text{O}$  concentration, which was quantified by linear regression using  $\sim 3$  min of data to maximize linearity of gas production (Davidson 1990).

### Statistical analysis

Microbial communities were annotated bioinformatically using the Quantitative Insights Into Microbial Ecology (QIIME) platform against the Greengenes database (Supplementary Material 2; Price et al. 2009; Caporaso et al. 2010; Edgar 2010; McDonald et al. 2012; Bokulich et al. 2013). To assess alpha diversity of the microbial community, we calculated the number of observed operational taxonomic units (OTUs) for each sample in QIIME and performed a three-way ANOVA including main and interactive effects of site, treatment, and time since wetting. If interactive effects were non-significant, we removed them and re-ran the model. We assessed beta diversity using non-metric multidimensional scaling (NMDS) of the weighted UniFrac distance calculated in QIIME, which incorporates information about phylogenetic distance between pairs of samples using the phylogenetic tree (Lozupone and Knight 2005). Differences in overall microbial community composition across site, treatment, and time points were assessed with permutational multivariate ANOVA (perMANOVA) implemented using the Vegan function ‘adonis’ in R (999 permutations) (McArdle and Anderson 2001). These steps were conducted twice: once for the overall microbial community (all taxa) and once for nitrifying taxa only (Nitrosomonadales, Nitrospira, Thaumarchaeota). To further analyze the nitrifying microbial community, we assessed the overall relative abundance of nitrifying taxa within each sample using an ANOVA approach, same as above. We also used an ANOVA to determine how short and long-term N additions influenced the abundance of AOA and AOB; specifically, we compared *amoA* gene abundances between sites, treatments, and timepoint (pre-wetup and 48 h only). Combined, these approaches allowed us to determine whether nitrifying microbial communities were significantly affected by any of the factors included in our study.

We related variations in microbial community composition to N fluxes within and across sites by regressing fluxes with the first NMDS axis of the weighted UniFrac distances, nitrifier community abundance, and *amoA* gene abundances to  $\text{N}_2\text{O}$  and  $\text{NO}_x$  fluxes. The BoxCox family of transformations was used to fulfill assumptions of normality and homoscedacity where necessary. All statistical analysis was performed in R version 3.2.1 (R Development Core Team 2015).

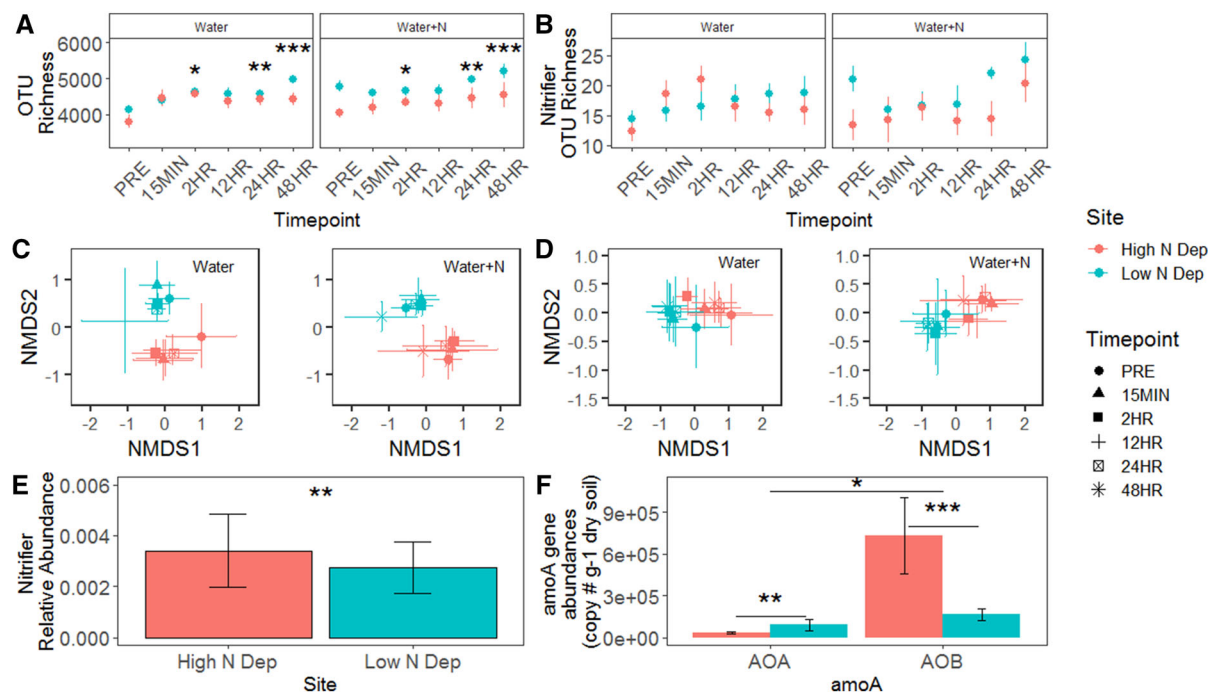
## Results

### Soil microbial dynamics

Microbial community diversity and composition differed between the High and Low N sites and in response to adding water and water + N. Overall richness of operational taxonomic units (OTUs) was higher at the Low than at the High N deposition site (site,  $p < 0.0001$ ; Fig. 1a). The site effect was more pronounced when comparing across the water + N treatment relative to adding only water (site  $\times$  treatment interaction,  $p = 0.04$ ; Fig. 1a). For the nitrifier community, OTU richness also differed by site, with the Low N deposition site harboring richer communities (site,  $p = 0.04$ ; Fig. 1b).

In addition to differences in richness, the microbial communities at both sites were distinct in composition. This was true when taking into account all taxa (site,  $p < 0.001$ ; Fig. 1c) or just the nitrifying taxa (site,  $p < 0.001$ ; Fig. 1d). Site also influenced how the microbial community responded to wetting soils with water or water + N (site  $\times$  treatment interaction,  $p = 0.004$ ), a pattern that was not observed for nitrifiers only (site  $\times$  treatment interaction,  $p > 0.05$ ). In contrast to nitrifier OTU richness, the relative abundance of nitrifiers was greater at the High than the Low N deposition site ( $p = 0.002$ ; Fig. 1e). The pattern was driven primarily by the higher relative abundance AOB over AOA, as the relative abundance of *Thaumarchaeota* was on average 149% lower at the High than at the Low N deposition site ( $p < 0.0001$ ). The absolute abundance of total *amoA* genes was also greater at the High than at the Low N deposition site ( $p = 0.03$ ). When separating AOA and AOB *amoA* gene abundances, AOA were greatest at the Low N





**Fig. 1** Microbial community responses to wetting with and without added N across High and Low N deposition sites: **a** overall and **b** nitrifier OTU richness (\* denote significant differences compared to pre-wetting samples); NMDS of weighted UniFrac distance for **c** overall and **d** nitrifier

community composition; **e** relative abundance of nitrifiers and **f** AOA and AOB amoA gene abundances averaged across time. For **a**, **b**, **e**, and **f** error bars =  $\pm 1$  SE of the mean. For **c** and **d**, error bars = 95% confidence intervals. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

deposition site ( $p = 0.01$ ), whereas AOB were greatest at the High N deposition site ( $p < 0.001$ ; Fig. 1f).

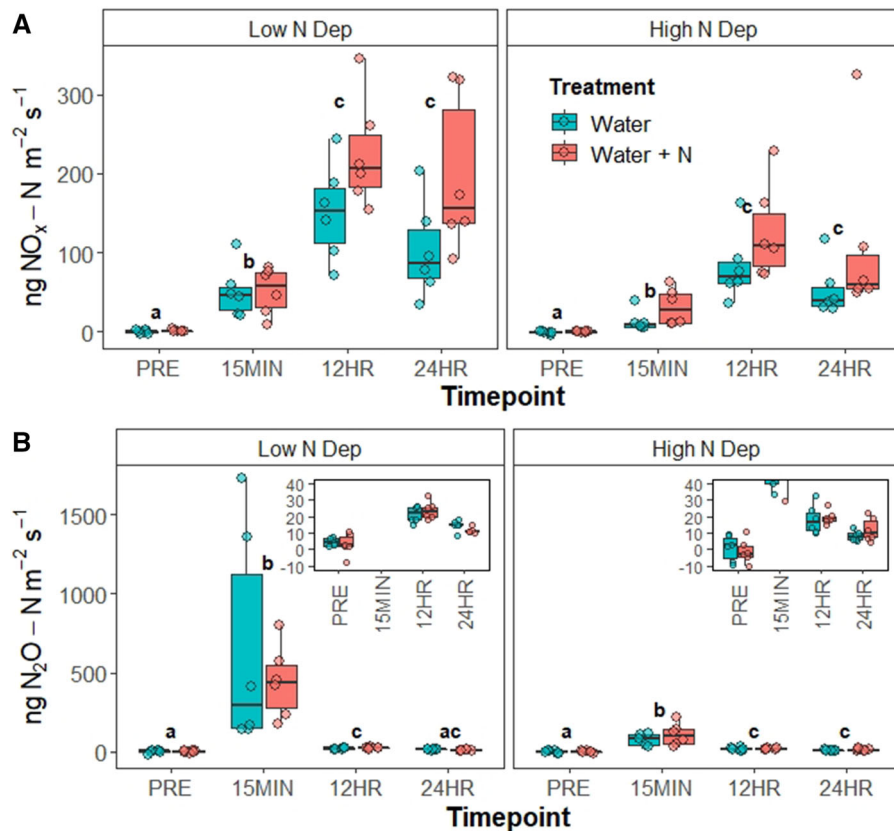
The effects of wetting soils on overall OTU richness varied according to site, with richness at the Low N deposition site (but not the High N deposition site) increasing more strongly after adding water + N than after adding water alone (site  $\times$  treatment interaction,  $p = 0.04$ ). Moreover, while overall OTU richness increased within two hours of wetting (for both water and water + N), and generally remained elevated compared to pre-wetting levels over time (time,  $p < 0.001$ ; Fig. 1a), this was not the case for nitrifier OTU richness (treatment,  $p > 0.05$ ; time,  $p > 0.05$ ; Fig. 1b) or the relative abundance of nitrifiers within the community (treatment,  $p > 0.05$ , time,  $p > 0.05$ ).

### 3.2. Soil N emissions.

$\text{NO}_x$  and  $\text{N}_2\text{O}$  emissions were low ( $0.8 \text{ ng NO}_x\text{-N m}^{-2} \text{ s}^{-1}$  and  $1.0 \text{ ng N}_2\text{O-N m}^{-2} \text{ s}^{-1}$ ) in dry soils and did not differ between the Low and High N deposition sites (Fig. 2;  $p > 0.05$ ). However, adding water produced both  $\text{NO}_x$  and  $\text{N}_2\text{O}$  emission pulses that differed in the amount of N emitted and the timing and duration

of peak emissions (Fig. 2).  $\text{NO}_x$  emission pulses were higher at the Low than at the High N deposition site ( $p < 0.0001$ ); they rose as high as  $345 \text{ ng NO}_x\text{-N m}^{-2} \text{ s}^{-1}$  within 12 h of wetting and remained elevated for at least 24 h at both sites. Experimentally adding water + N increased  $\text{NO}_x$  emissions at both the High ( $p = 0.003$ ) and Low ( $p = 0.03$ ) N deposition sites relative to adding water alone. In contrast to  $\text{NO}_x$  emissions,  $\text{N}_2\text{O}$  emission pulses were short-lived (Fig. 2). Peak  $\text{N}_2\text{O}$  emission rates were measured at the 15-min sampling timepoint post-wetting (up to  $1725 \text{ ng N}_2\text{O-N m}^{-2} \text{ s}^{-1}$ ) and declined within 12 h, although the fluxes after 12 h remained elevated relative to dry soils ( $p < 0.0001$ ). Consistent with  $\text{NO}_x$  emissions,  $\text{N}_2\text{O}$  emission pulses were lower at the High than at the Low N deposition site ( $p < 0.0001$ ). Unlike  $\text{NO}_x$  emissions, adding water + N did not change  $\text{N}_2\text{O}$  emissions relative to adding water alone at either the High ( $p = 0.49$ ) or Low ( $p = 0.2$ ) N deposition sites.

We did not detect correlations between the NMDS1 axis and  $\text{NO}_x$  or  $\text{N}_2\text{O}$  emissions within or across sites



**Fig. 2** Soil **a** NO<sub>x</sub> and **b** N<sub>2</sub>O emissions before (“Pre”) and after adding water and water + N at the Low and High N deposition sites. Inset shows the N emissions outside the 15-min peak. Box plots represent the median and interquartile range of

our measurements (circles). Letters represent significant differences between time points within a site for both treatments ( $p < 0.05$ )

nor did we detect correlations between *amoA* gene abundances and NO<sub>x</sub> or N<sub>2</sub>O emissions ( $p > 0.05$ ).

## Discussion

We designed our study to assess the magnitude of desert NO<sub>x</sub> and N<sub>2</sub>O emission pulses, whether the pulses were related to changes in microbial community diversity and richness, and whether N emissions were stimulated by N deposition. Wetting dry desert soils produced large emission pulses of NO<sub>x</sub> and N<sub>2</sub>O. These emission patterns could not be explained by the measured microbial metrics, despite strong differences in microbial community structure between sites and in response to wetting. Contrary to our hypothesis that increased N deposition would alleviate N limitation of emissions, we found that both NO<sub>x</sub> and N<sub>2</sub>O

emissions were lower at the High than at the Low N deposition site.

## Structural response of the soil microbial community to N deposition

We found support for our hypothesis that chronic N deposition would reduce microbial species richness, favor nitrifier communities, and increase the presence of AOB relative to AOA. In particular, the High N deposition site had lower OTU richness, consistent with a recent meta-analysis demonstrating that high N inputs reduce bacterial richness (Wang et al. 2018). We also observed that communities at the High N site had higher relative abundances of nitrifiers and greater *amoA* gene abundances with an increasing AOB/AOA ratio. Similar patterns have been reported elsewhere (Carey et al. 2016; Ying et al. 2017), and they suggest

an increased capacity for nitrification (Prosser and Nicol 2012) and potential to increase N emissions (Mushinski et al. 2019) with increasing N deposition. However, these differences could be either a direct response to N deposition or an indirect effect of other site differences, such as increased soil acidity that can result from N deposition (Fierer and Jackson 2006; Zeng et al. 2016; Li et al. 2018). While AOA would conceivably be favored at low pH (Zhang et al. 2012), changes in pH can have large and varied effects on microbial communities, including reducing microbial diversity (Zeng et al. 2016). The site-level differences in our study echo previous findings that suggest desert microbial communities are sensitive to N deposition.

Microbial structure also changed after experimentally adding water or water + N. Overall OTU richness increased by an average of 14% after 48 h following wetting. Measuring an increase in OTU richness after adding N is opposite to what we observed under long-term N deposition, and contrary to our hypothesis that N inputs reduce richness (Wang et al. 2018; Št'oviček et al. 2017). Possibly, a one-time 2-cm wetting event with and without 30 kg N ha<sup>-1</sup> is not a large enough disturbance to cause negative effects, instead releasing microbes from water and N limitation while increasing community richness. Alternatively, changes in richness may not be linear and 48 h may not be enough time to observe effects that emerge over longer time scales (Shade et al. 2012). Treatment-induced increases in richness were accompanied by broadscale shifts in microbial community composition (as shown by ordination and perMANOVA) at both sites, and an increase in AOA *amoA* gene abundances at the High N deposition site. However, in general, changes in community composition with time were not predictable and did not show a clear trend, implying that compositional turnover was not systematically following a trajectory after adding water and N.

#### Desert N emission responses to experimental wetting and N addition

The timing of peak emissions and response to experimentally adding N suggest different mechanisms governed NO<sub>x</sub> and N<sub>2</sub>O emission pulses. Two observations suggest microbial processes controlled NO<sub>x</sub> emissions following wetting: (1) the timing of peak NO<sub>x</sub> emissions occurred 12 h post-wetting, a

period consistent with the time required by microbes to recover from dormancy (Blagodatskaya and Kuzyakov 2013) and nitrify (Homyak et al. 2016), and (2) adding NH<sub>4</sub>NO<sub>3</sub> stimulated this response. In contrast to NO<sub>x</sub>, the nearly instantaneous N<sub>2</sub>O emission pulse, and insensitivity to adding NH<sub>4</sub>NO<sub>3</sub>, suggests processes other than nitrification and/or denitrification, perhaps abiotic, controlled these emissions. Rapid displacement of pore gas space has been suggested for some trace gas emission pulses, but in the sandy soils at our sites, the accumulation of enough N<sub>2</sub>O within the pore space to account for emissions is unlikely. Alternatively, chemodenitrification, the abiotic and non-enzymatic production of N trace gases from reactive N intermediates (Heil et al. 2016), can be an important pathway in drylands (Homyak et al. 2017; Wang et al. 2017) and may be responsible for the high rates of N<sub>2</sub>O produced within 15 min of wetting and their insensitivity to adding NH<sub>4</sub>NO<sub>3</sub>. While different mechanisms may have governed the production of NO<sub>x</sub> and N<sub>2</sub>O, both can be tied to microbial processes that either generate the N emissions directly or through the production of reactive N intermediates.

Although we measured differences in microbial communities between the Low and High N deposition sites and variable changes in microbial community structure within hours post-wetting, we found no correlations relating these community dynamics with NO<sub>x</sub> and N<sub>2</sub>O emissions. The lack of a relationship between microbial composition and NO<sub>x</sub> emissions does not mean the process is not biotically controlled; instead, it may reflect a temporal disconnect between changes in microbial community activity, process rates, and associated community composition, with the latter taking more time to accrue discernable shifts (Bier et al. 2015). In the case of N<sub>2</sub>O, this may be because emissions depend on the accumulation of reactive N intermediates (e.g. NO<sub>2</sub><sup>-</sup> and NH<sub>2</sub>OH), and their abiotic decomposition. Relationships between the dynamics of microbial community structure and N trace gas emissions may also be diluted because of relic and dormant DNA captured by the sequencing technology (Lennon and Jones 2011; Lennon et al. 2018). Evaluating combinations of mRNA expression activities and additional N cycling genes (Graham et al. 2016) may provide a more robust approach for linking microbial processes with N emissions.



## N deposition and N emissions: higher emissions at the low N deposition site

We found that N emissions were lower at the High N deposition site. While unexpected because of the general N limitation of dryland ecosystem processes and N emissions in particular, the lack of soil N emission stimulation by increased N inputs is consistent with previous studies showing encroachment by N-fixing trees did not increase N trace gas emissions (Soper et al. 2016). Possibly, differences in plant N uptake, soil pH, and/or microbial N use between sites may constrain N availability and help explain why higher rates of atmospheric N deposition did not translate into higher soil N emissions. The larger shrub cover and understory of herbaceous plants at the High N site, consistent with desert responses to elevated N deposition (Rao and Allen 2010), may have increased plant N uptake relative to the Low N site, lowering soil N availability and N emissions (Homyak et al. 2016). Alternatively, the lower pH at the High N deposition site, also consistent with soil responses to N deposition (Fenn et al. 1998), may reduce the availability of  $\text{NH}_3$  and constrain nitrification (Li et al. 2018). Finally, the greater relative abundance of nitrifiers measured at the High N deposition site may imply reactive N intermediates (e.g.  $\text{NO}_2^-$  and  $\text{NH}_2\text{OH}$ ) may be more efficiently transferred between nitrifiers, thereby minimizing their extracellular accumulation in drying soils and their potential to chemodenitrify (Homyak et al. 2017). While N deposition did not stimulate greater N emission pulses, indirect effects of N deposition along with site differences in plant biomass, soil acidity, and efficient transfer of reactive intermediates between nitrifiers may help explain the patterns we observed. A better characterization of N deposition effects on N emissions remains an important research need.

## Implications for desert N cycling

Our field measurements are among the largest instantaneous N emission rates observed from soils. Relative to other field studies (Supplementary Material 3), peak emissions of  $\text{N}_2\text{O}$  at our desert sites were greater than in grazing lands (Smith et al. 1994; Teh et al. 2011; Cowan et al. 2015), tropical rain forests (Hall and Matson 2003; Holtgrieve et al. 2006), tropical dry forests (Davidson et al. 1993); grasslands (Leitner

et al. 2017; Niboyet et al. 2011), and urbanized ecosystems (Hall et al. 2008; Townsend-Small and Czimczik 2010). The only higher peak emissions of  $\text{N}_2\text{O}$  we identified, were observed from ant refuse mounds in a tropical forest (Soper et al. 2019). Similarly, our peak  $\text{NO}_x$  emissions exceed those measured in tropical wet forests (Hall and Matson 2003; Holtgrieve et al. 2006), tropical dry forests (Davidson et al. 1991), urban soils (Hall et al. 2008), and grasslands (Leitner et al. 2017), but were exceeded by those measured in the Chihuahuan desert (Hartley and Schlesinger 2000), a chaparral ecosystem (Homyak and Sickman 2014) and especially by those from a high-temperature agroecosystem (Oikawa et al. 2015). These comparisons suggest peak N emissions after wetting dry soils can be substantial in deserts, a finding consistent with large denitrification potentials (Peterjohn and Schlesinger 1991) and NO emissions measured in other drylands (Hartley and Schlesinger 2000; Homyak and Sickman 2014). While it is clear these emissions can be substantial, their implications for ecosystem N budgets remain uncertain.

To assess the potential contribution of NO and  $\text{N}_2\text{O}$  emission pulses to desert N budgets, we used trapezoidal integration to calculate the average mass of N emitted over a 24 h period across our two sites (we excluded the water + N treatment from our calculations). Using this approach, we estimate 7.3 mg  $\text{NO}_x\text{-N m}^{-2}$  and 8.0 mg  $\text{N}_2\text{O-N m}^{-2}$  are emitted over 24 h after wetting dry soils. We then multiplied the mass of N emitted by the average number of precipitation events exceeding 5 mm in a year at our Low N deposition site (6.7 days using a 10-year record; Boyd Deep Canyon Research long-term weather data) yielding 0.049 g  $\text{NO}_x\text{-N m}^{-2} \text{ yr}^{-1}$  and 0.059 g  $\text{N}_2\text{O-N m}^{-2} \text{ yr}^{-1}$ . Relative to throughfall N deposition measured at our sites, these potential N emissions represent 49% of atmospheric N inputs at the Low N Deposition site and 9% of atmospheric N inputs at the High N deposition site. Relaxing these criteria by expanding the N emitted from soils to 48 h (and assuming emissions ceased at this time) and using a precipitation threshold of 2 mm, we estimate N emissions could remove 102% of atmospheric N inputs at the High N deposition site and 18% of atmospheric N inputs at the Low N deposition site. Improving this estimate will require better assessment of emissions across seasons, precipitation timing effects, temperature, and N deposition. Nevertheless,

this initial scaling suggests soil N emissions are large components of the desert N cycle consistent with predictions of desert N budgets (Peterjohn and Schlesinger 1990) and highlights the potential for N emissions to help explain widespread N limitation across drylands.

## Conclusions

We show that desert soils can produce large NO<sub>x</sub> and N<sub>2</sub>O emission pulses, and that while microbial communities respond as predicted to elevated N deposition, higher N inputs do not necessarily produce higher soil N emissions. This suggests soil N emissions are predominantly controlled by site-specific factors and not by direct stimulation of long-term N deposition. The site-specific factors (e.g. increased herbaceous plants and lower soil pH) may nevertheless still reflect ecosystem responses to elevated N deposition (Zeng et al. 2016) and complicate predictions of dryland N emissions. How biotic and abiotic processes interact to control N emission pulses is still not well understood and will require new techniques that evaluate the rates and magnitudes that microbes recover from drought stress and process N, as well as better characterizing emission pathways. Regardless of the underlying mechanism, large N emission pulses from dryland ecosystems may contribute to sustained N limitation with potential implications for atmospheric chemistry and Earth's climate.

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