Long-term fertilization increases soil but not plant or microbial N in a Chihuahuan Desert Grassland

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Abstract. Although the negative consequences of increased nitrogen (N) supply on plant communities and soil chemistry are well known, most studies have focused on mesic grasslands, and the fate of added N in arid and semi-arid ecosystems remains

10 unclear. To study the impacts of long-term increased N deposition on ecosystem N-pools, we sampled a 26-year-long fertilization (10 g N m^{-2} yr^{-1}) experiment in the northern Chihuahuan Desert at the Sevilleta National Wildlife Refuge (SNWR) in New Mexico. To determine the fate of the added N, we measured multiple soil, microbial, and plant N pools in shallow soils at three time points across the 2020 growing season. We found small but significant increases with fertilization in soil available (NO$_3^-$-N and NH$_4^+$-N), yet the soil microbial and plant communities do not appear to be taking advantage of the increased N-

15 availability, with no changes in biomass or N-content in either community. However, there were increases in total soil N with fertilization, suggesting increases in microbial or plant N earlier in the experiment. Ultimately, the majority of the N added in this multi-decadal experiment was not found in the shallow soil, nor the microbial or plant community, and is likely to have been lost from the ecosystem entirely.

1 Introduction

20 Agriculture and other anthropogenic activities have disrupted the nitrogen (N) cycle and increased N deposition in ecosystems worldwide (Vitousek et al., 1997). Reactive N released into the atmosphere has doubled over the last 50 years (Galloway et al., 2008). More than half of which has been attributed to crop production (Liu et al., 2022) and the southwestern United States has become a region of particular concern (Reed et al. 2013). Higher levels of atmospheric N deposition often increase plant biomass and alter soil chemistry in grasslands across the globe (Fay et al., 2015; Fenn et al., 2003), but the effects of chronic

25 N addition in arid ecosystems remain unclear. Although aridlands are known to be primarily water-limited ecosystems (Noy-Meir et al., 1973; Collins et al., 2008), multiple studies hint that a sequential limitation by water then N may exist (e.g., Hall et al., 2011; Ladwig et al., 2012; Rao and Allen 2010; Rao et al., 2011) and thus, increased N deposition in these ecosystems may result in shifts in ecosystem nutrient availability and cycling, as well as changes in plant and microbial biomass (Chalcraft et al., 2008; Stevens et al., 2010; Wei et al., 2013). Drylands represent Earth's largest biome (Schimel, 2010), support nearly

30 40% of the world's population (WRI, 2005), and are dominant drivers of interannual variability in the global terrestrial carbon

(C) sink (Poulter et al., 2014; Ahlström et al., 2015). Therefore, understanding alterations to ecosystem processes caused by increased levels of N deposition is critical.

There are a number of different ultimate sinks for N added to ecosystems—changing soil nutrient pools, uptake by organisms,
35   or potentially being lost from the system through leaching or volatilization (Peterjohn and Schlesinger, 1990; McCalley and Sparks, 2009; Brown et al., 2022). For example, nutrient cycling models indicate that N inputs of 20 to 35 kg per ha per year in semi-arid ecosystems lead to higher concentrations of NO_3^- leaching beyond the primary rooting zone, a decrease in NH_4^+ pools in soil, and increasing soil acidity (Fenn et al., 1996, 2003). The decrease in NH_4^+ pools may result from increases in net N mineralization and nitrification caused by N deposition, with the N cycle being NO_3^- dominated (Risch et al., 2019). Further,
40   N may be lost as nitric oxide emissions, which have been found in semi-arid or arid ecosystems to be primarily reemissions from atmospherically deposited N as a result of excess N deposition, especially following rewetting (Homyak et al., 2016).

Instead of being stored in soils or lost from the system, additional N in arid ecosystems may be taken up by organisms including soil microbes for their metabolic functions, particularly during periods of higher water availability. Drylands are known for
45   pulse-driven nutrient cycling, where biological activity and ecosystem processes are predominantly driven by precipitation and the lags between water pulses (Ogle and Reynolds, 2004; Schwinning and Sala, 2004; Collins et al., 2014). Studies, however, show a high variability in microbial responses to increased N deposition. On the one hand, small increases in N inputs can alleviate the limitation in the microbial pool, increasing soil organic matter (SOM) decomposition (Allison et al., 2009). In contrast, chronic N-enrichment leads to altered microbial community composition (Chen et al., 2015; Widdig et al.,
50   2020) and a reduction of microbial biomass and slower SOM decomposition (Frey et al., 2004; Forstner et al., 2019). These factors pose potential consequences for ecosystem nutrient cycling and climate regulation. Unfortunately, predictive models of ecosystem responses to N enrichment for other geographic regions are often not applicable to the southwestern US (Fenn et al., 2003).

55   The plant community, in competition with soil microbes, may also be taking up added N. Although secondary to water limitations, desert plants often exhibit N-deficiency (Peterjohn and Schlesinger 1990; Hooper and Johnson 1999; Elser et al., 2007; Le-Bauer and Treseder, 2008; Hall et al., 2011). Added N can stimulate plant growth (Baez et al., 2007) and increase biomass (Zeng et al., 2010), but can also cause significant losses in plant biodiversity (e.g., Sala et al., 2000; Fay et al., 2015; Midolo et al., 2018), with nitrophilic (Bobbink et al., 1988) and invasive (Brooks, 2003) plants often outcompeting native
60   species (Tilman, 1987). Additionally, accumulation of N in the soil can increase soil acidification which, in turn, can result in the loss of cations essential to plants (e.g., calcium) (Johnson et al., 1994) and increase the availability of toxic soil metals, such as aluminum (Lindsay and Walthall, 1995), further affecting productivity. These responses, however, seem to be highly variable within dryland ecosystems. While some studies report significant increases in net primary production (Luo et al., 2017), other studies suggest that plant species richness, foliar N concentrations and percent cover exhibited limited to no

65   response to increased N (McHugh et al., 2017). These inconsistencies highlight the need for further research to understand how chronic addition of high levels of N affect the nutrient dynamics of desert ecosystems.

To study the impacts of long-term increases in N availability on the ecosystem N budget, we sampled soils from a long-term fertilization experiment established in 1995 at the Sevilleta National Wildlife Refuge (SNWR), central New Mexico, USA.

70   We examined soil, microbial and plant N pools to determine the allocation of additional N in response to 26 years of N fertilization in a native northern Chihuahuan Desert plant community across the growing season. We hypothesized that 1) fertilized plots would exhibit higher N content in all pools relative to controls, and 2) that seasonal variation would play a bigger role in measured N than treatment.

2 Methods

2.1 Study Site

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The experiment was conducted at the Sevilleta National Wildlife Refuge (SNWR) located approximately 90 km south of Albuquerque, New Mexico, USA. Yearly temperatures range from 1.6° C in January to 33.4° C in June (Collins et al. 2008). This region receives an average of 234 mm annual precipitation, with the majority falling as large monsoon events occurring from July to September (Gosz et al. 1995; Pennington and Collins 2007). The SNWR is composed of several ecoregions,

80   including the northernmost extent of Chihuahuan Desert grassland that intergrades into shortgrass steppe to the north and east of the refuge (Ladwig et al. 2012). This research was conducted in a transition zone between these two ecoregions, containing a mixture of dominant grasses, such as black grama grass (Bouteloua eriopoda), blue grama grass (Bouteloua gracilis), and other perennial C4 grasses. The soil at the research site is a Typic Calciorthid sandy loam (Johnson et al., 2003).

2.2 Experimental Design

85   Twenty 10m x 5m plots (10 control and 10 treatment) were established in 1995 to examine the impacts of N enrichment on above- and belowground processes (Johnson et al. 2003). Every year since their establishment, the ten treatment plots have received 10g N m^{-2} as NH_4NO_3 each June prior to monsoon season to reflect the potential rates of atmospheric deposition near urban areas in the southwestern US; the remaining ten have served as ambient controls (Fenn et al. 2003b; Ladwig et al. 2012). In June of 2003, a management fire was allowed to burn through all the plots.

2.3 Sampling Protocol

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Soils were collected on three sampling dates: late June (pre-monsoon), early July (mid-monsoon) and early October 2020 (post-monsoon) after 26 years of N addition treatments. At each plot, five soil cores were collected near the base of individual clumps of grass and/or other dominant plants when grasses were not present from 0-10 cm in depth using a 2.5 cm wide soil auger. Soil samples were homogenized, passed through a 2mm sieve, and divided into subsamples for respective analyses.

95 Additionally, following fertilizer application in late June, three soil samples were collected as described above, this time using a 15 cm diameter bucket auger at a depth of 20 cm to quantify total root standing crop biomass. In early October, leaf tissue samples from Mesa dropseed (*Sporobolus flexuosus*), the only grass common in all treatment and control plots, were harvested from each plot. Leaf tissue samples were dried, ground, and analyzed for %C and %N as described below.

2.4 Soil pH, salinity, and organic matter content

100 Soil pH and salinity were measured by combining a 15g subsample of soil with 30mL of deionized water, stirring, and leaving to rest for 30 minutes before measuring pH and salinity using a monitoring sonde. Soil salinity was recorded as specific conductance (SPC) value. SOM was measured using the loss of ignition method (Rather, 1918). A subsample of 5g of soil was dried at 60° C for 48 hours. Once dry, the sample was combusted in a muffle furnace at 375-450° C for 16 hours. % SOM was calculated as the mass lost by ignition divided by the pre-ignition soil mass following Brown et al. (2022).

105 2.5 Soil N pools

One 5g subsample from each plot was oven dried at 65° C for 48 hours to calculate its soil/water ratio. For %C and %N, a subsample was dried, ground, rid of carbonates using acid fumigation (Harris et al., 2001) and combusted to determine %C and %N using a dry combustion C and N analyzer (ElementarPyroCube, Ronkonkoma, NY, USA). A 5g soil subsample per plot was extracted with 0.5M K_2SO_4 for 2 hours and vacuum-filtered through glass filter paper. Extracts were frozen at -20° C

110 until analyzed for nitrate (NO_3^--N) and ammonium (NH_4^+-N) content, extractable organic C, and extractable total N (EOC and ETN, respectively). Both NO_3^--N and NH_4^+-N were analyzed using colorimetric microplate assays (BioTEK Synergy HT microplate reader; BioTek Instruments Inc., Winooski, VT, USA). NO_3^--N was analyzed using a modified Griess reaction (Doane and Horwath 2003) and NH_4^+-N using the Berlethot reaction protocol (Rhine et al. 1998). EOC and ETN concentrations were determined in a minimum 10x dilution of the original extract using a Shimadzu EOC/TN analyzer with autosampler

115 (TOC-L CPH/CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Extractable organic N (EON) was calculated as the difference between ETN and the sum of inorganic pools (NO_3^--N and NH_4^+-N).

2.6 Microbial pools

Microbial biomass N (MBN) and C (MBC) were quantified using a modification of the chloroform fumigation-extraction technique (Brookes et al., 1985). One 5g subsample of fresh soil was combined with 2 mL of ethanol-free chloroform and

120 incubated at room temperature under a fume hood for 24 hours in a stoppered 250 mL Erlenmeyer flask. Following incubation, flasks were vented and extracted with 0.5 M K_2SO_4 following the same procedure described in the previous section. Fumigated samples were analyzed for EOC and ETN; MBN and MBC were calculated as the difference between ETN and EOC concentrations extracted from fumigated and non-fumigated samples.

2.7 Standing Crop Root Biomass

125 To determine root biomass, triplicate soil cores described above were combined and homogenized before measuring total soil volume using a graduated cylinder. Roots were collected by sieving the samples with a 2 mm sieve and hand-picking roots with forceps. Extracted roots were then washed free of soil and dried at 65° C for 48 hours before mass was determined. Root biomass (mg cm^{-3}) was calculated as root biomass per unit soil volume.

2.8 Statistical Analyses

130 Data underwent log$_{10}$ transformation to achieve a higher approximation to normality; normality was tested using Shapiro-Wilk test (p > 0.05) in R. Soil N pools were analyzed as a two-way analysis of variance (ANOVA) using the "aov" function, with the factors of "Treatment" and "Sampling date." Statistical significance was determined at α < 0.05. When statistically differences or interactions between factors were detected, Tukey tests were performed to find differences between specific groups using the "stats" R package. All analyses were performed using R 4.0.3 (R Core Team 2021).

3 Results

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We found that long-term N-addition had few effects on the measured physical soil properties. Soils from both control and fertilized plots had similar pH (8.79, 8.73, respectively), and soil organic matter content (1.05 and 0.97%, respectively) although fertilized plots had a higher salinity (106.51μS/cm SPC, SE= 3.25) than control plots (93.00 μS/cm SPC, SE= 4.40) (Table 1). In contrast, we found a strong effect of fertilization treatment on soil available N including higher NO_3^--N (Figure

140 1a, Table 1) and NH_4^+-N (Figure 1b, Table 1) in fertilized plots. Both NO_3^--N and NH_4^+-N also showed significant seasonal variation (Figure 1, Table 1) and there was no interaction between sampling month and fertilization treatment for either variable (Table 1).

EOC and ETN both showed significant increases with N-addition and responses to sampling date with no interaction between

145 the two (Figure 2a,b, Table 1). However, neither MBC (Figure 2c) nor MBN (Figure 2d) responded to N-treatment but did show significant seasonal variation (Table 1). Soil total % C showed no response to either treatment, or sampling date (Figure 2e, Table 1), while soil total % N significantly increased with both N-addition and varied across the season (Figure 2f, Table 1). There were no interactions between treatment and sampling date for either soil total %C or %N (Table 1).
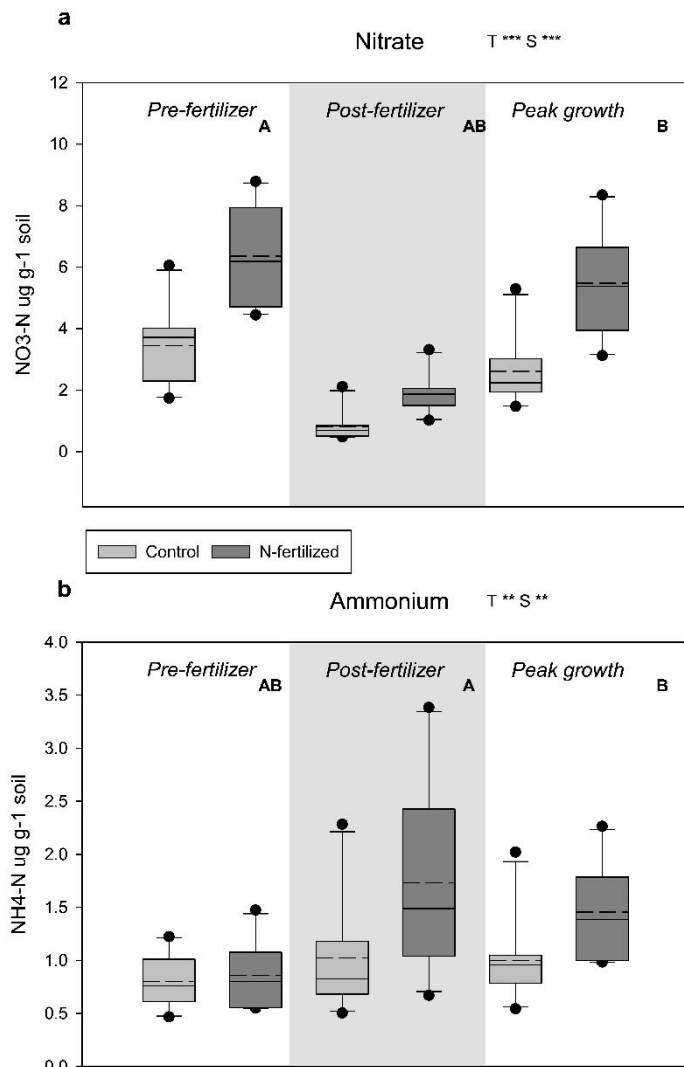
Figure 1. Box plot, including the mean (dashed line, n=10 and extreme values (black dots) of inorganic N pools: soil nitrate (a) and soil ammonium (b) in a 26-year southwestern US grassland fertilization experiment. Overall significance for a two-way ANOVA with the main factors of treatment (T) and sampling date (S) are shown (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Letters (A and B) indicate statistical difference between individual sampling dates ($p < 0.05$).

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We found no effects of fertilization on any of the plant variables measured. Root standing crop biomass (control 0.71 mg/cm³, fertilized 0.65 mg/cm³), as an estimate for below-ground productivity, did not show a significant response to fertilization ($F_{1,18}$ = 0.56, p = 0.46). Similarly, neither plant % C (control 45.20%; fertilized 45.12%) or %N (control 1.32%; fertilized 1.44%) showed responses to the N-addition treatment (Table 1). Above-ground biomass did not respond to fertilization treatments
180 either in the spring (t_{18}= -0.35, p=0.73), the fall (t_{18}= -1.01, p=0.33) or as annual biomass measures (t_{18}= -1.16, p=0.26) (Baur et al., 2021).

Table 1. Summary of two-way ANOVA for nutrient pools and soil properties across one growing season in a grassland fertilization experiment after 26 years (est. 1995) of annual N-addition in the form of NH_4NO_3. Significant values (p< 0.05) are in bold. *df* for all soil nutrient pool responses: treatment effect (1,54), sampling date and the interaction of treatment and sampling date (2,54). *df* for plant nutrient pools and all soil properties is (1,18).

	Fertilization		Sampling date		F x S	
Soil property	*F*	*p*	*F*	*p*	*F*	*p*
pH	3.02	0.1	-	-	-	-
Salinity	6.12	**0.02**	-	-	-	-
%SOM	0.39	0.54	-	-	-	-
Nutrient pool	*F*	*p*	*F*	*p*	*F*	*p*
NO3	71.50	**<0.01**	85.64	**<0.01**	0.65	0.52
NH4	11.36	**<0.01**	7.10	**<0.01**	1.93	0.15
MBC	0.17	0.68	27.18	**<0.01**	0.35	0.71
MBN	0.03	0.87	35.05	**<0.01**	0.14	0.87
EOC	6.16	**0.02**	19.71	**<0.01**	0.22	0.80
EON	0.30	0.59	8.44	**<0.01**	0.59	0.56
Soil %C	0.69	0.41	1.91	0.16	1.27	0.29
Soil %N	6.35	**0.02**	6.37	**<0.01**	1.11	0.34
Plant %C	0.17	0.69	-	-	-	-
Plant %N	0.34	0.26	-	-	-	-

Using the determined N-concentration in each pool above, we estimated the N-content per square meter to our sampling depth of 10 cm. All pools were estimated based on the October measurement, as this was the only sampling that plant N was measured. Bulk density did not vary between treatments (t_{18} = -1.22 p=0.24), and we used the average bulk density of all plots (1.31 g cm^{-3}) to determine N content in fertilized and unfertilized plots separately. The difference between these values was calculated by subtracting N of control plots from N of fertilized plots – in most cases this resulted in a positive number, indicating an accumulation of N in fertilized plots (Table 2). N content in the plant pools is only a rough estimate, as we only measured leaf N concentration for a single species and did not measure root N concentration. Using just the N-concentration of the measured species in each control and fertilized plots, and the fall above-ground biomass values (see above), we estimated the N-content of the above ground plants, and then calculated a difference as above (Table 2). For the root N-content, we also used the N-content of the above-ground tissue (this will be an overestimate) and calculated the N-pool for the volume within the top 10 cm of soil, using our root biomass measures (Table 2).

Table 2. Summary of N pools in soil and plants during a single post-monsoon sampling period in a grassland fertilization experiment after 26 years (est. 1995) of yearly N-addition in the form of NH_4NO_3. N in µg g^{-1} soil were measured on soil collected from the field, and then converted to g N g^{-1} soil using the soil bulk density (1.31 g cm^{-3}). Plant aboveground N pools were estimated using % N of a single species *(Sporobolus flexuosus)* collected from fertilized and unfertilized plots and calculated aboveground biomass. Belowground N pools were estimated using the measured above ground N-concentrations and measured belowground biomass to a 10cm depth.

	N Control (ug N g^{-1} soil)	N Fertilized (ug N g^{-1} soil)	N_{fert} - $N_{control}$ (ug N g^{-1} soil)	N_{fert} - $N_{control}$ (g N g^{-1} soil)
Soil N Pools				
NH4	1.00	1.43	0.43	0.06
NO3	2.61	5.48	2.86	0.38
EON	1.36	1.11	-0.25	-0.03
MBN	4.67	5.29	0.62	0.08
TN	310	330	20	2.62
Total				**3.10**
Plant N Pools				
NO3	3.40	2.72		-0.68
NH4	0.94	0.94		0.00

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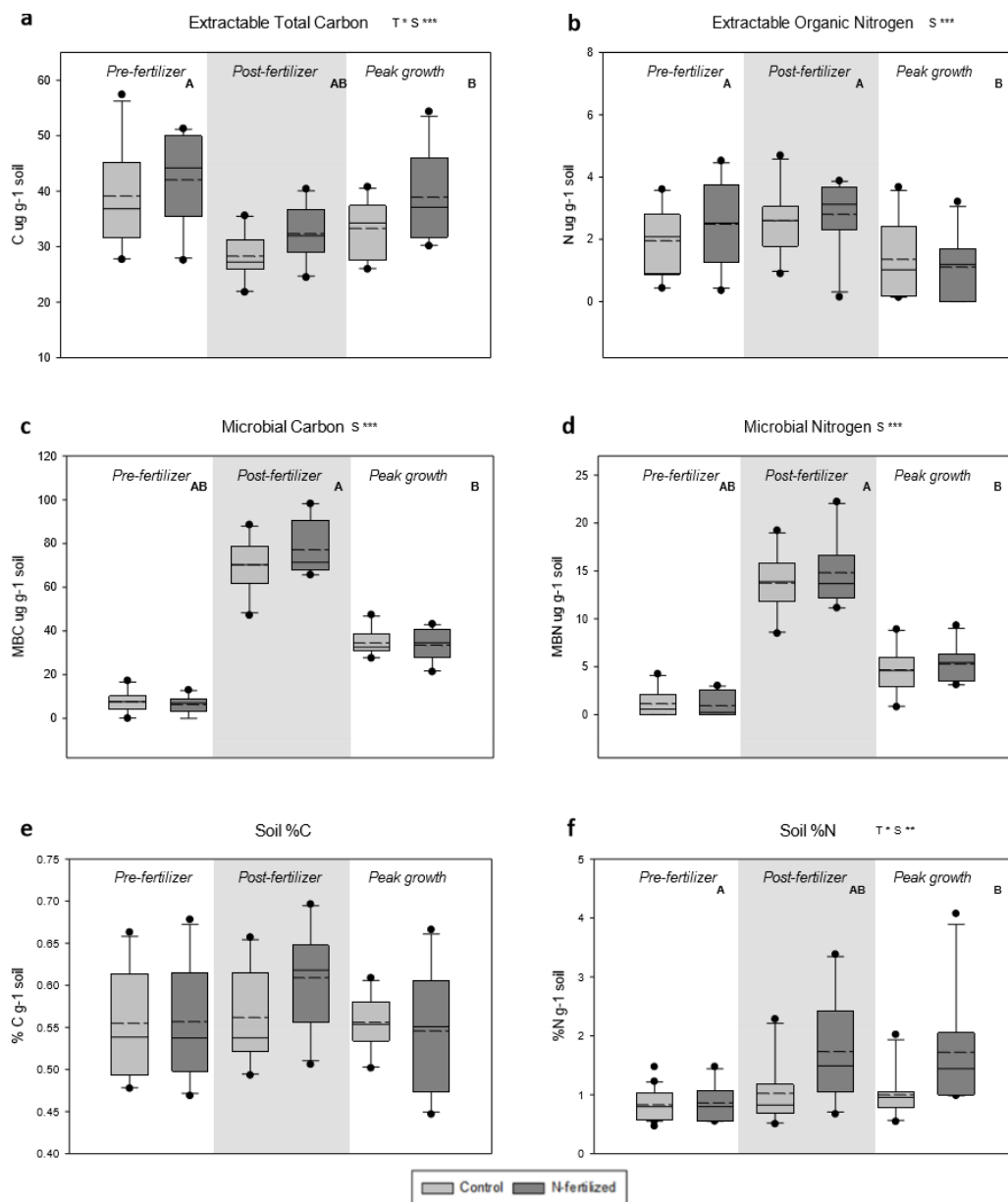
Figure 2. Distribution of data based on a five number summary, and mean (dashed line), of different analyzed C and N pools: (a) extractable organic C, (b) extractable organic N, (c) microbial C, (d) microbial N, (e) soil percent C and (f) soil percent N in a long-term fertilization experiment in the northern Chihuahuan Desert. Levels of overall significance for a two-way ANOVA with the main factors of treatment (T) and sampling date (S) are shown (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). Letters (A and B) indicate statistical difference between individual sampling dates ($p < 0.05$).

4 Discussion

220 We examined the effects of increased N inputs on plant, soil and microbial N pools in a Chihuahuan Desert grassland that has received annual additions of N for 26 years. We found small but significant increases with fertilization in most soil N pools, including available N (NO_3^--N and NH_4^+-N) (Figure 1) and soil total N (Soil %N, Fig. 2f), but not extractable organic N (EON, Fig. 2b). However, this did not translate to higher N pools within the organisms in this system. Neither soil microbial C nor N significantly increased with fertilization (Fig. 2c,d), and there was no effect on above- or below-ground plant biomass, nor N

225 content in plant leaf tissue of a common grass, *Sporobolus flexuosus*. Thus, despite significant inputs of N into this system (260 g m^{-2} of N) there were only small increases in soil pools. Further, the soil microbes and plant communities do not appear to be taking advantage of increased N-availability.

The N added to these plots did translate to increased inorganic N in the upper soil layers of fertilized plots. Nitrate and

230 ammonium are the primary sources of N for both soil microbes and plants (Harrison et al., 2007), suggesting annual fertilization increased N-availability to these organisms. Using some back-of-the envelope calculations, we determined that the additional inorganic N available in these plots is less than 0.5 g m^{-2} (Table 2), which is less than 5% of the 260 g added over the lifetime of the experiment. Our data are consistent with prior research demonstrating that arid ecosystems have low N retention (Peterjohn and Schlesinger 1990; McCalley and Sparks 2009). For instance, seven years of simulated N deposition at three

235 semi-arid grasslands resulted in rapid but ephemeral changes in soil inorganic N (Phillips et al., 2021), suggesting that the additional N quickly left the system.

Despite the additional inorganic N added to the ecosystem, the vegetation did not detectably respond to fertilization with either increased above- or belowground biomass or increased N content within that biomass. Although not significant, the average

240 above-ground plant biomass was actually higher in control (256.8 g m^{-2}) than fertilized plots (188.4 g m^{-2}). We found similar % N in the leaf tissue of one common grass species, therefore, N-content in above-ground tissue could not account for any of the additional N added to the fertilized plots. Below-ground biomass in control (0.71 mg cm^{-3}) and fertilized plots (0.65 mg cm^{-3}) are nearly identical, and if we assume that the % N in the root tissue is similar between the two treatments, as it is for the above ground tissue, then this would also not account for any of the 'missing N'. Other dryland N-addition experiments on

245 the Colorado Plateau (Phillips et al. 2021) and earlier work in this experiment also reported no plant response to fertilization, except ephemeral increases in years with above-average precipitation (Ladwig et al. 2012), further supporting the claim that N-limitation in drylands is less prominent than water stress (Carpenter et al., 1990; Hooper and Johnson, 1999; Brooks, 2003; Rao and Allen, 2010; Rao et al., 2011; Wang and Wen, 2023). Indeed, water stress may actually lead to further nutrient stress - the mobility of free ions in the soil is highly dependent on water status, particularly NO3- (Plett et al., 2020). Moreover, a

250 meta-analysis of dryland N-addition studies by Hooper and Johnson (1999) concluded that geographical and temporal variation

in precipitation significantly increased absolute plant production and ecosystem resource use efficiency, emphasizing plant co-limitation by multiple resources and the tight link between species shifts, changing resource levels and water availability.

Similar to the plant response, below-ground microbial communities did not accumulate biomass C (MBC) nor N (MBN) in
255   response to fertilization treatments. In contrast with these findings, a meta-analysis of 14 studies on N-deposition in drylands by Sinsabaugh et al. (2015) showed soil microbial communities to be sensitive to even low rates of N deposition and show positive microbial biomass and metabolic responses. However, Sinsabaugh et al. (2015) described various short-comings of their interpretations of soil microbial responses: dryland landscapes are highly heterogeneous, and N-addition studies are scarce, as well as differing in doses and duration. Nevertheless, individual studies have reported that the composition and
260   function of dryland microbiota are unresponsive to N additions such as the two-year N-deposition experiment in semi-arid grasslands reported by McHugh et al. (2017), or even negative after 5-years of N-addition (Li et al. 2010) suggesting contingent responses to similar N inputs across dryland ecosystems. Like plants, the unresponsiveness of the microbial community to additional N inputs may be attributed to a possible co-limitation by another resource, especially water but potentially soil phosphorus, potassium or even micronutrients that hinders the biota's capacity to take advantage of additional N (Ramirez et
265   al., 2010; Fay et al., 2015; Radujković et al. 2021; Choi et al., 2022). Thus, because N is not being immobilized by plants or microbes, it is likely being lost from the system through leaching during large rain events or volatilization when soils are hot and dry (McCalley and Sparks, 2009; Lovett and Goodale, 2011; Brown et al., 2022). Similar to our results, eight years of simulated increases on N deposition in the Colorado Plateau led to some short-term but no long-lasting effects on an array of soil, plant and microbial metrics, suggesting that serial or simultaneous limitation of multiple other resources may weaken the
270   direct effects of higher N levels on ecosystem processes in drylands (Osborne et al., 2021).

Even though we did not detect an increase in N accumulation by the plant or microbial community with fertilization, we still found an accumulation of organic N in the fertilized plot soils, with the majority of this found in the total N pool rather than extractable organic N. Despite a relatively small difference in the soil % N between control (0.031 %) and fertilized (0.033%)
275   plots, when accounting for the volume and mass of soil found in the top 10 cm of the soil, this would be an additional 2.6 g m-2 of N (Table 2) found in fertilized plots. Because the N added each year is in an inorganic form (NH4NO3), this increase in organic or total N over time suggests that the N had been converted to an organic form (through uptake and assimilation of N into microbial and plant biomass, as observed by Harrison et al. (2007)), and then deposited into the soil. This is supported by short-term studies which have reported only increases in plant- N, but no increases in soil N after five (Zeng et al., 2010) or
280   eight (Osborne et al. 2022) years of N addition, although other experiments with similar amounts of N added for six years were sufficient to increase total soil N content (Sha et al., 2021). Thus, over the duration of the experiment there were at least some years where there was higher plant or microbial biomass produced in the fertilized plots, such as when production increases during high precipitation years (Hall et al., 2011; Ladwig et al., 2012).

285 In agreement with our second hypothesis, the effect of seasonal variability on N pools was higher than differences between fertilization and control treatments. Sampling date effects were significant for every variable that was measured multiple times, except for soil %C which is unlikely to change much seasonally, but can be quite variable from year to year in response to precipitation variability (Hou et al., 2021). In general, drylands inherently have high temporal heterogeneity in rainfall, where large, infrequent precipitation events are often thought to regulate ecosystem structure and function (Noy-Meir, 1973),

290 including fluctuations in C and nutrient availability (Collins et al., 2014; Schimel, 2018). In particular, NO_3^- and NH_4^+ are known to show high intra-annual seasonality in many ecosystems, including drylands where N-availability is often quite low to begin with (Zak et al., 1994) and linked to the timing of rainfall (Cui and Caldwell, 1997; Dijkstra et al., 2012; Brown et al., 2022). Surprisingly, the effect of fertilization did not vary between sampling dates for any of the variables, even when including dates both before and after fertilization. Shorter term N-addition studies (McHugh et al., 2017; Phillips et al., 2021)

295 have reported only transient increases immediately after fertilizer addition. Yet in this study these available forms of N still showed persistent, likely cumulative, effects a year after fertilization, consistent with limited uptake of available nutrients by microbial and plant communities. Persistent effects on variables such as total soil N are less unexpected because they typically vary little over the short-term. Finally, this study only took place within a single year, but we would also expect high year to year variability in available resources and N pools. Fertilizer in this experiment is timed to occur close to the beginning of

300 monsoon rains although because of the unpredictability of the rains, the time between fertilizer application and the monsoon shifts each year. Timing of N-addition relative to precipitation can alter the ultimate effects of fertilizer addition in drylands (Stursova et al., 2006).

5 Conclusions

Ultimately, between the 0.5 g m^{-2} of additional inorganic N found in fertilized plots and the 2.6 g m^{-2} found in the soil total N

305 pool, much of the additional 260 g m^{-2} of N added over the lifetime of the experiment remains unaccounted for. Thus, a significant portion of the added N has been lost from the system. While the route by which N left the system is unknown, because N was not immobilized by plants or microbes, we can infer the N has either reached deeper soil profiles or lost via gaseous losses. Given the limited amount of yearly precipitation received by our site, we suspected that the likelihood of rainfall percolating deep enough to cause nutrient leaching would be low. However, McHugh et al. (2017) reported resin

310 capture results showing that N was moving down through the soil profile in a semi-arid grassland on the Colorado Plateau with similar annual precipitation, and nitrate in the subsoil of numerous arid regions indicates that leaching may indeed be common (Walvoord et al. 2003). Alternatively, N may have been lost from the system via volatilization, which has been described as a dominant mechanism for N-loss from ecosystems of the desert southwest (Peterjohn and Schlesinger, 1990), and supported in several recent studies (Homyak et al., 2016, McHugh et al. 2017). Together our results demonstrate that these

315 nutrient-poor ecosystems have limited capacity to retain N even under high rates of N deposition.

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Data Availability

The datasets generated during the current study will be made available on the EDI Data Portal (https://portal.edirepository.org) prior to publication.

Author Contributions

320 All authors contributed to study conception and design. Sample collection and lab analyses were performed by VMM, and all authors participated in statistical analyses. The first draft of the manuscript was written by VMM, and all authors commented on previous versions of the manuscript and approved the final manuscript.

Competing Interests

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