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2024

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The Effects of Altered Precipitation on Gross N Transformations in a Pinyon-Juniper
Dryland

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Environmental Sciences

by

Jamie Christine Irby

September 2024

Thesis Committee:

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ACKNOWLEDGEMENTS

I would like to thank the following people, without whom I would not have been able to complete this study. Thank you to Dr. Alex Krichels, who helped me develop the project and method the moment I began this journey, and has helped me since with patience and compassion. Thank you to my fellow lab mates in the Homyak lab – Aral, Chloe, Elizah, Ellen, Val, and Yareli – who have always been there to help me with experiments, encourage me when I doubted myself, and face challenges both of the expected and unexpected. Thank you to my peer mentor – Alyssa Valdez – who’s advice I will always cherish with me, and who could never fail to make me laugh even on days I didn’t think I could smile. Thank you to my committee members – Dr. Tamara Harms and Dr. Samantha Ying – for being incredible role models in this journey, and for providing guidance both academically and non-academically. And finally, thank you to Dr. Peter Homyak for seeing the scientist in me, trusting me with this spectacular project, and supporting me in every aspect of this adventure. It’s been an amazing two years and I owe much of my success to you all.

DEDICATION

I would like to dedicate this study to the people I cherish the most. First, to my dad and sister, who have been with me since day one (literally). Not only have they watched me grow into the person I am today, they both shaped my character through their unconditional love and support throughout all of the hardships we have faced together. Second, to my partner Branden, who was firsthand witness to each of my struggles, who was my pillar during times when I thought my world was crumbling, and who has been my cheerleader in every stage of this journey. And finally, I dedicate this work to my mother, who I know is watching over me and would be proud of what I've accomplished.

ABSTRACT OF THE THESIS

The Effects of Altered Precipitation on Gross N Transformations in a Pinyon-Juniper Dryland

by

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Master of Science, Graduate Program in Environmental Sciences
University of California, Riverside, September 2024
Dr. Peter Homyak, Chairperson

Future climate projections predict a global increase in precipitation variability as well as a trend towards aridity, which can influence how nitrogen (N) is cycled in terrestrial ecosystems. Increasingly arid conditions can alter N cycling by increasing soil inorganic N concentrations and making this N more susceptible to loss. Further, shifts in precipitation patterns in one season can establish effects on the N cycle that can “carry-over” into the following season, known as precipitation legacy effects. However, how current and historic precipitation patterns may alter soil N availability through changes in inorganic N supply—i.e., gross N mineralization and gross nitrification—is not well constrained. We measured the effects of altering precipitation variability on soil N cycling using an isotope pool dilution on soils from a Pinyon–Juniper dryland under a precipitation manipulation experiment. We found that wintertime drought, the most

extreme drought we imposed, reduced gross N mineralization by ~2.5x and gross nitrification rates by ~9.5x compared to ambient conditions, establishing a legacy effect that reduced gross N mineralization by ~2x compared to ambient conditions the following summer. As we observed an increase in soil N concentrations (~6x greater than ambient conditions) simultaneous with lower gross rates of inorganic N supply under drought, our results imply that dry conditions must have reduced N immobilization rates below those of inorganic N supply to account for the increased N availability. Our measurements show that extreme drought can have negative consequences on inorganic N supply that persist even after the drought ends, suggesting these responses may become more common as drought is expected to increase in frequency and severity across the globe.

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Introduction

Climate change is altering global precipitation patterns, increasing both the frequency and severity of drought and extreme rainfall events (Cook & Seager, 2013; Seager et al., 2007; IPCC, 2014). These global changes in precipitation can, in turn, alter the nitrogen (N) cycle in terrestrial ecosystems (Vitousek et al., 2022), as N is largely cycled through biotic processes that rely on the production and diffusion of inorganic N in soils, including: i) gross N mineralization—the release of inorganic N, mainly ammonium (NH_4^+) and nitrate (NO_3^-), from organic N—and ii) gross nitrification—the oxidation of NH_4^+ to NO_3^- by nitrifying bacteria—hereafter defined as “inorganic N supply” (Austin et al., 2004; Schwinning & Sala, 2004). Thus, in water-limited ecosystems (i.e., drylands), where drought restricts inorganic N supply (Stark & Firestone, 1995), N often limits primary productivity (Hooper & Johnson, 1999). However, N may also be in short supply as a result of uncontrollable N losses that occur when N sources and sinks decouple (Von Sperber et al., 2017), particularly during the first moments of wetting dry soils when soil inorganic N concentrations are at their highest (Davidson, Vitousek, et al., 1991; Eberwein et al., 2020; Homyak et al., 2016; Krichels, Greene, et al., 2023). While high soil N concentrations can help explain why large N losses occur after wetting dry soils, the microbial populations producing the N are well-known to be constrained by those same dry conditions (Moyano et al., 2013; Stark & Firestone, 1995), raising questions about the mechanisms governing N supply and N accumulation in soils.

To understand how inorganic N concentrations increase as soils dry, many studies have focused on measuring net rates of inorganic N supply – i.e., net N mineralization and net nitrification. Net N mineralization and nitrification are the result of a balance between two processes occurring simultaneously: gross N supply, which includes gross N mineralization and gross nitrification, and gross N consumption, which includes both plant and microbial N immobilization, as well as other processes that remove inorganic N via gaseous and hydrologic pathways (e.g., nitrification, denitrification, and leaching). Thus, while net rates can provide useful information about the N available in excess of biological demand, they can also be misleading indicators for responses of N cycling to changes in precipitation; a net rate of zero may result from a tradeoff in relatively large gross rates of N mineralization and immobilization (Homyak et al., 2017). For example, wet years can stimulate plant growth, in turn increasing soil organic N pools and, subsequently, gross N mineralization rates (Engelhardt et al., 2021). However, enhanced plant growth may also increase N immobilization rates, producing low net N mineralization and nitrification rates if N immobilization outpaces inorganic N supply (Parker & Schimel, 2011). In contrast, droughts may stress microbial communities that mineralize N while also limiting plant and microbial access to N due to diffusion constraints (Stark & Firestone, 1995). If inorganic N supply outpaces N immobilization, albeit at reduced rates, net N mineralization and nitrification rates may increase even if microbial activity is low, helping to explain why inorganic N concentrations increase in dry soil. Further, shifts in precipitation patterns may affect the microbial communities governing inorganic N supply in different ways, especially since nitrifiers may be more

sensitive to changes in soil moisture than the heterotrophic microorganisms responsible for N mineralization (Slessarev et al., 2021). Thus, to understand the mechanisms controlling soil N pools and whether they are changing as a function of higher inorganic N supply and/or N immobilization rates, measurements of gross N transformations and how they trade off are required.

In addition to tradeoffs in gross N transformations, other factors may influence whether soil inorganic N concentrations increase and for how long they remain elevated with changes in precipitation. This is because ecosystem responses to changes in precipitation can persist even after the patterns revert to antecedent conditions, otherwise known as precipitation legacies (Reichmann et al., 2013; Sala et al., 2012). Indeed, increases in soil inorganic N concentrations in response to drought have been observed to persist even after the drought ends (Krichels, Greene, et al., 2023), as past dry seasons can lower N immobilization rates and increase the fraction of soil N that carries over into the following season (i.e., the N carryover hypothesis; Krichels, Greene, et al., 2023; Sala et al., 2012; Shen et al., 2016). However, the mechanisms behind this legacy effect have not been fully explored. In some cases, less precipitation in one season can decrease microbial biomass the following season (Krichels, Greene, et al., 2023), limiting both inorganic N supply and N immobilization. In other cases, microbial communities may be well adapted to drought (Leizeaga et al., 2021), limiting the impact of changes in precipitation on N supply. Further, many other soil and environmental factors, such as vegetation and substrate competition, are influenced by precipitation (Kou et al., 2018) and thus may be influenced by precipitation legacies and their relative intensity (i.e.,

extreme vs moderate droughts or rain events), making it difficult to determine to what extent the patterns of past precipitation can shape N supply.

To determine how changes in precipitation and precipitation legacies may alter soil inorganic N supply and availability, an approach that combines seasonal changes in precipitation with experimental precipitation manipulations that integrate ecosystem responses to drought using indices like the isotopic composition of N in bulk soil (i.e., $\delta^{15}\text{N}$ enrichment), may offer helpful insights. For instance, some drylands experience precipitation during two distinct seasons: i) cool wet winters with low evaporative losses that keep soils relatively moist and, ii) hot summers, characterized by occasional monsoonal rains that interrupt otherwise hot and dry conditions (Ludwig et al., 1988; Spasojevic et al., 2022). By manipulating precipitation to impose a winter drought, followed by the typical dry summer, the microbial communities responsible for N supply would be exposed to a relatively “extreme drought”. In contrast, imposing a summer drought may not induce substantial water stress on microbial communities that are already adapted to the high heat and low precipitation typical of summer (De Nijs et al., 2019), constituting a “moderate drought.” Similarly, increasing precipitation during the already wetter and cooler winter would be expected to keep soils moist for a longer period relative to increasing precipitation in summer, which may have greater potential to influence biological processes (Wu et al., 2022). These changes in precipitation, and the potential legacies they promote, would be expected to affect how N is cycled, altering the isotopic composition of N in bulk soil that may be used to infer how N is cycled

(Brunello et al., 2024; Mao et al., 2024; Robinson, 2001; Von Sperber et al., 2017; Wang et al., 2014). While many factors control bulk soil $\delta^{15}\text{N}$ values (e.g., vegetation, mycorrhizal associations, atmospheric N deposition; Robinson, 2001), there is growing evidence that bulk soil $\delta^{15}\text{N}$ increases with increasing aridity (Von Sperber et al., 2017; Wang et al., 2014). Altogether, these observations lead us to ask: How does drought influence N accumulation in soils via inorganic N supply, and how do precipitation legacies affect gross N transformations?

We answered these questions by manipulating the amount and timing of precipitation in a Pinyon-Juniper dryland in southern California to understand the extent to which past and current precipitation patterns influence dryland inorganic N supply. Specifically, we measured gross rates of N mineralization and nitrification in the lab using an isotope pool dilution after wetting soils to stimulate microbial activity. While this approach cannot yield in-situ gross N transformation rates—wetting the soils was required to add the isotope label—it allowed us to make relative comparisons between treatments and assess the relative sensitivity of microbial communities governing gross N mineralization and gross nitrification to drought stress. We also measured bulk soil $\delta^{15}\text{N}$ as an index of ecosystem N retention in response to shifts in precipitation during both winters and summers. We hypothesized that: 1) N accumulates in dry soils because gross N mineralization and nitrification persist during drought; and 2) the effects of drought and precipitation persist through seasons because of a lag in microbial response to changes in precipitation between seasons (i.e., a precipitation legacy effect).

Methods

Site description

We conducted our study in the Pinyon Flats precipitation manipulation experiment in Southern California (33° 36 '36.7"N, 116° 27' 01.1"W), a pinyon-juniper dryland consisting of 24 plots, part of the University of California Philip L. Boyd Deep Canyon Desert Reserve. The site receives precipitation in two distinct seasons: i) cool wet winters from November to May, characterized by average low temperatures of 14.1 °C, average high temperatures of 25.9 °C, and average monthly precipitation of 24.1 mm, and ii) monsoonal summers from June through October, characterized by average low temperatures 24.7 °C, average high temperatures of 38.2 °C, and average monthly precipitation of 7.1 mm from 2000-2022. The aridity index for this site is estimated to be 0.22 (Zomer et al., 2022) and modeled total atmospheric N deposition is 3.6 kg N ha⁻¹ y⁻¹ (National Atmospheric Deposition Program, 2023). Meteorological data were obtained by averaging publicly available data from the year 2000 through 2022 (<https://deepcanyon.ucnrs.org/weather-data/>).

Precipitation has been experimentally added or excluded from 6.1m x 8.5m plots during the winter and summer at Pinyon Flats since 2018 (Fig. 1; Spasojevic et al., 2022) according to the following treatments (listed from driest to wettest): *Winter-* received ambient summer precipitation while winter precipitation was excluded. On March 7th, 2023 approximately 25 mm of rain entered one of our *Winter-* plots, forcing us to exclude it from our analysis. Therefore, n=3 for *Winter-* plots. *Summer-* (n=4) received ambient

winter precipitation while summer precipitation was excluded. *Control* (n=8) received both ambient summer and winter precipitation. *Summer+* (n=4) received ambient winter precipitation and received additional water during the summer (Table 1; 29 mm in 2018, 22 mm in 2019, 0 mm in 2020, and 15 mm in 2021, 3.7mm in 2022, and 36.5 mm in 2023). *Winter+* (n=4) received ambient summer precipitation and additional water during the winter (Table 1; 132 mm in 2019, 106 mm in 2020, 7.3 mm in 2021, 40 mm in 2022, and 21.9 mm in 2023).

For the exclusion treatments (*Winter-* and *Summer-* plots), precipitation was excluded using metal frames covered with polyethylene plastic rainout shelters (Tuff Lite IV 28' x 70' TER IR/AC manufactured by Berry Plastics, Evansville, IN). *Summer-* roofs were placed in early June and moved to *Winter-* roofs in mid-November (Fig. 2). During each season, precipitation was collected from the exclusion plots through a downslope PVC system filling four 1,500-gallon tanks. Water collected in the tanks was added to the water addition treatments (*Winter+* in the winter and *Summer+* in the summer) within a week of each precipitation event by pumping water from the tanks through 17-mm drip tubing (Netafim Irrigation, Inc., Fresno, CA). We used a data logger (Campbell CR1000x) to collect hourly meteorological information including air temperature (Campbell 108-L BetaTherm 100K6A1IA Thermistor), soil moisture (Campbell CS616 30cm Water Content Reflectometer), and precipitation (Campbell TE525MM-L Metric Rain Gage).

Soil collection and analysis

We collected soil from A horizons (top 10cm, ~500g) from each of the 24 plots in both the winter of 2023 (April, aimed at capturing peak plant activity) and the summer of 2023 (September, aimed at capturing summer dry soils before the onset of the wet season) using an aluminum corer. Soils were sieved (2 mm) and a subsample was oven-dried at 104°C for 24 hours to determine soil gravimetric water content (GWC). We also measured soil water holding capacity (WHC) as the water retained by water-saturated soils against gravity over an 8-hour period (Franzluebbers, 2020); soils were allowed to drain inside an airtight container to minimize evaporative losses. Soil extractable NO_3^- and NH_4^+ concentrations were measured by extracting 3 g of soil in 30 mL of 2M KCl, which was then shaken for 1 hour and filtered (Whatman 42 filter paper; 2.5 μm pore size). Colorimetric assays were used to measure extractable NO_3^- (SEAL cadmium-sulfanilamide method EPA-126-A, limit of quantitation (LOQ) = 0.03 mg L⁻¹) and NH_4^+ (SEAL phenol-hypochlorite method EPA-129-A, LOQ = 0.05mg L⁻¹).

Soil microbial biomass C and N were measured using a chloroform slurry extraction (Vance et al., 1987). Briefly, 10 g of soil was extracted in 40 mL of 0.5M K_2SO_4 , with or without 0.5 mL of chloroform, and shaken for 4 hours (Vance et al., 1987). The extractions were then filtered (Whatman 42 filter paper; 2.5 μm pore size) and analyzed for total organic C and N using a total organic C analyzer (TOC-L CPH, Shimadzu Scientific Instruments, LOQ = 4 μg L⁻¹) connected to a nitrogen monoxide chemiluminescence analyzer (TNM-L, LOQ = 30 μg L⁻¹). Microbial biomass C and N was calculated as the difference in total organic C or total organic N between the two

extractions with or without chloroform. Our measurements represent a flush of C and N rather than total microbial biomass, as we did not correct for extraction efficiency.

Gross N transformation rates

To measure soil gross N mineralization and gross nitrification rates, we performed isotope pool dilutions (Davidson, Hart, et al., 1991). Soil samples were stored in plastic bags at 4°C for no more than one week post sampling. To measure gross nitrification rates, we added $^{15}\text{N-KNO}_3^-$ to raise the isotopic composition of the NO_3^- pool to 10 atom%. Time 0 started when the label was added, which included wetting the soil samples to 60% WHC to stimulate a wetting event and to allow for even distribution of the isotope label. While wetting soils stimulates microbial activity, we used the pool dilutions to assess relative differences between treatments and infer how changes in antecedent moisture and soil N affected N dynamics. After adding the label and wetting the jars, they were mixed for approximately 3 mins to ensure even distribution of the label. After 15 minutes and 4 hours, 50 g of soil from the jars were extracted in 150mL of 2M KCl for subsequent measurement of NO_3^- and NH_4^+ concentrations and isotopic composition (Davidson et al., 2003). We chose to measure gross N transformations up until 4 hours post-wetting because previous research at the site demonstrated that much N-cycling activity occurs within 4 hours of wetting soils (Krichels, Greene, et al., 2023; Krichels, Jenerette, et al., 2023). To measure gross N mineralization rates, we used the same procedure outlined above but with 10 atom% $^{15}\text{NH}_4\text{Cl}$. The ^{15}N enrichment of the NH_4^+ was determined using acid trap diffusion (Brooks et al., 1989), where NH_4^+ in the

extract is volatilized to NH_3 by raising the pH with magnesium oxide, and the NH_3 is trapped onto a paper disk acidified with 2.5 M potassium bisulfate. The same method was used to measure the ^{15}N enrichment of NO_3^- pools with the addition of Devarda's alloy to first reduce NO_3^- to NH_3 . The paper disks were dried for 24 hours in a desiccator before analysis on a Costech elemental analyzer (Costech Elemental Combustion System, LOQ = $1\mu\text{g}$) coupled to an isotope ratio mass spectrometer (Delta V Advantage IRMS, $\delta^{15}\text{N}$ precision = 0.06 ‰) at the Facility for Isotope Ratio Mass Spectrometry at UC Riverside (FIRMS, <https://ccb.ucr.edu/facilities/firms>). Gross N mineralization and nitrification rates were calculated from the dilution of the enriched NH_4^+ and NO_3^- pools between 15 minutes and 4 hours after adding the ^{15}N label (Kirkham & Bartholomew, 1954). We used the timepoint at 15 minutes post label addition to account for the initial abiotic processing of ^{15}N commonly observed in pool dilution experiments (Davidson, Hart, et al., 1991).

Dissimilatory nitrate reduction to ammonium (DNRA) rates were calculated as the difference in ^{15}N NH_4^+ atom % between 15 minutes and 4 hours post label addition, multiplied by the mean NH_4^+ pool size during the interval, and corrected for the mean residence time of the NH_4^+ pool, calculated as the initial size of the NH_4^+ pool divided by the NH_4^+ consumption rate. This was then divided by the mean ^{15}N NO_3^- atom % during the interval to account for the isotopic composition of the source pool (Silver et al., 2001).

Long-term field net N mineralization, nitrification, soil extractable NH_4^+ and NO_3^- , organic N and natural abundance $\delta^{15}\text{N}$

We conducted quarterly assessments of in-situ soil extractable NH_4^+ and NO_3^- , as well as in-situ net N mineralization and net nitrification rates. At the start of each quarter (i.e., January for winter, April for spring, July for summer, and October for autumn), a 10-cm long PVC (4 cm diameter) core was installed in each plot. An initial soil sample from the upper 10 cm (A horizon) next to the PVC core was collected to determine initial concentrations of extractable NH_4^+ and NO_3^- . The PVC cores were capped with PVC lids to prevent N inputs from the atmosphere but allow gas exchange. After 30 days, the cores were retrieved for analysis of extractable NH_4^+ and NO_3^- . Net nitrification was calculated as the difference in the NO_3^- pool over the 30-day period and net N mineralization was determined as the difference in the inorganic nitrogen pool (NH_4^+ and NO_3^-) over the same duration. During summer 2023, wildlife disturbed the cores in multiple plots and are missing from our analysis, such that $n = 4$ in *Control*, $n = 2$ in *Summer-*, and $n = 3$ in *Winter-*, *Winter+*, and *Summer+*. Natural abundance $\delta^{15}\text{N}$ for bulk soil was measured using an elemental analyzer coupled to an isotope ratio mass spectrometer at FIRMS. We estimated organic N concentrations as the difference between total bulk soil N and extractable inorganic N per treatment.

Statistical analyses

All statistics were run using R version 4.4.0 (R Core Team, 2019). We compared gross N transformation rates and soil properties across seasons and between precipitation treatments using a linear mixed effects model. Treatment, season and their interaction were evaluated as fixed effects, and plot as a random effect, and gross N transformation rates and soil properties as response variables in separate models using nlme. When a significant interaction effect ($p < 0.05$) was detected, we applied post hoc pairwise comparisons using emmeans. Immediate effects were defined as significant effects of the treatment in the same season by comparing *Control* means to precipitation treatment means. Legacy effects were defined as a significant season and treatment interaction. We used least squares linear regressions to explore relationships among soil properties and gross N cycling rates across seasons and treatments. To understand the relationship between received precipitation and bulk soil $\delta^{15}\text{N}$, we graphed $\delta^{15}\text{N}$ from our soils collected since 2019 against cumulative precipitation of the water year per treatment at time of sampling. We used a polynomial mixed effects model to model the interaction of season, treatment, and precipitation; A quadratic term for rainfall (rain^2) was included to account for the observed non-linear relationship between $\delta^{15}\text{N}$ and precipitation. To account for the repeated measures taken from the same plots over multiple years, random intercepts were included for both plot and sampling date.

For all statistical tests, we evaluated model assumptions of normality and homogenous variance using plots of residuals. When assumptions were not met, we modeled heterogeneous variances using the nlme package.

Results

Soil gravimetric water content (GWC)

Average soil GWC was nearly 3x higher in winter than in summer and was sensitive to changes in precipitation imposed by our treatments (Fig. 3, $F_{4, 18} = 10.5$, $p < 0.0001$): in the winter, excluding precipitation significantly lowered soil GWC (*Winter-* = 2.44 ± 0.45 %) by nearly 2x relative to the *Control* (4.4 ± 0.88 %, $p = 0.0002$), and by nearly 2x times relative to treatments that received extra water (*Winter+*; 4.74 ± 0.97 %, $p = 0.0013$; *Summer+* = 4.73 ± 0.24 %, $p = 0.0067$). In the summer, excluding summer precipitation (*Summer-*; 0.33 ± 0.08 %) significantly lowered soil GWC relative to all other treatments (Fig. 3; $p < 0.04$), whereas adding extra water (*Summer+*; 3.50 ± 1.26 %) significantly increased GWC relative to all other treatments ($p < 0.03$). The legacies of past precipitation, however, had no effect on soil GWC (i.e., manipulating precipitation during the prior season had no effect on soil moisture, $F_{4,18} = 13.3$).

Soil extractable NH_4^+ and NO_3^-

We did not detect an immediate effect of excluding precipitation in the winter or summer on NH_4^+ concentrations (Fig. 4; *Winter-* in winter, $p = 0.9$; *Summer-* in summer, $p = 0.17$). However, the legacies of past precipitation did affect soil extractable NH_4^+

concentrations in the winter (Fig. 4A, $F_{4,18} = 5.88$); excluding precipitation in the summer (*Summer-*) increased extractable NH_4^+ concentration the following winter by approximately 2x over the control (*Summer-* = $1.7 \pm 0.4 \mu\text{g NH}_4^+\text{-N g}^{-1}$ soil; *Control* = $0.8 \pm 0.50 \mu\text{g NH}_4^+\text{-N g}^{-1}$ soil, $p = 0.045$). In the summer, manipulating prior winter season precipitation had no effect on NH_4^+ ; *Winter-* and *Winter+* treatments were no different than the control (Fig. 4B, $p > 0.6$).

In contrast to the observed effects on NH_4^+ , manipulating seasonal precipitation immediately affected soil extractable NO_3^- (Fig 4, $F_{4,18} = 6.31$, $p < 0.001$); excluding winter precipitation increased winter NO_3^- concentrations by nearly 6x over the control (*Winter-* = $2.6 \pm 0.3 \mu\text{g NO}_3^-\text{-N g}^{-1}$ soil; *Control* = $0.5 \pm 0.2 \mu\text{g NO}_3^-\text{-N g}^{-1}$ soil, $p = 0.0015$; Fig 4c). In contrast to the increase in NO_3^- from excluding winter precipitation, excluding summer precipitation lowered NO_3^- concentrations relative to the *Control* ($p = 0.03$). We note, however, this contrasting result was measured in soils sampled approximately one week after a large storm (Fig. 2), and it is possible surface runoff could have entered our precipitation exclusion plots. The legacies of past precipitation did not affect soil extractable NO_3^- (Fig. 4C-D); *Summer+* and *Summer-* were no different than the *Control* in the winter ($p > 0.9$), and *Winter+* and *Winter-* were no different than the *Control* in the summer ($p > 0.9$).

Gross N mineralization

Manipulating precipitation had both immediate and legacy effects on gross N mineralization rates as measured in the lab (Fig. 5A-B, $F_{4, 18} = 4.18$, $p < 0.04$).

Specifically, imposing extreme drought stress during the winter (*Winter-*) had immediate effects by lowering gross N mineralization rates ~2.5 times relative to the *Control* (Fig. 5A; $p = 0.04$; *Winter-* = $0.12 \pm 0.03 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$; *Control* = $0.30 \pm 0.08 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$). This extreme drought stress (*Winter-*) then induced a legacy effect the following summer by lowering gross N mineralization rates by ~2x relative to the *Control* (Fig. 5B; $p = 0.03$; *Winter-* = $0.19 \pm 0.03 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$ and *Control* = $0.41 \pm 0.19 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$).

Across our precipitation treatments, gross N mineralization in the wet winter had no relationship with soil GWC (Fig. 6A, $p > 0.3$). In contrast, gross N mineralization was positively correlated with soil GWC (Fig. 6B, $p < 0.0001$) in the relatively drier summer.

Gross Nitrification

Similar to gross N mineralization, manipulating precipitation in the winter had immediate effects on gross nitrification rates; inducing extreme drought by excluding winter precipitation (*Winter-* = $0.02 \pm 0.01 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$) significantly lowered gross nitrification rates by ~9.5x compared to the *Control* (Fig. 5C; *Control* = $0.19 \pm 0.05 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$, $p < 0.001$). In contrast to the winter season, manipulating summer precipitation had no immediate effect on gross nitrification rates (Fig. 5D; $p > 0.2$), where on average, we measured lower gross nitrification rates than in the wetter winter (summer season = $0.07 \pm 0.04 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$; winter season = $0.15 \pm 0.07 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$, $p < 0.001$). Moreover, gross nitrification rates in the *Summer-* plots during summer were ~13x lower than in winter, emphasizing the sensitivity of gross nitrification to drought stress (Fig.

5D; $p < 0.0001$; $0.20 \pm 0.05 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$ in winter compared to $0.02 \pm 0.02 \mu\text{g N g}^{-1} \text{ soil h}^{-1}$ in summer).

The sensitivity of gross nitrification to drought stress gave rise to a positive correlation between gross nitrification rates and soil GWC in the summer (Fig. 6D, $R^2 = 0.453$, $p < 0.0001$), but not in the winter when wetter soils would have minimized drought stress (Fig. 6C, $R^2 = 0.113$, $p = 0.117$). Consistent with this understanding, we found that soil GWC was not correlated with gross nitrification rates in the plots receiving extra water to limit drought stress (*Winter+* and *Summer+*); instead, they were positively correlated with substrate availability (i.e., soil extractable NH_4^+ ; Fig. S6A,C). In contrast, the opposite was true in the drier summer season where gross nitrification was not correlated with substrate availability (NH_4^+), but it was correlated with soil GWC (Fig. S6B,D).

Unlike the patterns observed with gross N mineralization, gross nitrification rates were not affected by the legacies of past precipitation (Fig. 5C-D, $F_{4,18} = 6.13$, $p > 0.3$); *Summer-* and *Summer+* rates were no different than the *Control* in the winter (Fig. 5C, $p > 0.3$) and *Winter-* and *Winter+* rates were no different than the *Control* in the summer (Fig. 5D, $p > 0.9$).

Dissimilatory nitrate reduction to ammonium (DNRA)

DNRA rates averaged $0.30 \pm 0.17 \mu\text{g N g}^{-1} \text{ soil d}^{-1}$ in the winter and $0.96 \pm 0.89 \mu\text{g N g}^{-1} \text{ soil d}^{-1}$ in the summer (Fig. S8) and were not affected by the immediate effects of manipulating precipitation ($p > 0.7$) nor by the legacies of past precipitation ($F_{4,18} =$

0.5, $p > 0.3$); *Summer+* and *Summer-* were not significantly different than *Control* in the winter ($p > 0.9$), and *Winter+* and *Winter-* were not significantly different than *Control* in the summer ($p > 0.3$).

In-situ net N mineralization, net nitrification, and microbial biomass C & N

Manipulating seasonal precipitation had no immediate effects on net N mineralization ($p > 0.8$) or net nitrification ($p > 0.3$) when compared to *Control* plots. However, inducing extreme drought in the winter (*Winter-* plots) significantly increased net nitrification rates by ~8x (Fig. 7C; *Winter-* = $0.06 \pm 0.04 \mu\text{g N g}^{-1} \text{ soil}$) when compared to *Summer-* treatments ($0.007 \pm 0.006 \mu\text{g N g}^{-1} \text{ soil}$, $p = 0.03$), opposite to the decrease in gross nitrification rates we observed in the lab (Fig. 5C). The legacies of past precipitation did not significantly affect either field net N mineralization (Fig. 7A-B) or net nitrification rates (Fig. 7C-D); *Summer-* and *Summer+* rates were no different than the *Control* in the winter ($p > 0.53$) and *Winter-* and *Winter+* rates were no different than the *Control* in the summer ($p > 0.14$).

Average microbial biomass C was $146 \pm 79 \mu\text{g C g}^{-1} \text{ soil}$ in the winter and $118 \pm 80 \mu\text{g C g}^{-1} \text{ soil}$ (Fig. S1A-B). Average microbial biomass N was $9 \pm 4 \mu\text{g N g}^{-1} \text{ soil}$ in the winter and $13 \pm 7 \mu\text{g N g}^{-1} \text{ soil}$ (Fig. S1C-D). We did not detect significant differences between seasons or treatments for either microbial biomass C ($F_{4,18} = 1.07$, $p > 0.8$) or N ($F_{4,18} = 2.05$, $p > 0.7$).

Soil Organic N and total C

Manipulating precipitation produced both legacy and immediate effects on soil organic N (Fig. S2, $F_{4, 18} = 4.27$, $p < 0.043$). Excluding winter precipitation produced a legacy effect the following summer by lowering soil organic N ($p = 0.04$); summer organic N in the *Winter-* treatments ($13 \pm 3 \mu\text{g N g}^{-1}$ soil) were $\sim 2\text{x}$ lower than the *Control* ($23 \pm 6 \mu\text{g N g}^{-1}$ soil). Besides legacy effects, excluding precipitation in the winter significantly reduced organic N by $\sim 2.5\text{x}$ compared to the *Control* (*Winter-* = $12 \pm 2 \mu\text{g N g}^{-1}$ soil; *Control* = $27 \pm 8 \mu\text{g N g}^{-1}$ soil h^{-1} , $p = 0.04$). In contrast, manipulating precipitation had no effect on total C ($p = 0.3$).

Bulk soil $\delta^{15}\text{N}$ Natural Abundance

Manipulating seasonal precipitation did not have a significant effect on bulk soil $\delta^{15}\text{N}$ regardless of sampling date (Fig. S3A, $p > 0.3$). We also did not observe a significant relationship between bulk soil $\delta^{15}\text{N}$ and annual cumulative precipitation (measured as cumulative precipitation per water year; Fig. S3B, $p > 0.3$).

Discussion

To understand how altered precipitation and the legacies of past precipitation affect inorganic N supply in soils (i.e., gross N mineralization and nitrification), we measured gross and net rates of soil N mineralization and nitrification and inorganic N concentrations after manipulating precipitation in a Pinyon–Juniper dryland. We

hypothesized that: 1) N accumulates in dry soils because gross N mineralization and nitrification persist during drought; and 2) the effects of drought and precipitation persist through seasons because of a lag in microbial response to changes in precipitation between seasons (i.e., a precipitation legacy effect). In alignment with our first hypothesis, we found that drought lowered both gross N mineralization and nitrification rates, but the rates remained measurable even after exposing soils to extreme drought, suggesting inorganic N supply can persist under drought stress. We also found that the effects of drought on inorganic N supply carried over between seasons, establishing precipitation legacies that reduced soil gross N mineralization rates, but did not affect gross nitrification rates, partially supporting our hypothesis. Further, we observed cases in which these precipitation legacies increased soil inorganic N pools, implying N immobilization rates must have decreased below those of inorganic N supply to allow N to accumulate in soils. Together, these immediate and legacy effects induced by shifts in precipitation help explain the mechanisms interacting to carry over N between seasons (i.e., the N carryover hypothesis; Krichels, Greene, et al., 2023; Sala et al., 2012; Shen et al., 2016). Moreover, they suggest that expected increases in drought frequency and severity can affect soil N cycling by reducing inorganic N supply rates but still increasing inorganic N pools, with these effects persisting after the drought ends.

Effects of reduced precipitation on soil inorganic N supply and concentrations

We found that the interaction between sustained rates of inorganic N supply coupled with reduced N immobilization rates can help explain why soil inorganic N pools increased under drought stress. Inducing extreme drought (*Winter-* in the winter season) significantly reduced both gross N mineralization and nitrification rates compared to ambient conditions, but the rates remained above zero (Fig. 5), consistent with NO_3^- accumulating under dry conditions (Fig. 4C). For NO_3^- to accumulate in soils when inorganic N supply decreased, a simultaneous reduction in N immobilization rates would have been required for at least two reasons: First, we observed positive in-situ net N mineralization and net nitrification rates under extreme drought (*Winter-* treatment in the winter; Fig. 7A, C), which can only occur if the rate of gross inorganic N supply exceeds N immobilization. Given that gross inorganic N supply rates persisted under dry conditions, albeit at reduced rates, N immobilization must have been inhibited to yield net positive N supply rates in the field. Second, our findings are consistent with dry soils restricting substrate diffusion and, therefore, constraining plant and microbial N immobilization (Dijkstra et al., 2015; Homyak et al., 2016; Stark & Firestone, 1995). In the treatments where we increased precipitation to favor diffusion (i.e., *Winter+* and *Summer+*), we observed a significant positive correlation between gross nitrification and NH_4^+ in the wet winter (Fig. S6C), implying substrate availability limits these transformations, not water. In contrast, in the drier summer, water availability was positively correlated with gross nitrification (Fig. S6B), implying a breakdown in diffusion and reduced N immobilization, consistent with winter drought substantially

reducing plant biomass (Spasojevic et al., 2022). Thus, our results suggest that drought stress may not completely shut down inorganic N supply, which together with relatively more restrictions on N immobilization rates and N uptake, inorganic N pools accumulate under increasingly arid conditions to carry over into the following seasons.

Precipitation manipulation effects on the N carryover hypothesis

Precipitation patterns from the previous season established precipitation legacy effects on soil inorganic N supply rates and N concentrations the following season. Specifically, the legacy effect of excluding precipitation in the winter (i.e., imposing an extreme drought) reduced gross N mineralization rates the following summer (Fig. 5B). Microbial activity has been observed to decrease under drought stress (Moyano et al., 2013; Reichman et al., 1966.; Stark & Firestone, 1995) helping to explain why extreme winter drought could have kept microbial activity low into the summer (Shen et al., 2016). However, we did not find a precipitation legacy effect on microbial biomass (Fig. S1), consistent with microbial biomass not changing or even increasing with drought stress (Parker & Schimel, 2011; Schaeffer et al., 2017), suggesting other factors beyond microbial activity may have controlled this legacy. In fact, soil organic N pools consistently decreased in the *Winter-* treatment in both seasons (Fig. S2), suggesting reduced substrate availability, presumably from reduced organic N inputs in response to reduced plant biomass at our site (Shen et al., 2016; Spasojevic et al., 2022), may have been the main factor constraining gross N mineralization rates.

The sensitivity of gross N mineralization and inorganic N pools to past precipitation supports the “N carryover” hypothesis, where prior dry periods increase N

availability into subsequent seasons (Krichels, Greene, et al., 2023; Shen et al., 2016). Our results reveal that this buildup of N in dry soils is due to N supply persisting even after the drought ends. Moreover, the decrease in N immobilization during drought, via the shut down of N sinks to increase available N (Dijkstra et al., 2015), likely allows N to carry over into the next season, as indicated by higher winter NH_4^+ concentrations when summer precipitation was excluded (Fig. 4A). Thus, our results reveal a sensitivity in gross N mineralization and inorganic N pools to precipitation legacies, fueling mechanisms behind the N carryover hypothesis observed in other studies.

In contrast to gross N mineralization, gross nitrification rates were not sensitive to the legacies of past precipitation. It is well established that substrate availability (i.e., NH_4^+) and substrate diffusion are key controls over nitrification (Stark & Firestone, 1995), suggesting that the carryover of NH_4^+ and/or soil moisture into the following season would be necessary to establish a precipitation legacy for nitrification (Shen et al., 2016). While soil moisture did not carry over from winter to summer (Fig. 3), likely due to fast evaporative losses (Reichmann et al., 2013; Shen et al., 2016), NH_4^+ was carried over from a moderate drought imposed the previous summer into the winter (Fig. 4A), likely due to diffusion constraints limiting NH_4^+ uptake (Stark & Firestone, 1995). We expected this NH_4^+ carryover into winter to establish a precipitation legacy effect on gross nitrification, but it did not (Fig. 5C), suggesting other factors beyond water and substrate availability were important. It is possible the wet conditions of winter may have increased competition for NH_4^+ by heterotrophic microbes, as nitrifiers are poor competitors for NH_4^+ (Recous et al., 1990; Schimel et al., 1989). Further, as nitrifiers

have a higher sensitivity to drought relative to microbes responsible for N mineralization (Stark & Firestone, 1995), the summer drought may have adversely impacted these nitrifying communities, thereby preventing the establishment of precipitation legacies on gross nitrification despite the high substrate availability. Overall, our results suggest that despite ample substrate supply for nitrification, precipitation legacies were not established the following season, likely highlighting the sensitivity of nitrifying communities to current soil moisture that supersede effects from precipitation legacies.

Broader ecosystem implications

The long-term effects of precipitation legacies on the soil N cycle are difficult to predict, in part because of the relatively limited number of observations available to calibrate models and increase their predictive power. However, our measurements lend support to developing model structures that can integrate the legacies of past precipitation into forecasts of ecosystem N availability. In particular, model structures that integrate the carryover of N between seasons (i.e., the N carryover hypothesis; Krichels, Greene, et al., 2023; Sala et al., 2012; Shen et al., 2016) may improve forecasts of ecosystem N availability and losses. For example, previous work at our site established strong links between the carryover of N between seasons and the emission of N gases upon rewetting dry soils (Krichels et al., 2023). As drying–rewetting cycles can promote N emissions due to asynchrony between N sources and sinks, the carryover of N due to persistent N supply relative to N immobilization may further enhance the capacity of drylands to lose N during these transitions. As precipitation becomes more variable in tandem with expected

increases in drought frequency and severity (Seager et al., 2007), the consequences of drying–rewetting cycles on soil N availability may be exacerbated in dryland ecosystems.

Whether shifts in precipitation patterns that favor the carryover of N, together with more frequent soil drying–rewetting events, may have long-term consequences on ecosystem N cycling, may be assessed by bulk soil $\delta^{15}\text{N}$ values as an index of overall N availability. We expected that manipulating precipitation over the course of five years would have altered bulk soil $\delta^{15}\text{N}$ values in alignment with observations from other studies (i.e., an increase in bulk soil $\delta^{15}\text{N}$ as drought favored N loss over N being recycled; Brunello et al., 2024; Mao et al., 2024; Von Sperber et al., 2017; Wang et al., 2014). However, even imposing our most extreme drought (*Winter-*) for five years did not alter $\delta^{15}\text{N}$ values relative to the *Control* (Fig. S3A), suggesting our treatments may not have altered aridity significantly enough to produce a response on their own. Nevertheless, we did observe the expected negative trend in which bulk soil $\delta^{15}\text{N}$ increased as cumulative annual precipitation decreased across all treatments (Fig. S3B), raising questions as to why bulk soil $\delta^{15}\text{N}$ increases with decreasing annual precipitation but not directly in response to our treatments. New studies that evaluate seasonal changes in precipitation relative to those experienced over multiple years may be required to understand bulk soil $\delta^{15}\text{N}$ dynamics in response to changes in precipitation.

Future climate projections predict an increase in drought frequency and intensity (i.e., longer durations; Seager et al., 2007), which can alter soil N transformations in drylands in unique ways relative to global patterns. On a global scale, the biological N cycling in arid regions are more sensitive to increases in precipitation than they are to

decreases in precipitation (Wu et al., 2022). This is likely because microbial populations in water-stressed regions are already adapted to drought and, therefore, show stronger drought resistance (Zhou et al., 2018). Consistent with these findings, drought did not lower microbial biomass at our site (Fig. S1). However, opposite to the prevailing global patterns, we found that decreases in precipitation had a greater effect on soil inorganic N supply and extractable N than increases in precipitation. This is likely because dryland primary productivity is co-limited by both water and N (Hooper & Johnson, 1999) and drought constrains both of these resources, whereas increasing precipitation alleviates drought stress and increases competition for N (Cregger et al., 2014). Thus, our findings highlight how global patterns may fail to predict dryland ecosystem function, suggesting how roughly 1/3 of the Earth's surface may respond in unique ways to shifts in precipitation patterns.

Conclusion

Future climate projections predict an increase in the frequency and intensity of drought (i.e., longer periods without precipitation) (Li et al., 2024; Trenberth, 2011; IPCC 2014). Our results show that extreme drought can reduce the rate of inorganic N supply to soils, as both gross N mineralization and nitrification rates decreased under drier conditions, but neither were completely shut down. Under persistent—albeit slow— inorganic N supply that outpaced N immobilization rates, we found that N carried over between seasons, as influenced by both immediate and legacy effects of changing precipitation patterns. With the global climate trending towards aridity, paired with the

projection of dryland expansion (Feng & Fu, 2013; Huang et al., 2016), our results contribute to growing evidence for how droughts can increase N availability, reflecting how drylands and the future of other ecosystems may respond under increasingly arid conditions.

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Tables

Table 1 – Annual precipitation (mm) at our study site separated by treatment and mean annual precipitation (MAP) for the length of our study since manipulation began in August of 2018. Data collected from on-site rain gage.

Treatment	2018 (mm)	2019 (mm)	2020 (mm)	2021 (mm)	2022 (mm)	2023 (mm)	MAP (2018- 2023)
Control	193.8	399.5	188.5	185.2	176.4	278.1	236.9
Summer-	103.4	326.4	188.5	127.5	49	170.9	160.9
Winter-	90.4	73.1	0	57.7	127.4	107.1	76.0
Summer+	222.98	421.1	188.5	199.8	180.1	314.6	254.5
Winter+	208.35	529.7	275.5	192.5	216.6	292.7	285.9

Figures

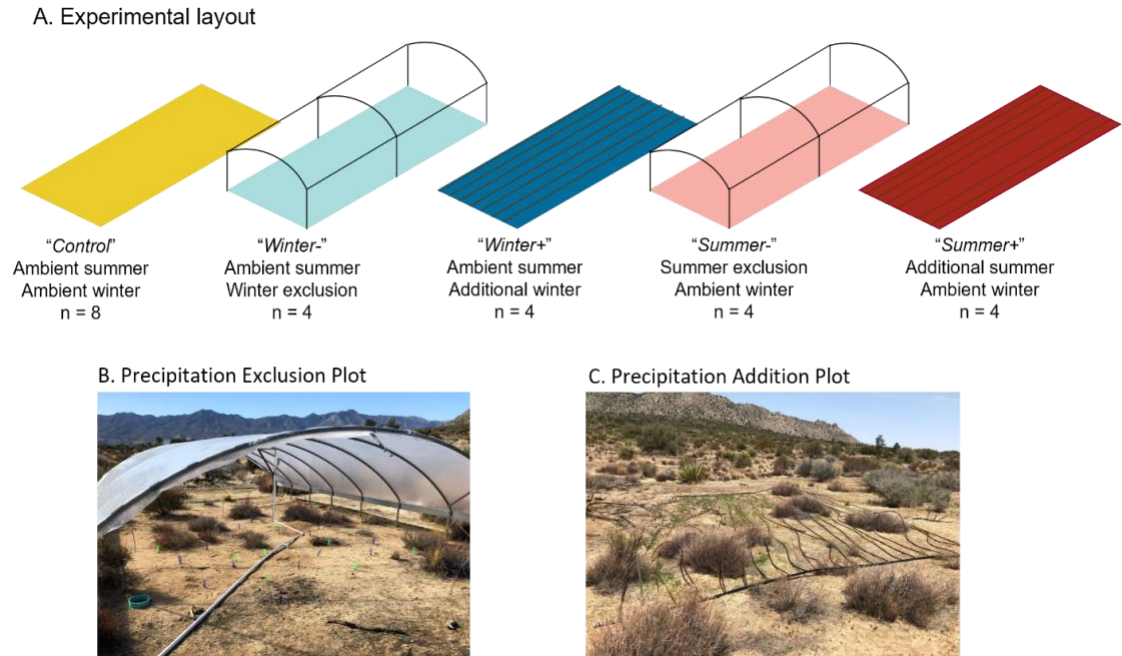


Figure 1 – **A)** Field experimental layout of our research site. **B)** Plastic roofing was used to exclude precipitation in *Winter-* and *Summer-* plots and **C)** irrigation lines were used to supplement precipitation in *Winter+* and *Summer+* plots.

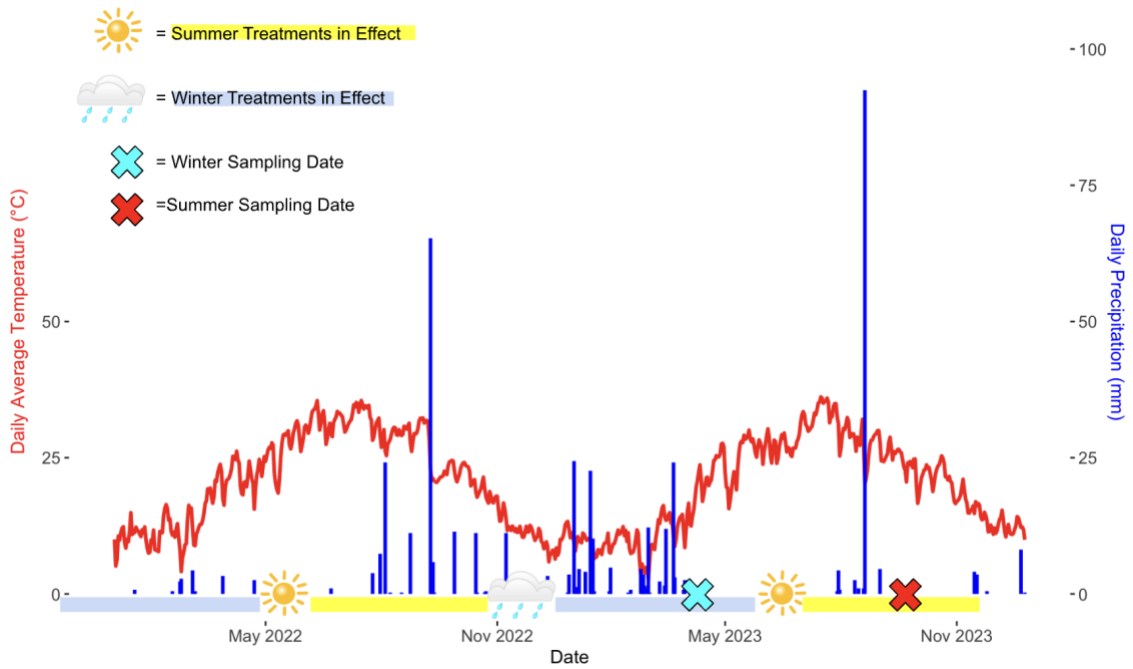


Figure 2 – Daily precipitation and air temperature measured at our field site. Symbols represent when winter (*Winter-* and *Winter+*) or summer treatments (*Summer-* and *Summer+*) are in effect and their duration. “X” denotes when soil samples were collected in winter and summer 2023.

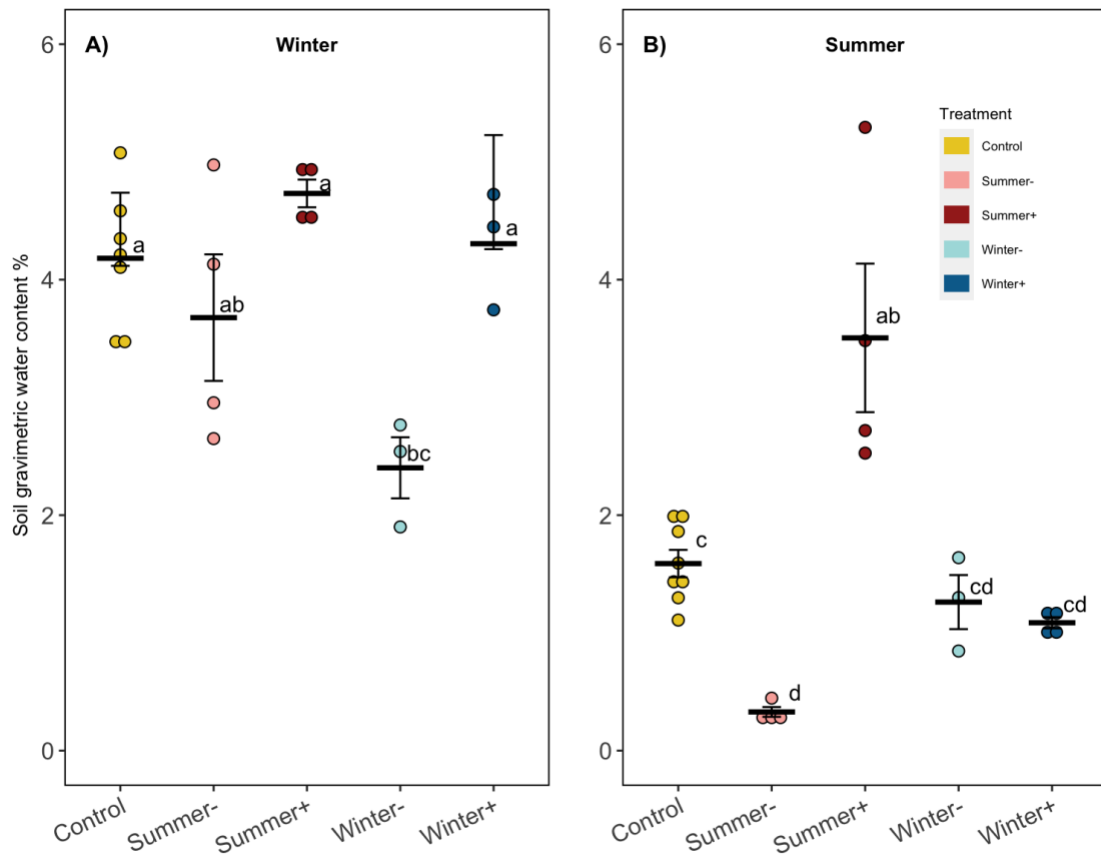


Figure 3 – Soil gravimetric water content (%; $g_{\text{water}} g_{\text{soil}}^{-1}$) for each treatment in the A) winter and B) summer of 2023. For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors ($n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments).

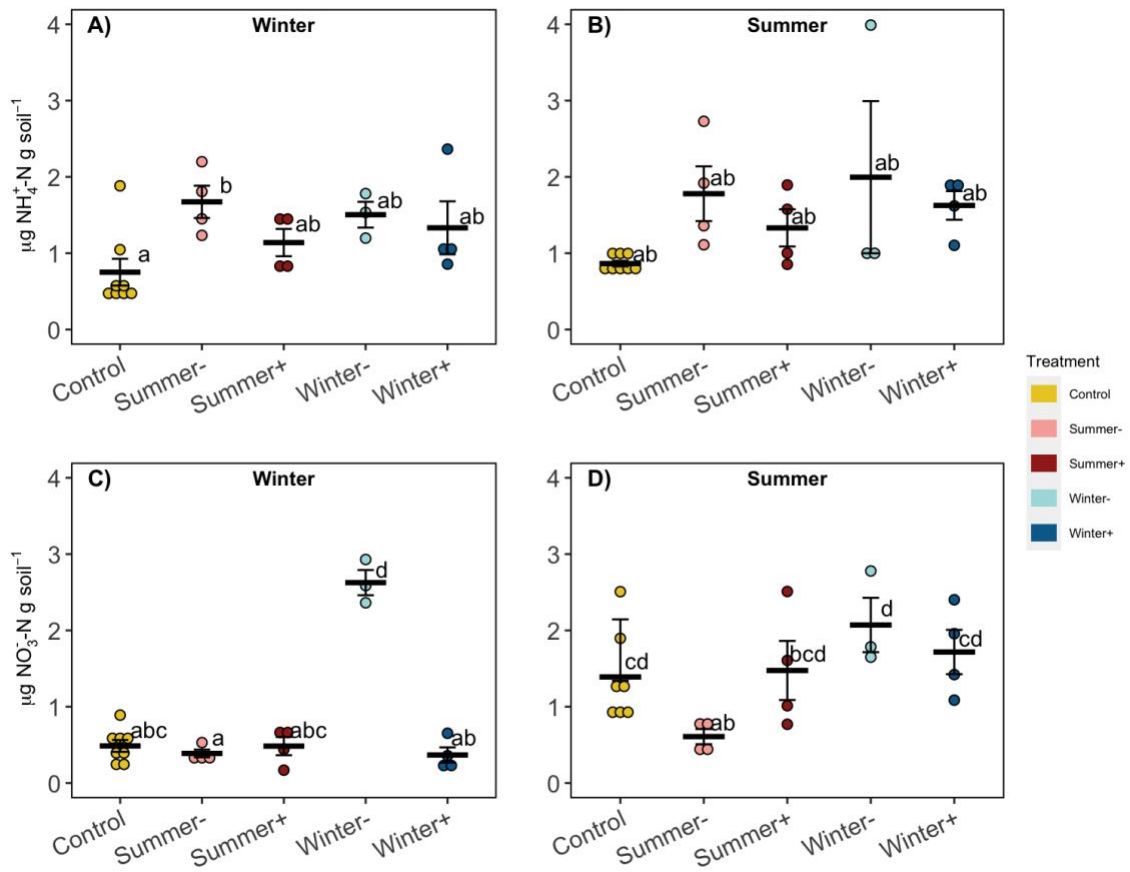


Figure 4 – Soil extractable ammonium (NH_4^+) and nitrate (NO_3^-) concentrations from our treatment plots during summer and winter 2023. For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors ($n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments).

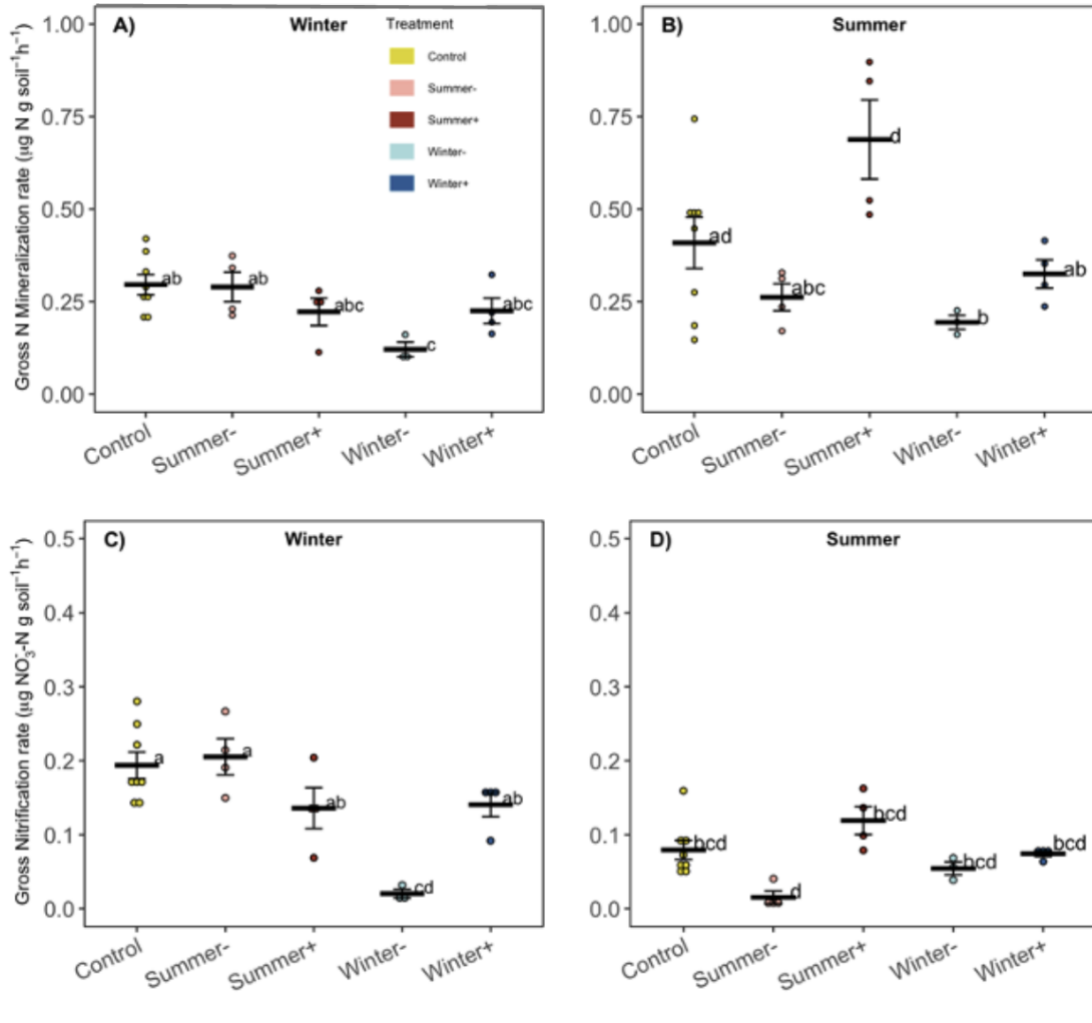


Figure 5 – Gross N mineralization and gross nitrification rates measured from soils collected at our site in winter and summer 2023. For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors ($n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments).

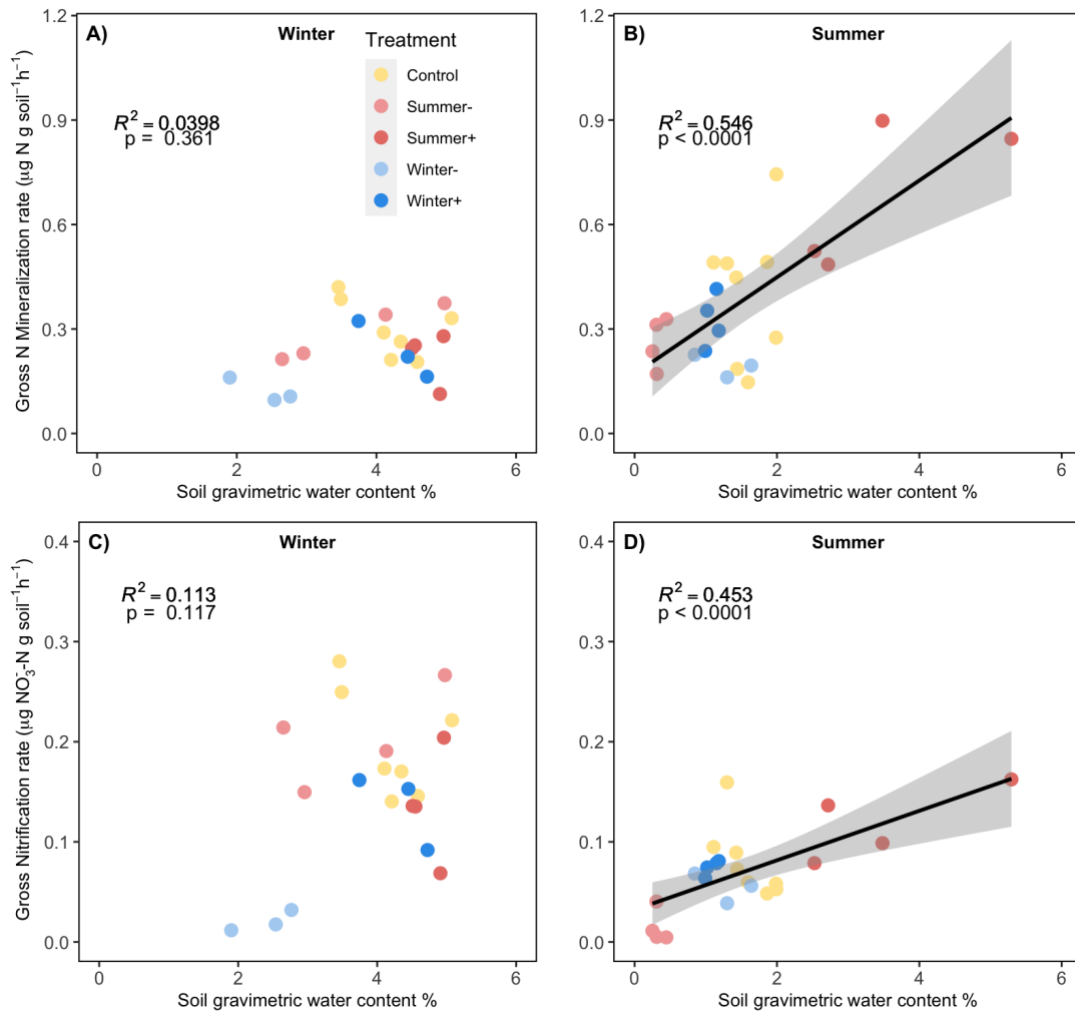


Figure 6 – Relationships between gross N transformation rates and soil gravimetric water content (%; $g_{\text{water}} g_{\text{soil}}^{-1}$) during both winter 2023 and summer 2023. The shaded area represents the 95% confidence interval around the trend line.

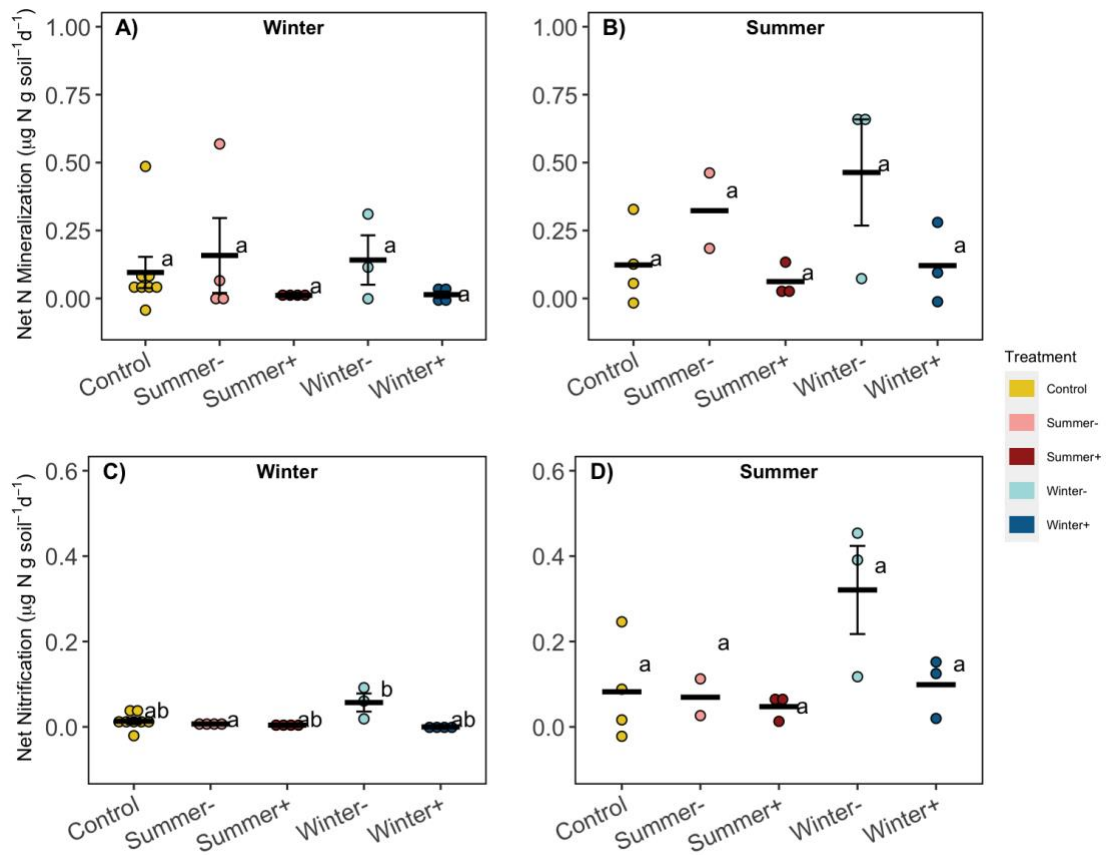


Figure 7– Net N mineralization (A, B) and net nitrification (C, D) rates from 30-day field incubations in winter (April) and summer (September) of 2023.

For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors (Winter: $n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments; Summer: $n = 3$ for *Control*, *Summer+*, *Winter+*, and *Winter-*, $n = 2$ for *Summer-*).

Supplemental Figures

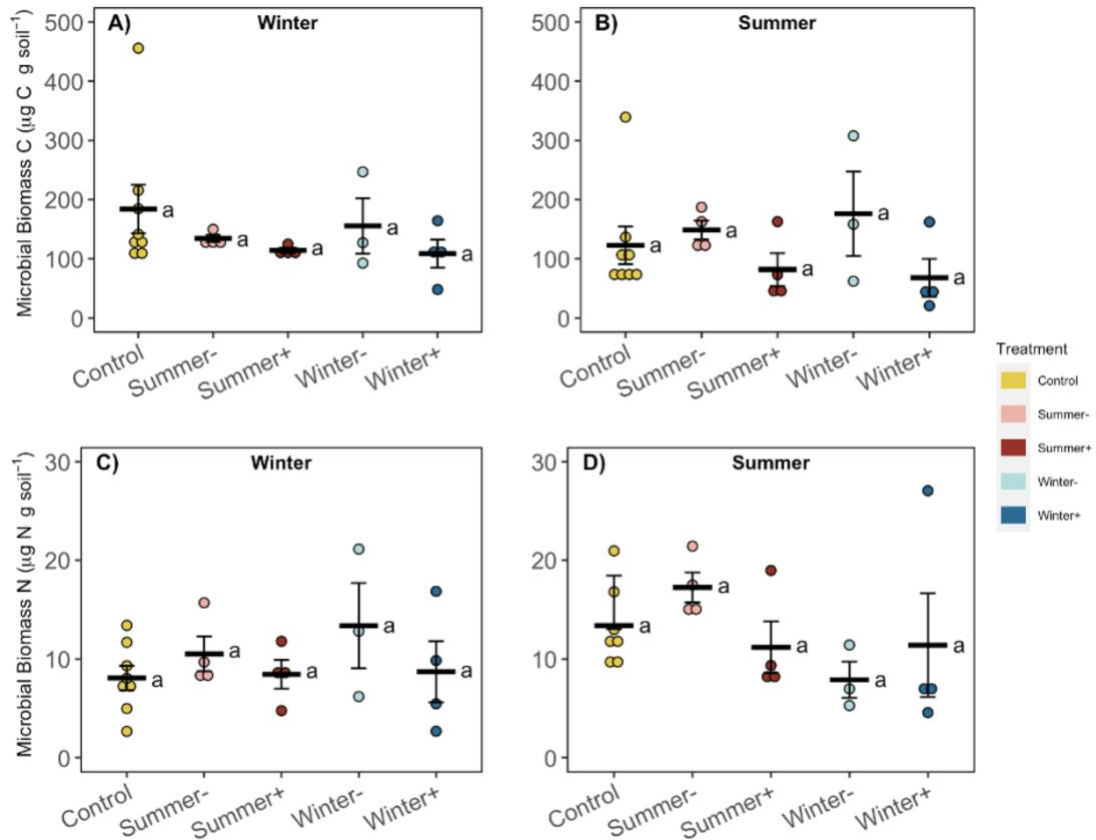


Figure S1 – Microbial biomass C and N during winter 2023 and summer 2023 at our site. For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors ($n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments).

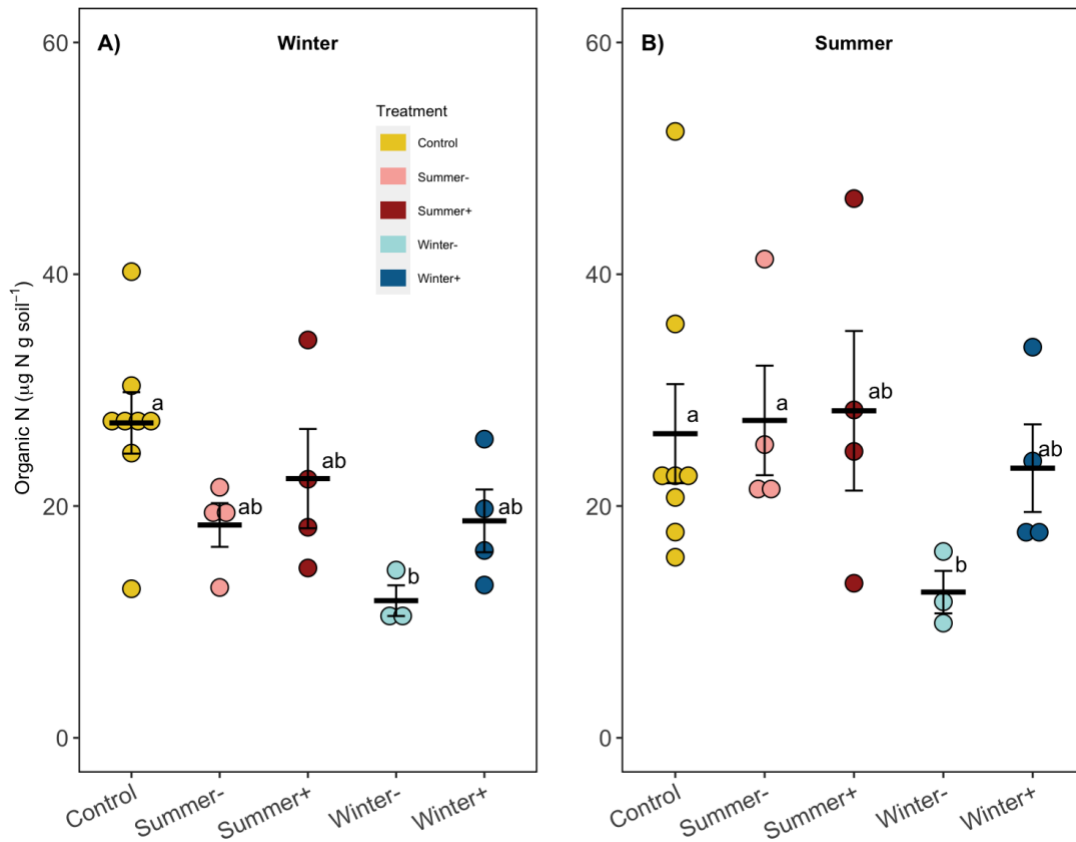


Figure S2 – Soil organic N during winter 2023 and summer 2023, estimated as the difference between total inorganic N and total N. For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors ($n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments).

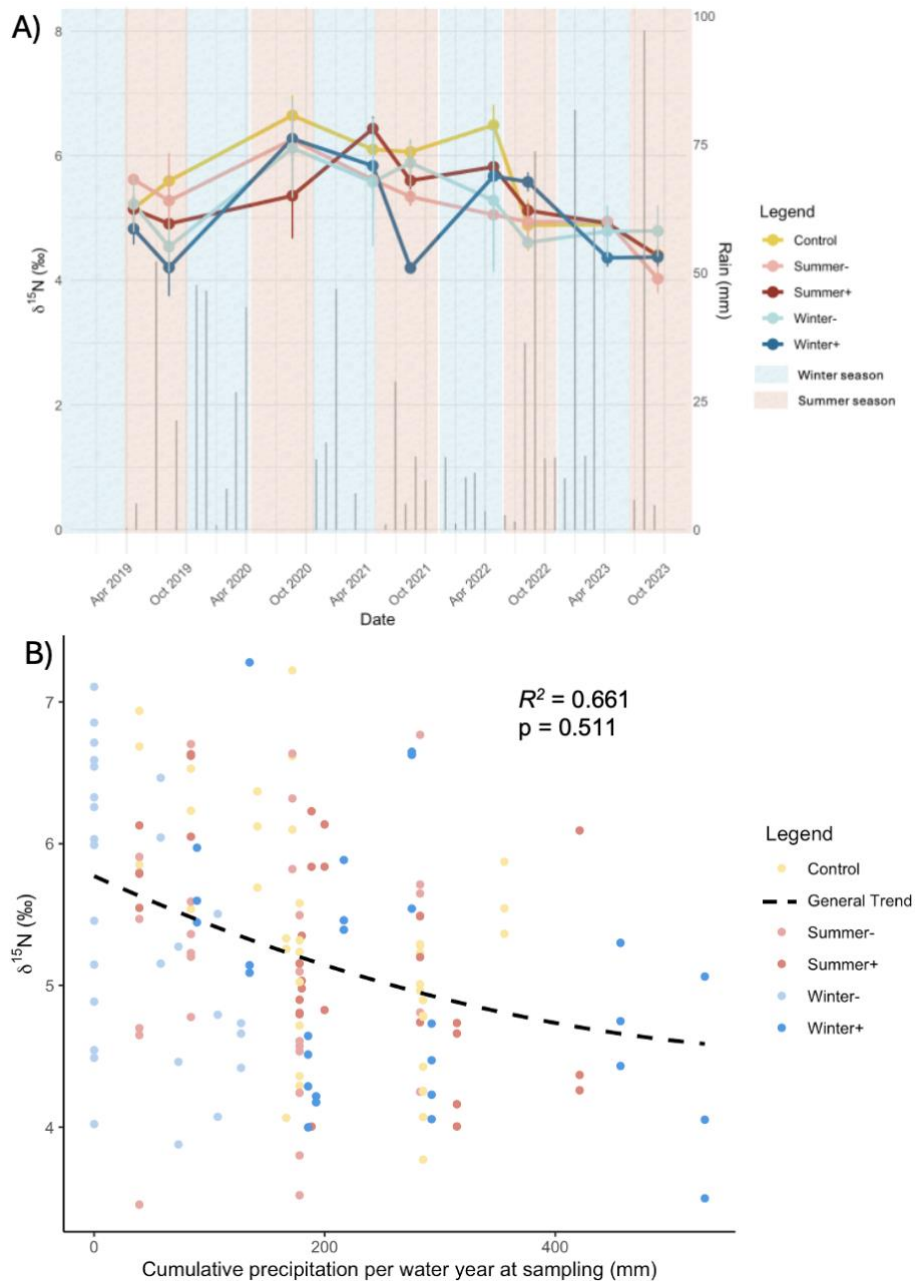


Figure S3 – A) Bulk soil $\delta^{15}\text{N}$ natural abundance (permil; ‰) and average monthly precipitation (mm) from April 2019 until September 2023. The wet winter season is represented by the blue background and the drier summer season by the red background. Circles represent the average bulk soil $\delta^{15}\text{N}$ and error bars represent standard errors ($n = 8$ for the *Control*, and $n = 4$ for all other treatments). For a description of treatments, see Fig. 1. **B)** Relationship between bulk soil $\delta^{15}\text{N}$ (permil; ‰) per treatment and cumulative precipitation for the water year at the time of sample collection (mm). The dotted line represents overall trend modeled by a linear mixed effects model.

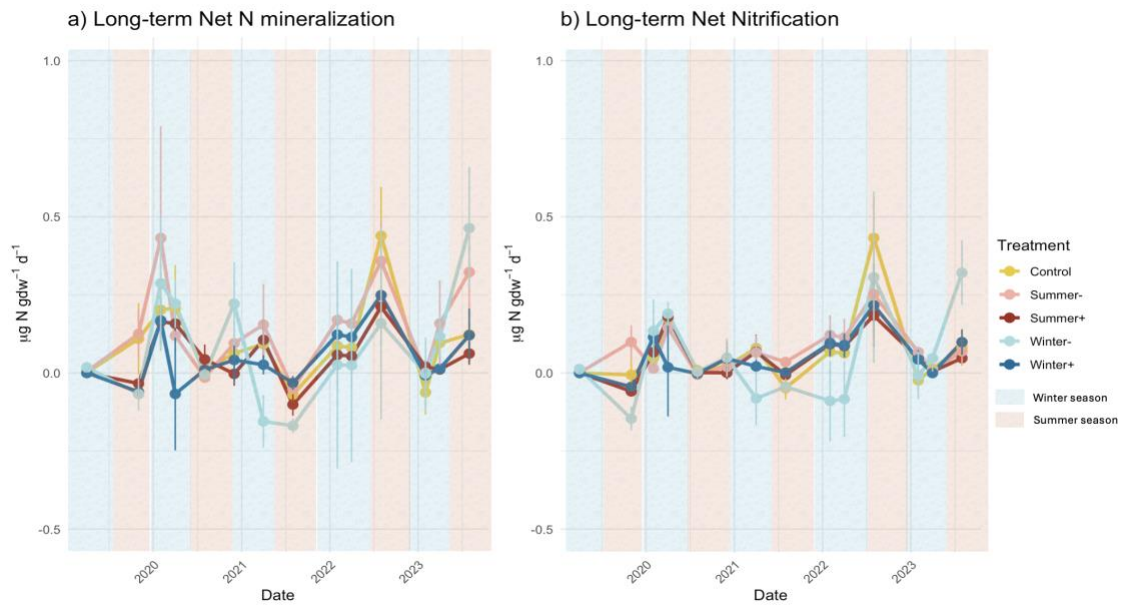


Figure S4 – In-situ net N mineralization and net nitrification rates over a ~ 30-day field incubation at our site from April 2019 until September 2023. The wet winter season is represented by the blue background and the drier summer season by the red background. For a description of treatments, see Fig. 1. Circles represent averages and vertical lines represent standard errors ($n = 8$ for the *Control*, and $n = 4$ for all other treatments).

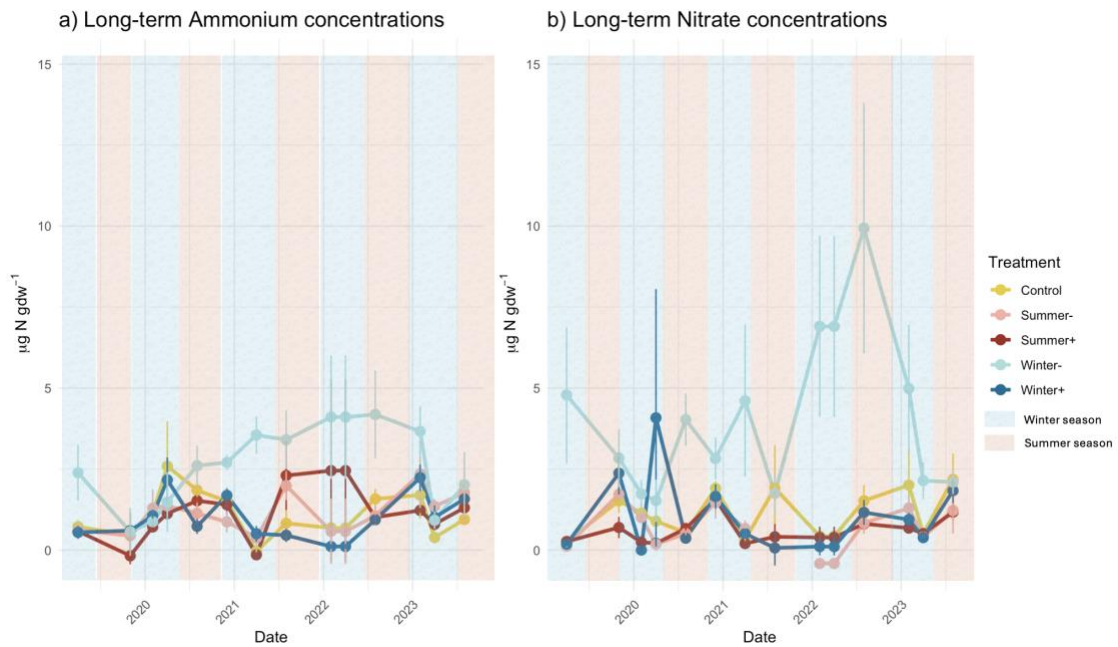


Figure S5 – In-situ a) ammonium and b) nitrate concentrations per treatment at our site from April 2019 until September 2023. Circles represent average N concentration at sample date, and vertical lines represent standard errors ($n = 8$ for the *Control*, and $n = 4$ for all other treatments). The wet winter season is represented by the blue background and the drier summer season by the red background. For a description of treatments, see Fig. 1

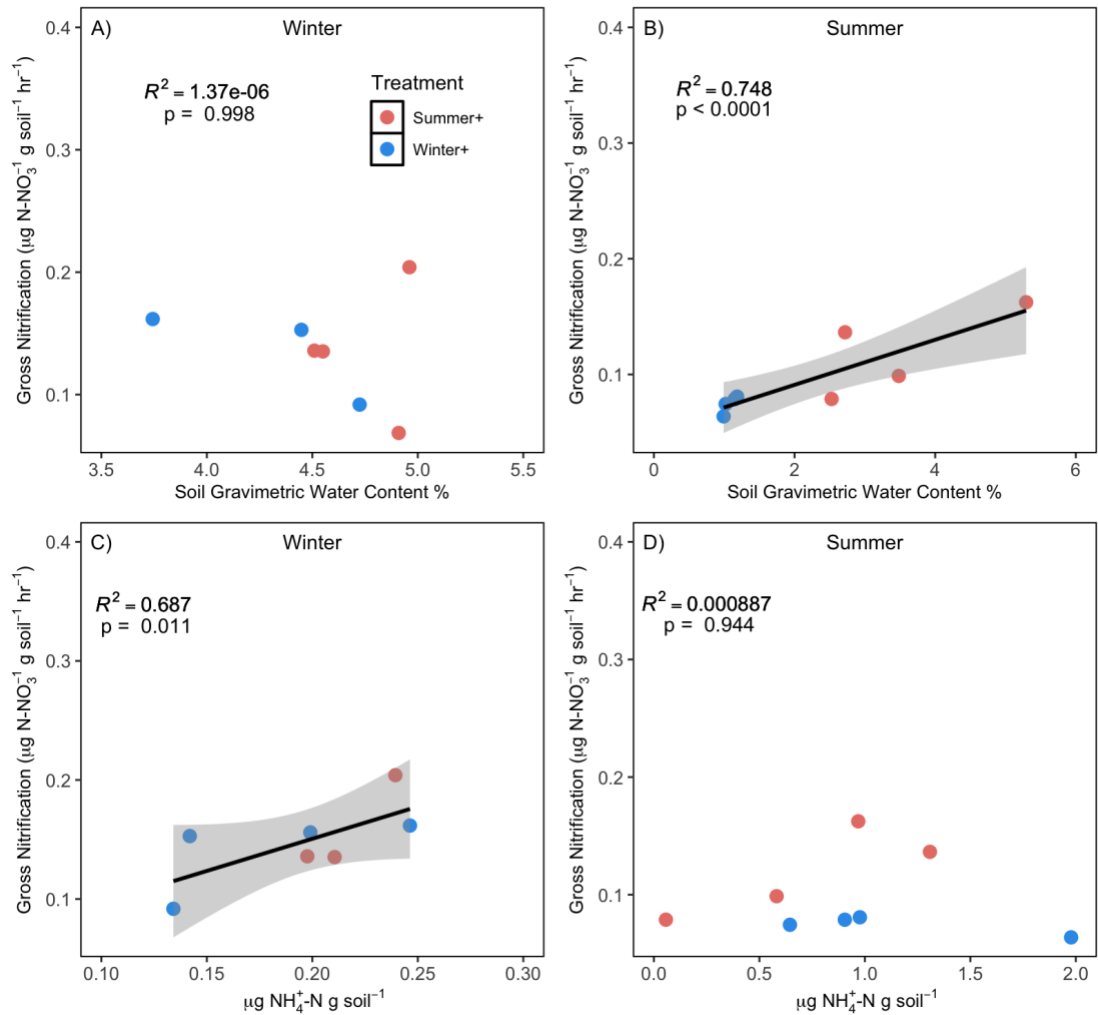


Figure S6 – Relationship between gross nitrification rates and soil gravimetric water content (%; $\text{g}_{\text{water}} \text{g}_{\text{soil}}^{-1}$) for water addition plots (*Summer+* and *Winter+*) during A) winter 2023 and B) summer 2023; or between gross nitrification rates and extractable NH_4^+ 15 minutes post-label addition during the isotope pool dilution in C) winter and D) summer 2023. The shaded area represents the 95% confidence interval around the trend line.

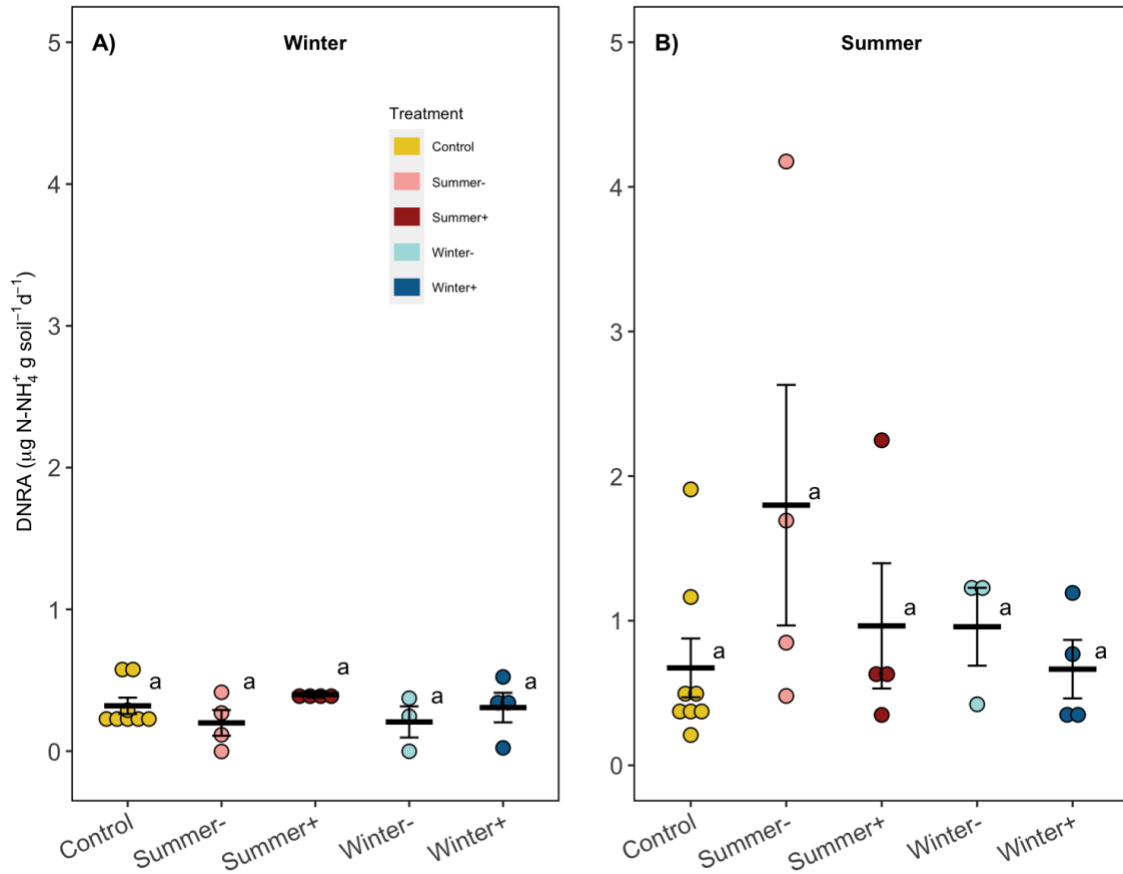


Figure S7 – Dissimilatory nitrate reduction to ammonium rates measured from soils collected at our site in A) winter and B) summer 2023. For a description of treatments see Fig. 1. Different letters denote statistically significant differences between treatments among seasons as determined by pairwise comparisons following a linear mixed effects model ($\alpha = 0.05$). Black horizontal lines represent average soil gravimetric water content, dots are individual observations, and error bars represent standard errors ($n = 8$ for the *Control*, $n = 3$ for *Winter-*, and $n = 4$ for all other treatments).