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Simulated nitrogen deposition and precipitation events alter microbial carbon cycling during early stages of litter decomposition

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**Abstract**

Plant litter decomposition is a major nutrient input to terrestrial ecosystems that is primarily driven by microorganisms. Litter quality is considered a key drive of decomposition; however, human-induced global disturbance like nitrogen deposition and increasing extreme precipitation events will shift nutrient availability during litter decomposition. Little is known about how shifting nutrient availability will impact dissolved organic matter concentrations and microbially driven carbon cycling that are critical to soil organic matter formation. This study investigated the effect of simulated nitrogen deposition and repeated precipitation events on microbially-driven carbon flow during short-term litter decomposition using a ‘common garden’ experiment with microcosms containing sand and blue grama grass litter inoculated with different microbial communities. Overall, nitrogen deposition decoupled respiration and dissolved organic carbon (DOC) by increasing respiration and not affecting DOC concentrations. Moreover, nitrogen deposition had no effect on microbial carbon use efficiency (CUE). Repeated simulated precipitation events significantly increased DOC concentrations, decreased microbial CUE, increased the microbial metabolic quotient ($qCO_2$), and altered microbial composition and diversity. These findings highlight the complex interactions and responses of surface litter decomposers to shifting nutrient availability and contradicts previous findings that nitrogen deposition will increase soil carbon sequestration from a larger supply of DOC and reduced respiration.

**Sustainability Statement**

This study into the impact of nitrogen deposition and repeated precipitation events on microbially-driven carbon flow during litter decomposition holds important implications for ensuring healthy soils under climate change. It is demonstrated in this controlled experiment that increases in soil carbon sequestration from nitrogen deposition are unlikely to be from a larger dissolved organic matter pool, which has implications on longer-term carbon storage through mineral-binding. These findings focused on understanding the impact of nutrient availability on microbially-driven carbon flow to the soil or the atmosphere support the United Natation’s sustainable development goals Life on Land and Climate Action.

## Introduction

Litter decomposition by microorganisms is an essential process to input carbon, nitrogen, and other nutrients into soil (Prescott 2010, Cotrufo et al. 2015). Dissolved organic carbon (DOC) from litter decomposition can be transported to deeper layers in the soil where it can be immobilized on mineral surfaces for long periods of time, serving as a carbon sink (Ahrens et al. 2015). Therefore, understanding the abiotic and biotic controls of DOC flux to soil during litter decomposition is an important step in developing management strategies for increasing soil carbon stabilization.

Historically, litter decomposition models have emphasized the role of climate, soil physical factors, and litter traits on litter decomposition rates (Krishna and Mohan 2017). Specifically, the initial litter chemistry (i.e. substrate quality) was considered the main control of litter-derived dissolved organic matter (DOM) concentrations (Scheibe and Gleixner 2014). However, research has discovered that during early stages of decomposition the decomposer community regulates nutrient loss and climate is the main regulator during later stages of decomposition (García-Palacios et al. 2016). More recently, microbial community composition was found to create significant variation in carbon flow (DOC and respiration) across litter types of varying substrate quality during short-term litter decomposition (Kroeger et al. 2021). These findings add to a growing body of literature (Albright, Thompson, et al. 2020, Albright, Johansen, et al. 2020, Albright et al. 2022, Campbell et al. 2022) that highlight the important influence of microbial community composition on carbon flow during early stages of litter decomposition. Furthermore, this microbially driven variation in carbon flow during litter decomposition also changes the chemical composition of the DOM (Campbell et al. 2022). In these previous studies, large microbiome effects were quantified unambiguously by holding environmental conditions constant, providing the same litter substrate, and isolating the litter microbiome as the single factor driving variation in carbon flow. However, because environmental conditions in nature routinely vary over space and time, it is imperative to understand the interaction of environmental factors with microbiome effects, especially in the context of global change (Cavicchioli et al. 2019).

Two global change factors that are expected to influence carbon flow are increased nitrogen deposition and extreme precipitation events. Anthropogenic nitrogen deposition is a contributor to global change, whereas increased intensity of precipitation events is a consequence of climate change with uncertain feedbacks (Tabari 2020, Zhang et al. 2021). The general effects of nitrogen deposition and precipitation on DOC concentrations are unclear. Some studies have found no effect of nitrogen addition on DOC concentration (Magill and Aber 2000, Tian et al. 2019, Wang et al. 2021), while others have observed positive effects (Sleutel et al. 2009, Fang et al. 2014), and some have found a negative effect (Park, Kalbitz and Matzner 2002, Lu et al. 2013). These variable effects may be caused by differences in the microbial communities and their response to the added nitrogen, with some communities potentially increasing activity through increased extracellular enzyme production (Keeler, Hobbie and Kellogg 2009, Lv et al. 2013, Jiang et al. 2014, Ullah et al. 2019), others decreasing activity due to acidification (Riggs and Hobbie 2016) or lower need for nitrogen mining from organic matter (Moorhead and Sinsabaugh 2006). The effect of precipitation on litter-derived DOC concentrations is clear with studies finding a positive relationship between rainfall frequency and intensity with DOC (Deng et al. 2018, Yang et al. 2020) likely caused by the (1) leaching of soluble plant litter compounds (Deng et al. 2017) and (2) physical removal of labile carbon compounds before microbial consumption. The effect of removing DOM via transport into the soil where it could bind to minerals on microbially-driven litter decomposition is less clear. On one hand, removal of labile carbon sources (e.g. sugars) from plant litter would be expected to decrease decomposer microbial activity, biomass, and respiration (Cotrufo et al. 2013). On the other hand, DOM removal may increase microbial activity if the DOM includes microbial inhibitors such as aliphatic acids and phenolic compounds (Jönsson and Martín 2016, Tramontina et al. 2020). Since precipitation events naturally transport DOM from the surface plant litter to the soil, simulated precipitation events can be used to further understand how litter decomposition microbial communities respond to shifting nutrient availability and the impact on microbially-driven carbon flow during the early stages of plant litter decomposition.

The objective of this study was to understand the effect of shifting nutrient availability through simulated nitrogen addition and precipitation events on microbially-driven carbon flow during early-stage litter decomposition. We hypothesized that increased nitrogen availability through nitrogen deposition would increase microbial activity while DOM removal via simulated precipitation events would decrease microbial activity during short-term litter decomposition. We expected that these shifts in nutrient availability would alter the disparate microbially driven carbon flow previously observed during short-term litter decomposition (Kroeger et al. 2021). To test these hypotheses, we used a 'common garden' experiment, where microcosms containing sterilized blue grama plant litter and sand were inoculated with twelve distinct soil microbial communities that were subjected to 4 different nutrient availability conditions: (1) both carbon and nitrogen limited, (2) carbon limited, (3) nitrogen limited, (4) no carbon or nitrogen limitation. To quantify the impact of nutrient availability on these communities, we measured respiration, microbial biomass, DOC, and total dissolved nitrogen along with microbial community structure in a subset of communities.

## Materials and Methods

### Microcosm Construction

Microcosms consisted of 7 g sterile sand and 0.1 g (dry weight) of 1 cm cut blue grama litter in a clear 125mL Wheaton serum bottle (DMK Life Sciences, Millville, NJ, USA) (Experiment 1 total microcosms = 156; Experiment 2 total microcosms = 44) (Supplemental Figure 1). Microcosms were constructed by first adding 7g of premium play sand (Quikrete, Atlanta, GA, USA) and then sterilized by autoclaving at 121°C for 1 hour. After cooling for 12 hours, the 0.1g (dry weight) plant litter was added on top of the sand and autoclaved 2 additional times for 1 hour with at least 12 hours between cycles. Blue grama plant litter was collected from the Valles Caldera National Preserve (Permit # VALL-2019-SCI-0021) (GPS coordinates: 35° 58' 28" N 106° 32'33" W, 2589 m elevation). The initial C:N ratio for the blue grama litter was 53.95 ± 0.83.

The microbial communities used to inoculate the microcosms (Experiment 1 = 12 communities, Experiment 2 = 4 communities) were chosen based on their DOC concentrations after ~6 weeks in previous experiments from microbial decomposition of blue grama litter. Communities were initially phenotyped as "low" or "high" DOC communities based on DOC concentrations that were ± 1 standard deviation from the mean in previous experiments. We extracted microbial communities from soil samples (n=12; Supplemental Table 1)) on the day of inoculation by suspending 2 g of soil into 18 mL of autoclaved milliQ water, then transferring 5ml of soil dilution to either 5ml of filter-sterilized $NH_4NO_3$ solution (stock concentration: 7.143 mg/mL, final solution concentration: 3.57 mg/mL) or 5ml of autoclaved milliQ water to create a 1:20 soil dilution. Negative control microcosms (n=12) had either autoclaved milliQ water or filter-sterilized 3.57 mg/mL $NH_4NO_3$ solution added to the microcosms.

Two treatments were tested in experiment 1 (Supplemental Figure 1): nitrogen addition (3.57 mg/mL $NH_4NO_3$) and weekly precipitation events. Therefore, all microbial communities had 12 replicates: 3 nitrogen addition with weekly precipitation events, 3 nitrogen addition with no precipitation event, 3 water control with weekly precipitation events, and 3 water control with no precipitation event. Each microcosm was inoculated with 1.3 mL of the appropriate 1:20 soil dilution or control solution. Microcosms were then sealed with a 20 mm Teflon-lined crimp cap and incubated at 25ºC in the dark for 48 days with replacement of air in the headspace as described below.

**Respiration Measurements**

On days 3, 8, 14, 21, 28, 35, 42, and 48, respiration was measured by gas chromatography using an Agilent Technologies 490 Micro GC (Santa Clara, CA, United States). After each measurement, the headspace air was evacuated with a vacuum pump and replaced with sterile filtered (0.2µm Millipore filters) air.

**Dissolved Organic Carbon and Dissolved Total Nitrogen**

To test the effect of nutrient availability (nitrogen and carbon) on microbially-driven carbon cycling, 3 microcosms from each microbial community with nitrogen addition and 3 without nitrogen addition had dissolved organic matter (DOM) removed weekly while the other half only had DOM removed at the end of the experiment (day 48). When DOM was removed weekly, we added 10 ml of sterilized milliQ water, mixed by gently swirling, then collected the water with sterile pipets. The collected liquid was then filter-sterilized using a 0.2 µm filter. Filtered extracts were analyzed for non-purgeable organic carbon and total nitrogen (TN) using a Shimadzu TOC-LCSH/TNM-L Carbon/Nitrogen Analyzer (Shimadzu Scientific Instruments, Inc. Columbia, MD) employing a catalytic combustion oxidation method and non-dispersive infrared detector (NDIR) for carbon and chemiluminescent detector for nitrogen. Samples were acidified with 5N HCL and purged with air to remove inorganic carbon. A sample aliquot was then injected onto a palladium catalytic bed held at 720 °C. The $CO_2$ and NO combustion products were then measured serially with the two detectors. The instrument was calibrated daily using a NIST-traceable standard of potassium hydrogen phthalate and potassium nitrate. Reagent water blanks were run periodically along with the check standards to monitor carry-over and instrument drift. During methods development a representative set of samples was analyzed by the method of standard additions to verify complete recovery of organic carbon and total nitrogen under the analysis conditions.

**Destructive Sampling**

On the final day (day 48) of the experiment after all the respiration measurements were taken, microcosms were divided into three subsamples. Prior to dividing the samples into subsamples stored in tin dishes, each tin dish was weighed to ensure accurate sample weights. Then, the sample was divided by first dividing the cut blue grama litter between each of the three subsample dishes to ensure all samples had equal plant litter, then adding sand until the entire sample was split into 3 subsamples (~2.5 g each). Then, each tin dish was measured and the weight recorded again. One subsample was used to determine moisture content while other two subsamples were used for microbial biomass measurements described below.

**Moisture Measurement**

For each microcosm, the subsample for moisture measurement was placed in a drying oven at 105°C. Samples were dried overnight (14 hours) which was sufficient to achieve a constant weight due to the small sample size (~2.5g) and consistency of only sand and plant litter. The samples were then cooled to room temperature and reweighed. The dry weights were recorded and the moisture content (%) was determined using this equation: ((wet weight (g) – dry weight (g))/ dry weight (g)) * 100. Although we did our best to collect as much liquid as possible during the weekly precipitation events, there was a discrepancy in moisture content (%) at the end of the experiment between the control (9.38% ± 1.07%) and weekly precipitation samples (13.5% ± 2.01) (Supplemental Figure 2). The moisture correction values were later used in microbial biomass measurements to determine biomass carbon per g of oven-dry "soil".

**Microbial Biomass Carbon and Nitrogen**

For each microcosm, the other two subsamples of the sand-litter mixture were used to determine microbial biomass carbon and nitrogen. Both subsamples were air-dried for 24 hours, which was sufficient for the small sample mass (~2.5g). One sample was fumigated as described previously (Allison, Czimczik and Treseder 2008) with some alterations. The subsample for fumigation was placed in a desiccator with a 50 ml beaker containing 20 ml of chloroform and boiling chips. The desiccator was evacuated four times until the chloroform boiled and then vented, except the desiccator was not vented the final time. The desiccator and samples incubated in the dark for 24 hours to ensure complete fumigation of the samples, which was sufficient for the small sample mass and mixture of sand/litter rather than real soil. After 24 hours, the vacuum was released and the chloroform beaker removed. Any excess chloroform fumes were removed through vacuum and venting prior to sample removal. After removing chloroform vapor, we extracted the DOC from the subsample with 10 ml of 0.5M $K_2SO_4$ by inverting 5 times and vortexing for 30 seconds at full speed. The supernatant was filtered through a 0.2 μm syringe filter and stored at -20ºC until analyzed for DOC and TN concentrations as described above. The non-fumigated subsample was processed like above except no chloroform fumigation occurred. Specifically, non-fumigated samples were air dried, DOC was extracted with 10 ml of 0.5M $K_2SO_4$, filtered, and stored at -20ºC. Biomass carbon and nitrogen measurements were calculated as previously described (Allison, Czimczik and Treseder 2008) using the following equations:

$$Microbial\ biomass\ Carbon = \frac{\left(\left(\frac{Fumigated\ C\ (mg)}{g\ ODeq\ soil}\right) - \left(\frac{Non-Fumiga\quad C\ (mg)}{g\ ODeq\ soil}\right)\right)}{0.45}$$

$$Microbial\ biomass\ Nitrogen = \frac{\left(\left(\frac{Fumigated\ N\ (mg)}{g\ ODeq\ soil}\right) - \left(\frac{Non-Fumigated\ N\ (mg)}{g\ ODeq\ soil}\right)\right)}{0.54}$$

ODeq = oven-dry equivalent soil based on moisture-correction

All values < 0 were excluded for downstream statistical analyses.

**Carbon Use Efficiency and Microbial Metabolic Quotient**

Carbon use efficiency (CUE) was calculated using the microbial biomass carbon measured by chloroform fumigation method (above) and the cumulative respiration measured throughout the experiment using gas chromatography and using the following equation:

$$CUE = (Biomass/(Biomass + Cumulative\ Respiration)$$

The microbial metabolic quotient ($qCO_2$) calculation was determined using the following equation:

$$qCO2 = Cumulative\ Respiration/Microbial\ Biomass\ Carbon$$

**DNA Extraction and Amplicon Sequencing**

A second smaller microcosm experiment used a subset of 4 soil communities (microcosm total = 44; Supplemental Figure 3) that were previously characterized as producers of low or high DOC concentrations from litter decomposition (2 each) were set up as described above except all of these received the nitrogen addition. Since all communities responded similarly to nitrogen addition and precipitation events in the first experiment, 4 communities were chosen as representatives of low and high DOC phenotypes in the follow-on experiment investigating the effect of repeated dissolved organic matter removal on the microbial community structure. At the end of the experiment, the samples were analyzed for microbial community composition using amplicon sequencing described in detail previously (Kroeger et al. 2021). Briefly, DNA was extracted using the standard protocol in the DNeasy PowerSoil 96-well HTP kit (Qiagen, Hilden, Germany) except that 0.3 g of material was used per extraction and all samples were eluted to a final volume of 30 µl and stored at -80°C until processed. DNA was quantified with the Invitrogen Quant-iT TM dsDNA Assay (HS) Kit (Invitrogen, Waltham, MA, USA) following manufacturer protocols on BioTek Synergy HI Hybrid Reader (BioTek, Winooski, VT, USA). Bacterial 16S rRNA (V3-V4 region) was amplified using 515f-R806 primers (Bates et al. 2011) and the D2 hypervariable region of the fungal 28S rRNA was amplified using LR22R primer and LR3 reverse primer (Mueller, Gallegos-Graves and Kuske 2015). The PCR and library preparation for Illumina MiSeq paired-end 300 bp reads were completed as described in detail previously (Kroeger et al. 2021) except amplicon clean-up was performed with a 0.9 ratio of AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA) following manufacturer's instructions and final elutions were performed with 30 µl of Elution Buffer. A final clean-up step was performed on pooled samples using a 0.9 ratio of AMPure XP beads. Samples were sequenced on an Illumina MiSeq platform with PE250 chemistry at Los Alamos National Laboratory. All unprocessed sequences are deposited in the NCBI Sequence Read Archive under Bioproject ID PRJNA851447.

## Microbial Community Sequence Analyses

Amplicon sequences were demultiplexed, pre-processed for quality control as described previously (Kroeger et al. 2021) using the UPARSE pipeline in usearch v11 (Edgar 2013) with paired ends merged with a 90% minimum similarity, truncating tails at the first base pair below 30, and a minimum merge length of 150 bp. Sequences were quality filtered for a maximum expected error rate of 1.0 and globally trimmed at 250 bp for fungal reads and 240 bp for bacterial/archaeal reads. Singletons were removed after dereplication and OTUs were picked using cluster_otus along with chimeric sequences being removed. OTU tables were generated using 97% similarity and bacterial and fungal OTUs were classified using the Ribosomal Database Project Naïve Baysian rRNA Classifier version 2.11 with training set 18 and 11, respectively (Wang et al. 2007).

Data was rarefied once to total OTU counts of 1562 for bacteria, and 1776 for fungi using the package vegan::rarefy (Oksanen et al. 2020). Four samples were not analyzed for bacteria because they had a total OTU count less than 1000; all fungal sequencing samples were kept. Alpha diversity metrics were quantified using the vegan::diversity function and species richness was determined using the vegan::specnumber function for each sample. Rarefied count data was then log-transformed using the package vegan::decostand to calculate the non-metric multidimensional scaling distances using the package vegan::metaMDS based on Bray-Curtis dissimilarity with 95% confidence ellipses calculated using the package vegan::ordiellipse. The effect of treatment and initial soil community on the final microbial community were assessed with a permutational multivariate analysis of variance using the vegan::adonis package with the formula (~ Treatment*Community) with 999 permutations on the Bray-Curtis dissimilarity matrix. Indicator species analyses were completed using the package indicspecies::multipatt (De Cáceres and Legendre 2009, De Cáceres, Legendre and Moretti 2010) to assess the association of certain taxa with treatment (simulated precipitation events) and initial soil community (4 communities) with 999 permutations. Only species with a *P* value less than or equal to 0.05 were kept.

**Statistics of nutrient pools**

All statistical analyses of carbon and nitrogen data were conducted in RStudio (v1.4.1717) with R (v4.1.1) (RStudio Team 2020, R Core Team 2021). All metrics were checked for normality using the Shapiro-Wilk test. Since the data were not normal, Kruskal-Wallis tests were used for comparing the medians between treatment groups. The Ansari-Bradley test was used to compare variance between treatments. Spearman correlations were used to assess the relationship between measured metrics. Figures were made using packages ggplot2 and ggpubr (Wickham. 2016, Kassambara 2020).

**Results**

**Influence of nutrient availability on carbon and nitrogen pools**

Overall, repeated DOM removal via simulated precipitation events did not significantly affect cumulative respiration (Kruskal-Wallis, p=0.72) (Figure 1A), but it did significantly increase the cumulative DOC (Kruskal-Wallis, p=7.701e-16) (Figure 1B). Nitrogen addition significantly increased cumulative respiration (Kruskal-Wallis, $p < 2.2e\text{-}16$) (Figure 1A) and had no effect on cumulative DOC (Kruskal-Wallis, $p>0.05$) (Figure 1B). Regardless of the initial microbial community inoculum, all microbial communities had increased respiration when nitrogen was added (Supplemental Figure 4) (Final: Kruskal-Wallis, p=7.72e-08; Weekly: Kruskal-Wallis, p=4.69e-10), but there was no effect on cumulative DOC (Supplemental Table

3). There was no significant difference in the cumulative $CO_2$ variance between nitrogen treatment or precipitation treatment (Ansari-Bradley: N treatment p=0.075, Precipitation treatment p=0.56). Furthermore, there was no significant difference in the cumulative DOC variance between either nitrogen or precipitation treatment (Ansari-Bradley: N treatment p=0.27, Precipitation treatment p=0.60). Nitrogen addition increased both microbial biomass carbon and nitrogen (Figure 2), whereas precipitation treatment, when we consider only the water controls, significantly decreased microbial biomass carbon (Figure 2A) but had no impact on microbial biomass nitrogen (Figure 2B). Overall, nitrogen addition increased cumulative respiration by an average of 104% (Figure 1A Final), microbial biomass carbon by an average of 112% (Figure 2A Final), microbial biomass nitrogen by an average of 292% (Figure 2B Final), and had no effect on DOC concentration (Figure 1B Final; Supplemental Table 3). While weekly precipitation events had no effect on cumulative respiration (Figure 1A Final vs Weekly Water) or microbial biomass nitrogen (Figure 2B Final vs Weekly Water), decreased microbial biomass carbon (average 36.9% decrease) (Figure 2A Final vs Weekly Water), and increased cumulative DOC (average 2536% increase) (Figure 1B Final vs Weekly Water) (Supplemental Table 3).

Carbon use efficiency (CUE), which was calculated by (microbial biomass carbon/(microbial biomass carbon + cumulative respiration)) was not affected by nitrogen addition (Figure 3), but did decrease overall with weekly precipitation events. However, when respiration was normalized to microbial biomass using the microbial metabolic quotient ($qCO_2$ = cumulative respiration/microbial biomass carbon), we observed a significant increase in the weekly precipitation treatment while nitrogen addition still had no significant effect (Figure 4).

## Nitrogen addition decouples $CO_2$ and DOC

Nitrogen addition decoupled $CO_2$ and DOC as seen by the lack of correlation in nitrogen addition samples and observed negative correlation in the water control samples (Figure 5). We found a strong positive correlation in $CO_2$ and total nitrogen (p=8.42E-5) from the nitrogen addition with weekly precipitation samples that disappeared when nitrogen was not added (Supplemental Table 4). In the water control with no precipitation event samples (Final), DOC positively correlated with total nitrogen (p=6.07E-3) and negatively correlated with MBC (2.62E-3) (Supplemental Table 4). We observed a weak positive correlation between MBC and MBN in the water controls with ($R^2$ = 0.16, p=0.038) and without ($R^2$=0.12, p=0.044) weekly precipitation events, but this relationship disappeared when nitrogen was added (Weekly: $R^2$=0.08, p=0.111, Final: $R^2$<0.01, p=0.8).

## Microbially driven differences in litter decomposition nutrient pools still observed under changing nutrient conditions

Although nitrogen and simulated precipitation treatments created significant changes in nutrient pools, microbially driven effects were still observed in some nutrient pools (Supplemental Figure 5). Specifically, in the precipitation treatment with (Kruskal-Wallis p=0.01) and without (Kruskal-Wallis p=0.031) nitrogen addition, the DOC concentration changed significantly between communities (Supplemental Figure 5). Interestingly, the expected DOC phenotype (high or low) based on previous experiments, did hold even with these changing nutrient conditions (Supplemental Figure 6). Respiration also significantly differed between communities in nitrogen limited contition (no nitrogen addition (N-limited) without precipitation treatment ) (Kruskal-Wallis p=0.012) and carbon limited condition (nitrogen added with precipitation treatment) (Kruskal-Wallis p=0.0082) (Supplemental Figure 5). Microbial biomass carbon did not significantly change between communities in any of the treatments, however, microbial biomass nitrogen did in the nitrogen limited condition (no nitrogen addition (N-limited) without precipitation treatment) (Kruskal-Wallis p=0.018) (Supplemental Figure 5). Cumulative total dissolved nitrogen only significantly changed in the carbon limited treatment (nitrogen added with precipitation treatment) (Kruskal-Wallis p=0.0072) (Supplemental Figure 5).

**Weekly precipitation events alter community composition and diversity**

For bacteria, final community composition was affected by both the community composition of the initial soil inoculum and the simulated precipitation treatment applied (weekly vs. final), though initial soil inoculum had a greater effect (Adonis: Treatment $R^2$ = 0.190, $P$ = 0.001; Community $R^2$ = 0.283, p = 0.001; Treatment:Community $R^2$ = 0.120, $P$ = 0.086) (Figure 6A). For eukaryotes, the composition of the initial inoculum had a much greater effect than treatment (Adonis: Treatment $R^2$ = 0.086, $P$ = 0.001; Community $R^2$ = 0.465, $P$ = 0.001; Treatment:Community $R^2$ = 0.144, $P$ = 0.001) (Figure 6B). The impact of initial inoculum on the final community composition can be seen in aggregate in all samples at the phyla level (Figure 6C, D, Supplemental Figure 7). From the initial inoculum, there was a decrease in the relative abundance of Firmicutes in both the precipitation treatment (65% reduction) and control (54% reduction) samples (Figure 6C). This shift was mostly caused by a reduction in the class Clostridia from 71% to 3.5% and 2.9% in precipitation and control samples, respectively. The weekly precipitation and control treatments both had a relative increase in the abundance of Proteobacteria (51%, 47%, respectively) and Bacteroidetes (17%, 9.2%, respectively) (Figure

6C). The main class changing relative abundance for Bacteroidetes was Chitinophagia that increased from 0.5% in the initial inoculum to 13% and 9.2% in the precipitation and control treatments, respectively. The eukaryotic community also shifted from the initial inoculum with a relative increase in Ascomycota (initial: 54%, precipitation: 79%, control: 82%) and Tubulinea (initial: 0.1%, precipitation: 7.0%, control: 3.8%) for both the precipitation treatment and control, while Metazoa (initial: 40%, precipitation: 13%, control: 12%) decreased in both conditions (Figure 6D).

Further highlighting the impact of initial community composition, indicator species analysis identified 211 bacterial OTUs and 41 eukaryote OTUs that were community specific. Only 16 bacterial OTUs and 2 bacterial OTUs were found to be significant ($p<0.05$) indicators of weekly vs final precipitation treatments, respectively (Supplemental Figure 8, Supplemental Table 5). Specifically, bacterial taxa from the orders Burkholderiales (n=3), Rhizobiales (n=5), Sphingomonadales (n=1), Spartobacteria (n=1), Cytophagales (n=1), Caulobacterales (n=1), Planctomycetales (n=1), Verrucomicrobiales (n=1), Nevskiales (n=1), and unclassified bacteria (n=1) were indicators of the precipitation treatment. The 2 bacterial OTUs associated with the final precipitation treatment are both Bacilliales, specifically Paenibacillus (Weekly = 0.83%; Final = 5.21%) and Peribacillus (Weekly = 0.14%; Final = 0.48%) (Supplemental Table 5). No eukaryotic taxa were found to be significant indicators of the precipitation treatment or the control. The effect of precipitation events on the bacterial community is further emphasized by the fact that weekly precipitation events significantly increased bacterial Shannon diversity ($p=0.0011$), but not eukaryote Shannon diversity ($p=0.87$) (Supplemental Figure 9, Supplemental Table 6). Eukaryote richness ($p=0.038$) and bacterial richness ($p=0.0011$) did increase significantly in the weekly precipitation treatment (Supplemental Figure 9).

## Discussion

Dissolved organic matter from litter decomposition is an important nutrient input to ecosystems and thought to contribute significantly to SOC stocks globally (Kaiser and Kalbitz 2012, Freeman et al. 2024, Nakhavali et al. 2021). Although microbial community composition has previously been shown to create significant variation in DOC during the early stages of litter decomposition (Kroeger et al. 2021, Albright et al. 2020), it was unclear prior to this study how shifting nutrient availability may alter these microbially driven effects. We predicted that increased nitrogen availability from nitrogen addition would increase DOC concentrations by stimulating microbial activity (e.g. extra-cellular enzyme production and deconstruction of litter biomass) while repeated DOM removal via simulated precipitation events would create a more carbon limited environment reducing microbial activity. However, our data does not fully support our expectations as (1) nitrogen addition did not increase DOC concentrations and (2) repeated DOM removal through simulated precipitation events did not decrease microbial respiration.

In the early phase (8 weeks) of litter decomposition, nitrogen addition generally doubled microbial respiration ($CO_2$) without altering DOC concentrations (Figure 1). This trend held regardless of microbial community composition (Supplemental Figure 4). Thus, nitrogen addition appears to decouple the observed negative linear correlation between cumulative $CO_2$ and DOC seen in control samples, regardless of whether DOC was removed weekly (Figure 5). The increased respiration from nitrogen deposition could be due to microbial community members decomposing a different pool of plant carbon compounds like cellulose, which is consistent with the "nitrogen mining" hypothesis (Craine, Morrow and Fierer 2007) and previous research that found a decrease in cellulose content across multiple litter sources during short-term litter decomposition with nitrogen addition (Magill and Aber 2000). This likely reallocation of resources from "nitrogen mining" (i.e. producing enzymes to break down more recalcitrant plant compounds for nitrogen) to metabolism of other carbon compounds could explain why microbial biomass and respiration significantly increased with nitrogen addition while DOC concentration did not change (Figures 1- 2). These findings are consistent with previous research which found that microbial biomass increases with nitrogen availability in soil (Hartman and Richardson 2013). Other studies that observed increases in DOC from nitrogen addition (e.g. Sinsabaugh et al. 2004; Sleutel et al. 2009), unlike our findings, may be due to differences in the nutrient demands of the microbial community and/or the plant litter quality. These differences could result from those decomposer communities continuing to allocate resources to nitrogen mining. Alternatively, those communities may have different metabolic rates and community structure leading to slower DOC consumption compared to the rate of production.

With nitrogen addition, we observed no significant difference in CUE (Figure 3). Based on a previous meta-analysis (Qiao et al. 2019), we expected CUE to decrease with nitrogen addition since previous studies observed a decrease on average of ~50% in soils supplied plant residue compared to no nitrogen addition. However, in our study there was no change in CUE with nitrogen addition since both microbial biomass and respiration increased. Since we only tested one litter type, blue grama grass, which has low lignin content (Pieper, Kelsey and Nelson 1974), future studies should investigate whether these findings hold with higher lignin content plant litter. Additionally, we suggest future studies need to investigate the composition of DOC under nitrogen deposition and determine the relative increase in microbial biomass and thus necromass to the native pool of DOC that could be altering carbon sorption/desorption from mineral surfaces along with increasing microbial respiration during litter decomposition and ascertain the broader impacts to SOC stocks versus $CO_2$ release.

Weekly removal of DOM (i.e. simulated precipitation events) did not significantly reduce respiration as expected, but did significantly increased cumulative DOC concentration, which could be due to (1) shifts in the microbial community towards non-soluble plant litter metabolism, (2) decrease in microbial antagonistic interactions, (3) shift in microbial resources toward cell maintenance rather than growth, and/or (4) physical removal of DOC from potential consumption by the decomposer community. Our findings support a combination of these hypotheses with a significant decrease in CUE (Figure 3), an increase in the microbial metabolic quotient (Figure 4), and clear changes in the microbial community composition and diversity (Figure 6, Supplemental Figures 7-9).

The microbial metabolic quotient ($qCO_2$), which is determined by the relationship between respiration and biomass has previously been used as an indicator of microbial stress (Leita et al. 1999, Hartman and Richardson 2013) and capacity of microbes to use organic matter (Cao et al. 2019). The repeated removal of DOM from the system is increasing nutrient stress likely leading to more microorganisms focusing on cell maintenance rather than growth, which will increase qCO2 (Figure 4). An alternative hypothesis is that the increase in $qCO_2$ with weekly precipitation events is caused by a shift in microbial community structure since previous research found that $qCO_2$ decreased when the fungal:bacterial ratio increased (Blagodatskaya and Anderson 1998). Although we did not directly measure the fungal:bacterial ratio in our samples, we did see a significant increase in bacterial Shannon diversity in the simulated precipitation samples (Supplemental Figure 9) while eukaryotic Shannon diversity did not change. This could be an indication of shifting fungal:bacterial ratios. Another potential cause of this shift in $qCO_2$ is due to increased extracellular enzymes as a means of decomposing the supplied plant material. Increased extracellular enzymes such as those necessary for bacterial decomposition of plant material force microbes to shift energy from growth to enzyme synthesis (Calabrese, Mohanty and Malik 2022).

Simulated precipitation events that removed nutrients (e.g. DOC, dissolved nitrogen) significantly affected microbial community composition, but the eukaryotic community composition was driven more by initial inoculum than precipitation treatment (Figure 6, Supplemental Figure 7, Supplemental Table 5). This more significant effect on the bacterial community is likely due to the fact that most fungi directly metabolize plant litter while many bacterial community members rely on the DOC produced from plant litter deconstruction and metabolism, such as microbial metabolites, necromass, or soluble plant components (Purahong et al. 2016). Many of the bacterial OTUs that were shifted with weekly simulated precipitation events are known to directly metabolize plant litter (e.g. Devosia, Steroidobacter, Prosthecobacter) (DeAngelis et al. 2011, Wilhelm et al. 2021, Levy-Booth et al. 2022); therefore, they would not be greatly affected by the removal of easily metabolized DOC. Moreover, there were multiple bacterial taxa (e.g. Bradyrhizobium, Beijerinckia, Mesorhizobium) in these more carbon limited communities that are known to be involved in nitrogen fixation, phosphorus mobilization, and/or methanol metabolism (Ye et al. 2024, Morawe et al. 2017). These microorganisms are likely indirectly involved in litter decomposition and may have formed close association with other microbes under these nutrient limited conditions in a potentially mutualistic relationship (Purahong et al. 2016). The two bacterial OTUs associated with the control (e.g. no simulated precipitation events) were Paenibacillus and Peribacillus. It is surprising that Paenibacillus is an indicator species of the control since this genus is known to have cellulolytic capabilities (Grady et al. 2016). However, it is possible that rather than carbon limitation, lack of other nutrients like phosphorus, caused a decrease in Paenibacillus abundance with repeated simulated precipitation events. We observed an overall increase in bacterial diversity and richness with carbon limitation. More competition for nutrients can lead to a more diverse community (Mello et al. 2016) since 'early-bird' species' have a reduced ability to dominate the community (Erez et al. 2020). Additionally, antagonistic interactions that rely on metabolites (e.g. quorum sensing, antimicrobials) that are likely being removed with simulated precipitation may further increase alpha diversity (Hibbing et al. 2010).

We discovered that even with the large changes in nutrient availability during this experiment, the previously observed microbially-driven effect on DOC concentration from short-term litter decomposition is still observed with the expected high DOC phenotype communities having significantly higher cumulative DOC compared to low DOC phenotype communities in all treatments (Supplemental Figure 6) (Kroeger et al. 2021, Albright et al. 2020). This supports the idea that microbial communities can create significant variation in carbon cycling during short-term litter decomposition even under changing environmental conditions. However, even with these differences between communities, the treatment effects (nutrient availability) appear to be a higher-order regulator of nutrient pools during litter decomposition with most communities responding similarly across treatments, although to varying degrees (Supplemental Figure 5). The variation seen between communities within treatments may explain the large variation previously observed in studies on litter decomposition rate . Interestingly, the two extreme nutrient conditions (N-addition without C-removal and C-removal without N-addition) created the largest variance in cumulative DOC between high and low DOC phenotype communities (>60%) whereas only N-limited or C-limited produced less variance between phenotypes (26-31%). These more extreme nutrient conditions are likely driving microbial community succession and niche partitioning as has been seen previously based on nutrient availability in different litter types (Yang et al. 2022, Buresova et al. 2019, Ge et al. 2013)

This study highlights the need for more research into the combined effects of global change scenarios on varying microbial communities and feedback to biogeochemical cycling. Through the use of a common garden experimental design, we clearly demonstrated the effect of nitrogen deposition and precipitation on microbial carbon cycling during short-term litter decomposition. Overall, nitrogen deposition decoupled $CO_2$ and DOC during short-term litter decomposition with respiration increasing and no effect on DOC concentration. Moreover, nitrogen deposition increased microbial biomass and had no effect on CUE due to the significant increase in respiration. Simulated precipitation events significantly increased DOC concentrations, decreased CUE, increased $qCO_2$, and greatly altered microbial composition and diversity. Furthermore, we observed that microbially-driven variation in carbon flow previously observed during litter decomposition does continue even with changing nutrient availability and may explain large variance observed when litter decomposition studies aggregate data. Through this research, we have outlined some key hypotheses for future studies to improve predictions of how microbial litter decomposition will shift under climate change including whether these findings will hold across litter types and longer time periods are necessary.

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**Author Contributions:** MEK designed the experiments, analyzed data, wrote and edited manuscript drafts, RZW analyzed data and edited manuscript, DS assisted with experiments and edited the manuscript, MBNA and JD assisted with experimental design, data interpretation, and edited the manuscript, TY assisted with data collection and edited the manuscript.

**Data Availability Statement:** All unprocessed sequences are deposited in the NCBI Sequence Read Archive under Bioproject ID PRJNA851447. All other raw data required for the statistical analyses are included in a github repository (https://github.com/mkroeger7/Kroeger_2024_Nitrogen_Precipitation). Any additional data or information will be provided upon request to the corresponding author.

## References

Ahrens, Bernhard, Braakhekke, Maarten C., Guggenberger, Georg, Schrumpf, Marion, and Reichstein, Markus, ‘Contribution of Sorption, DOC Transport and Microbial Interactions to the 14C Age of a Soil Organic Carbon Profile: Insights from a Calibrated Process Model’, *Soil Biology and Biochemistry*, 88 (2015), 390–402

Albright, Michaeline B. N., Gallegos-Graves, La Verne, Feeser, Kelli L., Montoya, Kyana, Emerson, Joanne B., Shakya, Migun, et al., ‘Experimental Evidence for the Impact of Soil Viruses on Carbon Cycling during Surface Plant Litter Decomposition’, *ISME Communications*, 2/1 (2022), 24

Albright, Michaeline B. N., Johansen, Renee, Thompson, Jaron, Lopez, Deanna, Gallegos-Graves, La V., Kroeger, Marie E., et al., ‘Soil Bacterial and Fungal Richness Forecast Patterns of Early Pine Litter Decomposition’, *Frontiers in Microbiology*, 11 (2020), 542220

Albright, Michaeline B. N., Thompson, Jaron, Kroeger, Marie E, Johansen, Renee, Ulrich, Danielle E M, Gallegos-Graves, La Verne, et al., ‘Differences in Substrate Use Linked to Divergent Carbon Flow during Litter Decomposition’, *FEMS Microbiology Ecology*, 96/8 (2020), fiaa135

Allison, Steven D., Czimczik, Claudia I., and Treseder, Kathleen K., 'Microbial Activity and Soil Respiration under Nitrogen Addition in Alaskan Boreal Forest: BOREAL MICROBIAL ACTIVITY UNDER N ADDITION', *Global Change Biology*, 14/5 (2008), 1156–68

Bates, Scott T, Berg-Lyons, Donna, Caporaso, J Gregory, Walters, William A, Knight, Rob, and Fierer, Noah, 'Examining the Global Distribution of Dominant Archaeal Populations in Soil', *The ISME Journal*, 5/5 (2011), 908–17

Blagodatskaya, Evgenia V., and Anderson, Traute-Heidi, 'Interactive Effects of pH and Substrate Quality on the Fungal-to-Bacterial Ratio and qCO2 of Microbial Communities in Forest Soils', *Soil Biology and Biochemistry*, 30/10–11 (1998), 1269–74

Bradford, Mark A., Berg, Björn, Maynard, Daniel S., Wieder, William R., and Wood, Stephen A., 'Understanding the Dominant Controls on Litter Decomposition', ed. by Will Cornwell, *Journal of Ecology*, 104/1 (2016), 229–38

Buresova, A., Kopecky, J., Hrdinkova, V., Kamenik, Z., Omelka, M., and Sagova-Mareckova, M., 'Succession of Microbial Decomposers Is Determined by Litter Type, but Site Conditions Drive Decomposition Rates', ed. by Claire Vieille, *Applied and Environmental Microbiology*, 85/24 (2019), e01760-19

Calabrese, Salvatore, Mohanty, Binayak P., and Malik, Ashish A., 'Soil Microorganisms Regulate Extracellular Enzyme Production to Maximize Their Growth Rate', *Biogeochemistry*, 158/3 (2022), 303–12

Campbell, Tayte P., Ulrich, Danielle E. M., Toyoda, Jason, Thompson, Jaron, Munsky, Brian, Albright, Michaeline B. N., et al., 'Microbial Communities Influence Soil Dissolved Organic Carbon Concentration by Altering Metabolite Composition', *Frontiers in Microbiology*, 12 (2022), 799014

Cao, Yingqiu, Xu, Li, Zhang, Zhen, Chen, Zhi, and He, Nianpeng, 'Soil Microbial Metabolic Quotient in Inner Mongolian Grasslands: Patterns and Influence Factors', *Chinese Geographical Science*, 29/6 (2019), 1001–10

Cavicchioli, Ricardo, Ripple, William J., Timmis, Kenneth N., Azam, Farooq, Bakken, Lars R., Baylis, Matthew, et al., 'Scientists' Warning to Humanity: Microorganisms and Climate Change', *Nature Reviews Microbiology*, 17/9 (2019), 569–86

Cotrufo, M. Francesca, Soong, Jennifer L., Horton, Andrew J., Campbell, Eleanor E., Haddix, Michelle L., Wall, Diana H., et al., 'Formation of Soil Organic Matter via Biochemical and Physical Pathways of Litter Mass Loss', *Nature Geoscience*, 8/10 (2015), 776–79

Cotrufo, M. Francesca, Wallenstein, Matthew D., Boot, Claudia M., Denef, Karolien, and Paul, Eldor, 'The Microbial Efficiency-Matrix Stabilization (MEMS) Framework Integrates Plant Litter Decomposition with Soil Organic Matter Stabilization: Do Labile Plant Inputs Form Stable Soil Organic Matter?', *Global Change Biology*, 19/4 (2013), 988–95

Craine, Joseph M., Morrow, Carl, and Fierer, Noah, ‘MICROBIAL NITROGEN LIMITATION INCREASES DECOMPOSITION’, *Ecology*, 88/8 (2007), 2105–13

De Cáceres, Miquel, and Legendre, Pierre, ‘Associations between Species and Groups of Sites: Indices and Statistical Inference’, *Ecology*, 90/12 (2009), 3566–74

De Cáceres, Miquel, Legendre, Pierre, and Moretti, Marco, ‘Improving Indicator Species Analysis by Combining Groups of Sites’, *Oikos*, 119/10 (2010), 1674–84

DeAngelis, Kristen M., Allgaier, Martin, Chavarria, Yaucin, Fortney, Julian L., Hugenholtz, Phillip, Simmons, Blake, et al., ‘Characterization of Trapped Lignin-Degrading Microbes in Tropical Forest Soil’, ed. by Christopher V. Rao, *PLoS ONE*, 6/4 (2011), e19306

Deng, Qi, Hui, Dafeng, Chu, Guowei, Han, Xi, and Zhang, Quanfa, ‘Rain-Induced Changes in Soil CO2 Flux and Microbial Community Composition in a Tropical Forest of China’, *Scientific Reports*, 7/1 (2017), 5539

Deng, Qi, Zhang, Deqiang, Han, Xi, Chu, Guowei, Zhang, Quanfa, and Hui, Dafeng, ‘Changing Rainfall Frequency Rather than Drought Rapidly Alters Annual Soil Respiration in a Tropical Forest’, *Soil Biology and Biochemistry*, 121 (2018), 8–15

Dinno, Alexis, ‘Dunn.Test: Dunn’s Test of Multiple Comparisons Using Rank Sums.’, 2017 <https://CRAN.R-project.org/package=dunn.test>

Edgar, Robert C, ‘UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads’, *Nature Methods*, 10/10 (2013), 996–98

Erez, Amir, Lopez, Jaime G, Weiner, Benjamin G, Meir, Yigal, and Wingreen, Ned S, ‘Nutrient Levels and Trade-Offs Control Diversity in a Serial Dilution Ecosystem’, *eLife*, 9 (2020), e57790

Fang, Huajun, Cheng, Shulan, Yu, Guirui, Xu, Minjie, Wang, Yongsheng, Li, Linsen, et al., ‘Experimental Nitrogen Deposition Alters the Quantity and Quality of Soil Dissolved Organic Carbon in an Alpine Meadow on the Qinghai-Tibetan Plateau’, *Applied Soil Ecology*, 81 (2014), 1–11

Freeman, Erika C., Emilson, Erik J. S., Dittmar, Thorsten, Braga, Lucas P. P., Emilson, Caroline E., Goldhammer, Tobias, et al., ‘Universal Microbial Reworking of Dissolved Organic Matter along Environmental Gradients’, *Nature Communications*, 15/1 (2024), 187

García-Palacios, Pablo, Shaw, E. Ashley, Wall, Diana H., and Hättenschwiler, Stephan, ‘Temporal Dynamics of Biotic and Abiotic Drivers of Litter Decomposition’, ed. by Johannes M H Knops, *Ecology Letters*, 19/5 (2016), 554–63

Ge, Xiaogai, Zeng, Lixiong, Xiao, Wenfa, Huang, Zhilin, Geng, Xiansheng, and Tan, Benwang, ‘Effect of Litter Substrate Quality and Soil Nutrients on Forest Litter Decomposition: A Review’, *Acta Ecologica Sinica*, 33/2 (2013), 102–8

Grady, Elliot Nicholas, MacDonald, Jacqueline, Liu, Linda, Richman, Alex, and Yuan, Ze-Chun, 'Current Knowledge and Perspectives of Paenibacillus: A Review', *Microbial Cell Factories*, 15/1 (2016), 203

Hartman, Wyatt H., and Richardson, Curtis J., 'Differential Nutrient Limitation of Soil Microbial Biomass and Metabolic Quotients (qCO2): Is There a Biological Stoichiometry of Soil Microbes?', ed. by Melanie R. Mormile, *PLoS ONE*, 8/3 (2013), e57127

Hibbing, Michael E., Fuqua, Clay, Parsek, Matthew R., and Peterson, S. Brook, 'Bacterial Competition: Surviving and Thriving in the Microbial Jungle', *Nature Reviews Microbiology*, 8/1 (2010), 15–25

Jiang, Xinyu, Cao, Lixiang, Zhang, Renduo, Yan, Linjie, Mao, Yi, and Yang, Yunwei, 'Effects of Nitrogen Addition and Litter Properties on Litter Decomposition and Enzyme Activities of Individual Fungi', *Applied Soil Ecology*, 80 (2014), 108–15

Jönsson, Leif J., and Martín, Carlos, 'Pretreatment of Lignocellulose: Formation of Inhibitory by-Products and Strategies for Minimizing Their Effects', *Bioresource Technology*, 199 (2016), 103–12

Kaiser, Klaus, and Kalbitz, Karsten, 'Cycling Downwards – Dissolved Organic Matter in Soils', *Soil Biology and Biochemistry*, 52 (2012), 29–32

Kassambara, Alboukadel, 'Ggpubr: "ggplot2" Based Publication Ready Plots.', 2020 <https://CRAN.R-project.org/package=ggpubr>

Keeler, Bonnie L., Hobbie, Sarah E., and Kellogg, Laurie E., 'Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition', *Ecosystems*, 12/1 (2009), 1–15

Krishna, M. P., and Mohan, Mahesh, 'Litter Decomposition in Forest Ecosystems: A Review', *Energy, Ecology and Environment*, 2/4 (2017), 236–49

Kroeger, Marie E., Rae DeVan, M., Thompson, Jaron, Johansen, Renee, Gallegos-Graves, La Verne, Lopez, Deanna, et al., 'Microbial Community Composition Controls Carbon Flux across Litter Types in Early Phase of Litter Decomposition', *Environmental Microbiology*, 23/11 (2021), 6676–93

Leita, L., De Nobili, M., Mondini, C., Muhlbachova, G., Marchiol, L., Bragato, G., et al., 'Influence of Inorganic and Organic Fertilization on Soil Microbial Biomass, Metabolic Quotient and Heavy Metal Bioavailability', *Biology and Fertility of Soils*, 28/4 (1999), 371–76

Levy-Booth, David J., Navas, Laura E., Fetherolf, Morgan M., Liu, Li-Yang, Dalhuisen, Thomas, Renneckar, Scott, et al., 'Discovery of Lignin-Transforming Bacteria and Enzymes in Thermophilic Environments Using Stable Isotope Probing', *The ISME Journal*, 16/8 (2022), 1944–56

Lu, X., Gilliam, F. S., Yu, G., Li, L., Mao, Q., Chen, H., et al., ‘Long-Term Nitrogen Addition Decreases Carbon Leaching in a Nitrogen-Rich Forest Ecosystem’, *Biogeosciences*, 10/6 (2013), 3931–41

Lv, Yanna, Wang, Congyan, Wang, Fangyuan, Zhao, Guiying, Pu, Gaozhong, Ma, Xu, et al., ‘Effects of Nitrogen Addition on Litter Decomposition, Soil Microbial Biomass, and Enzyme Activities between Leguminous and Non-Leguminous Forests’, *Ecological Research*, 28/5 (2013), 793–800

Magill, Alison H, and Aber, John D, ‘Dissolved Organic Carbon and Nitrogen Relationships in Forest Litter as A ected by Nitrogen Deposition’, *Soil Biology*, 2000, 11

Mello, Bruno L., Alessi, Anna M., McQueen-Mason, Simon, Bruce, Neil C., and Polikarpov, Igor, ‘Nutrient Availability Shapes the Microbial Community Structure in Sugarcane Bagasse Compost-Derived Consortia’, *Scientific Reports*, 6/1 (2016), 38781

Moorhead, Daryl L., and Sinsabaugh, Robert L., ‘A THEORETICAL MODEL OF LITTER DECAY AND MICROBIAL INTERACTION’, *Ecological Monographs*, 76/2 (2006), 151–74

Morawe, Mareen, Hoeke, Henrike, Wissenbach, Dirk K., Lentendu, Guillaume, Wubet, Tesfaye, Kröber, Eileen, et al., ‘Acidotolerant Bacteria and Fungi as a Sink of Methanol-Derived Carbon in a Deciduous Forest Soil’, *Frontiers in Microbiology*, 8 (2017), 1361

Mueller, Rebecca C, Gallegos-Graves, La Verne, and Kuske, Cheryl R, ‘A New Fungal Large Subunit Ribosomal RNA Primer for High Throughput Sequencing Surveys’, *FEMS Microbiology Ecology*, 2015, fiv153

Nakhavali, Mahdi, Lauerwald, Ronny, Regnier, Pierre, Guenet, Bertrand, Chadburn, Sarah, and Friedlingstein, Pierre, ‘Leaching of Dissolved Organic Carbon from Mineral Soils Plays a Significant Role in the Terrestrial Carbon Balance’, *Global Change Biology*, 27/5 (2021), 1083–96

Oksanen, J, Blanchet, FG, Friendly, M, Kindt, R, Legendre, P, McGlinn, D, et al., ‘Vegan: Community Ecology Package’, 2020

Park, Ji-Hyung, Kalbitz, Karsten, and Matzner, Egbert, ‘Resource Control on the Production of Dissolved Organic Carbon and Nitrogen in a Deciduous Forest ¯oor’, *Soil Biology*, 2002, 12

Pieper, Rex D., Kelsey, R. Joe, and Nelson, Arnold B., ‘Nutritive Quality of Nitrogen Fertilized and Unfertilized Blue Grama’, *Journal of Range Management*, 27/6 (1974), 470

Prescott, Cindy E., ‘Litter Decomposition: What Controls It and How Can We Alter It to Sequester More Carbon in Forest Soils?’, *Biogeochemistry*, 101/1–3 (2010), 133–49

Purahong, Witoon, Wubet, Tesfaye, Lentendu, Guillaume, Schloter, Michael, Pecyna, Marek J., Kapturska, Danuta, et al., ‘Life in Leaf Litter: Novel Insights into Community Dynamics of Bacteria and Fungi during Litter Decomposition', *Molecular Ecology*, 25/16 (2016), 4059–74

Qiao, Yang, Wang, Jing, Liang, Guopeng, Du, Zhenggang, Zhou, Jian, Zhu, Chen, et al., 'Global Variation of Soil Microbial Carbon-Use Efficiency in Relation to Growth Temperature and Substrate Supply', *Scientific Reports*, 9/1 (2019), 5621

R Core Team, 'R: A Language and Environment for Statistical Computing.' (Vienna, Austria, 2021) <https://www.R-project.org/>

Riggs, Charlotte E., and Hobbie, Sarah E., 'Mechanisms Driving the Soil Organic Matter Decomposition Response to Nitrogen Enrichment in Grassland Soils', *Soil Biology and Biochemistry*, 99 (2016), 54–65

RStudio Team, 'RStudio: Integrated Development for R. ', (Boston, MA, 2020) <http://www.rstudio.com/.>

Scheibe, Andrea, and Gleixner, Gerd, 'Influence of Litter Diversity on Dissolved Organic Matter Release and Soil Carbon Formation in a Mixed Beech Forest', ed. by Andrew Hector, *PLoS ONE*, 9/12 (2014), e114040

Sinsabaugh, R.L, Zak, D.R, Gallo, M, Lauber, C, and Amonette, R, 'Nitrogen Deposition and Dissolved Organic Carbon Production in Northern Temperate Forests', *Soil Biology and Biochemistry*, 36/9 (2004), 1509–15

Sleutel, S., Vandenbruwane, J., De Schrijver, A., Wuyts, K., Moeskops, B., Verheyen, K., et al., 'Patterns of Dissolved Organic Carbon and Nitrogen Fluxes in Deciduous and Coniferous Forests under Historic High Nitrogen Deposition', *Biogeosciences*, 6/12 (2009), 2743–58

Tabari, Hossein, 'Climate Change Impact on Flood and Extreme Precipitation Increases with Water Availability', *Scientific Reports*, 10/1 (2020), 13768

Tian, Jing, Dungait, Jennifer A. J., Lu, Xiankai, Yang, Yunfeng, Hartley, Iain P., Zhang, Wei, et al., 'Long-term Nitrogen Addition Modifies Microbial Composition and Functions for Slow Carbon Cycling and Increased Sequestration in Tropical Forest Soil', *Global Change Biology*, 25/10 (2019), 3267–81

Tramontina, Robson, Brenelli, Lívia Beatriz, Sodré, Victoria, Franco Cairo, João Paulo, Travália, Beatriz Medeiros, Egawa, Viviane Yoshimi, et al., 'Enzymatic Removal of Inhibitory Compounds from Lignocellulosic Hydrolysates for Biomass to Bioproducts Applications', *World Journal of Microbiology and Biotechnology*, 36/11 (2020), 166

Ullah, Sami, Ai, Chao, Huang, Shaohui, Zhang, Jiajia, Jia, Liangliang, Ma, Jinchuan, et al., 'The Responses of Extracellular Enzyme Activities and Microbial Community Composition under Nitrogen Addition in an Upland Soil', ed. by Jorge Paz-Ferreiro, *PLOS ONE*, 14/9 (2019), e0223026

Wang, Mengke, Zhang, Ziting, Wang, Yinghui, Mo, Xiaohan, Zhang, Qiang, Zhang, Peng, et al., ‘Characteristics of Dissolved Organic Matter and Dissolved Lignin Phenols in Tropical Forest Soil Solutions during Rainy Seasons and Their Responses to Nitrogen Deposition’, *ACS Earth and Space Chemistry*, 5/11 (2021), 3150–58

Wang, Qiong, Garrity, George M., Tiedje, James M., and Cole, James R., ‘Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy’, *Applied and Environmental Microbiology*, 73/16 (2007), 5261–67

Wickham., H, *Ggplot2: Elegant Graphics for Data Analysis* (New York, 2016) <https://ggplot2.tidyverse.org>

Wilhelm, Roland C., Pepe-Ranney, Charles, Weisenhorn, Pamela, Lipton, Mary, and Buckley, Daniel H., ‘Competitive Exclusion and Metabolic Dependency among Microorganisms Structure the Cellulose Economy of an Agricultural Soil’, ed. by Jennifer B. H. Martiny, *mBio*, 12/1 (2021), e03099-20

Yang, Kai, Zhu, Jiaojun, Zhang, Weiwei, Zhang, Qian, Lu, Deliang, Zhang, Yakun, et al., ‘Litter Decomposition and Nutrient Release from Monospecific and Mixed Litters: Comparisons of Litter Quality, Fauna and Decomposition Site Effects’, *Journal of Ecology*, 110/7 (2022), 1673–86

Yang, Xu, Szlavecz, Katalin, Pitz, Scott L., Langley, J. Adam, and Chang, Chih-Han, ‘The Partitioning of Litter Carbon Fates during Decomposition under Different Rainfall Patterns: A Laboratory Study’, *Biogeochemistry*, 148/2 (2020), 153–68

Ye, He, Tu, Nare, Wu, Zhendan, He, Shilong, Yue, Mei, and Hong, Mei, ‘Identification of Bacteria and Fungi Responsible for Litter Decomposition in Desert Steppes via Combined DNA Stable Isotope Probing’, *Frontiers in Microbiology*, 15 (2024), 1353629

Zhang, Qi, Li, Yanan, Wang, Mengru, Wang, Kai, Meng, Fanlei, Liu, Lei, et al., ‘Atmospheric Nitrogen Deposition: A Review of Quantification Methods and Its Spatial Pattern Derived from the Global Monitoring Networks’, *Ecotoxicology and Environmental Safety*, 216 (2021), 112180

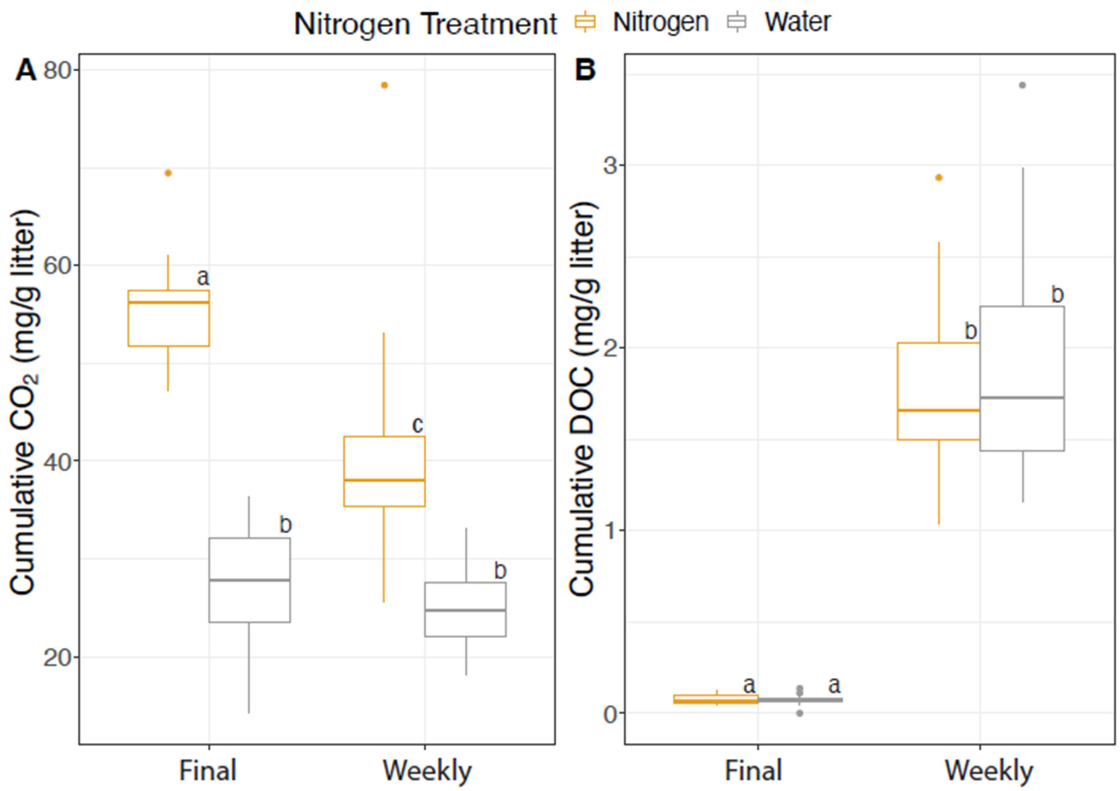


**Figure 1.** Cumulative $CO_2$ (A) and DOC (B) between nitrogen treatments (nitrogen addition = orange, water control = grey) and "precipitation" treatments (weekly = DOC removed weekly, final = DOC removed at the end of experiment). Letters above boxed denote significant differences between groups based on Dunn's test *(Dinno 2017)*.

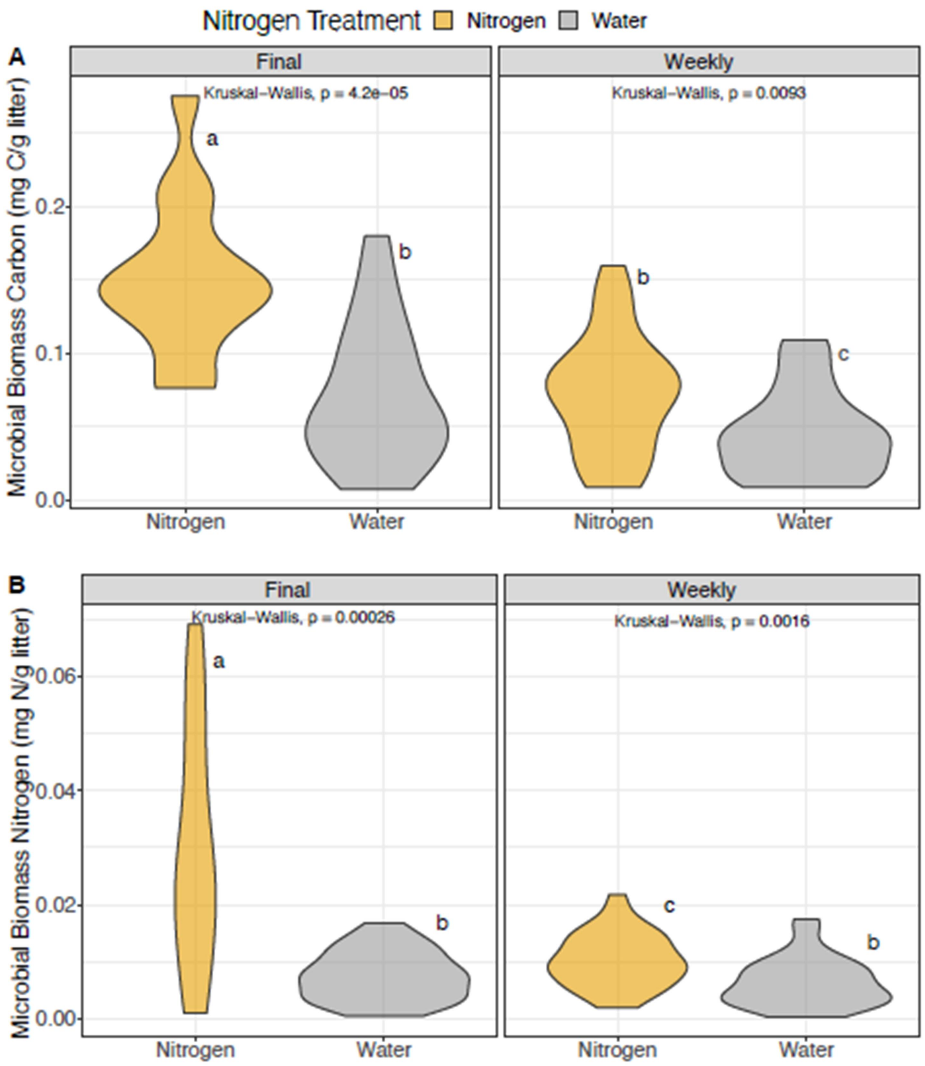


**Figure 2.** Microbial biomass carbon (A) and nitrogen (B) between nitrogen treatments (nitrogen addition = orange, water control = grey) and “precipitation” treatments (weekly = DOC removed weekly, final = DOC removed at the end of experiment). Kruskal-Wallis p-values are reported comparing the effect of nitrogen addition within precipitation groups (weekly and final). Letters above boxed denote significant differences between all groups (nitrogen and precipitation treatments) based on a Dunn’s test (Dinno 2017).

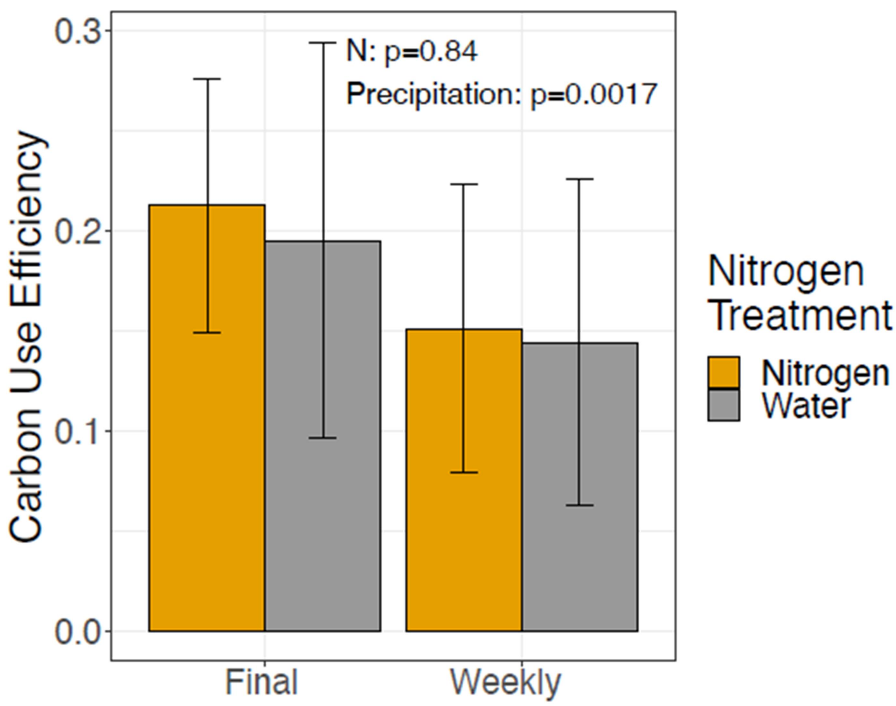


**Figure 3.** Carbon use efficiency (CUE) (ratio of biomass/(biomass+respiration)) between nitrogen treatments (nitrogen addition = orange, water control = grey) and "precipitation" treatments (weekly = DOC removed weekly, final = DOC removed at the end of experiment). P-values listed for nitrogen treatment (N) and "precipitation" treatment (DOC) on CUE from an ANOVA since CUE was determined to be normally distributed based on p-value >0.05 with a Shapiro-Wilk test of normality.

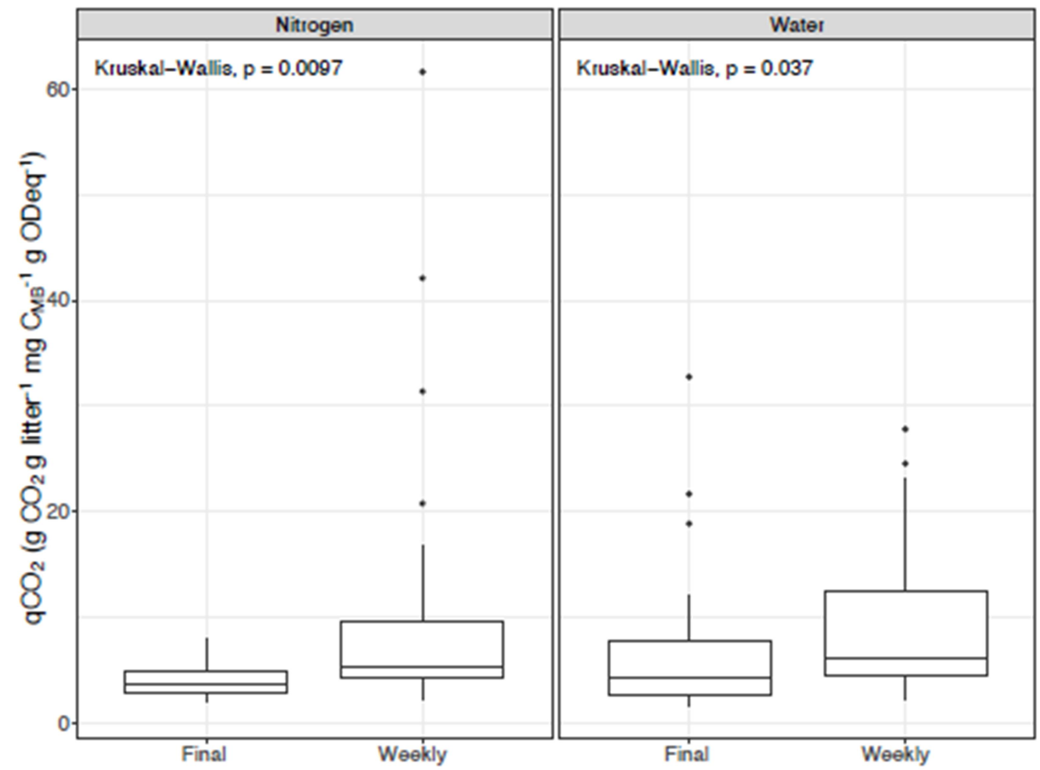


**Figure 4.** Microbial metabolic quotient ($qCO_2$) between "precipitation" treatments and nitrogen addition. P-value listed for "precipitation" treatments (weekly vs final DOC removal) on $qCO_2$ based on a Kruskal-Wallis test. ODeq = oven-dry equivalent "soil". MB = microbial biomass carbon.

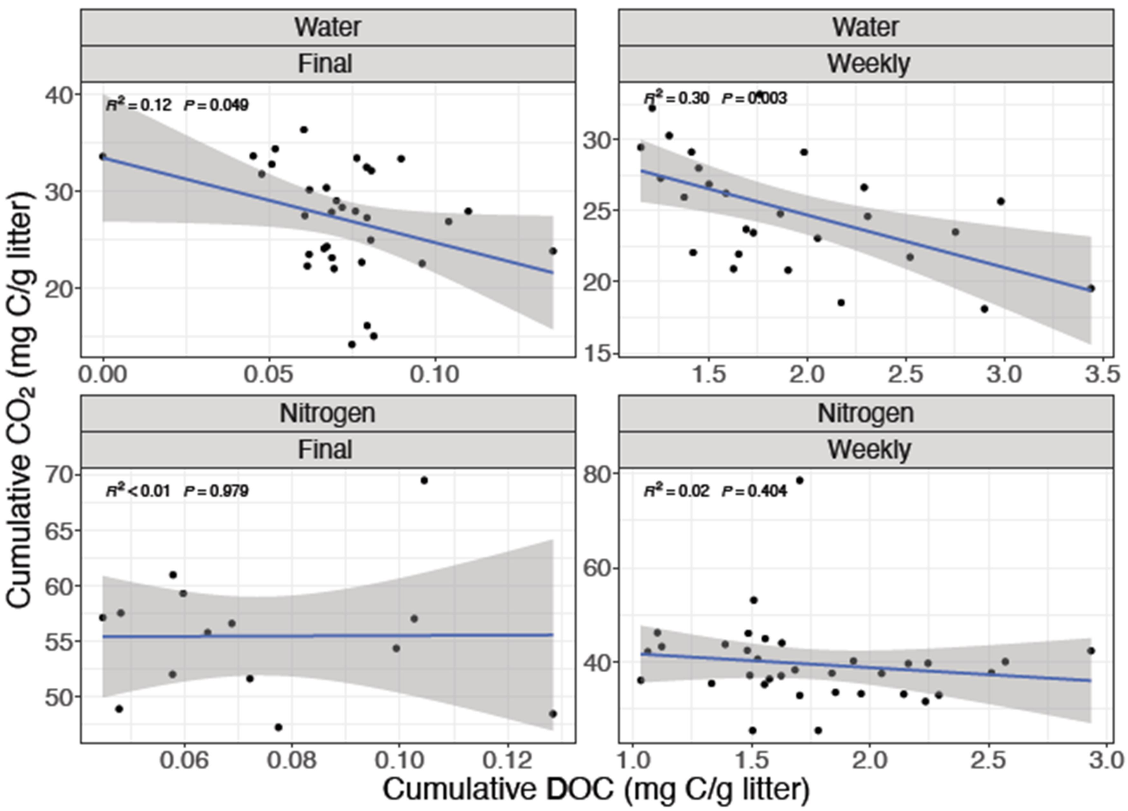


**Figure 5.** Linear regression between cumulative $CO_2$ and DOC within treatments (weekly = DOC removed weekly, final = DOC removed at the end of experiment, Nitrogen = nitrogen addition, Water = no nitrogen added). $R^2$ and p-values reported for Pearson correlations.

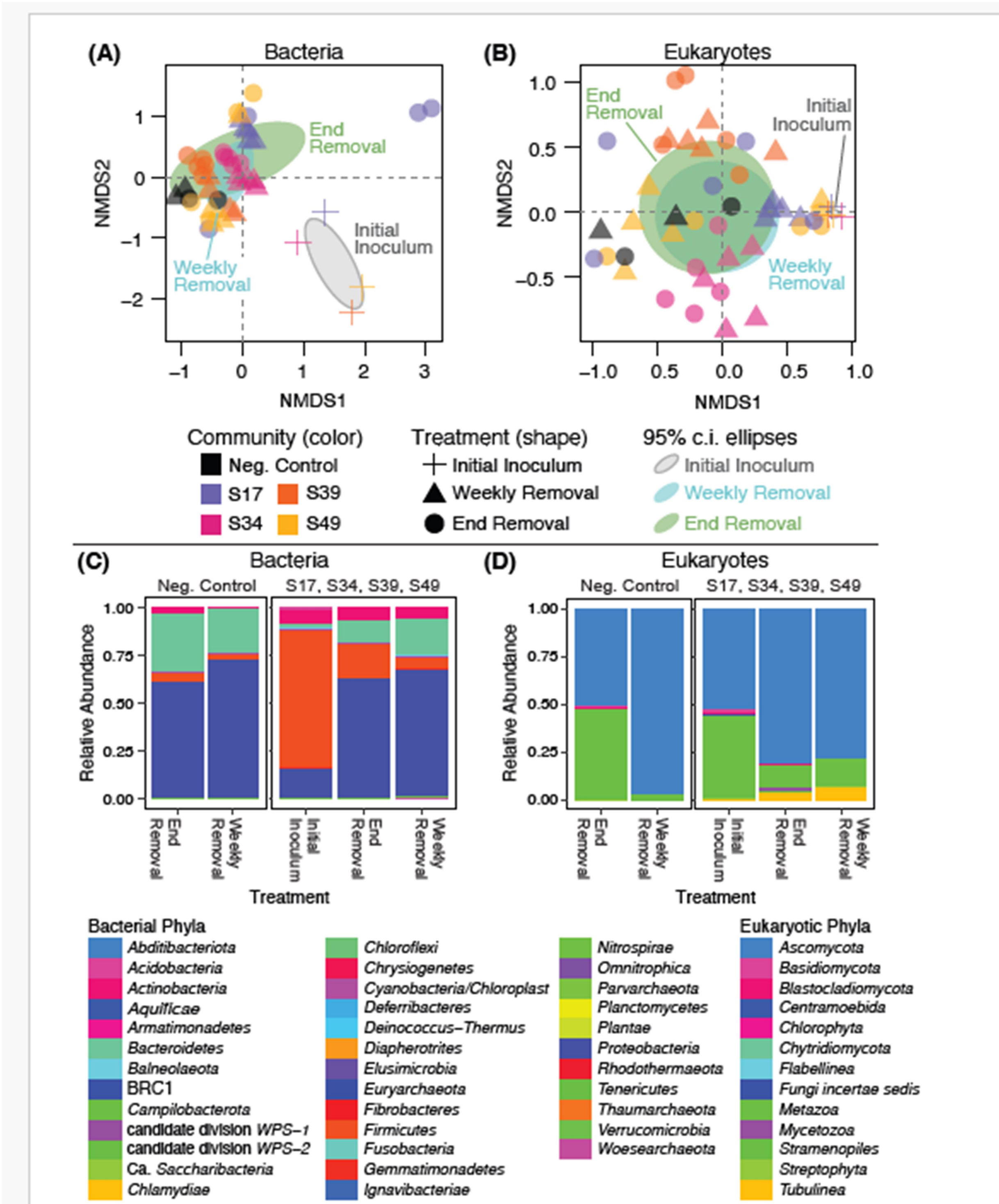


**Figure 6.** NMDS (non-metric multidimensional scaling) plots of (A) bacterial, and (B) eukaryotic community composition for different initial soil communities as indicated by colors (S17, S34, S39, S49, negative control). The treatment is indicated by shape with weekly removal

= triangle, end removal = circle, and initial inoculum = crosses. Weekly removal = weekly simulated precipitation treatment. End removal = control. Initial inoculum = initial community added to microcosms. 95% confidence ellipses show initial inoculum (gray) vs. weekly DOC removal (green) vs. end DOC removal (blue). Bar graphs showing the average relative abundance of bacterial (C) and eukaryotic (D) community members at the phylum level within each treatment (weekly removal = precipitation treatment, end removal = control), initial inoculum, and negative controls (no microbiome added, sterile blue grama grass litter and sand).