OIKOS

Research article

Seasonal soil moisture thresholds inhibit bacterial activity and decomposition during drought in a tallgrass prairie

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Oikos
2023: e10201
doi: 10.1111/oik.10201
Subject Editor: Deliang Kong
Editor-in-Chief:
Gerlinde B. De Deyn
Accepted 28 October 2023

Soil moisture reductions during drought often inhibit soil microbial activity and inhibit decomposition rates by reducing microbial biomass or by altering microbial communities. Evidence suggests that soil water must drop below a critical threshold to inhibit microbial activity. Thus, it is likely that the seasonal timing of drought will determine the extent to which belowground processes are adversely impacted by drought. Specifically, the effects of drought might be minimal during cool, wet periods typical of late spring but dramatic during hot summer months with high evapotranspiration rates that lower soil moisture levels below the critical threshold. Here, we present results from a study designed to quantify the effect of drought on soil microbial abundance, community composition, and soil water diffusion across four months, and to then assess how drought impacts the microbial decomposition of leaf matter. We imposed a season-long drought in a Wisconsin tallgrass prairie and measured soil moisture, bacterial composition and abundance, microbial respiration, and decomposition rates throughout the growing season. Bacterial communities varied considerably among dates, but drought did not affect either bacterial abundance or community composition. Microbial respiration declined significantly during periods of drought when soil pores likely became hydrologically isolated, ultimately reducing cumulative microbial respiration by 10%. The reduction in microbial activity in drought treatments caused a 50% decline in the decomposition of refractory material. Our study highlights that sublethal effects of drought on microbial communities, occurring only when soil moisture declined below a tolerance threshold, can have large impacts on microbial carbon release or decomposition, highlighting the need to incorporate such measures into future studies.

Keywords: 16S sequencing, bacteria, decomposition, genomics, microbiome, soil respiration

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Introduction

Soil microbial CO_2 respiration drives both regional and global carbon (C) cycling, releasing roughly ten times more C per year than fossil fuel combustion (Bond-Lamberty and Thomson 2010a, Hashimoto et al. 2015, Xu and Shang 2016, Marland et al. 2020). The amount of C respired by microbes depends on both soil temperature and water content (Hursh et al. 2017). Though temperature nearly always has a positive relationship with microbial respiration (Bond-Lamberty and Thomson 2010b, Hashimoto et al. 2015), the relationship between microbial respiration and soil moisture is more complex. Soil water content is frequently positively correlated with microbial respiration (Orchard and Cook 1983, Manzoni et al. 2012, Zhang et al. 2013), but the correlation can become negative in overly wet or humid ecosystems (Waring and Hawkes 2015). Regardless of the direction, however, the influence of soil water content on microbial respiration means that changes in local precipitation patterns can alter the global C system. Yet there is enormous variation in how changes in rainfall impact microbial composition, CO_2 respiration and microbial-driven decomposition both among and within ecosystems (Ren et al. 2018). This variability makes it difficult to predict, at local and global scales, how global change will impact microbial C cycling. Hence, there is an urgent need to understand how microbial communities and the dependent ecosystem processes will be affected by changes in precipitation, particularly drought, during the next century.

Droughts are already increasing in both severity and frequency across the world (Vicente-Serrano et al. 2014, Diffenbaugh et al. 2015, Parolari et al. 2016). The accompanying soil moisture reductions can impair microbial ecosystem function through a variety of non-mutually exclusive mechanisms. First, drought can alter the makeup of the bacterial community, either by reducing microbial biomass or changing community composition (Allison et al. 2013). Indeed, changes in microbial community composition during drought are common and typically reflect decreases in fast-growing Proteobacteria and increases in slower-growing Acidobacteria (Castro et al. 2010, Tóth et al. 2017, Ren et al. 2018). Second, drought can inhibit microbial activity by limiting water transport through soil pore spaces (Stark and Firestone 1995, Carson et al. 2010, Manzoni et al. 2012). Soil pores become hydraulically isolated when soil water potential reaches a 'tipping point' known as the diffusion limitation coefficient (ψ_{th}, Olesen et al. 2001). When soil water falls below this threshold, microbial activity is suppressed due to restricted dispersal of nutrient substrates like carbon and nitrogen or due to the accumulation of inhibitory compounds like antibiotic compounds and enzymes. This can lead to physiological stress (i.e. desiccation, Schimel et al. 2007) or nutrient deficiency (Manzoni et al. 2012). It is also possible that changes in microbial community composition only occur below ψ_{th}, which would suggest that ψ_{th} is a critical threshold for drought-altered microbial processes.

Because evidence suggests that soil water must drop below a critical threshold for drought effects to manifest, it is likely that the seasonal timing of drought will determine the extent to which belowground processes are adversely impacted by drought. Specifically, the effects of drought might be minimal during cool, wet periods typical of late spring but dramatic during hot summer months with high evapotranspiration rates that reduce soil moisture near the ψ_{th} threshold (Knapp et al. 2001, Cherwin and Knapp 2012, Hoover et al. 2014a). In other words, droughts during specific times of the growing season might push microbial communities beyond such thresholds into a state of lowered activity. Combined, these mechanistic changes induced by drought have consequences for belowground ecosystem function, as reductions in microbial activity and changes to the community composition can dramatically decrease decomposition rates (van der Heijden et al. 2008, Allison et al. 2013, Tóth et al. 2017). Therefore, examining the influence of drought across seasons and a range of abiotic conditions is essential to pinpointing when soil communities and their associated functions are most at risk. However, field studies rarely account for drought timing and seasonality, and the presence of such phenological 'tipping points' remains unknown.

Here, we present results from a study designed to quantify the effect of drought on soil microbial abundance, community composition, and soil water diffusion across four months, and to then assess how drought impacts the microbial decomposition of leaf matter. Our study system is a mesic tallgrass prairie that is subject to a wide range of soil moistures, from 5 to 40% VWC, enabling us to capture potential nonlinear effects of soil moisture on microbial processes. Specifically, we tested the following hypotheses:

H1. Drought inhibits microbial activity via either direct or indirect pathways

Specifically, we predicted that drought-imposed reductions in soil moisture would inhibit microbial activity, as measured by CO_2 respiration. There are three non-mutually exclusive mechanisms by which drought could reduce microbial activity:

H1a. Reduced soil moisture decreases bacterial abundance. As soil moisture declines, bacteria can die from desiccation or lower carbon use efficiency (Schimel 2018).

H1b. Reduced soil moisture alters bacterial community composition. In this case, reducing soil moisture would alter the relative abundances of bacterial taxa by favoring the survival of only slow-growing, stress-tolerant species.

H1c. Reduced soil moisture inhibits microbial activity via diffusive limitation. As Manzoni et al. (2012) reported, reduced water diffusion can cause a buildup of antimicrobial compounds, limit nutrient availability, or cause desiccation/dormancy of existing microbes. Any of these changes would lower activity and respiration rates.

H2. Drought inhibits microbial activity only when soil moisture declines below a threshold in the mid-to-late summer

We expected to find a non-linear relationship between soil moisture and microbial activity. Specifically, we predicted that soils would reach a threshold water content at which soil pores become hydraulically isolated, causing microbial activity to decline. Drought will keep soil water content closer to this threshold (ψ_{th}), causing reductions in activity to occur earlier and more frequently.

H3. Reduced microbial activity under drought inhibits decomposition

Ultimately, we predicted that a reduction in soil microbial activity in drought conditions, even briefly, would inhibit decomposition of either labile or refractory plant material throughout the growing season (Tóth et al. 2017).

Material and methods

Site description

We tested the effects of rainfall reduction on belowground processes at the University of Wisconsin – Milwaukee at Waukesha (UWM-W) field station in Oconomowoc, Wisconsin. The field station encompasses about 40 ha containing oak savannas, jack pine forests, maple forests, oak forests and tallgrass prairie. The tallgrass prairie is dominated by the C_4 grasses *Andropogon gerardii*, *Bouteloua cutripendula* and *Schizachyrium scoparium* and the forbs *Echinacea pallida*, *Ratibida paradoxa*, *Dalea* spp. and *Monarda fistulosa*. Soils are mineral soils with about 5% organic matter, 0.22% nitrogen and about 4.75 ppm phosphorus. Soil texture is 56% sand, 26% silt and 18% clay. The climate is relatively cool and mesic; from April – September, mean daily temperatures are ~20°C and mean precipitation is ~ 520 mm (Fig. 1). Precipitation occurs evenly from May–November (Supporting information). Southern Wisconsin experiences 1–2 severe droughts every 30 years, and the frequency of droughts is predicted to double by 2100 (Sheffield and Wood 2008). Furthermore, droughts at our site are typified by daily rainfall shortages that occur throughout the entire growing season from May to September (Supporting information).

Rainfall reduction treatment

We reduced rainfall using passive shelters following the established Drought-Net design (Yahdjian and Sala 2002). On 15 April 2020, we marked twenty 2 × 2 m experimental plots randomly assigned as either 'Ambient' or 'Drought' treatments (n = 10 per treatment). Ambient plots had no shelters and received normal growing season precipitation, while drought plots had rainout shelters installed to mimic drought. Using 127 years of daily recordings from a weather station (NOAA NCDC Station ID USC00478937, Waukesha WWTP, WI US), we determined that a 40% reduction in growing season precipitation fell below the 5th percentile for annual growing season rainfall and thus represented a severe drought at our study site (Fig. 1B).

Passive rainout shelters consisted of a 2 × 2 m wooden frame covering a 1 × 1 m measurement plot, allowing for a 0.5 m buffer on each side. Roofs were made of nine polycarbonate sheets (1.8 m long, 15 cm wide) evenly spaced to cover 40% of the 2 × 2 m plot. To verify our drought treatment, we measured soil volumetric water content (%VWC) every two weeks using a Field Scout TDR 150 with 12 cm probes. We recorded three %VWC measurements per plot and averaged the estimates to produce a single value per plot. We converted %VWC to soil water potential using a soil water release curve (Supporting information), measured by The METER Group. The METER group constructed the soil water characteristic curve using two devices, the Hyprop 2 measures soil water potential in the 0 to −100 kPa range using mini-tensiometers, while the WP4C measures soil water potential ranges from −100 to −100 000 kPA using a dewpoint sensor inside a sealed chamber. We calculated ψ_{th} by using the equation of Olesen et al. (2001), which uses clay fraction, silt fraction, and soil bulk density to determine the soil VWC at which diffusion limitation occurs. We then converted that level of VWC to soil water potential (ψ_{th}) using the soil water release curve.

Soil sampling

Beginning on 11 May 2020, we collected soil cores every other week until 1 September 2020. Specifically, we collected a 7–15 g soil core from the top 10 cm of soil using a 1 cm diameter soil corer (n = 5 randomly chosen plots per treatment). Cores were placed into sterile Whirl Pak bags, kept on ice, and transported to the lab. Once in the lab, soils were passed through a 1.5 mm mesh seive to remove roots, rocks, and other plant material. Sieves were washed with 70% ethanol between soil samples. Sieved soils were then partitioned into two batches (per core). One batch was allocated for immediate soil respiration analysis followed by soil organic content. A second batch was frozen at −70°C until processing for DNA. We extracted DNA from ~ 0.25 g soil from the second batch using DNeasy PowerSoil Pro kits (Qiagen). Extracted DNA was partitioned into two aliquots, one for qPCR and one for sequencing. Aliquots were stored at −70°C until analyses.

Bacterial abundance via qPCR

We estimated bacterial abundance using quantitative PCR. We first created five qPCR standards from a two-fold dilution series of pure *Escherichia coli* culture. We quantified the cell count of each standard using a Beckman Coulter CytoFLEX flow cytometer. We then multiplied the estimated *E. coli* cell count by seven, the number of 16S rRNA gene in *E. coli*, to provide a final estimate of 16S rRNA gene copy number per sample. Our qPCR protocol was based on Fierer et al. (2005) and Rousk et al. (2010). Specifically, we used a 25 µl PCR reaction

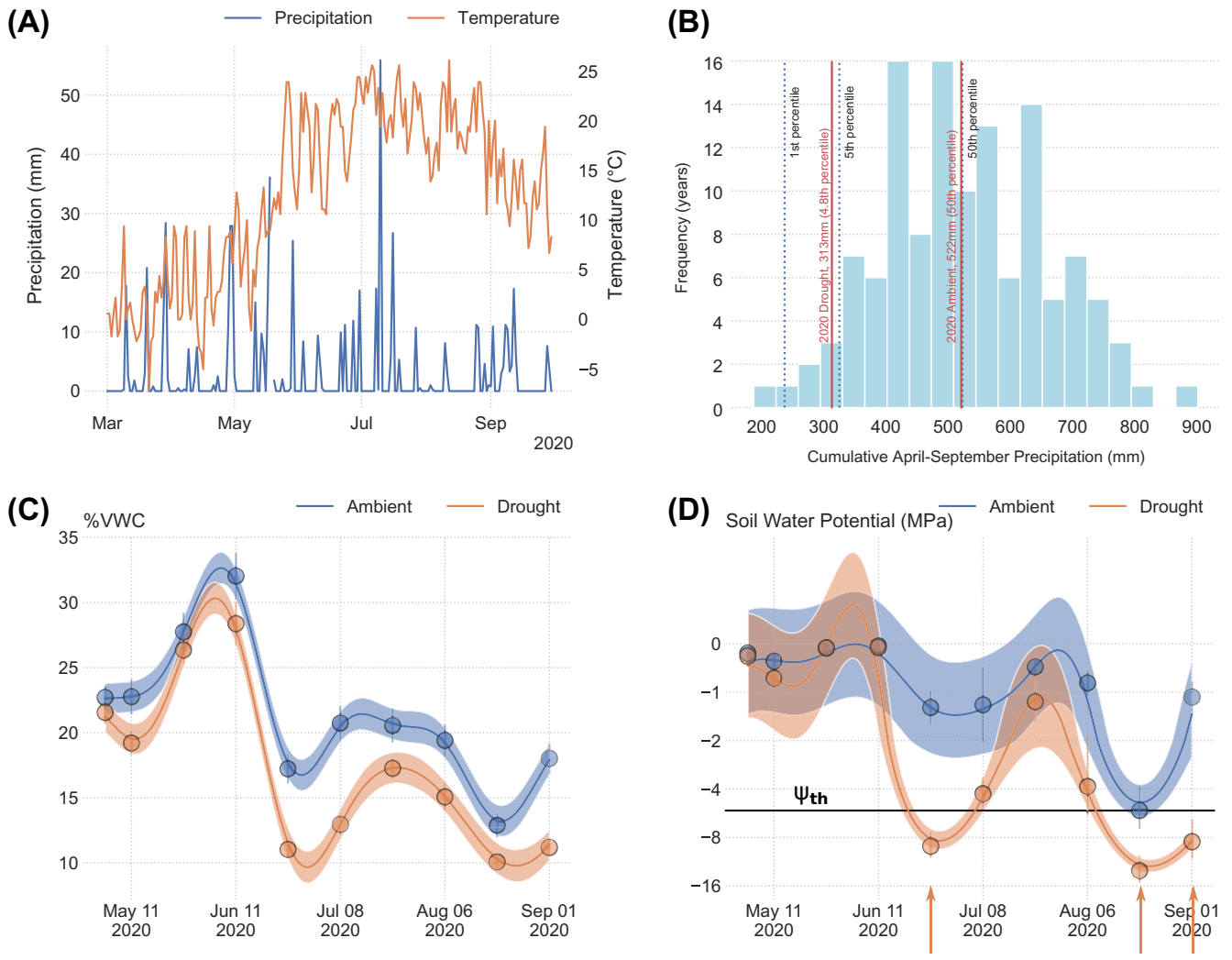

Figure 1. Weather, climate, and soil moisture profiles during our experiment. (A) Daily total precipitation and average temperature for our study site at Oconomowoc, WI during the experimental year of 2020. Precipitation data come from a long-term weather station in Waukesha, WI (USC00478937), and temperature data were obtained from a nearby weather station in Brookfield, WI (USC00471062). (B) Histogram of total growing season (April–September) precipitation, based on 127 years of data from Waukesha, WI (USC00478937). Dotted blue lines show the 50th, 5th and 1st percentiles of growing season precipitation. Red lines show growing season precipitation under ambient conditions and in our experimental manipulations (40% reduction from ambient). (C) Soil moisture profiles during the course of our experiment. Points and bars show means ± 1 SE, and trend lines were fitted via a Gaussian process model. Gaussian process model shows the mean trend ± 1 SE. (D) Soil water potential profiles during the course of our experiment, as estimated from a soil water release curve. Points and bars show means ± 1 SE, and trend lines were fitted via a Gaussian process model. Gaussian process model shows the mean trend ± 1 SE. Orange arrows show the dates at which soil water potential declined below ψ_{th}.

containing the following: 0.5 µl of forward primer Eub338 (10 µM concentration, ACTCCTACGGGAGGCAGCAG), 0.5 µl of reverse primer Eub518 (10 µM concentration, ATTACCGCGGCTGCTGG), 12.5 µl Maxima SYBR Green Flourescein qPCR Master Mix, 9.5 µl ddH2O, and 2.0 µl of template DNA. On each 96-well plate, we ran standards in duplicate, while samples and no-template controls (ddH2O) were run in duplicate or, where possible, triplicate. We used a Bio-Rad CFX96 qPCR machine with thermocycler conditions set to 95°C for ten minutes, followed by 40 cycles of 95°C for 15 s, 50°C for 30 s, and 72°C for 30 s. At the end of each qPCR run, we used melting curves to ensure that the flourescence was not due to primer dimers or other artifacts (Fierer et al. 2005). Following qPCR, we converted cycle numbers to '16S rRNA gene copy numbers per g dry soil', where the dry soil weight was the dry weight of the soil used to extract DNA for each sample.

Bacterial community composition

The second aliquot of extracted DNA samples were sent to Argonne National Laboratory for 16S rRNA gene amplicon sequencing. We amplified the V4 region of the 16S rRNA gene 515F (GTGYCAGCMGCCGCGGTAA) – 806R

(GGACTACNVGGGTWTCTAAT) using a 12-base barcode on the forward primer. The PCR mixture contained 9.5 µl of PCR water, 12.5 µl of Quantabio's Accustart II PCR ToughMix (2× concentration, 1× final), 1 µl of Golay barcode-tagged forward primer (5 µM concentration, 200 pM final), 1 µl reverse primer, (5 µM concentration, 200 pM final), and 1 µl template DNA. Thermocycler conditions were set to 94°C for 3 min to denature DNA, with 35 cycles at 94°C for 45 s, 50°C for 60 s, and 72°C for 90s, followed by a final extension of 10 min at 72°C. Samples were pooled into a single tube, quantified, and diluted to 2 nM. We then added 6.75 pM of 10% PhiX. Amplicons were sequenced on a 151-bp by 12-bp by 151-bp Illumina MiSeq run.

Raw sequence reads were demultiplexed using *idemp* (https://github.com/yhwu/idemp). Paired-end sequence reads were then processed in R (ver. 4.1.1, www.r-project.org) using the DADA2 pipeline (ver. 1.20.0, Callahan et al. 2016a). Briefly, sequences were filtered and trimmed (truncated to minimum 145 bp with maximum error rate of 2 for forward and reverse reads), an amplicon sequence variant (ASV) table was constructed, and chimeras were removed. Taxonomy was assigned using the 'DECIPHER' package (ver. 2.20.0, Wright 2016, Murali et al. 2018) with the Silva database (release ver. 138, Quast et al. 2012). DECIPHER was also used to align the DNA sequences for tree building and subsequent phylogenetic diversity metrics. Following alignment, the R package 'phangorn' (ver. 2.8.0, Schliep 2011) was used to build an initial neighbor-joining tree to use as a starting point for a GTR maximum likelihood tree (Callahan et al. 2016b). After taxonomy assignment, we filtered out only the ASVs belonging to the domain Bacteria and removed any sequences identified as chloroplasts or mitochondria.

Microbial respiration and soil organic content

We estimated microbial activity throughout the growing season in 'Ambient' and 'Drought' plots by measuring microbial CO_2 respiration using an incubation method. One batch of the sieved soil sample was weighed to approximately 5 g (± 0.2 g) and placed into a 100 ml clear glass bottle. Bottles were sealed and then flushed with CO_2-free air. Once flushing was complete, bottles were incubated in a Conviron GEN1000 growth chamber set to 25°C for one hour. After incubation, we recorded CO_2 concentrations (ppm) using an LI-850 CO_2/H_2O infrared gas analyzer modified for reading single samples. After respiration measurements, soils were dried at 60°C for 24–48 h until stable mass was achieved to determine dry weight. Finally, we standardized all measurements to CO_2 µg h^{-1} g dry $soil^{-1}$.

To ensure that changes in soil microbial activity were not caused by changes in soil carbon availability, we estimated soil organic matter content via loss-on-ignition. After respiration trials, soils were dried and weighed to obtain dry weights. The entire soil sample was then combusted at 550°C for four hours (Hoogsteen et al. 2015) and re-weighed. Organic content was calculated as the percent mass loss during combustion.

Decomposition

We quantified ecosystem-level decomposition rates using the tea bag method (Keuskamp et al. 2013). Prior to the experiment, we weighed out 2 g of dried green tea and dried rooibos (i.e. red) tea into nylon tea bags. Tea bags were then heat sealed. On 22 June 2020, we buried one green and one red tea bag in the center of each plot (n = 10 per treatment) 5–8 cm below the soil surface. On 6 September 2020, we retrieved tea bags. Large soil particles were removed from the outside of the bags, and we manually removed any small roots that had grown on or through the bags. One bag contained a hole that allowed soil to mix in with the tea and was excluded from analyses. After processing, the tea was removed, dried, and reweighed. Decomposition rate was calculated as the percent mass loss for each tea type.

Statistical analyses

We analyzed microbial community diversity by first converting the number of reads into relative abundances. We then removed rare taxa that occurred in fewer than 5% of samples and dropped any samples with fewer than 12 000 reads (less than 50% of the average) as unreliable. For each remaining sample, we calculated the exponential of the Shannon diversity index. Temporal trends in diversity and drought effects were modeled using a Gaussian process model. GPMs are commonly used for time-series analyses because they automatically incorporate temporal autocorrelation and allow for nonlinear trends (Rasmussen and Williams 2005, Roberts et al. 2013). The advantage of GPMs is that the Bayesian treatment smooths outliers and thus avoids overfitting and mistaking noise for a signal (Lemoine et al. 2016, Lemoine 2019). Both the response variables and predictor (week of experiment) were standardized to $N(0,1)$ prior to analysis.

Temporal or drought-driven changes in microbial community composition were assessed using multivariate ordination. As with diversity, we first dropped any rare ASVs that occurred in fewer than 5% of samples and removed any unreliable samples with fewer than 12 000 reads. The ASV abundance table was then subject to the Wisconsin transformation for ordination: the abundance table was square-root transformed, each column (ASV) divided by its maximum value, and finally relative abundance was calculated for each sample (Legendre and Legendre 1998). Using the Wisconsin matrix, we then calculated the Bray–Curtis (relative abundance) and Sørenson (presence/absence) pairwise distances for each sample. We also calculated the UniFrac weighted and unweighted pairwise distances using the 'phyloseq' package in R (www.r-project.org, Lozupone and Knight 2005, 2007, McMurdie and Holmes 2013). For each distance matrix, we analyzed differences in community composition using PERMANOVAs and visualized differences using NMDS. We analyzed taxon-specific patterns using differentially abundant taxa analyses based on beta-binomial regression (Martin et al. 2020). Following differentially abundant taxa analyses, we regressed relative abundance against log(VWC)

for all of the bacterial families exhibiting significant variability among sampling dates.

We analyzed temporal trends in both microbial abundance and respiration using Gaussian process models as described above. Given the known relationship between soil moisture and microbial activity, Gaussian process models for both microbial load and respiration included date, drought treatment, and measured VWC as predictors. We analyzed teabag decomposition with a Bayesian two-factor ANOVA that included both tea type, drought treatment, and their interaction as predictors. We placed a hierarchical $N(0,1)$ prior on the standard deviation of regression coefficients. The decomposition mass loss was standardize to $N(0,1)$ prior to analysis.

All analyses, with the exception of sequence processing, PERMANOVAs, and differentially abundant taxa analyses, were performed in Python ver. 3.8.13. Gaussian process models were fit using the *sckit-learn* module, and Bayesian models were fit using cmdSTAN 2.25.0 accessed via the *cmdstanpy* 0.9.67 module. PERMANOVAs and differentially abundant taxa analyses were conducted in R ver. 4.1.1 using the 'vegan' and 'corncob' packages. All raw data, cleaned data, Python scripts, and figures are available on Figshare (https://doi.org/10.6084/m9.figshare.24281992.v1).

Results

Rainout shelters simulated drought by reducing soil moisture

During 2020, our field site in Oconomowoc received 522 mm of rainfall (Fig. 1A). The 40% rainfall reduction imposed by our shelters simulated a drought falling just below the 5th percentile of growing season precipitation, based on 127 years of records (Fig. 1B). Our rainfall reduction treatment successfully reduced soil moisture in the middle and end of the growing season. In late spring, soil moisture was ~ 27% in both 'Ambient' and 'Drought' treatments (Fig. 1C). By early summer, soil moisture was ~ 5% lower in 'Drought' plots than in controls, though VWC remained above 25% in both treatments (Fig. 1C). By mid- and late-summer, VWC fluctuated between 15–20% in 'Ambient' plots and between 10–20% in 'Drought' plots (Fig. 1C). In fact, rainout shelters reduced VWC to 40% of the ambient value throughout most of the growing season, closely matching our 40% rainfall reduction (Fig. 1C).

As hypothesized, the reduction in soil VWC corresponded to a strong reduction in soil water potential (Fig. 1D). Based on our calculations, diffusion limitation (ψ_{th}) occurs when water potential falls below −5.44 MPa. Such severe reductions occurred only three times in our measurements: mid-June, mid-August, and late-August (Fig. 1D).

Drought did not reduce bacterial abundance

We predicted that drought might directly affect microbial communities by reducing bacterial abundance. However, bacterial abundance was unaffected by reductions in soil water content. Bacterial abundance was constant at an average of 11.14 ± 0.44 (mean ± 1 SE) × 10^6 16S rRNA gene copies per g dry soil across the entire soil moisture gradient ($p = 0.381$, Fig. 2A). Given the lack of relationship between soil moisture and bacterial abundance, drought treatments also did not affect soil bacterial abundance, which was also constant throughout the growing season (Fig. 2B). The only treatment difference appeared in late July, when bacterial abundance in 'Drought' plots briefly spiked above 'Ambient' plots (Fig. 2B), but it is unlikely that this difference was related to soil moisture (Fig. 2A).

Drought did not affect bacterial 16S rRNA gene composition

We hypothesized that drought alters ecosystem processes by changing the structure of microbial communities. However, drought had little effect on microbial community composition, although composition did vary throughout the growing season. Across the summer, diversity remained relatively stable between 320–360 equivalent ASVs, and drought did not alter diversity in any appreciable way (Fig. 3). As with diversity, community composition remained stable despite reduced soil moisture in 'Drought' plots, though communities did vary depending on time of sampling (Fig. 4A–C). Indeed, sampling date had a highly significant effect on microbial community structure for all of Bray–Curtis, UniFrac unweighted and UniFrac weighted distance metrics (Table 1). Most notably, bacterial community composition shifted markedly during a rainfall pulse on 11 June, but quickly returned to a relatively stable community composition for the remainder of the summer (Supporting information). However, drought had no impact on either Bray–Curtis or UniFrac weighted distances (Table 1, Fig. 4A–B). Results were similar for the Sørenson presence/absence distance metric, in which only Date had a significant effect (Supporting information). For the UniFrac unweighted distance, drought had a significant but weak effect ($p = 0.02$, Fig. 4C). Differentially abundant taxa analysis identified only two families out of 251 that exhibited slightly significant drought effects: Sphingomonadacaeae and the NS11-12 marine group (Supporting information). All other 249 identified families were unresponsive to drought.

The seasonal variation in community composition was caused by fluctuations in the relative abundance of two phyla. Across all dates, the most common phyla were Actinobacteria (~ 25% of reads), Proteobacteria (~ 22.5% of reads), Acidobacteria (~ 10.5% of reads), Verrucomicrobiota (~ 8% of reads), and Firmicutes (~ 8% of reads). The relative abundances of no phyla were significantly impacted by drought (Supporting information). However, Proteobacteria and Firmicutes both showed rainfall-dependent variation. Following a rainfall event in early June, where soil moisture reached the highest recorded levels (Fig. 1), the relative abundance of Proteobactera declined to 17.5%, while the relative abundance of Firmicutes spiked to 12% before declining back to nominal levels (Supporting information). Thus, these

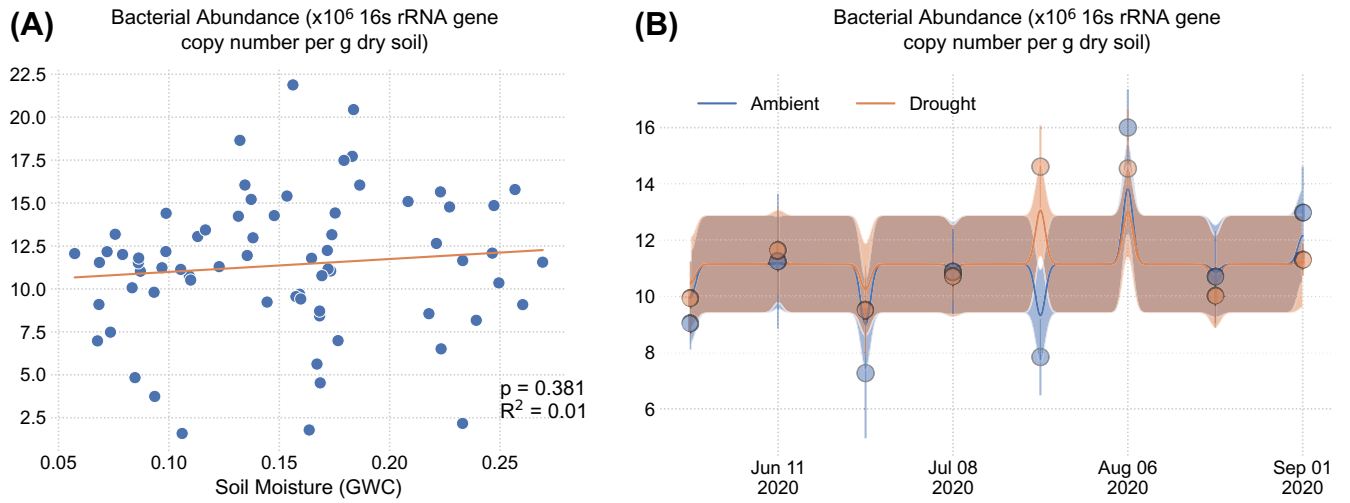

Figure 2. Drought did not affect bacterial abundance. (A) Scatterplot between bacterial load (×10⁶ 16 s copies per g dry soil) and gravimetric water content of the soils for which respiration was measured. (B) Time series of bacterial abundance (×10⁶ 16 s copies per g dry soil) during the course of our experiment. Points and bars show means ± 1 SE, and trend lines were fitted via a Gaussian pProcess model. Gaussian process model shows the mean trend ± 1 SE.

two phyla appear to fluctuate with rainfall and extreme wetting, but were relatively insensitive to soil drying.

Likewise, differentially abundant taxa analysis found 52 of 251 families exhibited significant variation by date (Supporting information). In particular, Micrococcaceae, Rhizobiaceae, Chitinophagaceae, Methyloligellaceae and Rubrobacteriaceae were among the most variable (Fig. 5). Interestingly, variability in abundance among sampling dates appeared to result from phenological changes in soil moisture, but only for some taxa (Supporting information). Examining the eight bacterial taxa that varied most among sampling dates revealed that common bacterial families, such as Bacillaceae, Chitinophagaceae and Rubrobacteriaceae, had strong relationships between abundance and soil moisture (Fig. 5), suggesting that seasonal changes in soil moisture drive the abundance of these bacterial groups. Abundances of rare taxa, like Micrococcaceae, Hyphomicrobiaceae and Methyloligellaceae, varied among dates but had no relationship with soil moisture (Fig. 5). Rare taxa might therefore exhibit stochastic fluctuation among dates, while the abundances of common taxa were determined to large extent by environmental drivers.

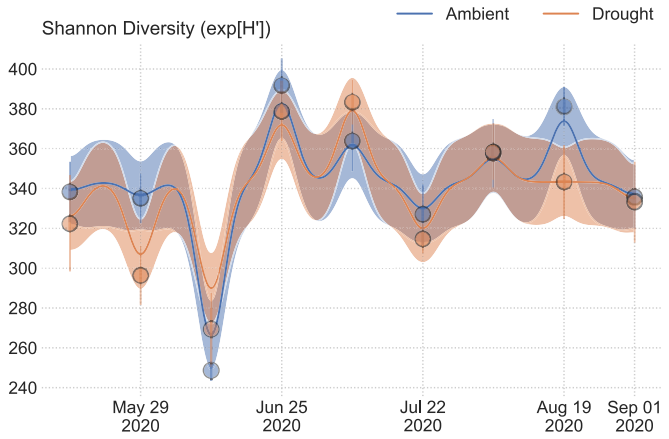

Figure 3. Drought did not affect bacterial diversity. Time series of bacterial diversity during the course of our experiment. Points and bars show means ± 1 SE, and trend lines were fitted via a Gaussian process model. Gaussian process model shows the mean trend ± 1 SE.

Drought-induced diffusion limitation reduced microbial respiration

Despite the stability of bacterial communities under drought, microbial respiration was highly dependent on soil moisture content. Microbial respiration exhibited a curvilinear relationship with VWC; respiration increased rapidly with increasing water content when soils were dry (< 20% VWC), but the relationship weakened in wetter soils (Fig. 6A). Likewise, soil water potential exerted a strong control over microbial activity. Respiration rates declined in a log-linear fashion with soil water potential (Fig. 6B). However, only a few points fell below ψ_{th}, nearly all from the 'Drought' plots (Fig. 6B). The dependence of microbial respiration on soil moisture led to strong drought effects on soil microbial respiration (Fig. 6C). Importantly, respiration was adversely affected by drought only at three time points: mid-June, mid-August, and late-August (Fig. 6C). During these time points, drought reduced soil microbial respiration by 20–30% (Fig. 6C). Importantly, these are the same time points at which drought reduced soil water potential below the ψ_{th} (Fig. 1D). In other words, despite drought reducing soil moisture throughout most of the growing season, adverse effects of drought on microbial respiration appeared only in the time points during which soil pores became hydraulically isolated. The strong reduction in microbial respiration during late summer ultimately reduced cumulative soil respiration throughout the growing season by ~ 10% (Fig. 6D).

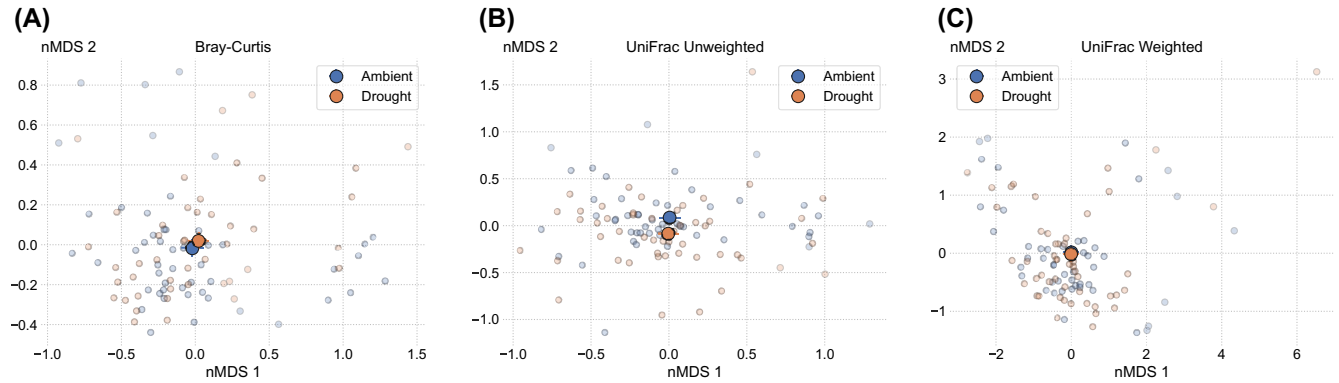

Figure 4. Drought did not affect bacterial community composition. Non-metric multidimensional scaling (NMDS) plots for bacterial community composition for three distance metrics: (A) Bray–Curtis, (B) UniFrac unweighted and (C) UniFrac weighted. Each small point shows a single sample, the large circles show the centroid ± 1 SE. Time series of NMDS profiles are available as Supporting information.

The seasonal and drought-driven changes in soil microbial respiration were not caused by changes in soil organic matter. Loss-on-ignition remained stable between 7–8% for the entire growing season, and drought had no effect on this estimation of organic matter content (Supporting information).

Drought reduced decomposition of refractory leaf material

Decomposition was affected by drought, but varied by type of tea (Pr(Interaction) = 0.95). As expected, green tea decomposed more readily than red tea (Pr(Green tea > Red tea) = 0.99, Fig. 7). However, drought had no effect on green tea decomposition: green tea lost 48 ± 3% of mass under ambient conditions and 45 ± 3% of mass in drought conditions (Pr(Drought < Ambient | Green tea) = 0.20, Fig. 7). Drought did significantly impact red tea decomposition, however. Under ambient conditions, red tea lost 23 ± 3% of mass. Drought reduced decomposition of red tea by almost half, to 12 ± 3% mass loss (Pr(Drought < Ambient | Red tea) = 0.99, Fig. 7).

Table 1. Results of PERMANOVAs for microbial community composition for each of the three pairwise distance metrics. ANOVA table shows type II sums-of-squares for main effects tested in the absence of an interaction, which was not significant for any distance metric. PERMANOVA were conducted using the '*adonis*' function in the package 'vegan' in R (www.r-project.org).

Factor	df	SS	MS	F	p
Bray–Curtis					
Date	**9**	**3.09**	**0.34**	**1.38**	**< 0.001**
Drought	1	0.28	0.28	1.14	0.115
Date × Drought	9	1.97	0.23	0.08	1.00
UniFrac unweighted					
Date	**9**	**1.85**	**0.21**	**1.36**	**< 0.001**
Drought	**1**	**0.19**	**0.19**	**1.26**	**0.02**
Date × Drought	9	1.24	0.14	0.90	1.00
UniFrac weighted					
Date	**9**	**0.19**	**0.02**	**4.24**	**< 0.001**
Drought	1	0.01	0.01	0.98	0.35
Date × Drought	9	0.03	0.003	0.04	1.00

Discussion

We tested the hypothesis that drought would reduce decomposition rates by inhibiting microbial activity below a threshold of soil water potential. Specifically, we predicted that drought would minimize or eliminate the 'hydraulic safety margin' in soils, thereby making microbial communities more susceptible to fluctuations in precipitation and temperature throughout the mid-to-late growing season. Though we found that drought had surprisingly little direct effect on bacterial abundance or composition, we did find that cellular respiration was water-limited only during time periods when soil water potential declined below ψ_{th}. Although sporadic throughout the growing season, these brief reductions in microbial activity translated into large impacts on ecosystem function, cumulative CO_2 release by microbes throughout the growing season declined by 10% and decomposition of refractory rooibos tea declined by 50%. Thus, it appears that drought inhibits microbial-driven ecosystem processes during even brief dry-down events in the mid- to late summer, which can have lasting effects on ecosystem function.

Our study demonstrates that drought encourages hydraulic isolation which can create a tipping point for microbial activity that can occur prior to changes in microbial abundance or composition. Soil microbial activity declined significantly only when drought drove soil water potential below ψ_{th} during the hottest, driest points of the year. There are many, non-mutually exclusive reasons why diffusion limitation might inhibit microbial activity. First, limited water flow might encourage the buildup of antimicrobial compounds or other enzymes that inhibit microbial activity. Many microorganisms, especially Actinobacteria, produce antibiotic enzymes that can accumulate in soil pores when water diffusion is limited (Bouskill et al. 2016). Second, diffusion limitation can also decrease nutrient supply to soil microbes, thereby limiting growth (Manzoni et al. 2012). Finally, reduced water availability can impose physiological costs, such as osmolyte accumulation or eventual desiccation, that reduce microbial activity and growth (Schimel et al. 2007). For all of these reasons, some researchers have suggested that

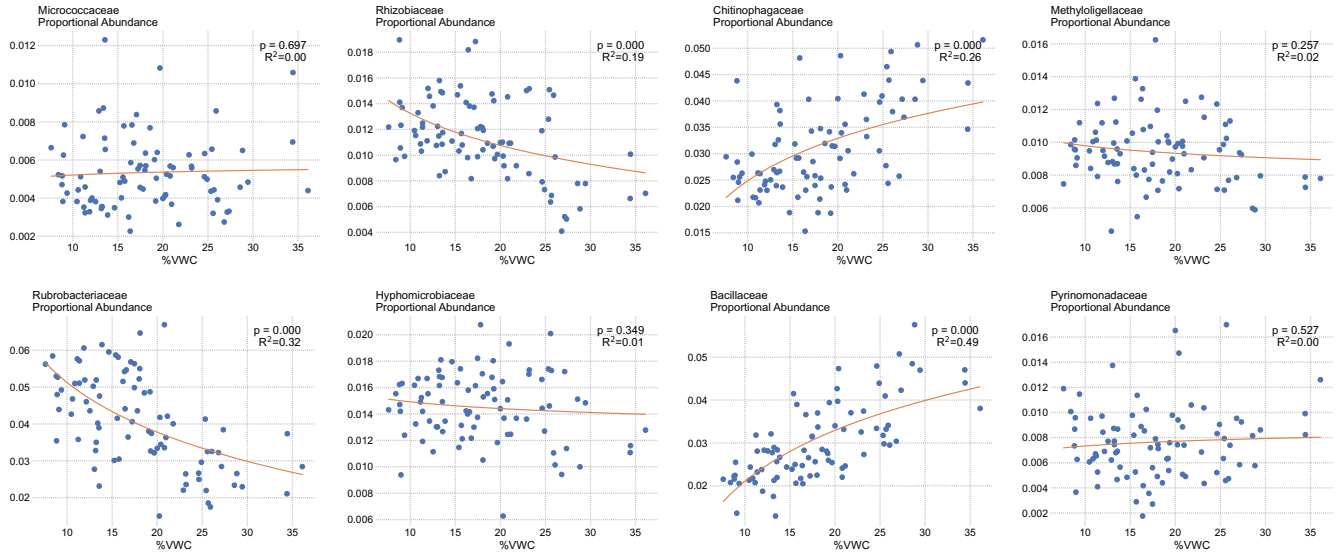

Figure 5. The relationship between soil moisture and relative abundance varied among bacterial families. Linear regressions between relative abundance and soil VWC for the eight bacterial families showing the strongest effect of date during differential analysis. Graphs for all other taxa are available on Figshare (https://doi.org/10.6084/m9.figshare.24281992.v1).

diffusion limitation might be the most important factor limiting microbial activity during soil drydown, especially for mineral soils like those at our study site (Stark and Firestone 1995, Carson et al. 2010).

It is interesting, however, that diffusion limitation did not inhibit decomposition of green tea, only of red tea. Red tea has a lower water content, higher insoluble fraction, and lower nitrogen content and is thus more refractory than green tea (Keuskamp et al. 2013). Green tea typically decomposes faster than red tea, but red tea decomposition is often affected more by environmental and biotic factors than green (Desie et al. 2023). These patterns would suggest that drought affects the decomposition of labile and refractory materials differently, and it is likely that some combination of both physiological stress and substrate limitation contribute to lower decomposition rates at our study sites. Microbial dormancy due to physiological stress could account for the impacts of drought on microbial physiological profiles (Preece et al. 2020), but we do not have those data to state how drought impacted microbial substrate use at our site. Regardless, our study confirms that neither microbial biomass nor diversity appear to be directly related to ecosystem processes (Balser and Firestone 2005), which are instead contingent on soil pore connectivity at certain time points throughout the year.

The overall stability of our studied microbial communities under drought conditions might reflect the fact that a 40% reduction in rainfall is still relatively wet, considering the Wisconsin climate. However, the 40% growing season rainfall reduction imposed here does represent an extreme drought for southern Wisconsin, confirmed by VWC of 10% during the driest points of the growing season (Fig. 1). Though 10% soil VWC might be wet in arid regions (Cherwin and Knapp 2012), soil moisture in wet prairies does not usually fall below 20% except during severe droughts (Knapp et al. 2001, Hoover et al. 2014b, Felton et al. 2019). In addition, the 10% VWC in the clay-rich soils at our study site translated to a soil water potential of −8 to −16 MPa (Fig. 1). These low water potentials resulted in hydraulic isolation of soil pores, which occurs at $\psi_{th} = -5.44$ MPa. Importantly, drought did not reduce soil water potentials below ψ_{th} for the entire growing season, but did so only during the hottest points in the middle to late summer months. Thus, drought kept soil water content closer to hydraulically tipping-points, wherein high temperatures increased evaporation and drove soil water below the hydraulic threshold. The relative stability of bacterial communities to drought mirrors the response of the aboveground communities at this site, where drought had no detectable impact on plant photosynthesis or primary production (Lemoine and Budny 2022). It is also worth noting that we did not measure fungal biomass or community composition. Fungal activity can account for a significant fraction of activity in restored tallgrass prairies (Bailey et al. 2002), although fungi rarely comprise more than 30% of microbial biomass in grasslands (de Vries et al 2006). Still, the role of fungi in driving belowground responses to drought in this ecosystem remains an important topic of further exploration.

Despite the extreme reduction in both soil VWC and MPa, bacterial communities remained numerically and compositionally unaffected by our drought treatment throughout the experiment. While unexpected, our results mirror other studies that report similarly drought-resistant bacterial communities. In some cases, microbial load as measured by either 16S copy number (via qPCR) or bulk microbial C (via fumigation) is unaffected by drought (Balser and Firestone 2005, Canarini et al. 2016, Jurburg et al. 2018). Likewise, the effects of drying on soil bacterial composition varies among studies (Schimel 2018), with many reporting no effect of changing soil moisture on bacterial community composition (Balser and Firestone 2005, Evans and Wallenstein 2012, Canarini et al. 2016). There are several potential reasons why

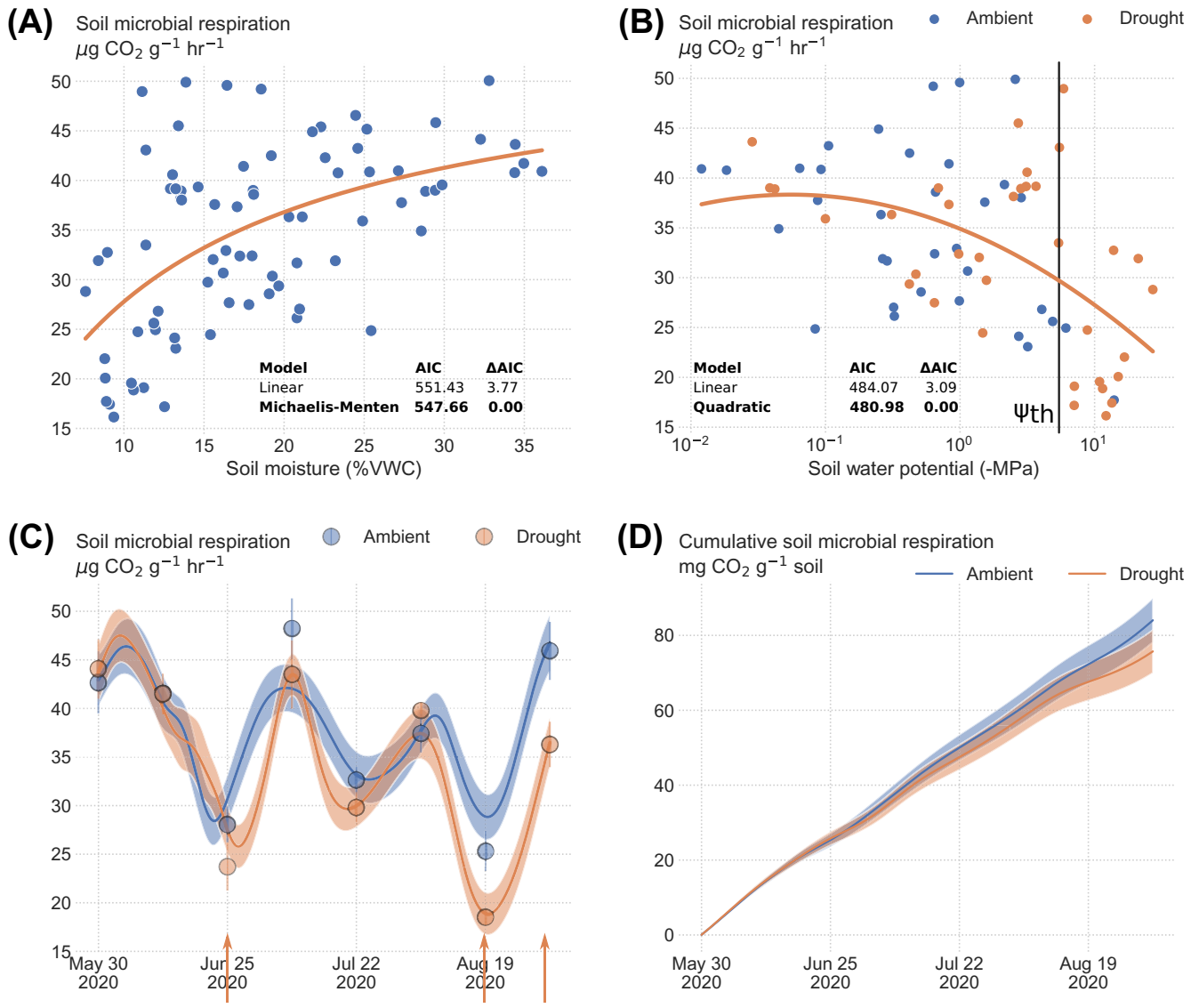

Figure 6. Drought reduced microbial activity during times of year when soil water potential was below ψ_{th}. (A) Scatterplot relating soil microbial respiration to plot-level soil water content. Orange line shows the best-fit line for a Michaelis–Menten model (Table 2). (B) Scatterplot relating soil microbial respiration to the $\log_{10}$ of plot-level soil water potential. Vertical bar represents ψ_{th}, and points are colored by treatment to illustrate that almost all measurements wherein soil water potential fell below ψ_{th} were drought plots. Note that water potential is represented as $-1 \times$ MPa, such that higher values indicate more negative (drier) soils. (C) Time series of soil microbial respiration during the course of our experiment. Points and bars show means ± 1 SE, and trend lines were fitted via a Gaussian process model. Gaussian process model shows the mean trend ± 1 SE. Orange arrows show the dates at which soil water potential declined below ψ_{th} as in Fig. 1D. (D) Cumulative soil microbial respiration (estimated from the Gaussian process model ± 1 SE) over the course of our experiment.

our bacterial communities were unaffected by drought. First, environmental context can dictate how some communities respond to drying, in some situations, soil microbiomes were only drought-sensitive in the presence of large grazing mammals (Jurburg et al. 2018), which are absent from our site. Second, our use of 16s rRNA gene amplicons for both qPCR and sequencing might make microbial communities appear more functionally stable than they actually are. For example, it is possible that portions of our community may have become dormant but were not eliminated across our study, but our metabarcoding process could not account for this. Other methods, such as 16S rRNA or phospholipid fatty acid profiles, might more accurately capture the composition of active bacterial communities (Schimel 2018, Osburn et al. 2022). However, less than 25% of DNA in soils is relic DNA, and relic DNA does not often influence estimates of microbial diversity, richness, or community composition (Lennon et al. 2018). Moreover, the fact that we captured variability in taxa abundances across high temporal resolution sampling dates suggests that our 16S sequencing was sensitive enough to identify short-term changes in community composition on the scale of two weeks. We should therefore have been able to

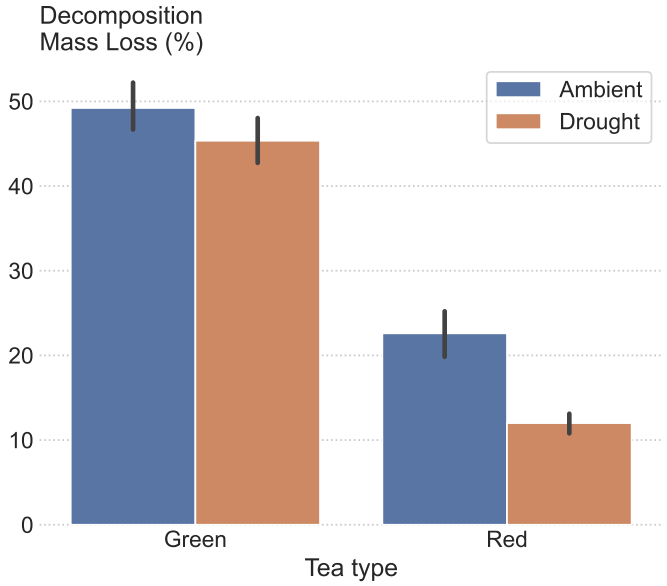

Figure 7. Drought inhibited decomposition of refractory material. Barplot showing the percent mass loss of both green and red tea by drought treatment. Bars and lines represent means ± 1 SE.

detect significant changes in community composition due to drought had they occurred. Instead, our results demonstrate that seasonal phenology, specifically weekly changes in rainfall, is a much stronger driver of bacterial community composition in our study system than a season-long drought. Other studies have reported remarkably stable microbial communities within a season in both grasslands (Lauber et al. 2013) and forests (Rasche et al. 2011), and our results challenge this common perception that microbial communities within a given site are relatively stable throughout the growing season (Kostin et al. 2021, Fox et al. 2022).

Seasonal precipitation patterns can influence microbial processes through drying-rewetting cycles. Variable soil moisture can stimulate microbial activity beyond what would be expected from a stable environment (Evans and Wallenstein 2012). It is unclear whether the dry-down or rewetting has a stronger influence on the pulse of microbial activity (Schimel 2018), but is highly likely that both the precipitation and freeze-thaw seasonality of our study site contributes to the season-long resistance of soil communities to drought (Fierer et al. 2003, Schimel et al. 2007). Given that the variability of precipitation is expected to increase as the climate changes, it is possible that the largest impact of climate change on belowground function at our study site will be the alteration of seasonal drying–rewetting cycles rather than season-long droughts.

More frequent and severe droughts are expected to disrupt ecosystem processes across the globe. Given their importance to global carbon cycling, we must develop a thorough understanding of how drought impacts soil microbial processes. Many studies focus on how drought affects soil microbial composition (Ochoa-Hueso et al. 2018) and/or physiological profiles (Preece et al. 2020). However, in order to understand how global change will impact belowground processes, we need a detailed understanding of how microbial communities, soil moisture, and ecosystem processes like decomposition are linked (Balser and Firestone 2005). Our study highlights that sublethal effects of drought on microbial communities can have large impacts on microbial carbon release or decomposition, and it is necessary incorporate such measures into future studies.

Acknowledgements – We would like to thank the UWM-Waukesha field station staff, Teresa Schueller and Marlin Johnson, for allowing us to work at the site and giving us an endless supply of natural history knowledge. We thank Stacia Peiffer and Kyle Leistikow for their help with qPCR. We also thank Jane Dorweiler and Anita Manogaran for training on and access to the flow cytometer. This research used the Raj high-performance computing facility funded by the National Science foundation award CNS-1828649 and Marquette University. *Funding* – NSF DEB 1941390 to NPL. NSF CNS 1828649 to Marquette University.

Author contributions

Nathan P. Lemoine: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Project administration (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Michelle L. Budny**: Data curation (supporting); Investigation (supporting); Methodology (supporting); Project administration (supporting). **Ethan Rose**: Formal analysis (supporting). **Jane Lucas**: Formal analysis (supporting); Methodology (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). **Christopher W. Marshall**: Conceptualization (supporting); Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Methodology (supporting); Writing – original draft (supporting); Writing – review and editing (supporting).

Data availability statement

Data are available from Figshare: https://doi.org/10.6084/m9.figshare.24281992.v1 (Lemoine et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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