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Land Use History Mediates Soil Biogeochemical Responses to Drought in Temperate Forest Ecosystems

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Abstract

Terrestrial ecosystems are experiencing increasing frequency and intensity of droughts as a result of climate change. Despite a wealth of previous studies investigating soil responses to drought, the importance of historical land use in mediating drought effects remains poorly understood. To identify interactions between drought and historical land use, we sampled soils from two adjacent forested watersheds at the USFS Coweeta Hydrologic Laboratory: a 'reference' forest that has remained undisturbed for approximately a century and a 'disturbed' forest with a history of conversion to pasture followed by abandonment to forest succession. We incubated intact soil cores in the laboratory under one of two moisture treatments: a constant moisture control and a drought-rewet treatment, which involved a six-week drought followed by rewetting and six additional weeks at constant moisture. We measured respiration and characterized soil C and N pools and microbial communities multiple times throughout the experiment. Soils exposed to drought-rewetting had higher cumulative respiration than control soils, which was driven by particularly strong respiration responses of disturbed soils. In addition, drought-rewetting reduced microbial biomass and increased total extractable N and NH_4^+, with greater responses in reference watershed soils. Microbial communities also exhibited responses, with increased biomass C:N, increased fungal:bacterial ratios, and decreased nitrifier abundance in response to drought-rewetting. Overall, our results show that drought responses of forests will vary among land use histories and among soil parameters, which should be considered when seeking to predict biogeochemical responses of temperate ecosystems to climate stressors such as intensifying droughts.

Key words: Soil; Drought; Forest; Carbon; Nitrogen; Nitrification; Microbial community; 16S; ITS; Respiration.

Highlights

- Respiration responses to drought were greater in historically disturbed forests.
- Soil N pool responses to drought-rewetting were

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Author Contributions EDO, BDS, and JEB conceived of and designed the study. EDO and JSS conducted the experiment. EDO conduced statistical analyses and wrote the manuscript. EDO, JSS, BDS, and JEB contributed to editing and revising the manuscript.

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greater in reference forests.
- Drought-rewetting reduced nitrifier abundance and NO3- pools in all forest soils.

Introduction

Ecosystems globally are experiencing an intensification of earth's hydrologic cycle, including increased intensity of rainfall events, but also increased frequency and duration of droughts (IPCC 2013). This intensification of drought-rewetting cycles will have far-reaching consequences for terrestrial ecosystem functioning. For example, desiccation and rewetting represent significant physiological stressors for soil microorganisms (Schimel and others 2007), which are the proximate drivers of element cycles in terrestrial environments (Falkowski and others 2008; Fierer 2017). Drought-rewetting will also alter soil physical processes such as soil organic matter (SOM) stabilization (Fierer and Schimel 2003; Schimel 2018), which has implications for the global carbon (C) cycle, as soils are the largest terrestrial stock of C (IPCC 2013). Further, these drought-sensitive elemental fluxes are key drivers of ecosystem services that support human well-being, including regulation of nutrient cycling (Millenium Ecosystem Assessment 2005), thus highlighting the need to understand effects of drought-rewetting on soil communities and processes.

Some effects of drought-rewetting cycles on soil have been long recognized—for example, drying reduces soil respiration, which is followed by a pulse of CO_2 production after rewetting (Birch 1958). This ''Birch Effect'' results in respiration rates that can be several times higher than basal rates. Though the mechanism of this phenomenon remains poorly understood, the CO_2 pulse likely results from mineralization of microbial necromass and/or intracellular osmolytes by rapidly resuscitating microbial communities (Fierer and Schimel 2003; Placella and others 2012). Additional C substrates fueling the CO_2 pulse may originate from SOM that has been destabilized by rapid rewetting of dry soil (Fierer and Schimel 2003; Schimel 2018). However, the CO_2 pulse is short-lived and may not balance out reductions in CO_2 emissions during dry periods (Borken and Matzner 2009), though soil C balance in response to drought-rewetting will vary among ecosystems and will depend on soil properties such as SOM content (Canarini and others 2017; Zhang and others 2020). Drought-rewetting has similar effects on soil nitrogen (N) cycling, including reduced rates of N-cycle processes (for example, N-mineralization, nitrification) during dry periods, followed by peaks in process rates and larger soil inorganic N pools following rewetting (Birch 1958; Fierer and Schimel 2002; Borken and Matzner 2009; Gao and others 2020). These biogeochemical drought responses are also associated with effects on microbial communities, including reduced microbial biomass, altered bacterial community structure, and increased activity of nitrifying taxa following drought-rewetting (Barnard and others 2013; Placella and Firestone 2013; Evans and Wallenstein 2014; De Vries and others 2018; Zhou and others 2018; Preece and others 2019).

Intensifying drought-rewetting cycles are not the only perturbation facing terrestrial ecosystems—these environments are also experiencing effects of historical and ongoing land use change. Indeed, land use change has modified about 75% of ice-free terrestrial ecosystems globally (Ellis 2011). Further, despite the wealth of previous studies investigating soil responses to drought-rewetting, the influence of land use change in mediating drought-rewetting responses is not well understood. Though drought-rewetting studies across varying land uses are not common, one previous study showed that natural grasslands had more drought-resistant microbial communities and more resistant C-and N-cycle processes compared to intensively cultivated systems (De Vries and others 2012). Another grassland study showed that microbial N metabolism was more sensitive to drought in managed grasslands compared to unmanaged grasslands (Fuchslueger and others 2019). Studies in forested systems have shown that ongoing management (that is, forest thinning) can increase the resilience of microbial community structure and function to drought (Bastida and others 2017, 2019), and that conversion of forests to cultivated or abandoned agricultural land can influence drought responses of some soil biogeochemical processes, such as microbial extracellular enzyme activities (Moreno and others 2019). Although these studies suggest that land use may play an important role in determining soil responses to drought, no studies to our knowledge have assessed the influence of long-term legacies of historical land use (that is, several decades previous) on the drought-rewetting responses of soils from currently unmanaged terrestrial ecosystems.

To assess potential influences of historical land use on soil biogeochemical responses to drought, we conducted a drought-rewetting experiment using intact soil cores sampled from two adjacent watersheds at the USDA Forest Service Coweeta

Hydrologic Lab. Though both watersheds are currently forested, they have distinct land use histories: one is an unmanaged reference site while the other experienced extensive disturbance associated with conversion to pasture in the mid-twentieth century. This land use history, similar to other historical disturbances in the region, has had well-documented long-term effects on various ecosystem properties, including altered vegetation (Elliott and Vose 2011), altered soil microbial communities (Osburn and others 2019; Osburn and Barrett 2020), increased soil nitrification rates (Keiser and others 2016), and increased watershed-scale N export (Swank and Vose 1997). However, the importance of these land use legacies in influencing responses to ongoing and future stressors associated with climate change has not been assessed. The goal of this study is to address the following questions: (1) are soil C losses following drought-rewetting (that is, the ''Birch Effect'') influenced by historical land use? (2) Does historical land use influence responses of soil C and N pools to drought-rewetting? And (3) do key microbial functional groups (for example, nitrifying taxa) respond differently to drought-rewetting across different historical land uses?

Material and Methods

Soil Sampling

We sampled soils at the Coweeta Hydrologic Laboratory, a USDA Forest Service experimental forest in North Carolina, USA (latitude 35°03′ N, longitude 83°25′ W). Annual rainfall is usually high at Coweeta, averaging ~ 1800 mm per year, though droughts are becoming increasingly common and are increasing in duration (Laseter and others 2012; Burt and others 2018). Within the Coweeta basin, we selected two forested watersheds for soil sampling (Figure 1A), one of which (watershed 6) was clear-cut and converted to pasture in 1958, which involved soil scarification, liming, and fertilizing. In 1966–67, the grass was herbicided and the watershed abandoned to forest succession. This history of agricultural abandonment is a common past land use in forested systems of the eastern US (Gragson and Bolstad 2006). The other watershed (watershed 14) is directly adjacent to watershed 6 (Figure 1A). This watershed has not been manipulated by the US Forest Service and has been undisturbed since at least 1923, when ~ 20% of the basal area of the Coweeta basin was cut and harvested (Elliott and Vose 2011). We refer to these alternative land use histories as 'reference' (that is, watershed 14) vs. 'disturbed' (that is, watershed 6), which reflects the more recent and intensive human activity that took place on the abandoned pasture site. Detailed watershed information is available in Table S1. In each watershed, we established six 1 m^2 plots, three at lower elevations near the outlet of the stream and three at upper elevations closer to the source of the stream, a total of 12 plots (Figure 1A). The three plots at each elevation were located at 5, 25, and 50 m along a transect running upslope starting from the stream channel. This sampling scheme is similar to previous studies at Coweeta (Keiser and others 2016) and is intended to incorporate spatial variation in soil properties present in these watersheds into our experiment.

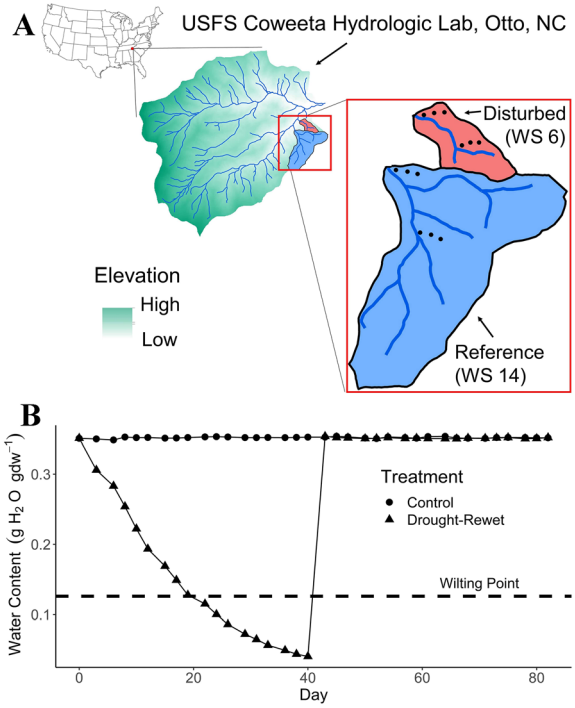

Figure 1. Map of reference and disturbed watersheds sampled for this study (**A**). Black dots represent locations of the 6 plots within each watershed. **B** Illustrates moisture treatments applied to intact soil cores sampled from the field plots. Water content at wilting point (−1500 kPa) was estimated using a pedotransfer function based on soil texture measurements averaged across all pre-treatment soils.

In each plot, we sampled thirteen 10 cm depth, 3.8 cm diameter mineral soil cores in PVC corers. Samples did not include O-horizon material and mineral soil samples at this depth generally include the entire A-horizon in these watersheds (Knoepp and Swank 1994). We focused on mineral soils because C and N pools are much larger in mineral layers than in organic layers at Coweeta (for

example, Knoepp and others 2018) and because organic matter is generally more stable long-term in mineral layers than in organic layers (for example, Hemingway and others 2019). Consequently, mineral soil C and N dynamics are more relevant to the long-term C and N balance of these forests and more effectively reflect the long-term biogeochemical legacies of historical disturbance. Therefore, mineral soils are appropriate for investigating potential influences of historical disturbance on soil biogeochemical responses to climate change factors. After sampling, one core from each plot was immediately processed for measurement of initial soil properties. We attached 25 µm nylon mesh to the bottom of the remaining cores to prevent soil spillage, transported the cores to Virginia Tech on ice, and stored them for one week at 4 °C until the start of the experiment.

Experimental Design

The twelve intact soil cores from each of the twelve field plots (144 cores total) were incubated in the original PVC corers at 20 °C under one of two moisture treatments: a constant moisture control and a drought-rewet treatment. Incubating intact cores minimized disturbances to the soil, allowing us to observe more realistic biological and chemical responses to experimental treatments. On day one of the experiment, all soil cores were adjusted to 0.35 g H_2O gdw^{-1} and soil cores assigned to the constant moisture control group were maintained at this water content throughout the duration of the twelve-week (84 day) experiment (Figure 1B). This water content is equivalent to the long-term average summer soil moisture previously measured at Coweeta (Helvey and Hewlett 1962), is also similar to recent measurements of summer soil moisture at Coweeta (Keiser and others 2016; Osburn and others 2019), and has been used as a control in previous moisture experiments using Coweeta soils (Knoepp and Swank 2002). Further, this water content is approximately equal to field capacity in these soils (~ -10 kPa), which we estimated using a pedotransfer function (Saxton and others 1986) based on soil texture measurements made on the pre-treatment soils using the hydrometer method (Robertson and others 1999). To monitor water content in each core, we weighed PVC cores before and after sampling soil and calculated the dry weight of soil in each core based on initial water content, which we determined for each core based on the water content of the pre-treatment soil sample from the same plot. We adjusted water content by gently pipetting DI H_2O evenly to the soil surface until reaching the calculated weight of the core at 0.35 g H_2O gdw^{-1}. Water additions were done three times per week, which is equal to the long-term average frequency of summer rainfall events at Coweeta (Burt and others 2018).

Soils assigned to the drought-rewet treatment did not receive water for the first six weeks of the experiment (Figure 1B), which was intended to simulate an extreme drought. Our experimental drought period was similar in duration to recent extreme droughts experienced regionally (Williams and others 2017) and at Coweeta specifically (Miniat and others 2017). By the third week of the experiment, soils in the drought-rewet treatment had reached wilting point (-1500 kPa, equivalent to $\sim$ 0.12 g H_2O gdw^{-1}) (Figure 1B). This water potential is also approximately equal to the threshold for inducing microbial osmotic stress previously reported for intact soil cores (Manzoni and others 2012). Soils in this treatment dried to 0.04 g H_2O gdw^{-1} on average by the end of the six-week drought period (Figure 1B). At the end of the six-week drought, we rewet these soils to 0.35 g H_2O gdw^{-1} and maintained them at this water content for the second six weeks of the experiment (Figure 1B). At six time points throughout the experiment, we destructively harvested one control and one drought-rewet core from each field plot (24 cores per time point): after the initial adjustment to 0.35 g H_2O gdw^{-1} (day 1), at the end of the drought (day 42), 24 h after re-wetting (day 43), 72 h after re-wetting (day 45), two weeks after re-wetting (day 56) and six weeks after re-wetting (day 84). This sampling plan allowed us to assess both immediate and longer-term effects of drought-rewetting on our soils. After harvesting, soils were sieved (4 mm), homogenized, and subsamples stored at − 20 °C (for DNA extraction) or 4 °C (for soil properties).

Soil Respiration

We measured soil respiration by capping cores with PVC end caps with butyl septa installed, flushing CO_2 from the headspace of the cores, and measuring accumulated CO_2 after incubating for 24 h using an infrared gas analyzer (Li-7000; Li-Cor Biosciences, Lincoln, Nebraska, USA), similar to the static incubation procedure described by Bradford and others (2008). We conducted respiration incubations twice during the first week of the experiment and twice during the week of the rewet—otherwise, respiration incubations occurred once per week. At each respiration measurement

time point, we randomly selected one control and one drought-rewet core from each field plot (that is, 24 cores measured each time) for measurement. We calculated cumulative respired C for each replicate by integrating under CO_2 evolution time series curves.

Soil Properties

We measured several chemical properties on all collected soils, including microbial biomass C and N, extractable organic C (DOC), total extractable N, NH_4^+–N, and NO_3^-–N. We determined microbial biomass C and N using a modified chloroform extraction procedure (Fierer and Schimel 2003). Briefly, duplicate subsamples of each soil were extracted with 0.5 M K_2SO_4 (1:5 soil:solution ratio) and shaken for four hours, one with 1 ml chloroform added and one without. All extracts were analyzed for DOC and total extractable N on an Elementar vario cube TOC/TNb (Elementar Americas Inc., Mt. Laurel, NJ, United States). Extracts that did not receive chloroform were also measured for NO_3^--N and NH_4^+-N on a Lachat QuikChem flow injection analyzer (Hach Company, Loveland, Colorado, United States). Extractable organic N (DON) was calculated as Total extractable N − (NO_3^-−N + NH_4^+−N). On pre-treatment soils, we additionally measured pH in a 1:1 soil:DI H_2O slurry (Allen and others 1974), using a Hach Sension+ pH meter (Hach company, Loveland, CO, USA) and total C and N on dried, milled samples using an Elementar vario MAX cube (Elementar Americas Inc., Mt. Laurel, NJ, United States). Gravimetric water content of all soils was measured by mass loss after drying at 105 °C for 24 h, and all soil properties are presented on an oven-dried basis.

Microbial Functional Groups

We assessed abundance of microbial functional groups using qPCR. We extracted DNA from 0.25 g soil using the Qiagen DNeasy PowerSoil kit (Qiagen, Valencia, CA, United States) and quantified extracts using a Qubit 2.0 Fluorometer (Thermo Fisher Inc., Waltham, MA, United States). We assessed total bacterial and total fungal abundance by amplifying the 16S rRNA and ITS regions, respectively. For ITS qPCR, we used the ITS1f/5.8 s primers while for 16S rRNA qPCR we used the EUB 338/515 primers (Fierer and others 2005). We also assessed abundance of *amoA* genes involved in nitrification from ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and complete ammonia-oxidizing bacteria (CAOB). For AOA we used the primer set Arch-amoAF/R (Francis and others 2005), for AOB we used the primer set amoA-1f/2r (Rotthauwe and others 1997), and for CAOB we used the comamoa F/R primer set (Zhao and others 2019). All qPCR reactions contained 10 µl Quantitect SYBR master mix (Qiagen, Valencia, CA, United States), 4 µl DNA extract, 0.25 µM (*amoA* genes) or 0.5 µM (16S and ITS) forward and reverse primers, and nuclease-free H_2O to 20 µl. For *amoA* genes, we used undiluted DNA extract (~ 40–300 ng DNA), while for 16S and ITS, we used 1:10 diluted extract (~ 4–30 ng DNA). Thermal cycling conditions for each gene are provided in Table S2. Standard curves for each gene were generated by amplifying serial dilutions of the target genes. Standards for AOA, AOB, and CAOB were synthesized DNA oligonucleotides of *amoA* gene sequences from *Nitrosospira multiformis*, *Nitrosotalea devannaterra*, and *Nitrospira inopinata*, respectively. 16S and ITS gene standards were from environmental (that is, soil) amplicons cloned into plasmids. Standard curves had amplification efficiencies of 80.5–97.5% and R^2 values > 0.99. Amplifications were performed in triplicate, and amplification specificity was assessed using melt curves. All gene abundances were corrected for dry soil mass and were log transformed prior to analysis. Fungal:bacterial ratios (ITS:16S) were calculated by dividing ITS gene copy numbers by 16S gene copy numbers (Fierer and others 2005).

Statistical Analysis

We performed all statistical analyses in R (R Core Development Team 2019) using the 'lme4' and 'emmeans' packages (Bates and others 2019; Lenth and others 2019). To assess effects of historical land use (that is 'disturbance') on pre-treatment soil properties, we used one-way analysis of variance (ANOVA). For soil variables measured throughout the experiment, we assessed effects of disturbance, drought treatment, and disturbance x drought interactions using linear mixed effects models ('lmer' function, lme4 package). Our mixed effects models included 'plot' as a random effect, which allowed us to account for repeated measurements of soil and microbial variables from the same field plots over time, plots being represented in the laboratory experiment by the intact cores sampled from each respective plot. We assessed assumptions of normality of residuals of our models by examining q-q plots, and when deviations from normality were observed, we used generalized linear mixed effects models ('glmer' function, gamma distribution and log-link function, lme4 package).

Though we were primarily interested in effects of disturbance and drought, for variables assessed at multiple time points we also included 'day' as a categorical fixed effect in our models, which allowed us to conduct pairwise comparisons (Tukey's HSD) between all treatments within each day using the emmeans package ('emmeans' and 'contrast' functions). We identified relationships between all variables using Spearman rank correlations, and we investigated potential drivers of soil NO_3^-–N pools using linear regression.

For all statistical analyses, plots were treated as independent replicates, similar to other recent watershed-scale studies in the region (Keiser and others 2016). Strictly speaking, our individual plots within watersheds do not represent true replicates of the land use treatments, as land use treatments were applied at the whole-watershed scale. However, because each plot occupies a unique elevation x slope location within each watershed, and because Coweeta watersheds exhibit high spatial variability in soil properties among these different elevations and slope positions (for example, Hawthorne and Miniat 2018; Knoepp and others 2018), our individual field plots are statistically independent.

Results

Pretreatment Soils

Pre-treatment soils collected from the reference and disturbed watersheds exhibited distinct soil properties, including significantly higher pH and ~ fourfold higher NO_3^-–N in the disturbed forest soils (Table 1). Reference forest soils, on the other hand, had ~ 52% higher DOC, ~ 116% higher microbial biomass C, ~ 24% higher DON, 24% higher extractable C:N, and ~ 25% higher total C:N (Table 1).

Soil Respiration

Respiration rates in constant moisture control soils exhibited little change over time and were not different between land uses at any measurement time point (Figure 2A, Table S3). In contrast, soils assigned to the drought-rewetting treatment exhibited significantly altered respiration dynamics—by week two of the drought period, these soils respired significantly less than control soils, with 50% lower respiration rates in drought-rewet soils by the end of the drought period on day 42 (Figure 2A, Table S3). Drought soils peaked in respiration after being rewet, respiring ~ threefold more than control soils on day 44 (Figure 2A, Table S3). These soils continued respiring more than control soils for ~ 3 weeks following the rewet, and then fell to levels statistically indistinct from the control soils for the final 3 weeks of the experiment (Figure 2A). The peak in respiration was larger in disturbed-drought soils, which had 40% higher respiration than reference-drought soils on day 44 (Figure 2A, Table S3). Integrating under time series curves showed that cumulative respiration did not differ between land uses in constant moisture control soils (Figure 2B, Table S4); however, we did detect a significant overall effect of drought on cumulative respiration, where soils exposed to drought-rewetting respired ~ 8% more C than control soils overall (Figure 2B, Table S4). This pattern was driven primarily by a significant pairwise difference in cumulative respiration between moisture treatments from the disturbed watershed, where

Table 1. Pre-treatment Soil Variables from Watershed 14 (Reference) and Watershed 6 (Disturbed)

Variable	Reference	Disturbed
pH	5.14 (0.20)	5.77 (0.06)*
NO_3^- (μg N gdw^{-1})	0.035 (0.015)	0.185 (0.080)†
NH_4^+ (μg N gdw^{-1})	0.762 (0.163)	0.923 (0.152)
Total Extractable N (μg N gdw^{-1})	40.1 (1.89)*	32.8 (1.47)
DON (μg N gdw^{-1})	39.3 (1.55)**	31.7 (1.77)
Extractable DOC (μg C gdw^{-1})	553 (36.6)***	363 (18.2)
Extractable C:N	13.8 (0.59)**	11.1 (0.44)
Microbial Biomass C (μg C gdw^{-1})	164 (24.5)*	75.9 (17.7)
Total C (mg C gdw^{-1})	44.3 (2.02)	37.1 (5.06)
Total N (mg N gdw^{-1})	2.27 (0.271)	2.31 (0.141)
Total C:N	19.9 (1.37)*	15.9 (0.41)

*Values are means with one SE shown in parentheses. Symbols indicate significantly higher values (ANOVA) at the following significance levels: † $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*

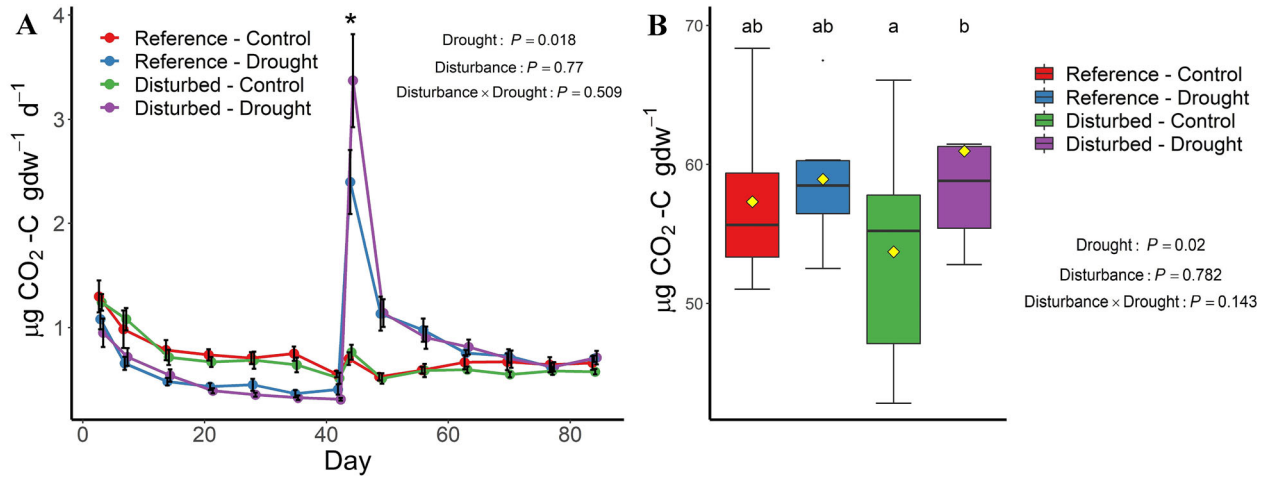

Figure 2. Respiration dynamics during the drought-rewetting experiment (**A**) and cumulative respiration integrated over the entire 84-day experiment (**B**). *P* values are from mixed effects models. The asterisk in (**A**) indicates significantly higher respiration in the Disturbed-Drought soil cores relative to the Reference-Drought soil cores immediately after the re-wet occurred. Yellow diamonds in (**B**) represent mean cumulative respiration for each treatment. Different letters in (**B**) represent significant pairwise differences between treatments (Tukey's HSD $P < 0.05$). Full statistical tables for mixed effects models and post hoc tests are shown for respiration dynamics and cumulative respiration in Tables S3 and S4, respectively.

drought soils had ~ 13% higher cumulative respiration than control soils (Figure 2B, Table S4).

Soil C and N pools and Microbial Functional Groups

We observed a significant overall effect of drought on microbial biomass C pools, where control soils had ~ 80% higher microbial biomass than drought soils at the end of the drought on day 42 (Figure 3A, Table S5). We also identified a significant drought x disturbance interaction, where soils from the reference watershed exhibited greater effects of drought on microbial biomass—reference-control soils had ~ 98% more microbial biomass than reference-drought soils on day 42 (Figure 3A, Table S5). This pattern held at other time points in the experiment—reference-drought soils again had significantly lower microbial biomass C than reference-control soils at day 56, while microbial biomass C in disturbed-drought soils were never significantly different from disturbed-control soils (Figure 3A, Table S5). Indicators of microbial community composition, that is, microbial biomass C:N and fungal:bacterial ratios (that is, ITS:16S), also exhibited effects of drought, though the effects were the same across both historical land uses (Figure 3B, C, Table S6, S7). Specifically, biomass C:N was ~ 92% higher in drought soils at the end of the drought on day 42, though biomass C:N quickly returned to control levels within days of re-wetting (Figure 3B, Table S6). Similarly, fungal:bacterial ratios were ~ 52% higher in drought soils at the end of the drought but did not show consistent patterns between moisture treatments thereafter (Figure 3C, Table S7). Fungal:bacterial ratios also exhibited clear land use effects throughout the experiment, being ~ 67% higher in reference soils than disturbed soils overall (Figure 3C, Table S7).

Extractable DOC declined with microbial activity—in constant moisture control soils, DOC had significantly reduced by day 42, whereas DOC remained high in drought soils at that time (Figure 4A, Table S8). After re-wetting, DOC rapidly declined in drought-rewet soils and reached control levels by day 56 (Figure 4A, Table S8). Total extractable N showed significant drought effects, with drought soils having ~ 75% higher N at the end of the drought on day 42, a pattern that persisted throughout the remainder of the experiment (Figure 4B, Table S9). Further, we identified a significant drought x disturbance interaction, where drought had particularly strong effects on extractable N in reference soils (~ 130% higher in drought soils, Figure 4B, Table S9). Because more than 90% of the extractable N is organic in our samples (Table 1), extractable organic N exhibited nearly identical responses (Figure S1). These extractable N responses are responsible for the drought responses we observed for extractable C:N, which was overall lower in soils exposed to

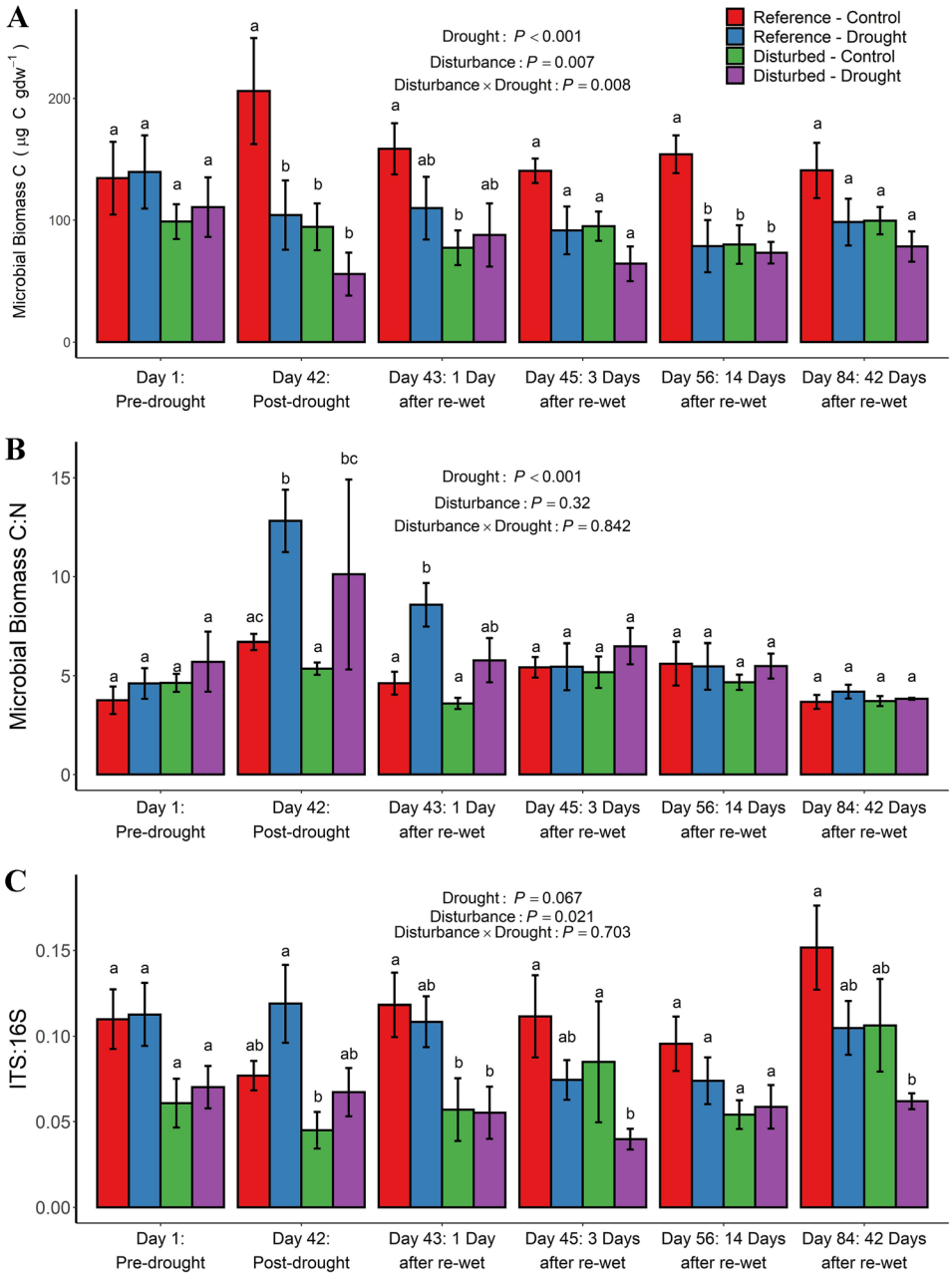

Figure 3. Microbial biomass C (**A**), Microbial Biomass C:N (**B**), and fungal:bacterial (ITS:16S) ratios (**C**) at key time points in the drought-rewetting experiment. *P* values are from mixed effects models, whereas different letters represent significant pairwise differences between treatments within each day (Tukey's HSD $P < 0.05$). Error bars represent ± one SE. Complete statistical tables for mixed effects models and post hoc tests are shown for Microbial biomass C, biomass C:N, and ITS:16S in Tables S5, and S6, and S7, respectively.

drought, but particularly in drought soils from the reference watershed (Figure S2). The response of NH_4^+–N was similar, though effects did not appear until the re-wet occurred on day 43, at which point drought soils had 173% higher NH_4^+-N than control soils, a pattern that persisted until the end of the experiment (Figure 4C, Table S10). Once again, we identified a significant drought x disturbance interaction, where reference soils showed particularly strong drought effects on NH_4^+ (~ 480% higher in drought soils, Figure 4C, Table S10).

NO_3^--N and nitrifying taxa exhibited clear land use effects throughout the experiment, where disturbed soils had ~ 12-fold higher NO_3^--N concentrations on average (Figure 5A, Table S11) and generally greater abundance of nitrifiers (Fig-

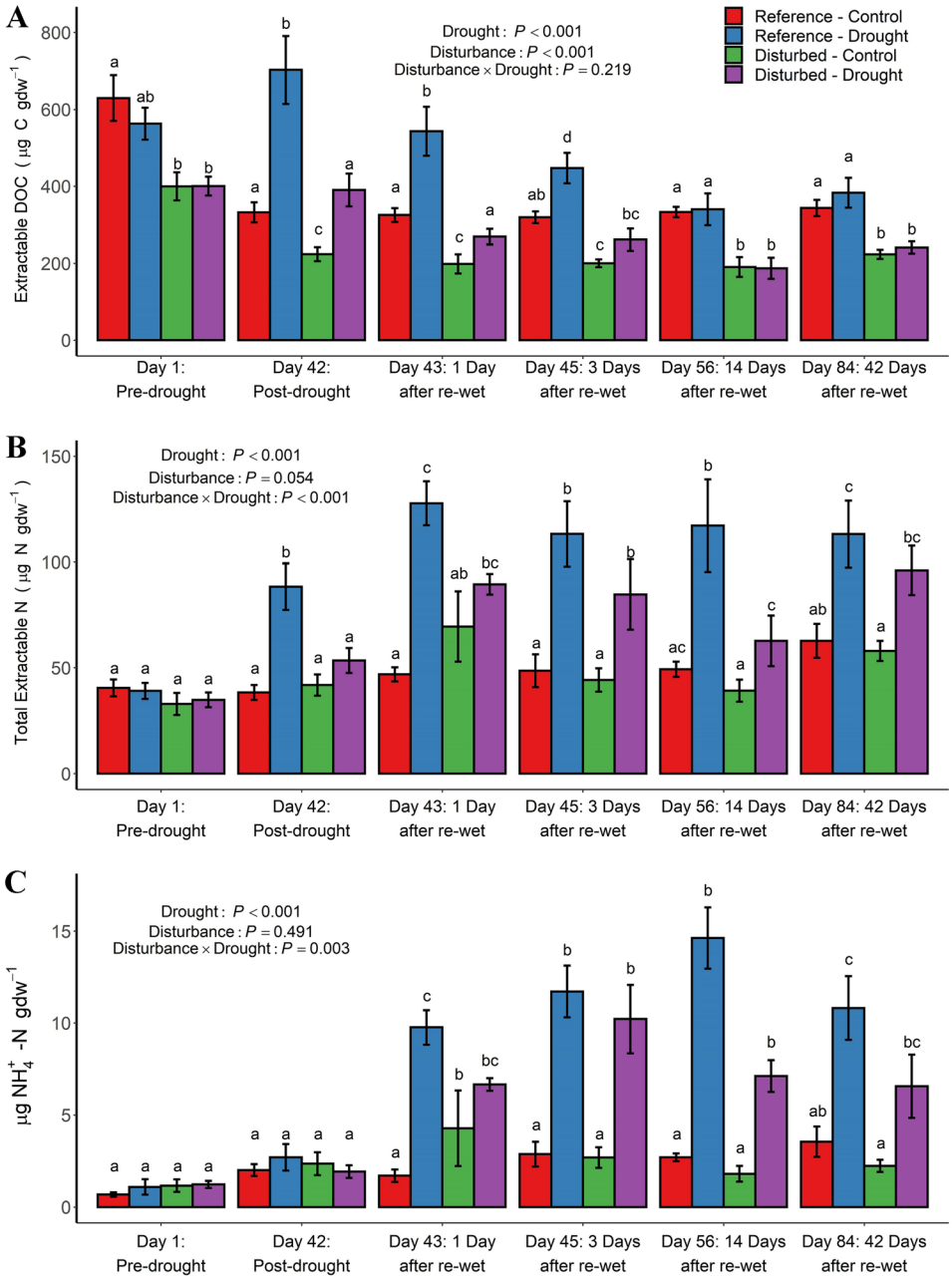

Figure 4. Soil extractable DOC (**A**) total extractable N (**B**) and NH_4^+-N (**C**) pools at key time points in the drought-rewetting experiment. *P* values are from mixed effects models, whereas different letters represent significant pairwise differences between treatments within each day (Tukey's HSD $P < 0.05$). Error bars represent ± one SE. Complete statistical tables for mixed effects models and post hoc tests are shown for DOC, total extractable N, and NH_4^+ in Tables S8, S9, and S10, respectively.

ure 5B, C, S3, Table S12, S13). NO_3^--N also showed significant drought effects, though they were opposite of NH_4^+-N; at the end of the drought on day 42, control soils had ~ fourfold higher NO_3^--N than drought soils, a pattern that held at most remaining time points (Figure 5A, Table S11). The NO_3^- response is likely related to the drought responses of nitrifying taxa; both AOA and CAOB had lower abundance in drought-exposed soils (Figure 5B, S3, Table S12), indicating inhibiting effects of drought-rewetting on nitrifier activity and growth. In contrast, AOB exhibited less clear drought effects; there were no clear patterns in AOB abundance immediately post drought-rewetting (Figure 5C, Table S13). However, by the end of the experiment AOB appeared to be growing in the

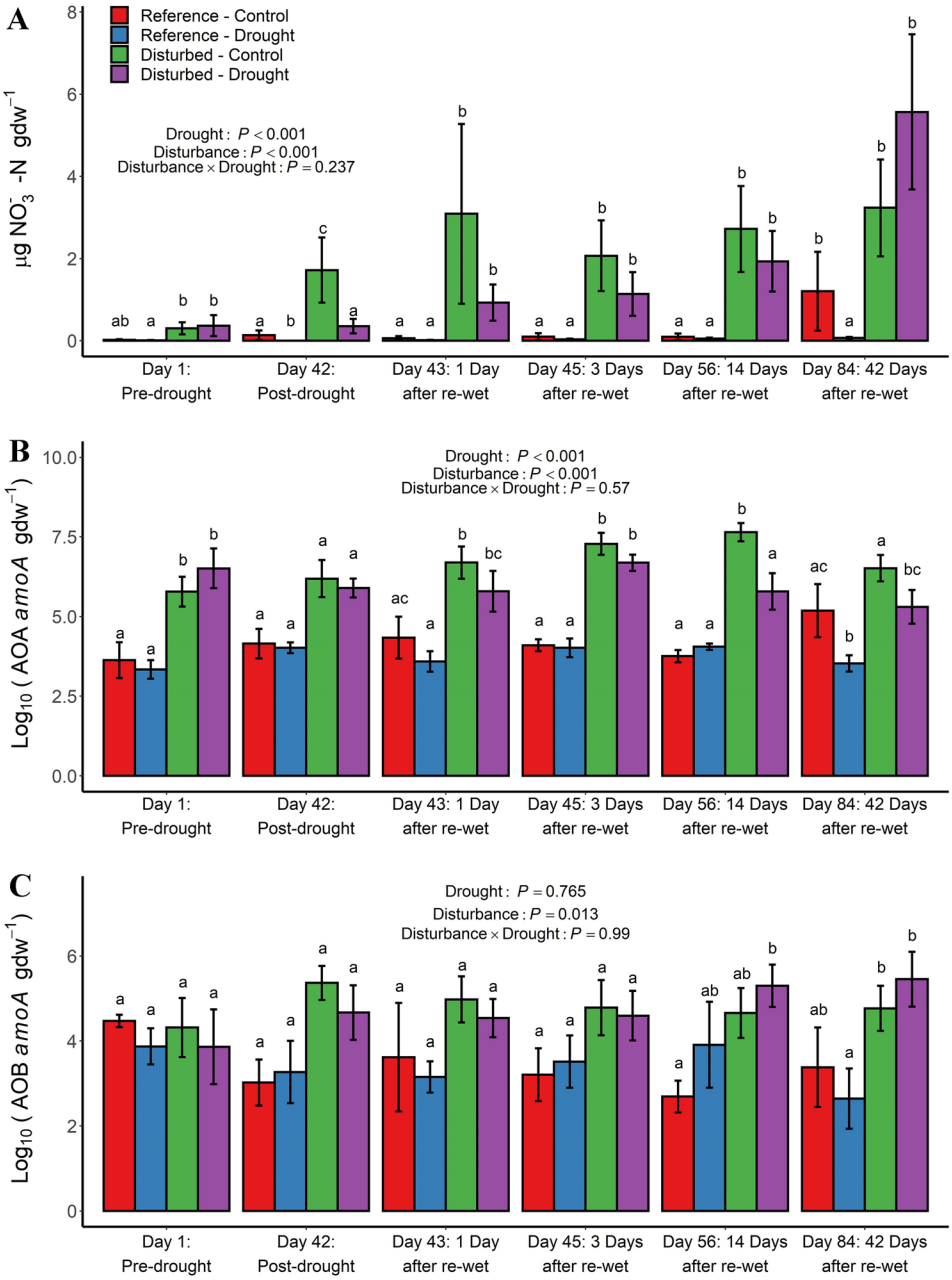

Figure 5. Soil NO_3^- (**A**), AOA *amoA* (**B**), and AOB *amoA* (**C**) at key time points in the drought-rewetting experiment. *P* values are from mixed effects models, whereas different letters represent significant pairwise differences between treatments within each day (Tukey's HSD $P < 0.05$). Error bars represent ± one SE. Complete statistical tables for mixed effects model and post hoc tests are shown for NO_3^-, AOA *amoA*, and AOB *amoA* in Tables S11, S12, and S13, respectively.

disturbed-drought treatment, which had higher AOB abundance than some other treatments on days 56 and 84 (Figure 5C, Table S13), which may be related to the peak in NO_3^- we observed on day 84 in disturbed-drought soils (Figure 5A, Table S11). Indeed, AOB abundance was a strong predictor of NO_3^- at that time point ($R^2 = 0.771$, Figure S4). Overall, across all samples, the combination of extractable C:N and AOA *amoA* gene abundance was strongly predictive of soil NO_3^- (adj. $R^2 = 0.599$, Figure 6), where high soil NO_3^- was associated with the co-occurrence of low C:N and high AOA *amoA* gene abundance (Figure 6, Table S14).

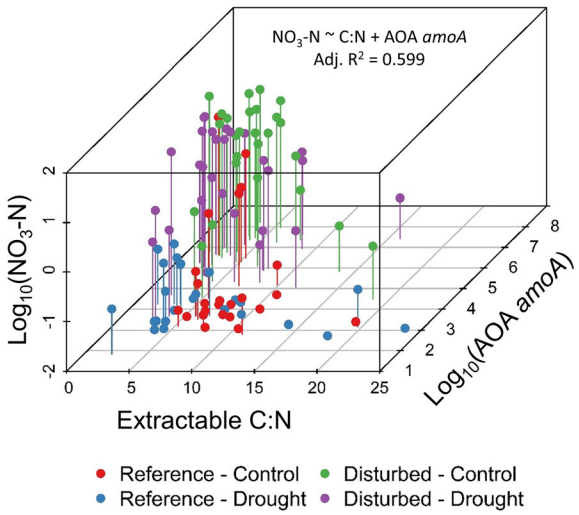

Figure 6. Relationships among soil extractable C:N, AOA *amoA* gene abundances, and soil NO_3-N concentrations. NO_3^- is in units of μg N and both NO_3-N and AOA *amoA* are expressed per gram of dry soil. The adjusted R^2 listed is for the multiple regression shown, predicting NO_3-N from extractable C:N and AOA *amoA* abundance. Table S14 shows complete statistical results for the multiple regression model shown.

Discussion

Forest soils of the southern Appalachian region may experience net respiratory C losses in response to drought-rewetting (Figure 2B), likely associated with the C-rich nature of these soils (Canarini and others 2017). These cumulative respiratory C losses appear to be greater in soils from forests with a history of intensive land use (that is, 'disturbed', Figure 2B), which is associated with the exaggerated Birch Effect we observed in these soils (Figure 2A). Several potential mechanisms may have contributed to the different respiration responses between land uses we observed. For example, increased C substrates from destabilization of SOM have been frequently proposed to be a potential mechanism for the Birch Effect (Fierer and Schimel 2003; Borken and Matzner 2009; Zhang and others 2020), and this destabilization effect may vary among land uses due to differences in soil physical properties (for example, aggregation) leading to differences in soil C protection in soils with distinct disturbance histories. SOM pool sizes also differ among land uses (that is, larger in reference watershed soils) (Keiser and others 2016), which may further contribute to SOM-related mechanisms that differ among historical land uses. However, SOM destabilization is thought to play a larger role when multiple repeated cycles of drought-rewetting take place (Miller and others 2005; Xiang and others 2008; Schimel 2018) and SOM destabilization is therefore a less likely source of C in our single drought-rewetting event.

Differences in resident microbial communities between historical land uses may also have contributed to the distinct respiration responses we observed; disturbed soils at Coweeta host higher relative abundance of r-selected bacterial taxa and higher microbial activity (that is, higher substrate-induced respiration) (Osburn and others 2019), both of which may have contributed to the amplified respiration response. In addition, the apparently greater drought-tolerance of microbial biomass in disturbed soils (Figure 3A) may have resulted in a larger active community in disturbed soils following drought, potentially contributing to the larger respiration responses we observed. The differences in drought-tolerance of microbial biomass between historical land uses may also be attributed to compositional differences in microbial communities. For example, reference soils from Coweeta have higher relative abundance of Acidobacteria (Osburn and others 2019), which have been previously shown to decline in abundance following drought (Barnard and others 2013), whereas disturbed soils host higher relative abundance of Actinobacteria (Osburn and others 2019), which have been shown to respond positively to drought (Barnard and others 2013; Preece and others 2019). Regardless, the reductions in biomass suggest that labile C from lysed microbial cells is a likely source of C fueling the CO_2 pulse we observed after rewetting. Rapid mineralization of intracellular osmolytes as microorganisms adapted to changing water potentials also likely played a role (Fierer and Schimel 2003; Schimel 2018).

Our results also illustrate effects of drought on broad indicators of microbial community composition. For example, we observed increased microbial biomass C:N in response to drought (Figure 3B), which has also been previously observed (Evans and Wallenstein 2012; Sun and others 2020b). This increased biomass C:N may be due in part to microbial physiological responses to drought. For example, fungal taxa accumulate polyol osmolytes (for example, glycerol, mannitol) in response to water stress, which do not contain N and might increase overall biomass C:N (Schimel and others 2007). Another potential mechanism is greater dominance of fungi, which have higher biomass C:N than bacteria (Cleveland and Liptzin 2007). This is supported by fungal:bacterial ratios, which were higher in soils exposed to drought at the end of the drought period (Figure 3C), a pattern that has also been observed in other studies (Evans and

Wallenstein 2012; Preece and others 2019; Sun and others 2020a). Indeed, in general, fungi are thought to be better adapted to water stress than bacteria (Schimel and others 2007) and previous studies have shown fungal communities to be more drought-tolerant relative to bacterial communities (De Vries and others 2012, 2018; Barnard and others 2013; Preece and others 2019). Nevertheless, more sophisticated molecular techniques (for example, amplicon sequencing) should be used in future studies to assess influences of past land use on drought responses on soil microorganisms at the community scale.

Because our experiment did not involve C additions, the reductions in DOC we observed over the course of the experiment (Figure 4A) were expected. C additions to mineral soil from O-horizon leachate and root exudates would likely influence drought responses, highlighting the need to perform additional studies under field conditions. The increases in NH_4^+ pools we observed following rewetting (Figure 4C) are a common result in drought-rewetting experiments, and are the product of a peak in N-mineralization rates after rewetting, that is, the N-cycle manifestation of the Birch Effect (Birch 1958; Borken and Matzner 2009; Gao and others 2020). However, the larger N-mineralization responses of reference soils were surprising, given the greater N-mineralization potential we have previously observed in disturbed soils from Coweeta (Osburn and others, unpublished manuscript). We considered the possibility that higher nitrification rates in disturbed soils may be responsible for the smaller apparent responses of NH_4^+ pools. However, examining total DIN (that is, $NH_4^+ + NO_3^-$) reveals that this is not the case—the DIN response of reference soils still appears larger (Figure S5). Instead, the larger NH_4^+ response of reference soils may be attributed to differences in microbial community structure—reference soils exhibit higher fungal:bacterial ratios (Figure 3C), and because fungi have lower N demands than bacteria (Cleveland and Liptzin 2007), lower microbial N uptake in fungal-dominated reference soils may explain the larger net increase in NH_4^+. The large NH_4^+ response of reference soils is also likely attributed to the large flush of mineralizable organic N that occurred in these soils after drought (Figure 4B, S1) and NH_4^+ pools were indeed strongly correlated with DON availability (Figure S6). Approximately half of the $\sim$ 50 μg N increase in DON that occurred in reference-drought soils can be accounted for by lysed microbial biomass N entering the DON pools (Figure S1, S7). After accounting for low extraction efficiency of microbial biomass N using chloroform-based methods ($\sim$ 50%, Joergensen and Mueller 1996), it is likely that the majority of the DON increase can be explained by lysed biomass N. Though less likely in our single drought-rewetting event, it is also possible that N from destabilized SOM contributed to the DON increase.

Multiple previous studies have documented rapid increases in activity of nitrifying taxa and increases in soil NO_3^- pools immediately after drought-rewetting (Fierer and Schimel 2002; Placella and Firestone 2013; Gao and others 2020). However, our experiment yielded different results—we observed reduced abundance of AOA and CAOB up to six weeks following re-wetting (Figure 5B, S3) and smaller NO_3^- pools in drought-rewet soils compared to control soils at most time points (Figure 5A). This result may reflect small resident populations of nitrifiers or poor drought-tolerance of nitrifying taxa in soils from this normally high-rainfall ecosystem. Indeed, a previous study from high-rainfall ecosystems also reported reduced nitrifier abundance after repeated drought and rewetting (Zhou and others 2016). Regardless, the inhibiting effects of drought-rewetting on nitrifying taxa may be short lived—AOB appeared to be growing in disturbed-drought soils by days 56 and 84 of the experiment (Figure 5C), and NO_3^- pools had peaked in those soils by day 84 (Figure 5A). Further, long-term effects of drought-rewetting on nitrifiers and NO_3^- pools will likely be mediated by effects of drought on soil C:N, which will alter competitive dynamics for NH_4^+ between heterotrophic microorganisms and autotrophic nitrifiers (Verhagen and others 1992; Booth and others 2005). Indeed, our results show that large soil NO_3^- pools are associated with the co-occurrence of low soil C:N and high nitrifier abundance (Figure 6), likely because C-limiting conditions promote increased nitrifier activity as a result of reduced heterotrophic N-demand (Booth and others 2005; Keiser and others 2016). This C:N–NO_3^- relationship is similar to previous reports (Taylor and Townsend 2010) and has implications for the long-term N-balance of these ecosystems, as NO_3^- is the dominant form of N loss from watersheds in the region (Webster and others 2016).

In addition to documenting influences of historical land use on effects of drought-rewetting, our study is somewhat unique in that the Coweeta basin is historically among the wettest locations in the eastern US (Laseter and others 2012). While many previous drought-rewetting studies have taken place in semi-arid environments with soils presumably pre-adapted to water stress (for exam-

ple, Fierer and Schimel 2002; Placella and others 2012; Barnard and others 2013; Bastida and others 2017; Guillot and others 2019; Moreno and others 2019), our results show that drought responses may be different in humid, historically high rainfall ecosystems. For example, though previous work has suggested that the Birch Effect generally does not make up for reduced respiration during dry periods (Borken and Matzner 2009), we documented larger respiratory C loss in drought-rewetting soils compared with control soils (Figure 2B). This is potentially due to the C-rich nature of our soils (Canarini and others 2017) or to resident microbial communities poorly adapted to stress associated with drought-rewetting cycles. Similarly, we observed N-cycle responses different from those reported from semi-arid environments (that is, reduced nitrification, Figure 5A) (Fierer and Schimel 2002; Placella and Firestone 2013). Our study also highlights opportunities for future work—for example, future studies should incorporate different drought durations, as different C and N dynamics may be observed under more moderate drought conditions that induce less extreme microbial water stress. Future investigations should also focus on identifying causal links among soil properties, microbial communities, and biogeochemical process rates in the context of drought and rewetting. Though our results show many strong correlations between variables (Figure S6) and suggest clear mechanistic links between soil C:N, nitrifier abundance, and soil NO_3^- pools (Figure 6, S4), our experiment does not directly assess causal mechanisms. Future studies should attempt to elucidate these mechanisms, potentially by employing ^{15}N and ^{13}C isotopes to track movement of C and N between various soil pools throughout the drought-rewetting process.

Finally, it should be noted that our study only explicitly addresses the influence of one type of historical land use, that is, forest conversion to agriculture, on drought responses of forest soils. However, the differences in soil properties we observed between land uses (Table 1) are similar to those reported from nearby sites across multiple different types of historical disturbance, including clear-cutting, cable logging, and conversion to pine monoculture (Keiser and others 2016; Osburn and others 2019). Further, microbial communities and biogeochemical cycles respond similarly to multiple different historical disturbance types in the region (Swank and Vose 1997; Keiser and others 2016; Osburn and others 2019). Therefore, not only do reference and disturbed soils exhibit distinct drought responses, but we also expect soils across multiple disturbance types to exhibit similar disturbance x drought interactions to those described here, at least within the context of forested ecosystems of the southern Appalachian region.

Conclusions

Responses of terrestrial ecosystems to drought will depend on the drought-tolerance of microbial communities, which are the proximate drivers of all soil biogeochemical processes. We observed greater drought-tolerance of microbial biomass in soils from historically disturbed forests, likely due to existing compositional differences in the communities as a result of past land use activities. This drought-tolerant microbial biomass contributed to an amplified Birch Effect and higher cumulative respiratory C loss following rewetting in disturbed soils. However, greater drought-tolerance of microbial biomass also appears to have resulted in less responsive soil N pools in disturbed soils relative to soils from reference forests following drought-rewetting. The complexity of these responses illustrates the possibility of alternative trajectories of different soil pools and processes, even when these parameters have common microbial drivers. Regardless, our results show that intensifying drought-rewetting cycles are likely to alter the C and N balance of temperate forest ecosystems. Additionally, our results illustrate that drought responses will likely vary among terrestrial environments with different historical land uses, which will be a critical consideration when assessing potential threats to ecosystem services due to climate change in ecosystems of the eastern US.

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DATA AVAILABILITY

Data are available at https://github.com/eosburn/Coweeta-Microbes/tree/master/CWT_Drought

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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