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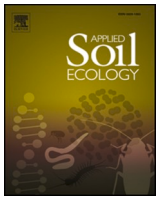

Drought, heat, and management interact to affect soil carbon and nitrogen losses in a temperate, humid climate

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ABSTRACT

Some areas with historically mesic climates are predicted to experience more climate extremes, including longer droughts combined with hotter days and more intense precipitation. Drought and rewetting are known to alter carbon (C) and nitrogen (N) cycling. However, little information is available on how the effects of drought on C and N cycling differ with temperature and land use in soils from humid regions. We evaluated several metrics of C and N cycling under drought with or without heat stress in a forest site and conventionally and organically managed arable sites. We sampled undisturbed soil cores from 0 to 10 cm and incubated them under either reference conditions (REF), drought (DRT), or drought combined with heat stress (D + H). Metrics of C and N cycling, including actual and potential mineralization, enzyme activities, microbial biomass, and dissolved organic C and N, and microbial community structure were assessed at the end of the stress period and 14 and 28 d after rewetting. We found that the effects of D + H differed in magnitude and direction from those of DRT: cumulative C and N mineralization followed the order DRT < REF ≤ D + H. Land management affected stress response: mineralization was always greater in the forest and organic sites than in the conventional site. Post-wet pulses of C and potential net N mineralization were 1.7 and 3.6 times higher, respectively, in the D + H soils than DRT soils, and were greatest at the forest site. Only the organic site was sensitive to DRT alone. Across sites, microbial biomass N was reduced more by stress than C, and only N-cycle parameters failed to reach reference levels after the recovery period. In agreement with previous studies, the N cycle was more affected than the C cycle. Our results suggest that climate change-induced heatwaves during drought have implications for ecosystem C and N balance in mesic climates.

1. Introduction

In many regions of the world, the International Panel on Climate Change predicts more frequent "compound climate hazards" such as concurrent drought and extreme heat events (Pörtner et al., 2022), potentially disrupting ecosystem functions. For agroecosystems, one of the most critical functions is the coupled cycling of carbon (C) and nitrogen (N). Tightly coupled C and N cycles are thought to be more efficient for N provision to plants and soil organic C (SOC) retention. Soils with a larger stock of slowly cycling organic matter will be better able to supply N to crops without losing N to the environment (Bowles et al., 2015), and C is most likely to be efficiently used by microbes when sufficient N is present (Buchkowski et al., 2019). However, environmental stress may contribute to decoupling these cycles, as evidence from wet-dry cycle laboratory assays and in situ climate manipulations suggest that drought stress and the attendant osmotic stress when dry soils are rewet affects N cycling processes more strongly than C cycling processes (Borken and Matzner, 2009; Fuchslueger et al., 2019; Schimel, 2018). Nitrogen cycle process rates (Maxwell et al., 2022), enzymes, (Brzostek et al., 2012) and genes (Zhang et al., 2021) have all been observed to increase during drought, although the direction and magnitude of changes depends on the severity and duration of the drought (Borken and Matzner, 2009). In contrast, C mineralization is generally depressed (Borken and Matzner, 2009). While C mineralization is stimulated after rewetting (the so-called "Birch effect"), this pulse often does not compensate for reduced respiration during the dry period

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(Harrison-Kirk et al., 2014).

The effects of wetting and drying cycles on microbial communities and function have been extensively studied; however, less is known about the compounded effects of heat and drought (Bérard et al., 2015). Existing research suggests that the effects of heat and drought are non-additive and non-linear, and may have important consequences for C storage and N availability (e.g., A'Bear et al., 2014; Acosta-Martinez et al., 2014, Luis Moreno et al., 2022; Philippot et al., 2021). Adding heat has been observed to both exacerbate (Bérard et al., 2011; Birch, 1960; Guillot et al., 2019) and reduce (A'Bear et al., 2014; Fuchslueger et al., 2019) the effects of drought. In order to better predict how compound climate hazards such as drought and heat waves will affect C and N cycling, more research is needed that tests their combined effects.

The magnitude and direction of the effect of compounded heat and drought stress on C and N cycling are affected by edaphic properties (Acosta-Martinez et al., 2014) and management (Fuchslueger et al., 2019; Guillot et al., 2019; Shen et al., 2022), as well as the severity of the stresses compared to normal site conditions (Brzostek et al., 2012). This is because microbial function under disturbance depends on characteristics of microbial communities, and external environmental characteristics such as climate history, soil resource availability, and pH influence those microbial characteristics (Bardgett and Caruso, 2020; de Vries and Shade, 2013). Resource-poor environments may select for communities dominated by K-strategists—organisms that are comparatively more resource-efficient but slower-growing—than by r-strategists, which have higher growth rates, fewer physiological adaptations to stressful environments, and lower efficiency (de Vries and Shade, 2013). The soil environment can also moderate the microbial community's effect on functional stability, in that soils with more resource availability may support faster recovery after a stress is lifted (de Vries and Shade, 2013). Therefore, land and crop management are likely to affect how microbial communities respond to stress (Guillot et al., 2019; Brangarí et al., 2022; Riah-Anglet et al., 2015; Zhang et al., 2019).

Most studies on soil responses to drought and combined drought and heatwave are performed on soils from semi-arid regions, and thus with microbial communities that are adapted to periodic drought. In these soils, a large body of literature suggests that soil microbial communities and functions are generally resilient even to extreme drought (e.g., Brown et al., 2021; Kaurin et al., 2018). In contrast, work with soils from more mesic climates suggests that drought will have stronger and more long-lasting effects in these non-adapted soils, with potential consequences for long-term C and N balance (Osburn et al., 2022b; Tiemann and Billings, 2012). However, we are not aware of any studies which have measured the combined effects of drought and heat stress events in soils from a humid climate although it is speculated that the effects may be more pronounced in these soils (e.g., Dacal et al., 2022).

In this study, our goal was to test how drought or combined drought and heat stress change C and N cycling functions and microbial community structure in a soil from a humid climate, and the extent to which those changes are affected by management. To this end, we took undisturbed soil cores from conventional and organic agricultural fields and a nearby strip of secondary forest which were all mapped as the same soil type. We subjected the cores to either a drought (DRT) or drought and heat (D + H) stress event of a regionally realistic magnitude and duration, and measured several C and N cycle indicators at maximum stress and 2 and 4 weeks after rewetting. Indicators assessed at each date included measurements of ammonium and nitrate-N, enzyme activity, microbial biomass C and N, and community profiles using phospholipid fatty acid (PLFA) analysis on the cores, as well as potential C and net N mineralization of sieved subsamples under optimum conditions. We hypothesized that: a) soils exposed to drought alone (DRT) will have lower C and N mineralization than soils exposed to heat and drought (D + H), and would return to reference levels more slowly b) Management that resulted in a microbial community more dominated by K-strategist groups (e.g. fungi, gram-positive bacteria) would be more resistant in both community structure and function to stress than a more r-strategist dominated community, and recover function more slowly and c) N cycle parameters would be more strongly affected than C cycle parameters for both stresses, particularly for the D + H treatment.

2. Methods

2.1. Site management history

Soils were taken from conventionally (CONV) and organically (ORG) managed agricultural soils, and from an unmanaged forested strip (FOR) in Robertson County, Tennessee, USA. The climate is subhumid tropical with a Köppen climate classification of Cfa. The normal annual precipitation (1991–2021) is 1380 mm and average annual temperature is 15 °C (NOAA). The CONV field (36°35′N, 86°36′W) was in corn (*Zea mays*, L.)-soybean (*Glycine max*, L. Merr.)-wheat (*Triticum aestivum*, L.) rotation. The site had been under no-till management for 12 yr. A grass-legume cover crop was grown approximately every 2 to 3 yr. The site received synthetic herbicides, pesticides, and fertilizers, with corn receiving N, P, and K and micronutrients at variable rates according to precision soil testing. The ORG field (36°35′N, 86°41′W) was in a 6-yr corn-soybean-pasture rotation for at least twenty years, with a multi-species cover crop grown after arable crops. The site received reduced tillage for cover crop and manure incorporation depending on the crop phase, but it had been under no-till management for the past 1.5 yr previous to sampling with the cover crop terminated by grazing. Both arable sites were limed as needed. The FOR site (36°35′N, 86°41′W) was a strip of unmanaged oak (*Quercus* spp.)- hickory (*Carya* spp.) secondary forest adjoining the ORG field.

2.2. Soil sampling

Undisturbed soil cores (10 cm depth, 5.08 cm diameter) were collected into polyethylene sleeves from each of the three sites on August 8th and 9th, 2021. Early August was chosen as being a time that is normally relatively hot and dry. The ORG and CONV soils were planted with corn, and both fields were in the reproductive phase. At each site, all cores (n = 55) were taken from an area approximately 10 m diameter within an area mapped as Pembroke silt loam (Fine-silty, mixed, active, mesic Mollic Paleudalfs; Soil Survey Staff, 2014). Cores were taken from interrow spaces away from tractor tracks in the crop fields, and from large trees at the FOR site. All residues were removed from the soil surface prior to sampling. Cores were capped, placed on ice, and kept at 4 °C until incubation. At each site, an additional composite sample of approximately 25 cores was taken with a 2-cm diameter probe to a depth of 10 cm throughout the sampling area. After homogenization, a subsample was immediately placed on dry ice, transported to lab, and stored at −80 °C for microbial community analysis.

2.3. Baseline analyses

Biomass of major microbial groups were estimated from phospholipid-fatty acid (PLFA) profiles, quantified from sieved soils that were stored at −80 °C and then freeze-dried. Phospholipid fatty acids extraction and data processing was performed by the University of Tennessee Biological and Small Molecules Lab (Supplemental Methods). Peaks which made up <0.5 % of total PLFA were discarded, and others were assigned to microbial groups according to current literature (Supplemental Table S1). As no reliable PLFA marker currently exists for arbuscular mycorrhizal fungi (AMF; Olsson and Lekberg, 2022), this class was not represented. Thus, estimates of the fungal to bacterial ratios may be underestimates.

Five randomly selected cores from each site were sieved to <4 mm. One subsample was stored at −20 °C for measuring the potential activity of four hydrolytic enzymes (β-glucosidase (BG), leucine aminopeptidase (LAP), *N*-acetyl-β-glucosaminidase (NAG), and β-xylosidase (XYL) using

the microplate fluorescence method (Bell et al., 2013). Gravimetric water content (GWC) was measured by drying field-moist soil for 24 h at 105 °C. Microbial biomass C and N were measured by chloroform fumigation extraction (Horwath and Paul, 1994). Briefly, one duplicate 5-g sample of field moist soil was extracted immediately with 25 mL 0.5 M K_2SO_4, and the other was fumigated with chloroform for 24 h, and then similarly extracted. Extracts from both were analyzed for extractable organic C (EOC) using a Total Organic C Analyzer (vario TOC cube, Elementar, Germany). On the same extracts, total extractable N was measured using alkali persulfate digestion (Cabrera and Beare, 1993) followed by colorimetric analysis for nitrate (NO_3-N) using a single reagent method (Doane and Horwáth, 2003). Microbial biomass C and N were calculated as the difference between C and N in fumigated and unfumigated soil extracts. No correction factor was used, as cytoplasm to membrane ratios may fluctuate with soil moisture (Kakumanu et al., 2013). Nonfumigated soil extracts were analyzed for ammonium (NH_4-N) using the salicylate method (Verdouw et al., 1978; Forster, 1995) and NO_3-N as previously described. Mineral N was calculated as the sum of NH_4-N and NO_3-N.

The remainder of the sieved soils were air-dried as a thin layer and sieved to <2 mm for baseline soil characterization. Organic C and total N were measured by dry combustion on a CN Analyzer (vario MAX cube, Elementar, Germany), after a dilute HCl test indicated that no carbonates were present (Nelson and Sommers, 1996). Available P was extracted with a Mehlich-1 extraction (Mehlich, 1953) and measured using Inductively Coupled Plasma Emission Spectroscopy at the University of Georgia Agricultural & Environmental Services Lab. pH was measured in a 2:1 water: soil slurry (Thomas, 1996). Water-holding capacity (WHC) was measured as the water content after saturated soil samples in a funnel were left to drain for an hour. Texture was measured using the hydrometer method (Gee and Bauder, 1996). Bulk density was calculated using the core volume and oven-dry soil mass.

2.4. Incubation

A 56-d aerobic incubation was performed to assess C and N cycling responses to DRT or D + H stress (Fig. 1), compared with reference cores (REF) maintained at a steady state. Stress conditions were based on a long drought and heat event that Tennessee, USA experienced in August 2007. All cores (5 replicate cores per treatment per sampling date) were brought to a water content of 0.3 g H_2O g^{-1} dry soil by injecting deionized water from a syringe equipped with a side-port needle to ensure uniform wetting. This amount of water represented 60 % WHC for CONV and ORG soils, and 60 % water-filled pore space (~70 % WHC) for the FOR soils, as preliminary tests showed in these very low bulk density soils, 60 % WHC was insufficient for uniform wetting. Cores were covered with plastic wrap pierced with holes and placed in plastic bins lined with moist paper towels to maintain humidity. Bins were covered with lids pierced with holes plugged with moistened melamine sponges, to enable gas exchange while limiting water loss. Bins were placed in 21 °C ventilated incubators and let to pre-incubate for 7 d, until respiration measurements had stabilized. Following this, the DRT and D + H cores were removed from the bins and allowed to air dry in the 21 °C incubator, while the REF cores were maintained at the initial water content. Water content was monitored by weighing and adding water drops as necessary for the REF cores. Weights were recorded. Change in water from stressed treatments was calculated as (Eq. (1)):

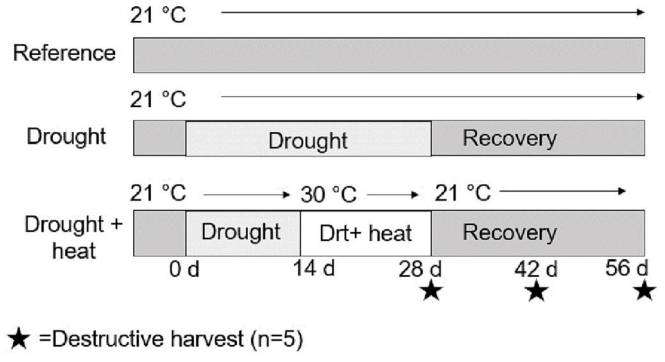

Fig. 1. Incubation treatments for undisturbed cores. Darker gray areas represent incubation under moist conditions.

$$\Delta = \left(\frac{T}{C} - 1\right) * 100 \quad (1)$$

where Δ is the percent change from the REF, T = treatment value, and C = REF value.

After 14 d of drying at 21 °C, D + H cores were dried at 30 °C for 14 days. After that all cores were again adjusted to their initial weights by injecting deionized water uniformly through the soil with a side-port needle and incubated at 21 °C for an additional 28 d (Fig. 1). From each site and treatment, 5 replicate cores were destructively harvested for C and N cycle assays directly prior to rewetting (Maximum Stress) and 14 and 28 d after rewetting (Recovery). These times were chosen based on the results of a similar experiment testing drought and heat stress on C and N cycling under Mediterranean conditions (Guillot et al. (2019), as times at which it would be expected that mildly stressed soils may have recovered normal C mineralization but severely stressed soils would not (14 d), and at which more severely stressed soils would have stabilized (28 d).

2.5. Respiration monitoring

Respiration was measured on days 2, 5, 12, 15, 18, 21, 28, 29, 30, 32, 32, 35, 41, 48 and 55 of incubation. At each sampling, five cores were randomly chosen from each site and treatment and placed in 2.37 L plastic jars with lids equipped with rubber septa for gas sampling and replaced in their appropriate incubators (21 °C or 30 °C). After 24 h headspace air was sampled into evacuated glass vials and analyzed for CO_2 on a gas chromatograph using a flame ionization detector (Shimadzu, Kyoto, Japan) and cores were placed back into their respective bins. On the day of each destructive sampling, (days 28, 42, and 56), the cores used for respiration monitoring were immediately harvested. After rewetting (day 28), a single set of randomly chosen cores were kept in jars for 96 h at 21 °C. Headspace CO_2 was measured at 12, 24, 48, 72, and 96 h, after each of which jars were aerated by vigorous fanning and immediately resealed. The respiration pulse after rewetting ("Birch pulse") was calculated as the cumulative 96-h respiration. All respiration measurements are reported as CO_2-C, calculated from headspace CO_2 using the ideal gas equation.

Over the whole incubation, respiration rates for each day were estimated as the average 24-h respiration of the two closest sampling dates, and the cumulative respiration was calculated as the sum of the estimated daily rates. Specific respiration was calculated as the cumulative respiration divided by the total soil organic C measured on the baseline soils. Daily respiration of DRT and D + H cores relative to the REF was calculated using Eq. (1).

2.6. C and N cycle assays

At each destructive harvest date, soil from each core was sieved to <4 mm and mixed well. Two 5-g subsamples were immediately stored at −20 °C and − 80 °C for enzyme and microbial community analysis, respectively. The GWC was determined on a 20-g sample by placing in a 105 °C oven for 24 h. Four 5-g subsamples of moist sieved soil were weighed into separate 40-mL glass vials. Two vials were immediately used for analysis of initial mineral N, MBC, MBN, EOC, and EON as described for the baseline soils. The proportion of NH_4-N in the mineral N (NH_4-N plus NO_3-N) was used as an indicator of nitrification activity

(Osburn et al., 2022b). Although some denitrification likely occurred, the fact that N_2O concentrations in the jar headspace measured by GC using an electron capture detector during respiration monitoring were several orders of magnitude lower than net mineralization suggests it was not a major loss pathway. Cumulative net N mineralization at each date was calculated as the difference between mineral N at that sampling date and average mineral N measured in the baseline cores. To assay C and net N mineralization potential, the other two vials of sieved soil were adjusted to 60 % WHC with deionized water and placed together uncovered into a 907-mL glass jar with an airtight lid fitted with a rubber septum for headspace sampling, and sealed jars were incubated for 7 d at 21 °C. Jar headspace CO_2-C was measured after 24 h, 72 h and 7 d as described above. Jars were aerated after each sampling event. After 7 d, pairs of vials were analyzed for mineral N as described above. Potential 7-d C and net N mineralization (Cmin7 and Nmin7) from soil organic matter were defined as the cumulative C evolved over the week or the difference between initial and final mineral N concentrations. The metabolic quotient (qCO2) was calculated as the cumulative CO_2 mineralized between 24 h and 7 d divided by the MBC.

2.7. Enzyme analysis and microbial community profiling

Potential enzyme activity and PLFA concentrations were measured on soils from each sampling date as described for the baseline soils in Section 2.3.

2.8. Statistical analysis

A principal components analysis was performed for all baseline properties using PROC PRINCOMP in SAS (SAS Corporation, Cary, North Carolina). Differences between soil baseline properties, PLFA groups, and C and N cycling indicators were determined using two-way ANOVA in PROC GLIMMIX in SAS, with post-hoc means separation using Tukey's HSD. Site and Treatment were considered to be fixed effects and replicate to be random effect. Assumptions were checked by Shapiro-Wilks' test for normality and visual inspection of the residual plots, and values were log-transformed if needed to meet assumptions. Microbial biomass C:N ratio did not meet assumptions after transformation. Differences between treatments for each site and date were assessed by Kruskal-Wallis' test in PROC NPAR1WAY in SAS, with post-hoc means comparisons using Dwass, Steel, Critchlow-Fligner Method.

3. Results and discussion

3.1. Baseline soil properties

Principal components analysis of initial soil properties revealed three distinct soil environments (Fig. 2). The CONV was site characterized by high mineral N and low SOC and microbial biomass, the ORG site by high SOC, available P, microbial biomass and activity, WHC and GWC at sampling, and the FOR site by low pH and bulk density, and high C:N ratio, NH_4-N as a proportion of the total N, and ratio of G+ to G− bacteria. Soil pH was 5.5, 6.4, and 6.7 at FOR, ORG, and CONV sites, respectively (Supplemental Table S2). Although classified as the same soil type, the texture differed slightly but significantly among the three sites, with clay decreasing as CONV > ORG > FOR and silt increasing in the same order ($p < 0.05$, Supplemental Table S2).

3.2. Water loss during the incubation

At the end of the stress phase, the FOR-DRT cores had lost more water relative to their REF than the cores from the other sites (Fig. 3a; $p < 0.05$). This may have been a function of their slightly lighter texture. However, D + H cores from all sites had statistically similar relative water losses. At maximum stress, cores from the D + H treatment had on average 30 % less water than cores from the DRT treatment (0.08 and 0.12 g H_2O g^{-1} dry soil, respectively; equivalent to 18 % and 24 % WHC).

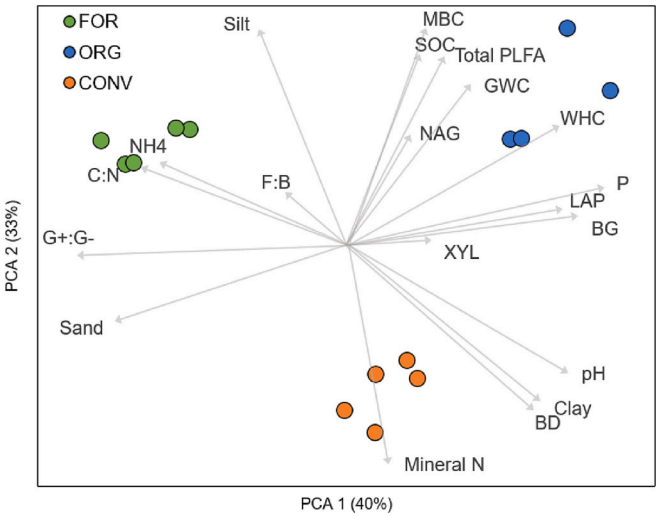

Fig. 2. Principal components analysis of selected forest (FOR), organic (ORG) and conventional (CONV) site baseline soil properties. WHC = water holding capacity; GWC = gravimetric water content at sampling; BD=Bulk density; Organic. C = soil organic C, MBC = microbial biomass C, P = Mehlich 1-extractable P, Mineral. N=NH_4-N + NO_3-N; BG, XYL, LAP, NAG = potential activities of β-glucosidase, β-xylosidase, leucine aminopeptidase, and *N*-acetyl-β-glucosaminidase, respectively. PLFA = total phospholipid fatty acids. G+:G− = ratio of PLFA markers from Gram positive to Gram negative bacteria, F:B ratio of fungal to bacterial PLFA markers.

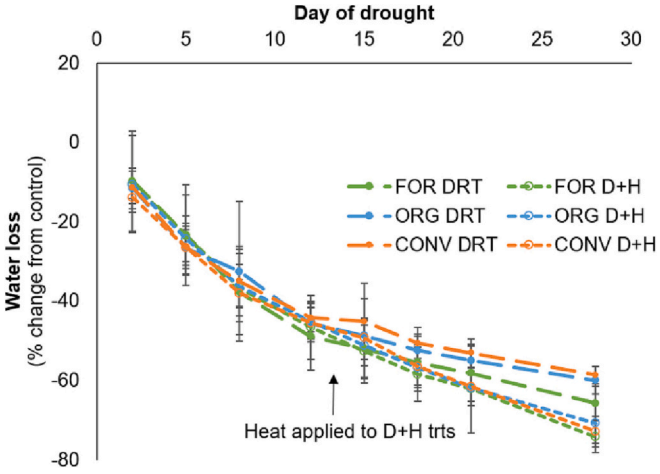

Fig. 3. Water loss from drought (DRT) and drought + heat (D + H) soils during the stress period, expressed relative to the water content of the reference (REF) treatment (0.3 g H_2O g^{-1} soil). FOR = forest, ORG = organic corn, CONV = conventional corn. Error bars represent the standard error of the mean (n = 15).

3.3. Carbon and nitrogen mineralization from undisturbed cores

Drought lowered core respiration relative to the steady-state REF treatment after 3 d for all sites (Fig. 4). Respiration temporarily increased in the D + H treatment when temperature was increased from 21 °C to 30 °C, compared to the DRT treatment maintained at 21 °C ($p < 0.01$ at Day 15). The difference was particularly strong in the FOR site, in which respiration from the D + H treatment was on average 132 % higher than the REF treatment. By Day 28, the final day of the stress period, respiration from both stressed treatments was on average 60 % lower than that of the REF treatment ($p < 0.05$) and did not differ

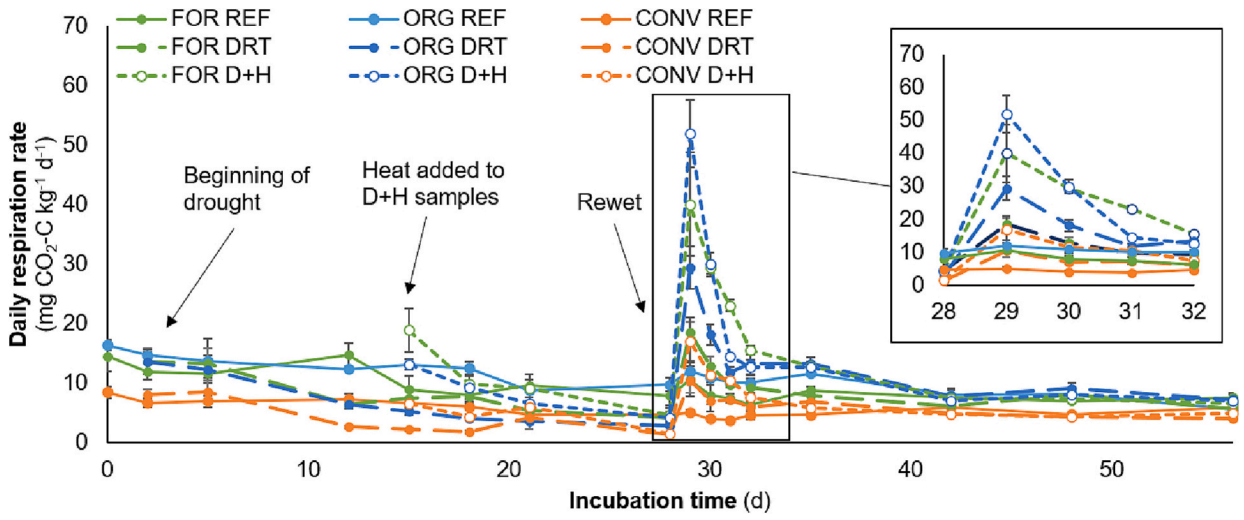

Fig. 4. Daily respiration rates in reference (REF), (DRT) and drought + heat (D + H) stressed samples. FOR = forest, ORG = organic corn, CONV = conventional corn. Error bars represent the standard error of the mean (n = 5). The inset shows daily rates measured at the end of the drought and the first 24, 48, 72, and 96 h after rewetting.

between stresses or sites.

Respiration and net N mineralization data from the undisturbed cores supported our hypothesis that the D + H treatment would trigger a greater post-wetting mineralization response than soils exposed to DRT alone (Figs. 4, 5). Across all sites, the size of the Birch pulse following rewetting was too low under the DRT treatment to compensate for the continuously reduced respiration during stress, leading to overall reduced respiration compared with the REF treatment (Fig. 5a, b). In contrast, the D + H treatment showed an immediate increase in respiration when first exposed to heat (Fig. 4) and had a larger Birch pulse than the DRT treatment (Fig. 5a). These additional pulses led total cumulative C mineralization from the D + H treatment to be similar to the REF treatment in all sites, and on average higher than that from the DRT treatment. While this trend occurred in all sites, it was only statistically significant in the FOR site (p = 0.01, Fig. 5b). Similarly, net N mineralization was reduced across sites in the DRT but not in the D + H treatment at maximum stress (Fig. 5c). However, net N mineralization two weeks after rewetting was higher in the D + H treated soils across

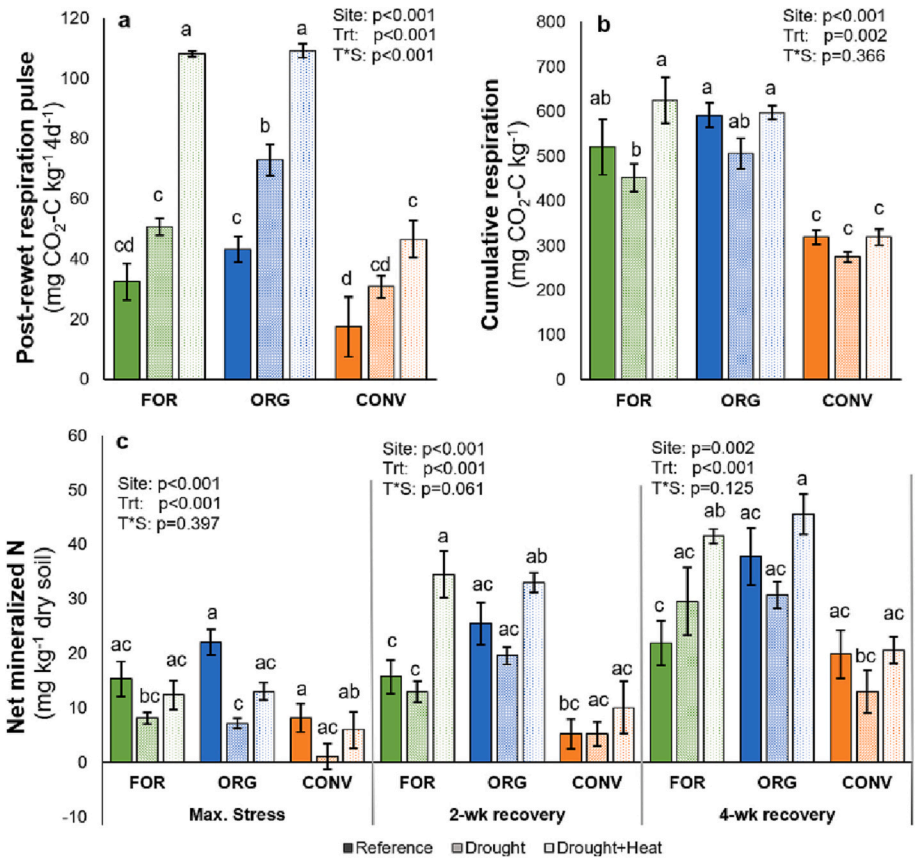

Fig. 5. a) Cumulative CO_2-C respired in the 4 d after rewetting ("Birch Pulse"); b) Cumulative CO_2-C respired over the entire incubation, and c) Cumulative net N mineralization directly before rewetting (Max. Stress) and after 2 and 4 wk. of incubation at optimum moisture and temperature. Different lowercase letters represent statistically different ($p < 0.05$) means according to a two-way ANOVA and post-hoc mean separation using Tukey's HSD. Error bars represent the standard error of the mean (n = 5).

sites than in either the REF or DRT treatments, which did not differ significantly. Like respiration, net N mineralization increased after rewetting to a much greater extent in the D + H than DRT treatment, particularly at the FOR site. After 4 wk., net N mineralization was on average higher in the D + H treatment than either DRT or REF (Fig. 5c). Within the FOR site, cumulative net N mineralization from D + H was 89 % higher than that of REF ($p = 0.027$).

Although the heat stress was moderate and was applied when the soil had already partially dried, our results showed that heat exerted an important influence in all sites compared to drought alone, and demonstrated that conclusions about the effects of dry-rewet cycles or heat on C and N mineralization must take into account the temperature at which they occur. The relatively increased respiration and N mineralization under D + H compared with DRT-treated soils supports the findings of Anjileli et al. (2021), who used high-frequency respiration measurements in natural systems across the United States and showed increased respiration under heatwave versus non-heatwave conditions in dry soils. However, the fact that cumulative C and N mineralization responses to D + H were significant in the FOR site but not the arable sites demonstrate that temperature responses are affected by land use.

Organic matter quantity and quality likely influenced the response to heat. The CONV response may have been lower because of its low organic matter and microbial population. Both FOR and ORG soils had greater SOC and MBC contents than the CONV soil, which also had a lower C and N mineralization response to D + H. An experiment that tested the responses of soils located at different distances from the tree row in an agroforestry system found that responses to D + H were much greater in soils from the tree row than from a cropped interrow (Guillot et al., 2019). The interrow, like the CONV site in our study, had the least SOC and MBC. However, the response did not seem to be entirely resource-dependent in our study, as the ORG site had higher SOC, MBC and potential enzyme activity than the FOR site, but their mineralization responses to D + H were similar in magnitude. The high net C and N mineralization per unit organic matter at the FOR site could be influenced by stoichiometry, in that the organic matter C:N ratio was significantly higher than at the ORG site and available P was extremely low. Stoichiometry that was less favorable to microbial growth may have led to a lower carbon use efficiency (Sinsabaugh et al., 2013). The microbial community at the high C:N ratio FOR site may also have had lower physiological N requirements than the manure-adapted ORG community, contributing to the relatively high net N mineralization in the FOR site (Lazicki and Geisseler, 2021).

In the FOR site, the DRT and REF cores tended to be very similar and there was a high ratio of Gram positive to Gram negative bacteria (Fig. 1; Supplemental Table S2). This was in line with our second hypothesis that sites with a more k-strategist dominated community would be less affected by drought, as G+ bacteria are thought to lead a more k-strategist lifestyle (Barnard et al., 2013; de Vries et al., 2012, 2018). We also expected that the DRT-resistant forest site would have had higher baseline fungal to bacterial ratio (F:B) than the arable sites, as fungi are thought to be more drought resistant than bacteria (de Vries et al., 2012), but our PLFA analysis did not support this (Fig. 1, Supplemental Table S2). However, the F:B ratio derived from PLFA analysis may not be reliable due to the lack of reliable AMF PLFA markers (Olsson and Lekberg, 2022).

Our DRT results differ from those of a recent study in Virginia which, employing a similar undisturbed core approach, found that drought tended to increase cumulative respiration in forest soils (Osburn et al., 2022a). As the Virginia soils in their natural state were nearly continuously moist whereas ours were not (GWC at sampling being similar to that observed after 28 d in the DRT treatment), our combined results are in line with the suggestion that the likelihood of increased mineralization under drought will increase with decreasing natural variability in soil moisture (Tiemann and Billings, 2012). This is further suggested by the fact that the ORG soil (which had the highest WHC and moisture content at sampling, indicating its historic exposure to drought may have been less severe) was the only site with a significant Birch pulse in the DRT treatment.

It is important to note that our study design, which allowed moisture to decrease with added heat, led to D + H soils being drier than DRT soils (18 % vs 24 % WHC, on average). Since the effects of drought increase with the amplitude of the soil moisture change between wet and dry phases (Harrison-Kirk et al., 2014), the stronger effects seen in the D + H soils were also due to a more severe drought as well as an increased temperature. We chose this approach as natural heatwaves do increase the severity of meteorological droughts (Kaurin et al., 2018), and adding water to maintain D + H cores at an equivalent moisture or bringing down both sets of cores to an extremely low uniform moisture would both have introduced factors that would make our findings less valid for a realistic drought in this study's temperate region. While our experiment is not designed to isolate the effect of heat from that of drought, the magnitude of the difference between DRT and D + H mineralization responses, particularly in the FOR soil, suggest that our observations cannot be explained solely by the greater amplitude of the wet-dry cycle.

3.4. Carbon and nitrogen cycling parameters and microbial community structure

To gain insight into the mineralization response, we measured a set of C and N cycle parameters throughout the incubation. We hypothesized that the magnitude and duration of stress effects would be greater for the D + H treatment than for DRT alone, and greater for N cycle than for C cycle parameters. This hypothesis was partially supported and depended on management and measured parameters in question.

The 7-d C mineralization in the FOR site, which tested respiration potential under optimum conditions, was greater in the D + H (but not DRT) treatment than the REF treatment (Fig. 6a). The almost identical potential C mineralization between the DRT and REF treatments measured in the FOR site confirmed the very low Birch pulse observed from the undisturbed cores under DRT alone. In the ORG site, mineralized C from both stress treatments was significantly greater than the REF, and in the CONV site, although the pattern was similar to that of the ORG, the treatments did not differ from each other. Conversely, for the ORG site alone after 2 wk. of incubation at optimum moisture and temperature, potential respiration from both REF and DRT treatments exceeded that of D + H (Fig. 6a). The trends in respiration potential at maximum stress were seen in reverse for MBC (Fig. 6b) but were similar to those of EOC (Fig. 6c). The fact that respiration under D + H stress was accompanied by a pulse of EOC and a decrease in MBC suggests that microbial lysis may have contributed to substrate pools. Similarly, Guillot et al. (2019) observed that a large Birch pulse under D + H was accompanied by a reduction in 16 s and 18 s copy numbers and an increase in EOC.

All measured pools and processes tended to be lowest at the CONV site, and absolute change in response to stress was correspondingly low. However, trends in the metabolic quotient (qCO_2; which we estimated as the difference between the 7-d and 24-h potential respiration per unit MBC measured from the undisturbed core at each destructive harvest) suggests that microbial physiological stress may have been greater. An elevated qCO_2 is often considered to be a sign of a stressed system (Anderson and Domsch, 1993). Across sites, at maximum stress qCO_2 was 77 % and 142 % higher in the DRT and D + H treatments than the REF treatment, respectively ($p < 0.001$). However, the difference between stressed and REF cores was significant only in the CONV site for both stresses ($p = 0.02$ for DRT and $p < 0.001$, and for the FOR site under D + H treatment ($p < 0.001$). At all samplings, qCO_2 on average was higher in the CONV than FOR or ORG sites ($p < 0.05$).

In accordance with our hypothesis, N cycle parameters were more affected by stress than C cycle parameters. The pulse of potential net N mineralization in the D + H treatment compared to the REF treatment after rewetting was 6 and 8 times larger than that of C mineralization for FOR and ORG sites, respectively (Fig. 7a). For all sites and both stresses,

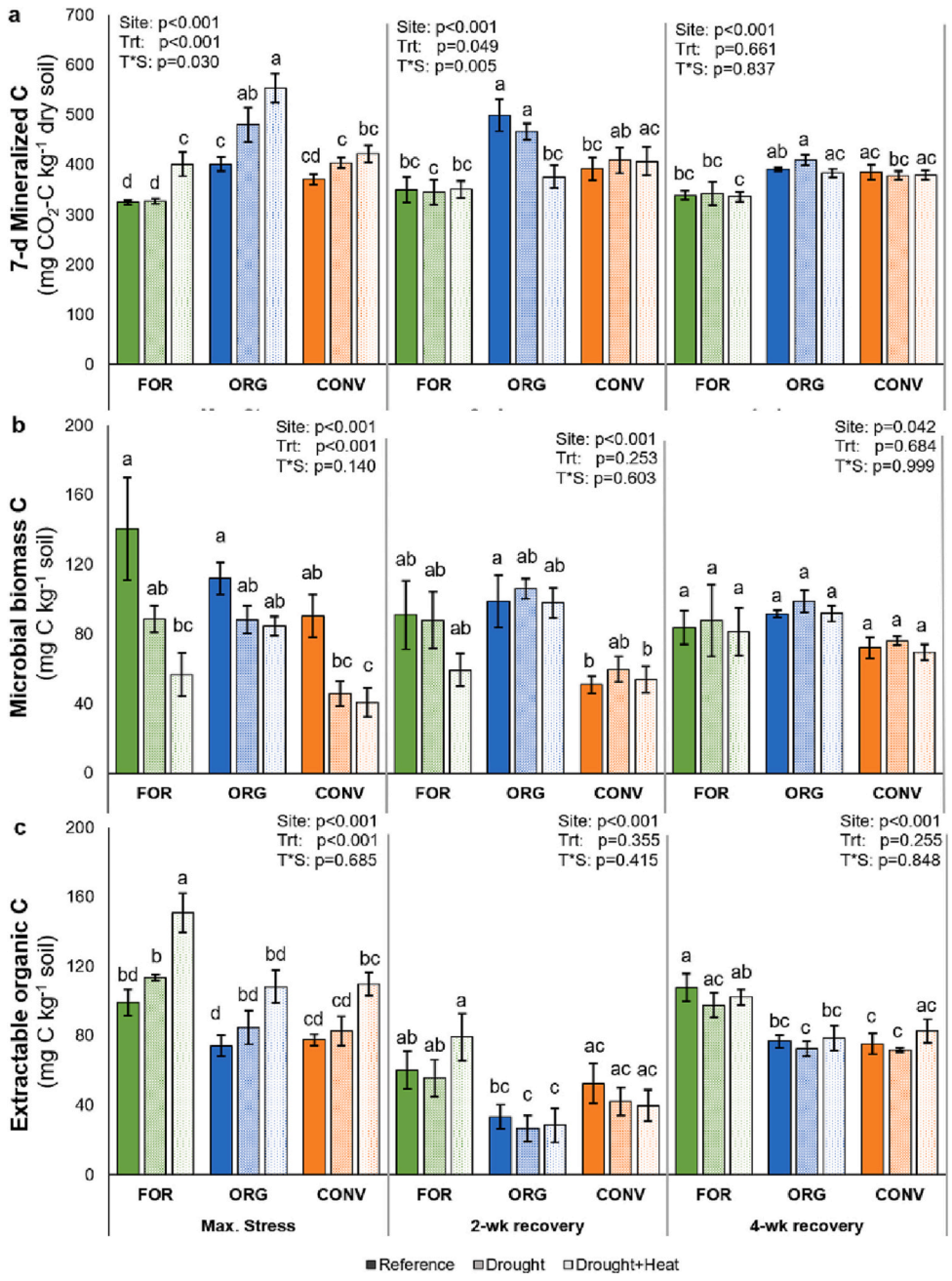

Fig. 6. a) Cumulative CO_2-C respired over 7-d incubation at 60 % WHC and 21 °C, b) microbial biomass C, c) K_2SO_4 -extractable organic C. Different lowercase letters represent statistically different ($p < 0.05$) means within a sampling date according to a two-way ANOVA and post-hoc mean separation using Tukey's HSD. Error bars represent standard error of the mean ($n = 5$).

stress reduced MBN more than MBC (Fig. 7b). The MBC:N was significantly higher in the D + H than either DRT or REF treatments in the ORG site and marginally higher in the D + H than REF treatment in the FOR site ($p = 0.05$). Additionally, MBN was one of the only parameters that was still affected by stress after 4 wk. of recovery: MBN was lower on average in the D + H treatment than in the REF treatment. Extractable organic N, like EOC, was elevated at maximum stress, and under D + H exceeded both the other treatments (Fig. 7c). However, unlike EOC, the EON was also on average higher in the DRT treatment than the REF treatment, and it remained elevated in the FOR site at the 2-wk recovery date.

A large increase in NH_4-N as a proportion of the total mineral N in the undisturbed cores suggested a decrease in nitrification in the DRT and especially D + H cores from all sites during stress (Fig. 8). At maximum stress, the proportion of NH_4-N was 2, 8, and 3 times more in the D + H treatment than the REF treatment in the FOR, ORG, and CONV sites, respectively. However, after rewetting, the nitrification process recovered quickly in the arable but not the FOR sites. Nitrification generally decreases in dry soils, likely due to reduced NH_4^+ diffusion (Stark and Firestone, 1995). When diffusion is the major limitation, it would be expected that nitrification would resume quickly once soils are rewet (Schimel, 2018). However, while this was the case in the arable soils, nitrification was slower to resume in the FOR soil subjected to D + H, suggesting that mortality of the nitrifier community may have been higher and recovery slower.

The microbial PLFA markers and enzyme activities differed strongly and consistently among sites and sampling dates but were generally less affected by treatment than the C and N cycle metrics discussed above

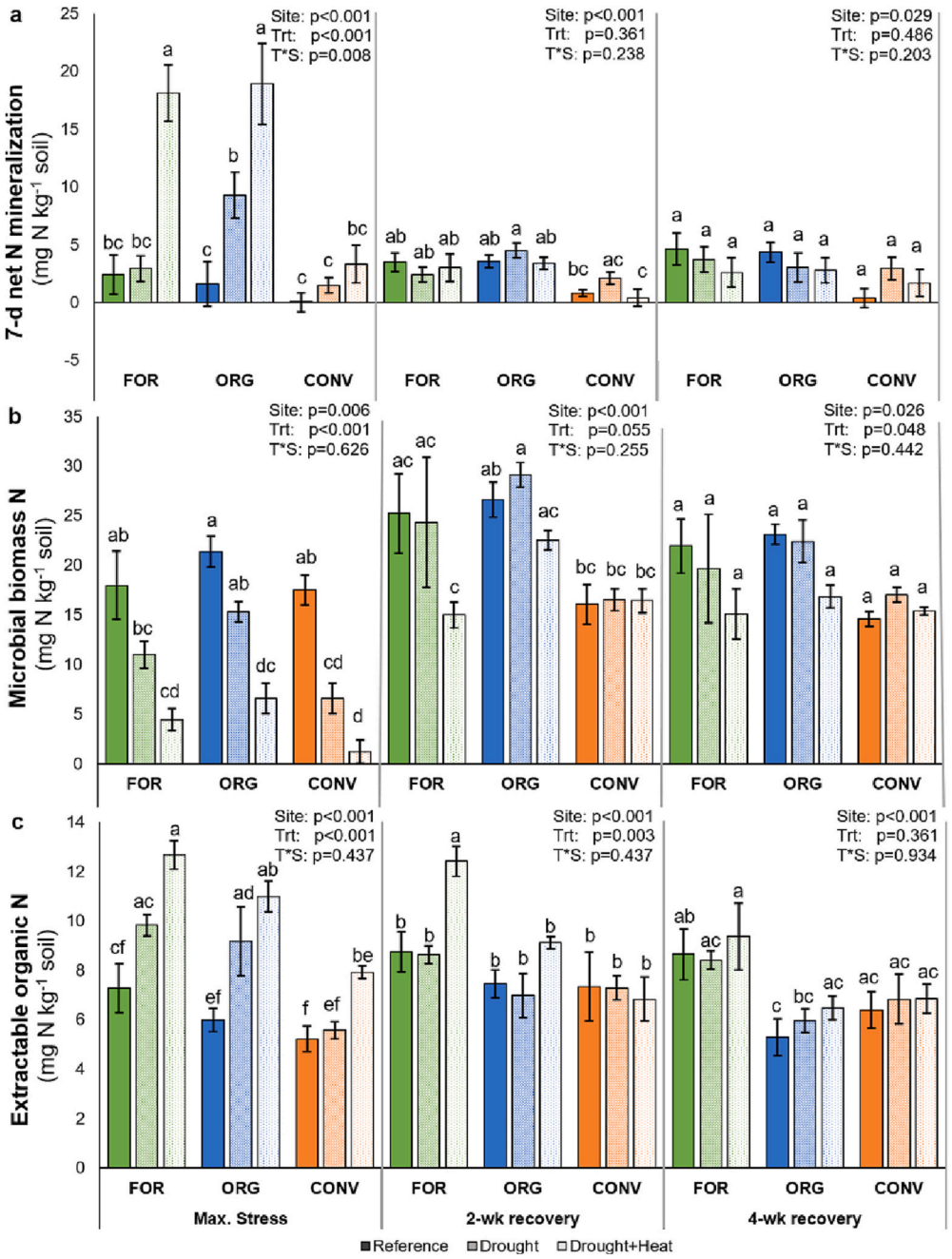

Fig. 7. a) Net mineral N change in a 7-d aerobic incubation of sieved soil at 60 % WHC and 21 °C, b) Microbial biomass N in undisturbed cores c) Organic N measured in 0.5 M K_2SO_4 extracts from undisturbed soils. Different lowercase letters represent statistically different ($p < 0.05$) means within a sampling date according to a two-way ANOVA and post-hoc mean separation using Tukey's HSD). Error bars represent standard error of the mean ($n = 5$).

(Table 1). In strong contrast to microbial biomass as measured by fumigation extraction, treatment did not affect total PLFA concentration at any date or site. However, there was both a general decrease of total PLFAs and a loss of differences among sites at the 2-wk recovery sampling date. Other studies have also noted a reduction in PLFA during the recovery period as compared to the drought period (Bérard et al., 2011; Brown et al., 2021). This may indicate that rewetting causes more mortality than drought (Bérard et al., 2011), and that the effect is general across management systems and microbial groups. Ratios of Gram-positive to Gram-negative bacteria tended to be the highest in the FOR site at all dates for all treatments. Contrary to our expectation, we found no evidence of higher fungal than bacterial mortality under D + H stress. Studies in Mediterranean climates have found fungi to be more sensitive than bacteria to heatwaves (Bérard et al., 2011; Guillot et al., 2019; Riah-Anglet et al., 2015). However, our heat stress of 30 °C, which is 5 °C above an expected August soil temperature at this site (https://www.greencastonline.com/tools/soil-temperature), is lower than the temperatures used in these studies (40–50 °C) as well as the temperature of 40 °C cited by Bérard et al. (2015) as being a threshold for microbial tolerance. We also did not observe an increase in F:B ratio under drought, in contrast to several studies which suggest that fungi are more competitive under drought than bacteria (de Vries et al., 2012; Osburn et al., 2022a, 2022b).

Interestingly, the different methods of measuring microbial biomass (PLFA and chloroform fumigation extraction) were similar in the baseline soils but responded differently to stress. This is likely because these two microbial biomass measurement methods target different components of microbial physiology (the membranes and cytoplasmic

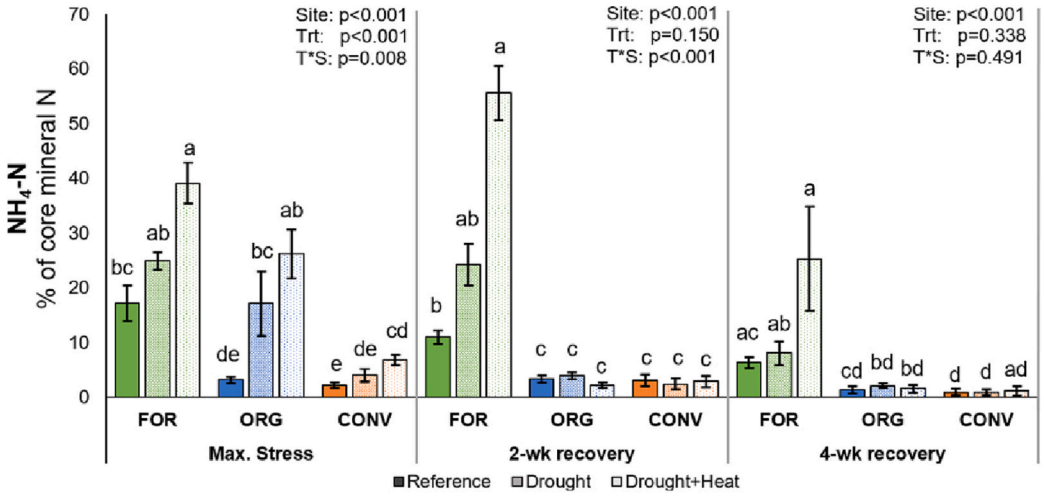

Fig. 8. The NH_4-N, as a proportion of mineral N, measured in the undisturbed cores. Different lowercase letters represent statistically different ($p < 0.05$) means within a sampling date according to a two-way ANOVA and post-hoc mean separation using Tukey's HSD). Error bars represent standard error of the mean ($n = 5$).

Table 1
Microbial community and activity indicators in forest (FOR), organic (ORG) and conventional (CONV) sites under reference (REF), drought (DRT) and drought+heat (D + H) treatments. PLFA = phospholipid fatty acid; G+:G− = ratio of Gram positive to Gram negative bacterial PLFAs; F:B = Ratio of fungal to bacterial PLFAs; BG, XYL, LAP, NAG = potential activities of β-Glucosidase, β-Xylosidase, Leucine Aminopeptidase, and *N*-acetyl-β-glucosaminidase. Within each date, different uppercase letters denote that sites are different within a treatment, while different lowercase letters denote that treatments are different within a site, with $p < 0.05$ determined by a two-way ANOVA with post-hoc means separation by Tukey's HSD.

Date	Site	Trt	Total PLFA	Fungi	Bacteria	G+:G−	F:B	BG	XYL	LAP	NAG
			nmol g^{-1}					nmol kg^{-1} h^{-1}			
Max. stress	FOR	REF	9735 AB	406 AB	2612 B	2.27 A	0.16	40.0 B	9.8	14.9 B	34.0 a
		DRT	9000 AB	343 AB	2511 B	2.69 A	0.14	31.5C	9.2	16.6 B	22.7
		D + H	9452 AB	320 AB	2682 B	2.66A	0.12	29.3 B	6.7	14.3 B	16.9 b
	ORG	REF	11,545 A	858 A	3362 A	1.19 B	0.24 aA	85.3 A	9.3	42.9 A	38.8
		DRT	12,135 A	635 A	3660 A	1.07 B	0.17 ab	73.4 A	10.5	49.7 A	37.3 A
		D + H	11,638 A	536 A	3588 A	1.19 B	0.15 b	58.6 A	8.2	40.3 A	24.2
	CONV	REF	7198 B	305 B	2213 B	1.67 B	0.13 B	71.0 a	7.4	35.2 A	26.5
		DRT	7058 B	263 B	2229 C	1.52B	0.12	52.0 bB	5.9	34.3 A	19.2 B
		D + H	6919 B	242 B	2205 B	1.67 B	0.11	53.2	6.5	25.1 A	17.8
2-wk recov.	FOR	REF	7271	313	2202	2.47	0.14	39.9 B	6.8	22.4 B	27.1
		DRT	7624	335	2297	2.26	0.14	34.8 C	7.0	16.2 C	27.8 B
		D + H	11,238	782	2947	1.91	0.28 A	35.0 B	8.4	16.8 B	18.5
	ORG	REF	9730	394	2888	2.06	0.13	72.7 A	5.4	47.0 A	31.5
		DRT	7997	386	2526	1.66	0.15	83.8 A	8.9	59.6 A	42.3 aA
		D + H	8120	286	2571	1.97	0.11 B	66.3 A	6.2	49.0 A	24.7 b
	CONV	REF	7908	258	2362	2.20	0.11	63.6	6.0	41.5	24.8
		DRT	8508	311	2644	1.89	0.12	57.7 B	5.4	38.4 B	20.0 B
		D + H	9154	323	2879	2.13	0.11 B	54.8	5.5	32.9 A	18.6
4-wk recov.	FOR	REF	10,789 A	512	2888	2.57 B	0.18 a	45.8 B	13.6	28.8 B	41.9
		DRT	11,288 A	480	2980	2.77 A	0.16 ab	50.9 aB	13.9	36.2 B	35.0
		D + H	9543	302	2674	2.95 A	0.11 b	35.2 bB	10.1	23.3 B	21.9
	ORG	REF	10,125 AB	510	3159	1.37 C	0.16	82.2 A	13.1	58.3 A	42.7
		DRT	10,092 AB	442	3209	1.37 B	0.14	80.2 A	12.4	59.7 A	40.7
		D + H	9882	473	3019	1.56 C	0.16	76.4 A	11.5	51.9 A	34.7
	CONV	REF	7762 B	366	2309	2.02A	0.16	66.8 A	10.4	45.2	26.8
		DRT	7328 B	301	2359	1.73 B	0.13	64.1	10.0	60.5	23.6
		D + H	7140	261	2312	1.98 B	0.11	67.9 A	10.3	54.1 A	24.2

contents, respectively) whose relationships are expected to change under stress (Kakumanu et al., 2013; Maxwell et al., 2022). The fact that MBC and especially MBN decreased under stress but PLFA did not suggest that organisms under D + H stress increased ratios of membrane components to cytoplasmic contents (Bérard et al., 2015; Kakumanu et al., 2013).

Contrary to our hypothesis, we did not see an increase in N cycle enzymes under stress. Potential activities of both BG and NAG were initially reduced by stress (REF > DRT = DH), and LAP and XYL were unaffected (Table 1). However, due to the decline in microbial biomass specific activities of all enzymes increased under D + H stress and specific LAP activity increased under both stresses. Averaged across sites, potential NAG activities in D + H treated soils were still lower than those from the REF soils after 4 wk. of recovery ($p < 0.0148$); the only measured property besides MBN still affected by stress at the end of the incubation.

A general increase in N cycle process rates has often been observed during drought (e.g. Borken and Matzner, 2009; Homyak et al., 2017; Gao et al., 2020; Maxwell et al., 2022), and increased proteolytic enzyme activities, and dissolved organic N and NO_3 leaching have also been measured under heat (Brzostek et al., 2012; Gao and Yan, 2019) but relatively few studies have looked at the combined effects of drought

and heatwave (that is, a sudden, temporary increase to above-normal temperatures, rather than a legacy effect of sustained warming). One such study, conducted in a Mediterranean climate and with a stronger drought and heat stress than in this experiment, also measured a larger decrease in MBN compared to MBC under combined D + H stress than under DRT alone (Guillot et al., 2019). An increased MBC:N ratio under drought stress, which we also observed under D + H in the FOR and ORG sites, has been attributed to the preferential accumulation of C-rich osmolytes (Osburn et al., 2022a), or a community shift to one with a lower N demand (Guillot et al., 2019; Kakumanu et al., 2013; Osburn et al., 2022a). An increased community demand for C relative to N could explain the increased potential net N mineralization we observed in the D + H treatment, and would be in line with the findings of Guillot et al. (2019), who noted marked alterations in community stoichiometry under D + H stress. However, it is an interesting question why organic N cycling processes often appear to be intensified under drought, if communities require less N. One explanation is that extracellular enzymes are no longer under metabolic control, and the increased process rates may be due to the concentration of N-rich substrate as the soil dries (Maxwell et al., 2022; Schimel, 2018). An increase in N-rich substrate for a community with lower N needs would also explain the disproportionately large pulses of net N mineralization observed on rewetting, and would be in line with our finding that enzymes did not decrease proportionately to MBC and MBN, leading to higher specific activities.

3.5. Study limitations

While the soils in our study were mapped as the same soil type, the slight but significant differences in texture may have affected C and N cycling response. Soil C and N mineralization during wet-dry cycles has been observed to increase with silt to clay ratio (Harrison-Kirk et al., 2014). The FOR soil had slightly more silt and sand and tended to lose water more rapidly during drying than both arable sites, which may have contributed to its high stress response compared to arable soils. Additionally, in the reference state, the FOR cores were kept at a proportionately higher WHC, as was needed to achieve uniform wetting. However, the low FOR soil C and N pulses under DRT alone suggests that any impact texture and wetting may have had operated differently across stresses and were not a major controlling factor in our results.

We performed this experiment on undisturbed soil cores to preserve field soil structure and spatial organization in a controlled lab setting. However, further work is necessary to test the applicability to field conditions. Excess N can accumulate in lab incubations when no plants are present, potentially changing microbial community structure, enzymatic investments, and decomposition rates (Rinkes et al., 2013). Further, living roots in field conditions can accelerate decomposition through priming (Adamczyk et al., 2019). Additionally, our method did not allow us to remove roots from the cores prior to incubation, so some of the observed mineralization from all treatments may have been from decomposing roots.

4. Conclusions

Our study showed that adding heat to drought stress increased post-wetting C and especially N mineralization compared to soils kept at a reference moisture or drought alone, and that the effect was particularly large in the FOR site. We found that stress had little effect on microbial community structure (as measured by PLFA), and most pools and processes returned rapidly to reference levels after both stresses. However, the slower return of MBN than MBC to reference levels after D + H stress suggests alteration in community stoichiometry. As our experimental conditions were based on a historical weather event our results suggest that such events, especially if accompanied by more intense rainfall events, may induce short-term C and N cycle decoupling and increase the risk of losses. Our results also demonstrated that predictions of C cycling under future droughts should take into account the temperature at which drought occurs, as the common assumption that post-rewet pulses do not compensate for low respiration during the stress is less likely to be accurate if the drought occurs at higher temperatures. Modeling of climate change's effects on C and N cycling should also take into account the effects of land management, and in particular, on pH, organic matter quality, and soil moisture. Overall, our study implies an increased potential for decoupled C and N cycling when drought occurs at high temperatures, especially in unmanaged systems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2023.104947.

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