

The carbon economy of drought: comparing respiration responses of roots, mycorrhizal fungi, and free-living microbes to an extreme dry-rewet cycle

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Abstract

Aims The effects of drying and wetting on soil carbon processes are regulated by the responses of plants, plant-associated microbes, and free-living microbes. Whether these groups respond similarly to drying and wetting is not clear, however, making it difficult to predict the net effect of drought on soil carbon processes.

Methods We imposed a drought-rewetting event on mesocosms planted with maple (*Acer saccharum* Marshall; arbuscular mycorrhizal fungi host) or oak (*Quercus alba* L.; ectomycorrhizal fungi host) saplings. In each mesocosm, we used mesh to create chambers separating roots, mycorrhizal fungi hyphae, and free-living microbes. We compared respiration and carbon degrading enzyme activity across chambers throughout the dry-rewetting cycle.

Results Respiration from all chambers declined during drought, and chambers differed in their drought-sensitivity. In maple mesocosms, respiration from the Hyphae+Microbes chamber was the most drought-resistant; in oak mesocosms respiration from the Microbes chamber was the most drought-sensitive.

Respiration did not recover after rewatering, indicating a persistent drought legacy. In contrast, enzyme activity returned to control functioning after 2 weeks of well-watered conditions.

Conclusions Our results suggest that belowground biota differ in their sensitivity to and recovery from drought, which affects the carbon processes differently. An improved ability to partition carbon fluxes into biotic sources can help to constrain predicted carbon fluxes under future climate scenarios.

Keywords CO₂ flux · Heterotrophic respiration · Autotrophic respiration · Mycorrhizal fungi · Plant roots · Soil enzyme activity

Introduction

Droughts are predicted to become more frequent and more extreme under future climate scenarios (IPCC 2012, 2014; Seidl et al. 2017). Since terrestrial carbon

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(C) stocks are highly sensitive to precipitation anomalies (Reichstein et al. 2013; Wolf et al. 2016; Zscheischler et al. 2014), future droughts are expected to have strong impacts on terrestrial C cycling. However, accurate predictions of C cycling under future environmental conditions are limited by uncertainty about the relative effects of drought on plants, plant-associated microbes (e.g. mycorrhizal fungi), and free-living microbes, as well as the contribution of these groups to soil carbon efflux. These biota mediate the effects of drought on C processes through their metabolic use of carbon and water (Manzoni et al. 2012 and citations therein), but differences among them in their drought sensitivity can have important repercussions for soil carbon gains and losses. Incorporating and improving the mechanistic representation of rhizosphere processes into earth system models is largely expected to improve their accuracy in predicting global C biogeochemistry under future climates scenarios (Bradford et al. 2016; Schlesinger et al. 2016; Warren et al. 2015). Indeed, models which do not explicitly include root processes perform poorly in predicting ecosystem-specific C fluxes during drought (Hanson et al. 2004), emphasizing the importance of studying belowground processes to understand ecosystem responses to environmental stressors.

Plant roots, mycorrhizal fungi, and free-living soil microbes interact via the exchange of C – the C economy. Photosynthetically fixed C is allocated by plants belowground to grow and maintain plant roots and mycorrhizal symbionts. Fixed C is also exuded from roots into the rhizosphere, where it stimulates microbial activity and decomposition (Kaiser et al. 2014). As roots, fungi, and microbes die, C stored in their tissues is incorporated into soils as necromass (Crowther et al. 2015; Liang et al. 2017; Schweigert et al. 2015). Soil organisms mechanically and enzymatically decompose labile necromass to gain access to C and other nutrients, releasing small monomers and mineral nutrients in the process (Schimel and Bennett 2004; Talbot et al. 2008). The growth and activity of all three biotic groups – plant roots, mycorrhizal fungi, and free-living soil microbes – releases CO₂ to the atmosphere through respiration.

During drought, the C economy slows as plants reduce photosynthesis (Ruehr et al. 2009). Bulk soil respiration is fundamentally limited by photosynthetically-derived C (Högberg et al. 2001; van der Molen et al. 2011), so drought-driven reductions in photosynthesis can cause cascading impacts on C transfer among rhizosphere biota (Clarholm 1981).

Drought has been shown to reduce the transfer of C from meadow plants to soil bacteria, but not soil fungi (Fuchslueger et al. 2014). Different plant strategies to balance water loss and C gain during drought (i.e. isohydry vs anisohydry; McDowell et al. 2008) likely play an important role in the extent to which rhizodeposition is maintained during drought (Preece and Peñuelas 2016) and the net effect of drought on the C economy. Respiration from plant roots ranges from ~10–80% of total respiration across global forests, and accounts for a relatively greater proportion as total respiration increases (Bond-Lamberty et al. 2004; Subke et al. 2006). Since plant root respiration is allometrically related to photosynthesis (Heilmeyer et al. 1997; Litton et al. 2007), reductions in C uptake during drought would also reduce root respiration. Mycorrhizal respiration is often included in estimates of root respiration due to sampling limitations, but disentangling the responses of mycorrhizal fungi from those of plant roots is necessary to mechanistically understand ecosystem C flux dynamics. Plants can allocate a substantial fraction (Franklin et al. 2014; Hobbie 2006), and up to 85%, of net photosynthate to mycorrhizal fungi (Treseder and Allen 2000), so reductions in C flux belowground are also likely to reduce mycorrhizal respiration (Talbot et al. 2008). However, differences in the C demand of mycorrhizal types likely affects their response to, and effect on, the altered C economy during drought (Read 1991). Finally, microbial biomass is positively correlated with net primary production, suggesting that microbial respiration may be also limited by photosynthate-C (Zak et al. 1994). Microbial respiration may decline during drought if root turnover and exudate production or microbial and mycorrhizal exoenzyme production is reduced (Kuzyakov and Domanski 2000; Schimel and Weintraub 2003; Wieder et al. 2013).

In addition to uncertainty surrounding the individual responses of roots, mycorrhizal fungi, and microbes to drought, their capacity to recover remains unclear. Rewetting following drought produces a pulse of high CO₂ efflux (Hagedorn et al. 2016), which, after a single extreme rain event, can account for up to 10% of annual net ecosystem CO₂ production (Lee et al. 2004). The source of this pulse is debated, but is hypothesized to be driven by increases in microbial respiration following an increase in labile C substrate availability (Schimel et al. 2010). These C substrates are derived either from soil organic matter that has been released from physico-chemical protection during soil rewetting, or from lysed

microbial cells (Fierer and Schimel 2002; Fierer and Schimel 2003). These different mechanisms of C loss after rewetting imply different repercussions for long-term terrestrial C storage, and the overall net effect of drought on the C economy (Kuzyakov 2006). Furthermore, the degree to which plant roots, mycorrhizal fungi, or soil microbes contribute to the observed CO₂ pulses following dry-rewetting cycles is not known. In the absence of plants (and presumably mycorrhizal fungi), CO₂ production was strongly correlated with new microbial DNA production 3 hours after rewetting in a laboratory incubation (Blazewicz et al. 2014). These findings suggest that part of the CO₂ flush observed after rewetting is correlated with the proliferation of microbial biomass. In addition, a ¹⁴C tracer study in root-free soil also found that the majority of C respired after rewetting came from microbial biomass (Fierer and Schimel 2003). However, in a field study with roots, mycorrhizal fungi, and free-living microbes, ectomycorrhizal respiration increased during rewetting relative to root and microbial respiration (Heinemeyer et al. 2007). This implies that mycorrhizal respiration may contribute non-negligibly to CO₂ pulses after rewetting events, and also underscores the need to disentangle the responses of different biota in order to gain mechanistic insight into an overall ecosystem-level response to drought and rewetting.

In this study, we examined belowground C processes (respiration and potential enzyme activity; EA) throughout an extreme drought (in terms of its severity and duration) and extreme rewetting event. In order to understand how plant roots, mycorrhizal hyphae, and free-living microbes independently and collectively mediate soil respiration in dry-rewet conditions, we used mesocosms with mesh barriers with different pore sizes to exclude the growth of different biotic groups (Fig. S1). To quantify soil C losses and transformations, we compared respiration and EA across the chambers in mesocosms planted with sugar maple saplings (*Acer saccharum* Marshall; which form associations with arbuscular mycorrhizal fungi), and in mesocosms with white oak saplings (*Quercus alba* L.; which form associations with ectomycorrhizal fungi). We measured photosynthesis throughout the dry and rewetting event to estimate photosynthetic C inputs. We asked: (1) How do belowground biotic groups (i.e. roots, mycorrhizal fungi, and microbes) respond to an extreme dry and rewetting event? (2) Do these groups differ in

their sensitivity to and recovery from drought, as evidenced by changes in respiration rate and EA? We hypothesized that both respiration rates and EA would decline across all chambers in response to drought.

Prior to drought, we expected that microbial respiration would be the largest source of CO₂, followed by mycorrhizal and plant root respiration in accordance with studies of forest soils (Fenn et al. 2010; Heinemeyer et al. 2007). In maple mesocosms, we expected that mycorrhizal fungi and microbes would be more sensitive to drought than plant roots, since their activity would be limited both by reduced photosynthate-C supply, and by the diffusion of C derived from organic matter degradation. In oak mesocosms, we expected that root respiration would be relatively less sensitive to drought since oaks maintain photosynthetic activity during drought (McDowell et al. 2008; Yi et al. 2017). We also expected that oak mycorrhizal respiration would be less sensitive to drought than microbial respiration, since ectomycorrhizal fungi have an especially high demand for C from the host plant (Read 1991).

Materials and methods

Experimental design

In May 2016, we collected *A. saccharum* and *Q. alba* seedlings from a mixed hardwood stand in Oak Ridge, TN, USA. To minimize potential genetic variability, seedlings were collected from a small area (~5 m radius) where both species co-occurred. Initial heights were approximately 15 cm for both species and seedlings were all estimated to be less than 1 year old. To preserve the native microbial community, seedlings were not washed prior to being transplanted into mesocosms.

We constructed 60 mesocosms (38 × 23.5 × 18 cm LxWxH or 15.14 L capacity) with mesh partitions that divided each mesocosm into three chambers (Fig. S1). Small holes (each ~0.75 cm diameter) at the bottom of each mesocosm prevented water logging. One tree seedling was planted into the center chamber of each mesocosm such that 40 mesocosms were planted with *A. saccharum* and 20 were planted with *Q. alba*. Seedlings were allowed to acclimate in potting mix (see below) for 2 months prior to being planted in mesocosms. The center chamber included plant roots,

mycorrhizal hyphae, and free-living microbes, and will hereafter be referred to as the Roots+Hyphae+Microbes chamber. One mesh partition (35 μm) excluded root growth, allowing only mycorrhizal hyphae and free-living microbes to colonize this chamber, which we hereafter refer to as the Hyphae+Microbes chamber. The second mesh partition (1 μm) excluded roots and mycorrhizal hyphae, allowing only free-living microbes to colonize this chamber which we hereafter refer to as the M chamber. We compare respiration across these chambers, but do not partition respiration into the individual biotic sources since competition among the biota would result in non-additive effects.

Each chamber had a schedule 40 PVC collar (10 cm height, 5 cm diameter) installed for gas sampling (see below). Twelve windows (each 3.7 cm diameter) were drilled into each PVC collar to allow biotic and hydrologic connectivity between soils in adjacent chambers and collars (Fig. S1). Mesocosms were filled with ~ 2.5 kg Fafard® 52 mixed with slow-release Osmocote Plus Fertilizer (15–9–12, NPK). Fafard® 52 is a commercially available potting soil mix of peat moss, bark, and vermiculate that is formulated for fast drainage of large interior pots. We used a common soil across chambers to eliminate potential differences in biogeochemistry associated with arbuscular mycorrhizal versus ectomycorrhizal fungi colonization (Phillips et al. 2013).

Seedlings in mesocosms grew and acclimated for 4 months prior to the start of the experiment. During this time, mesocosms were watered as needed. Mesocosms were divided evenly between treatment (drought) and control (well-watered) conditions ($N = 20$ *A. saccharum*; $N = 10$ *Q. alba* per treatment) and randomized on a bench in the greenhouse. The greenhouse was maintained between 18 and 21 °C throughout the duration of the experiment; 1000 watt HPS lights supplemented ambient sunlight to maintain the light intensity in the greenhouse above 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the day (06:00–22:00).

The experiment ran for 11 weeks from September – November 2016 (Chart S1). The pre-drought period (week 1) was followed by 7 weeks of drought (i.e. 49 days; weeks 2–8), and a 3-week recovery period (weeks 9–11). Oak Ridge, TN had not experienced a drought of at least this duration in 69 years; the longest drought (53 days with ≤ 1 mm precipitation) since 1948 occurred in fall 2016, while this experiment was ongoing (Menne et al. 2012a, b). During the pre-drought

period, all mesocosms were maintained under well-watered conditions. During the drought period, control mesocosms were watered generally twice per week to maintain target soil water status while treatment mesocosms were left un-watered and soils progressively dried down. Control mesocosms were initially maintained between -0.90 and -0.75 MPa (65–75% of field capacity, which was -0.45 MPa) during the drought period. To increase the difference in water availability between drought and control mesocosms, however, we increased water availability of control mesocosms and maintained them at -0.50 MPa ($\sim 80\%$ of field capacity) for the remaining 5 weeks of the drought period. During the recovery period, we watered control and treatment mesocosms to saturation, and then immediately allowed them to drain to field capacity. Because the drought increased the hydrophobicity of treatment soils, treatment mesocosms retained less water than control mesocosms after the first watering during the recovery period. Consequently, we re-watered all mesocosms to saturation for two consecutive days. For the first re-watering, we watered mesocosms in the evening and allowed them to drain overnight for 12 h prior to measuring soil respiration (see below) the following morning.

Soil water content

To maintain mesocosms at the target water contents, we weighed mesocosms, calculated the volumetric water content of each mesocosm (based on known mesocosm dry soil mass), and added water by hand at least weekly to bring water volume up to the target level. Dry soil mass was measured by destructively harvesting additional mesocosms. We constructed water release curves using soil subsamples that we progressively dried and re-measured in a WPC4 Water Potential Meter (Decagon Devices; Pullman, USA). We used these curves to estimate the water potential of each mesocosm based on its volumetric water content and known bulk density. Although water release curves constructed by drying wet soils may differ from those constructed by wetting dry soils, our rewetting event during the recovery period aimed to bring soils to full saturation rather than slowly increase soil water availability. For this reason, we did not construct separate water release curves to estimate soil water content during the recovery period. The presence of root and fungal biomass, as well as likely differences in soil structure (e.g. bulk density, water

content) among the three chambers of each mesocosm may have increased uncertainty of the actual water potentials in each chamber, although soil moisture measured gravimetrically did not differ among chambers of 12 supplementary mesocosms (ANOVA $p = 0.785$, data not shown). However, the integrated whole mesocosm estimate still provided a good guide to the relative water stress between mesocosms and should be considered in that context. Finally, the mesh partitions between chambers would continue to allow unsaturated and vapor flow of water from wetter chambers to drier chambers, and would contribute to the equilibration of water availability across chambers even if the root chamber dried at a faster rate. We report water availability as soil water potential (Ψ_{soil}), as calculated from abovementioned water release curves.

Respiration measurements

To assess soil respiration, we measured CO_2 efflux at least weekly by sampling the capped PVC collar headspaces (196 cm^3) installed in each chamber. PVC collars were capped with PVC caps fitted with butane septa. Caps were installed with needle vents to avoid headspace pressure changes during capping, and the headspace was mixed by pumping $3 \times 35 \text{ mL}$ with a syringe. The needle vent was removed from the septum and a 1-mL sample was drawn from the headspace and immediately injected into a Li-Cor 6252 infrared gas analyzer (Li-COR Inc., Lincoln, Nebraska, USA), modified to detect small volume CO_2 sample injections carried with an N_2 carrier gas. PVC collars were left capped for $\sim 1.5 \text{ h}$, and resampled after this incubation. To minimize diel variation in respiration rates, we sampled headspaces only in the morning (08:00–11:00), and sampled mesocosms and chambers in the same order every sampling date. At each sampling time, we used standard CO_2 gases to create a standard curve with which we calculated the CO_2 concentration in each sample's headspace. During the drought period, watering events of control mesocosms occurred no closer than 2 days prior to respiration measures to minimize any short term increases in respiration due to the watering event.

Enzyme activity assays

To assess differences in EA between the Roots+Hyphae+Microbes, Hyphae+Microbes, and Microbes chambers, we measured soil EA in all chambers from

a subset of mesocosms ($N = 5$ from each treatment) at the end of the pre-drought, drought, and recovery periods. Soil samples were stored at $4 \text{ }^\circ\text{C}$ and analyzed within 1 week of collection for EA according to Bell et al. (2013). We targeted four enzymes involved in C acquisition from organic matter degradation: α -glucosidase, β -glucosidase, cellobiohydrolase, and xylosidase. Briefly, we homogenized 1 g field moist soil in 70 mL of 50 mM sodium acetate buffer (pH 4) with an immersion blender. We incubated 800 μL soil slurry with 200 μL fluorescent substrate in 96-well deep-well plates for 2 hours at $25 \text{ }^\circ\text{C}$. We prepared separate standard curves for each soil sample by incubating 800 μL soil slurry with 200 μL standard (4-Methylumbelliferone, MUB) in deep-well plates with sample plates. After incubating, we inverted and centrifuged deep-well plates, and transferred 250 μL to black-bottom 96-well plates and read them on a fluorescence microplate reader (365 nm excitation, 450 nm emission). We report EA as the sum of all C degrading enzymes to reduce the number of statistical tests needed and to ease interpretation. See Table S1 for mean activity of individual enzymes.

Photosynthesis and leaf water potential

To assess belowground C dynamics in context of potential availability to roots or mycorrhiza, we measured photosynthesis on a subset of plants ($N = 5$ of each species in each treatment) with a Li-COR 6400 (Li-COR Inc., Lincoln, Nebraska, USA) at six time points throughout the experiment. One leaf was chosen per plant and measured at each time point. On one plant, the sample leaf abscised during the drought treatment; for all other samples, we measured the same leaf regardless of its wilting status.

To quantify the degree of plant water stress during the drought, we measured pre-dawn leaf water potential from all plants on the last day of the drought period. One leaf was collected from each plant before dawn, placed into a plastic bag with a small piece of slightly moistened paper towel and stored at $4 \text{ }^\circ\text{C}$ until analysis could be completed over the next several hours using a PMS Pressure Chamber Model 610 (PMS Instrument Co., USA).

Biomass

At the conclusion of the experiment, we harvested above- and belowground plant biomass from each

mesocosm. Root growth had filled full chambers, including inside the PVC collars. We excavated the remaining root biomass from the bulk chamber soil and rinsed it in water to remove excess soil. Aboveground and belowground biomass was dried at 70 °C until a constant mass, then weighed. Root:shoot ratios (R:S) were calculated as the root biomass divided by the shoot biomass.

Statistical analyses

Estimates of photosynthesis, soil water potential (Ψ_{soil}), leaf water potential (Ψ_{leaf}) and biomass were collected at the mesocosm-level (not at the chamber level). Biomass was non-normally distributed, so we compared biomass estimates (aboveground, belowground, and R:S) across treatments for each species using non-parametric Wilcoxon tests. Photosynthesis, Ψ_{soil} , and Ψ_{leaf} were measured multiple times on each mesocosm (or plant), so we used linear mixed effects models to compare differences across treatments (drought and control). We used the `lme()` function (nlme package version 3.1) in R (3.3.2; Team 2011) with mesocosm as a random effect and treatment (control or drought) and the predictor. We analyzed each species (maple or oak) separately. We report ANOVA results of the linear mixed effects models.

Potential C degrading EA was measured at the chamber-level. To examine how potential C degrading EA was affected by drought and rewetting we built linear mixed effects models. We analyzed each species (maple or oak) separately and tested for the effects of treatment (control or drought), chamber (Roots+Hyphae+Microbes, Hyphae+Microbes, or Microbes), and their interaction on EA at each time point using ANOVA tests. We report Tukey post hoc results.

Respiration was also measured at the chamber-level. To assess how respiration was impacted by drought and rewetting, we examined respiration in two ways. First, we compared the drought-sensitivity of each chamber within each tree species using the CO_2 fluxes from the pre-drought and drought time points. We built mixed effect models to explain Respiration during pre-drought and drought time points with mesocosm as a random effect and the following predictors: Ψ_{soil} , chamber type (Roots+Hyphae+Microbes, Hyphae+Microbes, or Microbes), and their interaction. We define drought-sensitivity as the estimated relationship between Ψ_{soil} and CO_2 flux (slope coefficient); significantly different

regression slopes among chambers (i.e. a significant interaction between Ψ_{soil} and chamber) indicates different drought-sensitivity between chambers. To account for potential differences in respiration due to biomass, we tested whether respiration drought-sensitivity was correlated with final biomass (aboveground, belowground, or R:S). Second, to examine the potential for respiration to recovery after rewetting, we compared the mean respiration from drought versus control chambers after re-watering. We compared respiration at each time point during the recovery period using ANOVAs and Tukey post-hoc tests. Finally, to examine whether drought had affected the underlying relationship between water availability and respiration, we used linear regressions to compare the relationship between CO_2 flux and Ψ_{soil} in control versus drought mesocosms (combining all chambers of both species). We examined these relationships both in the pre-drought and recovery periods.

Results

Plant biomass, photosynthesis, and water availability

Drought affected the aboveground and belowground biomass allocation of maples, but not of oaks (Fig. 1). Maples exposed to drought had significantly less aboveground biomass (Wilcoxon Rank Sum test $p = 0.020$) relative to control maples; this resulted in significantly higher R:S in drought maples than in control maples (Wilcoxon Rank Sum test $p = 0.018$). Oaks had higher R:S than maples ($R:S_{\text{Oak}} = 2.05 \pm 0.04$; $R:S_{\text{Maple}} = 0.86 \pm 0.02$; $F = 35.0$, Wilcoxon Rank Sum test $p < 0.001$).

Tree seedling photosynthetic rates were highly variable throughout the experiment. Across all experimental periods, photosynthetic rates differed significantly between species ($t = 5.58$, $p < 0.001$) but there were no significant differences between control and drought plants at any sampling time point. Maples and oaks in the drought treatment and during the drought period had mean photosynthetic rates of 0.43 ± 0.02 and $3.92 \pm 0.15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively. Maple and oak individuals in the control treatment had photosynthetic rates of 1.20 ± 0.08 and $5.00 \pm 0.15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ during the same time period. Although some plants abscised the individual leaves we used for photosynthesis measurements, all plants survived the drought.

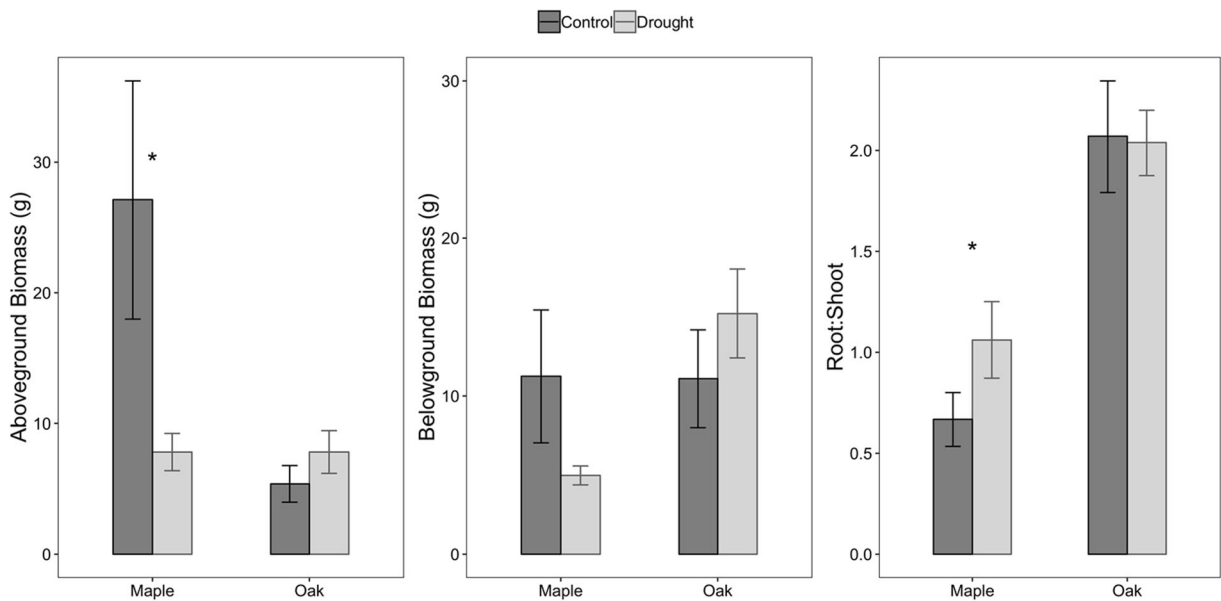


Fig. 1 Drought affected biomass allocation for Maples, but not Oaks. Significance, as computed through Wilcoxon Rank Sum tests, is indicated as follow: $p < 0.050$ is *, $p < 0.010$ is **, and $p < 0.001$ is ***

Pre-drought, Ψ_{soil} was -0.92 ± 0.01 in maple mesocosms and $-0.89 \pm <0.01$ MPa in oak mesocosms (Fig. S2) and did not differ between control and drought mesocosms. At the end of the drought, Ψ_{soil} differed significantly between control and drought mesocosms for both maples and oaks ($p < 0.001$ for both). At the end of the drought, Ψ_{soil} in drought mesocosms was -1.74 ± 0.01 MPa (maple) and -1.73 ± 0.02 MPa (oak), versus $-0.64 \pm <0.01$ MPa (maple) and -0.60 ± 0.01 MPa (oak) in control mesocosms. These differences in soil water availability corresponded to differences in the pre-dawn leaf water potential (Ψ_{leaf}) between control and drought mesocosms at the end of the drought period. For maple, Ψ_{leaf} of control and drought plants was -0.42 ± 0.04 MPa and -1.24 ± 0.24 MPa, respectively ($p = 0.003$). Oak Ψ_{leaf} of control and drought plants was -0.59 ± 0.09 and -1.34 ± 0.26 MPa ($p = 0.021$). After 2 weeks of well-watered conditions during the recovery period, differences in Ψ_{soil} between drought and control mesocosms were greatly reduced (Fig. S2) but persisted for maple mesocosms ($p = 0.023$) and was ameliorated for oaks ($p = 0.165$). At the end of the recovery period, Ψ_{soil} of control and drought mesocosms was -0.70 ± 0.01 MPa and -0.66 ± 0.01 MPa in maple mesocosms, and -0.66 ± 0.01 MPa and $0.69 \pm <0.01$ MPa for oaks.

Enzyme activity

Potential C degrading EA did not differ across chambers for either tree species, at any time period. Prior to drought, EA also did not differ between control and drought treatments (maple mesocosms: 0.20 ± 0.01 nmol C $\text{gds}^{-1} \text{h}^{-1}$; oak mesocosms: 0.19 ± 0.01 nmol C $\text{gds}^{-1} \text{h}^{-1}$; Fig. 2). At the end of the drought, potential C degrading EA in maple mesocosms was 50% lower in drought mesocosms (0.20 ± 0.02 nmol C $\text{gds}^{-1} \text{h}^{-1}$) compared to control mesocosms (0.37 ± 0.05 nmol C $\text{gds}^{-1} \text{h}^{-1}$; Tukey HSD $p = 0.007$; Fig. 2). For oaks, too, EA was lower in drought mesocosms (0.18 ± 0.03 nmol C $\text{gds}^{-1} \text{h}^{-1}$) than in control mesocosms (0.45 ± 0.05 nmol C $\text{gds}^{-1} \text{h}^{-1}$; Tukey HSD $p < 0.001$; Fig. 2). At the end of the recovery period (i.e. after 2 weeks of well-watered conditions) EA remained ~40% lower in the drought mesocosms relative to the control mesocosms (maple: Tukey HSD $p = 0.032$, oak: Tukey HSD $p = 0.015$; Fig. 2).

Respiration sensitivity to drought across biotic groups

Respiration varied over time and declined with the drought (Fig. 3). Pre-drought, respiration from maple mesocosms was higher from Roots+Hyphae+Microbes chambers (0.22 ± 0.02 mg C $\text{m}^{-2} \text{min}^{-1}$) than Microbes

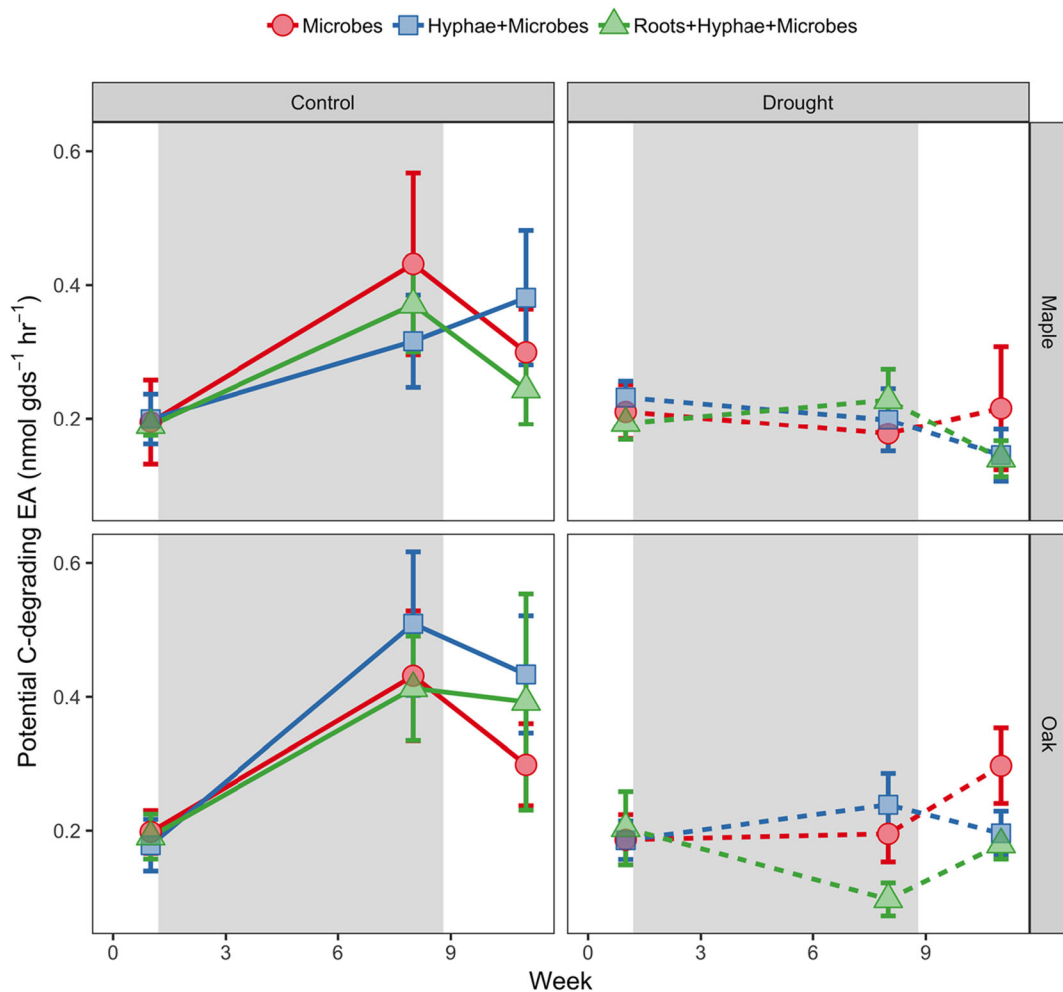


Fig. 2 Potential carbon degrading enzyme activity (EA) over a dry-rewetting cycle. EA did not differ among chambers, but differed between control and drought mesocosms at the end of the drought (weeks 2–8) and recovery periods (weeks 9–11). The grey

area denotes the drought period; the recovery period following the drought was initiated by rewatering all mesocosms 12 h prior to the week 9 measurements. See text for statistical details

chambers ($0.18 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$; $p < 0.001$) and did not differ between Roots+Hyphae+Microbes and Hyphae+Microbes chambers ($0.21 \pm 0.02 \text{ mg C m}^{-2} \text{ min}^{-1}$; $p = 0.386$). In oak mesocosms, respiration from Roots+Hyphae+Microbes chambers ($0.19 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$) was higher than respiration from both Hyphae+Microbes ($0.16 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$; $p = 0.007$) and Microbes chambers ($0.15 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$; $p = 0.001$). Respiration was not correlated with aboveground or belowground plant biomass in any chamber for either species. By the end of the drought, respiration from drought mesocosms was reduced to $<10\%$ of that of control mesocosms ($p < 0.001$ for both species).

Respiration from all chambers declined as soils dried, but chambers differed in their sensitivity to drought (i.e. regression slopes; Fig. 4). This resulted in differences in the relative C flux from each chamber relative to control mesocosms. In control mesocosms, the C flux from each chamber stayed remarkably constant over time at an approximate 1:1:1 ratio. In maple mesocosms, respiration from the Hyphae+Microbes chamber was less sensitive to drought (i.e. shallower slope; slope = 1.76) than the Roots+Hyphae+Microbes chamber (slope = 2.32) and the Microbes chamber (slope = 2.40; both $p = 0.001$; Fig. 4). By the last week of the drought, respiration from Hyphae+Microbes chambers of maple mesocosms in the drought treatment ($0.05 \pm 0.01 \text{ mg C}$

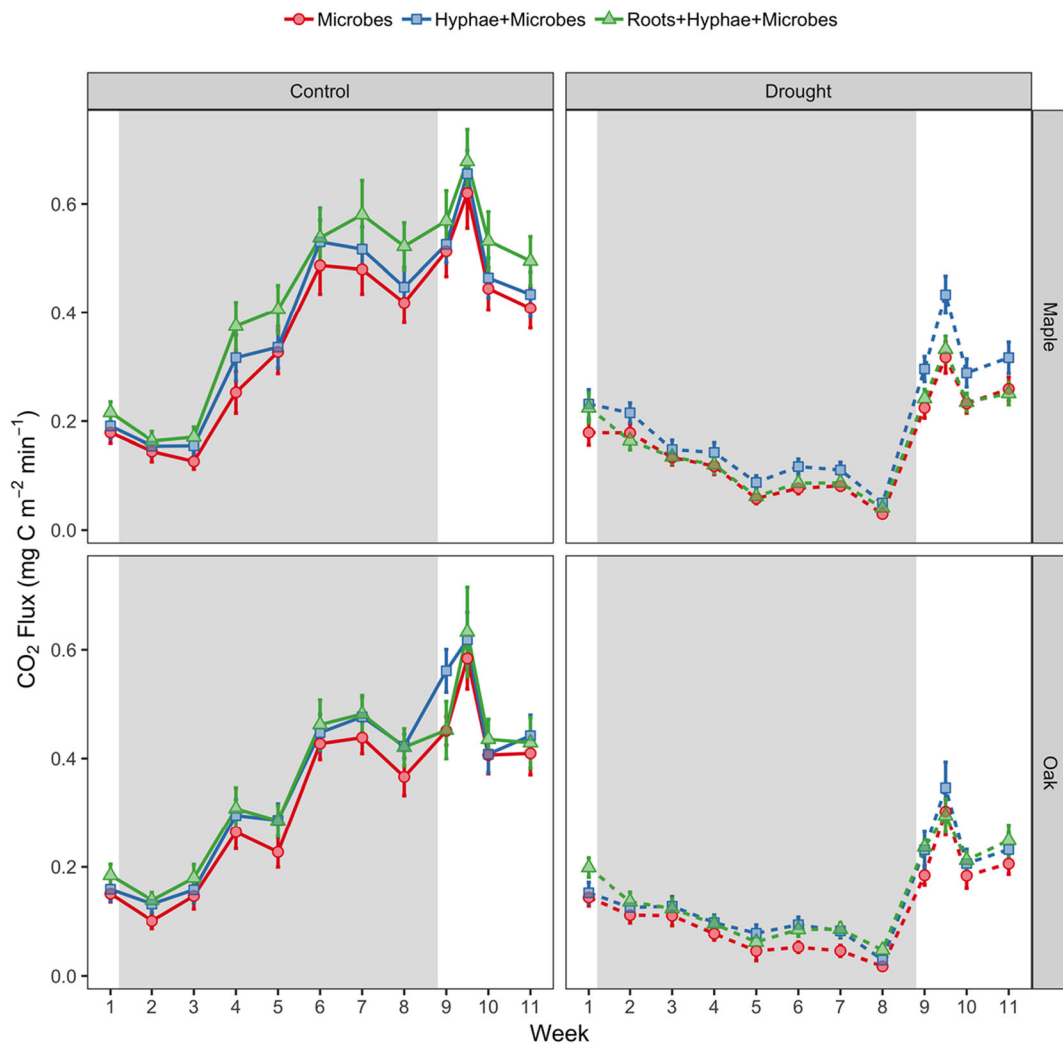


Fig. 3 Carbon flux from maple and oak chambers over an extreme dry-rewetting cycle. The grey area denotes the drought period; the recovery period following the drought was initiated

by rewatering all mesocosms 12 h prior to the week 9 measurements. See text for statistical details

$\text{m}^{-2} \text{min}^{-1}$) was $\sim 20\%$ higher than the Roots+Hyphae+Microbes chamber, and 40% higher than the Microbes chamber. In oak mesocosms, the Microbes chamber was significantly more sensitive to drought (i.e. steeper slope; slope = 2.97) than the Roots+Hyphae+Microbes ($p < 0.001$; slope = 1.97) and the Hyphae+Microbes (slope = 2.27; $p = 0.006$) chambers (Fig. 4). In oak mesocosms in the drought treatment, respiration from the Microbes chamber ($0.02 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$) was 40% lower than the Hyphae+Microbes chamber ($0.03 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$), and 60% lower than the Roots+Hyphae+Microbes chamber ($0.05 \pm 0.01 \text{ mg C m}^{-2} \text{ min}^{-1}$).

Respiration recovery from drought

To control for the effects of increasing water availability following the drought treatment, we saturated the soils in both control and drought mesocosms at the start of the recovery period. Immediately (12 h) after rewatering, respiration from control mesocosms increased by 20% , and respiration of drought mesocosms increased by nearly 550% (Fig. 5a). Maximum respiration values were observed 36 h after rewatering (i.e. week 9.5), but respiration from previously-droughted mesocosms was still lower than from control mesocosms despite being maintained under the same water availability

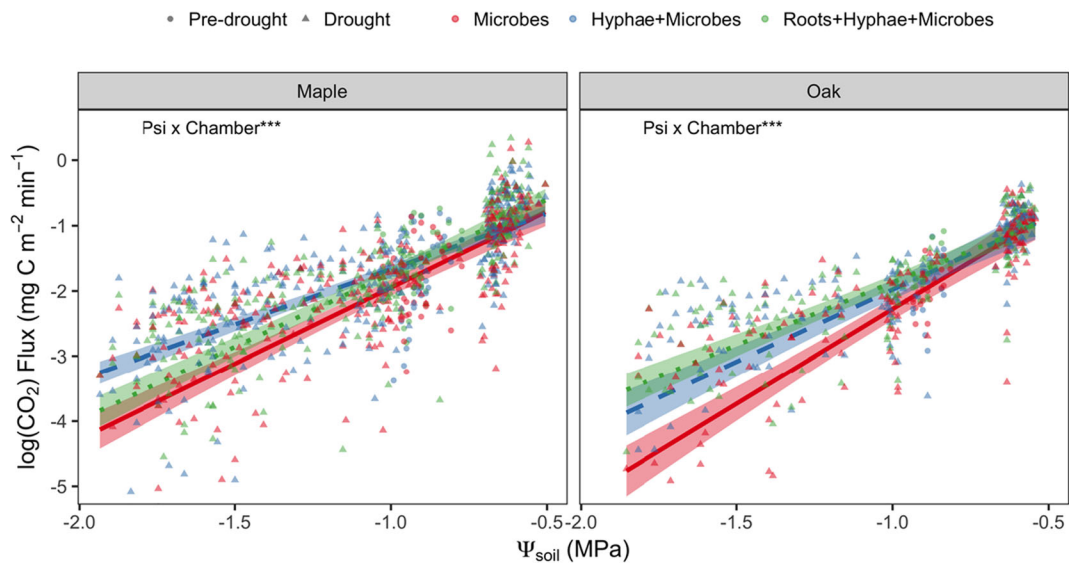


Fig. 4 Respiration-sensitivity differs across chambers for each species. Best models included a significant interaction term ($p < 0.001$ for each species) indicating that the regression line slopes differed across chambers. Solid, dashed, and dotted lines

correspond to the Microbes, Hyphae+Microbes, and Roots+Hyphae+Microbes chambers, respectively. See text for additional statistical details

(Tukey post-hoc test $p < 0.001$ for both species; Fig. 5b). During this respiration pulse, CO_2 flux from maple mesocosms was $0.65 \pm 0.03 \text{ mg C m}^{-2} \text{ min}^{-1}$ in the control treatment, and $0.36 \pm 0.02 \text{ mg C m}^{-2} \text{ min}^{-1}$ in the drought treatment. During this pulse, respiration did not differ across chambers for control maple mesocosms, but differed across chambers of drought maple mesocosms (Fig. 5a). Respiration from the Hyphae+Microbes chamber was ~ 1.3 times higher than the Roots+Hyphae+Microbes and Microbes chambers ($p < 0.001$ for both). Respiration from oak mesocosms was $0.61 \pm 0.04 \text{ mg C m}^{-2} \text{ min}^{-1}$ in the control treatment, and $0.31 \pm 0.02 \text{ mg C m}^{-2} \text{ min}^{-1}$ in the drought treatment. Respiration did not differ across chambers of either control or drought oak mesocosms during the respiration pulse (Fig. 5a).

Similar patterns persisted across the full recovery period. By the last week of the recovery period, respiration from control maple mesocosms was generally highest in the Roots+Hyphae+Microbes chamber (Roots+Hyphae+Microbes vs Hyphae+Microbes chamber $p = 0.074$; Roots+Hyphae+Microbes vs Microbes chamber $p = 0.013$; Fig. 5a). Respiration from previously-droughted maple mesocosms (i.e. drought treatment) however remained highest in the Hyphae+Microbes chamber (Hyphae+Microbes vs Roots+Hyphae+Microbes $p = 0.003$; Hyphae+Microbes vs

Microbes $p = 0.008$; Fig. 5a). Respiration did not differ across chambers of either control or drought oak mesocosms.

We compared the relationship of respiration and Ψ_{soil} between treatments in the pre-drought and recovery periods. The slope (Treatment* Ψ_{soil}) of the correlation between respiration and Ψ_{soil} did not differ between treatment groups (control vs drought) either in the pre-drought ($p = 0.704$) or recovery periods ($p = 0.656$) indicating no legacy effects on the overall effect of water availability on respiration (Fig. 5b). However, the intercept of the regression line differed significantly between treatment groups only in the recovery period (Fig. 5b; $p < 0.001$), suggesting that the drought resulted in a persistently reduced C flux 2 week even after ample water availability.

Discussion

Respiration from forests dominated by arbuscular mycorrhizal or ectomycorrhizal trees are controlled by different environmental factors (Vargas et al. 2010), indicating that plants with different mycorrhizal associations vary in their sensitivity to different stressors. In this study, we examined the responses of belowground respiration and potential carbon (C) degrading enzyme

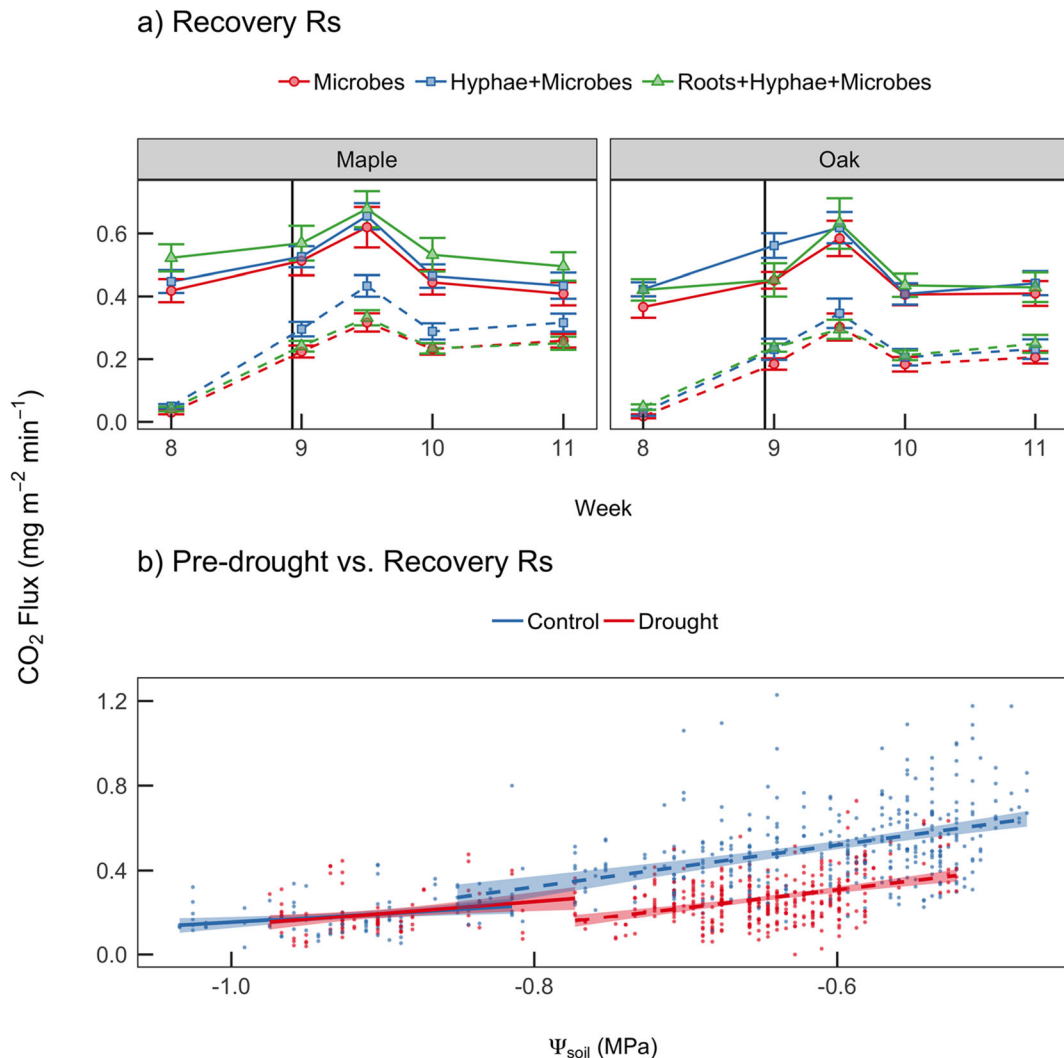


Fig. 5 Legacy of drought after re-watering. **a** Control (solid lines) and drought mesocosms (dashed lines) of both species experienced a spike in respiration rates 36 h after re-watering, but respiration remained significantly reduced in drought mesocosms relative to control ($p < 0.001$ for each time point). **b** The relationship between respiration and Ψ_{soil} did not differ between control

and drought mesocosms prior to drought (solid lines), but after re-watering, respiration from drought mesocosms remained lower than control mesocosms over the same Ψ_{soil} range (dashed lines). See text for statistical details. In panel (a), the black line indicates the time of re-watering, 12 h prior to the Week 9 measurement

activity (EA) throughout a cycle of extreme drought followed by saturated rewetting; we contrasted the responses among belowground biotic groups (i.e. roots, mycorrhizal fungi, and free-living microbes) in maple and oak mesocosms. We found that drought strongly reduced respiration and EA across all groups relative to control. While EA recovered somewhat after 2 weeks of well-watered conditions, respiration did not. We also observed different responses of respiration across biotic groups, particularly after the rewatering event. These asymmetrical responses of C processes over time and

across biota suggest that belowground biota may differ in their physiological responses to drought (Lennon et al. 2012).

Enzyme activity is resistant to drought

Despite the severity (minimum mean Ψ_{soil} of drought treatment was -1.73 ± 0.02 MPa) and duration of our extreme drought, all plants survived the experiment. Both respiration and C degrading potential decreased during the drought, and increased during rewatering, but neither

processes completely shut down. Furthermore, over the drought period, there appeared to be stronger increases in these processes in the control mesocosms compared to decreases in these processes in the drought mesocosms. Particularly for C degrading potential, differences in EA observed between control and drought mesocosms during the drought belied important temporal dynamics. That is, the differences between control and drought mesocosms during the drought and recovery periods are likely attributable to increases in EA in the control mesocosms (Henry 2012), more so than decreases in EA in drought mesocosms. Thus, we speculate that, while additional enzymes were not produced during the drought, enzymes were also not degraded throughout the drought in the drought mesocosms. We note, however, that other enzymes involved in C degradation, such as oxidases, were not measured and could respond differently to drought. If potential EA responds minimally to drought (Steinweg et al. 2013), enzymes may remain active in soil aggregates under dry conditions and continue degrading C substrates even as soils become hydrologically disconnected. Then, when soils rewet, the release of monomers from soil aggregates may be an important C source for microbial activity (Kieft et al. 1987), and has been hypothesized to contribute to the observed CO₂ pulse upon rewetting (Fierer and Schimel 2003).

Mycorrhizal fungi buffer soil respiration responses to drought

Belowground biota differed in their respiration-sensitivity to drought, and the relative performance of plant roots, mycorrhizal fungi, and free-living microbes differed in maple and oak mesocosms. We discuss these differences in the context of each mesocosm separately. We caution against drawing comparisons between the responses of maples (arbuscular mycorrhizal fungi hosts) and oaks (ectomycorrhizal fungi hosts), since we only examined two tree species which also differ in drought response strategy (i.e. isohydry vs anisohydry). In both maple and oak mesocosms, microbial respiration was the most sensitive to drying soils. This supports our original hypothesis that (free-living) microbial activity would be the most strongly C-limited during drought, since C supplies from root exudates and microbial access to soil organic matter are both reduced under drought. In addition, soil bacteria are thought to be less drought-tolerant than fungi (Yuste et al. 2011), since they have a more limited capacity to accumulate

osmolytes and scavenge for water than fungi (Schimel et al. 2007). However, because we did not assess biomass or composition of the microbial community, we cannot assess the extent to which drought caused a shift in fungal or bacterial abundances or composition.

Although we expected C limitation to also reduce the performance of arbuscular mycorrhizal fungi, in maple mesocosms respiration from the Hyphae+Microbes chamber was the least sensitive to drought. Arbuscular mycorrhizal fungi produce a protein, glomalin, which increases soil aggregation and is thought to enhance water holding capacity (Rillig 2004). The production of intracellular osmolytes is a C-expensive process (Schimel et al. 2007), so if microbial sensitivity to drought is buffered in the presence of arbuscular mycorrhizal fungi this may alter ecosystem C cycling dynamics in rhizosphere versus bulk soil. Although the production of glomalin provides a plausible mechanism to explain the sustained respiration of arbuscular mycorrhizal fungi relative to soil microbes, it fails to explain why the full chamber did not also exhibit sustained respiration. We speculate that, in the full chamber of maple mesocosms, increased competition by roots for water (Augé 2001) may have counteracted the positive effects of glomalin on soil water availability. Because we measured Ψ_{soil} at the mesocosm level, we cannot discount the possibility that chambers differed in water availability within a mesocosm.

In accordance with our expectations in oak mesocosms, respiration from the full and root-exclusion chambers were less sensitive to drought than respiration from the hyphal-exclusion chamber. We hypothesized that, because oaks are anisohydric and therefore maintain photosynthesis and C flux belowground, this would maintain a C supply for root and ectomycorrhizal respiration throughout a drought. Although we found that oaks had higher rates of photosynthesis than maples, unfortunately our ability to draw conclusions based on this variable is limited because droughted leaves senesced and prevented repeated photosynthesis measures during and after drought. However, a reduction in photosynthetic rate during drought is well documented, as is that photosynthesis of anisohydric plants, such as oaks, is relatively unresponsive to drought (McDowell et al. 2008; Yi et al. 2017). Additional research on the drought strategies of mycorrhizal fungi, in particular, can help to resolve their contribution to the relative performance differences observed among belowground biota.

Drought leaves a legacy on soil respiration

By the end of the drought, respiration was <10% that of the control mesocosms and C degrading potential was half that of control mesocosms. Respiration exhibited a strong pulse of high activity upon rewetting, but even when CO₂ fluxes were highest, respiration from the droughted mesocosms was still only ~50% that of the control mesocosms. We found a strong drought legacy that continued to suppress respiration even after 2 weeks of high water availability. After 2 weeks of well-watered conditions, respiration from previously-droughted mesocosms was 60% (for maple) and 50% (for oak) that of control mesocosms. This finding contradicts a number of studies that have found a rapid return to background respiration rates correlated with recuperating microbial activity (Barnard et al. 2013; Blazewicz et al. 2014; Placella et al. 2012), and suggests that patterns of respiration during dry-rewetting cycles cannot be attributed solely to free-living microbial activity. It is possible, however, that the extreme fluctuations in water availability between drought and recovery periods may have acted as consecutive stressors on the microbial community (Lennon et al. 2012) as opposed to a drought stress followed by the alleviation of that stress. Nevertheless, our finding may be explained in different ways. Rewetting may have flushed newly labile resources out of our mesocosms (Kieft et al. 1987), leaving behind a depleted resource supply for belowground biota relative to control mesocosms (He and Dijkstra 2014). If rewetting leaches resources from soils, the long-term performance of plants after drought may be influenced by nutrient-limitation. The rapid flushing of nutrients and ions from soils upon rewetting also has important implications for groundwater and aquatic ecosystem functioning (Evans et al. 2006). Alternatively, reduced respiration following drought may occur if the free-living microbial community composition shifted towards one that is more drought-tolerant (Evans and Wallenstein 2014), and therefore exhibited faster reactivation of microbial growth (Meisner et al. 2013, 2015; Ochoa-Hueso et al. 2018). A detailed assessment of microbial composition could help clarify these results.

Differences in the respiration recovery potential of belowground biota, especially in the context of competition, have important implications for predicting C fluxes across ecosystems (Hanson et al. 2004) and under different environmental stresses (Scott-Denton et al. 2006). These differences may be particularly important

under extreme droughts, when prolonged C limitation further reduces belowground respiration relative to plant productivity (Shi et al. 2014). Arbuscular mycorrhizal fungi are thought to have shorter turnover times (Soudzilovskaia et al. 2015; Staddon et al. 2003) than ectomycorrhizal fungi (Ekblad et al. 2016), and this may have contributed to the faster respiration recovery of the Hyphae+Microbes chamber of droughted maple mesocosms. These findings suggest that arbuscular mycorrhizal fungi can play an important role in the CO₂ pulses that occur following dry-rewetting cycles. We speculate that that recovery of arbuscular mycorrhizal activity could also help to initiate plant recovery following an extreme drought.

Conclusions

An improved understanding of the mechanisms underlying soil respiration is needed to refine predictions of terrestrial CO₂ emissions under future climate scenarios (Bradford et al. 2016; Friedlingstein et al. 2014). In particular, as extreme climatic events increase in severity and frequency, it is important to understand whether our current framework for understanding soil respiration can be extended to extreme environmental conditions (Vicca et al. 2014). Our results suggest that ecosystems dominated by different tree species and their associated mycorrhizal fungi may respond to and recover from drought differently in terms of respiration and enzyme activity. In addition to physiological differences between tree hosts, resource use patterns of belowground biota may influence respiration responses from rhizosphere and bulk soil. Incorporating such ecologically-relevant nuances into our understanding of the drought-responses of soil as a whole may improve the spatial accuracy, and thus our ability to scale up, estimates of terrestrial respiration.

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Author contributions Both authors designed the research, collected data, and wrote the manuscript. CDF analyzed data.

Data availability Data will be archived at <https://doi.org/10.3334/CDIAC/ormlfa.011> following acceptance.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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