



Cellular and extracellular C contributions to respiration after wetting dry soil

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Abstract Wetting of dry soil triggers a pulse of microbial respiration that has been attributed to two broad mechanisms: (1) recycling of microbial cellular carbon (C), and (2) consumption of extracellular organic C made available to microbes by wetting. We evaluated these two mechanisms by measuring cumulative CO₂ release, changes in the size and chemical composition of microbial biomass, and water-extractable organic carbon (WEOC) concentrations following artificial wetting of soil sampled from two depths at each of seven sites across California spanning a range of geologic parent materials. In samples collected from surface soil (0–10 cm depth), we found that cumulative CO₂ release after wetting in the laboratory was most strongly correlated with microbial biomass. In these samples, the relative

abundance of trehalose—a putative microbial osmolyte—decreased from 25% (SD = 12) to 16% (SD = 7) of the chloroform-labile fraction of the microbial biomass after wetting. This suggested a role for osmolyte consumption in generating the respiration pulse. In subsoil (40–50 cm depth, or sampled at contact with rock), however, the cumulative CO₂ release after wetting was unrelated to microbial biomass and more strongly related to WEOC. The concentrations of selected microbial biomass constituents (e.g. trehalose and amino acids) in WEOC were negligible (< 1%), suggesting that cell lysis was not important in generating WEOC in this study. The amount of WEOC relative to total organic C was greatest in subsoil, and negatively related to ammonium oxalate-extractable Fe (Pearson's $R = 0.42$, $p < 0.01$), suggesting a role for soil mineralogical properties in controlling WEOC release. Together,

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these findings suggest that microbial cellular C and extracellular C jointly contribute to the respiration pulse, and that their relative contribution depends on depth.

Keywords Soil carbon · Birch effect · Drought · Osmoregulation

Introduction

Wetting dry soil triggers a pulse of microbial respiration that can last for several days (Birch 1958). This respiration pulse has been attributed to two mechanisms: (1) stress-induced recycling of microbial biomass C, including both osmolyte consumption and cell lysis as C sources; and (2) enhanced microbial access to extracellular organic C (Borken and Matzner 2009; Kaiser et al. 2015; Schimel 2018). Variation in the relative importance of these two mechanisms may control the magnitude and dynamics of respiration after wetting. The source of this variation is largely unknown because studies of soil wetting typically involve detailed observation of at most a few soils (e.g. Fierer and Schimel 2002; Miller et al. 2005; Göransson et al. 2013). Differentiating these mechanisms across different soils and across depths within the soil is important because repeated pulses of respiration can consume a significant fraction of net ecosystem production (Xu et al. 2004; Kim et al. 2010; Lee et al. 2002), and thus might influence the capacity of soil to act as a long-term C sink.

Microbial biomass C may be recycled and respired as microbes acclimate, die, and grow in response to wetting (Kaiser et al. 2015; Kieft 1987; Bottner 1985; Blazewicz et al. 2014; Warren 2016). The direct contribution of microbial biomass C to the respiration pulse is supported by ^{14}C labeling experiments, which have shown that a substantial fraction of C released in the pulse can be extracted from the biomass before wetting occurs (Bottner 1985; Fierer and Schimel 2003). This C may be stored in microbial biomass in the form of inducible osmolytes: compounds that microbes synthesize to withstand low water potentials (Welsh 2000; Schimel 2018). After wetting, cells release or metabolize osmolytes to avoid osmotic shock (Schimel et al. 2007). Microbes synthesize a diversity of osmolytes in culture, including amino

acids, amino-derivatives, sugars, and sugar alcohols (Imhoff 1986; Killham and Firestone 1984; Kempf and Bremner 1998). Evidence for osmolyte accumulation in soil is however mixed, with some support from broad analyses (Warren 2016; Chowdhury et al. 2019), but little support from compound-specific searches (Boot et al. 2013; Williams and Xia 2009; Kakumanu et al. 2013; Kakumanu and Williams 2019). Alternatively, recycling of cellular C might operate at the population level as cells die and are replaced during cycles of drying and wetting (Lund and Goksøyr 1980; Kieft 1987; Blazewicz et al. 2014). The rapid increase in soil water potential after wetting may cause osmotically-induced lysis, releasing cellular material into the soil solution where it can be assimilated and respired by surviving microbes (Kieft 1987). While net changes in microbial biomass following wetting may be relatively small, mortality can be balanced by growth, which has been detected by quantifying ^{18}O incorporation into DNA within hours after wetting (Blazewicz et al. 2014).

Both osmolyte consumption and cell-lysis would provide sources of C that derive from recycling microbial biomass. Alternatively, wetting may mobilize extracellular organic C that is otherwise physically unavailable to microbes (Kaiser et al. 2015; Sørensen 1974; Adu and Oades 1978; Xiang et al. 2008; Homyak et al. 2018). Mobilization of extracellular organic C is consistent with observations of repeated wetting and drying cycles, which can drive net growth of microbial biomass (Fierer and Schimel 2002; Xiang et al. 2008), and yield more CO_2 than can plausibly be attributed to recycling mechanisms alone (Scheu and Parkinson 1994; Miller et al. 2005). Furthermore, radiocarbon measurements show that wetting a dry soil can generate CO_2 with a mean turnover time on the order of centuries (Schimel et al. 2011), even when soil CO_2 sampled at the same site under static wet conditions is modern (Fierer et al. 2005). This indicates that wetting dry soils links microbes with a pool of relatively less-available extracellular C.

Mobilization of extracellular organic C is indirectly supported by measurements of water-extractable organic carbon (WEOC), a proxy for in situ dissolved organic C concentrations. Dry soil typically yields more WEOC than soil under static moist conditions (Lundquist et al. 1999; Guo et al. 2012; Homyak et al. 2018). WEOC might increase in dry soil because

degradation of dead roots and other buried plant detritus continues in the absence of microbial uptake (Miller et al. 2005; Lawrence et al. 2009), although root litter degradation appears to be negligible under dry conditions (Homyak et al. 2018). WEOC might also integrate microbial cellular constituents that are released from the microbial biomass during wetting, potentially blending C released by recycling mechanisms (i.e. cell lysis) with other sources of extracellular C (Warren 2016). However, ^{14}C tracer experiments at a California grassland site showed that microbial cellular constituents were not detectable in soluble C extracted from dry soil (Fierer and Schimel 2003), suggesting that WEOC is not necessarily composed of microbial cellular constituents. Alternatively, WEOC might represent C that is associated with soil minerals: organic matter released from disruption of aggregates (Adu and Oades 1978; Kemper 1985; Deneff 2001), or C that is desorbed from minerals due to drying-induced changes in surface chemistry (Kaiser et al. 2015; Newcomb et al. 2017). In particular, drying changes pore configuration, increases ionic strength, and acidifies clay surfaces (Todoruk et al. 2003; Clarke et al. 2011). These changes may weaken bonds between organic C and clays, making C available to microbes.

Cellular and extracellular sources of C after wetting are not mutually exclusive. Some of the C released in the respiration pulse might be derived from recycling of microbial biomass via osmolyte consumption or lysis pathways, while the remainder may be derived from extracellular C. The relative contribution of these C sources at different depths and across soil profiles is largely unknown. In this investigation we ask: across different soil profiles, does depth influence the relative contribution of cellular versus extracellular C sources to respiration pulses after wetting? We hypothesize that recycling mechanisms dominate release of C after wetting at the soil surface, where microbial biomass is relatively abundant and microbes are exposed to repeated cycles of drying and wetting and thus might be expected to accumulate osmolytes. Conversely, release and consumption of extracellular mineral-associated C may be more important in subsoil where microbial biomass is scarce, abiotic conditions are more stable, and the potential for production and consumption of osmolytes and cell lysis is limited. We predict that respiration after wetting will scale with microbial biomass at the soil surface—where cellular

osmolytes may comprise a substantial fraction of that biomass under dry conditions—but will scale more closely with WEOC in subsoil. We also predict that the chemical composition of WEOC extracted from dry soil will reflect recycling mechanisms at the surface, with higher relative abundances of osmolytes and other microbial cellular constituents suggesting a greater contribution of cell lysis to WEOC.

We evaluated these predictions by quantifying correlations between the cumulative CO_2 released after wetting, microbial biomass, and WEOC extracted from dry soil in laboratory incubations drawn from 37 soil profiles sampled at two depths (0–10 cm and 40–50 cm, or at contact with hard rock). The soil profiles were located across seven sites within the Mediterranean portion of the California floristic province characterized by different geologic parent materials but a similar exotic annual grass vegetation cover. We incubated soils under laboratory conditions to quantify the cumulative CO_2 release following simulated wetting, as well as shifts in the size and chemical composition of microbial biomass and WEOC pools after wetting. We also evaluated relationships between WEOC and the solid-phase soil C pools that might generate WEOC (free plant detrital C versus mineral-associated C), plus soil physicochemical properties that broadly predict bulk C stocks across different soil types: texture, pH, reactive Fe and Al, and exchangeable cation stocks (Rasmussen et al. 2018). Incubating a variety of soils in the laboratory allowed us to observe their behavior under controlled—albeit physically disturbed—conditions, helping us to identify general patterns governing CO_2 release after wetting.

Methods

Field sampling

We sampled soils at seven sites across California (Table 1). All sampling was conducted in landscapes dominated by introduced annual grass species (*Avena barbata*, *Avena fatua*, *Bromus diandrus*, *Bromus hordeaceus*, *Taeniatherum caput-medusae*) with scattered oaks (primarily *Quercus douglassii*, with subdominant *Quercus lobata* and *Quercus agrifolia*). Rainfall and mean annual temperature vary across sites (Table 1), but the climate at all sites is

Table 1 Field sites

| Site | MAT (°C) | MAP (mm) | Parent material (n pits) |
|---|----------|----------|---|
| Sierra Foothill Research and Extension Center | 16 | 879 | Seafloor basalt (3 pits) |
| Sedgwick Reserve | 16.7 | 398 | Sandstone (3 pits); Serpentinite (3 pits); Mixed terrestrial sediments (3 pits) |
| Dye Creek Preserve | 16.6 | 751 | Mixed volcanoclastics (3 pits) |
| Hopland Research and Extension Center | 14.6 | 1223 | Sandstone (3 pits) |
| McLaughlin Reserve | 14.6 | 981 | Mudstone/sandstone (3 pits); Basalt (3 pits); Serpentinite (3 pits) |
| Hastings Natural History Reservation | 14.5 | 607 | Granodiorite (4 pits); Basalt (3 pits) |
| Blue Oak Ranch Reserve | 14.6 | 578 | Sandstone (3 pits) |

Mean annual temperature (MAT) and Mean annual precipitation (MAP) are 30-year means (1981–2010) obtained from PRISM at 4 km resolution (PRISM 2018)

Mediterranean, defined by winter-spring rainfall, high summer temperatures, and virtually no summer rainfall. At some sites closed-canopy forest grows on cooler north-facing slopes (i.e. Hopland, Hastings), in which case sampling was restricted to south or east-facing slopes in grassy areas that supported *Quercus douglasii*.

Replicate soil pits were excavated at each site to maximize the range of variation in soil properties across samples. The sites varied in geologic parent material, and individual sites could include more than one parent material (Table 1). When multiple parent materials were sampled within a site at least three soil pits were excavated in each parent material, and within a given parent material individual soil pits were positioned at least 200 m apart. A total of 37 soil pits were excavated across the seven sites, with 3–9 pits per site depending on the diversity of parent materials (Table 1). Sampling locations were situated in convex or planar slope positions. In general, soils were mapped as either Mollisols (Argixerolls and Haploxerolls) or Alfisols (Haploxeralfs), except in volcanic parent materials, which were generally rocky and too poor in amorphous minerals to form Andisols, and were mapped as Inceptisols (Haploxerepts) (Soil Survey Staff 2017).

Soil samples were collected during July of 2015 by excavating pits to a depth of 0.5 m. Two samples were collected at each of the 37 soil pits to represent surface and subsoil material, yielding a total of 74 (37*2) soil samples. Surface (A-horizon) soil was sampled at a fixed depth interval of 0–10 cm after removal of the

litter layer, and subsoil (typically B or BC horizons) at a depth of 40–50 cm. When contact with hard rock occurred at a depth of less than 40 cm, soil was sampled at the deepest possible 10 cm interval overlying the rock, always below a depth of 25 cm. Depth intervals guided sampling rather than horizon boundaries in order to maximize contrast between surface and subsoil samples. Sampling took place during the dry season when soil moistures in the field were low, even in subsoils. Soil samples were sieved through a 4 mm mesh in the field and soils were air-dried in the laboratory for several weeks prior to incubation.

Chemical and physical analyses

Total sand content (particles 0.05–2 mm) was analyzed via wet sieving. Soils were shaken with sodium hexametaphosphate solution (37.5 g L⁻¹) for 12 h at 140 rpm prior to sieving to 53 µm and the fraction retained in the sieve was oven dried at 110 °C and weighed. Total water holding capacity was obtained by saturating subsamples of each soil with water in a paper filter placed in a funnel and then allowing each sample to drain over 24 h. Water holding capacity was then assumed to equal the gravimetric water content of the drained soil.

Subsamples of each soil were ground to a fine powder by hand and total C and N were measured by combustion using an elemental analyzer (Fisons NA1500). Inorganic C was quantified by measuring CO₂ evolution following acid-digestion of finely-ground soil in a sealed jar (Lin et al. 2016). Free light

fraction (FLF) and occluded light plus heavy fraction (OLF + HF) organic C pools were separated by density using sodium polytungstate (SPT) at a density-cutoff of 1.6 g cm^{-3} (Cerli et al. 2012). The 1.6 g cm^{-3} cutoff is effective at separating minerals from macroscopic plant detritus in California grassland soils (Lin et al. 2018). Density fractions were obtained by mechanical dispersion of 5 g soil in 20 ml SPT by lightly vortexing the soil-SPT mixture in 50-ml centrifuge tubes. Dispersion was followed by centrifugation for 15 min at 180 revolutions per minute. Free light fractions were collected on glass-fiber filters after two dispersion cycles, and both free light and occluded light plus heavy-fractions were rinsed with DI water prior to drying and analysis. Total C in each fraction was analyzed with an elemental analyzer (Fisons NA1500). Total mass recovery was $101.9 \pm 2.5\%$ (mean \pm s.d.), recovery of C was $101.6 \pm 9.5\%$ (mean \pm s.d.). Total organic C and heavy-fraction organic C values were obtained by correcting for the contribution from inorganic C.

Exchangeable cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+ , and Al^{3+}) were quantified by extraction with 0.1 M BaCl_2 solution and analysis by ICP-OES (Perkin Elmer Optima 7300DV). Samples were extracted for 2 h on a reciprocal shaker, centrifuged for 10 min, and then filtered to $0.45 \mu\text{m}$ prior to analysis. Reactive iron and aluminum minerals were quantified by 0.1 M ammonium oxalate extraction at pH 3 for two hours and analyzed by ICP OES (Perkin Elmer Optima 7300DV) (Loeppert and Inskeep, 1996) at the UC Riverside Environmental Sciences Research Laboratory (<https://envisci.ucr.edu/environmental-sciences-research-laboratory-esrl>). Soil pH was measured in a 1:1 water slurry using an electrode (NRCS 2004).

Incubations

The soils were subjected to a controlled cycle of drying and wetting in the laboratory in fall-winter of 2015–2016. Physical disturbance can augment C release after wetting (Navarro-García et al. 2012), and so we wetted and incubated our sieved soil before drying it down a second time in the laboratory and conducting the main wetting experiment. This preliminary incubation was intended to expose the sieved soil to an initial simulated wetting event, allowing microbes to consume the C made available by destruction of aggregates during sieving. During the

preliminary incubation, each soil was wetted to 40% of its water-holding capacity and incubated in a sealed jar for 10 days. We cannot fully rule out sieving artifacts after this preliminary incubation: more intensive grinding of aggregates down to a 1-mm size class at one of our sites led to increases in respiration and microbial biomass of approximately 20% after one wetting cycle (Navarro-García et al. 2012)—albeit a shorter one than the 10-day preliminary incubation in this study. After the initial 10-day preliminary incubation, the soils were exposed to the lab air and allowed to dry under ambient laboratory humidity for six weeks at approximately 20°C . During the dry-down phase, soils were weighed to assess the rate of drying. All soils had reached a constant water content after three weeks, indicating that they equilibrated with the mean water potential of the laboratory air.

After six weeks, sub-samples of the dry soils were placed in glass Mason jars and wetted to 40% of their water holding capacity with deionized water. The jars were sealed and set to rest for 3 h to allow for equilibration of water throughout the soil and to permit sequential capping and wetting of all jars. CO_2 measurements were then made at 3, 6, 27 and 51 h after wetting. CO_2 concentrations were measured on small syringe samples collected through rubber septa and quantified using an infrared gas analyzer (LiCOR Biosciences; Li820). Average respiration rates were obtained for each time interval (3–6 h, 6–27 h, and 27–51 h) by dividing the cumulative mass of C evolved during each interval by the duration of the interval; these estimates represent time-integrated averages rather than estimates of the instantaneous respiration rate. To represent cumulative respiration after wetting (R_c), we calculated the total CO_2 -C evolved over the 3–51 h period following wetting. Respiration rates and R_c were normalized by the organic C content of each soil sample to isolate the magnitude of the wetting response from variation in total organic C content across soils.

Microbial biomass measurements

We evaluated correlations between cumulative CO_2 release after wetting and microbial biomass using measurements made on dry soil, and also evaluated net changes in microbial biomass after wetting. Microbial biomass was quantified in subsamples of each soil after the soil had dried to a constant moisture content

(after three weeks of dry-down), and again on a subsample of the soil 8 days after the simulated wetting event. Microbial biomass was quantified by two approaches: chloroform extraction (CE; Setia et al. 2012), and substrate-induced respiration (SIR, West and Sparling 1986; Fierer et al. 2003).

Chloroform extraction was performed by adding 0.5 ml of chloroform to an 8 g sub-sample of soil and extracting with 32 ml of deionized water on a reciprocal shaker for 4 h. Control extracts without chloroform were made in parallel, and total chloroform-extractable C was obtained by difference. Deionized water was used because the control extracts also functioned as extracts for quantifying WEOC. Extracts were sequentially filtered with glass-fiber filters followed by 0.45- μm nylon filters prior to analyses of biomass chemistry (see below). CE extracts were analyzed for total organic C with a TOC analyzer (Shimadzu TOC-V). Separate blanks were analyzed for fumigated and unfumigated extracts and were used to correct the data for background organic C concentrations. We did not apply a correction factor to relate chloroform-labile C to total biomass C to avoid assumptions related to the scaling factor; hence we report “CE-flush” -C and -N values rather than total microbial biomass C and N.

We found that CE produced low or negative results in subsurface samples because chloroform-amended and control extractions had similar concentrations; consequently we also estimated microbial biomass using the SIR method to provide a consistent method across depths. SIR was performed by agitating a slurry of soil and autolyzed yeast extract (12 g L⁻¹; 2:5 soil:liquid ratio by mass; Fierer et al. 2003) in a sealed jar fitted with a rubber septum and measuring the rate of CO₂ production over the period from 0.5–3 h using an infrared gas analyzer (LiCOR Biosciences; Li820).

CE flush and WEOC chemistry

We quantified a subset of the constituents of CE and WEOC extracts that might function as inducible osmolytes based on evidence from broad-spectrum chemical analysis of microbial biomass extracted from dry soil (Warren 2016). These included total reducing sugars, D-mannitol/L-arabitol, trehalose, and free amino acids. To quantify selected CE-flush constituents, analyses were conducted on water extracts of chloroform fumigated soils (see above) and

corrected for background concentrations in un-fumigated WEOC samples. When background concentrations were below the analytical detection limit (e.g. for D-mannitol/L-arabitol) no correction was performed. Chloroform-labile total reducing sugars were quantified using a colorimetric microplate method (Fursova et al. 2012). D-mannitol/L-arabitol and trehalose were quantified using commercial colorimetric kits based on compound-specific enzyme reactions (D-mannitol/L-arabitol: Sigma Aldrich, product code MAK096; Trehalose: Megazyme Incorporated, product code K-TREH). D-mannitol and L-arabitol were quantified together because the enzyme-based colorimetric assay reacts with both of these compounds (see Sigma Aldrich MAK096 Technical Bulletin). All colorimetric analyses were conducted on a microplate reader (Tecan Infinite M200). A total of 17 amino acids were quantified by reverse-phase high pressure liquid chromatography (alanine, arginine, aspartate, cysteine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine). Amino acids were quantified on a UPLC system using a fluorescence detector (Waters Aquity; see Supplementary Methods in Leitner et al. 2017). While we quantified 17 amino acids, in this analysis we focused on amino acids that were most abundant in chloroform extracts (glutamate and glycine) or amino acids putatively function as osmolytes (proline); the remaining 15 amino acids were summed with these to calculate the total amino acid pool.

Statistical analyses

We evaluated relationships between cumulative respiration following wetting (R_c) and the two variables that we hypothesized to represent the immediate controls on R_c : WEOC and microbial biomass. When fitting regression models, we represented microbial biomass using SIR because we judged CE to be unreliable in subsoil samples. Because we were interested in testing the hypothesis that both variables influence R_c , we included both WEOC and SIR as predictors in bivariate models and excluded CE because it was collinear with SIR and unreliable in the subsurface. Models were fit separately for surface and subsoil samples, under the assumption that the main drivers of R_c might vary with depth. All variables

were expressed on an organic C normalized basis before model fitting.

We controlled for grouping of soil pits at each field site and the grouping of pits within different rock types using linear mixed-effects models. Mixed-effects models were coded with random-intercept terms designating the field site and rock type as a nested factor within site. Models were fit using the R package lme4 (Bates et al. 2015) using the REML approach, and p values were obtained from t-tests using the Kenward-Rogers approximation for degrees of freedom using the R package lmerTest (Luke 2017; Kuznetsova et al. 2017). Model residuals were checked for normality by examining quantile–quantile plots, and models were checked for homogeneity of variance by plotting residuals versus the predictor variables.

We estimated changes in CE-flush-C and -N, SIR, and WEOC as well as the relative abundance of selected CE-flush and WEOC chemical constituents using linear mixed effects models. The models were devised to test the hypothesis that each quantity changed after wetting, and accounted for the paired structure of the data and the non-independence of observations within each site and rock type. For each quantity, we specified a response variable that included both the concentration before wetting and the concentration 8 days after wetting. We then fit a model testing a single fixed effect factor that encoded the water status of each observation (i.e. whether sampling occurred during the dry period or 8 days after wetting). The identity of each soil sample was included as a random-intercept term to account for the fact that observations made before and after wetting represented a paired comparison for each sample. To account for non-independence of observations within each site and rock type, we included random intercept terms encoding the site and rock type as a factor nested within site. Model fitting and evaluation were otherwise conducted using the same approach that was used for modeling R_c (see above).

Selected biomass and WEOC constituents (trehalose, D-mannitol/L-arabitol, total reducing sugars, total amino acids, glutamate, glycine, and proline) were evaluated after normalizing the total C contained in each constituent by the total C in each extract. For these selected constituents, we were interested in testing the hypothesis that one or more of the seven constituents responded to wetting, and so the p values

were evaluated using a probability threshold adjusted for the familywise error rate. The p values were adjusted using a Bonferroni correction, with the adjustment made separately when evaluating changes in selected CE and WEOC constituents. The sample size varied for different constituents because some constituents were not analyzed across all samples due to limited volume of archived extract (e.g. amino acids were only analyzed in 25 out of 37 samples).

We explored relationships between dry soil WEOC/OC, solid-phase C fractions, and other soil physicochemical variables by computing correlation coefficients across all samples. This analysis was intended to explore relationships in the data rather than test specific hypotheses, and so we used simple correlation analysis rather than employing more complex statistical approaches (e.g. multivariate mixed effects models). We computed Pearson correlation coefficients between WEOC/OC measured from extracts made from soil during the dry phase of the incubation and 12 variables: C-normalized density fractions (FLF C/OC and OLF + HF C/OC), sand percentage, exchangeable cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Al^{3+}), reactive metals (oxalate extractable Fe and Al), soil pH, and sampling depth. We evaluated the statistical significance of each correlation coefficient by computing t statistics and p values adjusted for the familywise error rate with a Bonferroni correction.

Results

Soil respiration dynamics

We first examined the shape of the respiration pulse after wetting after normalizing respiration rates by the organic C content of each sample (Fig. 1). The shape of the respiration pulse was virtually identical in all surface samples, following a pattern of monotonic decline (Fig. 1a). However, the shape of the respiration pulse varied in subsoil samples (Fig. 1b). Some subsoil samples showed a pattern of monotonic decline much like the surface samples—a pattern that has been identified with a “type I” response to wetting, in which microbial growth occurs immediately after water addition (Meisner et al. 2015, Fig. 1b). In contrast, respiration in the remaining subsurface samples increased over time in a pattern identified

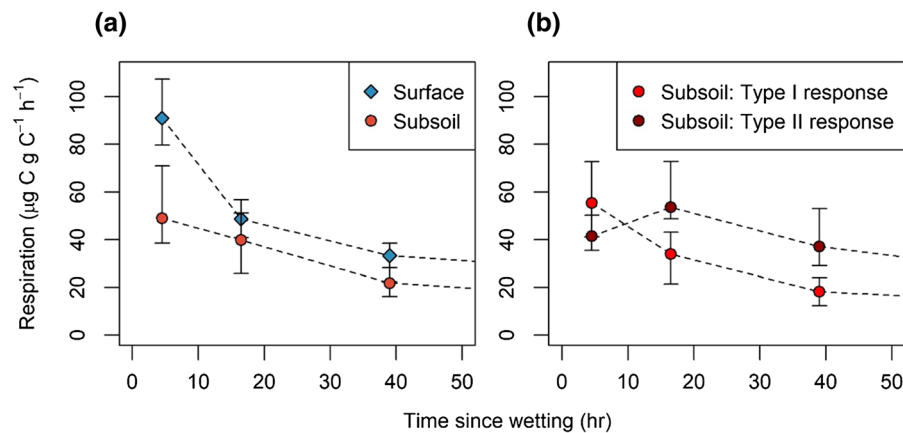


Fig. 1 Respiration pulse shape. Rates are normalized by total organic C content. Symbols show median values for each class of samples (e.g. surface versus subsoil) and error bars show 25th and 75th quantiles. **a** shows respiration over time in surface (blue diamonds) and subsurface soils (red circles), while **b** subdivides subsurface soils to show median values for

samples for which the average rate 3–6 h after wetting exceeded the rate 6–27 h after wetting (type I response; light-red circles) and samples for which the average rate 3–6 h after wetting was less than the rate 6–27 h after wetting (type II response; dark-red circles)

with a “type II” response to wetting, in which growth is delayed (Meisner et al. 2015). In these subsoil samples, the average respiration rate initially increased, such that the rates 6–27 h after wetting were higher than those 3–6 h after wetting (Fig. 1b). We also noted that the initial respiration rate in subsoil samples was often high relative to the theoretical maximum respiration rate defined by the SIR assay, which quantifies respiration under resource-saturating conditions; the respiration rate 3–6 h after water addition was on-average 56% (SD = 22) of the SIR rate in subsoil samples, versus 23% (SD = 5) of the SIR rate in surface samples.

Cumulative respiration

We identified relationships between the cumulative respiration after wetting (R_c), dry soil SIR, and dry soil WEOC (Fig. 2). Due to the inherent noisiness of the CE method, CE-flush-C was effectively undetectable in most subsurface samples, so instead we relied on SIR as a proxy for microbial biomass across depths. A positive relationship between SIR and R_c was statistically detectable in surface samples (Table 2), while in subsoil samples SIR biomass was typically very low and showed no relationship with R_c (Table 2, Fig. 2a). The relationship between R_c and WEOC sampled during the dry phase of the incubation also depended on sampling depth: WEOC extracted

from dry soil was not strongly correlated with R_c in surface samples, but was positively correlated with R_c in subsoil samples (Table 2, Fig. 2b) While we focused on C-normalized quantities in order to isolate relationships independent of total C content, we also plotted R_c versus SIR and WEOC without normalizing. We discovered that there was still no clear relationship between R_c and SIR in subsoil samples when the data were not C-normalized (Fig. 2c). The relationships between R_c , SIR, and WEOC appeared stronger in surface samples when the data were not normalized (Fig. 2d), partly reflecting the fact that respiration, WEOC, and microbial biomass all correlate with total organic C content at the surface.

We compared the magnitudes of R_c , CE-flush-C, and WEOC to evaluate whether there was sufficient C in these pools to account for the respiration pulse after wetting. The near-zero to negative CE-flush-C yields in subsoil suggested that the chloroform extraction method was approaching its detection limit at depth. Averaging CE-flush-C across all of the subsoil samples (including negative values) yielded a mean of $5 \mu\text{g C g soil}^{-1}$ (SD = 20) whereas the average value for R_c across subsoils was $12 \mu\text{g C g soil}^{-1}$ (SD = 6). This suggests that R_c across subsoils was substantially larger than the chloroform labile C pool, although the propagated uncertainty associated with this is very high, reflecting the fact that CE yields were unreliable in subsoil. We were better able to compare R_c with

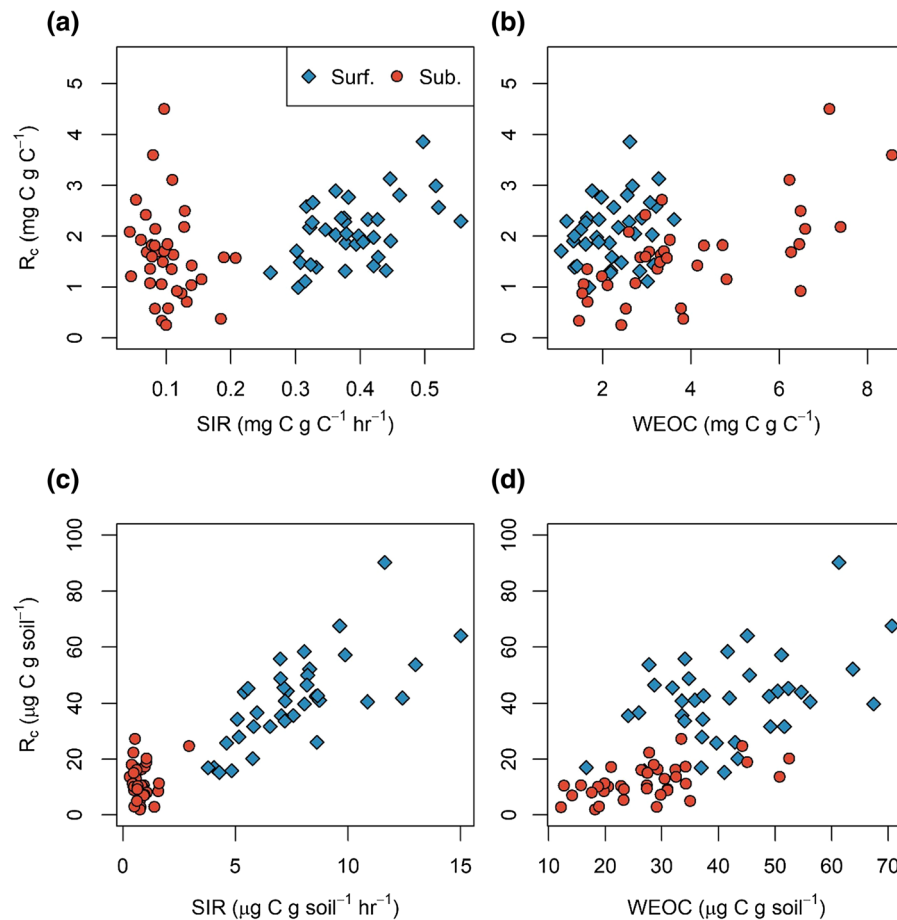


Fig. 2 Cumulative respiration 3–51 h after wetting (R_c). All variables in the upper two panels **a**, **b** are normalized by organic C content. **a** shows R_c versus SIR; **b** shows R_c versus WEOC. **c**, **d** show the same relationships without normalization. Surface samples are shown as blue diamonds, subsoil samples are shown

CE-flush-C in surface soil and discovered that R_c was consistently less than CE-flush-C, averaging 36% (SD = 22) of the chloroform labile C pool. We compared dry soil WEOC with R_c and found that R_c could equal or exceed the dry soil WEOC pool in surface samples, averaging 103% (SD = 38) of that pool. In subsoil samples R_c was smaller than the dry soil WEOC pool, averaging 45% (SD = 20) of WEOC.

Changes in microbial biomass and WEOC

We did not observe significant changes in microbial biomass after wetting surface samples regardless of whether biomass was represented using SIR, CE-

as red circles. Mixed effects models were used to identify a statistically significant positive relationship between R_c and SIR in surface samples, and a significant positive relationship between R_c and WEOC in subsoil samples based on C-normalized values (Table 2)

flush-C, or CE-flush-N (Table 3). In contrast, in subsoil samples SIR increased significantly after wetting (Table 3). Furthermore, we observed that the increase in subsoil SIR after wetting was related to the shape of the respiration pulse. In soils that experienced the largest increases in SIR, respiration tended to increase between the 3–6 h and the 6–27 h measurement intervals, indicating a “type II” response to wetting (Fig. 3).

We analyzed amino acids, trehalose, D-mannitol/L-arabitol, and total reducing sugars in chloroform extracts (i.e. released from microbial biomass) and water extracts of surface soil. Amino acids averaged 11% (SD = 2) of CE-flush-C during the dry phase, and 13% (SD = 4) of CE-flush-C 8 days after wetting. The

Table 2 Mixed effects models statistics relating R_c to WEOC and SIR

| | Estimate | SE | DF | t value | p value |
|-------------|----------|------|-------|---------|----------|
| Surface | | | | | |
| Intercept | − 5.27 | 7.36 | 26.45 | − 0.72 | 0.481 |
| Slope: WEOC | 0.28 | 0.17 | 19.62 | 1.63 | 0.11 |
| Slope: SIR | 5.34 | 1.78 | 16.83 | 3.01 | 0.008* |
| Subsoil | | | | | |
| Intercept | 8.89 | 3.61 | 22.13 | 2.47 | 0.022* |
| Slope: WEOC | 0.30 | 0.05 | 25.16 | 5.73 | < 0.001* |
| Slope: SIR | − 2.44 | 2.31 | 27.83 | − 1.05 | 0.302 |

Mixed effects models were coded to predict R_c as a function of WEOC and SIR, with random intercept terms controlling for site and rock type nested within site. All variables were normalized by the total organic C content of each sample prior to model fitting. Separate models were run for surface and subsurface samples. Columns show coefficient estimates

t values, and p values estimated for each fixed effect

The p values marked with an asterisk (*) are less than the nominal threshold of statistical significance ($\alpha = 0.05$)

SE standard error, DF Kenward-Rogers approximation of the effective degrees of freedom

most abundant amino acid, glutamate, comprised an average of 4% (SD = 2) of CE-flush-C before wetting and 5% (SD = 1) after wetting. Glycine became more concentrated in CE-flush-C 8 days after wetting, while glutamate, proline, and total amino acids showed no significant response to wetting (Table 3; Fig. 4a). In contrast, trehalose, D-mannitol/L-arabitol, and the total reducing sugar pool tended to decrease after wetting, but only trehalose changed significantly, decreasing from a mean value of 25% (SD = 12) to 16% (SD = 7) of CE-flush-C after wetting (Table 3; Fig. 4a). While the magnitude of the decrease in trehalose varied, the change was consistent: 34 of the 37 soil samples analyzed showed a decline in trehalose after wetting.

We also analyzed the relative abundance of amino acids, trehalose, D-mannitol/L-arabitol, and total reducing sugars in WEOC. WEOC concentrations declined consistently after wetting in both surface and subsurface samples (Table 3). The relative abundances of amino acids, trehalose, and D-mannitol/L-arabitol were much lower in WEOC extracts than in chloroform-amended extracts, both before and after wetting. Amino acids comprised less than 1% of WEOC both during the dry phase and 8 days after wetting (Fig. 4b). Neither trehalose nor D-mannitol/L-arabitol were detectable in WEOC. In contrast, reducing sugars comprised a substantial fraction of WEOC sampled during the dry phase, and the relative

abundance of reducing sugars in WEOC increased after wetting (Fig. 4b, Table 3). The relative abundance of glutamate in WEOC also increased significantly after wetting (Table 3), but glutamate-C comprised less than 0.5% of WEOC (Fig. 4b).

Correlation analysis

We conducted an exploratory correlation analysis to identify C fractions and soil physicochemical variables that might be associated with WEOC extracted during the dry phase of the incubation. We first computed Pearson correlation coefficients between dry soil WEOC / OC and each of 12 variables (Table 4). Dry soil WEOC was negatively correlated with ammonium-oxalate extractable Fe (Fe-ox)—a measure of amorphous Fe mineral content. The relationship between dry soil WEOC and Fe-ox appeared concave curvilinear, with the highest dry-soil WEOC yields occurring in soils with low Fe-ox content (Fig. 5a). Higher dry soil WEOC values were also obtained in subsoils (Fig. 5b, Table 4).

Discussion

Our results indicate that both recycling of microbial biomass and mobilization of extracellular C contribute to the respiration pulse after wetting, and that the

Table 3 Mixed-effects model statistics comparing dry versus wet soil

| Variable | Estimated change after wetting | SE | n | DF | t value | p value |
|--|--------------------------------|------|----|----|---------|---------|
| Surface (% of CE flush C) | | | | | | |
| Glycine | 0.06 | 0.01 | 50 | 24 | 4.41 | < 0.01* |
| Proline | 0.07 | 0.04 | 50 | 24 | 2.03 | 0.38 |
| Glutamate | 0.49 | 0.44 | 50 | 24 | 1.12 | 1.00 |
| Total AA | 1.75 | 1.00 | 50 | 24 | 1.76 | 0.64 |
| Trehalose | – 8.22 | 1.85 | 74 | 36 | – 4.43 | < 0.01* |
| Red. Sugar | – 5.14 | 3.65 | 60 | 29 | – 1.41 | 1.00 |
| D-mannitol/L-arabitol | – 0.36 | 0.21 | 56 | 27 | – 1.70 | 0.70 |
| Surface (% of WEOC) | | | | | | |
| Glycine | 0.01 | 0.02 | 52 | 25 | 0.31 | 1.00 |
| Proline | 0.01 | 0.01 | 52 | 25 | 0.53 | 1.00 |
| Glutamate | 0.02 | 0.01 | 52 | 25 | 3.02 | 0.03* |
| Total AA | 0.43 | 0.19 | 52 | 25 | 2.31 | 0.15 |
| Red. Sugar | 9.28 | 2.72 | 60 | 29 | 3.41 | 0.01* |
| Surface (bulk) | | | | | | |
| CE flush C ($\mu\text{g g soil}^{-1}$) | – 0.5 | 5.8 | 74 | 36 | – 0.08 | 1.00 |
| CE flush N ($\mu\text{g g soil}^{-1}$) | 1.9 | 0.7 | 74 | 36 | 2.50 | 0.07 |
| SIR ($\mu\text{g C g soil}^{-1} \text{ h}^{-1}$) | 0.1 | 0.1 | 74 | 36 | 0.79 | 1.00 |
| WEOC ($\mu\text{g g soil}^{-1}$) | – 17.9 | 1.3 | 74 | 36 | – 13.52 | < 0.01* |
| Subsoil (bulk) | | | | | | |
| SIR ($\mu\text{g C g soil}^{-1} \text{ h}^{-1}$) | 0.3 | 0.1 | 74 | 36 | 6.83 | < 0.01* |
| WEOC ($\mu\text{g g soil}^{-1}$) | – 16.7 | 1.4 | 74 | 36 | – 11.55 | < 0.01* |

Mixed effects models were coded to predict each variable as a function of water status, with random intercept terms controlling for sample identity, site, and rock type nested within site. Columns show parameter estimates

t values, and p values estimated for water status. Parameter estimates represent the estimated mean change in each quantity 8 days after wetting. Sample sizes (n) reflect the total number of observations included in the comparison (e.g. if the comparison was made across 37 soils n = 74). For bulk constituents separate models were run for surface and subsurface samples. A Bonferroni correction was applied to p values within each family of comparisons (i.e. within each subheading listed in bold)

The p values marked with an asterisk (*) are less than the nominal threshold of statistical significance ($\alpha = 0.05$)

SE standard error, DF Kenward-Rogers approximation of the effective degrees of freedom

relative contributions of these mechanisms vary by depth. In surface soil, microbial biomass was relatively abundant and was most strongly correlated with C release after wetting; furthermore, shifts in the chemical composition of the microbial biomass suggested a role for recycling of microbial C via osmolyte consumption at the surface. In contrast, cumulative C release after wetting was unrelated to microbial biomass estimated by SIR in subsoils and the chloroform-labile fraction of the biomass in subsoils was near the limit of detectability, suggesting that the C released in the pulse may be decoupled from the amount of microbial biomass at depth. Furthermore,

cumulative respiration after wetting was most strongly correlated with WEOC in deeper soil horizons; hence physicochemical controls on WEOC release may be the stronger control at depth.

Recycling mechanisms

In surface soil, microbial biomass was abundant enough to potentially supply all of the C released after wetting, which averaged 36% of the chloroform-labile C pool. Although we did not observe net changes in total microbial biomass measured by CE or SIR in surface soil, we did observe consistent shifts in

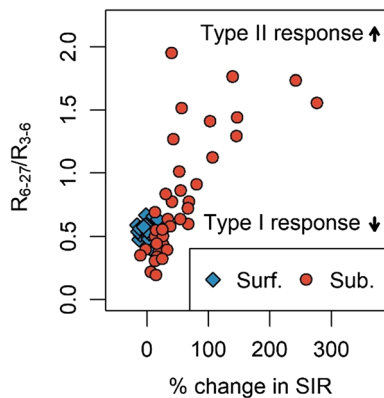


Fig. 3 Respiration pulse shape versus net increase in SIR. The vertical axis shows the ratio of the respiration rate 6–27 h after wetting (R_{6-27}) to the average respiration rate 3–6 h after wetting (R_{3-6}); low values are diagnostic of a “type I response” while high values are diagnostic of a “type II response”. The % change in SIR is the relative difference between SIR measured on air-dried soil and SIR measured on soil 8 days after wetting. Surface samples are shown as blue diamonds, subsoil samples are shown as red circles

the chemical composition of the biomass that suggested a role for biomass recycling in generating the pulse. Some of these shifts—in particular the decrease in the sugar trehalose—were consistent with the

hypothesis that microbes synthesize osmolytes to survive dry periods, and that these are then metabolized by microbes after wetting. By contrast, proline and glutamate—which are also thought to function as osmolytes (Welsh 2000; Schimel 2018)—did not accumulate in dry soil. This finding corroborates existing data from one of our field sites, where putative amino-acid osmolytes did not increase as soils dried (Boot et al. 2011). A large array of compounds are thought to function as inducible osmolytes, but many of these compounds have multiple functions (Welsh 2000). It is thus unsurprising that evidence for osmolyte accumulation is compound specific.

Given the diversity of potential osmolytes, it is challenging to evaluate their total contribution to cellular water potential. However, we can consider the role of trehalose alone, given that it comprised a substantial fraction (25%) of the chloroform-labile C pool in dry soil. We estimate that the average water-volume in microbial biomass in surface soils was $0.92 \mu\text{l H}_2\text{O g soil}^{-1}$ using measured chloroform labile-C values scaled by a factor of 0.45 (Joergensen 1996), and a factor of $2.8 \text{ nl H}_2\text{O } \mu\text{g biomass C}^{-1}$ (Manzoni et al. 2014; Bratbak and Dundas 1984). Given this estimate, cellular trehalose concentrations

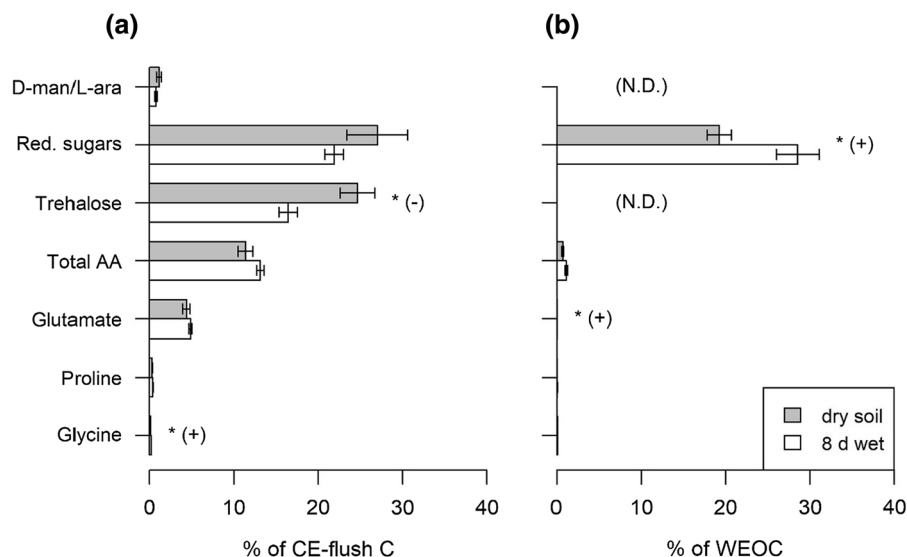


Fig. 4 Changes in selected microbial biomass constituents after wetting. Bars show the mean concentrations of each constituent before wetting and 7 days after wetting in both CE flush C (a) and WEOC (b). Error bars show standard error. Sample sizes varied: $n = 25$ for amino acids, $n = 28$ for D-mannitol/L-arabitol, $n = 30$ for total reducing sugars, and $n = 37$ for

trehalose. All data are from surface soils because selected constituents were not quantified in subsoils. An asterisk (*) identifies a statistically significant change between dry soil and soil 8 days after wetting derived from mixed effects models (Table 3), while signs in parentheses (\pm) indicate the direction of change after wetting

Table 4 Correlation coefficients relating WEOC/OC to other variables

| Variable | Pearson's R | p value |
|---------------|-------------|---------|
| % sand | 0.18 | 0.49 |
| Fe-ox | − 0.42* | < 0.01 |
| Al-ox | − 0.31 | 0.07 |
| Mg-X | − 0.07 | 1.0 |
| Ca-X | − 0.21 | 0.41 |
| K-X | − 0.25 | 0.22 |
| Na-X | 0.2 | 0.45 |
| Al-X | 0.07 | 1.0 |
| pH | − 0.03 | 0.07 |
| OLF + HF C/OC | 0.31 | 0.21 |
| FLF C/OC | − 0.26 | < 0.01 |
| Depth | 0.56* | 1.0 |

The WEOC values used in computing correlations were measured on air-dried soil

R values marked with an asterisk (*) achieved the nominal threshold of statistical significance ($\alpha = 0.05$)

Fe-ox NH₄ oxalate extractable iron, Al-ox NH₄ oxalate extractable aluminum, -X exchangeable cation obtained by 0.1 M BaCl₂ extraction

in dry soil averaged 0.25 M, which corresponds to an osmotic potential of -603 kPa (osmotic potential = $-MiRT$; molarity $M = 0.25$; ionization constant $i = 1$; gas constant $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$; temperature $T = 293^\circ\text{K}$). This value is less negative

than total water potentials measured on a subset of the soils before wet up, which ranged from -2.1 to -8.2 MPa when measured by thermocouple psychrometer. Thus, although trehalose comprised a substantial fraction of chloroform extractable C, trehalose alone could not balance the low water potentials during the incubation. Instead, we speculate that trehalose may have other roles in helping microbes survive drought; for instance, trehalose is a molecular stabilizer that increases the free energy of protein denaturation (Kaushik and Bhat 2003), inhibits abiotic degradation of proteins by minerals (Reardon et al. 2018), and maintains function of core metabolic enzymes and cell membranes during dehydration (Wiemken 1990; Sampredo et al. 1998; Sun and Davidson 1998). Trehalose and other compounds might therefore function as “osmo-protectants” rather than osmolytes, allowing microbial cells to survive dormancy under dry conditions by minimizing damage to enzymes and other cellular machinery, rather than by maintaining hydration.

Cell lysis is a second pathway through which microbial recycling might fuel respiration after wetting. Microbial C mobilized by lysis would also presumably contribute to WEOC; consequently, we hypothesized that WEOC extracted from dry soils might contain microbial cellular material released by lysis of cells during water extraction. Contrary to this hypothesis, we saw little evidence that WEOC is augmented by microbial cellular material. The relative

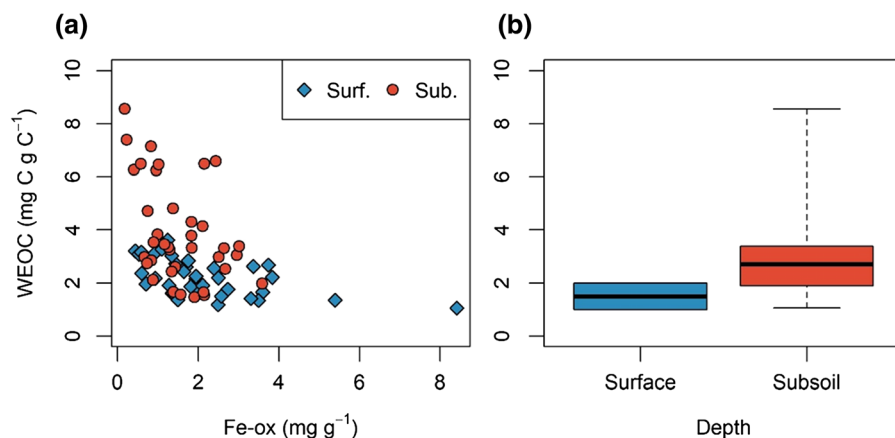


Fig. 5 Factors correlated with WEOC. WEOC / OC was negatively related to Fe-ox (a) and positively related to sampling depth (b). Pearson's correlation coefficients for each variable are listed in Table 4. When computing correlations, we used the actual sampling depth, which ranged between 30 and 50 cm in

subsoils; for clarity subsoil samples are grouped together here. Surface samples are shown in blue ($n = 37$), while subsoil samples are shown in red ($n = 37$). In panel b the central line in each box is the median, the boundaries of the box show the 1st and 3rd quartiles, and the whiskers show the range of the data

abundances of trehalose, D-mannitol/L-arabitol, and total amino acid were low or below analytical detection limits in WEOC extracts, despite the fact that these selected constituents represented 37% (SD = 17) of the CE-flush-C pool. Cell lysis cannot have contributed substantially to WEOC unless these cellular constituents were transformed during the extraction procedure. However, molecular evidence indicates that cellular turnover is accelerated by wetting even when total microbial biomass shows no net change, implying a major role for lysis (Blazewicz et al. 2014). We are unsure how to relate this finding to the data presented here. It is possible that the duration and severity of drought and the rate of wetting might influence the potential for lysis, making the importance of lysis highly dependent on experimental design (e.g. length of dry period, rate of wetting). Studies that apply isotope tracer measurements of dissolved C in-situ at high temporal resolution (e.g. via microdialysis) could help to resolve this.

Extracellular C mobilization

Although microbial biomass recycling can explain the wetting respiration pulse in surface soil, it may have limits in biomass-poor subsoils. It was challenging to quantify microbial biomass C directly in subsoil samples given that chloroform extracts and water extracts from the same soil yielded similar C concentrations, resulting in near-zero or negative estimates of biomass C. Averaging these measurements suggests that C released after a single wetting event may equal or exceed the size of the chloroform-labile C pool in subsoils—although methodological limitations of chloroform extraction make this estimate uncertain. If we were to assume that R_c did roughly equal the microbial biomass pool in subsoils, recycling mechanisms alone would then require turnover of 100% or more of the chloroform-labile C after wetting to generate the pulse. If such high levels of biomass turnover occurred during the pulse, it seems that they would have required a large subsidy of non-microbial C to support the increases in biomass (estimated by SIR) that we observed in subsoils. Consequently, recycling of biomass C alone—either via osmolyte metabolism or cell lysis—likely do not fully account for the respiration pulse in subsoil.

Unlike microbial biomass C, WEOC was comparatively abundant in dry subsoil and was also correlated

with cumulative respiration after wetting, suggesting a more-central role for WEOC in fueling the pulse. Furthermore, the changes in microbial biomass and the dynamics of respiration show that subsoils experience a distinct burst of growth that we did not observe in surface samples—perhaps reflecting the increased importance of WEOC in subsoils. Subsoil SIR values increased after wetting, and increases in SIR were associated with a delay in the maximum respiration rate after wetting. The delayed pulse of respiration is characteristic of a “type II” growth response, which is defined by a period of stasis followed by an exponential growth phase (Göransson et al. 2013; Meisner et al. 2015). While we did not track microbial growth dynamics directly, we can infer that near-exponential growth dynamics may have occurred in subsoils from the fact that the initial respiration rate approached the theoretical maximum set by the SIR rate. In aggregate, these details suggest that wetting can saturate the microbial biomass with resources and fuel net growth.

While the respiration data support the idea that WEOC is a proxy for C availability, the nature of WEOC is challenging to determine from the data we collected. We found that a fraction of WEOC at the surface is comprised of reducing sugars—potentially labile plant or microbial products—but we have little basis for inferring the ultimate source of WEOC from this single bulk chemical measurement. However, despite the limits imposed by our analytical approach, exploratory correlation analysis did suggest factors that may control WEOC mobility. The fraction of total C extractable as WEOC was negatively related to the amount of Fe-ox, which represents an amorphous mineral pool that contributes disproportionately to mineral surface area and reactivity, and thus has a high capacity to immobilize C (Torn et al. 1997; Kramer et al. 2012; Coward et al. 2018). The relationship between WEOC and Fe-ox suggests that metal (oxy)hydroxides mediate exchange of C with the soil solution on wetting, but this pattern gives no clue regarding the ultimate source of the C that is mobilized.

Sampling depth also emerged as an important predictor of WEOC concentrations: WEOC comprised a higher fraction of total organic C in subsoil samples than in surface samples. How does this relatively abundant water-soluble organic C accumulate in subsoils without being consumed? Much of this soluble C must somehow be inaccessible to microbes

under static moisture conditions. That deep soluble C is relatively inaccessible to microbes is suggested by the “old” radiocarbon values found in water extracts and tension-lysimeter samples from the B horizon of a mesic California grassland (Sanderman and Amundson 2008; Sanderman et al. 2008); both the WEOC and in situ DOC pools had to cycle slowly. A significant fraction of the DOC in deeper soil horizons can be locally derived from exchange with mineral-associated soil organic matter (Sanderman et al. 2008; Leinemann et al. 2018). Wet-dry cycles may mobilize some of this C by altering the associations between specific chemical moieties and mineral surfaces: for instance, drying may favor inner-sphere complexation of some organic classes while possibly displacing others (Olshansky et al. 2018). Wet-dry cycles may also redistribute C between soil pores via mass flow. Soluble organic matter held in fine pores can be chemically distinct from organic matter in coarse pores but it remains biochemically labile, suggesting that isolation from microbial consumers may ultimately limit its consumption (Bailey et al. 2017). As a consequence, the low frequency or intensity of wet-dry cycles in subsoil may lead to WEOC accumulation because C remains physically isolated from microbes rather than because of specific mineral protection mechanisms (Homyak et al. 2018; Schimel 2018).

This interpretation implies that the nature of wetting may affect the potential or C-subsidy: fast wetting events may drive mass-flow and redistribution, while slower, diffusive wetting events may not have this effect. In our laboratory incubation, we first sieved soil samples and followed that with a relatively severe cycle of drying and abrupt wetting. Wetting of dry soil in the field—especially at depth—is likely to occur slowly via capillary flow, and the rate and mechanism (e.g. mass flow vs. capillarity) of wetting have been shown to influence the amount of C mobilized during wetting events (Smith et al. 2017). Both sieving and rapid water addition seem likely to increase soluble C mobilization, possibly increasing R_c and exaggerating the importance of WEOC relative to field conditions. Aggregate destruction in particular may increase respiration and microbial biomass by approximately 20% after a single wetting event (Navarro-García et al. 2012). The patterns we observed are thus unlikely to apply directly to intact soils in natural systems. Furthermore, if Fe-ox limits WEOC mobility, the capacity of microbes to utilize

WEOC may be limited in wetter climates, where amorphous Fe concentrations tend to exceed those observable in the semi-arid climate zone we sampled (Chadwick et al. 2003; Graham and O’Geen 2010). However, we suspect that the basic mechanisms we identified still apply in the field: respiration after wetting may scale with either microbial biomass or WEOC depending on which C source is more abundant.

Conclusions

Our data show that respiration after wetting can be derived from both recycling microbial biomass and mobilizing extracellular C. In surface horizons where microbial biomass is plentiful, recycling of microbial biomass may be the dominant contributor to respiration after wetting—particularly metabolism of cellular osmolytes, including trehalose. In deeper soil horizons the contribution from recycling of microbial biomass is necessarily small because microbial biomass is scarce, and respiration dynamics reflect a response to increased extracellular C availability. Respiration due to extracellular C mobilization may occur in surface soils but may be masked in the field because microbial biomass is typically abundant at the surface. However, extracellular organic C may be mobilized simultaneously and resupply C lost from the biomass due to recycling mechanisms. Identifying the fraction of respiration that is derived from extracellular C could be especially important for modeling soil C storage if that extracellular C is mineral associated or physically occluded and consequently relatively slow cycling under static moisture conditions.

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Author contributions EWS was responsible for study design, field sampling, sample analysis, and writing. YL and BYJ contributed to field sampling, laboratory incubations, and manuscript revision. PMH contributed to chemical analyses and manuscript revision. OAC, CMD, and JPS contributed to study design, data interpretation, and manuscript revision.

References

- Adu JK, Oades JM (1978) Physical factors influencing decomposition of organic materials in soil aggregates. *Soil Biol Biochem* 10(2):109–115
- Bailey VL, Smith AP, Tfaily M, Fansler SJ, Bond-Lamberty B (2017) Differences in soluble organic carbon chemistry in pore waters sampled from different pore size domains. *Soil Biol Biochem* 107:133–143
- Bates D, Maechler M, Bolker B, Walker S (2015) lme4: linear mixed-effects models using Eigen and S4. R package version 1(1–7):2014
- Birch HF (1958) The effect of soil drying on humus decomposition and nitrogen availability. *Plant Soil* 10(1):9–31
- Blazewicz SJ, Schwartz E, Firestone MK (2014) Growth and death of bacteria and fungi underlie rainfall-induced carbon dioxide pulses from seasonally dried soil. *Ecology* 95(5):1162–1172
- Boot CM, Schaeffer SM, Schimel JP (2013) Static osmolyte concentrations in microbial biomass during seasonal drought in a California grassland. *Soil Biol Biochem* 57:356–361
- Borken W, Matzner E (2009) Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob Change Biol* 15(4):808–824
- Bottner P (1985) Response of microbial biomass to alternate moist and dry conditions in a soil incubated with ¹⁴C- and ¹⁵N-labelled plant material. *Soil Biol Biochem* 17(3):329–337
- Bratbak G, Dundas I (1984) Bacterial dry matter content and biomass estimations. *Appl Environ Microbiol* 48(4):755–757
- Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012) Separation of light and heavy organic matter fractions in soil—testing for proper density cut-off and dispersion level. *Geoderma* 170:403–416
- Chadwick OA, Gavenda RT, Kelly EF, Ziegler K, Olson CG, Elliott WC, Hendricks DM (2003) The impact of climate on the biogeochemical functioning of volcanic soils. *Chem Geol* 202(3–4):195–223
- Chowdhury TR et al (2019) Metaphenomic responses of a native prairie soil microbiome to moisture perturbations. *mSystems* 4(4):e00061-19
- Clarke CE, Aguilar-Carrillo J, Roychoudhury AN (2011) Quantification of drying induced acidity at the mineral-water interface using ATR-FTIR spectroscopy. *Geochim Cosmochim Acta* 75(17):4846–4856
- Coward EK, Ohno T, Plante AF (2018) Adsorption and molecular fractionation of dissolved organic matter on iron-bearing mineral matrices of varying crystallinity. *Environ Sci Technol* 52(3):1036–1044
- Denef K, Six J, Bossuyt H, Frey SD, Elliott ET, Merckx R, Paustian K (2001) Influence of dry–wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biol Biochem* 33(12–13):1599–1611
- Fierer N, Schimel JP (2002) Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biol Biochem* 34(6):777–787
- Fierer N, Schimel JP (2003) A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Sci Soc Am J* 67(3):798–805
- Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem* 35(1):167–176
- Fierer N, Chadwick OA, Trumbore SE (2005) Production of CO₂ in soil profiles of a California annual grassland. *Ecosystems* 8(4):412–429
- Fursova O, Pogorelko G, Zabolina OA (2012) An efficient method for transient gene expression in monocots applied to modify the *Brachypodium distachyon* cell wall. *Ann Bot* 110(1):47–56
- Göransson H, Godbold DL, Jones DL, Rousk J (2013) Bacterial growth and respiration responses upon rewetting dry forest soils: impact of drought-legacy. *Soil Biol Biochem* 57:477–486
- Graham RC, O’Geen AT (2010) Soil mineralogy trends in California landscapes. *Geoderma* 154(3–4):418–437
- Guo X, Drury CF, Yang X, Reynolds WD, Zhang R (2012) Impacts of wet–dry cycles and a range of constant water contents on carbon mineralization in soils under three cropping treatments. *Soil Sci Soc Am J* 76(2):485–493
- Homyak PM, Blankinship JC, Slessarev EW, Shaeffer SM, Manzoni S, Schimel JP, (2018) Effects of altered dry-season length and plant inputs on soluble carbon. *Ecology*. <https://doi.org/10.1002/ecy.2473>
- Imhoff JF (1986) Osmoregulation and compatible solutes in eubacteria. *FEMS Microbiol Rev* 2(1–2):57–66
- Joergensen RG (1996) The fumigation-extraction method to estimate soil microbial biomass: calibration of the kEC value. *Soil Biol Biochem* 28(1):25–31
- Kaiser M, Kleber M, Berhe AA (2015) How air-drying and rewetting modify soil organic matter characteristics: an assessment to improve data interpretation and inference. *Soil Biol Biochem* 80:324–340
- Kakumanu ML, Cantrell CL, Williams MA (2013) Microbial community response to varying magnitudes of desiccation in soil: a test of the osmolyte accumulation hypothesis. *Soil Biol Biochem* 57:644–653
- Kakumanu ML, Ma L, Williams MA (2019) Drought-induced soil microbial amino acid and polysaccharide change and their implications for CN cycles in a climate change world. *Sci Rep* 9(1):10968
- Kaushik JK, Bhat R (2003) Why is trehalose an exceptional protein stabilizer? An analysis of the thermal stability of proteins in the presence of compatible osmolyte trehalose. *J Biol Chem* 278(29):26458–26465
- Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch Microbiol* 170(5):319–330

- Kieft TL (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biol Biochem* 19(2):119–126
- Killham K, Firestone MK (1984) Salt stress control of intracellular solutes in streptomycetes indigenous to saline soils. *Appl Environ Microbiol* 47(2):301–306
- Kim DG, Mu S, Kang S, Lee D (2010) Factors controlling soil CO₂ effluxes and the effects of rewetting on effluxes in adjacent deciduous, coniferous, and mixed forests in Korea. *Soil Biol Biochem* 42(4):576–585
- Kramer MG, Sanderman J, Chadwick OA, Chorover J, Vitousek PM (2012) Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. *Glob Change Biol* 18(8):2594–2605
- Kuznetsova A, Brockhoff PB, Christensen RHB (2017) lmerTest package: tests in linear mixed effects models. *J Stat Softw.* <https://doi.org/10.18637/jss.v082.i13>
- Lawrence CR, Neff JC, Schimel JP (2009) Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. *Soil Biol Biochem* 41(9):1923–1934
- Lee MS, Nakane K, Nakatsubo T, Mo WH, Koizumi H (2002) Effects of rainfall events on soil CO₂ flux in a cool temperate deciduous broad-leaved forest. *Ecol Res* 17(3):401–409
- Leinemann T, Preusser S, Mikutta R, Kalbitz K, Cerli C, Höschel C, Mueller CW, Kandeler E, Guggenberger G (2018) Multiple exchange processes on mineral surfaces control the transport of dissolved organic matter through soil profiles. *Soil Biol Biochem* 118:79–90
- Leitner S, Homyak PM, Blankinship JC, Eberwein J, Jenerette GD, Zechmeister-Boltenstern S, Schimel JP (2017) Linking NO and N₂O emission pulses with the mobilization of mineral and organic N upon rewetting dry soils. *Soil Biol Biochem* 115:461–466
- Lin Y, Prentice SE III, Tran T, Bingham NL, King JY, Chadwick OA (2016) Modeling deep soil properties on California grassland hillslopes using LiDAR digital elevation models. *Geoderma Reg* 7(1):67–75
- Lin Y, Slessarev EW, Yehl ST, D'Antonio CM, King JY (2018) Long-term nutrient fertilization increased soil carbon stocks in California grasslands. *Ecosystems* 22(4):754–766
- Loeppert RH, Inskeep WP (1996) Iron. In: Sparks DL (ed.) *Methods of soil analysis part 3: SSSA Book Ser. 5. SSSA, Madison* pp 639–664.
- Luke SG (2017) Evaluating significance in linear mixed-effects models in R. *Behav Res Methods* 49(4):1494–1502
- Lund V, Goksøyr J (1980) Effects of water fluctuations on microbial mass and activity in soil. *Microb Ecol* 6(2):115–123
- Lundquist EJ, Jackson LE, Scow KM (1999) Wet–dry cycles affect dissolved organic carbon in two California agricultural soils. *Soil Biol Biochem* 31(7):1031–1038
- Manzoni S, Schaeffer SM, Katul G, Porporato A, Schimel JP (2014) A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. *Soil Biol Biochem* 73:69–83
- Meisner A, Rousk J, Bååth E (2015) Prolonged drought changes the bacterial growth response to rewetting. *Soil Biol Biochem* 88:314–322
- Miller AE, Schimel JP, Meixner T, Sickman JO, Melack JM (2005) Episodic rewetting enhances carbon and nitrogen release from chaparral soils. *Soil Biol Biochem* 37(12):2195–2204
- Navarro-García F, Casermeiro MÁ, Schimel JP (2012) When structure means conservation: Effect of aggregate structure in controlling microbial responses to rewetting events. *Soil Biol Biochem* 44(1):1–8
- Newcomb CJ, Qafoku NP, Grate JW, Bailey VL, De Yoreo JJ (2017) Developing a molecular picture of soil organic matter–mineral interactions by quantifying organo–mineral binding. *Nature communications* 8(1):396
- Olshansky Y, Root RA, Chorover J (2018) Wet–dry cycles impact DOM retention in subsurface soils. *Biogeosciences* 15:821–832
- PRISM (2018) PRISM Climate Group. Oregon State University, Corvallis, OR
- Rasmussen C, Heckman K, Wieder WR, Keiluweit M, Lawrence CR, Berhe AA, Blankinship JC, Crow SE, Druhan JL, Hicks Pries CE, Marin-Spiotta E, Plante AF, Schädel C, Schimel JP, Sierra CA, Thompson A, Wagai R (2018) Beyond clay: towards an improved set of variables for predicting soil organic matter content. *Biogeochemistry* 137(3):297–306
- Reardon PN, Walter ED, Marean-Reardon CL, Lawrence CW, Kleber M, Washton NM (2018) Carbohydrates protect protein against abiotic fragmentation by soil minerals. *Scientific reports* 8(1):813
- Sampedro JG, Guerra G, Pardo JP, Uribe S (1998) Trehalose-mediated protection of the plasma membrane H⁺-ATPase from *Kluyveromyces lactis* during freeze-drying and rehydration. *Cryobiology* 37(2):131–138
- Sanderman J, Amundson R (2008) A comparative study of dissolved organic carbon transport and stabilization in California forest and grassland soils. *Biogeochemistry* 89(3):309–327
- Sanderman J, Baldock JA, Amundson R (2008) Dissolved organic carbon chemistry and dynamics in contrasting forest and grassland soils. *Biogeochemistry* 89(2):181–198
- Scheu S, Parkinson D (1994) Changes in bacterial and fungal biomass C, bacterial and fungal biovolume and ergosterol content after drying, remoistening and incubation of different layers of cool temperate forest soils. *Soil Biol Biochem* 26(11):1515–1525
- Schimel JP (2018) Life in dry soils: effects of drought on soil microbial communities and processes. *Annu Rev Ecol Evol Syst* 49:409–432
- Schimel J, Balser TC, Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88(6):1386–1394
- Schimel JP, Wetterstedt JM, Holden PA, Trumbore SE (2011) Drying/rewetting-Cycles mobilize old C from deep soils from a California annual grassland. *Soil Biol Biochem* 43(5):1101–1103
- Setia R, Verma SL, Marschner P (2012) Measuring microbial biomass carbon by direct extraction–comparison with chloroform fumigation-extraction. *Eur J Soil Biol* 53:103–106
- Smith AP, Bond-Lamberty B, Benscoter BW, Tfaily MM, Hinkle CR, Liu C, Bailey VL (2017) Shifts in pore connectivity from precipitation versus groundwater rewetting

- increases soil carbon loss after drought. *Nat Commun* 8(1):1335
- Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. (2017) Web soil survey. <https://websoilsurvey.sc.egov.usda.gov/>.
- Sørensen LH (1974) Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. *Soil Biol Biochem* 6(5):287–292
- Sun WQ, Davidson P (1998) Protein inactivation in amorphous sucrose and trehalose matrices: effects of phase separation and crystallization. *Biochim Biophys Acta (BBA)* 1425(1):235–244
- Todoruk TR, Langford CH, Kantzas A (2003) Pore-scale redistribution of water during wetting of air-dried soils as studied by low-field NMR relaxometry. *Environ Sci Technol* 37(12):2707–2713
- Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. *Nature* 389(6647):170
- Warren CR (2016) Do microbial osmolytes or extracellular depolymerisation products accumulate as soil dries? *Soil Biol Biochem* 98:54–63
- Welsh DT (2000) Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate. *FEMS Microbiol Rev* 24(3):263–290
- West AW, Sparling GP (1986) Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents. *J Microbiol Methods* 5(3–4):177–189
- Wiemken A (1990) Trehalose in yeast, stress protectant rather than reserve carbohydrate. *Antonie Van Leeuwenhoek* 58(3):209–217
- Williams MA, Xia K (2009) Characterization of the water soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie. *Soil Biol Biochem* 41(1):21–28
- Xiang SR, Doyle A, Holden PA, Schimel JP (2008) Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biol Biochem* 40(9):2281–2289
- Xu L, Baldocchi DD, Tang J (2004) How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. *Glob Biogeochem Cycles*. <https://doi.org/10.1029/2004GB002281>

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