

Spatial access and resource limitations control carbon mineralization in soils

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ABSTRACT

Core-scale soil carbon fluxes are ultimately regulated by pore-scale dynamics of substrate availability and microbial access. These are constrained by physicochemical and biochemical phenomena (e.g. spatial access and hydrologic connectivity, physical occlusion, adsorption-desorption with mineral surfaces, nutrient and resource limitations). We conducted an experiment to determine how spatial access and resource limitations influence core-scale water-soluble SOM mineralization, and how these are regulated by antecedent moisture conditions. Intact soil cores were incubated at field-moist vs. drought conditions, after which they were saturated from above (to simulate precipitation) or below (to simulate groundwater recharge). Soluble C (acetate) and N (nitrate) forms were added to some cores during the rewetting process to alleviate potential nutrient limitations. Soil respiration was measured during the incubation, after which pore water was extracted from the saturated soils and analyzed for water soluble organic carbon concentrations and characterization. Our results showed that C amendments increased the cumulative CO₂ evolved from the soil cores, suggesting that the soils were C-limited. Drought and rewetting increased soil respiration, and there was a greater abundance of complex aromatic molecules in pore waters sampled from these soils. This newly available substrate appeared to alleviate nutrient limitations on respiration, because there were no further respiration increases with subsequent C and N amendments. We had hypothesized that respiration would be influenced by wetting direction, as simulated precipitation would mobilize C from the surface. However, as a main effect, this response was seen only in the C-amended soils, indicating that surface-C may not have been bioavailable. At the pore scale (pore water samples), drought and the C, N amendments caused a net loss of identified molecules when the soils were rewet from below, whereas wetting from above caused a net increase in identified molecules, suggesting that fresh inputs stimulated the C-and N-limited microbial populations present deeper in the soil profile. Our experiment highlights the complex and interactive role of antecedent moisture conditions, wetting direction, and resource limitations in driving core-scale C fluxes.

1. Introduction

In soils, microbial access to carbon (C) occurs at the pore scale but this proximity to substrate governs the overall decomposition of C at larger spatial scales. At the pore or micro-scale, decomposition depends on the co-location of competent microorganisms, carbon, and other

resources (Dungait et al., 2012). Processes such as microbial motility, oxygen diffusion, and transport of substrates and resources regulate the availability of C for microbial degradation (Ebrahimi and Or, 2015). Local chemical conditions such as pH, moisture content, and ionic strength influence organo-mineral interactions, including adsorption-desorption processes that confer some protection to soil

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Table 1

Cumulative CO₂ evolved from the cores over a 24-h period. Antecedent moisture (M) conditions were significant for unamended soils, but not for C- or N-amended soils.

	Cumulative CO ₂ -C flux, mg gC ⁻¹		
	unamended	+C	+N
FM			
Precipitation	73.18 ± 32.93	460.58 ± 183.17	303.24 ± 82.36 *
Groundwater	115.36 ± 26.67	268.21 ± 47.75 *	147.65 ± 40.97
DROUGHT			
Precipitation	370.13 ± 28.76	235.6 ± 29.9 *	333.8 ± 58.3
Groundwater	284.85 ± 62.71	368.72 ± 46.64	184.42 ± 37.1
ANOVA results			
ANOVA:	ANOVA:	ANOVA:	
Moisture	Moisture non-sig	Moisture non-sig	
significant			
Wetting non-sig	Wetting non-sig	Wetting significant	
Interaction non-sig	Interaction non-sig	Interaction non-sig	

* represents a significant difference from the unamended. Statistical results are reported for ANOVA of $\log(\text{WSOC}) \sim M + W + M:W$ for each suction/Amendment combination.

organic C (Newcomb et al., 2017; Pan et al., 2010; Wagai and Mayer, 2007). Coarser scale processes that influence water content, including wetting direction (precipitation vs. groundwater rise) alter the accessibility of C to decomposers by opening or closing hydrological conduits between substrates, enzymes and microorganisms (Burns et al., 2013; Smith et al., 2017). Despite the importance of micro-scale environments and the tightly interwoven nature of micro- and macro-scale processes governing the fate of C in soils, we have not yet been able to effectively incorporate micro-scale processes into landscape-scale models (Baveye et al., 2018).

The challenge of incorporating microscale processes into larger-scale models is partly due to the complexity at which micro-scale environments shift in response to differences in soil moisture (e.g. drought, flood) and wetting conditions (e.g., precipitation and groundwater rise), soil structure (e.g., heterogeneous vs. homogeneous pore structures), and resource availability (e.g., C and nitrogen, N) (Chen et al., 2014; Soong et al., 2019). Drought alters soil chemistry at the pore scale, as the

high ionic strength in dry soils drives desorption of complex aromatic compounds (Kaiser et al., 2015; Newcomb et al., 2017; Patel et al., 2021b; Reemtsma et al., 1999). Microbial solutes (e.g. stress osmolytes, necromass) may accumulate under dry conditions and can subsequently become solubilized when these dry soils are rewet (Blazewicz et al., 2014; Chowdhury et al., 2011). Rewetting soil may also trigger an abrupt release of osmolytes as the osmotic balance causes microbial cells to rupture (Fierer and Schimel, 2003; Halverson et al., 2000; Warren, 2014). All of these contribute to the CO₂ flush seen when these dried soils are rewet, the “Birch Effect” (Bailey et al., 2019; Fierer and Schimel, 2003; Unger et al., 2010).

The process by which water enters soils (precipitation or groundwater rise) results in differential wetting pathways that can create hot-spots of C turnover (i.e. bioavailable C) throughout the soil matrix (Smith et al., 2017; Todoruk et al., 2003). When soils are wet from above, gravity guides water, C and other resources through soil pores by saturating the largest pores first. Conversely, capillary force drives the movement of water and solutes through the finest pores first as soils are wet from below, such as with groundwater rise or subsurface lateral water flow (Yang et al., 2014).

The objective of this research was to determine how physicochemical mechanisms (spatial access, adsorption, occlusion) and/or biochemical, resource limitations control C-mineralization in a sandy soil. Specifically, is the substrate physically/spatially isolated from the microbes; or are the microbes co-located with the C, but limited in terms of other resources; and how might this be influenced by micro-environment changes due to antecedent moisture conditions and wetting direction? We hypothesized that: (H1) Adding soluble C and N would alleviate substrate and resource limitations, resulting in increased microbial activity, i.e., greater CO₂ production, and a relative depletion in complex/aromatic compounds; (H2) Soils subjected to drought would show increased respiration when rewet due to desorption of organic substrate from mineral surfaces. This “fresh” substrate would alleviate substrate limitations, and therefore C, N additions would not increase respiration in drought-incubated soils; and (H3) Soils wet from above (precipitation) would exhibit greater respiration than soils wet from below (groundwater recharge), because of redistribution of C from the surface soil layers. However, the effect of C amendments would be greater in cores wet from below, due to C limitation at greater soil depths. We tested these hypotheses by incubating soil cores under field moist and

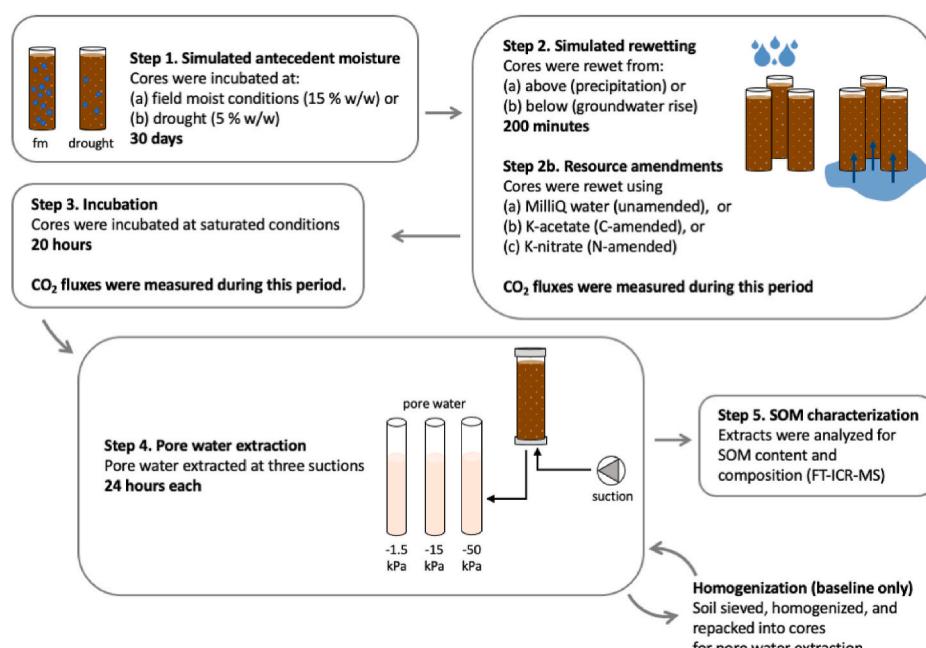


Fig. 1. Experimental design. Intact soil cores from the Disney Wilderness Preserve, FL were incubated at field moist vs. drought conditions and then rewet from above vs. below with unamended MilliQ water vs. potassium acetate vs. potassium nitrate. The cores were incubated at saturated conditions for 20 h at 22 °C and then pore water was extracted and analyzed. We refer to the field-moist incubated, unamended cores as “baseline” cores for their respective wetting directions. After extracting pore water, the baseline soils were sieved and homogenized and repacked into cores for pore water extraction.

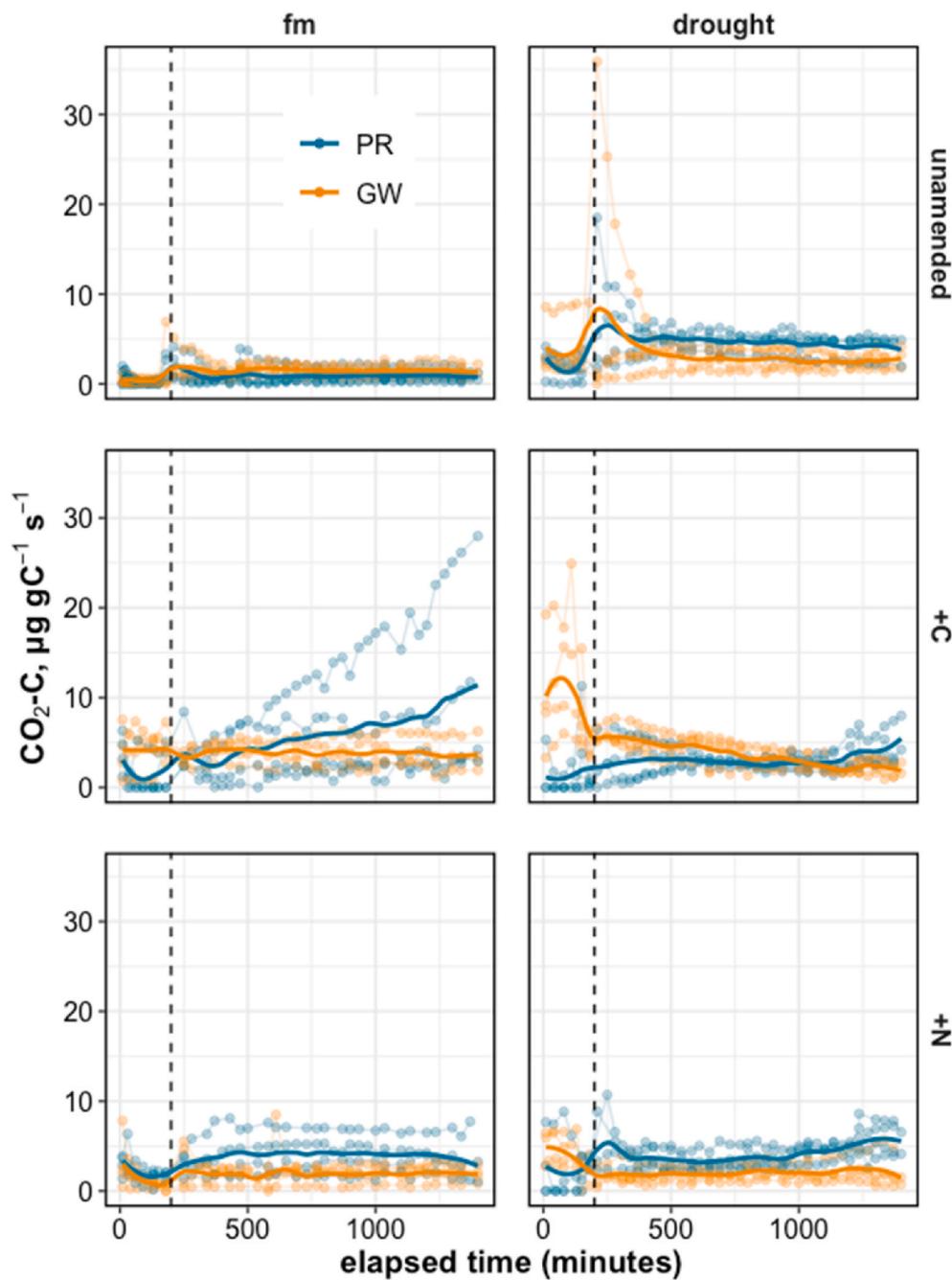


Fig. 2. Temporal progression of CO₂ flux over the 24-h period. The x-axis represents time since wetting began. The blue points represent the cores wet from above (precipitation, PR) and the orange points represent the cores wet from below (groundwater, GW). The lines represent LOESS regression lines over time. The vertical dashed line represents the end of the wetting treatment (200 min), after which the cores were held at saturated conditions for an additional 20 h. Wetting direction (PR vs. GW) influenced respiration only in the C-amended cores, especially in the drought-incubated cores: when wet from below, these cores showed an initial increase in CO₂-C, which then declined over time. The initial bump in respiration suggests that the carbon in micropores was being consumed for respiration. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

drought-simulating conditions for 30 days and then saturating the cores from above vs. below, measuring respiration through this event and including treatments of dissolved C and N amendments. Once saturation was achieved, we immediately extracted soil pore water to determine the forms of soluble C associated with these rapid soil moisture changes. Soluble C was characterized using high-resolution mass spectrometry.

2. Methods

2.1. Site description

The soil used in this experiment is characterized as a sandy, siliceous, hyperthermic Arenic Alaquod in the Immokalee Series (Soil Survey Staff, 1999) and was collected from a pine flatwood stand located within the Disney Wilderness Preserve (DWP), Florida, USA (28.105°, -81.419°). Vegetation at this part of the Preserve is dominated by long leaf pine

(*Pinus palustris*), saw palmetto (*Serenoa repens*), and wiregrass (*Aristida stricta*). We sampled soils from DWP because, in addition to site access and permissions in place, we have been studying these soils for several years (Bailey et al., 2017; Patel et al., 2021; Smith et al., 2017; Yan et al., 2018) and we have a strong foundational understanding of this system. Additionally, as a sandy soil, it was less likely to be confounded by anaerobic microsites and pore limitations, when incubated in the laboratory. Soil characterization is provided in Appendix A1.

Sixty-four intact cores (3 cm diameter, 15 cm height) were sampled in September 2014, in four groups of 16 from 0.25 m² areas, located 2 m apart; soils were sampled from the interplant spaces where soil was exposed. Soil cores were stored at -20 °C for 48 h per USDA-APHIS requirements, and then shipped to the laboratory overnight on blue ice. The cores were stored at 4 °C until 48 h prior to starting the experiment, when they were held at 22 °C.

Table 2

Water soluble organic carbon (WSOC) for the soils, combined across all three suctions/pore water size classes. Antecedent moisture (M) conditions were significant for unamended soils, but not for C- or N-amended soils.

	Water Soluble Organic Carbon, $\mu\text{g g}^{-1}$		
	unamended	+C	+N
FM			
Precipitation	0.25 ± 0.03	$985.72 \pm 314.24^*$	0.49 ± 0.15
Groundwater	0.32 ± 0.09	$255.51 \pm 66.81^*$	0.33 ± 0.22
DROUGHT			
Precipitation	0.66 ± 0.14	$917.11 \pm 556.88^*$	1.44 ± 0.49
Groundwater	0.46 ± 0.04	$367.52 \pm 115.62^*$	0.29 ± 0.11
ANOVA results			
Moisture significant	Moisture non-sig	Moisture non-sig	
Wetting non-sig	Wetting significant	Wetting non-sig	
Interaction non-sig	Interaction non-sig	Interaction non-sig	

* represents a significant difference from the unamended. Statistical results are reported for ANOVA of $\log(\text{WSOC}) \sim M + W + M:W$ for each suction/Amendment combination.

2.2. Experimental approach (Fig. 1)

Treatment combinations were established to vary antecedent soil moisture, rewetting direction, and resource amendments (C, N, or unamended) for each soil core. Four replicates of each combination were included for a total of 48 soil cores. We refer to the unamended, field-moist incubated cores as “baseline” cores for their respective wetting directions.

2.2.1. Antecedent soil moisture conditions

Intact soil cores were either maintained at field moisture content (~15% w/w moisture or ~25% water-filled pore space) or conditioned to laboratory-induced drought conditions ($n = 24$ cores). Drought was simulated by placing intact cores on a dry ceramic pressure plate (1 bar Tempe Pressure Cell, Soil Moisture Equipment Corp. Goleta, CA, USA) and allowing water to evaporate until the cores reached constant weight, ~5% w/w moisture in an environmental growth chamber set at 22 °C and 60% humidity (approx. 30 days) (BDW80, Conviron, Winnipeg, Manitoba, Canada). Field moist cores were maintained at their original moisture content.

2.2.2. Rewetting direction

Cores were rewet via simulated precipitation (rewet from above) or groundwater rise (rewet from below). To simulate groundwater rise, i.e., fine-to-coarse-pore wetting, soil cores were placed in water and allowed

to freely imbibe water for 200 min. To simulate precipitation, i.e., coarse-to-fine-pore wetting, deionized water was delivered at a rate of 0.53 mL min⁻¹ for the first 30 min and then at 0.08 mL min⁻¹ for 170 min using a peristaltic pump (Cole Palmer, Vernon Hills, IL, USA). These rates were based on a preliminary investigation of natural imbibition rates (groundwater rise) determined from a separate set of six cores placed in water (not included in this experiment). After the 200-min rewetting, the cores were held at saturation for an additional 20 h. Thus, the soils had a total incubation time (wetting + saturated) of ~24 h. We did this to allow for hydrologic pore connectivity within the cores, without turning the system anoxic. Headspace methane fluxes were measured during the incubation to check if the system turned anoxic. Methane fluxes remained more or less constant and did not increase over time, suggesting that the systems did not turn anoxic during the 24-h incubation (Appendix A4).

2.2.3. Resource amendments

To investigate how resource limitations (C, N) affected microbial consumption of C, intact soil cores received one of three solutions during the first 10 min of rewetting: Milli-Q deionized water (unamended group), 5 mL of 0.1 M potassium acetate, CH₃COOK (C-amended group), and 5 mL of 2.0 M potassium nitrate, KNO₃ (N-amended group). Amounts of C and N were chosen based on preliminary measurements from surface soils (0–15 cm) collected from DWP, as 10% of total C or N for the average core.

2.3. Flux measurements

To capture the immediate respiratory response of the soil to rewetting, we measured headspace concentrations of CO₂ during rewetting (200 min) and for 20 h post-rewetting using a Picarro G2301 Gas Concentration Analyzer (Picarro, Sunnyvale, CA, USA). Fluxes were computed from the concentration changes as: where A is the flux ($\mu\text{mol g soil}^{-1} \text{s}^{-1}$), dC/dt the rate of change in gas concentration (mole fraction s^{-1}), V the total chamber volume (cm^3), M dry soil mass (g), Pa atmospheric pressure (kPa), R the universal gas constant ($8.3 \times 10^3 \text{ cm}^3 \text{kPa mol}^{-1} \text{K}^{-1}$), and T air temperature (K). Picarro data were processed using the *picarro.data* R package (Bond-Lamberty, 2020). Fluxes were then normalized to soil C. Cumulative CO₂-C was calculated as the sum of linearly interpolated flux rates between measurement dates over the entire incubation (200 min +20 h).

2.4. Pore water collection and characterization

Using a novel method (Bailey et al., 2017), modified from (Lentz, 2006), pore water was collected sequentially at suctions of -1.5, -15 and -50 kPa using a dual valve pressure controller (Alicat Scientific,

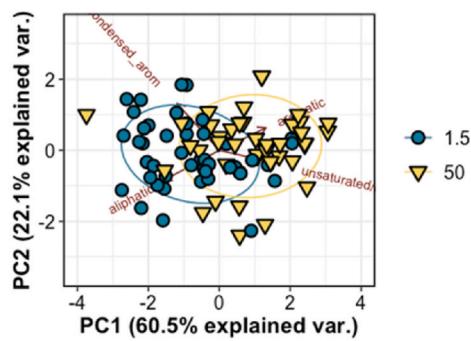
Table 3

FTICR total formulae. For unamended soils, Moisture (M) and wetting direction (W) were important only in the fine pores. But in amended soils, Moisture was significant for coarse pores and Wetting direction was significant for fine pores.

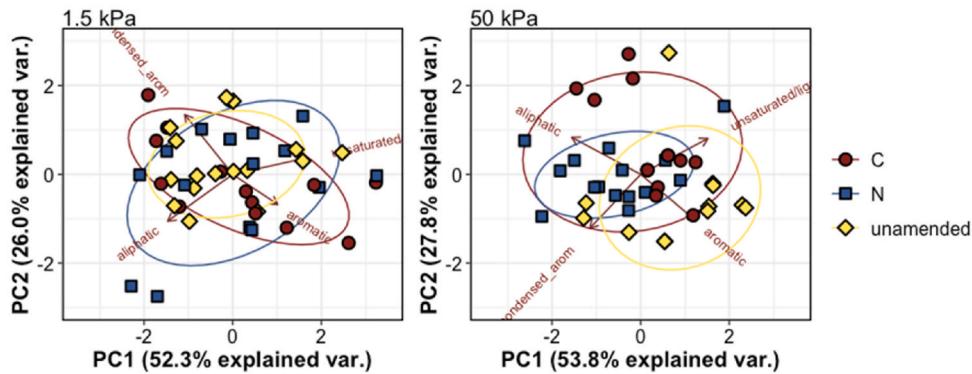
	1.5 kPa (coarse)			50 kPa (fine)		
	unamended	+C	+N	unamended	+C	+N
FM						
Precipitation	345 ± 41	$602 \pm 56^*$	289 ± 12	608 ± 80	$2386 \pm 115^*$	$988 \pm 12^*$
Groundwater	550 ± 32	$270 \pm 15^*$	$293 \pm 36^*$	1089 ± 262	767 ± 63	611 ± 74
DROUGHT						
Precipitation	637 ± 143	$1313 \pm 72^*$	924 ± 38	1231 ± 88	$2648 \pm 0^*$	1030 ± 65
Groundwater	400 ± 75	$1509 \pm 170^*$	881 ± 369	826 ± 0	1426 ± 432	777 ± 69
ANOVA results	M non-sig W non-sig M:W non-sig	M sig W sig M:W sig	M sig W non-sig M:W non-sig	M sig W sig M:W sig	M non-sig W sig M:W non-sig	M non-sig W sig M:W non-sig

Statistical results are reported for ANOVA of $\log(\text{counts}) \sim M + W + M:W$ for each suction/Amendment combination. Grand means are reported for each Suction/Amendment grouping, pooled across the Moisture and Wetting types. * represents a significant difference from the unamended within each suction type.

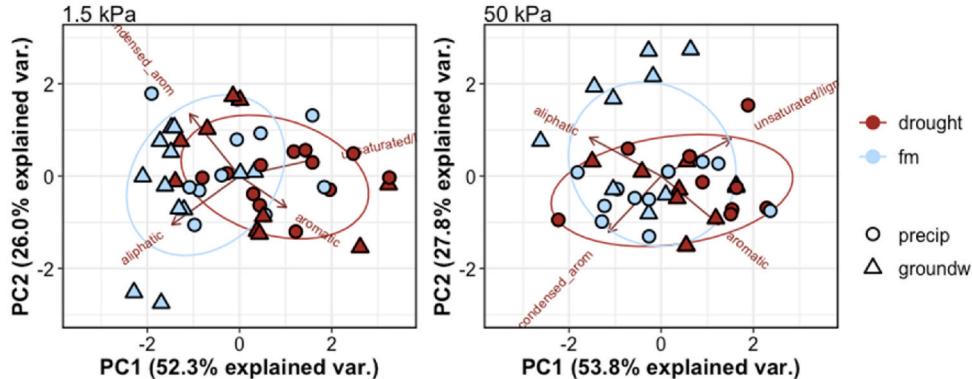
A



B



C



Tucson, AZ, USA) using individual 100 kPa Tempe Pressure Cell units (Soil Moisture Equipment Corp. Goleta, CA, USA). This pore water collection technique requires that the soil cores be saturated, in order to maintain sufficient suction to initiate pore water drawdown. Each suction, or pore size domain, was pulled for 24 h, starting with the lowest strength (-1.5 kPa). Water collected at -1.5 , -15 , and -50 kPa suctions represents water contained within soil pore spaces restricted by channels, or pore throats, approximately $>200\text{ }\mu\text{m}$, $20\text{--}200\text{ }\mu\text{m}$, and $6\text{--}20\text{ }\mu\text{m}$ diameter (Bailey et al., 2017; Marshall et al., 1996). For clarity, these three pore water fractions will be referred to by their suction strength or collectively referred to as “effective pore size domains”. The pore water samples collected were stored at $-20\text{ }^{\circ}\text{C}$ until further analysis.

Water-soluble concentrations of organic C (WSOC) were determined via combustion catalytic oxidation from pore water samples using a TOC-5000A TOC Analyzer (Shimadzu, Columbia, MD, USA). The WSOC content of each core was calculated as the sum of WSOC from the three

Fig. 3. PCA biplots of FT-ICR-MS data showing separation of samples by (A) Suction/pore-water throat, (B) Amendments, and (C) Moisture. Fine pores had a greater proportion of aromatic and lignin-like molecules, and coarse pores had a greater proportion of aliphatic molecules. In the coarse pores, drought-incubated soils had a greater proportion of aromatic and lignin-like molecules. In the fine pores, there wasn't a clear separation by antecedent moisture, although the fm-incubated soils wet from below showed more aliphatic molecules than the other soil types. Data are not separated by Amendments for these figures.

pore fractions. Because little to no pore water was collected at higher suctions (-15 and -50 kPa), replication was reduced for select pore water analyses. For low volume pore water samples, we prioritized characterizing the molecular composition of C in pore water rather than measuring WSOC due to technical limitations in measuring low volume samples.

The molecular composition of the C dissolved in the pore water was characterized by electrospray ionization (ESI) coupled with Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) located at the Environmental Molecular Sciences Laboratory (EMSL), a DOE user facility, located in Richland WA. Molecular characterization was done for the -1.5 and -50 kPa pore water samples, which represented the largest ($>200\text{ }\mu\text{m}$) and smallest ($6\text{--}20\text{ }\mu\text{m}$) pore domains sampled. Pore water samples were desalinated and concentrated by solid phase extraction using PPL cartridges (Agilent, Santa Clara, CA, USA) (Dittmar et al., 2008) and were eluted in methanol prior to direct

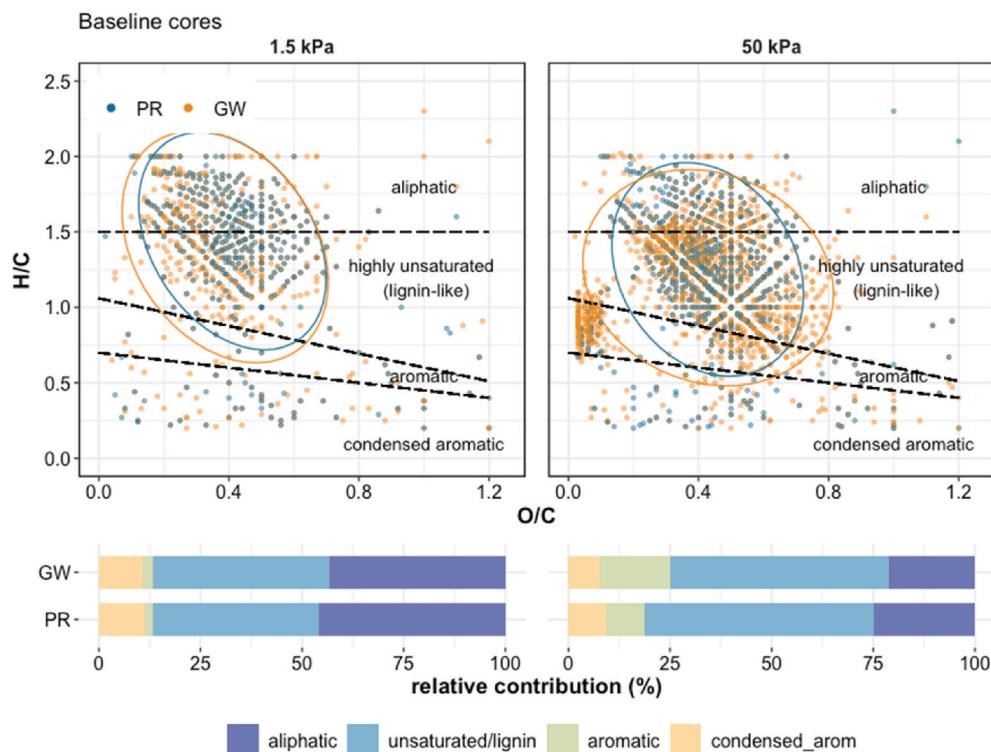


Fig. 4. Van Krevelen plots for baseline (fm, unamended) cores, separated by suction (pore size class). Molecules are plotted using the molecular H/C and O/C ratios. Ellipses represent 90% confidence intervals. The horizontal bar plots represent relative abundances of the compound classes, based on identified peaks.

injection on a 12 T Bruker SolariX FT-ICR-MS spectrometer; see (Tfaily et al., 2015, 2017) for detailed methods. Ninety-six individual scans were averaged for each sample and internally calibrated using organic matter (OM) homologous series separated by 14 Da ($-\text{CH}_2$ groups). The mass measurement accuracy was less than 1 ppm for singly charged ions across a broad m/z range (i.e., 200, $<\text{m/z}<1200$). Chemical formulae were assigned using the Formularity software, including only peaks with a signal/noise ratio >7 (Kujawinski and Behn, 2006; Tolić et al., 2017), with the restrictions C₁₋₁₃₀, H₁₋₂₀₀, O₁₋₅₀, N₀₋₃, S₀₋₃, P₀₋₂.

Further processing was done using the *fticrr* package in R (Patel, 2020). FT-ICR-MS-resolved peaks were analyzed only on a presence/absence basis, due to established issues with intensities and ionization efficiencies in complex matrices using ESI (Kujawinski, 2002; Ohno et al., 2016). Only peaks identified in $\geq 2/3$ of the replicates were considered present within that treatment (Payne et al., 2009; Sleighter et al., 2012). The modified Aromaticity Index (AI_{mod}), used to classify identified molecules, was calculated from (Koch and Dittmar, 2016) as: where C, H, O, N, S, P represent the stoichiometric element numbers for each formula. The identified peaks were assigned to one of the following classes following the method of (Seidel et al., 2014, 2017): (1) polycyclic condensed aromatics ($\text{AI}_{\text{mod}} > 0.66$); (2) highly aromatic compounds, which include polyphenols and polycyclic aromatic compounds with aliphatic chains ($0.66 > \text{AI}_{\text{mod}} > 0.50$); (3) highly unsaturated compounds, which include phenols such as soil-derived products of lignin degradation ($\text{AI}_{\text{mod}} \leq 0.50$ and $\text{H/C} < 1.5$); and (4) aliphatic compounds ($\text{H/C} \geq 1.5$), including unsaturated aliphatics, N-containing aliphatics, and saturated compounds including fatty and sulfonic acids, and/or carbohydrates. The FT-ICR-MS compound classes are tentative classifications as they are solely based on the indices (O/C and H/C ratios and AI_{mod} values) from the molecular formula, not the molecular structure. Relative abundance values were calculated from count values associated with each observed biomolecule group normalized by the total number of C molecules identified.

2.5. Homogenization

In addition to the experimental treatments outlined above, we performed an additional homogenization on the baseline cores to see if the physical disturbance of sieving and mixing would release any new C. After extracting pore water, the baseline cores were deconstructed, sieved through 2 mm mesh and homogenized, and then repacked into the same cores. These homogenized cores were then rewet (from above vs. from below) with deionized MilliQ water. Soil respiration and WSOC analyses were performed on these cores following the procedures described above and are provided in Appendix A2.

2.6. Data and statistical analysis

We used univariate linear mixed-effects models (LME) and analysis of variance (ANOVA) to determine significant differences among treatments for respiration, WSOC concentrations, and FT-ICR-MS peak counts. Antecedent moisture (field moist vs. drought), wetting direction (precipitation vs. groundwater), and amendments (unamended vs. C vs. N) were used as main effects, along with their interactions up to two levels. FT-ICR-MS data were analyzed using permutational multivariate analysis of variance (PERMANOVA) and principal components analysis (PCA). Due to a significant effect of pore-size class (see Results), the 1.5 kPa and 50 kPa pore size classes were analyzed separately. All data analyses were performed in R version 4.0.2 (2020-06-22) (R Core Team, 2020) using primarily the *dplyr v1.0.1* (Wickham et al., 2020) and *vegan v2.5-6* (Oksanen et al., 2019) packages for data processing and analysis, and *ggplot2 v3.3.2* (Wickham, 2016), *ggbiplot v0.55* (Vu, 2011), and *PNWColors* (Lawlor, 2020) packages for data visualization. The data and R scripts are available online at https://github.com/kaizadp/TES_spatial_access_2021 (DOI: 10.5281/zenodo.5522938) and are archived on ESS-DIVE (DOI: 10.15485/1821491).

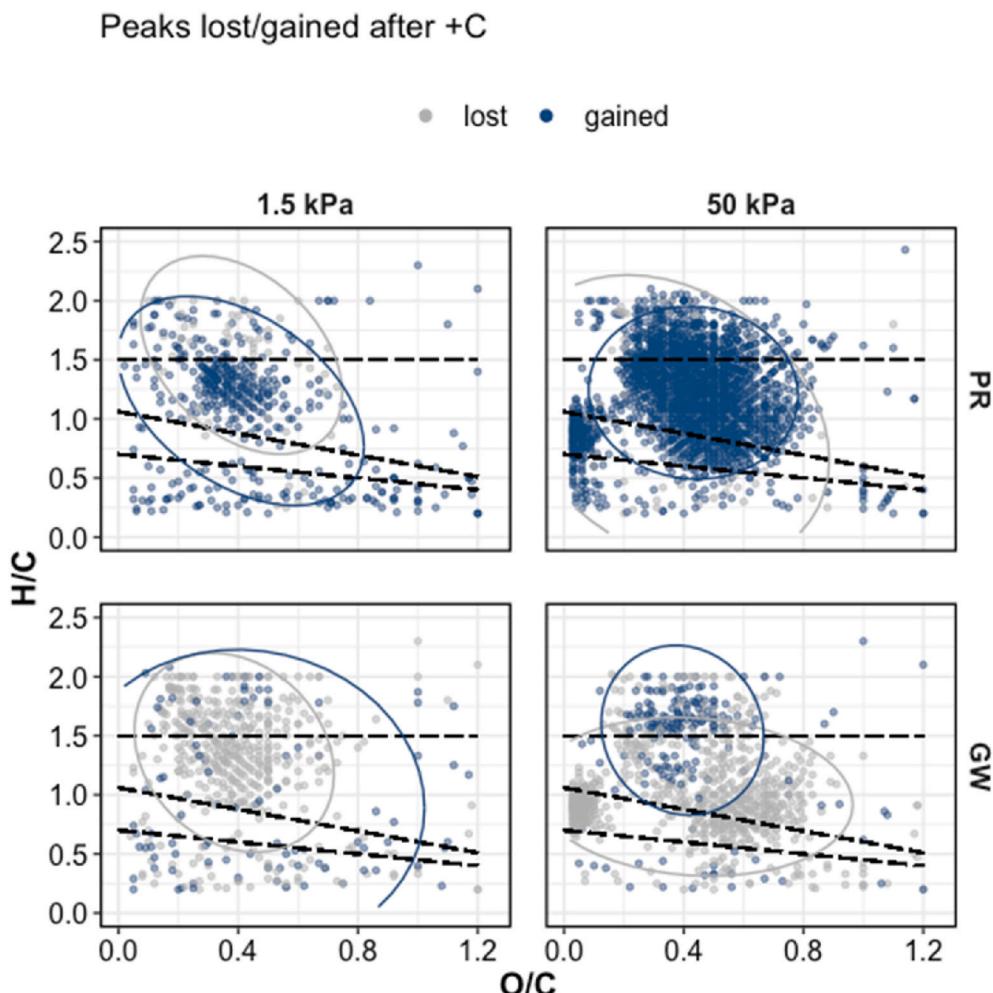


Fig. 5. Van Krevelen plots for C-amended fm cores, compared with the baseline. Molecules are plotted using the molecular H/C and O/C ratios. Ellipses represent 90% confidence intervals.

3. Results

3.1. Core-scale responses (soil respiration)

Respiration was strongly influenced by Amendments and Antecedent Moisture (ANOVA, interaction $F = 7.0770$, $P = 0.0026$). In the fm-incubated cores, C amendments increased respiration compared to the unamended soils (Table 1); N amendments increased respiration only in the soils wet from above (precipitation). Overall, drought-incubated soils showed greater respiration than fm-incubated soils after rewetting and did not show increased respiration after C/N amendments (Table 1).

Wetting direction influenced respiration only in the C-amended cores (Fig. 2), mainly in the drought-incubated cores, and to a lesser extent in the fm-incubated cores. For C-amended cores, fm-incubated cores wet from above showed a progressive increase in $\text{CO}_2\text{-C}$ over time. Drought-incubated cores wet from below showed an initial increase in $\text{CO}_2\text{-C}$, which then declined over time. The initial bump in respiration suggests that the carbon in micropores was being consumed for respiration, because of preferential flow paths as soils are wet from below (Todoruk et al., 2003).

3.2. Pore-scale responses (WSOC chemistry by FT-ICR-MS)

Overall, the WSOC from C-amended soils (Table 2) was three orders of magnitude greater than the WSOC from unamended and N-amended

soils (mean values 631 (+C) vs. 0.4 (unamended) and 0.6 (+N) $\mu\text{g C g}^{-1}$), a direct effect of the C-substrate added to these cores. Antecedent moisture influenced WSOC concentrations only in the unamended soils, with WSOC in drought-incubated soils nearly double that in fm-incubated soils. Wetting direction influenced WSOC concentrations only in the C-amended soils, with precip-wet soils showing $\sim 2.5\text{--}4$ times the WSOC in groundwater-wet soils.

We determined overall trends in WSOC composition by representing the multivariate FT-ICR-MS data in a two-dimensional ordination space. Principal Components Analysis (PCA) showed that FT-ICR-MS-resolved molecules separated primarily by Suction (pore throat size class) along PC1, with coarse pores dominated by aliphatic molecules and fine pores dominated by aromatic and lignin-like molecules (Fig. 3A). We therefore performed subsequent statistical analyses separately for the 1.5 kPa and 50 kPa pore waters. Although PERMANOVA results (Appendix A3) showed a significant effect of Amendments on pore water chemistry, there were no clear separation trends from the PCA plots (Fig. 3B), however, we detected strong interactions with Moisture and Wetting direction. The coarse pore chemistry showed a further separation by antecedent moisture (drought vs. fm), with drought-incubated soils dominated by aromatic and lignin-like molecules (Fig. 3C). There was no such separation in the fine pores, as most samples clustered toward the complex molecule classes, except for fm-groundwater (mostly of the C-amended soils), which were dominated by simple aliphatic molecules. There was a strong interaction of moisture conditions and wetting direction in the fine pores (Fig. 3C, PERMANOVA $F = 6.04$, $P = 0.005$).

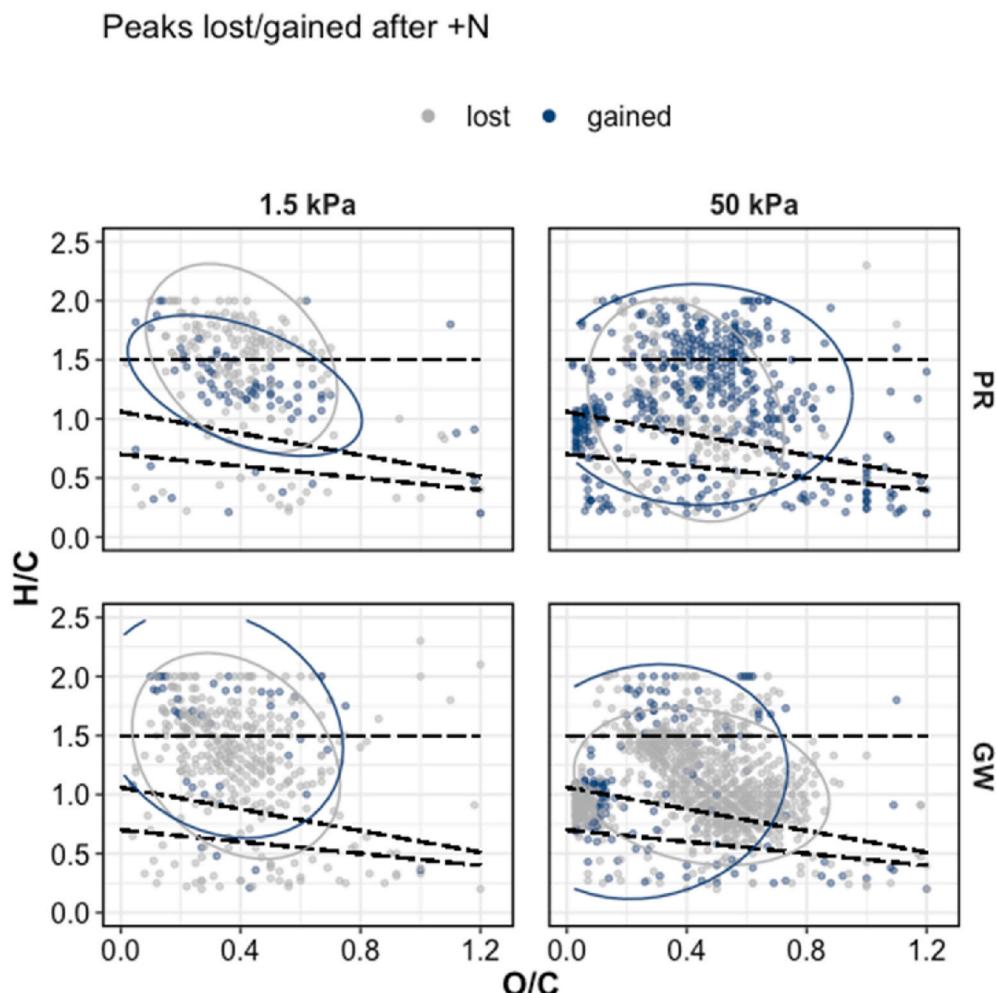


Fig. 6. Van Krevelen plots for N-amended fm cores, compared with the baseline. Molecules are plotted using the molecular H/C and O/C ratios. Ellipses represent 90% confidence intervals.

In order to isolate the various mechanisms at play in our soils, we compared specific subsets of the experimental units, depicted in the Van Krevelen plots in Figs. 4–7.

- **Baseline.** The baseline soils (fm + unamended; Fig. 4) were dominated by aliphatic molecules in the coarse pores (~50% of total identified molecules) and by complex lignin-like and aromatic molecules in the fine pores (~75% of total identified molecules). Wetting direction did not influence WSOC chemistry, in terms of total peaks (Table 3) or identified compounds (Fig. 4).
- **Baseline vs. amended.** In contrast to the baseline soils, the C-amended soils (fm-incubated) showed strong influence of wetting direction (Table 2, Fig. 5). When wet from above (precipitation), there was a net increase in peak counts, primarily aliphatic and lignin-like. The C-amended soils wet from below (groundwater recharge) showed a net loss of peaks, mostly aliphatic and lignin-like, despite the addition of fresh C. The N-amended cores showed similar patterns in chemical shifts, although to a lesser extent than the C-amended cores (Table 3, Fig. 6). Peaks were lost mainly from the lignin-like and aliphatic regions (Fig. 6). Overall, there was little change in the total number of peaks identified, although the precipitation-fed cores showed an increase in peaks after N amendments in the fine pores (Table 3, Fig. 6).
- Baseline vs. drought. Compared to the baseline cores, drought-induced cores showed a significant increase in identified peaks, mainly lignin-like, when wet from above (Table 3, Fig. 7). But when

wet from below, there was a net loss of peaks, mostly from the lignin-like and aliphatic regions.

- Baseline vs. homogenized. Homogenized cores showed a large increase in compounds identified, compared to the baseline soils. These new peaks were mainly in the aliphatic and lignin-like regions for the coarse pores, and across the aliphatic, lignin-like, and aromatic regions for the fine pores. Additionally, the homogenized soils also experienced significantly greater respiration and pore water DOC concentrations compared to baseline cores (Appendix A2).

4. Discussion

The goal of this experiment was to investigate controls on soil respiration, and the results we describe here allow us to disentangle some physicochemical and biochemical controls on C mineralization in soils (spatial access, adsorption to mineral surfaces, occlusion within aggregates, and nutrient/resource limitations). There were strong interactive effects among our treatments, particularly with wetting direction. This is not unexpected, as wetting can increase hydrologic connectivity along preferential flow paths/pore networks, increasing microbial access to substrates (Dungait et al., 2012; Ebrahimi and Or, 2015; Yan et al., 2016). The separation of pore water chemistry by pore size domains is consistent with prior findings (Bailey et al., 2017), as the well-connected, coarse pores would be generally enriched in more labile, fresh litter substrates, (Negassa et al., 2015; Six et al., 1998). As amendments or soil moisture conditions (e.g., drought) alter the

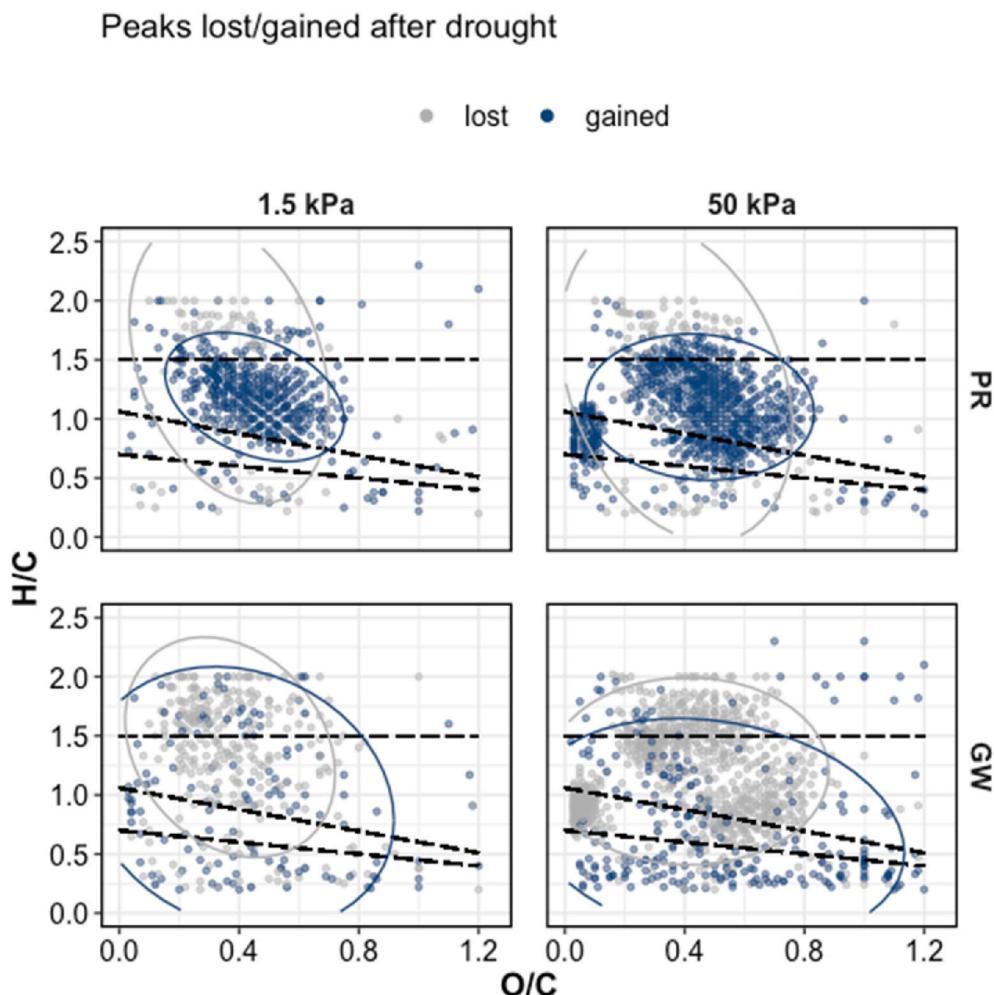


Fig. 7. Van Krevelen plots for drought-incubated, unamended cores, compared with the baseline. Molecules are plotted using the molecular H/C and O/C ratios. Ellipses represent 90% confidence intervals.

chemistry in the fine and coarse pores, simulated precipitation vs. groundwater would therefore improve microbial accessibility to different molecules.

4.1. C and N amendments alleviated nutrient limitations

Consistent with hypothesis H1, the increased respiration following C amendment suggests a C-limitation in the field moist (fm)-incubated soils, which was alleviated by the added acetate. The CO₂ evolution in C-amended cores was ~150–400 mg/gC greater than in the unamended soils, an order of magnitude greater than the C amendments themselves (20 mg acetate-C per gram of soil C). This indicates that even if all the acetate added was respired, the microbial community was stimulated to metabolize and respire additional substrate present in the soil (priming).

4.1.1. Interaction with wetting direction

Contrary to our expectations (H3), wetting direction did not influence respiration or WSOC composition in baseline soils. Given the strong C gradient with depth (1.0% ± 0.01% at the top vs. 0.1% ± 0.001 at the bottom), we had expected that simulated precipitation would mobilize and redistribute C from the surface, increasing WSOC concentrations as well as molecules identified. That this did not happen suggests some kind of physicochemical or biochemical protection. This could perhaps also be explained by an insufficient duration for the precipitation to solubilize organic compounds (e.g., from POM, which is present in larger proportions in the upper layer (Liebmann et al., 2020)).

In contrast, for the C-amended cores, the WSOC results (Table 2) suggest that the surface C was mobilized when the cores were wet from above. Added acetate may have (a) disrupted organo-mineral interactions, releasing previously protected C (Clarholm et al., 2015; Keiluweit et al., 2015); or (b) stimulated microbial activity either by co-metabolism or assimilation into microbial biomass (Kuzyakov et al., 2000). Acetate has a relatively high carbon use efficiency and low adsorptivity (Sokol et al., 2019), and thus the latter mechanism is likely the dominant one, and it is possible that the stimulated microbes were able to metabolize the surface C.

Similarly, in the C-amended soils, aliphatic and lignin-like peaks were lost when the cores were wet from below. We suggest that as water moved up through the low-C (C-limited) regions at the bottom of the cores, the fresh C amendments stimulated the microbes to consume the organic substrate in the fine pores (primarily complex molecules), as these pores were wet first. This is consistent with the temporal progression of respiration in these cores – respiration was high at the start of the wetting process, as the fine pores were being wet. Similarly, the N-amended cores also experienced a net loss of peaks when wet from below, suggesting that microbial activity in the fine pores was N-limited, and the nitrate amendments may have stimulated the microbes to consume the lignin-like and aliphatic molecules.

4.2. Drought destabilized mineral-bound organic substrate

Consistent with hypothesis H2, the drought-incubated cores

experienced increased soil respiration compared to the baseline soils. Further, the lack of respiration response to amendments in the drought-incubated cores suggests that the drought + rewetting may have alleviated some of the nutrient limitations seen in the baseline soils, perhaps by destabilizing previously adsorbed (protected) C (Bailey et al., 2019; Kaiser et al., 2015), or by the release of osmolytes or microbial necromass under drought stress (Fierer and Schimel, 2003; Warren, 2014), which would serve as fresh substrate for C mineralization. Indeed, the increases in WSOC concentrations and increases in peaks identified in the drought soils indicate a substantial release of organic molecules, mostly lignin-like and aromatic, when the soils were dried (Table 2, Table 3, Fig. 7).

Compounds adsorbed to mineral surfaces are generally protected from microbial degradation, and must be desorbed/destabilized to become bioavailable (Bailey et al., 2019). Adsorption-desorption dynamics are largely influenced by the ionic strength of the soil solution: under wet/saturated conditions, with low ionic strength, weak outer-sphere complexation (e.g., with carboxyl-containing aromatic compounds) dominates the mineral-associated SOM. In contrast, under high ionic strength (e.g. during drying), these weak bonds are broken, likely because of compression of the diffuse double layer (Stumm and Morgan, 2012), and the complex aromatic compounds are desorbed from the mineral surface (Aubry et al., 2013; Newcomb et al., 2017). Drought-affected soils may therefore have a larger proportion of complex compounds in soil solution (Kaiser et al., 2015), and the results we report here are consistent with this mechanism.

4.2.1. Interaction with wetting direction

Cumulative CO₂ evolution decreased in the drought-incubated cores wet from above (Table 1), which suggests a negative priming from the acetate amendments, at least for the 24-h incubations conducted in our experiment. The temporal respiration trends in Fig. 2B, however, show that in these cores, CO₂ flux was initially low, but increased over time. This lag may be a function of drought-induced chemical changes as well as preferential flow paths as the cores were wetted. Under simulated precipitation, the macropores are preferentially wetted, and these pores are typically dominated by simple aliphatic molecules (Bailey et al., 2017). Drought increased the availability of complex molecules in the pore waters, and it is possible that microbes that were adapted to degrade simple aliphatic molecules in the macropores were now inhibited by the sudden availability of more complex substrate. Our experiment focuses on short-term responses, but these results indicate the need for longer-term monitoring to capture the different phases of chemically controlled (sorption-desorption) and microbially mediated (decomposition) processes in future studies.

The patterns observed in the drought soils show an interesting similarity to the soils that received C amendments, where groundwater soils saw a net loss/consumption of peaks. Both these sets of results lead us to believe that the sandy baseline soils may have been C-limited, and that these limitations were alleviated by the addition of fresh substrate, either from the C amendments or from the drought-induced C destabilization.

4.3. Homogenization released carbon from aggregates

Aggregation in intact cores may serve as a spatial barrier, as

substrate trapped within aggregates may not be available for microbial consumption (Jastrow and Miller, 1997; Kravchenko et al., 2015). In addition, the lack of oxygen within aggregates may also contribute to SOM protection (Wang et al., 2019). Breaking up of the aggregates (e.g., by homogenization) would therefore allow microbes to access the substrate within, while also increasing oxygen availability. The results from our homogenized cores (Appendix A1) suggest that a substantial portion of the WSOC was protected within aggregates, which was released when the soils were sieved and homogenized.

We did not expect much effect of wetting direction in homogenized cores, as homogenization would have disturbed the existing soil architecture and caused a redistribution of the organic compounds. Indeed, in the coarse pores, there was no appreciable difference in newly detected compounds in precipitation vs. groundwater cores. In the fine pores, although there was much overlap in the WSOC composition, the cores wet from below did have a greater proportion of more-oxidized lignin-like molecules.

5. Conclusions

The mechanisms we outline here represent some of the physico-chemical and biochemical processes occurring in soils that govern C mineralization dynamics. Although general soil science theory can explain some of these observations, these processes, and their relative importance to soil C persistence, are dependent on soil properties, e.g., the amount and type of C, the microbial community, the soil texture and structure, mineralogy, etc. Larger-scale (landscape/regional) properties, e.g., climate, moisture history, etc. may also drive many of the soil processes, and it is therefore difficult to identify universal responses to moisture changes and fluctuations across different soil types and regions (Patel et al., 2021a). However, this research allows us to put forward a framework that begins to quantify and prioritize some of the important soil properties and processes that collectively govern the persistence and vulnerability of soil carbon.

Declaration of competing interest

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

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Appendix A1. Characteristics of the soil used in this experiment

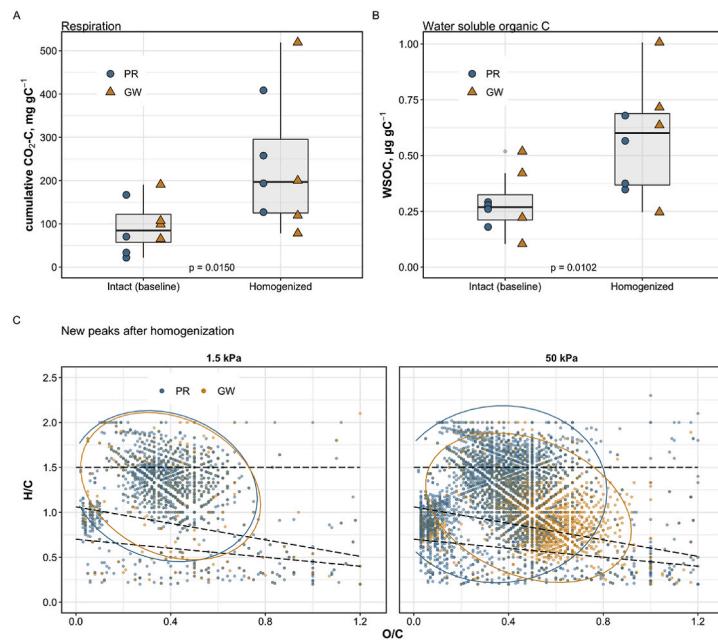
Values are reported as mean ± standard error. Data obtained from Patel et al., (2021b).

soil series	Immokalee
total C (%)	0.98 ± 0.1
total N (%)	0.04 ± 0
total organic C (%)	0.88 ± 0.11
pH	4.79 ± 0.11
sand (%)	90.8 ± 1.74
silt (%)	3.8 ± 0.66
clay (%)	5.4 ± 1.29
texture	sand

*Total C showed a strong depth gradient within the cores, with $1.0\% \pm 0.01$ at the top compared to $0.1\% \pm 0.001$ at the bottom (Smith et al., 2017).

Appendix A2. Homogenized Cores

FM-incubated, unamended cores were sieved through 2 mm mesh and homogenized, and then repacked into the same cores. The homogenized cores were then rewet (from above vs. from below) and incubated for 20 h, after which pore water was extracted as before.



(A) Respiration in intact vs. homogenized cores. Homogenized cores showed significantly greater respiration compared to the intact cores. PR = precipitation (wet from above), GW = groundwater (wet from below) **(B) Pore water WSOC in intact vs. homogenized cores.** Homogenized cores showed significantly greater WSOC than the intact cores. **(C) Van Krevelen plots showing newly identified FT-ICR-MS peaks after homogenization.** In fine pores, groundwater-fed cores showed increased presence of oxidized lignin-like molecules.

Appendix A3. PERMANOVA results for FT-ICR-MS data

term	df	SumsOfSqs	MeanSqs	F.Model	R2	p.value
1.5 kPa						
Amendments	2	0.0163	0.00816	0.746	0.0235	0.607
Moisture	1	0.113	0.113	10.3	0.162	0.001
Wetting	1	0.0377	0.0377	3.45	0.0542	0.024
Amendments:Moisture	2	0.0538	0.0269	2.46	0.0773	0.049
Amendments:Wetting	2	0.0831	0.0415	3.79	0.119	0.009
Moisture:Wetting	1	0.00923	0.00923	0.844	0.0133	0.467
Residuals	35	0.383	0.0109	NA	0.550	NA
50 kPa						
Amendments	2	0.236	0.118	7.64	0.233	0.001
Moisture	1	0.0660	0.0660	4.26	0.0649	0.016
Wetting	1	0.0385	0.0385	2.49	0.0379	0.097
Amendments:Moisture	2	0.0522	0.0261	1.69	0.0513	0.169
Amendments:Wetting	2	0.0349	0.0174	1.13	0.0343	0.325

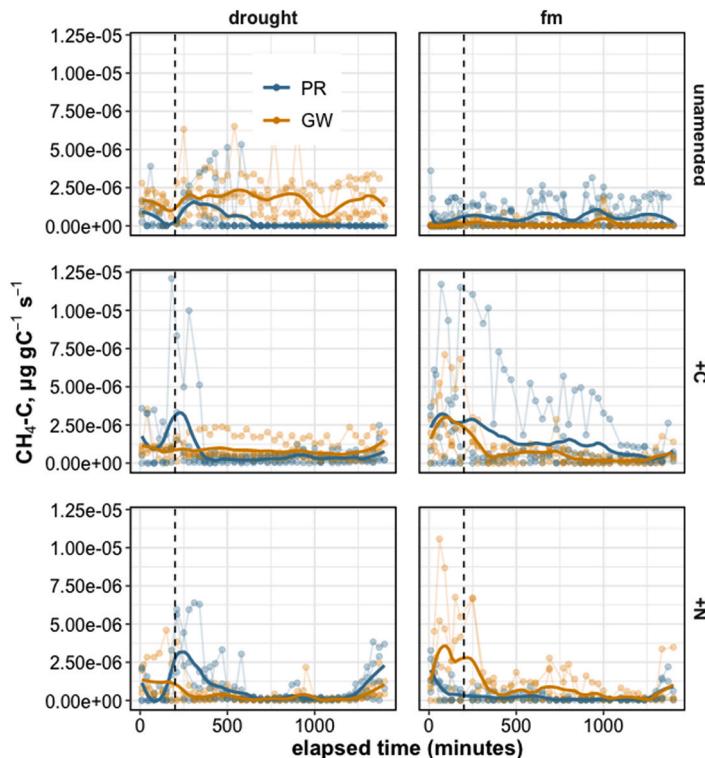
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term	df	SumsOfSqs	MeanSqs	F.Model	R2	p.value
Moisture:Wetting	1	0.0935	0.0935	6.04	0.0919	0.005
Residuals	32	0.495	0.0155	NA	0.487	NA

Appendix A4. Headspace methane fluxes measured during the 24-h incubation

The x-axis represents time since wetting began. The blue points represent the cores wet from above (precipitation, PR) and the orange points represent the cores wet from below (groundwater, GW). The lines represent LOESS regression lines over time. The vertical dashed line represents the end of the wetting treatment (200 min), after which the cores were held at saturated conditions for an additional 20 h. Methane fluxes remained low throughout the 24-h incubation, suggesting that the systems had not turned anoxic.



Data availability

The data and R scripts are available online at https://github.com/kaizadp/TES_spatial_access_2021 (DOI: 10.5281/zenodo.5522938) and are archived on ESS-DIVE (DOI: 10.15485/1821491).

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