Title: One thousand soils for molecular understanding of belowground carbon cycling

Authors: Maggie M. Bowman[1,*], Alexis E. Heath[2], Tamas Varga[1], Anil K. Battu[1], Rosalie K. Chu[1], Jason Toyoda[1], Tanya E. Cheeke[3], Stephanie S. Porter[4], Kevan Moffett[4], Brittany Letendre[4], Odeta Qafoku[1], John R. Bargar[1], Douglas Mans[1], Nancy Hess[1], Emily B. Graham[1]

Affiliation:

1) Pacific Northwest National Laboratory, Richland, WA, 99354, USA
2) Bowling Green State University, Bowling Green, OH, 43403, USA
3) School of Biological Sciences, Washington State University, Richland, WA, USA
4) School of the Environment, Washington State University, Vancouver, WA 98686, USA

Abstract:

While significant progress has been made in understanding global carbon (C) cycling, the mechanisms regulating belowground C fluxes and storage are still uncertain. New molecular technologies have the power to elucidate these processes, yet we have no widespread standardized implementation of molecular techniques. To help address this gap, we have developed a crowdsourced soil core research program for analyzing molecular and microstructural data that describe soil structure, soil organic matter (SOM) chemistry, and soil microbiology. Known as the 1,000 Soils Pilot and based at the Environmental Molecular Sciences Laboratory (EMSL), we use Fourier-transform ion cyclotron resonance- (FTICR) and liquid chromatography- (LC) mass spectrometry (MS); X-ray Computed Tomography (XCT), water retention curves; metagenomic sequencing; and standard biogeochemical measurements to enable new insights into soil C cycles. To emphasize cross-site comparability, we provide standardized sampling materials and protocols, and all data are generated on dedicated instruments with optimized settings. Here, we describe the 1000 Soils Pilot and present a use case describing differences in SOM chemistry, soil structure, and chemical and biological properties across forest soils exposed to differing wildfire regimes. Data and analytic workflows from the 1000 Soils Pilot will populate a unique continental-scale database of soil molecular properties, as part of the EMSL Molecular Observation Network (MONet) user program.

Introduction

Soil organic matter (SOM) is a critical part of the global carbon (C) cycle. Belowground ecosystems contain more C than stored in terrestrial vegetation and the atmosphere combined, (Crowther et al., 2019; Crowther et al., 2016; Jobbágy and Jackson, 2000), and SOM is the largest and most biologically active portion of soil C. SOM decomposition is regulated by a complex and interacting set of factors including soil structure, moisture distribution, temperature, pH, and nutrient status; collectively, these factors determine accessibility, bioavailability, and rate kinetics of SOM (Graham and Hofmockel, 2022). Despite the importance of SOM in the global C cycle, the drivers of SOM decomposition from molecular to continental scales are not well understood.

New research suggests that standardized, spatially resolved, and high-resolution data may be critical to reducing the uncertainty surrounding estimates of belowground C dynamics under future climate scenarios (Lehmann and Kleber, 2015; Viscarra Rossel et al., 2019). For example, pore-scale structural characterization could reveal spatial factors that inhibit SOM decomposition, and molecular chemistry may provide insight into SOM reactivity that cannot be observed from bulk measurements. As of yet, such information is lacking from soil databases (e.g., Web Soil Survey, International Soil Radiocarbon Database, and Soils Data Harmonization), and no widespread implementations of standardized high-resolution techniques simultaneously investigate the myriad factors influencing SOM decomposition. Consequently, soil C cycles are poorly represented in biogeochemical and Earth System Models (ESMs) that predict changes in the global climate (Bradford et al., 2016). For example, most ESMs simulate changes in belowground C concentration within coarsely defined pools (i.e., agnostic of SOM composition) rather than representing chemical and microbial reactions within SOM pools (Bond-Lamberty et al., 2020; Malhotra et al., 2019; Rasmussen et al., 2018; Waring et al., 2020). The dearth of soil molecular and microphysical/chemical information presents a major limitation to improving models at regional and Continental United States (CONUS) scales, where impacts of climate change are manifested and can be understood.

Led by a team at the Environmental Molecular Science Laboratory (EMSL), a new decadal initiative is developing a CONUS-scale database of soil compositional, physical, and genomic measurements that are open and FAIR (Findable, Accessible, Interoperable and Reusable), termed the Molecular Observation Networks (MONet) program. Its ultimate goal is to facilitate data syntheses and translation of experimental and modeling approaches to the regional scale to reduce uncertainty in soil C simulation at regional and CONUS scales. As a pilot effort in MONet, the 1000 Soils Pilot focuses on understanding the drivers and fluxes of belowground C cycles through standardized molecular measurements on soil cores. In this article, we describe and present a use case from the 1000 Soils Pilot to highlight the utility of high-resolution measurements for understanding the impacts of wildfire on soil structure, molecular SOM composition, and SOM decomposition.

Approach

Through partnerships with individual researchers and ecological networks (Figure 1A), the 1000 Soils Pilot collected soil cores using a standardized sampling kit and field protocols, provided by EMSL. Collaborators collected 30-cm (3-inch dia.) replicate intact cores using a slide hammer core, as well as four 10 cm (2-inch dia.) soil cores using a wooden block and mallet. Other field-based measurements included soil temperature, moisture, and infiltration rate. A step-by-step sampling protocol is available in the SI and as a video at https://www.emsl.pnnl.gov/monet. After sample collection, all data was generated by EMSL or EMSL-contracted vendors.

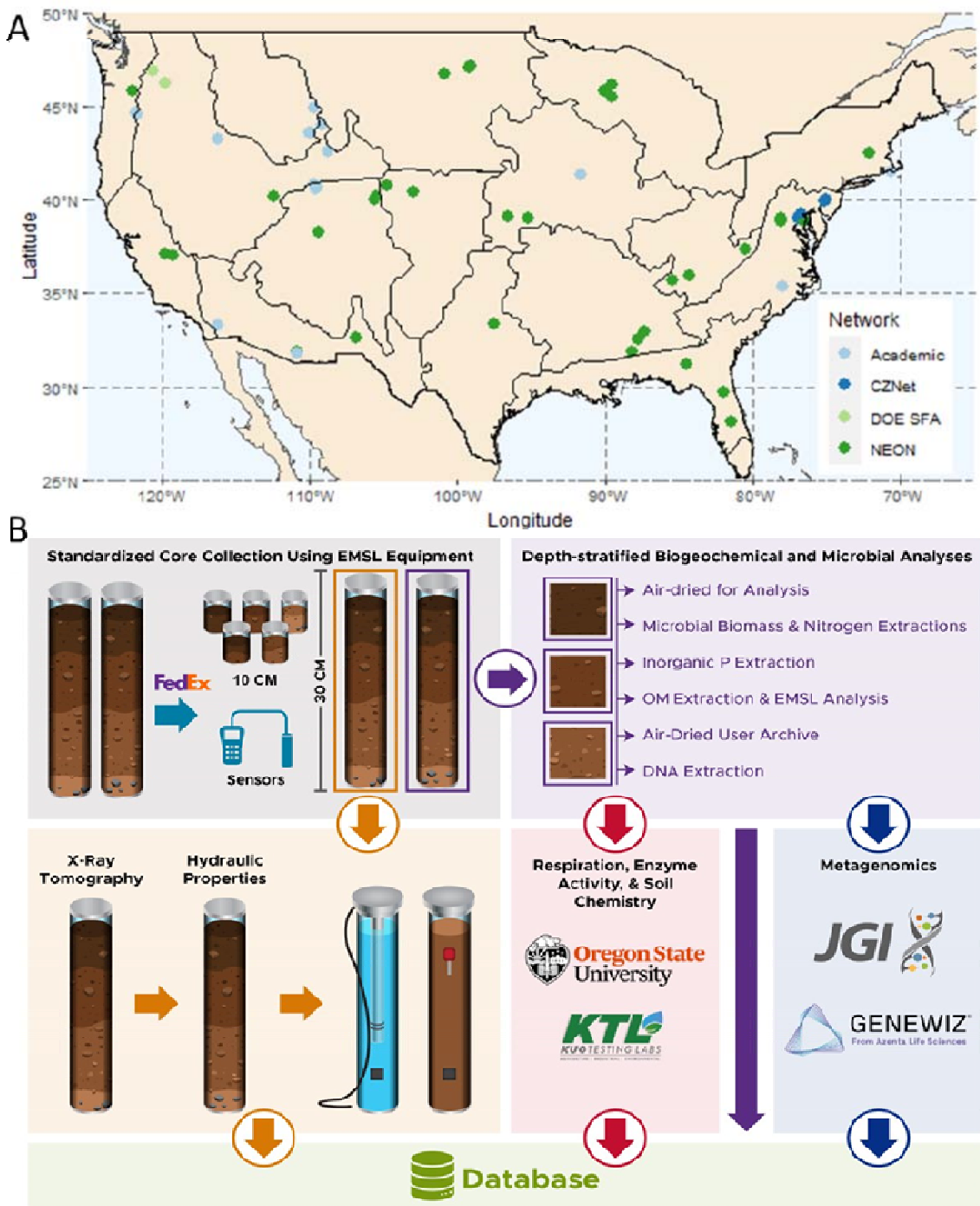

Figure 1: (A) Sites included in the 1000 Soils Pilot overlain on National Ecological Observatory Network (NEON) eco-domains. The color of the symbol indicates site affiliation as Academic (light blue), CZNet (dark blue), DOE Science Focus Area (light green), and NEON (dark green). (B) Soil core processing workflow at the Environmental Molecular Sciences Laboratory (EMSL).

Data Generation Overview.

Collaborators shipped all cores to EMSL on ice within 24 hr of core collection for immediate processing. One 30-cm core was dedicated to a biotic workflow for time sensitive measurements, and the other 30-cm core was dedicated to an abiotic workflow for structural and hydraulic characterization (Figure 1B). To account for surface heterogeneity, three of the 10 cm cores were homogenized with the top 10 cm of the larger biotic core. A full list of data types generated by the 1000 Soils Pilot is available in SI Table 1.

Immediately on arrival (<48 hr.), we processed the biotic core by separating it into 10 cm depth intervals, which were then sieved at 4 mm. The middle section was archived, and all analyses were conducted on homogenized top (0-10 cm) and bottom (deepest 10 cm) sections as described below. We measured microbial biomass C and N via chloroform fumigation (Brookes et al., 1985; Witt et al., 2000; Zhao et al., 2022). Aliquots of K_2SO_4 extractions from the initial (background) steps of microbial biomass measurements were archived at- 20˚ C for later analysis of NO_3 via Cd-reduction method and NH_4 via spectroscopic determination (Bremner, 1965; Dahnke and Johnson, 1990). Inorganic P was extracted from each soil section via Bray or Olsen extraction (depending on pH) and subsequent analysis via methods outlined in (Bray and Kurtz, 1945; Olsen et al., 1982). We collected sub-samples of soil for DNA extraction and subsequent metagenomic sequencing on the Illumina NovaSeq platform (stored at -80˚ C). We measured soil pH using a 1:1 soil to water ratio using a calibrated pH probe. Gravimetric water content was determined via oven drying at 60˚ C. Remaining soil from each section was air dried prior to the analyses below. Beta-glucosidase potential activity and respiration rates were measured using colorimetric assays and the CO_2 burst method. Cation concentrations from 1:10 NH4OAc extraction (K, Ca, Mg, Na) and 1:2 soil to DPTA extraction solution (Zn, Mg, Cu, Fe, B, SO_4^{2-} - S) extraction were measured with Inductively coupled plasma mass spectrometry (ICP-MS), while SOM concentration was determined by TOC-TN analyzer and composition from LC-MS, and FTICR-MS analysis. Details on SOM characterization are provided in the following sections.

The abiotic core was used to determine soil structure and hydraulic properties. We used X-ray Computed Tomography (XCT) to measure soil pore size, connectivity, distribution, and volume, as detailed below. In addition, soil hydraulic properties were measured using a series of instruments by The Meter Group (KSat, Hyprop, WP4C).

Experimental Methodology for Soil Physical Characterization by X-ray Computed Tomography.

The intact abiotic soil core was imaged using XCT on an X-Tek/Metris XTH 320/225 kV scanner (Nikon Metrology, Belmont, CA, USA). Data was collected at 110 kV and 325 µA X-ray power. A 0.5-mm thick Cu filter was used to enhance image contrast by blocking out low-energy X-rays. The core was rotated continuously during the scans with momentary stops to collect each projection (shuttling mode) while minimizing ring artifacts. A total of 2000 projections were collected over 360˚ rotation recording 2 frames per projection with 500 ms exposure time per frame. Image voxel size was 70.1

microns. The whole core was imaged in two segments/tiles to increase spatial resolution.

Higher resolution images were collected by zooming in on both the top and bottom sections 10 cm of the core. These data were also collected at 110 kV and 325 μA X-ray power with a 0.5-mm thick Cu filter. A total of 3142 projections were collected over 360˚ rotation recording 2 frames per projection with 708 ms exposure time per frame. Image voxel size was 33.6 microns. Data analysis procedures for XCT are described in the SI.

Detailed methods for FTICR-MS and XCT are included in the supporting information.

Case Study: Impacts of Wildfire Regimes on Soil; Warm Springs, Oregon

Dataset Description.

Our primary intent in this paper is to stimulate discussion and interest in large-scale collaborative efforts by introducing the 1000 Soils Pilot. To facilitate this discussion, we present initial results from three of the first cores in the Pilot project, collected in November 2021 in partnership with the Confederate Tribes of Warm Springs in Warm Springs, OR, USA. The high frequency burn (HB) site had moderate severity burns in 2003, 2014 and 2020; the moderate frequency burn (MB) site had moderate severity burns in 2014 and 2020; and the unburned (UB) site had no recent burn history within the last 20 years.

This subset of data is intended as a proof of concept of the utility of high-resolution data in understanding impacts of fire burn histories on soil physio-chemical properties and microbial functions. Though the dataset is small and does not allow for robust statistics (n = 3), it provides a depth of information that is unfeasible with traditional biogeochemical approaches. With these differences in mind, we compared FTICR-MS and XCT across burn histories and soil sections to show the differences in molecular SOM composition and soil structure.

Soil Biogeochemistry and Microbial Activity.

We first compared soil biogeochemical properties including, β-glucosidase activity (BG), microbial biomass C (MBC) and N (MBN), and respiration at 24 and 96 hr for evidence of differences among soil sections and burn histories (Fig 2A and 2B). Elevated microbial biomass concentrations, respiration rates, and potential enzyme activities in the unburned site are consistent with increased biological availability when compared to the burned sites (Fairbanks et al., 2020). We found the highest respiration rate at UB followed by the MB, with the lowest respiration at HB (Figure 2B). This is consistent with other literature, showing the sterilizing effects of high frequency fires on microbial respiration (Ajwa et al., 1999; Lombao et al., 2021). Across the first 24 hours after re-wet, the rate of CO_2 produced was greater in the top 10 cm vs. the lowest 10 cm across all cores. However, after 96 hours the rate of CO_2 production in soils from the bottom 10 cm was greater than the top 10 cm for all sites (Fairbanks et al., 2020). Similarly, β-glucosidase potential activity and microbial biomass C and N were highest at UB (SI Figure 1A and Figure 2B respectively). Lower microbial activity at the burned sites may

be related to chemical recalcitrance or physical protection of SOM related to the fires (Ajwa et al., 1999; Lombao et al., 2021). For all the sites, respiration increased between 24 and 96 hours in the bottom 10 cm, possibly reflecting less bioavailable SOM in deeper soils than in surface soils (Fang and Moncrieff, 2005). Corresponding changes in soil chemistry are show in SI Figure 2.

X-ray Computed Tomography.

To examine the pore space heterogeneity across soil sections and burn histories, we used XCT to interrogate the top and bottom 10 cm of each soil core. We compared total porosity, pore connectivity, and mean pore volume between the top and bottom sections of cores from UB, MB, and HB. Wildfire can impact soil structure from up to 10 cm depth depending on its duration, frequency, and intensity (Doerr et al., 2006). For UB and HB, we observed higher porosity and pore connectivity in the top sections when compared to the bottom sections, this is likely related to the higher abundance of root systems in the top 10 cm. However, there was minimal difference between total porosity or connectivity between the top and bottom section of the MB site, possibly due to a large root section resulting in a larger mean pore size (SI Figure 5 and SI Table 2). Across all cores, total porosity was more variable in the top than bottom section (8.82-20.08% vs 6.52-8.49%; SI Table 2). Pore connectivity was highest in the top section at UB (96.41%) and lowest in the bottom section at UB (70.12%); potentially indicating a homogenizing effect of wildfire on pore network structure at MB and HB.

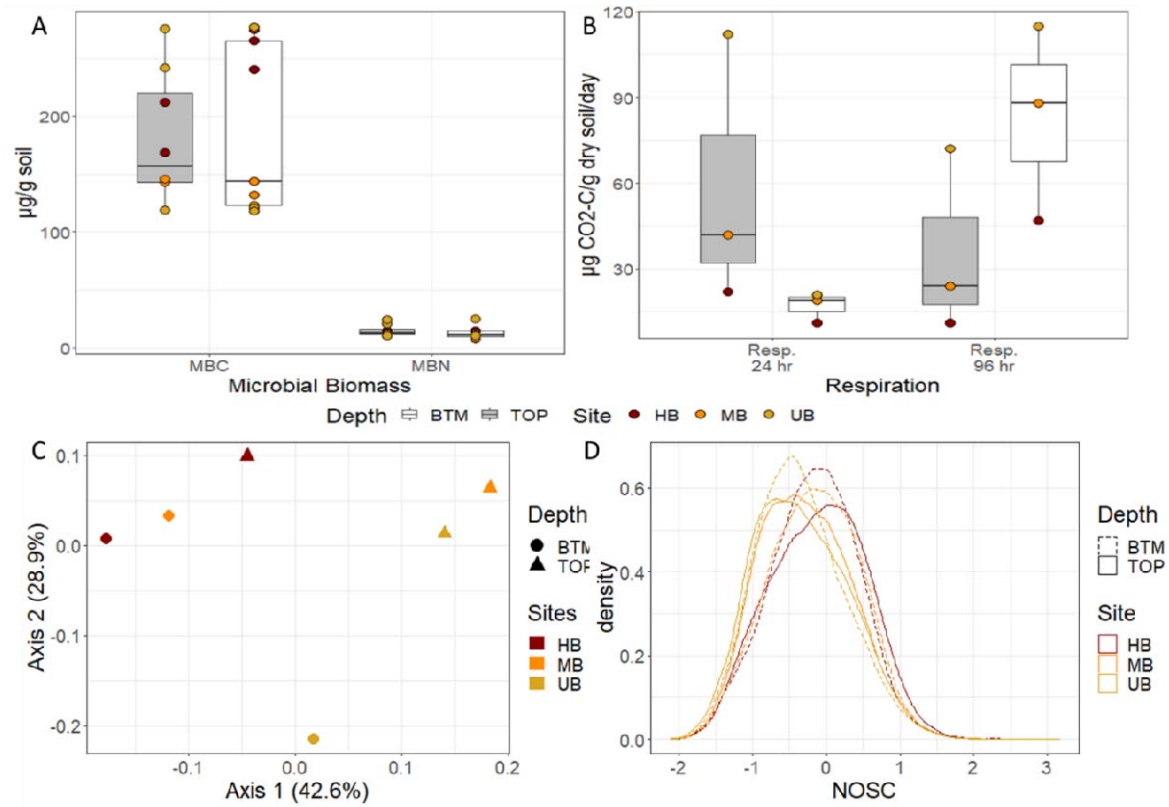

Figure 2: A) Microbial biomass C (MBC) and N (MBN) concentrations, B) respiration (24 and 96 hour; µg CO_2-C/g dry soil/day), C) SOM composition, and D) NOSC of SOM

profiles. A and B are boxplots where the median is represented by a line, the edges of the boxes reflect 25th and 75th percentile, and the end of the whiskers reflect the minimum and maximum. C shows a principal component analysis (PCA) of SOM composition, and D displays a density curve of NOSC values for each sample. For all plots, UB, MB, and HB are denoted in yellow, orange, and red respectively. In A and B, data from top and bottom sections are denoted by grey and white boxes respectively. In C, section is denoted by shape, and in D, section is denoted by dashed vs. solid lines.

Soil Organic Matter Composition.

Soil organic matter is derived from chemical, physical, and biological processes that decompose plant and animal residues into microbial metabolites and other byproducts (Jobbágy and Jackson, 2000; Köchy et al., 2015; Lehmann and Kleber, 2015; Schmidt et al., 2011; Tarnocai et al., 2009). This generates a heterogenous mixture of compounds in SOM pools. We used FTICR-MS to distinguish differences in SOM chemistry along depth profiles and across the three wildfire regimes. There was a clear separation in water-extractable SOM composition between the top and bottom sections of each core as well as across burn regimes, as indicated by principal component analysis (PCA) (Figure 2C). Additionally, the bottom sections exhibited more similar SOM pools (i.e., tighter clustering) than the top sections, possibly signifying stronger impacts of wildfire on surficial soils.

Since fire results in the oxidation of SOM to CO_2, we hypothesized that the extent of SOM oxidation, as indicated by NOSC, would be related to burn regime. In the top section, the average NOSC of SOM increased with stronger burn regimes (-0.34, -0.29, and -0.11 respectively for UB, MB, and HB), indicating more oxidization, while there was a binary effect of burning on NOSC in the bottom section. MB and HB had similar mean NOSC that was higher than UB (-0.16 vs -0.35). The presence of more oxidized SOM in burned soils, are likely related to the incomplete oxidation of SOM and the addition of charred materials from wildfire (Schmidt and Noack, 2000; Velasco-Molina et al., 2016; Watson et al., 2005). While limited to three sites, changes in NOSC could be an indicator of previous fire exposure, with the NOSC becoming more oxidized with increased high frequency fire exposure that persists even into deeper soil horizons.

Inferred Biochemical Transformations and Microbial Metabolism of SOM.

We also analyzed FTICR-MS spectra to understand potential biochemical transformations and microbial pathways involved in SOM decomposition. Commonly inferred biochemical transformations such as the addition of methyl chain (CH_2) groups were abundant at all sites and depths. We also note that one of the most abundant transformations observed was the transformation of carboxylation groups to CO_2 (greater than 1400 occurrences in each site and depth). The transformations of carboxylation groups to CO_2 are likely associated as a combustion byproduct associated with incomplete oxidation of OM during fire exposure. Within each core, the oxidation and/or addition of CO_2 accounted for a greater proportion of transformations at depth when compared to the top section at the same site (Hockaday et al., 2007). This

is potentially related to the translocation of water-soluble oxidized OM to deeper soil sections.

Additionally, we mapped compounds detected by FTICR-MS to microbial reference pathways in KEGG to infer mechanisms of SOM decomposition. Across all samples, nearly 50% of all mapped metabolites were detected in a set of eight metabolic pathways. These included ‘biosynthesis of secondary metabolites’ (map01110, 11.7%), ‘metabolic pathways’ (map01100, 8.4%), ‘biosynthesis of antibiotics’ (map01130, 7.5%), and ‘microbial metabolism in diverse environments’ (map01120, 4.9%) among other pathways (SI Table 4). Notably, the degradation of aromatic compounds (map012220) and the polycyclic aromatic hydrocarbons (PAH) degradation (map00624) are also among pathways with the highest number of mapped metabolites (16th and 19th most metabolites). The degradation of aromatic compounds was the most commonly mapped in the core with high frequency burns (HB). The degradation of PAHs was most common in the MB site; 1.61% of metabolites mapped to metabolic pathways in the moderate frequency burned core were associated with the degradation of PAHs in comparison to <1% of mapped metabolites in the unburned core.

Towards a CONUS-scale high resolution molecular SOM database

Earth system models currently rely on soil attributes such as soil texture, moisture, and C concentration to parameterize C fluxes. This highly simplified approach results in high levels of uncertainty at the pore to core scale. Molecular information has the potential to reduce this uncertainty through rapidly improving multiscale models (Blankinship et al., 2018; Feng et al., 2021; Kramer and Chadwick, 2018; Nave et al., 2021; Quesada et al., 2020; Rasmussen et al., 2018; Viscarra Rossel et al., 2019; Von Fromm et al., 2021; Yu et al., 2021).

To achieve these goals, MONet is developing a database of high-resolution organic matter, genomic, and soil structure preservation from soil cores across the CONUS. We collaborate with existing databases and networks, as well as with individual researchers, using standardized sample collection and high throughput processing pipelines, metadata documentation, and raw and processed open data publication. The focus of our data generation is on variables that are key to the parameterization of hierarchical multiscale models beginning with microbially explicit models and culminating in Earth system models.

In this article, we present the 1000 Soils Pilot from the first year of MONet and a use case to highlight the utility of high-resolution measurements for understanding the impacts of wildfires on soils. While the statistical analysis is limited by the small sample number (n=3), we observed trends in soil biogeochemical values, soil structure, and SOM composition related to fire history and soil section that are consistent with existing literature. We are currently accepting requests for collaboration in 2023 and anticipate that data from the first set of ~75 cores will be available in early 2023. With the support and collaboration of the scientific community, we hope to facilitate the next generation of fundamental knowledge and model representations of soil C cycling, which in turn constrain uncertainties in the global climate.

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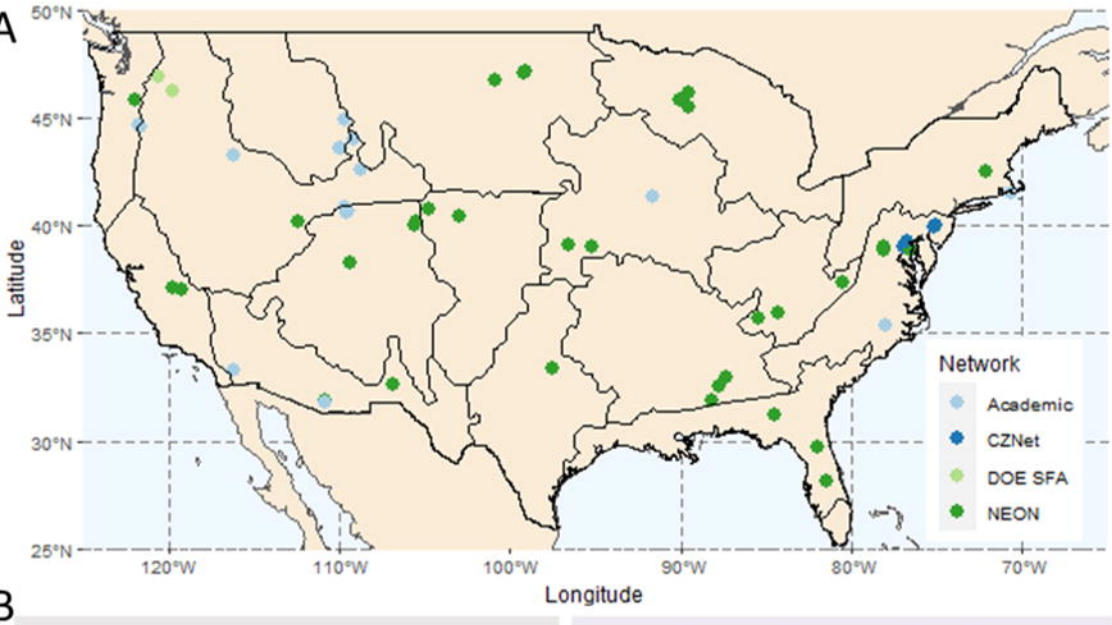
A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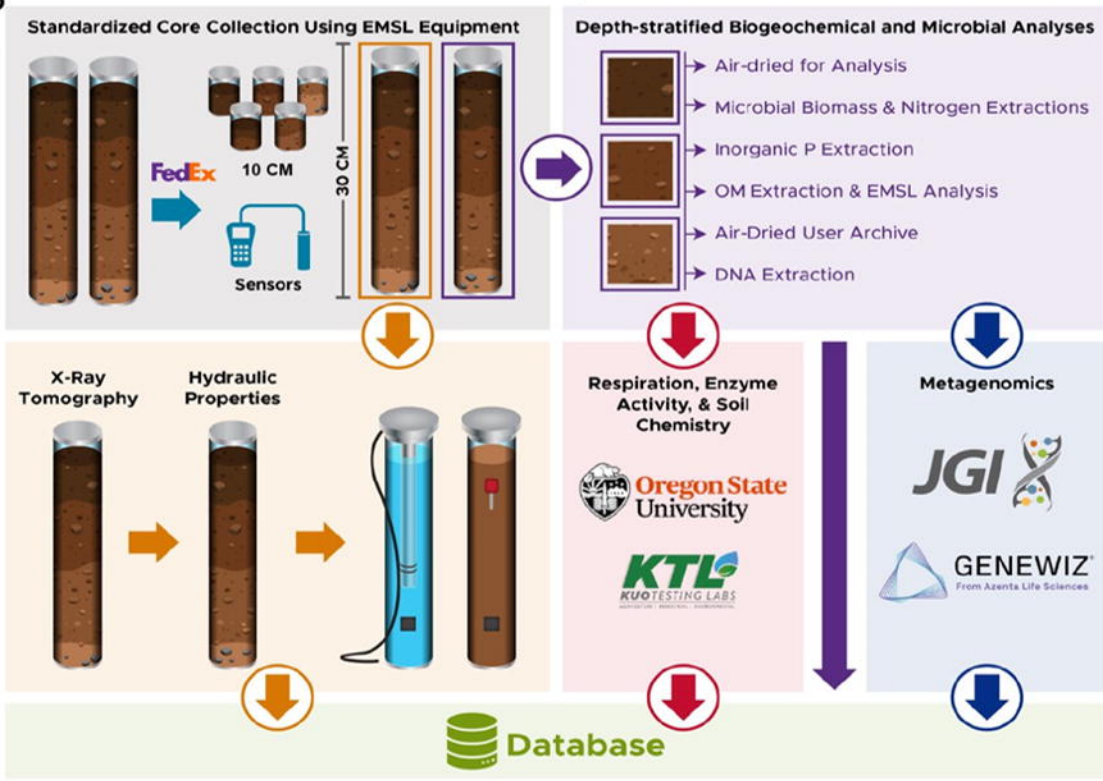
Standardized Core Collection Using EMSL Equipment
FedEx
10 CM
30 CM
Sensors
Depth-stratified Biogeochemical and Microbial Analyses
Air-dried for Analysis
Microbial Biomass & Nitrogen Extractions
Inorganic P Extraction
OM Extraction & EMSL Analysis
Air-Dried User Archive
DNA Extraction
X-Ray Tomography
Hydraulic Properties
Respiration, Enzyme Activity, & Soil Chemistry
Oregon State University
KTL
KUO TESTING LABS
Metagenomics
JGI
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From Azenta Life Sciences
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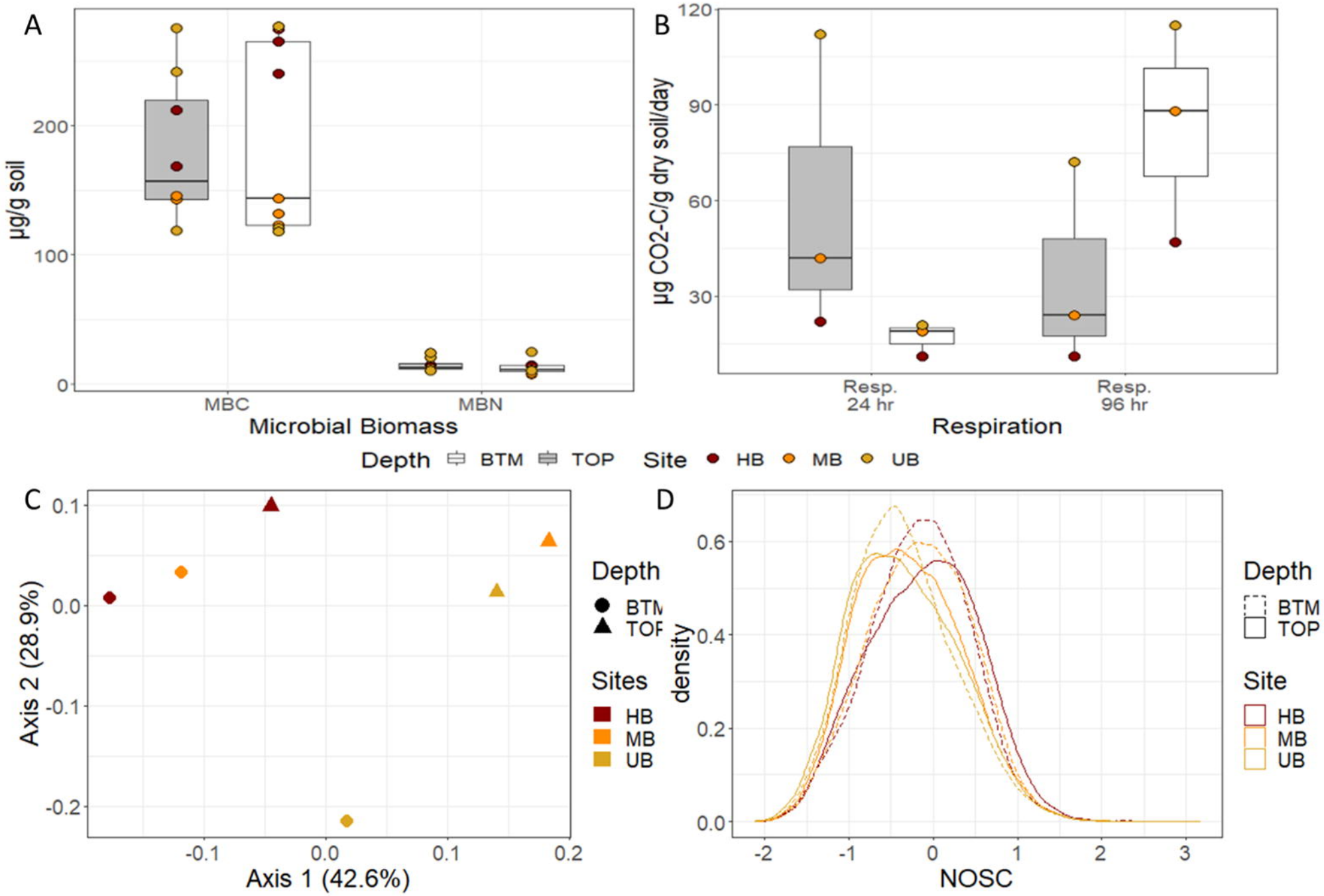
A
µg/g soil
0
100
200
MBC
MBN
Microbial Biomass
B
µg CO2-C/g dry soil/day
30
60
90
120
Resp. 24 hr
Resp. 96 hr
Respiration
Depth BTM TOP
Site HB MB UB
C
Axis 2 (28.9%)
0.1
0.0
-0.1
-0.2
Axis 1 (42.6%)
-0.1
0.0
0.1
0.2
Depth
BTM
TOP
Sites
HB
MB
UB
D
density
0.0
0.2
0.4
0.6
NOSC
-2
-1
0
1
2
3
Depth
BTM
TOP
Site
HB
MB
UB