January 25, 2024

Interrelationships among methods of estimating microbial biomass across multiple soil orders and biomes: Supporting data

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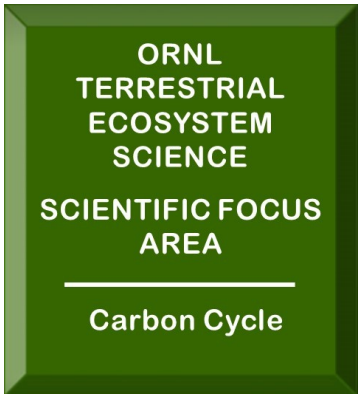

1. Data Set Overview:

Summary:

This dataset contains environmental and soil measurements from 18 different locations across the globe including the SPRUCE experiment site and multiple sampling depths, with 17 of these locations having samples processed between 2012-2013 and one location (SPRUCE) collected in 2021 and processed in 2022. Environmental measurements include: mean annual temperature, mean annual precipitation, and 30-day presampling temperature. Soil physicochemical measurements include: particle size analysis (PSA), pH, gravimetric moisture content (GMC), bulk soil carbon (C) and nitrogen (N), total organic C and N, C:N ratio, and dissolved organic carbon (DOC). Soil biological measurements include: microbial biomass carbon (MBC) measured through chloroform fumigation extraction (CFE), gene copy numbers (GCN) of bacteria, fungi, and archaea measured through quantitative polymerase chain reaction (qPCR), DNA yield measured through Nanodrop spectrophotometry, and phospholipid fatty acids (PLFA) of bacteria and fungi measured through PLFA analysis. This data set contains one file in comma separate (*.csv) format.

Relevant Publication:

These data were used in the following publication:
Buell, Z.W., Dabbs, J., Steinweg, J.M., Kluber, L.A., Phillips, J.R., Yang, Z.K., Miller, R.M., Gutknecht, J.L.M., Schadt, C.W., and Mayes, M.A. **Interrelationships among methods of estimating microbial biomass across multiple soil orders and biomes.** [Manuscript in Preparation]

Data Citation:

Cite this data set as follows:
Buell, Z.W., J. Dabbs, J.M. Steinweg, J.R. Phillips, L.A. Kluber, Z.K. Yang, R.M. Miller, J.L.M. Gutknecht, C.W. Schadt, and M.A. Mayes. 2024. **Interrelationships among methods of estimating microbial biomass across multiple soil orders and biomes: Supporting data.** Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/ornlsfa.031/2274949.

Related Data Sets

The present dataset summarizes selected data from the following data sets:

Buell, Z., J. Philips, S. Ottinger, K. Lowe, C.W. Schadt, & M.A. Mayes. 2024. **Chloroform Fumigation Extraction for Microbial Biomass and Dissolved Organic Carbon from SPRUCE, 2021-2022.** Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.109/1998876.

Buell, Z., M. Felice, J. Philips, S. Ottinger, K. Lowe, & J.L.M. Gutknecht. 2024. **SPRUCE Phospholipid Fatty Acid (PLFA) Abundances, August 2021-June 2022**. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.112/1998897.

Roth, S., Z. Buell, B. Kristy, J. Philips, S. Ottinger, K. Lowe, M.A. Mayes, & C.W. Schadt. 2024**. SPRUCE Quantitative PCR of microbial gene copy numbers, 2021-2022**. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.110/1998878.

2. Data Characteristics:

This data set contains one comma separated (*.csv) file:

- **TESSFA_SMB_proxy.csv**: Contains soil physicochemical and biological measurements from 16 sites.

Spatial Coverage

Measurements were taken from a total of 16 locations: Lavras, Brazil; Kakamega, Kenya; the Boston Area Climate Experiment (BACE) site, MA; the Prairie Heating and Carbon Dioxide Enrichment Experiment (PHACE) site, WY; the Missouri Ozark Forest AmeriFlux (MOFLUX) site, MO; Marcell Experimental Forest (MEF) and S1 Bog Spruce and Peatland Responses Under Changing Environments (SPRUCE) site, MN; Loma Ridge, CA; Melton Branch, TN; Walker Branch, TN; Big Ridge, TN; Fermi, IL; Critical Zone, PA; Fairbanks, AK; Barrow, AK

Temporal Coverage

The majority of soils were collected and shipped to ORNL for processing between 2012-11-1 and 2013-08-16. Peat samples from the SPRUCE site were collected 2021-08-24.

Data File Description

These data are considered at **Quality Level 1**. Level 1 indicates an internally consistent data product that has been subjected to quality checks and data management procedures.

Missing numeric data are indicated by -9999.

Data Dictionary for *TESSFA_SMB_proxy.csv*

Column Name	Units	Description
Site_Id		3 letter code to denote sampling location
Site_location		Name of country/region/site where sample was taken
Latitude	Decimal degrees	Approximate site latitude
Longitude	Decimal degrees	Approximate site longitude
Soil_order		Singular (-sol) classification of soil characteristics
Soil_horizon		Layer of soil profile in which sample was taken
Climate		Broad climate type in which sampling site is located
Temp_annual	(°C)	Mean Annual Temperature in °C of sampling location
Precip_annual	(cm)	Mean Annual Precipitation in cm of sampling location
Temp_30d_mean	(°C)	Mean temperature of the 30 days leading up to sampling at location
GMC	(g water g-1 dry soil)	[[mass of moist soil - mass of dried soil] / mass of dried soil]
pH		pH of sample dissolved in Milli-Q water
PSA_sand	%	Proportion of sample mineral content determined as sand using particle size analysis
PSA_clay	%	Proportion of sample mineral content determined as clay using particle size analysis
PSA_silt	%	Proportion of sample mineral content determined as silt using particle size analysis
Bulk_soil_C	%	Amount of total carbon measured in soil using Leco Combustion Analyzer
Bulk_soil_N	%	Amount of total nitrogen measured in soil using Leco Combustion Analyzer
TOC	%	Amount of total carbon measured in soil using Leco Combustion Analyzer after acid treatment
TN	%	Amount of total nitrogen measured in soil using Leco Combustion Analyzer after acid treatment
TIC	%	Amount of total inorganic Carbon. Bulk_Soil_C - TOC

CN_ratio		Ratio of C to N in bulk soil sample
DOC_mean	ug C g-1 dry soil	Mean dissolved organic carbon [Unfumigated TOC×[Volume of K2SO4 / dry weight]] of 3 soil subsamples
DOC_SE	ug C g-1 dry soil	Standard error of dissolved organic carbon of 3 soil subsamples
MBC_mean	ug C g-1 dry soil	Mean microbial biomass carbon [[Fumigated DOC - Unfumigated DOC] / 0.45] of 3 soil subsamples
MBC_SE	ug C g-1 dry soil	Standard error of microbial biomass carbon of 3 soil subsamples
Bacteria_copy_dry	gene copy number g-1 dry soil	qPCR bacterial gene copy number per gram of dry soil
Fungi_copy_dry	gene copy number g-1 dry soil	qPCR fungal gene copy number per gram of dry soil
Archaea_copy_dry	gene copy number g-1 dry soil	qPCR archaea gene copy number per gram of dry soil
Total_copy_dry	gene copy number g-1 dry soil	Sum of qPCR bacterial and fungal gene copy numbers per gram of dry soil
DNA_yield_dry	ng g-1 dry soil	Concentration of microbial DNA in ng per gram of dry soil
Fung_bact_copy_ratio		Ratio of fungal gene copies to bacterial gene copies within a sample
Bacteria_copy_dry_clay	gene copy number g-1 dry soil	qPCR bacterial gene copy number per gram of dry soil with clay correction factor applied
Fungi_copy_dry_clay	gene copy number g-1 dry soil	qPCR fungal gene copy number per gram of dry soil with clay correction factor applied
Archaea_copy_dry_clay	gene copy number g-1 dry soil	qPCR archaea gene copy number per gram of dry soil with clay correction factor applied
Total_copy_dry_clay	gene copy number g-1 dry soil	Sum of qPCR bacterial and fungal gene copy numbers per gram of dry soil with clay correction factor applied
DNA_yield_dry_clay	ng g-1 dry soil	Concentration of microbial DNA in ng per gram of dry soil with clay correction factor applied
PLFA_total	nmol g-1 dry soil	Nanomoles of total microbial lipids per gram of sample
PLFA_bacteria	nmol g-1 dry soil	Nanomoles of total bacterial lipids per gram of sample
PLFA_fungi	nmol g-1 dry soil	Nanomoles of total fungal lipids per gram of sample
PLFA_fung_bact_ratio		Ratio of fungal lipids to bacterial lipids within a sample

3 Applications and Derivation

This dataset contains diverse microbial and environmental data from a broad range of geographical areas and soil types. Numerous comparisons can be made between these various

metrics, allowing for the potential substitution of methods for each other and/or within ecosystem models.

4. Quality Assessment:

These data are considered at **Quality Level 1**. Level 1 indicates an internally consistent data product that has been subjected to quality checks and data management procedures. Established calibration procedures were followed.

5. Data Acquisition Materials and Methods:

Study Sites

Fresh soils were collected from a variety of climate regions and soil orders, including Aridisol (tropical), Oxisol (tropical), Ultisol (tropical and temperate), Inceptisol (temperate), Mollisol (temperate), Alfisol (temperate and boreal), Histosol (southern boreal) and Gelisol (sub-Arctic and Arctic). For most collection locations, a surface and a subsurface horizon were sampled for this analysis, e.g. A and B horizons from mineral soils, although in some cases only an A horizon was collected. For the Marcell Experimental Forest (MEF) bog Histosol, a sample from near-surface and at depth were collected. For permafrost soils, only the surface active layer was represented. In total, 18 surface and 15 subsurface soil samples were used for this comparison.

Methods

Soil treatment and characterization

Soil samples were stored at -20 ºC for up to five days before being sieved (2 mm) and subsampled (n = 3) for analyses of dissolved organic carbon (DOC), MBC, dry weight, and extraction of DNA. MEF bog qPCR and PLFA subsamples were stored at -80 ºC prior to analysis.

Gravimetric moisture content (GMC) was calculated by allowing 5 g of field moist soil to air dry for three days and using the equation:

$$\theta_{dw} = \frac{m_f - m_d}{m_d}$$

where m_f is the mass of fresh field moist soil and m_d is the mass of the air dried soil. Total bulk soil C (TC) and nitrogen (TN) were determined by the Leco Combustion Analyzer (Leco Corp., St. Joseph, MI) (Table 2). Total organic C (TOC) concentrations were determined by the same method after treating samples with 3*M* HCl for 1 hr. Particle size analysis for soil texture was

evaluated with the Bouyoucos hydrometer method (Gee and Or, 2002). Soil pH was determined by shaking 1 part soil in 2 parts Milli-Q water and measuring the pH of the supernatant.

Subsamples (n = 3) of 7 g fresh soil were used to measure DOC for each soil type. The soil was combined with 0.035L of 0.5 M K_2SO_4, and the samples were shaken on an orbital reciprocating shaker for 1 hour. Afterward, the soil suspensions were gravity filtered with Whatman No. 42 filter paper, and the extracts were immediately stored at -20° C. The extracts were analyzed using the Shimadzu Total Organic Carbon Analyzer (Shimadzu Corp., Kyoto, Japan) to obtain values for DOC.

Microbial community characterization

Microbial biomass by chloroform fumigation-extraction

Subsamples (n = 3) of 7 g fresh soil were thawed from -20° C and fumigated with chloroform for a total of 48 h at 25° C. The fumigated soil was then combined with 0.035 L of 0.5 M K_2SO_4, and treated identically to the DOC extractions in Section 2.1. On a subset of soils we measured MBC on fresh, never frozen soil samples and obtained similar values within standard error of the samples frozen at -20°C prior to fumigation.

Estimates for MBC were then calculated using the equation

$$MBC = \frac{K_C}{E_C}$$

where K_C is the difference between extractable carbon before and after fumigation and E_C is the extraction efficiency coefficient (Fierier et al., 2009). Although extraction efficiency will vary by individual soil, an E_C of 0.45 was applied here, as this is a standard value typically applied in the literature for mineral soils (Fierier et al., 2009; Vance at al., 1987).

PLFA analysis

Lipid analysis was performed according to an adaptation of the Bligh and Dyer (1959) method outlined in Gray et al. (2011). Extractions were performed on freeze-dried 2-g subsamples using a 1:2:0.8 ratio of chloroform, methanol, and phosphate buffer (pH 7.4). Water and chloroform were then added after three hours to produce a phase separation isolating total lipids in the chloroform layer. Phospholipids were separated using silicic acid column chromatography, then saponified and methylated using an alkaline solution to produce fatty acid methyl esters (FAMEs). Dried phospholipids were then reconstituted using a known concentration of FAME 19:0 as an internal standard to quantify PLFA concentrations. Samples from the Marcell Bog were processed using a modified Bligh and Dyer (1959) method modified for peat extractions (Blake, 2017). In contrast to the previously described method (Gray et al., 2011), a 1:1:0.9 ratio of chloroform, methanol, and citrate buffer (pH 4.0) was used to isolate total lipids and samples were not saponified prior to methylation.

Nomenclature of fatty acids adheres to Frostegård et al. (1993). Specific PLFAs were used to quantify relative abundance of bacterial and fungal biomass. Calculations of total PLFAs comprise fatty acids with less than 20 Cs (Zelles, 1999). Bacterial biomass calculations included the sum of fatty acids 14:0, i15:0, a15:0, 15:0, i16:0, 16:0, 16:1w7, 10me16:0, i17:0, a17:0, cy17:0, 18:2w7, 18:0, 10me18:0, cy19:0a (Zelles, 1999), while fungal biomass is represented by the sum of 18:2w6 (Zelles, 1999) and 18:1w9 (Zak et al., 1996).

Microbial DNA yield and gene copy numbers with qPCR

Subsamples (n = 3) of each soil type were stored at -80° C, and microbial DNA was extracted from 0.25 g of soil using the PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc., CA, USA) or the E.Z.N.A. Soil DNA Kit (Omega Biotek, Norcross, GA, USA) for the SPRUCE peat histosols. The concentration and purity of the extracted DNA was measured using the NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.). The same general approach outlined by Fierer et al., (2005) was then used to quantify GCN on a dry weight basis for bacteria, fungi, and archaea (Table 3). Analyses for each soil were conducted in analytical triplicate and set up in clear 96-well plates. Each qPCR reaction consisted of 19 µl of MasterMix—5 µl H_2O, 2 µl of forward primer, 2 µl of reverse primer, and 10 µl of SYBR Green SuperMix (Bio-Rad Laboratories, Inc.)—combined with 1 µl of extracted microbial DNA.

Pure culture standards of Escherichia coli, Saccharomyces cerevisiae and Methanococcus maripaludis of known DNA concentration were diluted to 1:10, 1:100, 1:1,000, and 1:10,000 and were used to generate a standard linear curve relating the log of the GCN to the measured threshold value (C_t). This standard curve was then used to convert sample threshold values measured on a CFX96 Real-Time System (Bio-Rad Laboratories, Inc., CA, USA) into GCN g^{-1} dry soil.

6. Related References:

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7. Data Access:

Get Data

For public access to data from the US Department of Energy Terrestrial Ecosystem Science Scientific Focus Ares (TES-SFA) please visit: **https://tes-sfa.ornl.gov/node/80**