Soil Respiration and Microbial Biomass from Soil Incubations with ^{13}C Labeled Additions

ORNL
TERRESTRIAL
ECOSYSTEM
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SCIENTIFIC FOCUS
AREA

Soil Carbon
Decomposition
Experiments

Summary:

This data set provides the respiration and soil microbial biomass data from a series of short and long-term laboratory incubation experiments with ^{13}C labeled substrates to examine how plant communities (forest vs. grassland), edaphic properties, and microbial communities influence C cycling and the long-term fate of C in soil systems. Paired forest and grassland soils from four locations were incubated with ^{13}C labeled glucose in a short-term (144 hours) study while ^{13}C labeled cellulose was added to soils used in the long-term (729 days) study. Soil respiration, microbial biomass, and soil ^{13}C values were monitored throughout the study.

In addition to improving the conceptual understanding of microbially-facilitated decomposition, these experiments were designed to parameterize and test the Microbial ENzyme Decomposition (MEND) model (Wang et al., 2013, 2014, 2015).

This data set contains one *.csv file with soil respiration rates, soil microbial biomass, and $\delta\,^{13}C$ values of CO_2 and soil.

Figure 1. Soils were collected from paired forest and grassland plots within each of four different soil types. Image on the right shows incubation setup.

Data and Documentation Access:

Get Data: https://tes-sfa.ornl.gov

Companion Documentation: https://tes-sfa.ornl.gov/node/80

Data Citation:

Cite this data set as follows:

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1. Data Set Overview:

This data set accompanies a laboratory incubation of 8 individual soils (4 locations x 2 types). Aerobic microcosms were constructed and incubated at room temperature and maintained at 30% gravimetric water content. Short term (144 hour) incubations included ^{13}C labeled glucose and control treatments. Long term (729 day) incubations included ^{13}C labeled cellulose and control treatments. Soil respiration, microbial biomass, and soil ^{13}C were monitored periodically over the duration of the studies.

2. Data Characteristics:

Spatial Coverage:

Soils were collected from paired forest and grassland locations in the following states in the United States of America: Tennessee (TN), Ohio (OH), Iowa (IA), and Missouri (MO).

Table 1: Sampling locations and taxonomy of soils used in the incubation study

State	Location	Owner/Manager	Soil Series	Soil Taxa
TN	35.93 N -84.31W	Oak Ridge National Laboratory	Etowah	Typic Paleudults
OH	39.32 N -82.12 W	Ohio University	Westmoreland	Ultic Hapludalfs
IA	41.79 N -93.43 W	Chichaqua Bottoms Greenbelt	Coland	Cumulic Endoaquolls
MO	38.74 N -92.19 W	University of Missouri	Kesswick	Aquertic Chromic Hapludalfs

Temporal Coverage:

The paired soil samples were collected in the fall of 2014. Data herein correspond with a series of laboratory incubations that began in the 2014 and continued through 2017. Short term incubations were conducted for 144 hours while long term incubations were conducted over a period of 729 days.

Data File Description:

There is one archived file provided in comma separated value (*.csv) format.

Soil_Respiration_Microbial_Biomass_From_Soil_Incubations_20170518.csv

Missing Values:

Blank cells represent missing values because a given analysis was not performed at a given time point.
ND is used to indicate when measurements failed due to analytical issues.

Data Dictionary:

Column	Column Name	Units / Format / Range	Additional Description
1	Sample_ID		Code used for laboratory purposes
2	Experiment		Indicates whether data correspond to long or short term study
3	TimePoint		Time point for sample collection
4	Time_units		Hour units for short term study Day units for long term study
5	DeltaTime		Hours since previous sampling. Used in cumulative respiration calculations
6	State		State of soil origin
7	Veg_Type		Vegetation type where soil originated: Forest or Grassland

Column	Column Name	Units / Format / Range	Additional Description
7	Amendment		Microcosm amendment: ^{13}C-Cellulose ^{13}C-Glucose Control (no addition)
9	Rep		Laboratory replicate: 3 replicates at each time point
10	CO2_delta13C	per mil	Isotopic signature of respired CO_2
11	Resp_rate	μg CO_2-C g^{-1} hr^{-1}	Respiration rate
12	Soil_N	%	Soil N content (%)
13	Soil_C	%	Soil C content (%)
14	Soil_delta15N	per mil	Isotopic signature of soil N
15	Soil_delta13C	per mil	Isotopic signature of soil C
16	DOC	μg C g^{-1} soil	Dissolved organic carbon
17	MBC	μg C g^{-1} soil	Microbial biomass carbon

3. Data Application and Derivation:

In addition to improving the conceptual understanding of microbially-facilitated decomposition, these experiments were designed to parameterize and test the Microbial ENzyme Decomposition (MEND) model (Wang et al., 2013, 2014, 2015).

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:

Site Description and Soil Sampling:

Soils were collected in the fall of 2014 from paired forest and grassland plots within each of four different soil types. Sampling locations are provided above in Table 1 under Spatial Coverage. The paired forest and grassland sites were located within 1 mile of each other and mapped to the same soil series, according to the U.S. Department of Agriculture Natural Resources Conservation Service (NRCS) soil surveys (https://soilseries.sc.egov.usda.gov/osdname.aspx). Forested sites were mature (>60 yrs old) mixed eastern deciduous forests and grassland sites were minimally managed grasslands with no recent fertilization or grazing, although all sites were mowed semi-annually or annually.

At each location, three representative locations were identified for subsamples. Litter and plant material was removed to reveal the mineral soil and the upper 15 cm was collected from an area of ~90 cm^2. Samples were transported on ice and stored at 4 °C.

Soil Characterization:

Properties of soils used for the incubation experiment are provided in Table 2a, 2b, and 2c. These data represent the natural/starting state of soils prior to the experiment.

Dried soil samples were submitted to EcoCore Analytical Laboratory at Colorado State University and analyzed for C and N content and isotopic signature (^{13}C and ^{15}N) with a Costech ECS 4010 elemental analyzer (Costech International, Pioltello (MI) Italy) coupled to a Thermo Fisher Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher, Bremen Germany). The pH of the samples was determined by shaking 1 parts soil to 2 parts MilliQ water and measuring the pH of the supernatant (Thomas, 1996). Dissolved organic carbon (DOC) and microbial biomass carbon (MBC) were determined using the K_2SO_4 extraction and chloroform fumigation-extraction method (Vance et al., 1987). Briefly, to determine DOC, 7 g soil was combined with 35 ml of 0.5M K_2SO_4 and placed on a shaker for 1 hour then filtered through Whatman #1 filters. A second set of soils were placed in a desiccator with 20 ml chloroform under a vacuum of 11 atm and fumigated for 48 hours prior to extraction with K_2SO_4 (as described above). Filtrate C contents of both samples were determined with the combustion catalytic oxidation method on a Shimadzu Corporation Total Organic Carbon-L analyzer (Kyoto, Japan). The MBC is calculated as the difference between fumigated and unfumigated (i.e., DOC) samples divided by the extraction efficiency (0.45 following Fierer et al., 2009 and Vance et al., 1987). Soil texture was determined by the Bouyoucos hydrometer method (Gee and Or, 2002).

The particulate organic matter (POM, plant residues + sand-sized particles) was separated from the mineral-associated organic matter (MOM, silt- and clay-sized fractions) by size-based fractionation (Allision and Jastrow, 2006). Briefly, 25 g of soil sample was mixed with 100 ml of sodium hexametaphosphate solution and shaken for 16 hours. Then the suspension was sieved through 53 µm sieve to retrieve the POM on the sieve, while the MOM fraction passed through the sieve and dried at 60°C. Further, the sand particles and plant residues were separated using density fractionation method by using sodium polytungstate solution (Magid et al., 1996). Briefly, sodium polytungstate solution (density = 1.8 g cm^{-3}) was added to POM fractions in 1:4 (POM:solution) ratio and shaken for 2 hours. The light plant residues were decanted on a filter paper using vacuum pump. This was repeated until no more POM material was seen floating on the sodium polytungstate solution. The filter paper retrieving the POM material was washed properly and then dried at 60°C. Dried POM and MOM samples were analyzed using a Leco combustion analyzer (Leco Corp., St. Joseph, MI) (Nelson and Sommers, 1996).

Table 2a. Carbon content, isotopic signature of C (δ^{13}C), nitrogen content, pH, dissolved organic carbon (DOC) and microbial biomass carbon (MBC) of soils used in the incubation experiments. Data represent the mean and standard deviation from 4 analytical replicates.

		Carbon (%)	δ^{13}**C**	**Nitrogen** (%)	**pH**	**DOC** (ugC g^{-1} soil)	**MBC** (ugC g^{-1} soil)
Tennessee							
	Forest	5.71 (0.22)	-26.76 (0.15)	0.47 (0.07)	7.56	176.95 (54.28)	765.79 (77.26)
	Grassland	3.08 (0.16)	-21.06 (0.11)	0.33 (0.03)	7.29	96.28 (9.15)	517.88 (142.323)
Ohio							
	Forest	4.14 (0.06)	-25.64 (0.72)	0.31 (0.01)	5.50	271.45 (52.69)	380.54 (245.51)
	Grassland	2.18 (0.08)	-25.41 (0.02)	0.23 (0.01)	6.36	66.51 (14.34)	623.56 (45.41)
Iowa							
	Forest	3.17 (0.29)	-26.05 (0.24)	0.27 (0.01)	6.56	50.83 (3.22)	221.00 (11.01)
	Grassland	3.13 (0.62)	-23.41 (0.29)	0.25 (0.01)	6.45	44.11 (2.83)	455.80 (17.46)
Missouri							
	Forest	4.42 (0.62)	-26.84 (0.19)	0.22 (0.13)	5.89	76.95 (1.72)	464.04 (22.05)
	Grassland	2.21 (0.08)	-25.96 (0.10)	0.20 (0.01)	5.57	67.91 (3.23)	187.87 (11.38)

Table 2b. Texture of soils used in the incubation experiments.

		Sand (%)	**Silt** (%)	**Clay** (%)
Tennessee				
	Forest	39.16	50.05	10.78
	Grassland	43.81	46.05	10.12
Ohio				
	Forest	23.46	67.79	8.73
	Grassland	16.97	66.48	16.53
Iowa				
	Forest	43.49	31.99	24.51
	Grassland	17.89	36.58	45.51
Missouri				
	Forest	14.18	70.21	15.60
	Grassland	25.85	63.37	10.77

Table 2c. Amount of particulate organic matter (POM), and mineral-associated organic matter (MOM) as a percentage of total soil mass; and the percentage of carbon and nitrogen content in each fraction (C-POM, N-POM, C-MOM, N-MOM) of the soils used in the incubation experiments.

		POM (%)	C-POM (%)	N-POM (%)	MOM (%)	C-MOM (%)	N-MOM (%)
Tennessee							
	Forest	3.66	5.59	0.50	96.72	2.64	0.21
	Grassland	0.58	7.31	0.89	99.41	1.18	0.06
Ohio							
	Forest	3.16	7.01	0.44	97.16	2.00	0.16
	Grassland	1.20	6.87	0.84	98.74	0.77	0.01
Iowa							
	Forest	1.60	12.94	0.94	98.38	1.28	0.05
	Grassland	2.44	4.89	0.39	97.60	1.27	0.07
Missouri							
	Forest	4.66	2.41	0.16	95.69	1.56	0.09
	Grassland	0.69	1.70	0.16	99.51	0.60	bdl

Microcosm Setup:

The three subsamples from each of the eight soils were combined, homogenized, and sieved to 4 mm. Soil water content was adjusted to 30 % gravimetric water content (GWC) and soils were pre-incubated at 22 °C for 3 days before incubations began. Microcosms were constructed with 30 g soil in pre-weighed specimen cups, additions were added to the soils then cups were then placed in 1 L glass mason jars for the incubation experiment. The use of specimen cups aided in sample handling during additions and monitoring water content throughout the incubations.

Microcosms received the equivalent of 1 % of the total carbon content as either ^{13}C labeled glucose (short term study) or ^{13}C labeled cellulose (long term study). To prepare the additions, 99 atom percent ^{13}C labeled glucose (Sigma Aldrich, USA) and cellulose (Cambridge Scientific, USA) were diluted with unlabeled glucose and cellulose such that final additions were labeled at 15 atom %. Glucose solutions were prepared with sterile DI water such that 1 ml glucose solution was added to each microcosm. Because cellulose is insoluble, dry additions prepared by mixing dried, ground soil (same as used in microcosms) with cellulose such that final 1 g dried soil/cellulose mixture was added to each microcosm. 1 ml sterile DI water was added to each microcosm after mixing soil/cellulose addition with soils to moisten the dried soils and obtain reach the desired 30% GWC target. Control microcosms for the short-term incubations were prepared by adding 1 ml sterile DI water, while control microcosms for the long-term incubations were prepared by adding 1 g dried ground soil and 1 ml sterile DI water. For each of soil x treatment combination, 16 replicate microcosms were prepared for short term incubations and 32 microcosms were prepared for long term incubations. In all, 768 microcosms were constructed (265 short term, and 512 long term) to allow for destructive

harvesting throughout the experiments and surplus microcosms to ensure enough samples in the event of laboratory errors or accidents.

All microcosms were incubated in the dark at 22 °C and loosely capped to allow for oxygen exchange while preventing drying. Soil moisture content was monitored regularly and sterile DI water was added as needed to maintain 30% GWC. Short term incubations were conducted over 144 hours with 7 gas and 4 destructive samplings. Long term incubations were conducted over 729 days with 18 gas and 9 destructive samplings (see Tables 3a and 3b).

Table 3a: Sampling schedule for short term incubation experiment.

Short Term Incubation Sampling Schedule								
Hour	**0**	**2**	**4**	**8**	**24**	**48**	**72**	**144**
Gas sampling		X	X	X	X	X	X	X
Soil sampling	X				X		X	X

Table 3b: Sampling schedule for long term incubation experiment.

Day	**0**	**1**	**2**	**4**	**6**	**11**	**20**	**39**	**64**	**90**	**120**	**151**	**229**	**323**	**390**	**480**	**571**	**665**	**729**
Gas sampling		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Soil sampling	X	X		X			X		X			X		X		X			X

Microcosm Sampling and Analysis:

At each time point, three replicates of each sample type were randomly selected. Jars were flushed with room air and capped with airtight lids fitted with a septum for 2 to 24 hours prior to sampling (dependent on expected activity). At the time of sampling, headspace gas was mixed and 15 ml gas samples were taken with airtight plastic syringes and injected into pre-evacuated 12 ml Exetainer vials (Labco Limited, UK). For destructive harvesting, once gas samples were taken, jars were opened and soils were partitioned into subsamples for analysis. Approximately 5 g were oven dried at 65 °C (dry archive), ~2 g were placed in microcentrifuge tubes and stored at -80 °C to preserve for molecular analysis, ~15 g were frozen at -20 °C for MBC and DOC analysis, and the remaining soil was frozen in a separate bag and stored at -20 °C as a frozen archive.

Gas samples were submitted to EcoCore Analytical Laboratory at Colorado State University and analyzed for CO_2 concentration and isotopic signature (^{13}C-CO_2) with a modified Precon device coupled to a Thermo Fisher Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher, Bremen Germany). Soil respiration flux (F) was calculated as $F = \frac{\partial C}{\partial t} \frac{V}{M} \frac{P}{RT}$ where $\frac{\partial C}{\partial t}$ is the change in headspace CO_2 concentration (ppm) over the measurement period. V is microcosm volume, M is the soil dry mass, P is the atmospheric pressure (1 atm), R is the universal gas constant (82.05 mL atm mol^{-1} K^{-1}), and T is the air temperature. The final respiration rate is expressed on a dry soil basis as μg CO_2-C g^{-1} hr^{-1}.

The respiration data and isotopic signatures provided in the data product can be used to calculate the percent of CO_2-C coming from the added substrate with the following mixing model

$\%C_{sub} = \left[\frac{(\delta C - \delta T)}{(\delta C - \delta S)}\right] 100$ where δC is the $\delta^{13}C$ value of the respired CO_2 from the control soil, δT is the $\delta^{13}C$ value of the respired CO_2 from the treated soils and δS is the $\delta^{13}C$ value of the added substrate (12,636.4). Further, priming can be calculated as the increase in total respiration following substrate addition minus the amount of C respired from the added substrate and is expressed as a percent where 100% represents doubling of SOM-C respiration.

As described above, dried soil samples were analyzed for C and N content and isotopic signature (^{13}C and ^{15}N). Due to high cost of analysis, only a subset of soil samples was analyzed (starting soils, 144 hours, 151 days, and 729 days). Microbial biomass carbon (MBC) and DOC on selected destructive harvest samples (Tables 3a, 3b) were also analyzed as described above.

6. References:

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7. Data Access:

For public access to ORNL TES SFA data please visit the TES SFA Web Site: https://tes-sfa.ornl.gov/home

Contact for Data Access Information: https://mnspruce.ornl.gov/contact