TropSOC Database

2.5.2. Forest – Soil experiments – Microbial biomass and enzyme experiments

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Introduction

The dataset comprises a unique plot identifier and a unique sample identifier and 26 additional variables that provide information regarding soil microbial activity and traits (extracellular enzyme activity, extracellular polymeric substances, dissolved and available carbon and nitrogen fractions). Missing values are indicated by –9999. Note: details regarding plots and plot design where soil samples were collected can be found in *2_forest.pdf.*

Data structure

No.	Variable	Explanation	Unit
1	plotID	unique identifier of each plot and point where data were collected	-
2	sampleID	unique identifier of any soil or vegetation sample taken in the field	-
3	BG_1	β-glucosidase, four days after re-wetting at the start of the incubation experiment	nmol g^{-1} h^{-1}
4	BG_2	β-glucosidase, at the end of the incubation experiment (120 days)	nmol g^{-1} h^{-1}
5	CB_1	Cellobiohydrolase, four days after re-wetting at the start of the incubation experiment	nmol g^{-1} h^{-1}
6	CB_2	Cellobiohydrolase, at the end of the incubation experiment (120 days)	nmol g^{-1} h^{-1}
7	AP_1	Acid phosphatase, four days after re-wetting at the start of the incubation experiment	nmol g^{-1} h^{-1}
8	AP_2	Acid phosphatase, at the end of the incubation experiment (120 days)	nmol g^{-1} h^{-1}
9	NAG_1	N-acetylglucosaminidase, four days after re-wetting at the start of the incubation experiment	nmol g^{-1} h^{-1}
10	NAG_2	N-acetylglucosaminidase, at the end of the incubation experiment (120 days)	nmol g^{-1} h^{-1}
11	LAP_1	Leucine-amino-peptidase, four days after re-wetting at the start of the incubation experiment	nmol g^{-1} h^{-1}
12	LAP_2	Leucine-amino-peptidase, at the end of the incubation experiment (120 days)	nmol g^{-1} h^{-1}

13	SACCH_1	EPS-saccharide, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
14	SACCH_2	EPS-saccharide, at the end of the incubation experiment (120 days)	mg kg^{-1}
15	PROT_1	EPS-protein, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
16	PROT_2	EPS-protein, at the end of the incubation experiment (120 days)	mg kg^{-1}
17	MBC_1	microbial biomass C, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
18	MBC_2	microbial biomass C, at the end of the incubation experiment (120 days)	mg kg^{-1}
19	TDN_1	total dissolved nitrogen, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
20	TDN_2	total dissolved nitrogen, at the end of the incubation experiment (120 days)	mg kg^{-1}
21	NH4_1	ammonium, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
22	NH4_2	ammonium, at the end of the incubation experiment (120 days)	mg kg^{-1}
23	NO3_1	nitrate, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
24	NO3_2	nitrate, at the end of the incubation experiment (120 days)	mg kg^{-1}
25	DOC-nf_1	dissolved organic carbon, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
26	DOC-nf_2	dissolved organic carbon, at the end of the incubation experiment (120 days)	mg kg^{-1}
27	DOC_1	dissolved organic carbon, four days after re-wetting at the start of the incubation experiment	mg kg^{-1}
28	DOC_2	dissolved organic carbon, at the end of the incubation experiment (120 days)	mg kg^{-1}

Methods

Heterotrophic extracellular enzymatic activity: As part of our experiments to assess heterotrophic respiration (see Bukombe et al. 2021), heterotrophic extracellular enzymatic activity (EEA) was measured at the start and end of our incubation experiment. Sampling at the start of the experiment was done after four days of pre-incubation where dried samples were re-wetted and equilibrated. Sampling at the end of the experiment was done when CO_2 measurements showed a stabilization of respiration at a low level. This stabilization was achieved when the difference of measured respiration between three replicates was greater than the difference of these replicates across three time points (approximately 120 days for forest soils).

The activity of five extracellular enzymes produced by soil microorganisms were measured fluorometrically during the incubation experiments following German et al. (2011). The activity was measured for carbon acquisition (cellobiohydrolase CB and β-glucosidase BG), nitrogen acquisition (N-acetylglucosaminidase NAG, leucine-aminopeptidase LAP) and phosphorus acquisition (acid phosphatase AP). Briefly, 1 g of 2 mm sieved soil sample was treated with 100 ml of 50 µM sodium acetate trihydrate buffer. To break up any aggregates, this mixture was then sonicated using an ultrasonic-homogenisator (HD 3100, Sonopuls, Bradlin, Inc., Germany) for 87 seconds. 4-methylumbelliferone (MUB, 0.625, 0.125, 2.5, 5, 10, 25, 50, and 100 µM) and 7-amino-4-methylcoumarin (AMC, 0, 0.156, 0.325, 0.625,

1.25, 2.5, 5, and 10 µM) of increasing concentrations were used as calibration standards for the fluorimeter. Five fluorogenic substrates were used to stimulate fluorescence of the investigated enzymes and to measure CB, BG, NAG, AP, and LA : MUB: β-D-cellobioside (200 µM), β-D-glucopyranoside (200 µM), N-acetyl-β-D-glucosaminide (200 µM), phosphate (400 µM) and AMC: L-Leucine-7-amido-4-methylcoumarin (100 µM) respectively.50 µl Standards , 50 µl substrates , a 200 µl soil suspension , 50 µl acetate buffer and 200 µl water were pipetted into black, 96-well microplates for analyses with four replicates for each soil sample. The substrate microplate contained sample essays (substrate + slurry), slurry controls (buffer + slurry), substrate controls (water + substrate) and buffer controls (water + buffer). The standard microplate contained the buffer, MUB + slurry or AMC + slurry and MUB + water or AMC + water. Before measurements, each microplate was incubated at 30 °C for one hour to allow the substrate to transform into fluorescent products (i.e. enzyme activity). Then, fluorescence was measured using a fluorescence microplate reader (Synergy HTX Multi-Mode Reader, Bio-Tek Instruments, Inc., USA) at an excitation wavelength of 360 nm and an emission wavelength of 450 nm (Deng et al. 2011). The EEA normalized to dry soil weight (nmol g^{-1} h^{-1}) was calculated as the difference in fluorescence between sample essay and controls related to the slope and the intercept of the standard curves (German et al. 2011).

Extracellular polymeric substances: As part of our experiments to assess heterotrophic respiration (see Bukombe et al. 2021), extracellular polymeric substances (EPS) were measured at the start and end of our incubation experiment. Sampling at the start of the experiment was done after four days of pre-incubation where dried samples were re-wetted and equilibrated. Sampling at the end of the experiment was done when CO_2 measurements showed a stabilization of respiration at a low level. This stabilization was achieved when the difference of measured respiration across three replicates was greater than the difference of these replicates across three time points (approximately 120 days).

Extracellular polymeric substances (EPS) were extracted using the cation exchange resin approach following (Frølund et al. 1996, Redmile-Gordon et al. 2014). First, soluble microbial products (SMP) were removed by adding 25 ml of 0.01 M $CaCl_2$ to 2.5 g of 2 mm sieved soil contained in 50 ml Falcon centrifuge tube. Sample and centrifuge tube was placed on a shaker (at two cycles per second at 4 °C for 30 minutes) and then transferred to a pre-cooled (4 °C) centrifuge (at 3200 x g for 30 minutes) before the SMP containing supernatants were pipetted. Following SMP removal, Dowex Cation Exchange Resin (Marathon C Sodium form (91973-1KG-F) Na^+ form, strongly acidic, and 25 ml of phosphate-buffered saline were added to the centrifuge tube for EPS extraction. The tube was placed on an end-over-end shaker for two hours at 4 °C before it was transferred to a centrifuge at 4200 x g for 20 minutes to separate soil and resin from the supernatant. Then, supernatants containing EPS were transferred to a new centrifuge tube by pipetting and frozen at − 20 °C until measurement.

EPS extracts were filtered using syringe filters (CHROMAFIL ® PET-45/25, Polyester) and measured as EPS-saccharides and EPS-proteins. EPS-saccharides were quantified by measuring carbohydrates with the sulfuric acid-phenol method using D(+)-Glucose (Roth, PN X997) as a calibration standard (Dubois et al. 1956). Briefly, 1 ml of EPS extract, 25 µl of 80% phenol as well as 2.5 ml of 95 - 98% sulfuric acid was added to a glass test tube and placed in a warm water bath (90 °C). Following a 10-minute warm bath, the tube was placed in a cool water bath at room temperature, before 200 µl were transferred to a 96-well microplate in triplicates for absorbance measurements. Absorbance was measured using a microplate reader (Synergy HTX Multi-Mode Reader, Bio-Tek Instruments, Inc., USA) at 480 and 490 nm emission wavelengths.

To measure EPS-proteins, bovine serum albumin (BSA) and humic acid (HA) were used as the calibration standards for the measurement of proteins and humic compounds following Lowry'S method modified by Frølund's (Lowry et al. 1951, Frølund et al. 1995). In this method, protein (0, 5, 10, 15, 20, 25 ppm) and humic acid (0, 80, 160, 240, 320, 400 ppm) calibration standards were prepared in 50 µm phosphate-buffered saline (PBS). Then, two Lowry reagents were prepared. Reagent 'A' was made of 3.5 g $CuSO_4 \cdot 5H_2O$ 100 mL^{-1} H_2O, 7 g NaK tartrate 100 mL^{-1} H_2O, and 70 g Na_2CO_3 L^{-1} 0.35 N NaOH which were sequentially combined in proportions of 1:1:100 (v:v:v), respectively. Reagent 'B' was made in the same way except that $CuSO_4 \cdot 5H_2O$ was excluded and its volume substituted by deionised water. Reagent A or B (100 µl) was added to 100 µl of EPS extracts or standard solutions using a 96-well microplate in four technical replicates before incubating in the dark at room temperature for 10 minutes. Folin-phenol (2 N diluted 10-fold in H_2O) reagent was prepared before the end of the first incubation, and subsequently 100 µl were injected into all wells. Microplate wells were then incubated in the dark at room temperature for an additional 30 minutes. The absorbance was measured using a microplate reader (Synergy HTX Multi-Mode Reader, Bio-Tek Instruments, Inc., USA) at 750 nm and 660 nm and measurement speed was 150 ms/well. Two absorbance measurements were obtained per sample for the respective reagents: 'Abs_A' and 'Abs_B'. Absorbance due to the presence protein ($Abs_{protein}$) and absorbance due to the presence humic substance (Abs_{humic}) were calculated using equations 1 and 2 (Frølund et al. (1995).

$$Abs_{protein} = 1.25\,(Abs_A - Abs_B) \quad (1)$$

$$Abs_{humic} = Abs_B - 0.2\,Abs_{protein} \quad (2)$$

Microbial biomass carbon: As part of our experiments to assess heterotrophic respiration (see Bukombe et al. 2021), microbial biomass carbon (MBC) was measured at the start and the end of our incubation experiment. Sampling at the start of the experiment was done after four days of pre-incubation, where dried samples were re-wetted and equilibrated. Sampling at the end of the experiment was done when CO_2 measurements showed a stabilization of respiration at a low level. This stabilization was achieved when the difference of measured respiration across three replicates was greater than the difference of these replicates across three time points (approximately 120 days). MBC was measured by the chloroform fumigation-extraction method (Vance et al. 1987). Briefly, two 5 g aliquots of 2 mm sieved soil were taken from each sample. One of these two aliquots was then fumigated using ethanol-free chloroform while the other was kept untreated (i.e. non-fumigated). The fumigated aliquot was incubated for 24 h at 25 °C, before chloroform was allowed to evaporate in the fume hood for 30 minutes. In a second step, 20 ml of 0.05 M K_2SO_4 was added to both the fumigated and non-fumigated aliquots and these mixtures were shaken for 60 minutes using an end-over-end shaker at 35 reversals min^{-1}. Following shaking, extracts were filtered through Whatman grade 42) filter paper. Then, dissolved organic carbon (DOC) content of both extracts was measured in triplicates using a vario TOC cube. MBC was then calculated as the difference in extractable DOC content between fumigated and non-fumigated samples, considering an extraction efficiency factor of 0.45 calculated using equation 1 (Beck et al. 1997).

$$MBC = (DOC_{fum} - DOC_{unfum}) / kEC \quad (3)$$

where *MBC* is microbial biomass carbon; DOC_{fum} is dissolved organic carbon (DOC) extracted after fumigation; DOC_{unfum} is DOC extracted without fumigation; and *kEC* is the efficiency of extraction of microbial biomass.

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