

Supplementary Materials

Soil organic matter properties drive microbial enzyme activities and greenhouse gas fluxes along an elevational gradient

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Supplementary Tables

Supplementary Table S1. Enzyme activities measured, substrate and measurement details. Artificial substrates used, Enzyme Commission number (EC) and final incubation concentrations used at substrate saturation. MUF: methylumbelliferyl. AMC: 7-amino-4-methylcoumarin. Wavelengths for fluorescence or absorbance measurements are also indicated.

Extracellular enzyme	Artificial substrate	EC number	Incubation concentration (mM)	Spectrometry method	Wavelength (nm)
β -glucosidase	MUF- β -d-glucoside	3.2.1.21	0.3	fluorescence	365/455 excitation/emission
cellobiohydrolase	MUF- β -d-cellobioside	3.2.1.91	0.3	fluorescence	365/455 excitation/emission
α -glucosidase	MUF- α -d-glucoside	3.2.1.20	0.3	fluorescence	365/455 excitation/emission
β -xylosidase	MUF- β -d-xyloside	3.2.1.37	0.3	fluorescence	365/455 excitation/emission
N-acetyl-glucosaminidase	MUF-N-acetyl-glucosaminide	3.2.1.52	0.3	fluorescence	365/455 excitation/emission
Phosphatase	MUF-phosphate	3.1.3.1–2	0.3	fluorescence	365/455 excitation/emission
Leucine aminopeptidase	AMC-leucine	3.4.11.1	0.3	fluorescence	364/445 excitation/emission
Phenol oxidase	L-3,4-dihydroxyphenylamine	1.14.18.1	1.5	absorbance	460

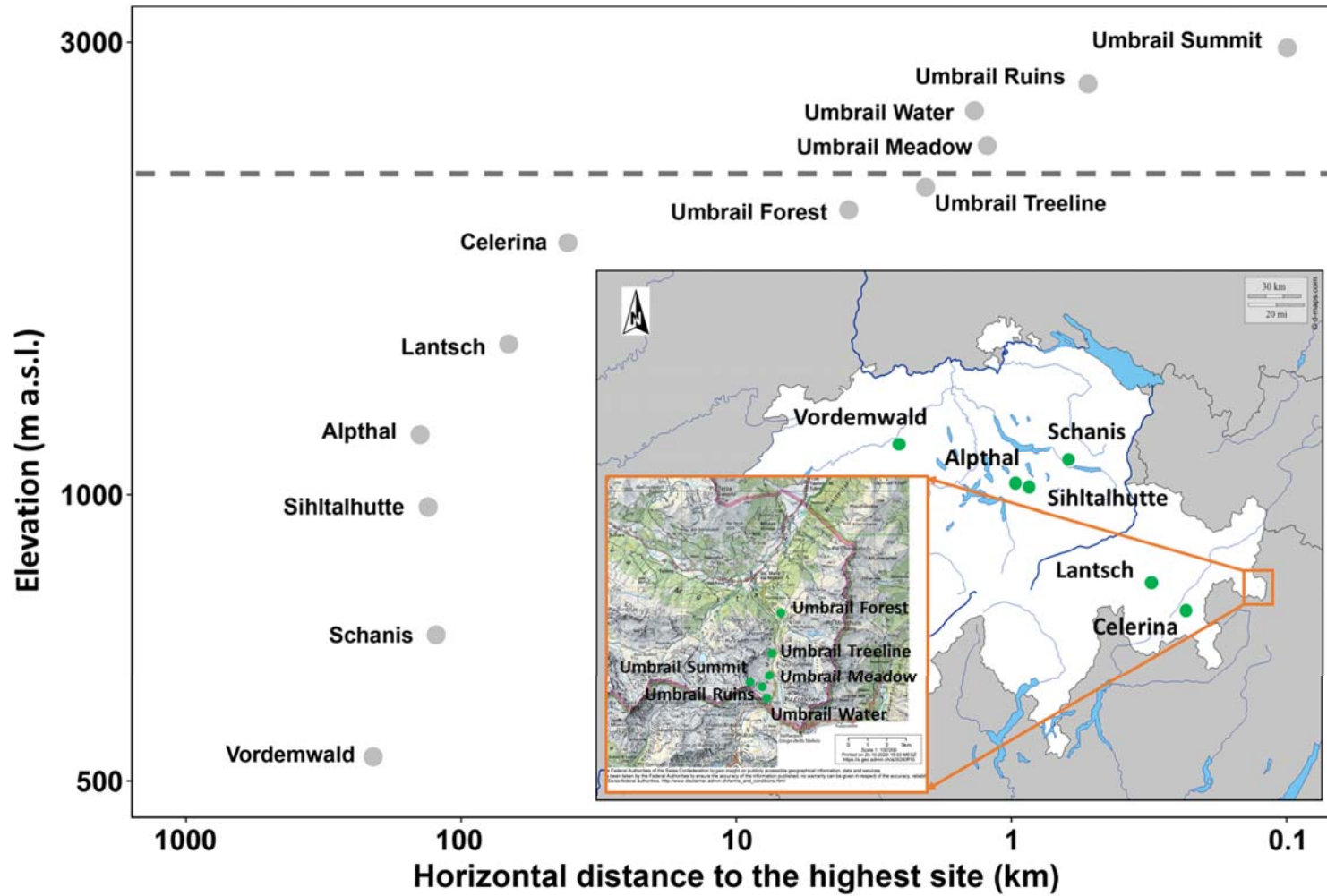
Supplementary Table S2. Incubation conditions for each sample for the extracellular enzyme activities assay. The extracellular enzyme activities incubations were performed using standard *in situ* approach, i.e. at the same soil pH and temperature measured in the field (Burns et al., 2013). The pH for each incubation was decided according to the pH value from each sample, previously measured. Temperature for the incubations matched the soil temperatures measured in the sites at the moment of the field sampling.

Sample	Hour of incubation with artificial substrates		Buffer composition (pH)	Temperature (°C)
	Hydrolytic enzymes	Phenol oxidase		
Umbrail Summit	1,13	2,04	Tris(hydroxymethyl)aminomethane (pH 7)	10
Umbrail Ruins	1,10	2,04	Tris(hydroxymethyl)aminomethane (pH 7)	10
Umbrail Water	1,07	1,94	Tris(hydroxymethyl)aminomethane (pH 7)	11
Umbrail Meadow	1,03	1,94	Tris(hydroxymethyl)aminomethane (pH 8)	14
Umbrail Treeline	1,08	1,94	Tris(hydroxymethyl)aminomethane (pH 7)	11
Umbrail Forest	1,20	2,08	Tris(hydroxymethyl)aminomethane (pH 7)	13
Celerina	1,08	1,99	Maleate (pH 6)	10
Lantsch	1,05	1,99	Tris(hydroxymethyl)aminomethane (pH 8)	14
Alpthal	1,05	1,94	Tris(hydroxymethyl)aminomethane (pH 7)	11
Sihltalhütte	1,08	2,13	Maleate (pH 6)	14
Schänis	1,07	2,19	Maleate (pH 6)	14
Vordemwald	1,15	2,17	Maleate (pH 6)	14

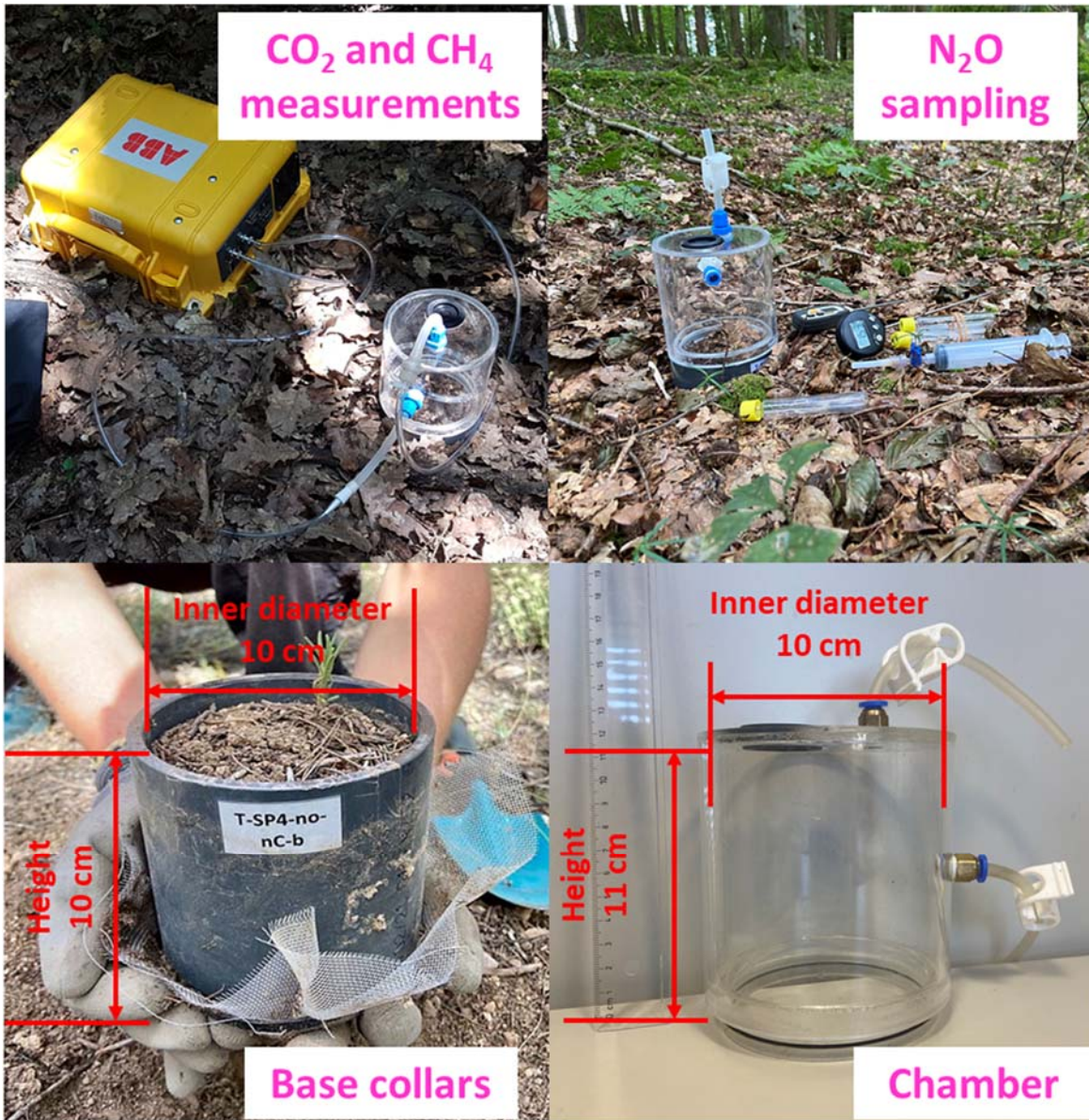
Supplementary Table S3. qPCR conditions for the quantification of each of the target genes.

	16S rRNA gene	Fungal ITS2	<i>mcrA</i>	<i>pmoA</i>	<i>nifH</i>	Archaeal <i>amoA</i>	Bacterial <i>amoA</i>	<i>nosZ</i>	<i>norB</i>	<i>phoD</i>
Stage 1: 1 cycle (denaturation)	2 min at 95 °C									
Stage 2: 40 cycles (amplification)	40s at 95 °C 40s at 58°C 1min at 72 °C	40s at 95 °C 40s at 58°C 1min at 72 °C	45s at 95 °C 45s at 50°C 45s at 72 °C	45s at 95 °C 45s at 56°C 45s at 72 °C	45s at 95 °C 45s at 55°C 30s at 72 °C	45s at 95 °C 45s at 53°C 45s at 72 °C	45s at 95 °C 45s at 55°C 45s at 72 °C	45s at 95 °C 45s at 55°C 45s at 72 °C	45s at 95 °C 45s at 57°C 45s at 72 °C	30s at 95 °C 30s at 58°C 30s at 72 °C
Stage 3: 1 cycle (melting curve)	15s at 95 °C 15s at 60°C 15s at 95 °C	15s at 95 °C 15s at 60°C 15s at 95 °C	15s at 95 °C 15s at 50°C 15s at 95 °C	15s at 95 °C 15s at 60°C 15s at 95 °C	15s at 95 °C 1min at 50°C 15s at 95 °C	15s at 95 °C 15s at 60°C 15s at 95 °C	15s at 95 °C 15s at 60°C 15s at 95 °C	15s at 95 °C 1min at 50°C 15s at 95 °C	15s at 95 °C 15s at 55°C 15s at 95 °C	15s at 95 °C 15s at 60°C 15s at 95 °C
Stage 4: cooling	4 °C									

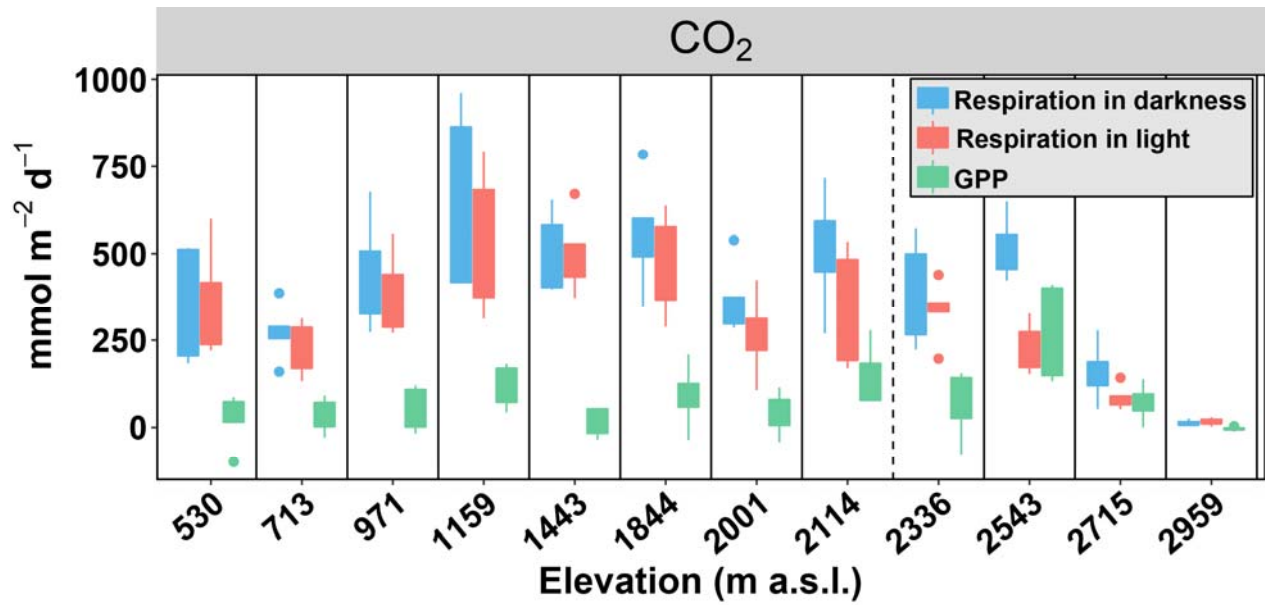
Supplementary Figures



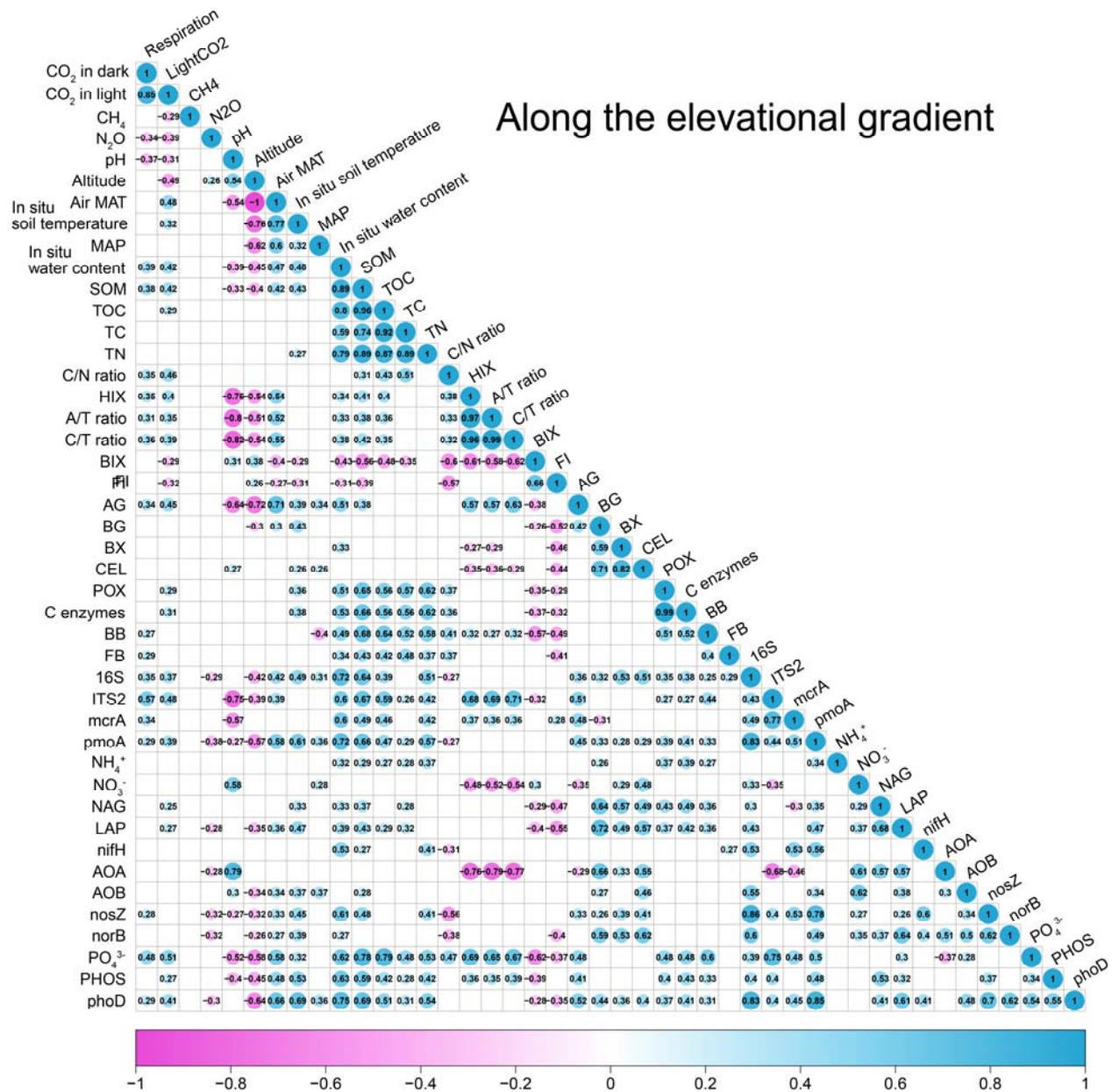
Supplementary Fig. S1. Map of sampling sites. The dashed line indicates the treeline. X and Y axes are log₁₀ scaled. Swiss map was adapted from https://www.d-maps.com/carte.php?num_car=2648&lang=en.



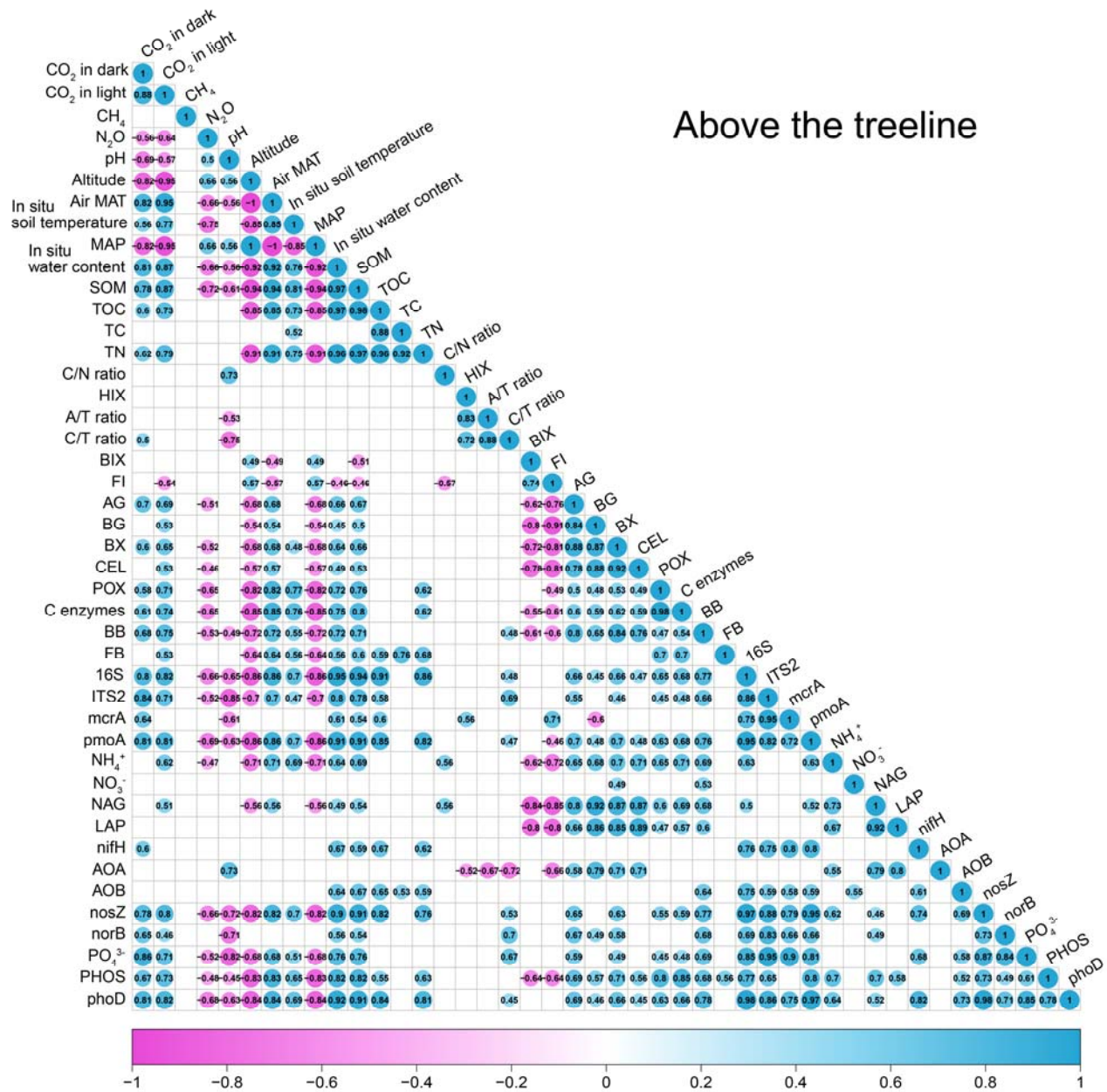
Supplementary Fig. S2. Pictures of soil core, gas chambers, and gas flux measurements.



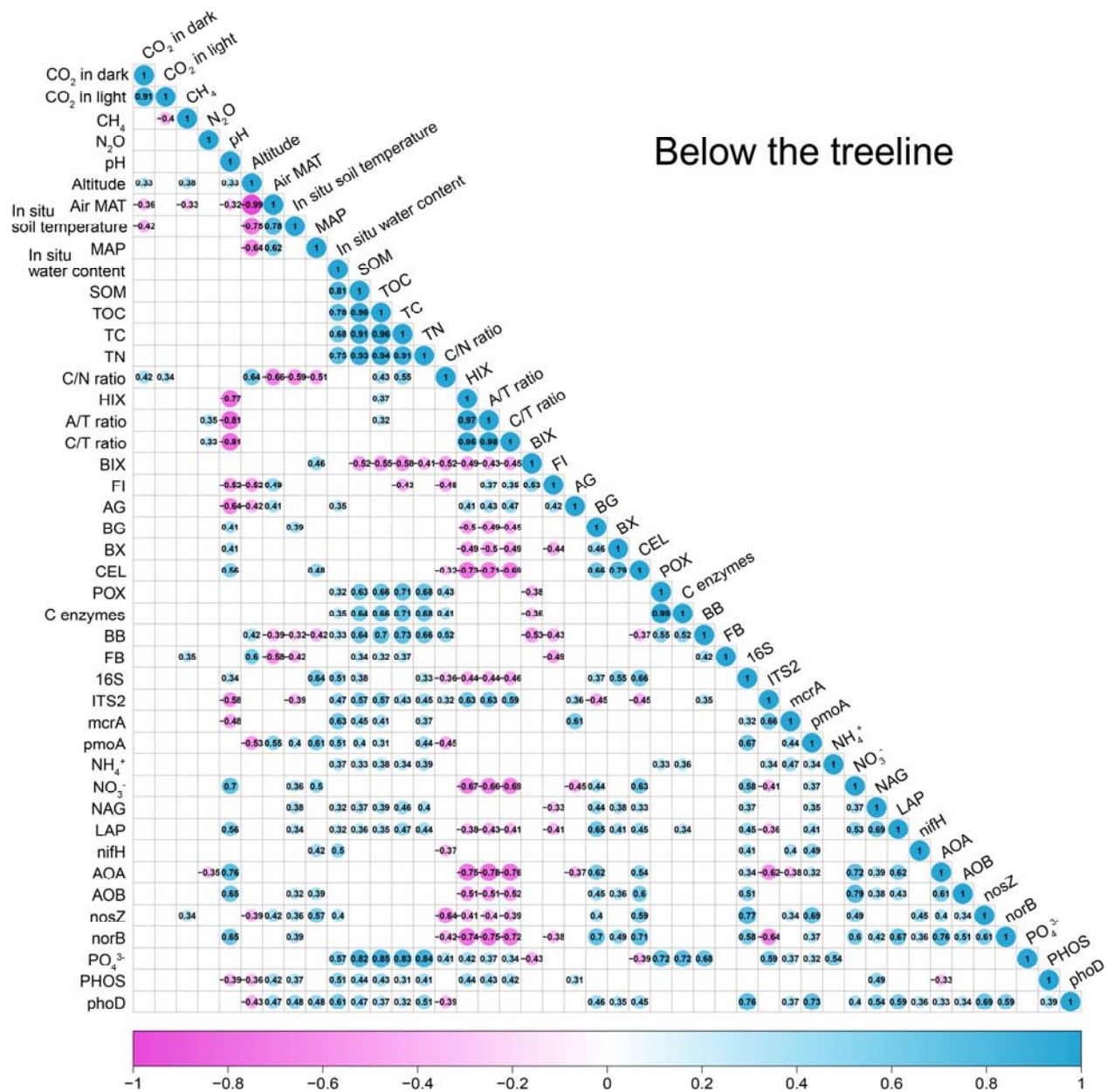
Supplementary Fig. S3. Soil respiration fluxes in darkness and light, and gross primary production (GPP) across the studied sites. Altitude increases from left to right. Each bar was calculated by five replicates. The grey dashed line indicates the treeline.



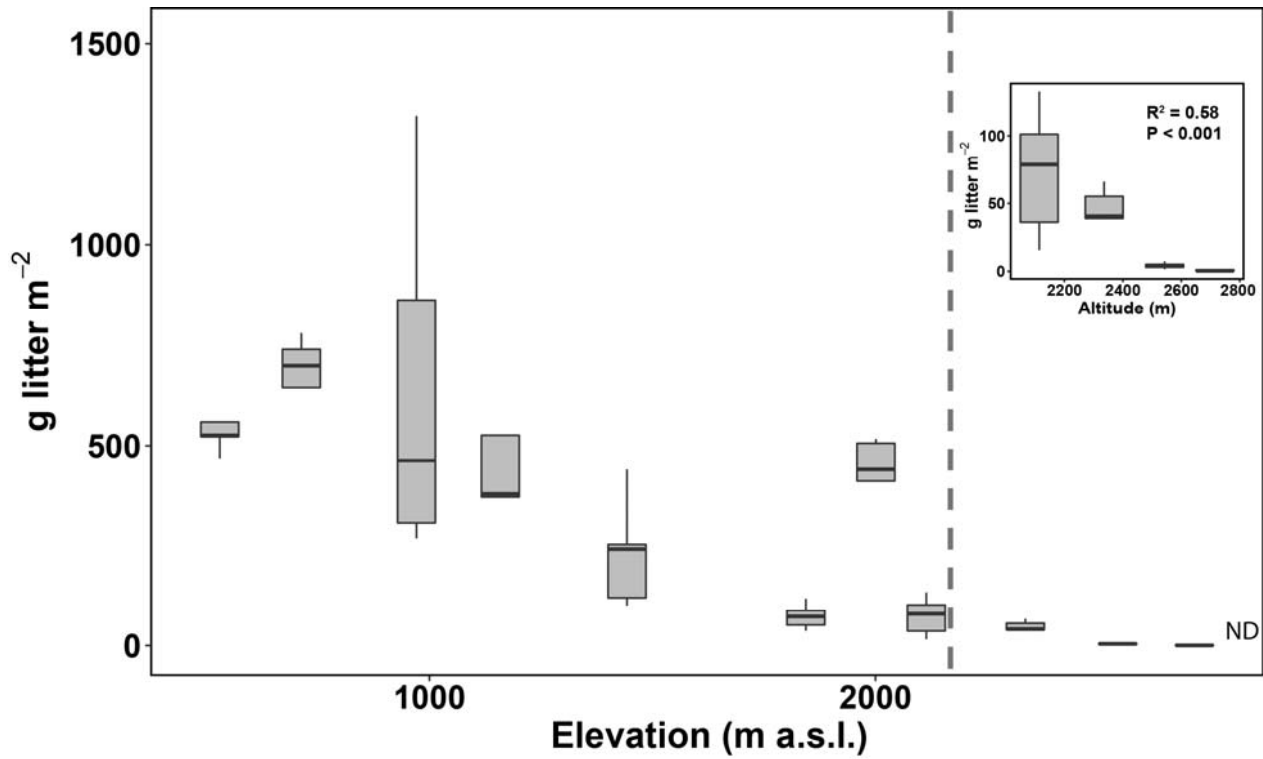
Supplementary Fig. S4. Spearman correlations between GHG fluxes, environmental and microbial variables along the elevational gradient. Only significant correlations are shown in the heatmap ($P < 0.05$). Green and purple represent positive and negative correlations, respectively. The size of the circle indicates the coefficient values, corresponding to the number in the circle. Humification index (HIX); biological index (BIX); fluorescence index (FI); α -glucosidase (AG); β -glucosidases (BG); β -xylosidase (BX); Cellobiohydrolase (CEL); Phenol oxidase (POX); C enzymes = AG + BG + BX + CEL + POX; BB: Bacterial biomass; FB: Fungal biomass; 16S: 16S rRNA genes; β -glucosaminidase (NAG); Leu-aminopeptidase (LAP); AOA (*Archaeal amoA*); AOB (*Bacterial amoA*); Phosphatase (PHOS).



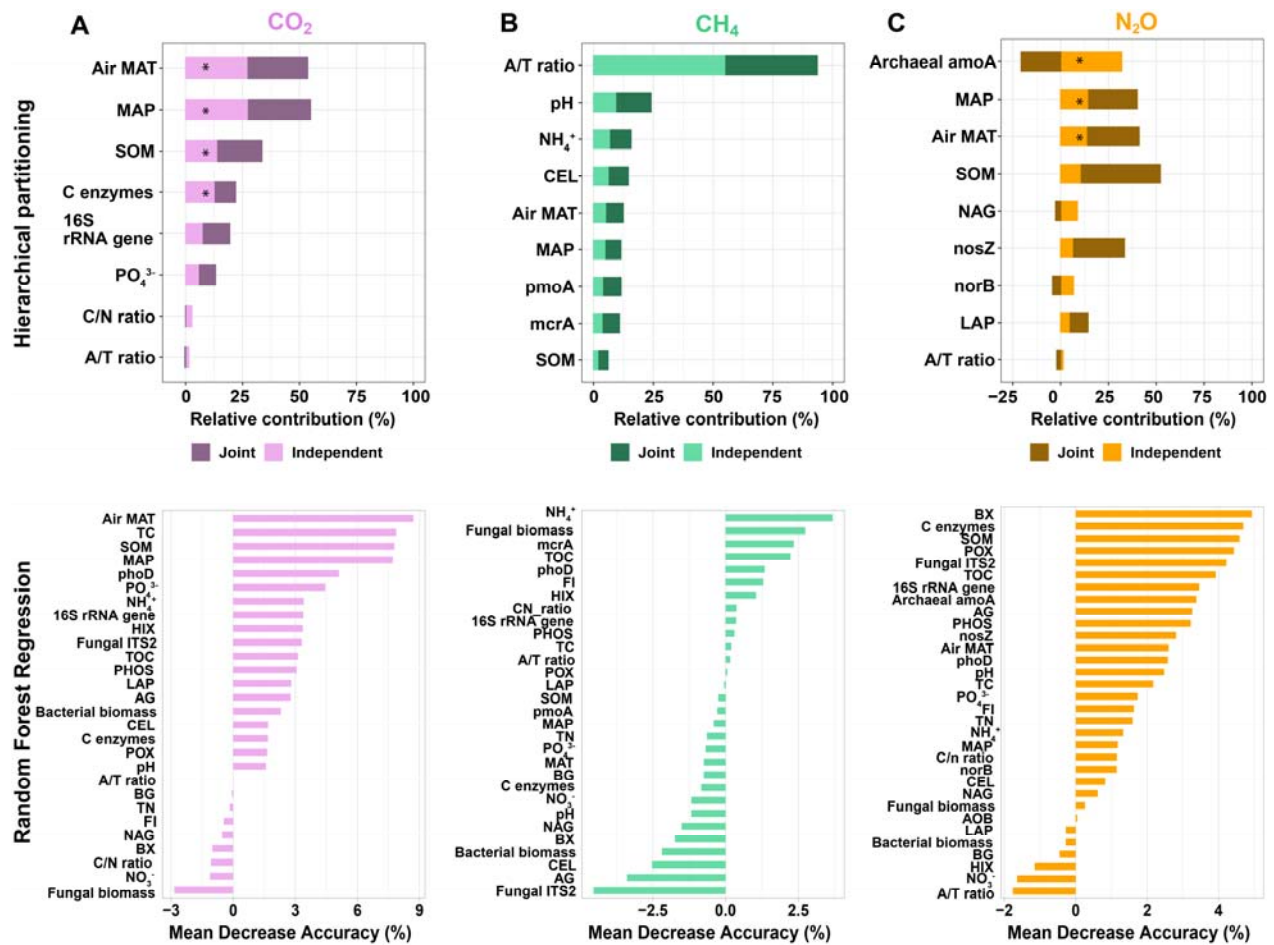
Supplementary Fig. S5. Spearman correlations between GHG fluxes, environmental and microbial variables above the treeline. Only significant correlations are shown in the heatmap ($P < 0.05$). Green and purple represent positive and negative correlations, respectively. The size of the circle indicates the coefficient values, corresponding to the number in the circle. Humification index (HIX); biological index (BIX); fluorescence index (FI); α -glucosidase (AG); β -glucosidases (BG); β -xylosidase (BX); Cellobiohydrolase (CEL); Phenol oxidase (POX); C enzymes = AG + BG + BX + CEL + POX; BB: Bacterial biomass; FB: Fungal biomass; 16S: 16S rRNA genes; β -glucosaminidase (NAG); Leu-aminopeptidase (LAP); AOA (Archaeal *amoA*); AOB (Bacterial *amoA*); Phosphatase (PHOS).



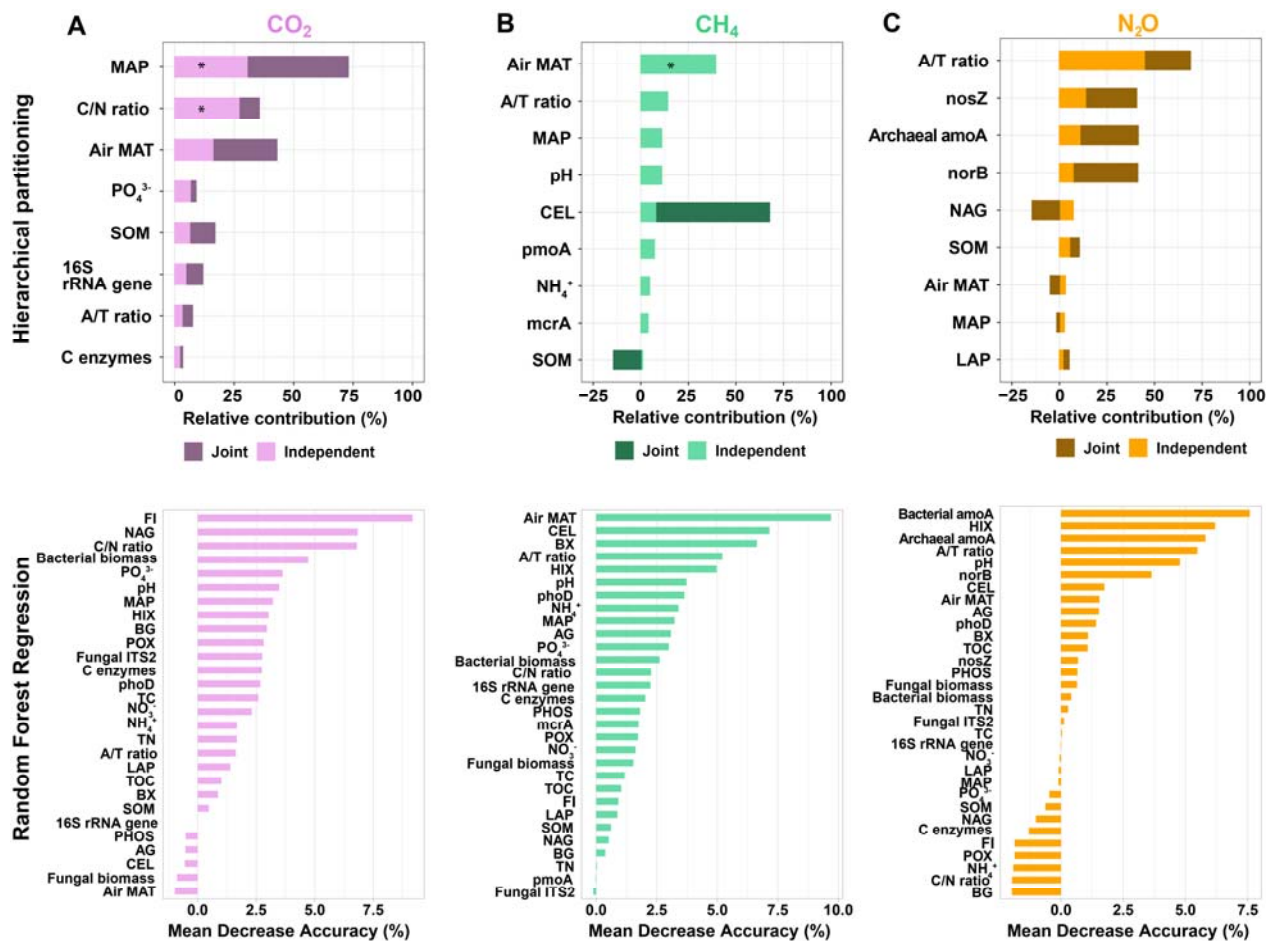
Supplementary Fig. S6. Spearman correlations between GHG fluxes, environmental and microbial variables below the treeline. Only significant correlations are shown in the heatmap ($P < 0.05$). Green and purple represent positive and negative correlations, respectively. The size of the circle indicates the coefficient values, corresponding to the number in the circle. Humification index (HIX); biological index (BIX); fluorescence index (FI); α -glucosidase (AG); β -glucosidases (BG); β -xylosidase (BX); Cellobiohydrolase (CEL); Phenol oxidase (POX); C enzymes = AG + BG + BX + CEL + POX; BB: Bacterial biomass; FB: Fungal biomass; 16S: 16S rRNA genes; β -glucosaminidase (NAG); Leu-aminopeptidase (LAP); AOA (Archaeal *amoA*); AOB (Bacterial *amoA*); Phosphatase (PHOS).



Supplementary Fig. S7. Litter biomass along the elevational gradient. Inlet graph shows the linear negative relationship of litter biomass with altitude from the treeline site. The dashed line indicates the altitude of the treeline. (n=5). Note: The mountain summit site has no litter biomass due to nearly none vegetation cover. ND: non-detectable.



Supplementary Fig. S8. Structural equation model, Hierarchical partitioning and Random Forest Regression showing the impacts of soil characteristics and microbial activities on GHG fluxes (Column A: CO₂; B: CH₄; C: N₂O) above the treeline (n = 20). In Structural equation model (top row), the numbers next to the arrows indicate the strength of standardized path coefficients (***P < 0.001; **P < 0.01; *P < 0.05). Only significant impacts are shown. Green arrows represent positive effects and red arrows negative effects. The fraction of explained variation (R²) for each endogenous variable is highlighted below the variable. In Hierarchical partitioning (middle row), * = P < 0.05 for significant independent effect. In Random Forest Regression (bottom row), % = Variable importance. Variables with individual importance over 4.0% and cumulative importance over 70.0% were considered as the most influential factors. SOM was applied as soil OM quantity, A/T ratio as OM quality and C-acquiring enzymes (AG + BG + BX + CEL + POX) for enzyme activities. Humification index (HIX); biological index (BIX); fluorescence index (FI); α-glucosidase (AG); β-glucosidases (BG); β-xylosidase (BX); Cellobiohydrolase (CEL); β-glucosaminidase (NAG); Leu-aminopeptidase (LAP); Phosphatase (PHOS); Phenol oxidase (POX). C enzymes = AG + BG + BX + CEL + POX.



Supplementary Fig. S9. Structural equation model, Hierarchical partitioning and Random Forest Regression showing the impacts of soil characteristics and microbial activities on GHG fluxes (Column A: CO₂; B: CH₄; C: N₂O) below the treeline (n = 40). In Structural equation model (top row), the numbers next to the arrows indicate the strength of standardized path coefficients (**P < 0.01; *P < 0.05). Only significant impacts are shown. Green arrows represent positive effects and red arrows negative effects. The fraction of explained variation (R²) for each endogenous variable is highlighted below the variable. In Hierarchical partitioning (middle row), * = P < 0.05 for significant independent effect. In Random Forest Regression (bottom row), % = Variable importance. Variables with individual importance over 4.0% and cumulative importance over 70.0% were considered as the most influential factors. SOM was applied as soil OM quantity, A/T ratio as OM quality and C-acquiring enzymes (AG + BG + BX + CEL + POX) for enzyme activities. Humification index (HIX); biological index (BIX); fluorescence index (FI); α -glucosidase (AG); β -glucosidases (BG); β -xylosidase (BX); Cellobiohydrolase (CEL); β -glucosaminidase (NAG); Leu-aminopeptidase (LAP); Phosphatase (PHOS); Phenol oxidase (POX). C enzymes = AG + BG + BX + CEL + POX.

Reference

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., . . . Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology & Biochemistry*, 58, 216-234. doi:10.1016/j.soilbio.2012.11.009