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Supplement of

Temperature sensitivity of dark CO_2 fixation in temperate forest soils

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Supplementary methods

Determination of natural ^{14}C isotope signatures of the beech and spruce soil.

The radiocarbon signature of the bulk soil samples was measured using the accelerator mass spectrometry (AMS) with a 3-MV Tandetron ion accelerator (HVEE, Amersfoort, Netherlands) according to Steinhof et al. (2017). As with the ^{13}C isotope ratios, radiocarbon ratios were reported as the ‰ deviation of the ^{14}C/^{12}C ratio but from the international oxalic acid universal standard (ox1) in Δ^{14}C. All Δ^{14}C value of the sample was then corrected appropriately as previously described (Trumbore, 2009; Mook and Van der Plicht, 1999). The measured bulk ^{14}C values of the beech and spruce soils are described in Table S1.

$$\Delta^{14}C = \left[\frac{\frac{^{14}C}{^{12}C}sample - 25}{0.95\frac{^{14}C}{^{12}C}ox1 - 19 \times exp^{\left(y-\frac{1950}{8267}\right)}}\right] \times 1000$$

(S1)
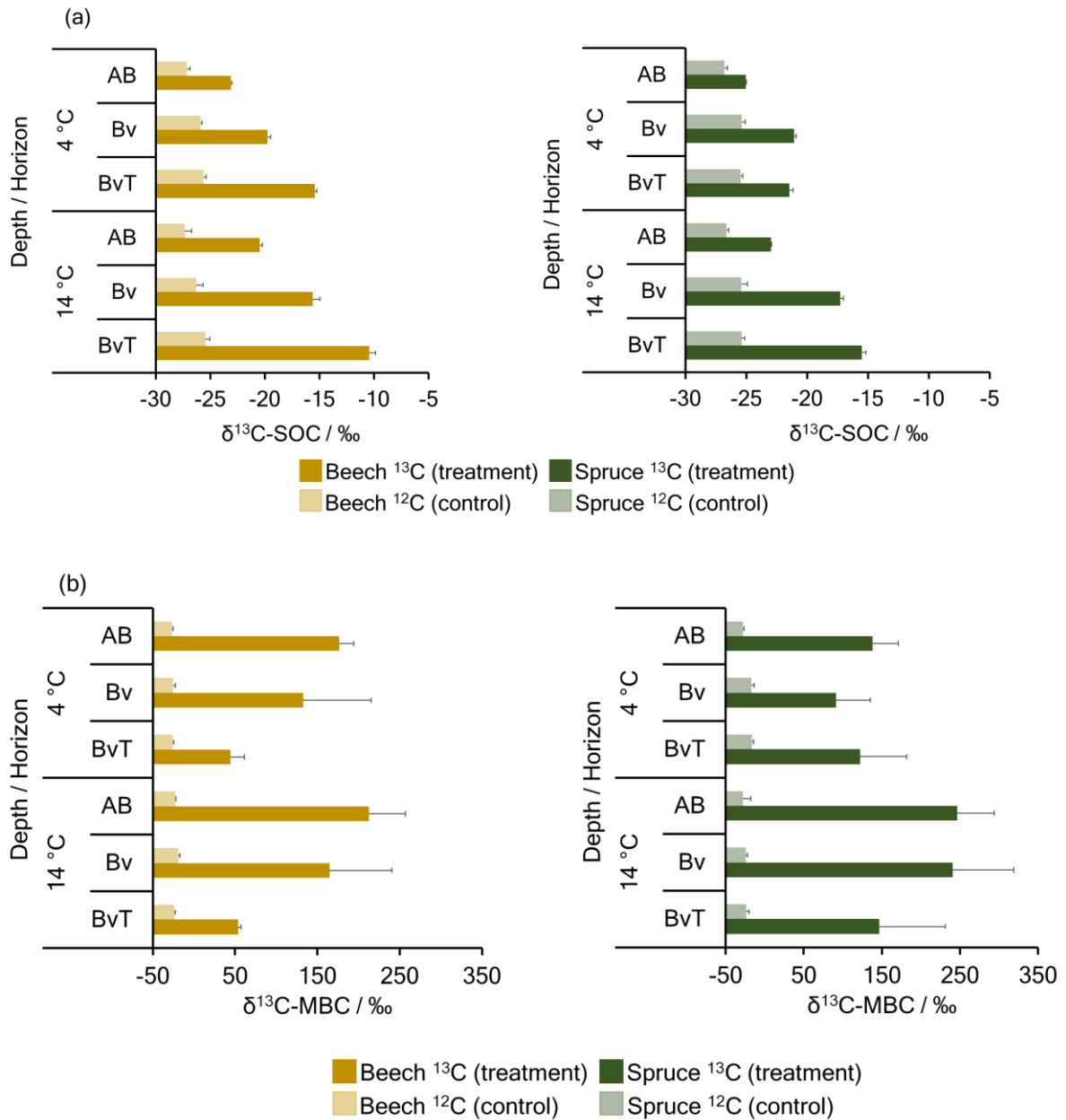
Figure S1: δ¹³C enrichment of SOC and MBC in soil microcosms supplemented with 2% ¹³CO₂ together with ¹²CO₂ labelled controls at 4 and 14 °C. Shown are (a) ^{13}C signal in SOC and (b) ^{13}C signal in MBC after 21 days of incubation with 2% $^{13}CO_2$ at 4 and 14°C across three horizons in beech (yellow bars) and spruce (green bars) soils. Incubations with ^{13}C labelled CO_2 (treatment) are denoted with filled bars while incubations with ^{12}C (control/natural abundance) are denoted by shaded bars. Error bars indicate the standard deviation of incubations from three replicate soil cores.
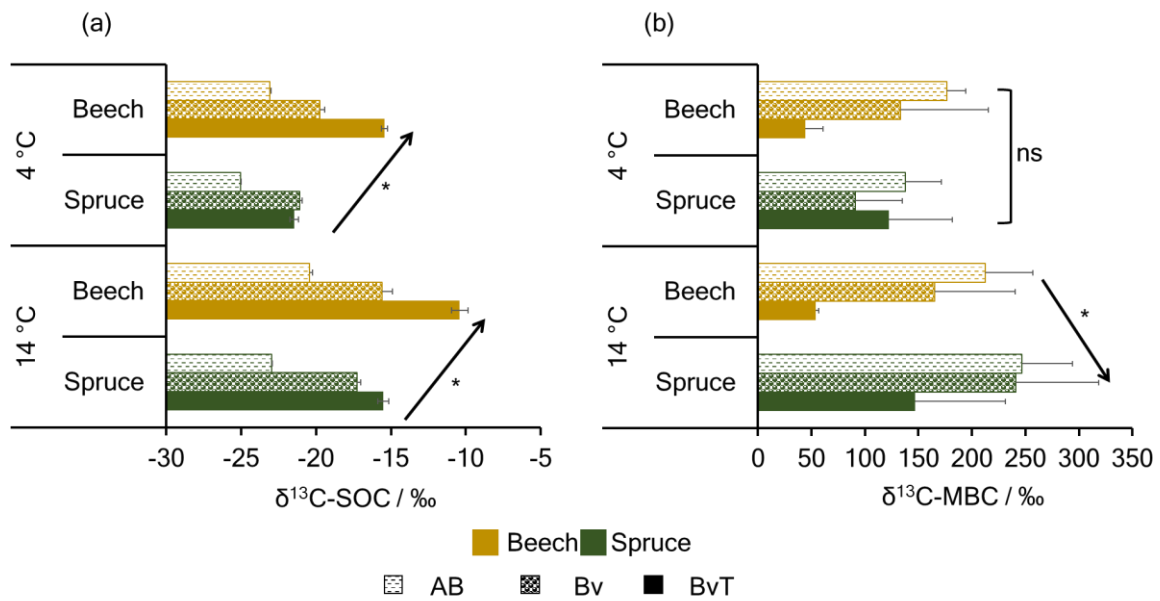
Figure S2: δ^{13}C signals of SOC and MBC in soil microcosms supplemented with 2% $^{13}CO_2$ at 4 and 14 °C from beech and spruce soils. Shown are (a) ^{13}C signal in SOC and (b) ^{13}C signal in MBC after 21 days of incubation with 2% $^{13}CO_2$ at 4 and 14°C across three horizons in beech (yellow bars) and spruce (green bars) soils. Error bars indicate the standard deviation of incubations from three replicate soil cores. * denote $p < 0.05$, ns denote not significant.
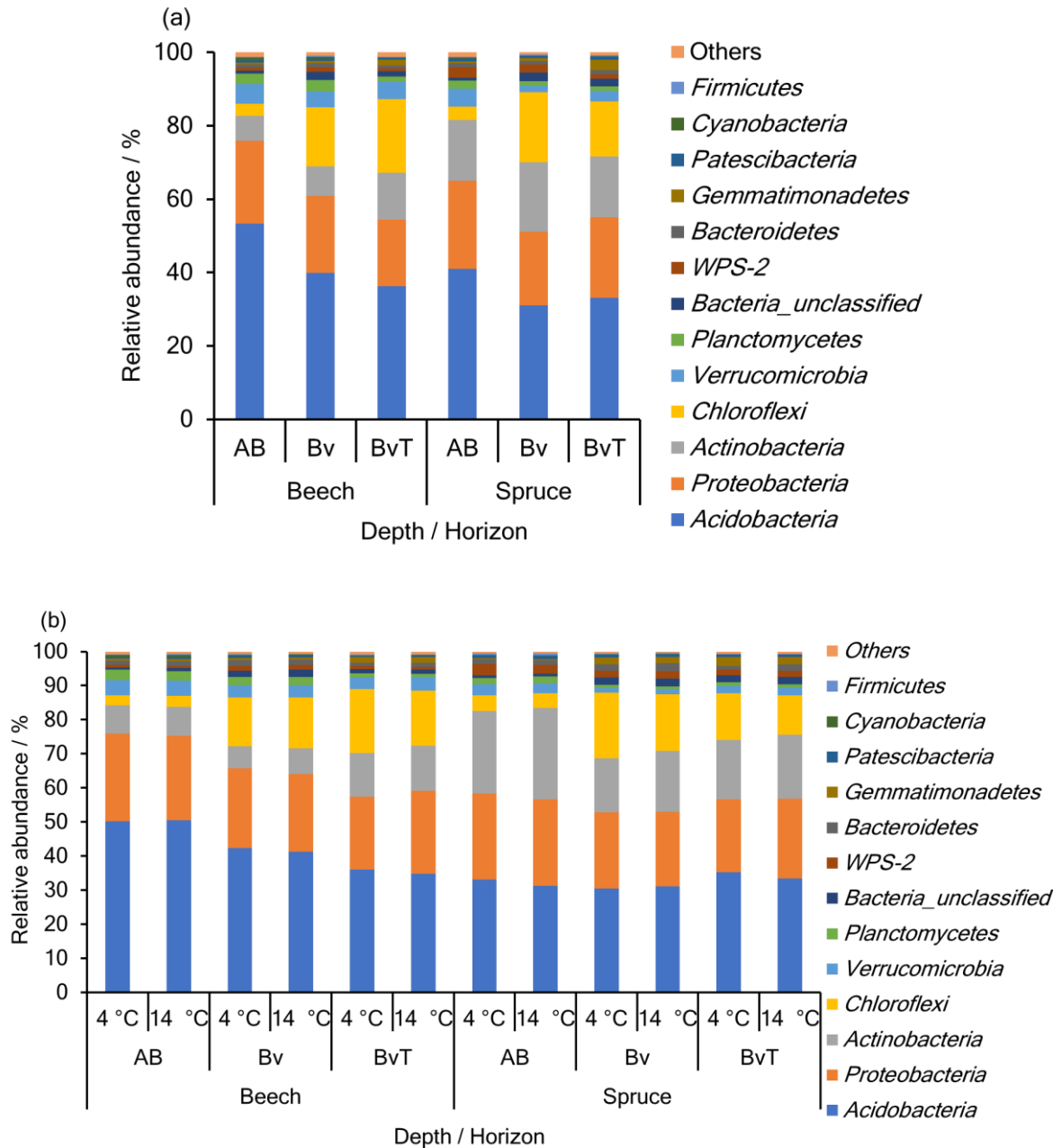
Figure S3: Bacterial community composition from beech and spruce soil. Shown are phylum-level relative abundances of assigned sequences for (a) the beech and spruce bulk soils and for (b) beech and spruce soils incubated with 2% $^{13}CO_2$ at 4 and 14°C. Bar plots are represented by three replicate soil cores per depth. Taxonomic assignment of OTUs is based on the SILVA database implemented on the MOTHUR sequence analysis pipeline. Data represent 3 replicate soil cores per depth for the beech and spruce soils.

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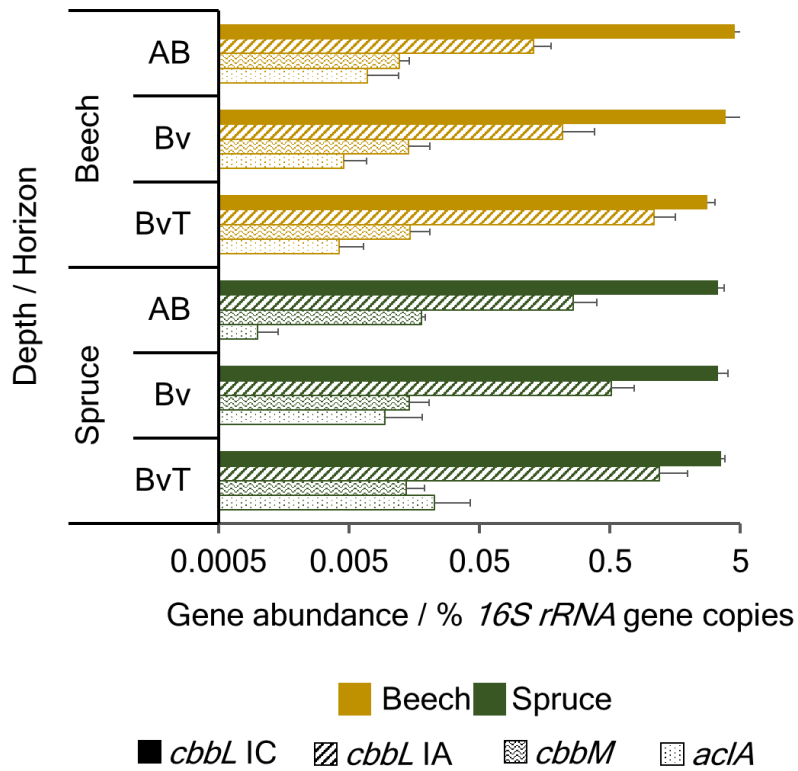
Figure S4: Abundance of chemolithoautotrophic marker genes potentially involved in dark CO₂ fixation in the beech and spruce bulk soi profiles. Shown are the abundances of RuBisCO (*cbbL* IA, *cbbL* IC, and *cbbM*) and ATP citrate lyase (*aclA*) genes relative to total bacterial abundance (*16S rRNA* gene copies) in the beech and spruce bulk soils. Data acquired by qPCR. The scale on the x-axis is logarithmic (base 10). Error bars indicate the standard deviation of incubations from three replicate soil cores.

Table S1: The Δ^{14}C signatures of SOC (‰) measured for soil cores obtained from beech and spruce soil plots at the Hummelshain forest. Each reported value represents the mean of three replicate soil cores taken from bulk soils during the sampling campaign.

Plot	Depth (Horizon)	Δ^{14}C-SOC (‰)
Beech	AB	-2.68 ± 7.48
	Bv	-61.00 ± 30.96
	BvT	-170.10 ± 0.75
Spruce	AB	16.64 ± 18.18
	Bv	-92 .98 ± 28.82
	BvT	-201.89 ± 76.59

Table S2: Geochemical properties of soil cores obtained from beech and spruce soil plots at the Hummelshain forest measured under two temperature conditions. Soil organic carbon (SOC), Total nitrogen (TN), carbon/nitrogen (C/N) ratio, microbial biomass carbon (MBC), Moisture, and *16S rRNA* gene copies reported for 3 depths definitions for the beech and spruce soils at 4 and 14 °C. Each reported value represents the mean of three replicate soil cores taken after the soil incubation period.

Depth (Horizon)		Beech			Spruce		
		AB	Bv	BvT	AB	Bv	BvT
SOC (%)	**4 °C**	0.89 ± 0.09	0.27 ± 0.10	0.12 ± 0.02	1.50 ± 0.05	0.23 ± 0.04	0.30 ± 0.14
	14 °C	0.89 ± 0.1	0.29 ± 0.11	0.13 ± 0.03	1.56 ± 0.04	0.25 ± 0.05	0.30 ± 0.14
TN (%)	**4 °C**	0.04 ± 0.003	0.03 ± 0.004	0.02 ± 0.002	0.07 ± 0.01	0.03 ± 0.002	0.03 ± 0.005
	14 °C	0.04 ± 0.001	0.03 ± 0.003	0.02 ± 0.003	0.08 ± 0.002	0.03 ± 0.002	0.03 ± 0.003
C/N ratio	**4 °C**	20.82 ± 0.64	10.12 ± 3.24	5.22 ± 0.51	19.99 ± 1.96	8.74 ± 1.37	9.32 ± 3.24
	14 °C	19.81 ± 2.54	10.62 ± 2.82	5.16 ± 0.93	19.23 ± 0.39	9.12 ± 1.44	9.25 ± 3.55
MBC (μg C gdw^{-1})	**4 °C**	54.91 ± 4.91	24.04 ± 2.61	12.72 ± 1.78	101.53 ± 19.5	28.97 ± 9.56	27.61 ± 8.50
	14 °C	47.61 ± 2.95	23.90 ± 7.06	15.81 ± 2.51	73.69 ± 17.81	13.12 ± 3.95	26.62 ± 5.86
Moisture (%)	**4 °C**	8.92 ± 1.68	11.09 ± 1.32	11.96 ± 2.48	7.71 ± 1.68	11.23 ± 2.83	11.29 ± 1.97
	14 °C	7.49 ± 1.3	7.31 ± 0.44	10.65 ± 1.31	7.12 ± 1.00	7.42 ± 0.75	10.72 ± 1.07
16S rRNA (copies/gdw^{-1})	**4 °C**	2.96 x 109 ± 9.69 x 108	8.49 x 108 ± 5.50 x 108	1.17 x 108 ± 4.27 x 108	2.19 x 109 ± 5.68 x 108	2.76 x 108 ± 1.32 x 108	2.92 x 108 ± 1.89 x 108
	14 °C	3.23 x 109 ± 7.41 x 108	8.67 x 108 ± 4.62 x 108	1.52 x 108 ± 1.02 x 108	2.54 x 109 ± 8.84 x 109	2.74 x 108 ± 1.06 x 108	2.98 x 108 ± 1.74 x 108

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Table S3: Derived decomposition rates and the Q_{10} for the beech and spruce soils across depth. Decomposition rates (at 4 and 14 °C) were derived by adding the respective measured CO_2 fixation rates with the net respiration rates for all samples while the Q_{10} values were calculated as similarly done for the CO_2 fixation rates and the net respiration rates (Eq. (6) in method section 2.4). Each reported value represents the mean of three replicate soil cores taken after the soil incubation period. ND denotes values that were "not determined".

Plot	Depth (Horizon)	Decomposition rates (μg C g^{-1} (dw) soil d^{-1})		Q_{10}
		4 °C	14 °C	
Beech	AB	1.28 ± 0.63	2.92 ± 1.42	2.29 ± 0.02
	Bv	0.58 ± 0.49	1.23 ± 0.93	3.37 ± 1.34
	BvT	ND	0.14 ± 0.09	ND
Spruce	AB	0.91 ± 0.39	2.34 ± 0.92	2.59 ± 0.11
	Bv	0.13 ± 0.11	0.34 ± 0.01	3.89 ± 3.28
	BvT	0.41 ± 0.12	1.07 ± 0.07	2.63 ± 0.29

Table S4: Primers and adapter sequences used for two-step barcoding approach for Illumina MiSeq sequencing of the bacterial *16S rRNA* genes.

Primer	Sequences (5' – 3')		References
1st PCR step			
Bact_341F	[TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG] CCTACGGGNGGCWGCAG		Klindworth et al., 2013
Bact_785R	[GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG] GACTACHVGGGTATCTAATCC		
2nd PCR step			
Index 1	CAAGCAGAAGACGGCATACGAGAT GTCTCGTGGGCTCGG	[i7]	Illumina®
Index 2	AATGATACGGCGACCACCGAGATCTACAC TCGTCGGCAGCGTC	[i5]	

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