



Supplement of

Soil profile connectivity can impact microbial substrate use, affecting how soil CO₂ effluxes are controlled by temperature

Frances A. Podrebarac et al.

Correspondence to: Susan E. Ziegler (sziegler@mun.ca)

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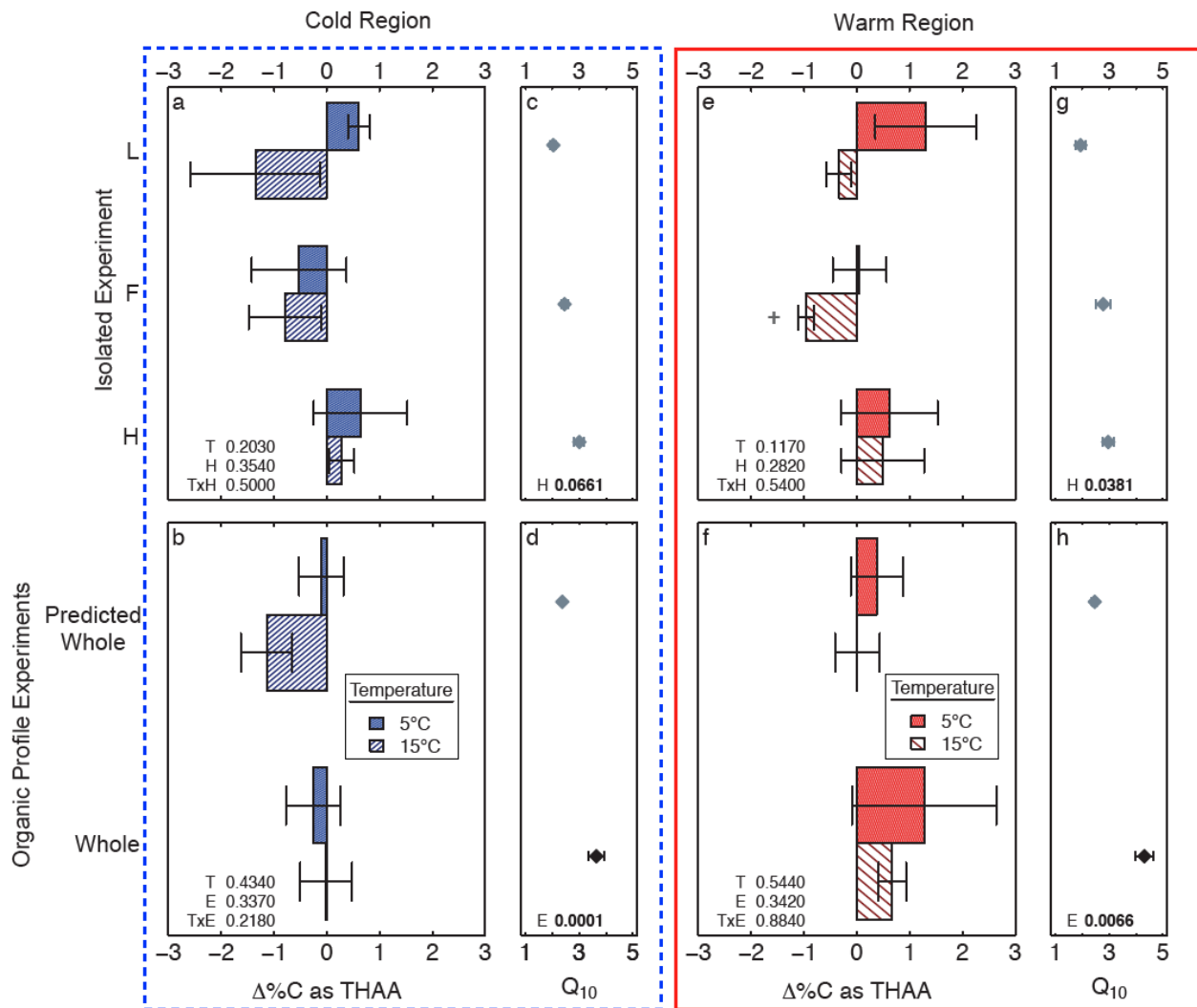
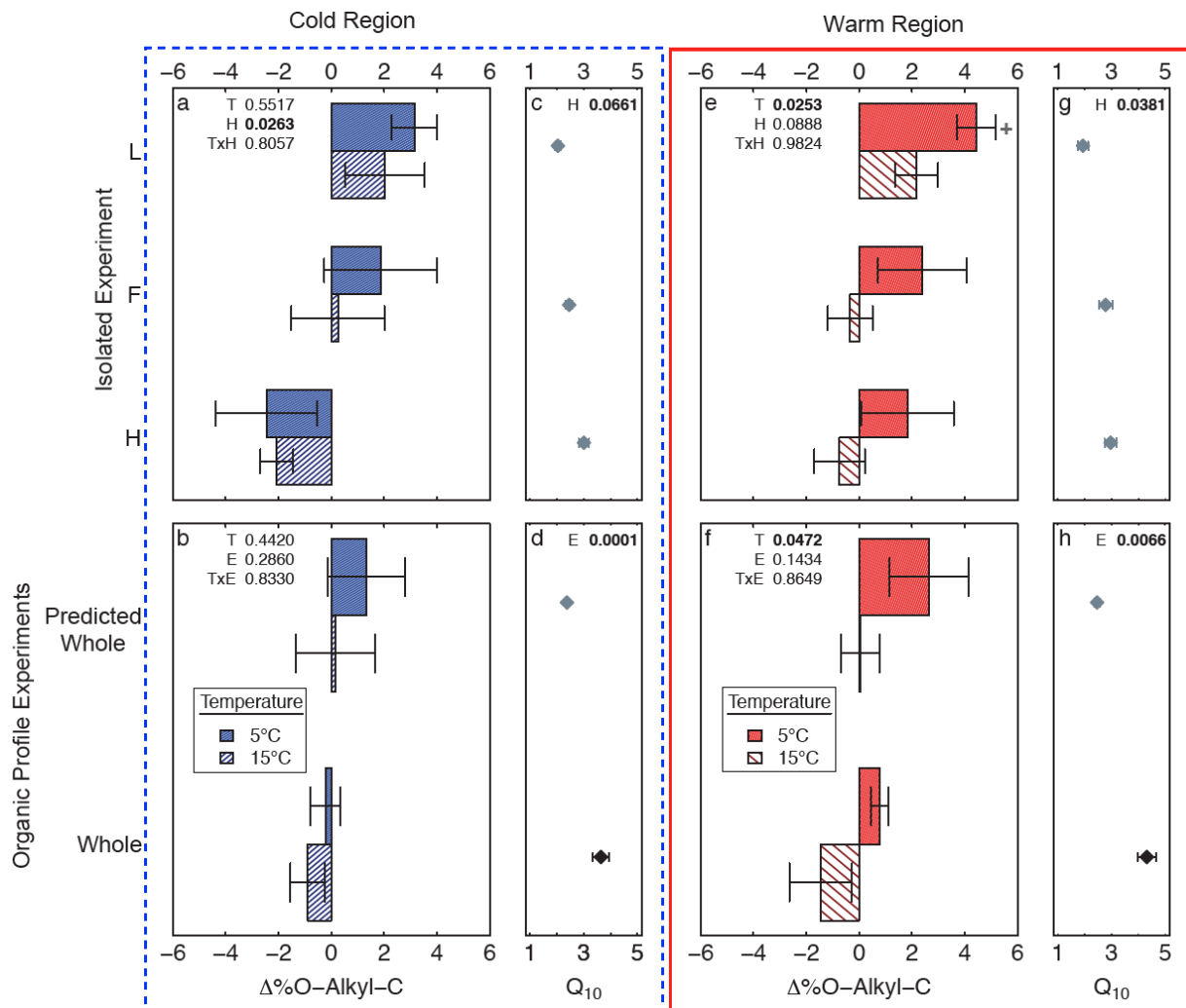


Figure S1. The change in %C as total hydrolyzable amino acid (Δ %C THAA) given as the difference between the absolute final minus initial values for the soils incubated as individual horizons in isolation from each other (upper panels labeled by horizon; L, F and H) and those calculated as whole profile values based upon those same isolated horizon results (predicted), and those measured directly from the incubation of whole organic profiles (measured). These results are given for both the cold (left, blue) and warm regions (right, red) with the corresponding temperature sensitivity (Q_{10}) of cumulative respiration (c,d and g,h, respectively). All values provided are the mean of the three sites \pm the standard error with a significant change from 0 denoted by symbol "+". Any significant ($\alpha \leq 0.05$) effect of temperature (T), horizon (H) or experiment (E) or interaction term (TxH or TxE) is denoted in bold. A significant effect of temperature within horizon or within experiment is denoted by an asterisk.

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30 **Figure S2.** The change in %Aromatic-C (Δ % Aromatic-C) given as the difference between the absolute final minus initial values for the soils incubated as individual horizons in isolation from each other (upper panels labeled by horizon; L, F and H) and those calculated as whole profile values based upon those same isolated horizon results (predicted), and those measured directly from the incubation of whole organic profiles (measured). These results are given for both the cold (left, blue) and warm regions (right, red) with the corresponding temperature sensitivity (Q_{10}) of cumulative respiration (c,d and g,h, respectively). All values provided are the mean of the three sites \pm the standard error with a significant change from 0 denoted by symbol "+". Any significant ($\alpha \leq 0.05$) effect of temperature (T), horizon (H) or experiment (E) or interaction term (TxH or TxE) is denoted in bold. A significant effect of temperature within horizon or within experiment is denoted by an asterisk.

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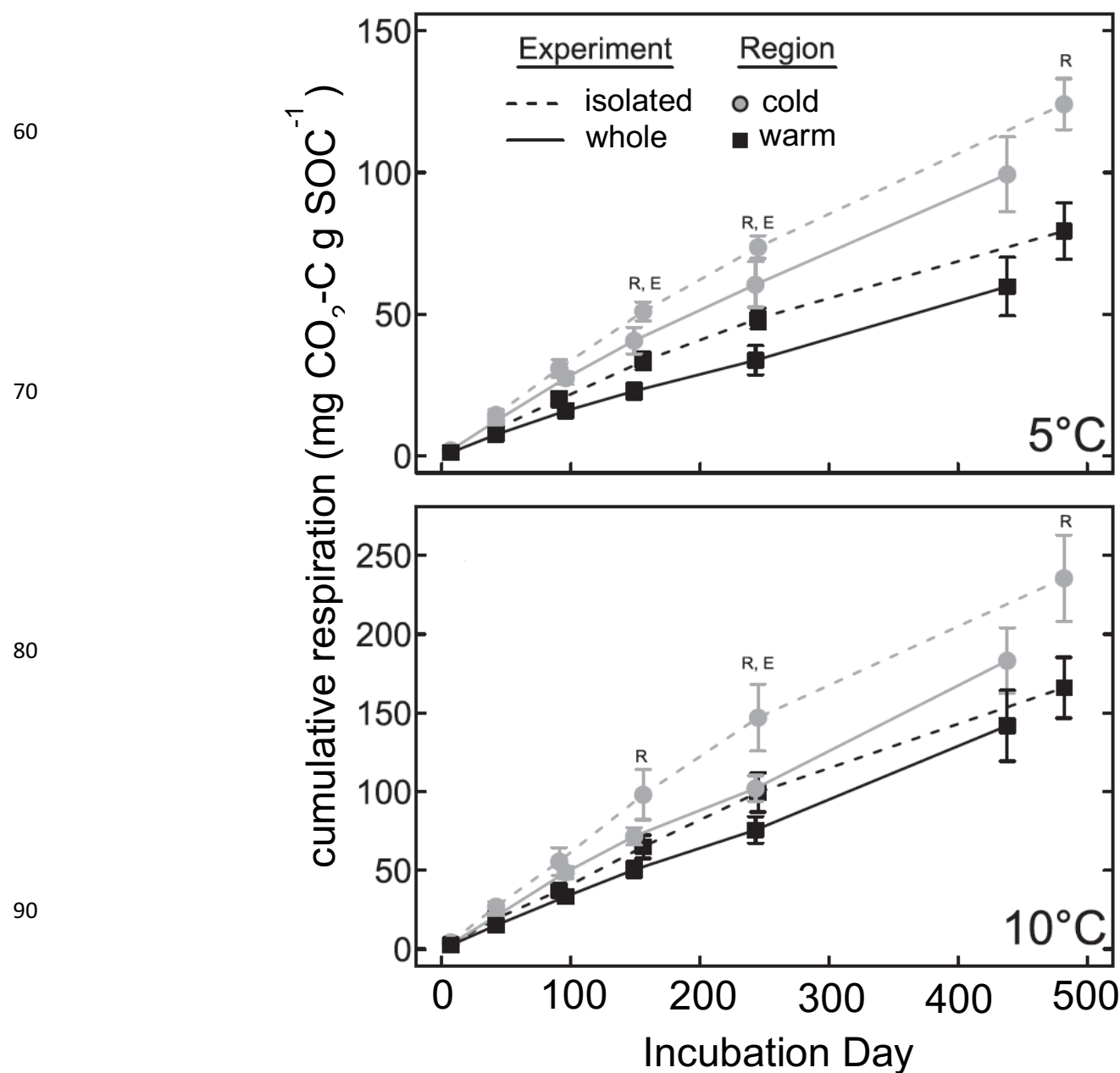


Figure S3. The cumulative soil respiration rate measured in both the whole (solid line) and the isolated (dashed line) experiment at each of the six time points over the entire incubation conducted at 5°C (top) and 10°C (bottom). Linear interpolation of instantaneous respiration rates was used to estimate respiration between time points and obtain cumulative values over the incubation. To enable a direct comparison between the two experiments, the isolated experiment rates are given as the sum of the isolated L, F and H horizons where each is in the same proportions of soil organic carbon (SOC) as incubated in the whole experiment. The effects of region (R) or experiment (E) are denoted by time point where they were found to be significant ($\alpha=0.05$). No interaction effects were noted. Figure modified from Podrebarac et al. (2016).

Table S1. Location and characteristics of field sites studied herein including mean annual temperature (MAT), mean annual precipitation (MAP), potential evapotranspiration (PET), annual litter fall input, tree basal area across each site, soil organic carbon (SOC) in the organic layer (LFH), and LFH depth. Table adapted from Kohl et al., (2018) and Ziegler et al., (2017).

Region	Site	Latitude	Longitude	Elevation (m)	MAT ¹ (C)	MAP ¹ (mm a ⁻¹)	PET ¹ (mm a ⁻¹)	Litterfall ² (kg ha ⁻¹ yr ⁻¹)	Basal area ² (m ² ha ⁻¹)	SOM in LFH ² (kg SOMC m ⁻²)	LFH depth ² (cm)
Eagle River (Cold)	Muddy Pond (MP)	53°33'01"N	56°59'13"W	145	0.0	1074	432	1815	37.2	2.43	9.8
	Sheppard's Ridge (SR)	53°03'25"N	56°56'02"W	170	0.0	1074	432	1992	a 50.1	a 2.16	a 7.9
	Harry's Pond (HP)	53°35'12"N	56°53'21"W	136	0.0	1074	432	2380	38.2	1.95	7.4
Salmon River (Mid)	Hare Bay (HB)	51°15'21"N	56°8'18"W	31	2.0	1224	489	4686	45.4	3.13	9.9
	Tuckamore (TM)	51°9'51"N	56°0'15"W	16	2.0	1224	489	3213	ab 39.2	a 3.15	ab 8.7
	CatchAFeeder (CF)	51°5'21"N	56°12'16"W	38	2.0	1224	489	19421 ³	34.0	2.51	9.7
Grand Codroy (Warm)	Slug Hill (SH)	48°00'39"N	58°54'16"W	215	5.2	1505	608	4562	48.3	2.88	8.1
	Maple Ridge (MR)	48°00'28"N	58°55'14"W	165	5.2	1505	608	4007	b 44.7	a 3.23	b 7.9
	O'Reagan's (OR)	47°53'36"N	59°10'28"W	100	5.2	1505	608	5374	50.1	2.91	8.3

¹ MAT; mean annual temperature; MAP, mean annual precipitation; PET annual potential evapotranspiration. Meteorological data represent climate normals from 1981-2010 from Cartwright, NL; Main brook, NL; and Doyles, NL weather stations (Environment Canada and Climate Change). Potential evaporation was calculated according to Xu and Singh (2001) based on monthly temperature and precipitation normals.

² Letters indicate significant differences among transect regions. Litterfall (collected from June 2011 to June 2013) and soil C stocks are taken from Ziegler et al., 2017.

³ Field sites affected by extreme windfall event.

Table S2. The mean (\pm standard error) of the change in the soil stable carbon and nitrogen composition ($\Delta \delta^{13}\text{C}$ and $\Delta \delta^{15}\text{N}$, respectively) over the incubation where individual horizons were incubated in isolation from each other (isolated; a), calculated as whole organic profiles values based upon those isolated horizon results (predicted whole; b) and the directly measured incubation results for whole organic profiles (whole; b). The results of the analysis of variance used to determine the effect of region (R), horizon (H), temperature (T), and the interaction terms are provided for the isolated horizon experiment (a) and the effects of R, experiment (E), T and the interaction terms are provide for the predicted whole and whole experiments. Significance ($\alpha=0.05$) is denoted in bold.

Experiment	Horizon	$\Delta \delta^{13}\text{C}$				$\Delta \delta^{15}\text{N}$			
		Cold region		Warm region		Cold region		Warm region	
		5°C	15°C	5°C	15°C	5°C	15°C	5°C	15°C
a. Isolated	L	-0.55 (0.11)	-0.53 (0.18)	-0.21 (0.15)	-0.26 (0.13)	0.76 (0.35)	0.37 (0.30)	0.30 (0.19)	0.26 (0.09)
	F	-0.72 (0.67)	-1.78 (0.76)	-0.59 (0.32)	-0.58 (0.34)	0.84 (1.17)	-0.40 (0.66)	0.45 (0.35)	-0.95 (1.47)
	H	-1.05 (0.59)	-0.66 (0.11)	-0.38 (0.21)	-0.59 (0.36)	-0.67 (0.66)	0.38 (0.19)	0.15 (0.13)	-0.23 (0.32)
	Effects	<i>F</i>		<i>p</i>		<i>F</i>		<i>p</i>	
	R	3.85		0.0615		0.34		0.5680	
	H	1.81		0.1849		0.76		0.4800	
	T	0.42		0.5235		1.19		0.2860	
	R x H	0.23		0.7930		0.20		0.8200	
	R x T	0.09		0.7664		0.32		0.5770	
	H x T	0.69		0.5114		1.72		0.2000	
R x H x T	1.16		0.3300		0.52		0.6010		
b. Predicted Whole	organic profile	-0.75 (0.37)	-1.32 (0.47)	-0.49 (0.26)	-0.52 (0.30)	0.55 (0.72)	-0.12 (0.40)	0.37 (0.18)	-0.60 (1.01)
	Whole organic profile	-0.79 (0.28)	-1.07 (0.32)	-0.24 (0.07)	-0.49 (0.01)	-0.12 (0.16)	0.09 (0.16)	0.15 (0.13)	0.46 (0.10)
	Effects	<i>F</i>		<i>p</i>		<i>F</i>		<i>p</i>	
	R	6.98		0.0178		0.00		0.9950	
	E	0.30		0.5411		0.08		0.7810	
	T	1.89		0.1879		0.70		0.4140	
	R x E	0.002		0.9665		0.93		0.3490	
	R x T	0.50		0.4897		0.02		0.8870	
	E x T	0.02		0.8941		2.58		0.1280	

R x E x T

0.39

0.5392

0.09

0.7690

106 **Table S3.** The mean and standard error (SE) of the initial soil carbon to nitrogen ratio (molar; C:N),
 107 percent N as total hydrolysable amino acids (%N as THAA), % alkyl-C, %di-O-alkyl-C and ratio of alkyl
 108 to O-alkyl-C for the separated organic layer horizons (L, F, H) and the whole organic layer (LFH) from
 109 both the forest sites located in the cold and warm regions.
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Region	Horizon	C:N		%N as THAA		mol% glycine		% alkyl-C		%di-O-alkyl-C		A:O-A ratio	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	S
Cold	L	54.4	2.2	42.8	1.4	12.9	0.2	24.8	1.0	9.1	0.4	0.76	0
Cold	F	42.3	1.9	42.2	1.8	14.2	0.5	28.7	2.4	8.2	0.5	0.88	0.
Cold	H	42.8	1.9	38.5	1.9	16.3	0.4	27.5	1.4	9.2	0.3	0.76	0.
Cold	LFH	44.6	1.3	41.6	1.3	14.3	0.3	27.8	1.9	8.6	0.4	0.84	0.
Warm	L	39.5	0.8	43.9	0.7	13.9	0.9	27.5	0.9	8.4	0.2	0.96	0.
Warm	F	34.5	0.6	43.5	1.8	14.0	0.5	27.5	2.1	7.8	0.6	0.94	0.
Warm	H	31.8	1.2	37.9	1.4	19.8	0.6	33.5	1.2	7.1	0.4	1.25	0.
Warm	LFH	34.9	0.6	42.5	1.4	15.0	0.3	28.6	1.6	7.8	0.5	1.00	0.

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142 **Table S4.** The change in the soil carbon to nitrogen ratio (Δ C:N), percent N as total hydrolysable
 143 amino acids (Δ %N as THAA), and change in % alkyl-C (Δ % alkyl-C) over the course of the 5°C and
 144 15°C incubation reported here for the individual separate horizons (L, F, H), the predicted values for
 145 the total organic layer based upon those separate horizons incubated in isolation (predicted LFH), and
 146 for the treatment where those same horizons were incubated together as a whole organic layer
 147 (measured LFH). Values are all given as the mean and standard error (SE) of three sites from the
 148 given region (cold, warm).
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Region	Horizon/Experiment	Temperature	Δ C:N		Δ %N as THAA		Δ %alkyl-C	
			mean	SE	mean	SE	mean	SE
Cold	L	5	-8.86	1.56	0.65	4.18	-4.47	0.55
Cold	F	5	-3.63	0.81	-0.79	2.03	-3.68	0.77
Cold	H	5	-3.24	2.25	4.45	8.57	3.82	5.06
Cold	LFH predicted	5	-4.51	1.05	0.43	2.08	-2.46	1.29
Cold	LFH measured	5	-4.24	1.08	-0.72	1.92	-1.75	0.67
Cold	L	15	-14.24	2.50	-7.51	1.09	-4.57	1.54
Cold	F	15	-8.28	1.11	-7.70	3.14	-3.13	2.41
Cold	H	15	-7.74	0.87	-3.95	3.55	0.40	2.05
Cold	LFH predicted	15	-9.27	1.23	-6.98	2.82	-2.75	2.07
Cold	LFH measured	15	-9.89	0.55	-3.44	1.62	1.09	1.02
Warm	L	5	-4.43	0.83	6.52	8.07	-7.07	0.41
Warm	F	5	-2.05	2.36	-1.84	6.72	-4.29	2.17
Warm	H	5	-0.62	0.83	3.47	4.09	-2.84	1.88
Warm	LFH predicted	5	-2.22	1.51	0.65	5.66	-4.53	1.70
Warm	LFH measured	5	-2.47	0.39	7.04	10.19	-0.38	0.77
Warm	L	15	-9.27	2.46	-12.39	0.78	-1.04	3.16
Warm	F	15	1.05	4.61	-7.18	6.19	0.46	1.68
Warm	H	15	-2.44	0.42	1.05	7.03	2.11	1.10
Warm	LFH predicted	15	-1.46	2.62	-6.63	4.08	0.49	0.83
Warm	LFH measured	15	-5.72	0.65	-0.91	1.96	2.06	1.15

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Table S5. The $\delta^{13}\text{C}$ of the soil respired CO_2 at the start of the experiment (initial) and the $\delta^{13}\text{C}$ of total cumulative respired CO_2 at the end of the incubation conducted at both 5°C and 15°C (5, 15) measured from the individual separate horizons (L, F, H), the predicted values for the total organic layer based upon those separate horizons incubated in isolation (LFH predicted), and from the treatment where those same horizons were incubated together as a whole organic layer (LFH measured). Values are all given as the mean and standard error (SE) of three sites from the given region (cold, warm).

Region	Horizon/Experiment	Temperature	$\delta^{13}\text{C}$ of respired CO_2	
			mean	SE
Cold	L	initial	-29.34	0.16
Cold	F	initial	-27.82	0.48
Cold	H	initial	-27.32	0.28
Cold	LFH predicted	initial	-28.01	0.32
Cold	LFH measured	initial	-28.01	0.32
Cold	L	5	-28.63	0.55
Cold	F	5	-27.12	0.07
Cold	H	5	-29.17	0.39
Cold	LFH predicted	5	-28.11	0.08
Cold	LFH measured	5	-24.17	0.45
Cold	L	15	-25.86	0.22
Cold	F	15	-25.59	0.03
Cold	H	15	-24.74	0.09
Cold	LFH predicted	15	-25.48	0.08
Cold	LFH measured	15	-24.93	0.20
Warm	L	initial	-29.31	0.12
Warm	F	initial	-28.61	0.17
Warm	H	initial	-27.76	0.20
Warm	LFH predicted	initial	-28.58	0.15
Warm	LFH measured	initial	-28.58	0.15
Warm	L	5	-27.84	0.06
Warm	F	5	-25.59	0.68
Warm	H	5	-24.80	0.91
Warm	LFH predicted	5	-26.15	0.59
Warm	LFH measured	5	-23.70	0.31
Warm	L	15	-25.67	0.20
Warm	F	15	-25.16	0.55
Warm	H	15	-24.29	0.54
Warm	LFH predicted	15	-25.09	0.48
Warm	LFH measured	15	-24.26	0.36

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