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

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Figure S1. The change in %C as total hydrolyzable amino acid (Δ %C THAA) results given as the final minus initial values comparing the experiment where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) with the corresponding temperature sensitivity (Q_{10}) of cumulative respiration for the soils from both the cold (a-d) and warm regions (e-h). Values provided are the mean of the three sites ± standard error. For the effect of temperature (T), horizon (H) and their interaction term (T x H) significance ($\alpha \le 0.05$) is denoted in bold. Significance within horizon or within experiment is denoted by an asterisk, note that no such effects were detected here.



Figure S2. The change in %mol glycine (Δ %mol glycine) given as the final minus initial values comparing the experiment where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) with the corresponding temperature sensitivity (Q₁₀) of cumulative respiration for the soils from both the cold (a-d) and warm regions (e-h). Values provided are the mean of the three sites \pm standard error with a significant change from 0 denoted by the symbol "+" (a, e). For the effect of temperature (T), horizon (H) and their interaction term (T x H) significance ($\alpha \le 0.05$) is denoted in bold. Significance within horizon or within experiment is denoted by an asterisk.



Figure S3. The change in alkyl-C:O-Alkyl-C (Δ A:O-A) given as the final minus initial values comparing the experiment where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) with the corresponding temperature sensitivity (Q₁₀) of cumulative respiration for the soils from both the cold (a-d) and warm regions (e-h). Values provided are the mean of the three sites ± standard error with a significant change from 0 denoted by the symbol "+" (a, e). For the effect of temperature (T), horizon (H) and their interaction term (T x H) significance ($\alpha \le 0.05$) is denoted in bold. Significance within horizon or within experiment is denoted by an asterisk, note that no such effects were detected here.



Figure S4. The change in %O-alkyl-C (Δ %O-Alkyl-C) given as the final minus initial values comparing the experiment where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) with the corresponding temperature sensitivity (Q₁₀) of cumulative respiration for the soils from both the cold (a-d) and warm regions (e-h). Values provided are the mean of the three sites ± standard error with a significant change from 0 denoted by the symbol "+: (a, e). For the effect of temperature (T), horizon (H) and their interaction term (T x H) significance ($\alpha \le$ 0.05) is denoted in bold. Significance within horizon or within experiment is denoted by an asterisk, note that no such effects were detected here.



Figure S5. The change in %Di-O-Alkyl-C (Δ %Di-O-Alkyl-C) given as the final minus initial values comparing the experiment where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) with the corresponding temperature sensitivity (Q₁₀) of cumulative respiration for the soils from the cold (a-d) and warm regions (e-h). Values are the mean of the three sites ± standard error with a significant change from 0 denoted by the symbol "+" (a, e). For the effect of temperature (T), horizon (H) and their interaction term (T x H) significance ($\alpha \le 0.05$) is denoted in bold. Significance within horizon or within experiment is denoted by an asterisk.



Figure S6. The change in %Aromatic-C (Δ % Aromatic-C) given as the final minus initial values comparing the experiment where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) with the corresponding temperature sensitivity (Q₁₀) of cumulative respiration for the soils from both the cold (a-d) and warm regions (e-h). Values are the mean of the three sites ± standard error with a significant change from 0 denoted by the symbol "+" (a, e). For the effect of temperature (T), horizon (H) and their interaction term (T x H) significance ($\alpha \le 0.05$) is denoted in bold. Significance within horizon or within experiment is denoted by an asterisk.

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)	MAT¹ (C)	MAP¹ (mm a ¹)	PET¹ (mm a ¹)	Litterfa (kg ha ¹ y	nll ² /r ¹)	Basal ar (m² ha¹	ea² ')	SOM in (kg SON	I LFH² IC m ²)	LFH dept	ן h ²
Eagle	Muddy Pond (MP)	53°33'01''N	56°59'13''W	145	0.0	1074	432	1815		37.2		2.43		9.8	,
River	Sheppard's Ridge (SR)	53°03'25''N	56°56'02''W	170	0.0	1074	432	1992	а	50.1	а	2.16	а	7.9	ab
(Cold)	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	Hare Bay (HB)	51°15'21"N	56°8'18"W	31	2.0	1224	489	4686		45.4		3.13		9.9	
River	Tuckamore (TM)	51°9'51"N	56°0'15"W	16	2.0	1224	489	3213	ab	39.2	а	3.15	ab	8.7	а
(Mid)	CatchAFeeder (CF)	51°5'21"N	56°12'16"W	38	2.0	1224	489	19421 ³		34.0		2.51		9.7	
Grand	Slug Hill (SH)	48°00'39''N	58°54'16''W	215	5.2	1505	608	4562		48.3		2.88		8.1	
Codroy	Maple Ridge (MR)	48°00'28''N	58°55'14''W	165	5.2	1505	608	4007	b	44.7	а	3.23	b	7.9	b
(Warm)	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 frpm 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 (standard error) of the $\Delta \delta^{13}$ C and $\Delta \delta^{15}$ N over the incubation where individual horizons were incubated in isolation from each other (isolated experiment) to both the calculated whole organic profiles values based upon those isolated horizon results (predicted whole experiment) and the actual measured incubation results for whole organic profiles (whole experiment) (a) the isolated experiment with an analysis of variance utilized to determine the effect of region (R), horizon (H), and interaction term; (b) organic profile experiments of predicted whole and whole experiments with an analysis of variance utilized to determine the effect of R. Significance (α =0.05) is denoted in bold.

			$\Delta \delta^{13}$ C			$\Delta \delta^{15} N$					
	Experiment	Horizon	Cold	region	Warm	region	Cold r	egion	Warm region		
			5°C	15°C	5°C	15°C	5°C	15°C	5°C	15°C	
a.	Isolated	L	-0.55	-0.53	-0.21	-0.26	0.76	0.37	0.30	0.26	
			(0.11)	(0.18)	(0.15)	(0.13)	(0.35)	(0.30)	(0.19)	(0.09)	
		F	-0.72	-1.78	-0.59	-0.58	0.84	-0.40	0.45	-0.95	
			(0.67)	(0.76)	(0.32)	(0.34)	(1.17)	(0.66)	(0.35)	(1.47)	
		Н	-1.05	-0.66	-0.38	-0.59	-0.67	0.38	0.15	-0.23	
			(0.59)	(0.11)	(0.21)	(0.36)	(0.66)	(0.19)	(0.13)	(0.32)	
		Effects		F)		F		0	
		R	3.85		0.0615		0.34		0.5680		
		Н	1.	81	0.1849		0.76		0.4800		
		Т	0.	42	0.5	0.5235		1.19		0.2860	
		RхH	0.23		0.7930		0.20		0.8200		
		RхT	0.09		0.7664		0.32		0.5770		
		НхТ	0.	69	0.5114		1.72		0.2000		
		R x H x T	1.	16	0.3	300	0.52		0.6010		
b.	Predicted Whole	organic	-0.75	-1.32	-0.49	-0.52	0.55	-0.12	0.37	-0.60	
		profile	(0.37)	(0.47)	(0.26)	(0.30)	(0.72)	(0.40)	(0.18)	(1.01)	
	Whole	organic	-0.79	-1.07	-0.24	-0.49	-0.12	0.09	0.15	0.46	
		profile	(0.28)	(0.32)	(0.07)	(0.01)	(0.16)	(0.16)	(0.13)	(0.10)	
		Effects F		F	p			F	p		
		R	6.	98	0.0	178	0.	00	0.9	950	
		E	0.	30	0.5	411	0.	08	0.7	810	
		Т	1.	89	0.1	879	0.	70	0.4	140	
		R x E	0.0	002	0.9	665	0.93		0.3490		
		RхT	0.	50	0.4	897	0.	02	0.8	870	
		ЕхТ	0.	02	0.8	941	2.	58	0.1	280	
		R x E x T	0.	39	0.5	0.5392		09	0.7690		

Table S3. The mean and standard error (SE) of the initial soil carbon to nitrogen ratio (molar; C:N), percent N as total hydrolysable

amino acids (%N as THAA), % alkyl-C, %di-O-alkyl-C and ratio of alkyl to O-alkyl-C for the separated organic layer horizons (L, F, H) and the whole organic layer (LFH) from both the forest sites located in the cold and warm regions.

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Region	Horizon	C:N		%N as T	%N as THAA		mol% glycine		% alkyl-C		%di-O-alkyl-C		atio
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Cold	L	54.43	2.21	42.80	1.44	12.91	0.23	24.84	0.98	9.10	0.35	0.76	0.08
Cold	F	42.34	1.91	42.18	1.80	14.16	0.46	28.70	2.43	8.22	0.46	0.88	0.15
Cold	Н	42.78	1.90	38.51	1.85	16.29	0.43	27.51	1.44	9.17	0.32	0.76	0.09
Cold	LFH	44.62	1.27	41.62	1.31	14.32	0.33	27.78	1.87	8.55	0.38	0.84	0.12
Warm	L	39.46	0.78	43.88	0.65	13.89	0.90	27.47	0.91	8.35	0.21	0.96	0.06
Warm	F	34.54	0.59	43.47	1.75	13.99	0.51	27.53	2.08	7.83	0.64	0.94	0.11
Warm	Н	31.76	1.19	37.87	1.36	19.76	0.64	33.49	1.18	7.11	0.43	1.25	0.09
Warm	LFH	34.93	0.62	42.52	1.36	15.02	0.33	28.60	1.58	7.80	0.51	1.00	0.10

Table S4. The change in the soil carbon to nitrogen ratio (Δ C:N), percent N as total hydrolysable amino acids (Δ %N as THAA), and change in % alkyl-C (Δ % alkyl-C) over the course of the 5°C and 15°C incubation reported here for the individual separate horizons (L, F, H), the predicted values for the total organic layer based upon those separate horizons incubated in isolation (predicted LFH), and for the treatment where those same horizons were incubated together as a whole organic layer (measured LFH). Values are all given as the mean and standard error (SE) of three sites from the given region (cold, warm).

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Region	Horizon/Experiment	Temperature	Δ C:N		Δ %N as 1	ГНАА	∆ %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	Н	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	Н	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	Н	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	Н	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 δ^{13} C of the soil respired CO₂ at the start of the experiment (initial) and the δ^{13} C of total cumulative respired CO₂ 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).

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Region	Horizon/Experiment	Temperature	δ^{13} C of respired C	δ^{13} C of respired CO ₂		
			mean	SE		
Cold	L	initial	-29.34	0.16		
Cold	F	initial	-27.82	0.48		
Cold	Н	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	Н	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	Н	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	Н	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	Н	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	Н	15	-24.29	0.54		
Warm	LFH predicted	15	-25.09	0.48		
Warm	LFH measured	15	-24.26	0.36		