Supplement of

Soil profile connectivity can impact microbial substrate use, affecting how soil CO$_2$ effluxes are controlled by temperature

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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 ± 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.
**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 ± the standard error with a significant change from 0 denoted by symbol “+”. Any significant (α ≤ 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.
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 (α=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            |
| Grand Codroy (Warm) | CatchAFeeder (CF)     | 51°5'21''N    | 56°12'16''W   | 38            | 2.0           | 1224                      | 489                       | 19421\(^3\)                     | 34.0                            | 2.51                            | 9.7               |
|            | 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 (± standard error) of the change in the soil stable carbon and nitrogen composition (Δ δ\(^{13}\)C and Δ δ\(^{15}\)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 (α=0.05) is denoted in bold.

| Experiment | Horizon | 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 | -0.21 | 0.15 | 0.76 | 0.35 | 0.30 | 0.09 |
|            | F       | -0.72 | 0.67 | -0.59 | 0.32 | 0.84 | 0.35 | 0.45 | 0.95 |
|            | H       | -1.05 | 0.59 | -0.38 | 0.01 | -0.67 | 0.66 | 0.15 | -0.23 |
| Effects    | F       | 3.85  | 0.0615 | 0.34  | 0.5680 |
|            | p       | 0.0615 | 0.1849 | 0.19  | 0.8200 |
|            | F       | 0.42  | 0.5235 | 1.19  | 0.2860 |
|            | p       | 0.1849 | 0.7619 | 0.70  | 0.4800 |
|            | 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
| Horizon | Cold region | Warm region | Cold region | Warm region |
|---------|-------------|-------------|-------------|-------------|
|         | 5°C | 15°C | 5°C | 15°C | 5°C | 15°C | 5°C | 15°C |
| 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 |
| 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       | 6.98  | 0.0178 | 0.00  | 0.9950 |
|         | p       | 0.0178 | 0.5411 | 0.08  | 0.7810 |
|         | R       | 1.89  | 0.1879 | 0.70  | 0.4140 |
|         | E       | 0.30  | 0.5411 | 0.08  | 0.7810 |
|         | T       | 0.90  | 0.70  | 0.4140 | 0.0120 |
|         | 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 |
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.

| 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        | 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        | 0.0         |
| Cold   | F       | 42.3| 1.9        | 42.2        | 1.8       | 14.2         | 0.5         | 28.7        | 2.4        | 8.2        | 0.5        | 0.88        | 0.0         |
| Cold   | H       | 42.8| 1.9        | 38.5        | 1.9       | 16.3         | 0.4         | 27.5        | 1.4        | 9.2        | 0.3        | 0.76        | 0.0         |
| Cold   | LFH     | 44.6| 1.3        | 41.6        | 1.3       | 14.3         | 0.3         | 27.8        | 1.9        | 8.6        | 0.4        | 0.84        | 0.0         |
| Warm   | L       | 39.5| 0.8        | 43.9        | 0.7       | 13.9         | 0.9         | 27.5        | 0.9        | 8.4        | 0.2        | 0.96        | 0.0         |
| Warm   | F       | 34.5| 0.6        | 43.5        | 1.8       | 14.0         | 0.5         | 27.5        | 2.1        | 7.8        | 0.6        | 0.94        | 0.0         |
| Warm   | H       | 31.8| 1.2        | 37.9        | 1.4       | 19.8         | 0.6         | 33.5        | 1.2        | 7.1        | 0.4        | 1.25        | 0.0         |
| Warm   | LFH     | 34.9| 0.6        | 42.5        | 1.4       | 15.0         | 0.3         | 28.6        | 1.6        | 7.8        | 0.5        | 1.00        | 0.0         |
Table S4. The change in the soil carbon to nitrogen ratio (ΔC: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).

| Region | Horizon/Experiment | Temperature | Δ C:N  | Δ %N as THAA | Δ %alkyl-C |
|--------|--------------------|-------------|--------|--------------|------------|
|        |                    |             | mean   | mean         | mean       |
|        |                    |             | SE     | SE           | SE         |
| Cold   | L                  | 5           | -8.86  | 0.65         | -4.47      |
| Cold   | F                  | 5           | -3.63  | -0.79        | -3.68      |
| Cold   | H                  | 5           | -3.24  | 4.45         | 3.82       |
| Cold   | LFH predicted      | 5           | -4.51  | 0.43         | -2.46      |
| Cold   | LFH measured       | 5           | -4.24  | -0.72        | -1.75      |
| Cold   | L                  | 15          | -14.24 | -7.51        | -4.57      |
| Cold   | F                  | 15          | -8.28  | -7.70        | -3.13      |
| Cold   | H                  | 15          | -7.74  | -3.95        | 0.40       |
| Cold   | LFH predicted      | 15          | -9.27  | -6.98        | -2.75      |
| Cold   | LFH measured       | 15          | -9.89  | -3.44        | 1.09       |
| Warm   | L                  | 5           | -4.43  | 6.52         | 8.07       |
| Warm   | F                  | 5           | -2.05  | 1.84         | 6.72       |
| Warm   | H                  | 5           | -0.62  | 3.47         | 4.09       |
| Warm   | LFH predicted      | 5           | -2.22  | 0.65         | -4.53      |
| Warm   | LFH measured       | 5           | -2.47  | 7.04         | 4.09       |
| Warm   | L                  | 15          | -9.27  | -12.39       | -1.04      |
| Warm   | F                  | 15          | 1.05   | 6.19         | 0.46       |
| Warm   | H                  | 15          | -2.44  | 7.03         | 2.11       |
| Warm   | LFH predicted      | 15          | -1.46  | -6.63        | 0.49       |
| Warm   | LFH measured       | 15          | -5.72  | -0.91        | 2.06       |
Table S5. The $\delta^{13}$C of the soil respired CO$_2$ at the start of the experiment (initial) and the $\delta^{13}$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 | $\delta^{13}$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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